Molecular Characteristics and Changing Trend of Methicillin-Resistant Staphylococcus Aureus From Invasive Infections between 2012 and 2018

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Research

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Abstract

Background Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major global problem. The analysis of the molecular characteristics and changing trend of MRSA is essential for the control and treatment of diseases caused by the pathogen.

Methods A total of 162 MRSA isolates from invasive infections between 2012 and 2018 were collected, molecular typing and antimicrobial susceptibility tests to explore its molecular epidemiologic change in a hospital were performed.

Results All of the 162 MRSA isolates (86.4% HA-MRSA and 13.6% CA-MRSA) were divided into 16 different ST and 30 Spa types. The major STs were ST5 (96/162, 59.3%) and the predominant spa type was t311 (83/162, 51.2%). Five SCCmeC types were found and the most common SCCmeC type was type II (101/162, 61.7%). The prevalence of ST5 MRSA gradually declined from 2014 to 2018 but the prevalence of ST59 MRSA significantly increased. At the same time, livestock-associated methicillin-resistant *S. aureus* ST239 and ST9 were detected. 28 isolates were Panton-valentine leucocidin (*pvl*) gene positive (28/162, 17.3%). The most prevalent *pvl*-positive clone was ST59-IVa-t437. Comparing with HA-MRSA, CA-MRSA had a lower probability of ST5 (9.1% vs. 67.1%, P=0.000) but a higher probability of ST59 (63.6% vs. 11.4%, P=0.000), not only that, it was more likely to carrying *pvl*-positive gene (36.4% vs. 14.3%, P=0.028).

Conclusions The molecular types of MRSA were getting complex over time. ST5-II-t311 was the predominant clone of MRSA isolate with a downward incidence from 2012 to 2018. ST59 MRSA strains, which is thought community related strain are spreading into hospitals and has an upward incidence during the investigational period.

Background

*Staphylococcus aureus* is a notorious pathogen which is able to cause widespread infections such as pneumonia, sepsis, endocarditis, toxic shock syndrome, or necrotizing fasciitis. Besides, Methicillin-resistant *S. aureus* (MRSA) infections, which threaten the public health safety of the world, are associated with higher mortality and higher health care costs than infections caused by methicillin-susceptible *S. aureus* (MSSA). According to the surveillances, the prevalence of MRSA in hospital is as high as 70%-80% in many Asian countries. In China, although a decrease of MRSA prevalence was observed in recent years, the high prevalence around 30% of MRSA were still causes major problems. Not only that, due to the continuous evolution of strains, the constant change of medications and the increasing frequency of personnel migration among regions, the MRSA infection becomes more complicated and diverse, which may bring new challenges to the infection management.

By the microbiological and clinical characteristics, community-acquired (CA)-MRSA infection differed from healthcare-associated (HA)-MRSA infection. Over the past decade, CA-MRSA infection have been increasing while HA-MRSA infection declined. In terms of the molecular epidemiology, most CA-MRSA isolates carry SCCmeC IV or V compared with HA-MRSA. And CA-MRSA are considered more virulent because they possess specific virulence factors including Panton-Valentine leucocidin (*pvl*). In addition, the molecular characteristics of MRSA also have significant differences in different regions and change over time. For example, in the United States, ST8 (USA300) has been the most prevalent type while in many Asian countries, ST5 and ST239 were the most common clones which have been observed. Since 2000, the Asian-dominant HA-MRSA ST239 clones have persisted and adaptively evolved in hospital environments for decades. However, a previous study conducted in a general teaching hospital in Shanghai, China has demonstrated that the predominant HA-MRSA clones, ST239-t030 and ST239-t037, were being replaced by the continually growing ST5-t2460 clone in 2017. Therefore, it is valuable to keep abreast of the prevalent strains, the changing trend and drug resistance of MRSA infection.

In this study, we reported an in-depth epidemiological and genomic investigation of MRSA from invasive infections between 2012 to 2018 in a teaching hospital in east China. The aim of this work was to explore the molecular characteristics and changing trend of MRSA isolates so that provide a basis for prevention and treatment of MRSA infections.

Methods

Bacterial Isolates
This study was conducted at the First Affiliated Hospital, College of Medicine, Zhejiang University, a 2500-bed teaching hospital in Eastern China. A total of 162 isolates collected between January 2012 to December 2018 were isolated from invasive infections, including the blood (n = 111), pleural effusion (n = 11), abdominal fluid (n = 19), cerebrospinal fluid (n = 5) and other sterile sources (n = 16). All isolates were confirmed to be \textit{S. aureus} by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and were defined as methicillin resistance by cefoxitin disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) standards\textsuperscript{14}. The investigation was a retrospective study and all patients were anonymized; informed consent was waived.

According to the Centers for Disease Control and Prevention(CDC) criteria of CA-MRSA, cases of CA-MRSA infection defined as cultured <48h after hospital admission or in the outpatient setting, having no history of MRSA infection or colonization, admission to healthcare facility, dialysis, surgery or insertion of indwelling devices in the past one year. Patients were defined as HA-MRSA infection when cultured >48h after hospital admission and contacted with previous healthcare which means MRSA linked to a hospitalization but presenting in the community or at hospital readmission\textsuperscript{15,16}.

\textbf{Antimicrobial susceptibility testing}

The antimicrobial susceptibility of all isolates was evaluated using the microdilution broth method. Results were interpreted in accordance with the CLSI guidelines\textsuperscript{14}. The antimicrobial agents tested included clindamycin (CLI), erythromycin (ERY), gentamicin (GEN), ciprofloxacin (CIP), levofloxacin (LVX), moxifloxacin (MXF), rifampin (RIF), tetracycline (TCY), trimethoprim/sulfamethoxazole (SXT), linezolid (LZD), tigecycline (TGC), vancomycin (VAN). \textit{S. aureus} ATCC 29213 and \textit{S. aureus} ATCC 25923 were used for quality control.

\textbf{DNA extraction}

DNA extraction was performed from pure \textit{S. aureus} cultures after 24 h of incubation at 37°C on Columbia agar + 5% sheep blood (bioMérieux) using QIAamp DNA Mini Kit (QIAGEN, CA, USA) according to the manufacturer’s specifications. Extracted DNA was stored at −20°C for further analyses. All isolated DNA was used as template for all PCR reactions.

\textbf{Confirmation of MRSA and detection of \textit{pvl}}

All \textit{S. aureus} isolates confirmed to be methicillin-resistant by cefoxitin disk diffusion method were collected to test the presence of \textit{mecA} or \textit{mecC}gene as previous described by the means of PCR\textsuperscript{17,18}. All isolates were screened for the Panton-Valentine leucocidin (\textit{PVL}) using the PCR method as previous described\textsuperscript{19}.

\textbf{Molecular typing methods}

Three typing methods were used for all MRSA strains. MLST was carried out by PCR amplification and sequencing of 7 housekeeping genes (\textit{arcC, aroE, glpF, gmk, pta, tpi, yqiL}) according to the method as previous described\textsuperscript{20,21}. The sequences of PCR products were compared with existing sequences available on the MLST website (http://\textit{saureus.mlst.net}) for \textit{S.aureus}, and the allelic number was determined for each sequence. Clustering of related STs that were defined as cloned complexes (CCs) was performed by using the program eBURST algorithm\textsuperscript{22}.

We used a multiplex PCR method to determine the SCC\textit{mec} types\textsuperscript{17}. SCC\textit{mec} types were assigned in accordance with the combination of the cassette chromosome recombinase (\textit{ccr}) type and \textit{mec} class. All MRSA isolates which could not be confirm with any type from I to V were defined as non-typable (NT). \textit{Spa} typing was carried out by amplification and sequencing of polymorphic X region of the protein A gene using the method of PCR\textsuperscript{23}. \textit{Spa} types were assigned by \textit{spa} database website (http://spa.ridom.de) .

\textbf{Statistical Analysis}

Statistical analyses were performed using SPSS Version 23.0 (IBM Corporation, Armonk, NY, USA). The chi-square test was used to compare the proportion of ST59 and ST5 strains, the probability of bloodstream source infection and distribution of virulence genes between CA-MRSA and HA-MRSA. P-values ≤0.05 were considered significant.

\textbf{Results}
Antimicrobial susceptibility

A total of 162 MRSA isolates (140 HA-MRSA and 22 CA-MRSA) were tested for antimicrobial susceptibility. The results showed that all of the isolates were susceptible to vancomycin, tigecycline, and linezolid, high resistance to erythromycin, fluoroquinolones, tetracycline, and clindamycin, lower resistance to gentamicin, rifampin, and sulfamethoxazole/trimethoprim (Table 1).

Among the 162 MRSA isolates, 118 (72.8%) strains were resistant to \( \geq 3 \) antibiotics. 74 (45.7%) strains were resistant to \( \geq 5 \) antibiotics, 11 (6.8%) strains were resistant to \( \geq 7 \) or more antibiotics. Almost 67.7% (65/96) ST5 strains were found to be MDR, defined as having resistance to more than three classes of antibiotics, but less resistance to ST239; ST 59 strains only 8 (8/30, 26.7%) isolates were found to be MDR.

MLST, spa, and SCCmec typing

The 162 MRSA isolates were divided into 16 STs. More and more ST types were found over time, in which the ST types in 2012 and 2018 were 4 and 10, respectively. The three major STs were ST5 (96/162, 59.3%), ST59 (30/162, 18.5%) and ST398 (8/162, 4.9%). Only was 3 ST239 (the former predominant ST type in China) strain detected. 3 ST239 and 1 ST9 (livestock-associated methicillin-resistant \textit{S. aureus}) were also detected. Overall, five SCCmec types, namely, types I, II, III, IVa and V were found. The most common SCCmec type was type II (100/162, 61.7%), followed by SCCmec IVa (32/162, 19.6%). And 22 untypied isolates (21/162; 13.0%) by the multiplex SCCmec typing method were defined as NT. The ST5 most commonly carried with SCCmec II, whereas ST59 carried with SCCmec IVa. Besides, all isolates yielded 30 spa types. The predominant spa type was t311 (83/162, 51.2%), followed by t437 (22/162, 13.6%) and t034 (8/162, 4.9%). The predominant combinations were ST5-t311 (83/162, 51.2%), ST59-t437 (22/162, 13.6%) and ST398-t034 (8/162, 4.9%). Above all, the most common genotype was ST5-II-t311 (81/162, 50.0%), followed by ST59-IVa-t437 (18/162, 11.1%) and ST398-NT-t034 (7/162, 4.3%) (Table 2).

At the same time, a change trend of ST type from 2012 to 2018 was observed. The prevalence of ST5 gradually declined from 2014 (72.2%) to 2018 (32.1%), while the prevalence of ST59 obviously increased from 2014 (5.6%) to 2018 (28.6%) (Figure 1).

Prevalence of \textit{pvl} gene

Only were 28 isolates \textit{pvl} positive (28/162, 17.3%) (Table 1). The most common specimen type was blood (20/28, 71.4%). Among 28 \textit{pvl}-positive isolates, the most prevalent clone was ST59-IVa-t437 (11/28, 39.3%). (Table 2).

Comparison of CA-MRSA and HA-MRSA

CA-MRSA and HA-MRSA in this study showed different molecular epidemiology distribution. 54.3% (76/140) HA-MRSA strains were recognized as MDR while only 27.3% (6/22) CA-MRSA isolates meeting the requirements of MDR. Most of the HA-MRSA strains were SCCmec II (98/140, 70%) while CA-MRSA strains were SCCmec IVa (14/22, 63.6%). Compared with HA-MRSA strains, CA-MRSA was associated with a lower probability of carrying ST5 (9.1% vs. 67.1%, \( P=0.000 \)) and a higher probability of carrying ST59 (63.6% vs. 11.4%, \( P=0.000 \)). Moreover, the probability of having the PVL gene was seen to be significantly higher in patients with CA-MRSA than HA-MRSA (36.4% vs. 14.3%, \( P=0.028 \)) (Table 3).

Discussion

MRSA represented a major problem for public health systems, as they resulted in high incidences both in hospital and community settings\(^{24}\). Previous studies have shown that MRSA infections were mainly caused by a few predominant clones causing outbreak in hospital\(^{25}\). Not only that, the predominant MRSA clones have their regional characters and can change over time\(^{13,26}\). Therefore, the studies analyzed the molecular typing and changing trend of MRSA can clarify the local epidemic clones and provide a basis for rational treatment and effective control of drug resistance.

In this study, one of the most important findings was the molecular type changed among MRSA isolates. The most common MRSA clone was ST5-II-t311 in our study, but the prevalence of ST5 is getting down; at the same time, ST59 is increasing gradually, and ST239 is getting rare.
ST5-II MRSA (NEW York/Japan) clone was one of the most predominant genotypes in many countries\textsuperscript{27,28}. Also, previous study showed that ST5 was the predominant HA-MRSA clone in Zhejiang, China from 2012 to 2013\textsuperscript{29}. Moreover, some investigators have expressed different opinions on the most main spa type of ST5. It has been reported that t002 was main spa type in ST5 clone isolates in previous study\textsuperscript{30}, but ST5-II-t311 has become the predominant HA-MRSA clone in Hangzhou, Zhejiang Province between 2012 to 2013. In our study, we demonstrated that t311 to be dominant in ST5 clonal isolates while t002 was the second spa type, which was similar to previous study\textsuperscript{31}. Notably, the prevalence of ST5-II-t311 decreased from 2014 (12/18, 66.7%) to 2018 (6/28, 21.4%) while ST5-II-t2460 slightly increased. Li et al.\textsuperscript{32} even showed that ST5-t2460 was the most common clone in \textit{S. aureus} causing bloodstream infection, which had never been reported in China before. Therefore, whether the ST5-II-t2460 clone has been conferred more competitive advantages remains bewildering.

ST59 was a major CA-MRSA and the second common MRSA in ours results. Our finding indicated the distinctions between CA-MRSA and HA-MRSA isolates have become blurred and they could be a mutual integration\textsuperscript{29}. Previous studies indicated that ST59 MRSA might spread into hospital from community\textsuperscript{29, 33} . Similarly in the study, the ST59 clone showed a gradually increasing trend to replace ST5, higher SCC\textit{mec}IV/V and \textit{pvl} positive prevalence (14/28, 50%) in ST59 fatherly confirmed the stipulation\textsuperscript{34}. More attention to the dissemination of ST59 in hospitals need.

Of note, previous studies have demonstrated ST239-III-t030 and ST239-III-t037 to be the most prevalent clones of MRSA in China\textsuperscript{34}. However, in our study, we only found 3 isolates with ST239. Similarly, some scholars have found that ST239 was gradually decreasing in China\textsuperscript{31}. The reasons need to be elucidated. Some studies inferred that ST239-t030 MRSA displayed lower growth rate and lower competitive advantage compared to ST59-t437 MRSA. These may also be one of the reasons why ST239 gradually decreased and ST59 gradually increased, although ST239 is a high resistant strain.

A number of STs that were rare in previous reports also caught our attention. As we all know, MRSA sequence type ST398 referred to as LA-MRSA (livestock-associated methicillin-resistant \textit{S. aureus}), from pigs, farmers and environment\textsuperscript{35} has been reported in Europe\textsuperscript{36} and North America\textsuperscript{37}. Interesting, we found eight ST398 (4.9%) isolates identified in our study, which was the third highest among all STs. Not only that, infections caused by ST9 which also belonged to LA-MRSA were seldom reported from human, but mostly in pigs. However, one ST9 isolate were identified by molecular typing in our study, which may suggest that cross-species transmission of the emerging ST9 MRSA between swine and humans.

We acknowledge several limitations in our study. As it was a retrospective study, this study had not collected the clinical data including prognosis, severity of disease and others, which may cause that this study had no information about the relationship between clinical data and molecular characteristics. Secondly, the single center isolates analyzed may influence the representativeness of the study. Finally, we could do more in-depth analysis such as WGS (Whole Genome Sequencing) for special strains including ST398 and ST9.

\textbf{Conclusion}

In conclusion, ST5-II-t311 was the predominant clone of MRSA isolate in our hospital, but showed a downward trend generally from 2012 to 2018. Community-associated ST59 MRSA strains were revealed to have spread into hospitals and there may be an upward trend in the future. Some LA-MRSA may spread into human being. We should regularly monitor the prevalence and drug resistance of methicillin-resistant \textit{Staphylococcus aureus} and take effective measures to prevent and control the occurrence of nosocomial infection.

\textbf{Declarations}

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Author contributions: THX designed the study. YYZ conducted the correlation analysis and prepared the drafts of the manuscript. YW, HX and TTX provided assistance with the study design and statistical analysis. KY, STZ YZZ were responsible for the results interpretation and manuscript review. JRJ and PS helped the identification of bacteria. All authors agree to be accountable for all aspects of the work.

Ethics: Ethics approval was submitted and approved through Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. The consent to participate was waived by our institutional review board since this study was retrospective data collection.

Transparency declarations: No potential conflict of interest was reported by the author(s).

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Tables

Table 1 Antibiotic resistance of methicillin-resistant *Staphylococcus aureus* isolates by ST types

| Molecular type | Isolates (n) | Resistant rates |
|----------------|-------------|-----------------|
|                | CLI (%)     | ERY (%)         | GEN (%) | CIP (%) | LVX (%) | MFX (%) | RIF (%) | TCY (%) | SXT (%) |
| ST5            | 96          | 20.8            | 100.0   | 16.7    | 99.0    | 99.0    | 99.0    | 3.1     | 54.2    | 0.0     |
| ST59           | 30          | 73.3            | 76.7    | 0.0     | 20.0    | 6.7     | 6.7     | 0.0     | 30.0    | 0.0     |
| ST398          | 8           | 25.0            | 50.0    | 0.0     | 12.5    | 12.5    | 12.5    | 12.5    | 12.5    | 0.0     |
| ST630          | 5           | 0.0             | 40.0    | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     |
| ST965          | 4           | 25.0            | 100.0   | 25.0    | 50.0    | 50.0    | 0.0     | 25.0    | 25.0    | 0.0     |
| ST293          | 3           | 66.7            | 100.0   | 66.7    | 66.7    | 66.7    | 66.7    | 66.7    | 33.3    | 0.0     |
| ST1            | 2           | 0.0             | 100.0   | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     |
| ST188          | 2           | 50.0            | 100.0   | 50.0    | 100.0   | 100.0   | 100.0   | 0.0     | 50.0    | 50.0    |
| ST6            | 1           | 0.0             | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     |
| ST7            | 1           | 0.0             | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 100.0   | 0.0     |
| ST9            | 1           | 100.0           | 100.0   | 100.0   | 100.0   | 100.0   | 100.0   | 0.0     | 100.0   | 100.0   |
| ST22           | 1           | 0.0             | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     |
| ST573          | 1           | 0.0             | 100.0   | 0.0     | 100.0   | 100.0   | 100.0   | 0.0     | 0.0     | 0.0     |
| ST764          | 1           | 100.0           | 100.0   | 100.0   | 100.0   | 100.0   | 100.0   | 0.0     | 100.0   | 0.0     |
| ST3193         | 1           | 100.0           | 100.0   | 0.0     | 100.0   | 0.0     | 0.0     | 0.0     | 0.0     | 0.0     |
| Total          | 162         | 31.5            | 88.3    | 14.2    | 69.8    | 66.0    | 66.7    | 3.7     | 42.6    | 3.1     |

*a* Antibiotic-resistant strains included strains that tested as intermediate and resistant by the cefoxitin disk diffusion method. All 162 isolates were susceptible to vancomycin, tigecycline, and linezolid. Abbreviations: CIP, ciprofloxacin; CLI, clindamycin; ERY, erythromycin; GEN, gentamicin; LVX, levofloxacin; MFX, moxifloxacin; RIF, rifampin; TCY, tetracycline; SXT, trimethoprim/sulfamethoxazole

Table 2 Molecular characteristics of 162 MRSA isolates over 7 years.
| ST-SCCmec-spa Type* (strain number) | Year | strain number | 2012 (6) | 2013 (31) | 2014 (18) | 2015 (30) | 2016 (28) | 2017 (21) | 2018 (28) | Total (162) | PVL positive (28) |
|----------------------------------|------|---------------|----------|-----------|-----------|-----------|-----------|-----------|-----------|-------------|------------------|
| ST5(96)                          |      |               | 3(50%)  | 21(67.7%) | 13(72.2%) | 21(70%)  | 19(67.9%) | 10(47.6%) | 9(32.1%)  | 96(59.3%)  | 5(17.9%)         |
| ST5-II-t311(81)                  |      |               | 3(50%)  | 18(58.1%) | 12(66.7%) | 18(60%)  | 16(57.1%) | 8(38.1%)  | 6(21.4%)  | 81(50.0%)  | 4(14.3%)         |
| ST5-II-t002(5)                   |      |               | 1(3.2%) | 1(5.6%)   | 1(3.3%)   | 1(3.6%)  | 1(4.8%)   |           |           | 5(3.1%)    |                 |
| ST5-II-t2460(3)                  |      |               | 1(3.6%) |           |           |           | 2(7.1%)   | 3(1.9%)   | 1(3.6%)   |                 |
| ST5-II-t494(1)                   |      |               | 1(3.2%) |           |           |           |           |           | 1(0.6%)   |                 |
| ST5-II-t2731(1)                  |      |               | 1(3.6%) |           |           |           |           |           | 1(0.6%)   |                 |
| ST5-II-t3235(1)                  |      |               |         |           |           |           |           |           | 1(3.6%)   | 1(0.6%)    |                 |
| ST5-NT-t311(2)                   |      |               |         |           |           |           |           | 2(6.7%)   |           | 2(1.2%)    |                 |
| ST5-IVa-t693(1)                  |      |               |         |           |           |           |           |           | 1(4.8%)   | 1(0.6%)    |                 |
| ST5-V-t319(1)                    |      |               | 1(3.2%) |           |           |           |           |           |           | 1(0.6%)    |                 |
| ST59(30)                         |      |               | 1(16.7%)| 7(22.6%)  | 1(5.6%)  | 2(6.7%)  | 5(17.9%)  | 5(23.8%)  | 8(28.6%)  | 30(18.5%)  | 14(50%)          |
| ST59-IVa-t437(18)                |      |               | 5(16.1%)| 1(3.3%)   | 4(14.3%) | 4(19.0%) | 4(14.3%)  | 18(11.1%) | 8(28.6%)  |                 |                 |
| ST59-IVa-t172(4)                 |      |               | 1(3.2%) | 1(3.3%)   | 1(4.8%)  | 1(3.6%)  | 4(2.5%)   | 1(3.6%)   |           |                 |                 |
| ST59-IVa-t441(2)                 |      |               | 1(5.6%) |           |           |           |           |           | 1(3.6%)   | 2(1.2%)    | 1(3.6%)         |
| ST59-IVa-t163(1)                 |      |               | 1(3.6%) |           |           |           |           |           |           | 1(0.6%)    |                 |
| ST59-NT-t437(3)                  |      |               | 1(16.7%)|           |           |           |           |           | 2(7.1%)   | 3(1.8%)    | 2(7.1%)         |
| ST59-V-t437(1)                   |      |               | 1(3.2%) |           |           |           |           |           | 1(0.6%)   | 1(3.6%)    |                 |
| ST59-V-t4135(1)                  |      |               | 1(3.2%) |           |           |           |           |           | 1(0.6%)   | 1(3.6%)    |                 |
| ST398-NT-t034(7)                 |      |               |         | 3(10%)    | 1(3.6%)  | 3(14.3%) |           |           |           | 7(4.3%)    |                 |
| ST398-II-t034(1)                 |      |               |         |           |           |           |           | 1(3.6%)   | 1(0.6%)   | 1(3.6%)    |                 |
| ST88-NT-t2310(1)                 |      |               |         |           |           |           |           | 1(3.6%)   | 1(0.6%)   | 1(3.6%)    |                 |
| ST88-NT-t7637(1)                 |      |               |         | 1(5.6%)   |           |           |           |           | 1(0.6%)   | 1(3.6%)    |                 |
| Strain   | No. (%) of each molecular type in the year |
|----------|------------------------------------------|
| ST88-II-t15074(1) | 1(3.6%) 1(0.6%) |
| ST88-III-t7637(1) | 1(5.6%) |
| ST88-V-2526(1) | 1(3.6%) 1(0.6%) 1(3.6%) |
| ST630-II-t4549(4) | 2(6.7%) 1(4.8%) 1(3.6%) 4(2.5%) |
| ST630-V-t4549(1) | 1(5.6%) |
| ST965-NT-t062(2) | 1(16.7%) 1(4.8%) 2(1.2%) |
| ST965-IVa-t062(1) | 1(3.6%) 1(0.6%) 1(3.6%) |
| ST965-II-t062(1) | 1(3.6%) 1(0.6%) |
| ST239-III-t030(2) | 1(3.3%) |
| ST239-IVa-t421(1) | 1(3.6%) 1(0.6%) 2(1.2%) |
| ST1-IVa-t127(1) | 1(3.2%) |
| ST1-NT-t127(1) | 1(3.6%) 1(0.6%) |
| ST188-II-t189(1) | 1(16.7%) |
| ST188-IVa-t3887(1) | 1(3.6%) |
| ST6-NT-t701(1) | 1(5.6%) |
| ST7-IVa-t091(1) | 1(3.6%) 1(0.6%) |
| ST9-NT-t899(1) | 1(3.2%) |
| ST22-NT-t309(1) | 1(3.6%) 1(0.6%) |
| ST573-IVa-t127(1) | 1(4.8%) 1(0.6%) |
| ST764-II-t045(1) | 1(3.6%) |
| ST3193-IVa-t172(1) | 1(3.3%) |

*No. (%) of each molecular type in the year; Abbreviations: NT Non-typeable; PVL, Panton-Valentine leucocidin

Table 3 Molecular epidemiological distribution of CA-MRSA and HA-MRSA
|                                | HA-MRSA (n = 140) | CA-MRSA (n = 22) | P-values |
|--------------------------------|-------------------|------------------|----------|
| ST5, n (%)                     | 94 (67.1%)        | 2 (9.1%)         | 0.000    |
| ST59, n (%)                    | 16 (11.4%)        | 14 (63.6%)       | 0.000    |
| PVL-positive, n(%)             | 20 (14.3%)        | 8 (36.4%)        | 0.028    |
| bloodstream infection, n(%)    | 93 (66.4%)        | 18 (81.8%)       | 0.217    |

Data are expressed as numbers (%) unless otherwise stated; Abbreviations: HA, healthcare associated; CA, community-acquired.