

## Original Article

# Molecular characterization of community-acquired methicillin-resistant *Staphylococcus aureus* isolated from patients with skin and soft tissue infection in a teaching hospital

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**Abstract:** *Staphylococcus aureus* (*S. aureus*) particularly community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is the predominant cause of skin and soft tissue infections (SSTIs) worldwide. This study examined the molecular characteristics and genotypes of CA-MRSA isolated from patients suffering from SSTIs. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were separated from secretions or pus of skin lesions of patients between 2008 and 2011 from Sun Yat-Sen Memorial Hospital. Identification of *S. aureus* isolates was performed by using a Vitek®2 microbial identification system. Genotypic analysis included pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and Panton-Valentine leukocidin (*pvl*) gene detection. Overall, 588 *S. aureus* from 516 patients were enrolled. 13 MRSA isolates (13/516, 2.5%) were CA-MRSA. The PFGE analysis grouped 13 isolates into two clusters (pulsotype A and B). Sequence type (ST) 239 was the most prevalent among the 6 observed STs, accounting for 46.2% (6/13, 46.2%), respectively. The other STs were ST59 (3/13), ST7 (1/13), ST338 (1/13), ST45 (1/13) and ST398 (1/13). SCC*mec* typing revealed that the common SCC*mec* type was type III (6/13) and type IV (5/13, including type IVa 2 isolates and type IVd 3 isolates). 53.8% (7/13) of isolates carried *pvl* gene. These data indicate ST239-SCC*mec* III and ST59-SCC*mec* IV was the most prevalent clone isolated from patients suffering from SSTIs. It suggested that hospital-acquired MRSA may be slowly spreading from hospital to community. We strongly advocate for more surveillance studies on MRSA in the community.

**Keywords:** MRSA, multiple drug resistance, community-acquired infections

## Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), which can colonize and cause various infections, has been considered as an important multidrug-resistant organism clinically since the 1980s. MRSA strains were previously known for being associated with hospital-acquired MRSA (HA-MRSA). However, it is reported that community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA), which was recognized worldwide in the late 1990s, is more variable than HA-MRSA because of the small staphylococcal cassette chromosome *mec* (SCC*mec*) types (types IV or V) in MRSA [1]. Therefore, further epidemiology monitoring of the CA-MRSA would be necessary

in China. Among CA-MRSA infections, the most common type was skin and soft tissue infection (SSTIs).

Skin is a barrier that maintains homeostasis and prevents microorganism invasion. Many local and systemic infections following trauma are often related to loss of skin barrier. Numbers of reports even supposed that the most common isolate in wound infection was *S. aureus* which accounted for 20-40% of the nosocomial infection [2, 3]. The emergence of multi-drug resistant *S. aureus* even MRSA has made the wound healing increasingly worrisome.

Molecular typing of *S. aureus* is also helpful for supporting infection control measures, investi-

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gating suspected outbreaks, and preventing nosocomial transmission. Analysis of the genotypic characteristics of MRSA clones usually uses methods including pulsed-field gel electrophoresis patterns (PFGE), carriage of the genes encoding Panton-Valentine leukocidin (*pvl*), *SCCmec* typing [3], Staphylococcus protein A gene (*spa*) typing and multilocus sequence typing (MLST) [4]. In the current study, we applied PFGE, MLST, *SCCmec* and *pvl* in CA-MRSA isolates collected from patients suffering from SSTIs.

### Methodology

#### *Clinical isolates and culture conditions*

This study was conducted at the Sun Yat-Sen Memorial Hospital, Sun Yat-sen University, a teaching hospital located in Guangzhou, China. 588 *S. aureus* were isolated from secretions or pus of 516 patients with SSTIs between 2008 and 2011.

A standardized questionnaire was used to collect patient demographics, history, and underlying medical conditions. CA-MRSA infection was defined by outpatient presentation and absence of healthcare exposures including history of hospitalization, surgery, or dialysis within the past 12 months; presence of any dwelling catheter or percutaneous device (e.g., urinary catheter) at the time of presentation; and residence in a nursing home. Patients with any of the above healthcare risk were designed as HA-MRSA infection. The specimens were processed at the hospital laboratories according to standard methods. The wound swabs were obtained and cultured on blood agar plates. Gram-positive,  $\beta$ -hemolytic, and coagulase-positive isolates were confirmed as *S. aureus* using a Vitek®2 microbiology analyzer (bioMérieux, Marcy l'Etoile, France). All isolates were stored at -80°C in a fat-free milk preservation medium until analyses were performed.

#### *Antimicrobial susceptibility testing*

Antimicrobial susceptibility testing was performed by the disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI; <http://clsi.org>) recommendation (M100-S23). Inducible clindamycin resistance was tested by the D-zone disk diffusion test. Methicillin resistance was screened

by cefoxitin discs, and confirmed by *mecA* polymerase chain reaction (PCR). All disks were obtained from Oxoid Ltd., and *S. aureus* ATCC 25923 was used as a quality control strain.

#### *DNA extraction*

Genomic DNA was extracted from MRSA isolates and stored at -80°C prior to testing by using genomic DNA extraction kit (TIANGEN Biotech, Beijing, Co., Ltd) according to the manufacturer's instructions.

#### *Detection of *mecA* gene and virulence *pvl* gene*

PCR amplifications was performed for detecting both *mecA* gene and virulence *pvl* gene using a procedure previously described [5].

#### *Staphylococcal cassette chromosome *mec* (*SCCmec*) typing*

*SCCmec* typing of MRSA isolates was performed using eight unique pairs of primers specific for *SCCmec* types and subtypes I, II, III, IVa, IVb, IVc, IVd, and V, as described previously [6]. Positive control strains for *SCCmec* types I (NCTC 10442), II (N315), III (85/2082), and IVa (JCSC 4744), were kindly provided by Dr. Fangyou Yu of the Department of Laboratory Medicine, the First Affiliated Hospital of Wenzhou Medical College.

#### *Multilocus sequence typing (MLST)*

13 MRSA isolates were investigated by MLST. MLST was performed as described previously [7], and the sequences of the PCR products were compared with an MLST database (<http://saureus.mlst.net>). eBURST software was used to cluster related sequence types, which were defined as clonal complexes (CCs) ([http://eburst.mlst.net/v3/enter\\_data/single](http://eburst.mlst.net/v3/enter_data/single)). STs that grouped together with  $\geq 70$  % bootstrap support were considered part of the same CC.

#### *Pulsed-field gel electrophoresis (PFGE)*

The clonal relationship of 13 isolates was assessed by PFGE using *SmaI* enzyme as previously described [8]. The genotypes were given in alphabetical order. The PFGE types were defined according to the criteria of Tenover *et al.* [9]. Salmonella serotype Braenderup H9812 was the reference strain. The isolates with

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**Table 1.** Clinical and molecular features of 13 cases with CA-MRSA infections

| Case | Age  | Gender | Infection             | Specimen type | Resistance profile     | <i>pvl</i> | SCC <i>mec</i> | MLST | CC  |
|------|------|--------|-----------------------|---------------|------------------------|------------|----------------|------|-----|
| 1    | 70 y | Male   | Cellulitis            | Pus           | ERY, DA                | -          | III            | 239  | 8   |
| 2    | 56 y | Male   | Pemphigus             | Secretion     | ERY, DA, SXT, CIP, GEN | +          | III            | 239  | 8   |
| 3    | 45 y | Male   | Pemphigus             | Secretion     | ERY, DA, SXT, CIP, GEN | -          | III            | 239  | 8   |
| 4    | 71 y | Female | Wound infection       | Abscess       | ERY, DA                | -          | IVd            | 338  | 59  |
| 5    | 81 y | Male   | Eczema                | Secretion     | ERY, DA, SXT, CIP, GEN | -          | III            | 239  | 8   |
| 6    | 7 y  | Female | Atopic dermatitis     | Secretion     | ERY, DA                | -          | IVa            | 7    | 7   |
| 7    | 36 y | Female | Pyoderma gangrenosum  | Secretion     | ERY, DA, SXT, CIP, GEN | -          | III            | 239  | 8   |
| 8    | 58 y | Female | Pemphigus             | Secretion     | ERY, DA                | +          | IVd            | 59   | 59  |
| 9    | 37 y | Female | Subcutaneous granulom | Abscess       | ERY, DA                | +          | IVd            | 59   | 59  |
| 10   | 38 y | Female | Wound                 | Abscess       | ERY, DA                | +          | I              | 45   | 45  |
| 11   | 70 y | Male   | Cellulitis            | Pus           | ERY, DA                | +          | IVa            | 59   | 59  |
| 12   | 11 d | Female | Cellulitis            | Pus           | ERY, DA, CIP           | +          | I              | 239  | 8   |
| 13   | 57 y | Female | Cellulitis            | Pus           | ERY, DA, SXT           | +          | III            | 398  | 398 |

*pvl*, Panton-Valentine leukocidin; SCC*mec*, staphylococcal cassette chromosome *mec*; MLST, multilocus sequence typing; CC, clonal complex; CIP, Ciprofloxacin; DA, Clindamycin; ERY, Erythromycin; GEN, Gentamicin; SXT, Trimethoprim/sulfamethoxazole. -, negative; +, positive.

>75% similarity were clustered in patterns. The results were also analyzed by the BioNumerics 5.01 statistical software, generating dendrograms according to a simple matching coefficient and the unweighted pair group method with arithmetic mean (UPGMA) algorithm.

### Result

#### Demographics

588 *S. aureus* isolates were obtained from 516 patients with SSTIs, including 402 MSSA from 370 patients, 186 MRSA from 146 patients. MRSA was isolated from 21.2% (31/146) of abscesses, 51.4% (75/146) of infected wounds, and 27.4% (40/146) of cellulitis with purulent discharge. According to the epidemiological definition, CA-MRSA infection was defined as MRSA infection occurring in the community or less than 48 hour after hospital admission in patients without healthcare-associated (HA) risk factors [10]. 13 CA-MRSA isolates were screened out for this study. The 13 patients with CA-MRSA infections occurred during work or daily activities without any identifiable risk factors. All of the 13 patients were treated with antibiotics and surgical drainage. 12 cases were cured, only a 57-year-old female who developed spontaneous abscesses on her chest expired 12 days after admission. The clinical and molecular characteristics of 13 CA-MRSA isolates are shown in **Table 1**.

#### Antimicrobial susceptibility

All of 13 MRSA isolates in this study possessed *mecA* genes. All clinical isolates were susceptible to vancomycin, teicoplanin, rifampicin and Linezolid, and resistant to erythromycin and clindamycin. Rates of resistance to gentamicin, ciprofloxacin and sulfamethoxazole/trimethoprim were 30.8%, 30.8% and 38.5%, respectively (**Figure 1** and **Table 1**).

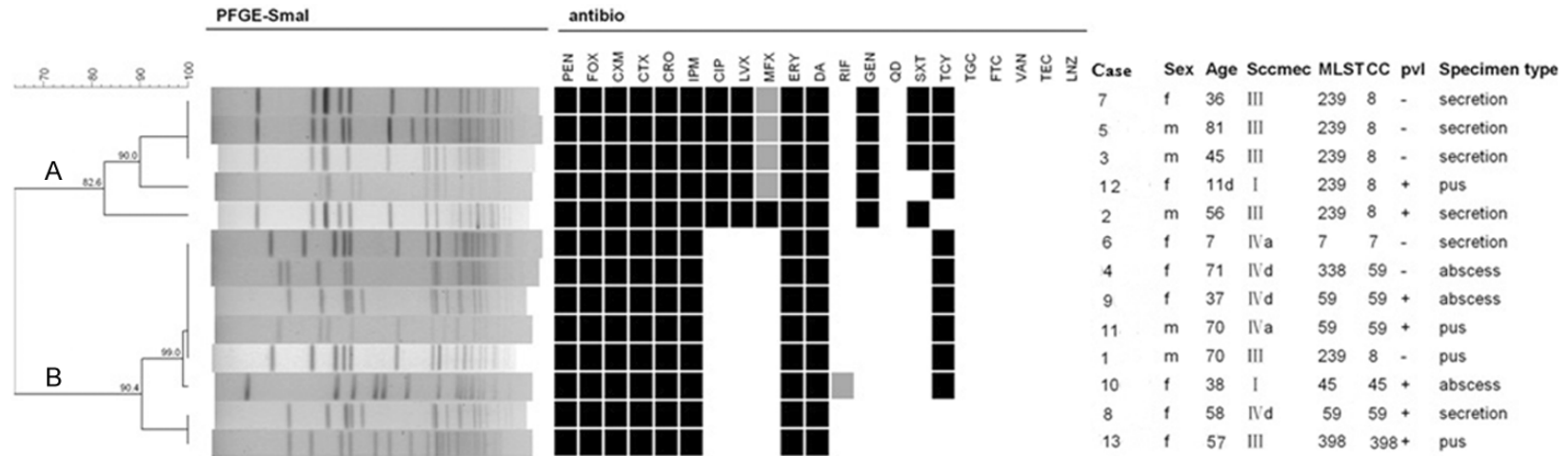
#### Expression of *pvl* gene

All of 13 MRSA isolates, 53.8% (7/13) isolates carry *pvl* gene. Among all five SCC*mec* IV MRSA isolates, 60.0% (3/5) isolates (including 1 SCC*mec* IVa and 2 SCC*mec* IVd) carry *pvl* gene. Among six SCC*mec* III MRSA isolates, 33.3% (2/6) isolates carry *pvl* gene. The other two isolates carrying *pvl* gene are belong to SCC*mec* I (**Table 1**).

#### SCC*mec* typing and MLST

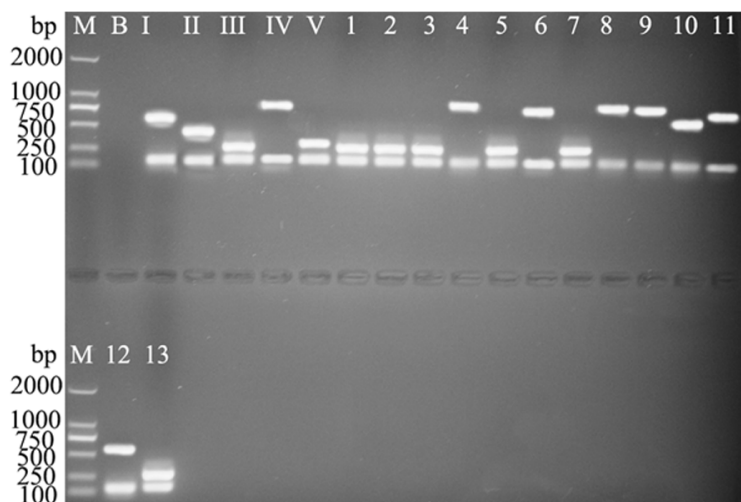
All of 13 MRSA isolates, two (15.4%, 2/15) isolate carried a type I SCC*mec* element, six (46.2%, 6/13) isolate carried a type III SCC*mec* element, while the other five (38.5%, 5/13) isolates carried a type IV SCC*mec* element (including 2 SCC*mec* IVa and 3 SCC*mec* IVd). None of the MRSA isolates carried a type V SCC*mec* element (**Figure 2** and **Table 1**).

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**Figure 1.** Genetic relatedness among 13 CA-MRSA isolates from patients with SSTIs. Dendrogram based on PFGE SmaI restriction pattern analysis of 13 CA-MRSA isolates. Similarity analysis was performed with Dice's coefficient, and clustering was done by using the unweighted-pair group method using average linkages (UPGMA) method. The scale at the top shows percentages of similarity. Further information is shown on the right, including the antibiotics, case, sex, age, staphylococcal cassette chromosome *mec* (SCC*mec*), multilocus sequence typing (MLST), clonal complex (CC) and Specimen types. Antimicrobial susceptibility tests (AST): black indicates resistance, grey indicates intermediate, and white indicates susceptibility. Abbreviations are as follows: PEN, penicillin; FOX, ceftazidime; CXM, cefuroxime; CTX, cefotaxime; IPM, imipenem; CIP, ciprofloxacin; LVX, levofloxacin; MFX, moxifloxacin; ERY, erythromycin; DA, clindamycin; RIF, rifampicin; GEN, gentamicin; QD, quinupristin/dalfopristin; SXT, trimethoprim/sulfamethoxazole; TCY, tetracycline; VAN, vancomycin; TEC, teicoplanin; LNZ, linezolid.

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**Figure 2.** Multiplex PCR assay identifies SCCmec types and subtypes I, II, III, IVa, IVb, IVc, IVd, and V. Simultaneously the *mecA* gene was detected. The following DNA templates were used for PCR (by lane): M, marker; B, blank; I-V, Control strains (SCCmec type I-V); 1-13, isolate No. 1-13 correspond **Table 1**.

Multilocus sequence typing of 13 MRSA strains revealed six different STs and five CCs. 6 isolates were defined as CC8 composed of ST239, and 3 isolates were defined as CC59 (including 2 ST59 and 1 ST338 strains), respectively. ST45, ST7 and ST398 were three distinct single clone in this study (**Table 1**).

The dominating clone was ST239-SCCmec III (38.5%, 5/13), followed by ST59-SCCmec IV (30.8%, 4/13).

### PFGE

PFGE grouped 13 MRSA isolates into 2 pulsotypes, data are shown in **Figure 1**. The mapping of DNA fingerprints revealed that 5 MRSA isolates (including NO. 2, 3, 5, 7, 12) had 82.6% similarity and belonged to the same cluster (designated pulsotype A), while eight other isolates belonged to another cluster (pulsotype B) with 90.4% similarity.

### Discussion

MRSA is considered as one of the most significant multidrug-resistant organisms causing nosocomial infections in mainland China, thus the HA-MRSA was studied by many experts in hospitals of China [4, 11, 12]. Recent years, infections caused by CA-MRSA have been reported worldwide. In this study, we have

found the prevalence of CA-MRSA was low among patient with SSTIs. This coincides with the recorded prevalence among adults in Beijing (3.0%) [13]. However, the confusing thing is ST59-SCCmec IV and ST239-SCCmec III MRSA which was the most prevalent clone among this study.

The most frequent CA-MRSA clone is ST59, which is related to the clonal complex 59 (CC59). In China, most of the CA-MRSA strains causing SSTIs belong to ST59-SCCmec IV [12, 14]. ST59-SCCmec V (*pvl*-positive) was the most dominant CA-MRSA genotype in Taiwan, accounting for 69-84% of CA-MRSA isolates in 1997-

2005 [15]. ST59-SCCmec IV was also the most dominant strain in East Asia according to other reports [16]. In the present study, four isolates (including 2 ST59-SCCmec IVa, 1 ST59-SCCmec IVd and 1 ST338-SCCmec IVd) were belonged to CC59. ST338 represents a distinct branch within CC59, which was found in CA-MRSA isolates from Taiwan and the southern region of China [17]. It was different compared with Western Europe and the USA, whose most of CA-MRSA isolates were CC80 (ST80) and CC8 (ST8) respectively [18]. Though ST45 was found in this study, which is an European clone isolated first in Germany and the Netherlands [19]. Apparently, the difference is more obvious of the type of CA-MRSA in different regions.

CC8 (ST239), as one of the most prevalent CCs of HA-MRSA worldwide [18], spread from Thailand to China in 1990s [20]. In China, HA-MRSA has been extensively studied during the past years with ST239-SCCmec III as the predominant clone [21, 22]. Nevertheless, in different geographical locations, it is known that the genetic backgrounds of MRSA strains are distinct, and that different genetic backgrounds can exist within a small area [23]. Song *et al.* found that previously established nosocomial MRSA strains including ST239 and ST5 clones were found among CA-MRSA isolates from patients without any risk factors for HA-

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MRSA infection [16]. CA-MRSA clones such as ST59, ST30 and ST72 were also isolated from patients with HA infections in many Asian countries [16]. In China, a prospective cohort of adults with SSTI was established between January 2009 and August 2010 at 4 hospitals in Beijing, both ST239-SCCmec III and ST239-SCCmec I were found in the CA-MRSA [13]. It shows that ST239-SCCmec III and ST239-SCCmec I begin to appear in the community in China. In our study, the most prevalent CCs was CC8 (ST239-SCCmec III). Our previous study found that having family members in the healthcare profession was a significant risk factor for *S. aureus* nasal carriage in the community group [24]. *S. aureus* nasal carriers may 'impose' their carrier status upon other household members, and the bacterium can be reintroduced into the hospital by intra-familial spread to and from healthcare workers. In the study, two ST239-SCCmec III isolates have showed a high level of genetic similarity based on PFGE typing (Figure 1). Case 5 was isolated from an 81-year-old male who lives with his daughter, a nurse works in the hospital. Case 7 was isolated from a 36-year-old female, and her husband is a dermatologist. These findings may indicate that healthcare personnel could contribute to the dissemination of ST239 strains between hospitals and within the community, which should be investigated further.

CA-MRSA may be going to replace HA-MRSA [11], on the other hand, HA-MRSA should spread from hospital to community in order to obtain competitive environment [25]. Ma *et al.* found that the major CA-MRSA clone in South Korea, ST72-SCCmec IVa/ *pvl*-negative, is different from those that have spread in Asia or internationally [26]. In fact, both ST239 and ST72 are distinct branch within clonal complex CC8 [27], so it is not contradictory to found a ST239 isolate contains with small SCCmec cassette (type IV or V). Actually Jae-Hoon Song *et al.* had reported that ST239-IV was found in one of the 98 HA-MRSAs in 2011 [16].

ST398 is frequently associated with animal infection, and individuals in close contact with animals are more likely to harbor ST398 isolates [13, 28]. We confirmed the presence of ST398 clone in the community. However, we did not find any association between livestock contact and ST398 carriage about the patient.

Similar findings came from European researchers who found the percentage of ST398 in MRSA increased from 0 in 2002 to 30% in 2007 in Netherlands and from 13% in 2005 to 22.4% in 2008 in Germany [29, 30], suggesting both regional variation as well as evolution in *S. aureus* ST398 over time. Whole genome sequencing of European ST398 has revealed that it lacked virulence factors such as enterotoxins and phage-encoded toxins [31]. Our findings are novel because we find a strains harbored *pvl* gene.

In this current study, most of the MRSA isolates presented multi-resistant phenotype. As we know that CA-MRSA isolates often show resistance to macrolide, lincosamides and tetracyclines besides  $\beta$ -lactam antibiotics [32]. All of five ST239 isolates were also resistant to another three antibiotics, including quinolones, aminoglycoside and sulfanilamide. According to the antibiotic resistant profiles, the five isolates may spread from hospital to community. Multidrug resistance in CA-MRSA isolates will appear more frequent in the future attribute to the increasing possibility of transfer of mobile genetic elements encoding antimicrobial resistance between CA-MRSA and HA-MRSA isolates both in the community and in hospitals [16].

It is reported that the SCCmec type IV cassette and the *pvl* locus as only two genes were unique to CA-MRSA isolates [33, 34]. *pvl* as one of virulent genes present in isolates, is associated with an enhanced inflammatory response and localized infections [34]. 7 strains found in this study carried *pvl* locus and two of them was ST59-SCCmec IV, the major type from strains of children with skin and subcutaneous tissue infection in China as previously described [17]. ST239-SCCmec III has been found as the predominant clone in HA-MRSA during the past years in China [21, 22]. It is rarely reported to carry *pvl* gene. Song *et al.* reported the genes for *pvl* were present in only 5.7% HA-MRSA isolates in his study [16]. A molecular epidemiology study demonstrated that the majority of HA-MRSA strains before 1985 in Japan were ST30, possessed SCCmec IV or SCCmec I, produced type 4 coagulase, and carried *pvl* gene at a high frequency, but the study was covering 1979-1999 [35]. It demonstrates that as time goes by, the genetic structure of MRSA

change. In our study, we found three isolates, such as ST239-SCCmec III, ST239-SCCmec I and ST398-SCCmec III isolates, also carried *pvl* gene. Apparently, there are significant differences between different regions. The diversity of *pvl*-positive strains might be attributed to the fact that the genes are localized on phages, which assist with the spread of the *pvl* genes through *S. aureus* populations [36].

In this report, we describe the distribution of various genotypes and *pvl* genes among 13 CA-MRSAs isolated from patients with SSTIs. The molecular type ST239-SCCmec III was the most prevalent clone among these isolates. MRSA clones may be going to spread between the community and hospitals. Further surveillance of these MRSAs should be conducted to elucidate the current status of MRSAs in the community.

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### Disclosure of conflict of interest

None.

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