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# Molecular characterization and genotyping of methicillin-resistant *Staphylococcus aureus* in nasal carriage of healthy Iranian children

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**Abstract**

**Purpose.** Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has become a considerable public health concern in both developed and developing countries due to the rapid spread of this bacterium around the world, also the epidemiology of MRSA has changed, as the isolation of MRSA strains is not limited to health-care settings or patients with predisposing risk factors. Therefore, the objective of this study is to determine the genetic diversity and antibiotic resistance profile of CA-MRSA nasal carriage in Iranian children.

**Methodology.** A cross-sectional study was conducted from April 2013 to March 2014. A total of 25 CA-MRSA were isolated from the anterior nares of 410 preschool children with no risk factors. All MRSA isolates were characterized by detection of the Panton–Valentine leukocidin (*pvl*) and γ-hemolysin genes, staphylococcal cassette chromosome *mec* (SCC*mec*) typing and multi-locus sequence typing (MLST).

**Results.** In 25 CA-MRSA isolates, *Pvl* and γ-hemolysin genes were detected in one (4%) and 18 (72%) isolates; respectively. Overall, 92% (23/25) of isolates belonged to SCC*mec* type IV and 8% (2/25) of them had SCC*mec* type V profile. Using MLST, the 25 isolates were grouped into six clonal complexes (CC) and eight sequence types (ST) (CC5/ST6, CC22/ST22 and ST217, CC30/ST30 and ST1107, CC78/ST859, CC398/ST291 and CC97/ST405). The ST859/SCC*mec* IV (11/25, 44%) was the predominant clone among the isolates. ST859-MRSA-IV-*pvl*-negative (resistant to tetracycline) have successfully adapted to the Iranian preschool children population.

**Conclusion.** Our results suggest that the genomic diversity was observed among the CA-MRSA. In addition, the current study demonstrates that *pvl* is not a reliable marker for CA-MRSA in our region.

## INTRODUCTION

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has become a considerable public health concern in both developed and developing countries due to the rapid spread of this bacterium around the world [1]. Recent studies have demonstrated that the epidemiology of MRSA has changed, as the isolation of MRSA strains is not limited to health-care settings or patients with predisposing risk factors [2].

Epidemiologically, CA-MRSA is defined as clinical MRSA isolated from out-patients or collected up to 48 h after hospital admission. However, in patients with risk factors for hospital-acquired MRSA (HA-MRSA), time-based criteria are not considered. The majority of CA-MRSA strains tend to harbour staphylococcal cassette chromosome *mec*type IV (SCC*mec* IV) and SCC*mec* V. In addition, these strains are non-multi-antibiotic resistant and have different genotypes from the local HA-MRSA [3]. In previous studies, Panton–Valentine leukocidin (*pvl*) expression was also considered as another genetic characteristic associated with CA-MRSA strains [4].

Multi-locus sequence typing (MLST) has shown that different genetic backgrounds are associated with CA-MRSA strains

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**Abbreviations:** CA-MRSA, community-associated methicillin-resistant *Staphylococcus aureus*; CC, clonal complex; LA-MRSA, livestock-associated MRSA; MLST, Multi-locus sequence typing; PCR, polymerase chain reaction; *pvl*, Panton-Valentine leukocidine; SCC*mec*, staphylococcal cassette chromosome *mec*; STs, sequence types.

and different sequence types (STs) are associated with different geographical areas. While a few pandemic clones cause most HA-MRSA infections [5], a considerably multiple CA-MRSA clone has been documented. The predominant CA-MRSA clones in different parts of the world include: MRSA-ST8-IV(USA300) and ST1-IV (USA400) in North America, ST80-IV (European clone) in Europe, North Africa and the Middle East, ST59-V (Taiwan clone) in Taiwan, ST93-IV (Queensland clone) in Australia, ST30-IV [South West Pacific (SWP) CA-MRSA] in the Western Pacific, and ST772-V (Bengal Bay clone) in India and Bangladesh [6].

In the last few years, isolation of CA-MRSA has been more frequent, especially in geographical areas with a high prevalence, where these strains have also started to replace HA-MRSA in hospital settings [7]. Epidemiological data on CA-MRSA carriage and infection and clonal diversity of CA-MRSA in our region are low [8, 9].

In the current study, we aimed to determine the prevalence of the virulence genes, SCC*mec* typing and molecular characteristics of nasal carriage CA-MRSA isolates from healthy preschool children in Isfahan, Iran.

## METHODS

### Bacterial strains

In a cross-sectional study conducted on 410 healthy 2- to 6-year-old preschool children in Isfahan, Iran, we detected 25 CA-MRSA isolates using the *mec*A gene PCR. In addition, the antibiotic resistance profile of these isolates was evaluated [1]. In the present study, all of the 25 detected CA-MRSA isolates were included. This study was approved by the ethics committee of Isfahan University of Medical Sciences and Social Welfare Organization under which the private and public day-care nurseries or kindergartens are organized and operate (Grant No. 392062). A parent or guardian of any child participant provided informed consent on their behalf.

### DNA extraction

The genomic DNAs of CA-MRSA isolates were extracted by simple boiling method. Briefly, 50 mg of bacterial biomass was suspended in 400 µl of TES [50 mM Tris hydrochloride (pH 8.0), 5 mM EDTA, 50 mM NaCl], and the suspension was heated at 95 °C for 7 min and centrifuged at 10000 ***g*** for 10 min. The supernatant was taken as DNA lysate and was kept at −20 °C for the molecular assays.

### Molecular assays

#### Virulence gene detection

The presence of Panton–Valentine leukocidin (*pvl*) and γ-hemolysin genes was tested by PCR assay according to the protocol of Lina *et al.* [10].

#### SCC*mec* typing

The structure of the SCC*mec* elements was determined using the multiplex PCR strategy developed by Boye *et al.* [11].

#### MLST

MLST analysis was performed as previously described [12] and STs were attributed by submitting the obtained DNA sequences to the online MLST database available at http://www.mlst.net/. CC was determined using the program BURST v3 based on related STs (http://eburst.mlst.net/).

## RESULTS

Among the 25 CA-MRSA isolates, only one (4%) isolate was positive for the *pvl* gene, whereas the γ-hemolysin gene was detected in 20 (80%) isolates (Fig. 1). Two SCC*mec* types were identified. In total, 23 strains harbour SCC*mec* type IV and two strains harbour SCC*mec* type V (Fig. 1). We detected eight different STs including: ST859, ST291, ST405, ST1107, ST217; and three major international epidemic *S. aureus* (E-SA) lineages ST6, ST22 and ST30 (Fig. 1). Using thee BURST v3 algorithm, STs were grouped into six CCs including: CC5, CC22, CC30, CC78, CC398, CC97 (Fig. 2). Among the eight ST-SCC*mec* genotypes, the predominant type was ST859-IV (44%; 11/25) followed by ST30-IV (16%; 4/25), ST22-IV (12%; 3/25), ST6-IV (12%; 3/25), ST1107-V (4%; 1/25), ST217-V (4%; 1/25), ST405-V(4%; 1/25) and ST291-V(4%; 1/25) (Fig. 1).

### Clonal complex 78

In the current study, the predominant clonal complex was CC78. This CC included 11*pvl*-negative/γ-haemolysin-positive/ST859-IV strains with high prevalence of tetracycline resistance (91%; 10/11). Furthermore, these strains showed low to intermediate oxacillin MICs (6–48 µg ml$^{-1}$) (Figs 1 and 2).

### Clonal complex 30

Three *pvl*-negative/ST30-IV strains and one *pvl*-negative/γ-haemolysin-positive /ST1107-V strain belonged to CC30. These strains had low oxacillin MICs (3–6 µg ml$^{-1}$) (Fig. 2).

### Clonal complex 5

CC5 contained three strains including: one *pvl*-positive/ST6-IV and two *pvl*-negative /ST6-IV. These strains had low oxacillin MICs (3–8 µg ml$^{-1}$) (Fig. 2).

### Clonal complex 22

CC22 contained three *pvl*-negative/ST22-IV strains, resistant to clindamycin and erythromycin/susceptible to other non-β-lactam antibiotics and one *pvl*-negative/ST217-V resistant to erythromycin. These strains had low oxacillin MICs (6–8 µg ml$^{-1}$).

### Clonal complex 97

CC97 included one *pvl*-negative/ST405-V strain. This strain had low oxacillin MIC (3 µg ml$^{-1}$) and was resistant to erythromycin.

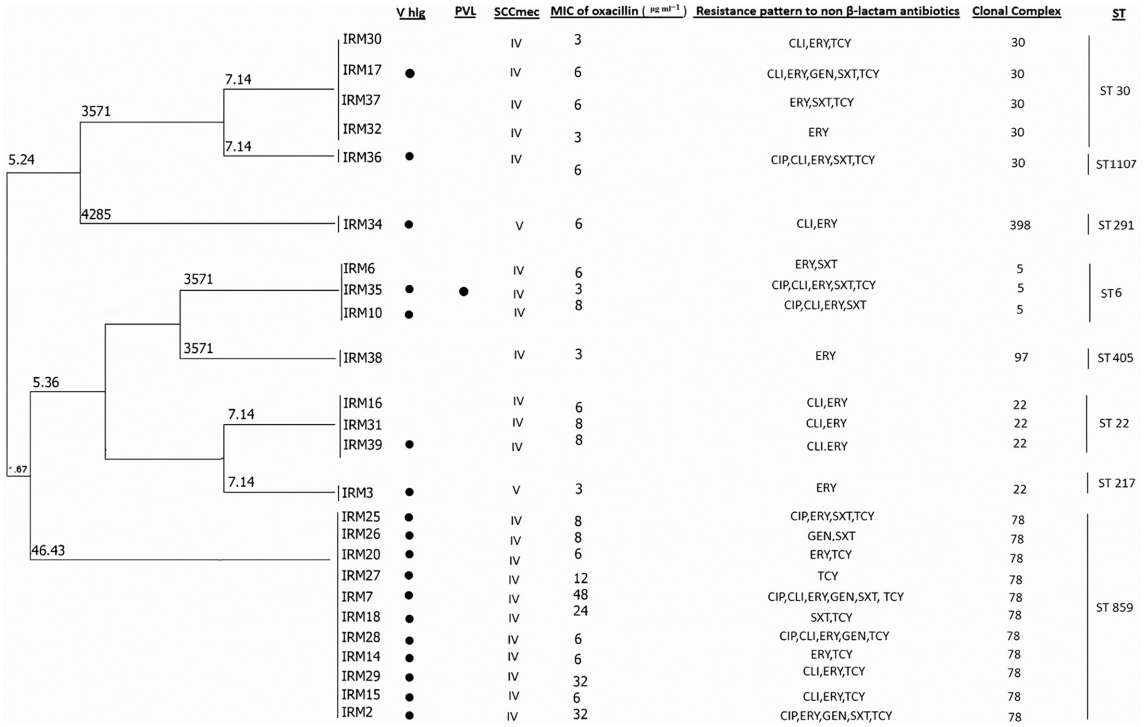


**Fig. 1.** Phylogenies of concatenated sequences from the 25 isolates of MRSA. Each isolate was presented by the type of SCC*mec*, STs and CCs. Bullets identify isolates that are γ-haemolysin and *pvl* gene positive. Vertical bars on the far right identify groups of isolates with the same STs. The phylogenetic tree was inferred according to the unweighted pair group method (UWPGM) with arithmetic mean using the matrix of pairwise differences.

### Clonal complex 398

CC398 contained one *pvl*-negative/γ-haemolysin-positive / ST291-V strain. This strain had low oxacillin MIC (6 μg ml$^{-1}$) and resistant to erythromycin and clindamycin.

## DISCUSSION

Among 25 CA-MRSA isolates, only one (3.3%) isolate was *pvl*-positive. In a study conducted by Havaei *et al.*, in our region, among 17 CA-MRSA isolates, they detected two (11.7%) *pvl*-positive isolates [13]. This study showed the relative low prevalence of *pvl*-positive strains in our area. These findings agree with other reports stating that carriage of the *pvl* gene cannot be used as a sole marker of CA-MRSA [3, 14, 15]. Unlike the low prevalence of the *pvl* gene in CA-MRSA isolates, γ-haemolysin gene prevalence was high (80%) in our study. Prevalence of γ-haemolysin gene in CA-MRSA has been less studied, although some studies claimed that most of the *S. aureus* isolated from human harbour the γ-haemolysin encoding gene [16].

Our molecular findings showed that most of the MRSA isolates carried type IV SCC*mec*, and two strains only carried type V. The isolates used in this study were isolated from healthy children and were considered CA-MRSA. According to previous studies, SCC*mec* type IV predominated among both CA-MRSA and HA-MRSA, but SCC*mec* type V was detected only among CA-MRSA strains [11, 17].

In the current study, eight different STs and six CCs profiles were identified by MLST. The predominant genetic background type belonged to CC78, which included ST859-IV (44%; 11/25). These isolates were *pvl*-negative, but all of them harboured the γ-haemolysin gene. Unfortunately, there is no detailed information about the CA-MRSA genetic background in Iran. In the study conducted on the clinical isolates by Havaie *et al.*, ST859 was one of the most commonly identified STs, and most of them were identified as HA-MRSA (four isolates from five) [13]. In another study carried out by Japoni-Nejad *et al.*, one isolate with ST859-IV profile was detected from the seven MRSA isolated from anterior nares of healthy students [8]. Goudarzi *et al.* reported that the ST859-IV was the second most-common MRSA clone identified in their study (8/70; 11.4% CA-MRSA and 8/70; 11.4% HA-MRSA) [17]. Ohadian Moghadam *et al.* detected two CA-MRSA belonging to ST859-IV from the health-care workers [18]. It seems that the ST859-IV strains are the common STs in both community and hospital MRSA strains in Iran and are actively circulating.

In the present study, the second frequent clonal complex was CC30, which included four isolates belonging to ST30-IV and one isolate belonging to ST1107-IV. During the 1950s

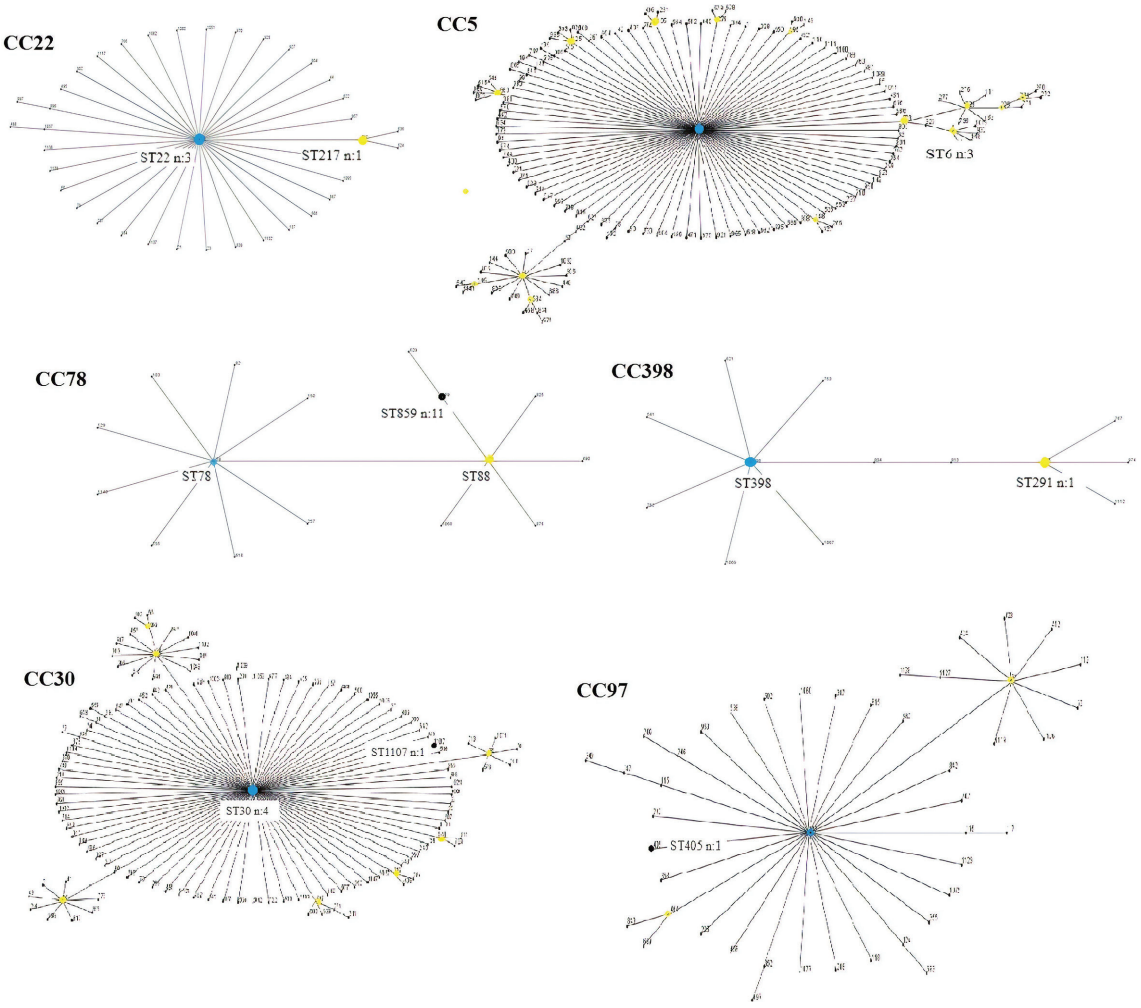


**Fig. 2.** Population snapshot of CA-MRSA lineages associated with their CCs. All STs identified in this study are marked, together with their CCs. The number of isolates (*n*) belonging to each ST is included. Primary and subgroup ancestors are coded by blue and yellow, respectively.

and 1960s, a ST30 methicillin-susceptible *S. aureus* (MSSA), *pvl*-positive strain was a virulent nosocomial pathogen in the United States. In later years, this MSSA strain changed to MRSA strain ST30 clones and has been reported as a CA-MRSA from many parts of the world including the USA, Europe, western Pacific area, Japan, Turkey and the Middle East [4, 19]. In two studies in Iran, ST30 isolates were also reported. However, in one of the studies, all isolates were MSSA [13].

One of the biggest *S. aureus* epidemic clones is CC5, which was identified in different parts of the world. ST6 strains may be created by a large-scale chromosomal replacement with a parental strain belonging to CC5(20). We detected three isolates belonging to ST6-IV, which were placed in CC5. The only *pvl*-positive isolates in this survey belonged to the ST6-IV. ST6 was previously reported in Iran. However, in one study, all reported isolates were MSSA [13], and in another study, most of the isolates were HA-MRSA [17].

CC22 is a frequent and wide-spread clonal complex. ST22-IV is a pandemic strain and one of the major epidemic MRSA (EMRSA) lineages which is also known as UK-EMRSA-15; and it was first reported in the UK in 1991 [20]. At first, this clone was considered a typical HA-MRSA strain; however, it changed to CA-MRSA, which may show the ability of transmitting the strains between the community and health-care settings. Like other parts of the world, ST22-IV was previously reported in Iran as three isolates were detected in this study. However, we detected one ST217-V isolate belonging to CC22, which had not been reported from Iran before. ST217 is a single-locus variant of EMRSA-15 belonging to CC22. Vignaroli *et al.* isolated one ST217-V strain from the nasal swap sample of a child, who had been admitted to a pediatric hospital 2 months later [21]. Very few data are found about ST217-V in the literatures, and more investigation is necessary.

Strains belonging to CC398 lineage are livestock-associated MRSA (LA-MRSA); however, they are able to cause human infections [20, 22]. ST291 strain is a double-locus variant of CC398, but based on the whole genome sequencing data, it is not closely related to the LA-MRSA CC398 group. The geographical distribution and epidemiology of these strains are more connected to CA-MRSA human lineage [23]. In this study, we detected one ST291-V isolate. ST291 has also been reported in Iran [13, 17] and other parts of the world [23].

Another clonal complex isolated in this study was CC97. Like CC398, CC97 have livestock origin. CC97-MSSA is often isolated from animals and humans, but MRSA strains are rare [20]. We detected one ST405-IV isolate belonging to CC97-MRSA. This ST has not been reported in Iran before. Unfortunately, we do not have any information about the livestock or animal contact of the child from whom the strain was isolated. However, CC97 was isolated from healthy people in Spain and Ghana [24, 25].

In conclusion, our study revealed that the CA-MRSA clones isolated from healthy preschool children belonged to diverse genetic backgrounds. Even some LA-MRSA lineages were observed. In addition, the current study demonstrates that *pvl* is not a reliable marker for CA-MRSA in our region.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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