Epidemiological and Genetic Diversity of *Staphylococcus aureus* Causing Bloodstream Infection in Shanghai, 2009-2011

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Abstract

**Objectives:** *Staphylococcus aureus* or methicillin-resistant *Staphylococcus aureus* (MRSA) has been an important pathogen causing bloodstream infections. Our study aimed to investigate the epidemiological and genetic diversity of clinical *S. aureus* isolates from patients with bloodstream infection in four hospitals of Shanghai from 2009 to 2011.

**Methods:** A collection of *S. aureus* isolates causing bloodstream infection from four hospitals in the central part of Shanghai was carried out. Antimicrobial susceptibility testings of collected isolates were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, and spa-type, multi-locus sequence typing, *agr* type and toxin gene profiling were performed to explore the molecular diversity. Moreover, MRSA strains were also characterized by Staphylococcal cassette chromosome *mec* (SCCmec) typing.

**Results:** The drugs such as linezolid, teicoplanin and vancomycin were efficacious for treating *S. aureus* including MRSA bloodstream infection. Methicillin-sensitive *Staphylococcus aureus* ( MSSA) strains displayed distinct diversity in molecular characterization and toxin genes, and three virulent MSSA strains encoding at least five toxins were detected. Five community-associated MRSA (CA-MRSA) strains were found, but the majority (88.7%) of MRSA strains belonged to two epidemic clones (ST239-MRSA-III and ST5-MRSA-II) with different toxin gene profiles among patients with bloodstream infection.

**Conclusions:** Healthcare-associated MRSA (HA-MRSA) strains were still the main pathogen causing bloodstream infections in spite of the emergence of CA-MRSA strains in hospital setting.

Introduction

*Staphylococcus aureus* or methicillin-resistant *Staphylococcus aureus* (MRSA) has been a major cause of nosocomial infections [1], and during the last two decades it increasingly causes infections in the community [2]. In the healthcare setting, *S. aureus* may cause wound infections, catheter-related infections, pneumonia, urinary tract infections and bacteremia [1,3], while in the community it usually results in skin and soft-tissue infections (SSTIs), and occasionally necrotizing pneumonia, necrotizing fasciitis and sepsis [2–4]. The first case of MRSA was reported in the 1960s, and now MRSA has reached a high prevalence of invasive infections globally. To make matter worse, patients with invasive MRSA infections such as bloodstream infection (BSI) show a high mortality [5–6]. Therefore, molecular epidemiology, antibiotic resistance pattern, virulence factors and clinical information of *S. aureus* bloodstream infection should be provided to clinicians or healthcare workers to improve prevention, control and treatment.

It is known that various MRSA clones circulate in different countries or regions, and that they differ in antimicrobial resistance pattern, molecular characterization and virulence factors [7]. A variety of genotyping methods have been developed to discriminate strains and understand the epidemiology of MRSA strains [8]. The common methods include multi-locus sequence typing (MLST), Staphylococcal cassette chromosome *mec* (SCCmec) typing.
tying, Staphylococcus protein A gene (spa) typing, accessory gene regulator (agr) typing and toxin gene profiling [9–12]. Above all, a thorough knowledge of S. aureus bloodstream infection will contribute to the clinical practice and outcome of patients.

As reported, the Brazilian or Hungarian clone (ST239) and the New York/Japan clone (ST15) were prevalent in most Asian countries, such as China, Korea and Japan [6]. Also, ST239-MRSA-III and ST3-MRSA-II clones usually cause healthcare-associated infections in China [9–11]. Recently, several studies have focused on the invasive MRSA infections, especially bloodstream infection [5,6,10–12]. However, in Shanghai, one of the major metropolises with a large population of residents and visitors in China, only Song et al analyzed the molecular epidemiology of 103 S. aureus isolated from blood in a hospital during six years [11]. There is a need for more data about the bloodstream infections. In the present study, S. aureus isolates causing bloodstream infections from four hospitals in Shanghai were collected. Antimicrobial susceptibility testing, molecular characterization and toxin gene profiling of these isolates were performed, and the relationship between epidemiological classification of bloodstream infection episodes and corresponding strain type were investigated.

Methods

Study design

From 2009 to 2011, clinical S. aureus isolates which caused a bloodstream infection were collected in four hospitals. The four hospitals (Hospital A to D) located in four different administrative districts in the central part of Shanghai, serving for a total population of around 3.3 million, approximately equivalent to fifteen percent of the whole population in Shanghai. Hospital A, B and C were comprehensive tertiary teaching hospitals, and Hospital D was a tertiary children’s hospital. Totally, in these four hospitals from 2009 to 2011 S. aureus bloodstream infection represented a proportion of 5.7% (327 out of 5712 cases from medical records), among which 108 cases (all available cases during this period) were enrolled in this study. All these isolates were recovered to determine the antimicrobial resistance pattern, spa-type, sequence type (ST), agr type and toxin gene profiling, and the SCCmec type of MRSA strains were also determined to know the circulating clone. This study was approved by Ruijin Hospital Ethics Committee (Shanghai Jiao Tong University School of Medicine), and the Review Board exempted the need for informed consent because this retrospective study mainly focused on bacteria and did no interventions to patients.

Definitions

The S. aureus bloodstream infection was defined by the isolation of S. aureus from blood cultures of patients with/without clinical signs or symptoms, such as fever, chills and sweats. The bloodstream infections were epidemiologically classified as i) healthcare-associated community-onset infection [HACOI], ii) healthcare-associated hospital-onset infection (HAHOI) and iii) community-associated infection (CAI) according the standards of the U.S. Centers for Disease Control and Prevention [5] by checking clinical data. MRSA strains were those expressing meca or another mechanism of methicillin resistance such as changes in affinity of penicillin-binding proteins for oxacillin, and were classified as healthcare-associated MRSA (HA-MRSA) strain type or community-associated MRSA (CA-MRSA) strain type according to their genetic features [13]: According to the situation of Shanghai, HA-MRSA was defined as strains possessing SCCmec I, II or III, and CA-MRSA was defined as strains possessing SCCmec IV or V in this study.

Pathogen, identification and antimicrobial susceptibility testing

Collected isolates were checked by Vitek-2 system and/or phenotypic tests as previously described [4,9,14], and the isolates were recovered to conduct antimicrobial susceptibility testing with the disk diffusion method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) [15]. The drugs tested included penicillin (10 units), oxacillin (1 μg), cefoxitin (30 μg), gentamicin (10 μg), tobramycin (10 μg), erythromycin (15 μg), clindamycin (2 μg), sulfamethoxazole/trimethoprim (25 μg), rifampicin (5 μg), linezolid (30 μg), mupirocin (5 μg), fusidic acid (10 μg) and teicoplanin (30 μg). Minimum inhibitory concentration (MIC) of vancomycin was determined by the agar dilution method [15]. Screening tests for β-lactamase production were performed by the penicillin zone-edge test and nitrocefin-based test if the zone diameter of penicillin indicated sensitivity, and inducible resistance to clindamycin was tested by the D-test. MRSA strains were screened by cefoxitin disk, and high-level mupirocin resistance was screened by the 200 μg mupirocin disk. S. aureus ATCC25923 and ATCC259213 were included for the quality control of the disk diffusion test and MIC detection respectively.

Detecting molecular epidemiologic characters

Bacterial DNA was extracted with the simplified alkaline-lysis method [14]. MRSA strains were verified by the detection of the meca gene [16,17]. The spa repeat region of all isolates was amplified and sequenced [18], and the spa-type was gained via the online database (http://www.spaserver.ridom.de/). The sequence type (ST) was characterized by multi-locus sequence typing (MLST), and the products of seven house-keeping gene fragments were sequenced (Sangon Biotech, Shanghai) and compared with allele profiles from database of S. aureus (http://saureus.mlst.net/). SCCmec typing of MRSA strains was conducted as previously described [14].

Grouping of agr allele and toxin gene profiling by polymerase chain reaction

The accessory gene regulator alleles (agr I–IV) were determined by a reported method [19]. Toxin gene profiles of all isolates were elucidated by detecting a variety of clinically significant toxin genes encoding staphylococcal enterotoxins (sea–see and ase–ase), exfoliative toxin (eta and etb), toxic shock syndrome toxin 1 ( tst) and Panton-Valentine leukocidin (pvl) [19].

Statistical analysis

Statistical data were processed in Excel format, and the univariate comparison was performed using the chi-square or Fisher’s exact test as appropriate. All statistical analysis was conducted by SAS 8.2 (SAS Institute Inc., Cary, NC, USA). It was considered statistically significant if the two-sided P-value <0.05.

Results

Collected isolates and classification of bloodstream infection

A total of 108 non-duplicated S. aureus isolates from 108 patients with bloodstream infection (one isolate from one patient) were enrolled in this study, including 54 from Hospital A, 12 from Hospital B, 26 from Hospital C, 16 from Hospital D. By
retrospectively reviewing clinical data, of these 108 BSI episodes 100 episodes were defined as healthcare-associated infections including 77 episodes as healthcare-associated hospital-onset infection (HAHOI) and 23 episodes as healthcare-associated community-onset infection (HACOI), and 8 episodes were defined as community-associated infection (CAI) according the standards of the U.S. Centers for Disease Control and Prevention as previously described [5].

Antimicrobial susceptibility testing

Sixty-two (57.4%) out of 108 S. aureus isolates were identified as MRSA by cefoxitin disk screening and mecA gene confirming. As shown in Table 1, all isolates were sensitive to linezolid, teicoplanin and vancomycin. Among MSSA and MRSA the minimum inhibitory concentration (MIC) of vancomycin both ranged from 0.5–2 µg/ml, meanwhile MIC50 and MIC90 values of MSSA were both 1 µg/ml, which were the same as those of MRSA (Table 1). Only 3 isolates were sensitive to penicillin with β-lactamase negative. The rates of resistance to other drugs varied from 11.1% to 62.0%. Moreover, inducible resistance to clindamycin was found among 6 isolates (5.6%) by the D-test, and also 7 isolates were showing high-level mupirocin resistance (Details in Table 1).

Strain type and its relationship with classification of BSI

Among 62 (57.4%) mecA-positive MRSA, the SCCmec typing revealed four types of SCCmec including SCCmec II (18, 29.0%), SCCmec III (39, 62.9%), SCCmec IV (2, 3.2%) and SCCmec V (3, 4.8%), thus indicating that 5 CA-MRSA strains and 57 HA-MRSA strains were detected according to genetic features as described previously. Furthermore, the relationship between epidemiological classification of 108 bloodstream infection episodes and corresponding strain type was explored and displayed in Table 2. HAHOI episodes were caused by 52 HA-MRSA strains, 1 CA-MRSA strain and 24 MSSA strains, while HACOI episodes were brought by 5 HA-MRSA strains, 3 CA-MRSA strains and 15 MSSA strains, respectively. CAI was found to be caused by 1 CA-MRSA strain and 7 MSSA strains.

Molecular epidemiological characteristics and toxin genes

A total of 39 different spa-types and 23 sequence types (STs) were found among all isolates. Grouping of agr allele indicated that agr I to IV was detected in 68, 27, 6 and 2 isolates respectively, and 5 isolates were negative for all the four agr alleles. By detecting 13 kinds of clinically significant toxin genes, the prevalence of these genes among S. aureus isolates was obtained (Table 3). The sei, seg, set genes were most prevalent (37.0%, 25.0% and 20.4%, respectively), and only one MSSA isolate was found both eta- and etb-positive. Thirteen (12.0%) isolates including 3 MSSA and 10 MRSA carried a t1 genotype, and three isolates (2 MSSA and 1 MRSA) were found to be pvl-positive. One toxin gene (lgt) was not detected among all collected isolates, and other four toxin genes (sed, sej, eta and etb) were not found in the MRSA strains. Statistical analysis suggested that there was no significant difference among detected toxin genes between MSSA and MRSA strains.

The combinative data of phenotypic resistance pattern, spa-type, sequence type (ST), agr allele and toxin gene profile of MSSA strains indicated that MSSA exhibited great diversity in both genotypes and toxin genes (Table 4). Thirty spa-types were detected in 46 MSSA strains, and nearly one combination of phenotypic resistance pattern, spa-type, agr allele and toxin gene profile corresponded to one strain. Still, three MSSA strains (ST30-t318, ST5-t045 and ST5-t306) were more outstanding by encoding at least five toxins. Meanwhile, one eta- and etb-positive MSSA strain (a new spa-type designated t11685, ST2073 with agr IV) was found (Table 4).

And in Table 5, the phenotypic resistance pattern indicated that 61 out of 62 (98.4%) MRSA strains were resistant to at least three classes of antibiotics, which were termed multi-drug resistance. To further explore epidemiological MRSA clones among patients with bloodstream infection, analysis of combined molecular data showed nine MRSA clones circulating among patients with bloodstream infections and that 88.7% of MRSA isolates belonged to two prevalent MRSA clones (ST239-MRSA-III and ST5-MRSA-II). Interestingly, the two major clones were prevalent among Hospital A–C except Hospital D, and diverse MRSA clones (ST59-MRSA-II, ST239-MRSA-III, ST5-MRSA-IV and ST7-MRSA-V) were observed in the paediatric Hospital D. In five CA-
MRSA strains/clones detected in this study, only one was found \( pvl \)-positive, which was t437-ST59-MRSA-V (\( agr \) I). Given the toxin gene profile, MRSA strains harboring SCC\textit{mec} II mainly secreted enterotoxin G and I, while those harboring SCC\textit{mec} III were apt to express enterotoxin A and I. Worth to be noted, among MRSA the \( tst \) gene was only found in t002-ST5-MRSA-II strains, which belonged to a epidemic clone from patients with BSI.

## Discussion

\textit{Staphylococcus aureus} or methicillin-resistant \textit{Staphylococcus aureus} has been a leading cause of infections in both hospital and community settings, and bloodstream infection caused by MRSA was one of the most serious infections with high mortality [20–22]. In China, \textit{S. aureus} was one of the leading pathogens isolated from bloodstream, and the average prevalence of MRSA among \textit{S. aureus} BSI isolates reached up to 40% [10–11]. Among the four hospitals included in this study the prevalence of \textit{S. aureus} from patients with bloodstream infection was about 6%, and the high percentage of multi-drug resistant MRSA isolated from blood warned narrow spectrum of antibiotics for empirical treatment. Antimicrobial susceptibility test of all collected isolates suggested that linezolid, teicoplanin and vancomycin were efficacious drugs for treating \textit{S. aureus} including MRSA bloodstream infections. However, by reviewing clinical data, the drugs used as empirical treatment for bloodstream infections displayed great diversity according to different physicians from varied wards or hospitals. Glycopeptides such as vancomycin were still the most widely used antibiotic for confirmed MRSA bloodstream infection, as recommended by the IDSA guidelines [23]. Despite sequential reports on linezolid-resistant \textit{S. aureus} [24–26], no isolates were found to be resistant to linezolid in our study, implying that linezolid may be still effective for treating MRSA infections in Shanghai. On other hand, linezolid resistance should be monitored in case of the emergency.

Exploration of the relationship between epidemiological classification of 108 bloodstream infection episodes and corresponding strain type revealed that HA-MRSA strains were still the main pathogen causing healthcare-associated hospital-onset infection (HAHOI), and only one episode of HAHOI was found to be caused by CA-MRSA, suggesting CA-MRSA was not prevalent in hospitals for all its emergence in hospital settings. MSSA strains could lead to both healthcare-associated and community-associated infections. Nevertheless, MSSA strains displayed great diversity in both genotypes and toxin genes. By detection of the \textit{mec}A gene, 62 MRSA strains were found. Molecular characterization analysis was further performed to shed light on the molecular epidemiology of MRSA strains causing bloodstream infection. Five CA-MRSA strains (2 SCC\textit{mec} IV and 3 SCC\textit{mec} V) were found, which meant CA-MRSA strains emerged in hospital setting in Shanghai, and the five CA-MRSA strains showed genetic diversity in clones (ST59-MRSA-IV, ST3-MRSA- IV, ST3-MRSA-V, ST59-MRSA-V and ST7-MRSA-V). The main genotype among SCC\textit{mec} II strains was ST5 (t002, \( agr \) II), whilst ST239 (t030 or t037, \( agr \) I) was the main genotype among SCC\textit{mec} III strains. Song et al analyzed the 103 \textit{S. aureus} isolated from blood in a hospital of Shanghai during six years, and concluded that the percentage of ST5 and ST239 decreased among MRSA strains and that of new MSSA clonal types increased [11]. Nevertheless, the predominance of two MRSA clones (ST239-MRSA- III and ST5-MRSA- II) was observed in this study, which was consistent with a recent study in Taiwan [27]. Li et al reported that a new mobile genetic element-encoded gene (\textit{sasX}) played an important role in MRSA epidemic (especially ST239 clone) and \textit{sasX} acted as a virulence determinant [28]. By checking the carriage of this new gene, we found that 15 out of 108 isolates possessed the \textit{sasX} gene. Moreover, of the 15 \textit{sasX}-positive isolates, 13 isolates belonged to ST239 MRSA, which confirmed the report by Li et al [28]. Virulence gene profiling of MRSA strains showed that five kinds of toxin genes (\textit{sed, see, sje, eta} and \textit{etb}) were not found. Notably, the \textit{tst} gene responsible for fatal toxic shock syndrome (TSS) was only found in t002-ST3-SCC\textit{mec} II MRSA strains, suggesting that patients infected with t002-ST3-SCC\textit{mec} II MRSA may have a greater potential for developing TSS.

### Table 3. Prevalence of toxin genes among \textit{S. aureus} causing bloodstream infection.

| Toxin gene | No. of positive isolates (% of 108) | No. distributing in | P-value |
|------------|-----------------------------------|--------------------|--------|
| \textit{sea} | 22 (20.4) | MSSA (n = 46) 7 15 | 0.2521 |
| \textit{seb} | 7 (6.5) | MRSA (n = 62) 2 5 | 0.7035 |
| \textit{sec} | 16 (14.8) | MSSA (n = 46) 8 8 | 0.5162 |
| \textit{sed} | 2 (1.9) | MRSA (n = 62) 2 0 | 0.1791 |
| \textit{see} | 0 (0.0) | - | - |
| \textit{seg} | 27 (25.0) | MSSA (n = 46) 11 16 | 0.8222 |
| \textit{seh} | 7 (6.5) | MRSA (n = 62) 3 4 | 1.0000 |
| \textit{sei} | 40 (37.0) | MSSA (n = 46) 15 25 | 0.4117 |
| \textit{sej} | 2 (1.9) | MRSA (n = 62) 2 0 | 0.1791 |
| \textit{eta} | 1 (0.9) | MSSA (n = 46) 1 0 | 0.4259 |
| \textit{etb} | 1 (0.9) | MRSA (n = 62) 1 0 | 0.4259 |
| \textit{tst} | 13 (12.0) | MRSA (n = 62) 3 10 | 0.1292 |
| \textit{pvl} | 3 (2.8) | MRSA (n = 62) 2 1 | 0.7924 |

\( \text{sea-see and seg-sej, gene encoding staphylococcal enterotoxins SEA-SEE and SEG-SEJ; eta and etb, gene encoding exfoliative toxin A and B; tst, gene encoding toxic shock syndrome toxin 1; pvl, gene encoding Panton-Valentine leukocidin. P-value, two-sided P-value calculated by the chi-square or Fisher’s exact test as appropriate.} \)

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Table 4. Phenotypic resistance pattern, spa-type, sequence type (ST), agr allele and toxin gene profiles among MSSA strains.

| Phenotypic resistance pattern | spa-type | ST | agr allele | Toxin gene profile | No. of strains |
|------------------------------|----------|----|------------|--------------------|----------------|
| None                         | t267     | 97 | I          | none               | 1              |
| None                         | t11666   | 1956 | IV | none       | 1              |
| E,CC                         | t321     | 1  | III        | sea,sec,seh        | 1              |
| P                            | t062     | 5  | II         | seg,sei           | 1              |
| P                            | t078     | 25 | I          | seb,sec           | 1              |
| P                            | t084     | 15 | II         | none               | 1              |
| P                            | t164     | 20 | I          | sei                | 2              |
| P                            | t189     | 188 | I          | seb                | 1              |
| P                            | t195     | 20 | I          | sei                | 1              |
| P                            | t304     | 6  | I          | sea                | 1              |
| P                            | t318     | 30 | III        | sec,seg,sei,tst,pvl| 1              |
| P                            | t701     | 6  | I          | sea                | 1              |
| P,CC,RA                      | t11687   | 573 | II         | sec,sei           | 1              |
| P,CC,RA                      | NT       | 1667 | NT        | none               | 1              |
| P,CC,RA,TOB,MUP,FD           | t5554    | 630 | I          | none               | 1              |
| P,CC,RA,TOB,E,CC             | t571     | 398 | I          | none               | 1              |
| P,CC,RA,TOB,E,SXT            | t037     | 241 | I          | sea                | 1              |
| P,E                          | t034     | 2077 | I         | pví                | 1              |
| P,E                          | t164     | 20 | I          | sei                | 1              |
| P,E                          | t491     | 15 | II         | none               | 1              |
| P,E                          | t571     | 2077 | I         | none               | 1              |
| P,E                          | t701     | 6  | I          | sea                | 1              |
| P,E                          | t11687   | 573 | II         | sec,sei           | 1              |
| P,E                          | NT       | 1667 | NT        | none               | 1              |
| P,E                          | t045     | 5  | II         | sea,sec,seg,sei,tst| 1              |
| P,E                          | t803     | 15 | II         | none               | 1              |
| P,FD                         | t377     | 630 | I          | none               | 1              |
| P,MUP                        | t034     | 398 | I          | none               | 1              |
| P,RA,MUP                     | NT       | 30 | III        | sec,seg,sei,tst    | 1              |
| P,RA,MUP                     | t062     | 5  | II         | seg,sei           | 1              |
| P,RA,MUP,FD                  | t548     | 5  | II         | sec,seg,sei,sej   | 1              |
| P,SXT,RA,MUP                 | t377     | 1821 | I         | none               | 1              |
| P,SXT,RA,MUP                 | t1346    | 72  | I          | sec,sei           | 1              |
| P,TOB                        | t084     | 15 | II         | none               | 1              |
| P,TOB                        | t091     | 7  | I          | none               | 2              |
| P,TOB                        | t091     | 306 | I          | sea                | 1              |
| P,TOB                        | t2616    | 7  | I          | none               | 1              |
| P,TOB                        | t3386    | 630 | I          | none               | 1              |
| P,TOB                        | t377     | 630 | I          | none               | 1              |
| P,TOE                        | t091     | 7  | I          | sea                | 1              |
| P,TOE                        | t306     | 5  | II         | sec,seg,sei,sej   | 1              |
| P,TOE                        | t571     | 398 | I          | none               | 1              |
| P,TOE,CC                     | t571     | 398 | I          | none               | 1              |
| Total                        | 46       |     |            |                    |                |

P, penicillin (10 units); OX, oxacillin (1 μg); CX, cefoxitin (30 μg); CN, gentamicin (10 μg); TOB, tobramycin (10 μg); E, erythromycin (15 μg); CC, clindamycin (2 μg); SXT, sulfamethoxazole/trimethoprim (25 μg); RA, rifampicin (5 μg); LZD, linezolid (30 μg); MUP, mupirocin (5 μg); FD, fusidic acid (10 μg); None, sensitive to all tested drugs.

*spa*, Staphylococcus protein A gene; *agr*, accessory gene regulator; NT, not-typeable; *sea*, *sec* and *seg*, gene encoding staphylococcal enterotoxins SEA–SEE and SEG–SEJ; *eta* and *etb*, gene encoding exfoliative toxin A and B; *tst*, gene encoding toxic shock syndrome toxin 1; *pví*, gene encoding Panton-Valentine leukocidin; none, no detection of above toxin genes.

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In the present study about one third (108/327) of S. aureus from 2009 to 2011 were included, and the isolates was not proportional to the number of cases in each hospital, therefore accurate prevalence rate of MRSA bloodstream infection cannot be concluded owing to the potential bias. Our study just illustrated the overall bloodstream infection caused by S. aureus or MRSA in the central part of Shanghai; two main MRSA clones (ST239-MRSA-III and ST5-MRSA-II) were prevalent among patients

| ST1-SCCmecII/t127 (1)       | Phenotypic resistance pattern | agr allele | Toxin gene profile | No. of strains |
|-----------------------------|------------------------------|------------|--------------------|----------------|
| ST239-SCCmecII/t030 (22)    | P.OX,CX                      | III        | sec,seh            | 1              |
|                            | P.OX,CX,TOB,EC              | I         | none               | 8              |
|                            | P.OX,CX,TOB,EC              | I         | sea                | 1              |
|                            | P.OX,CX,TOB,EC              | I         | sea,sei            | 1              |
|                            | P.OX,CX,TOB,EC,RA,MUP       | I         | none               | 1              |
|                            | P.OX,CX,TOB,EC              | I         | none               | 1              |
|                            | P.OX,CX,TOB,RA              | I         | sea                | 1              |
|                            | P.OX,CX,TOB,RA              | I         | sea,sei            | 4              |
|                            | P.OX,CX,TOB,RA              | I         | seg                | 1              |
|                            | P.OX,CX,TOB,RA              | I         | none               | 1              |
|                            | P.OX,CX,TOB,RA              | I         | sea                | 1              |
|                            | P.OX,CX,TOB,RA              | I         | sea,sei            | 4              |
|                            | P.OX,CX,TOB,RA              | I         | seg                | 1              |
|                            | P.OX,CX,TOB,RA              | I         | none               | 1              |
|                            | P.OX,CX,TOB,RA              | I         | sea                | 1              |

| ST239-SCCmecII/t037 (13)    | P.OX,CX,EC                  | I         | none               | 1              |
|                            | P.OX,CX,TOB,EC              | I         | seq,seh            | 1              |
|                            | P.OX,CX,TOB,EC              | I         | none               | 3              |
|                            | P.OX,CX,TOB,EC              | I         | sea,sei            | 3              |
|                            | P.OX,CX,TOB,EC              | NT        | none               | 2              |
|                            | P.OX,CX,TOB,EC              | I         | none               | 1              |
|                            | P.OX,CX,TOB,EC              | I         | sea                | 1              |
|                            | P.OX,CX,TOB,EC              | I         | none               | 1              |
|                            | P.OX,CX,TOB,EC              | I         | sea,sei            | 1              |
|                            | P.OX,CX,TOB,EC              | I         | none               | 1              |
|                            | P.OX,CX,TOB,EC              | I         | seq,seh            | 1              |
|                            | P.OX,CX,TOB,EC              | I         | seq,sei            | 1              |
|                            | P.OX,CX,TOB,EC              | I         | seq,sei, tst       | 2              |
|                            | P.OX,CX,TOB,EC              | I         | seg,sei            | 1              |
|                            | P.OX,CX,TOB,EC              | I         | seq,sei, tst       | 4              |
|                            | P.OX,CX,TOB,EC              | NT        | sec                | 1              |
|                            | P.OX,CX,TOB,EC              | II        | seb,seg,sei        | 1              |
|                            | P.OX,CX,TOB,EC              | II        | sec,seg,sei,tst    | 2              |
|                            | P.OX,CX,TOB,EC              | II        | seg,sei            | 1              |
|                            | P.OX,CX,TOB,EC              | II        | seq,sei, tst       | 4              |
|                            | P.OX,CX,TOB,EC              | II        | seb,seg,sei        | 1              |
|                            | P.OX,CX,TOB,EC              | II        | seq,seg,sei,tst    | 1              |
|                            | P.OX,CX,TOB,EC              | II        | seq,seg,sei,tst    | 1              |

| ST5-SCCmecII/t463 (1)       | P.OX,CX,TOB,RA,FD           | II         | sei                | 1              |
|                            | P.OX,CX,TOB,RA              | II         | seg,sei            | 1              |
|                            | P.OX,CX,TOB,RA              | II         | seb                | 1              |
|                            | P.OX,CX,TOB,RA              | II         | sei                | 1              |
|                            | P.OX,CX,TOB,RA              | II         | none               | 1              |
|                            | P.OX,CX,TOB,RA              | I         | seb,pvl            | 1              |

Clone, ST-SCCmec; ST, sequence type by multi-locus sequence typing; SCCmec, Staphylococcal cassette chromosome mec; spa, Staphylococcus protein A gene; agr, accessory gene regulator; NT, not-typeable.

P, penicillin (10 units); OX, oxacillin (1 μg); CX, cefoxitin (30 μg); CN, gentamicin (10 μg); TOB, tobramycin (10 μg); E, erythromycin (15 μg); CC, clindamycin (2 μg); SXT, sulfamethoxazole/trimethoprim (25 μg); RA, rifampicin (5 μg); LZD, linezolid (30 μg); MUP, mupirocin (5 μg); FD, fusidic acid (10 μg).

sea-see and seg-sej, gene encoding staphylococcal enterotoxins SEA-SEE and SEG-SEJ; eta and etb, gene encoding exfoliative toxin A and B; tst, gene encoding toxic shock syndrome toxin 1; pvl, gene encoding Panton-Valentine leukocidin; none, no detection of above toxin genes.

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In the present study about one third (108/327) of S. aureus from 2009 to 2011 were included, and the isolates was not proportional to the number of cases in each hospital, therefore accurate prevalence rate of MRSA bloodstream infection cannot be concluded owing to the potential bias. Our study just illustrated the overall bloodstream infection caused by S. aureus or MRSA in the central part of Shanghai; two main MRSA clones (ST239-MRSA-III and ST5-MRSA-II) were prevalent among patients
with bloodstream infections, whilst five CA-MRSA clones (ST59-MRSA-IV, ST5-MRSA-IV, ST33-MRSA-V, ST59-MRSA-V and ST7-MRSA-V) were found. HA-MRSA strains were still the main pathogen causing healthcare-associated bloodstream infections, despite the emergence of CA-MRSA strains in hospital setting. The antibiotics such as linezolid, teicoplanin and vancomycin were efficacious drugs for treating S. aureus including MRSA bloodstream infections.

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Author Contributions

Conceived and designed the experiments: LZH XC. Performed the experiments: XC YL HZ JT QZL YCH. Analyzed the data: WKW YXN. Contributed reagents/materials/analysis tools: LLo (Queen Mary hospital, The University of Hong Kong) for their kind support and cooperation in collecting clinical data. The authors also would like to thank Pak-Leung Ho and Wai-U Lo (Queen Mary hospital, The University of Hong Kong) for their professional suggestions.

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