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 (SCCmec), 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 bha genes, negative for vanA, sec, and tsa1, and belonged to agr I, SCCmec 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 (SCCmec) into the S. aureus genome [4]. The antibiotic resistance associated with SCCmec 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 SCCmec elements have been identified [7, 8]. There are five predominant SCCmec 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].
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, SCCmec 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], SCCmec 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 DISKSTM) 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 and arca-Reverse: 5’-GTTAAGCTTATTAGACAATCG-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 SCCmec and agr typing.** SCCmec 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.** spa 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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CONFLICT OF INTEREST

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

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