Original Article

Isolation of *PVL/ACME*-Positive, Community Acquired, Methicillin-Resistant *Staphylococcus aureus* (USA300) from Iran

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Introduction: Methicillin-Resistant *Staphylococcus aureus* (MRSA) is responsible for an increasing number of serious hospital- and community-acquired infections. USA300 is known to be the most common cause of community-acquired infections, but recently there have been some reports on hospital-acquired infections caused by this strain. **Methods:** Totally 171 isolates of *S. aureus* were collected from different clinical samples in selected university hospitals in the cities of Mashhad, Tehran, and Isfahan. Then, they were assessed by agar screening and disk diffusion methods to determine their resistance to Methicillin. The isolated MRSA strains were confirmed by detection of *mecA* gene. The staphylococcal cassette chromosome *mec* (SCC*mec*), *agr*, and *spa* typing and also detection of Panton-Valentine leukocidin (*PVL*) and arginine catabolic mobile element (*ACME*) genes were performed on *mecA* harboring isolates. Multilocus sequence typing was performed on *PVL/ACME* positive MRSA strains. **Results:** We found a *PVL/ACME* positive MRSA isolate. Genetic evaluation results for this isolate produced the following profile: positive for *mecA*, *pvl*, *arcA*, and *hla* genes, negative for *vanA, sec*, and *tst1*, and belonged to *agr* I, SCC*mec* IV, sequence type 8 (ST8), and *spa* t008. **Conclusion:** Our results suggest a finding of USA300CA-MRSA isolate in Mashhad, Iran. This is an uncommon finding, because USA300 is routinely found in areas other than Middle East. A notable point about these isolates is that they belong to American Endemic clones. *J Med Microbiol Infec Dis, 2014, 2 (3): 100-104.*

Keywords: *Staphylococcus aureus*, Methicillin-Resistant, Panton-Valentine leukocidin, Iran.

INTRODUCTION

Staphylococcus aureus is responsible for a wide range of community- and hospital-acquired infections, ranging from simple skin and soft tissue infections to life-threatening infections, such as toxic shock syndrome, endocarditis, *etc.* [1-3]. Resistance to antimicrobial agents emerged soon after the first use of antibiotics to treat *staphylococcal* infections [1]. The mechanism of resistance to beta-lactam antibiotics includes integration of the staphylococcal cassette chromosome *mec* (SCC*mec*) into the *S. aureus* genome [4]. The antibiotic resistance associated with SCC*mec* is caused by altered penicillin binding protein 2a (PBP2a), which is encoded by the *mecA* gene. This molecule has a low affinity for beta-lactam antibiotics [5, 6]. So far, 11 different SCC*mec* elements have been identified [7, 8]. There are five predominant SCC*mec* types of Methicillin-resistant *Staphylococcus aureus* (MRSA) in Iran (types I to V). Types I to III are typically considered as hospital-associated MRSA (HA-MRSA), and types IV and V are commonly linked to community-associated MRSA (CA-MRSA) [9-12].

MRSA has been a common pathogen in health care settings since 1960 [13]. Since the late 1990s, the emergence of CA-MRSA outside health care settings has been increasingly reported [14].

Multiple CA-MRSA clones have been identified according to Pulsed-field Gel Electrophoresis (PFGE) patterns. These clones were found to be responsible for outbreaks of MRSA in the United States and also other parts of the world. Among the CA-MRSA lineages, USA1000 displays a "sporadic" phenotype, USA1100 exhibits a "local outbreak" phenotype, and USA300 displays an "epidemic" phenotype, capable of wide spread [15-17].

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USA300 is the main type of MRSA strain causing community-acquired infections in the United States. However, it is becoming a common cause of MRSA infection in health care facilities [18, 19].

To date, isolation of USA300 CA-MRSA has been increasingly reported in different parts of the world [17, 20-24]. It has been associated with skin, soft tissue, and also invasive infections in previously healthy people [25]. USA300 typically carries Panton-Valentine leukocidin (*PVL*)-encoding genes, SCC*mec* element type IV [17], and arginine catabolic mobile element (*ACME*) [26].

USA300 isolates were initially classified based on PFGE profiles. Today, using molecular methods, they are commonly characterized as multilocus sequence type 8 (ST8) [27, 28], *spa* type t008 [27, 28] or t121 [29], SCC*mec* IV [23, 27, 28, 30, 31], *PVL*-positive [23, 27, 28, 32, 33], and possessing *ACME* [23, 27, 34]. Some previous studies proved that molecular determinant such as Multilocus ST, *Spa* type, and presence of *PVL* and *ACME* can be used for characterization of USA300 isolates with high sensitivity and specificity, similar to PFGE [27]. In the present study, we isolated USA300 CA-MRSA for the first time in Iran, and it can be speculated that this successful international clone can have been imported and be in circulation in Iran.

S. aureus was isolated from wound exudates of a 31-year-old woman who suffered from recurrent exudative lesions. Analysis of the isolated *S. aureus* showed that it was a *mecA* positive and *PVL*/*ACME* harboring strain.

MATERIAL AND METHODS

In our study, all strains of *S. aureus* isolated from patients admitted in selected hospitals in Mashhad, Isfahan, and Tehran in autumn has been evaluated. These hospitals are large reference hospitals in these cities, which were chosen due to the coverage of a wide variety of patients of various races and lifestyles. A total of 171 isolates of *S. aureus* were obtained from patients between September 2011 and December 2011 at Al-Zahra hospital in Isfahan, Emam Reza Hospital in Mashhad, and Dey Hospital in Tehran, Iran. Clinical samples, such as urine, sputum, blood, abscess, eye, throat, wound, nose, and respiratory specimens were included in this study. *S. aureus* isolates were identified by Gram staining, catalase, coagulase, DNase, and mannitol fermentation tests. The classification of isolates into community- and hospital-acquired MRSA, was performed according to criteria set by Center of Disease Control and prevention (CDC) [4, 6]. Patients' data such as history of hospitalization, surgery, antibiotic use, time of MRSA isolation, *etc.* were collected using questionnaire forms.

Screening for methicillin and vancomycin resistance. All *S. aureus* isolates were screened for oxacillin and vancomycin resistance by agar screening method. The isolates that had grown in vancomycin agar screening medium were tested with E-Test method for MIC determination.

Antimicrobial susceptibility test. Antibiotic susceptibility testing was performed using oxacillin, minocyclin, levofloxacin, ciprofloxacin, tetracycline, co-trimoxazol, gentamicin, clindamycin, and rifampicin antimicrobial disks for disk diffusion method (MAST DISKS™) according to Clinical Laboratory Standards Institute (CLSI) guidelines [35, 36]. *S. aureus* ATCC 25923 was used as control.

Genomic DNA extraction. Genomic DNA of *S. aureus* isolates were extracted using QIAamp Blood DNA mini kit. According to the manufacturer's protocol, we added lysostaphin enzyme at a final concentration of 30 µg/mL per extraction tube.

PCR assay. PCR reaction was performed using a TaKaRa TP600 thermal cycler (TaKaRa, Japan) in a volume of 50 µl. We used an EmeraldAmp Max PCR Master Mix for all PCRs.

(i) **PCR identification of the *mecA* and *vanA* genes.** The presence of the *vanA* and *mecA* genes was determined by PCR as previously described [1, 37].

(ii) **Multiplex PCR for detection of toxin genes.** The presence of the *Toxic Shock Syndrome Toxin-1 (tst 1), enterotoxin C* (*etc*), *alpha Hemolysin* (*hla*), and *PVL (lukS-PV and lukF-PV)* genes was determined using PCR reaction as previously described [1, 37]. These genes were selected because they represent well-characterized virulence factors that are not uniformly distributed in the *S. aureus* population.

(iii) **PCR identification of *arcA* gene (*ACME* marker).** The presence of *arcA* gene was determined by PCR reaction using the following primers: *arcA*-Forward: 5´-TCATCCACAGACACTTCATCG-3´ and *arcA*-Reverse: 5´-GGTAACGCTTTAGGACAATCG-3´. PCR was performed using the following thermal settings: 5 min at 94°C for initial enzyme activation followed by 40 cycles of amplification consisting of denaturation at 94°C for 40 s, annealing at 60°C for 40 s, and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The 731 bp PCR product was detected by 1% agarose gel electrophoresis and ethidium bromide staining.

(iv) **Multiplex PCR for SCC*mec* and *agr* typing.** SCC*mec* and agr typing were performed as previously described [1, 38].

MLST. Multilocus sequence typing was performed by PCR and sequencing of the internal fragments of *arc, aro, glp, gmk, pta, tpi,* and *yqi* genes of *S. aureus* [39].

***spa* typing.** s*pa* typing was performed by PCR and sequencing of polymorphic X region of *spa* gene as previously described [40].

Nucleotide sequencing. The PCR products were purified by a QIAquick Gel extraction kit. The purified PCR products were sequenced using an ABI 3730XL DNA analyzer (Applied Biosystems) in both directions.

RESULTS

Antibiogram analysis by disk diffusion method showed that our isolate was susceptible to most of the used antibiotic disks. Susceptibility to oxacillin and vancomycin was also evaluated by agar screening method. After all phenotypic

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tests for *S. aureus* reconfirmation were repeated, MICs were determined by the E-test method. The results of the antimicrobial susceptibility testing were as follows: the strain was resistant to oxacillin (MIC, 128 µg/ml), clindamycin, and rifampicin and susceptible to vancomycin (MIC, 2 µg/ml), minocycline, levofloxacin, ciprofloxacin, tetracycline, co-trimoxazol, and gentamicin. Moreover, genetic evaluation results for this strain yielded the following profile: positive for the *mecA*, *pvl*, *arcA*, and *hla* genes and negative for *vanA*, *sec* and *tst1*, and belonged to *agr* I, SCC*mec* IV, ST8, *spa* t008.

DISCUSSION

USA300 CA-MRSA is highly virulent and associated with skin and soft tissue infections and also invasive and sometimes fatal infections, such as bacteremia, endocarditis, necrotizing pneumonia, and osteomyelitis in previously healthy people [17, 25].

Outbreaks of CA-MRSA infections have been reported all over the world and successful clones are usually associated with specific geographic areas [41-43]. Clones with multilocus ST1 (USA400) and ST8 (USA300) are mostly reported in the United States and Canada [44], multilocus ST80 in Europe [44], multilocus ST59 in the Asia-Pacific region, and multilocus ST30 worldwide, including the United States, Europe, Western pacific area, and Japan [45-47]. These five clones are responsible for most of the CA-MRSA worldwide.

Unfortunately, there is not enough information about the molecular epidemiology of CA-MRSA in Iran. Japoni Nejad *et al*. reported that the most prevalent CA-MRSA clone in their study was t790/ST22/SCC*mec* IV. St22 has previously reported as a dominant CA-MRSA clone in Germany [12]. The most predominant clones in other Asian countries includes: ST59/spa t437/Scc*mec* IV in Vietnam, Hong Kong, Sri Lanka, and Taiwan; ST30/spa t019/ Scc*mec* IV in the Philippines, and ST72/spa t324/Scc*mec* IV in Korea [12].

USA300 has been identified as the predominant CA-MRSA genetic background in North America [27] and has been reported as a cause of clinical infection in many countries [17, 48, 49]. Previous studies suggested that the most prevalent CA-MRSA clones in Asian countries including Iran were related to European clones [12], but in the present study, we could isolate USA300 CA-MRSA (an American clone) for the first time in Iran. It is alarming, because up to now USA300 clone has been only found in Japan and Korea [50, 51], and our finding could be a sign of an increasing incidence of this clone in other Asian countries. Increasing reports of USA300 isolation in various parts of the world can introduce this clone as a successful worldwide disseminated clone. Due to the very low number of studies on molecular epidemiology of CA-MRSA in Iran, more extensive studies are required in various geographic regions in Iran to determine the prevalence of MRSA clones.

Given the high transmissibility of USA300 and increasing number of reports on USA300 isolation in various parts of the world, it is possible that this clone becomes a major problem worldwide.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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