Molecular characterization and antimicrobial susceptibility of the CA-MRSA isolated from healthcare workers, Tehran, Iran

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INTRODUCTION: Methicillin resistant Staphylococcus aureus (MRSA) has become as a nosocomial pathogen worldwide. Considering the importance of MRSA typing for understanding the evolution and dissemination of these strains, we studied the molecular characteristics of MRSA colonized healthcare workers (HCWs).

METHODOLOGY: All MRSA isolated from HCWs, were genotyped using staphylococcal cassette chromosome mec (SCCmec) with multiplex PCR assay, multilocus sequence typing (MLST) and spa typing. Then antibiotic susceptibility pattern and presence of pvl genes were evaluated in MRSA isolates.

RESULTS: Cluster analysis by eBURSTv3 showed that MRSA isolates belonged to two major clonal complexes (CC); CC88 (ST88, ST825, ST859) and CC30 (ST39, ST2, ST24) and five singletons. The most prevalent SCCmec type was type IV (70.59%) followed by type V (29.41%). Totally 11 different spa types were discriminated among which type t186 was predominant. All of the MRSA tested (100%) were susceptible to teicoplanin, linezolid and fusidic acid. Totally 52.94% of isolates were positive for pvl genes.

CONCLUSIONS: The ST88-MRSA-IV accounted for most colonized MRSA isolates. We documented a different molecular epidemiology of MRSA nasal colonization in hospitals under studied, due to the introduction of epidemic clones (ST88, ST39, ST2235, ST80, ST813, ST398, ST825, ST24, ST22, ST859 and ST2).

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transferred to transport medium and sent to the TUMS molecular laboratory for cultivation attempts. Identification of *S. aureus* was carried out by confirmatory tests [Gram’s stain, catalase, coagulase and DNase tests and mannitol fermentation on mannitol salt agar (MSA)]. Only one isolate per person was involved in this study. Resistance to methicillin was determined using disc diffusion method on Muller-Hinton agar medium containing 4% NaCl and oxacillin disk (1 μg oxacillin; MAST Diagnostics, Merseyside, U.K.) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines; 2011 [4]. Then meca gene was detected in DNA extracts by PCR assay as described previously [5].

2.2. Antibiotic susceptibility test

The antibiotic resistance pattern was performed by a disc diffusion method on Mueller-Hinton agar using ciprofloxacin (5 μg), chloramphenicol (30 μg), erythromycin (30 μg), fusidic acid (5 μg), gentamicin (10 μg) linezolid (30 μg), mupirocin (5 μg), rifampin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), tetracycline (30 μg) and teicoplanin (30 μg) (MAST Diagnostics, Merseyside, U.K.) according to the CLSI guidelines [4]. The antibiotic resistance pattern was performed by a disc diffusion method on Mueller-Hinton agar using ciprofloxacin (5 μg), chloramphenicol (30 μg), erythromycin (30 μg), fusidic acid (5 μg), gentamicin (10 μg) and teicoplanin (30 μg) (MAST Diagnostics, Merseyside, U.K.) according to the CLSI guidelines [4]. Resistance to methicillin was determined using disc diffusion method on Mueller-Hinton agar medium containing 4% NaCl and oxacillin disk (1 μg oxacillin; MAST Diagnostics, Merseyside, U.K.) according to the CLSI guidelines; 2011 [4]. Then meca gene was detected in DNA extracts by PCR assay as described previously [5].

2.2. Molecular typing

2.2.1. DNA extraction. DNA extraction was performed using Dneasy kit (Qiagen, Valencia, CA) as recommended by the manufacturer, with the modification that 1.5 μl lysostaphin (5 mM) (Sigma-Aldrich, St. Louis, MO) was added to the bacterial suspension and incubated for 30 min at 37 °C. Then the purified DNA was used for molecular analyses.

2.2.1.2. Multiplex PCR for SCCmec typing. Different SCCmec types determined by 9 pairs of primers including the unique and specific primers for SCCmec types I, II, III, IV and V, and the primers for the meca gene which were described by Zhang et al. [6]. The SCCmec types were determined on the basis of the band pattern obtained.

2.2.1.3. spa typing. Amplification of the polymorphic X region of the protein A gene (spa) was performed according to the method of Moodley et al. [7]. Purified spa PCR products were sequenced (DNA Sequencer ABI, model 3730-XL) commercially (Takapouzist, Iran), and spa types were assigned by using the spa database website (http://www.ridom.de/spaserver). *S. aureus* ATCC 25923 was used as positive control, while sterile deionized water was used as negative control.

2.2.1.4. Multi-locus sequence typing (MLST). MLST was accomplished according to Enright et al. [8] which encompassed of PCRs for seven housekeeping genes in *S. aureus* (arcC, arnE, glp, gmk, pta, tpi, and yqIL) accompanied by DNA sequencing. Then their allelic profile and sequence type (ST) were determined using database accessible via http://saureus.mlst.net/.

2.2.1.5. Detection of Panton-Valentine leukocidin (PVL) genes. PCR assay was used to detect the Panton-Valentine leukocidin (pvl) genes (lukS-PV and lukF-PV) as described previously by Lina et al. [9]. *S. aureus* NCTC 13300 was used as standard strain and distilled water as negative control.

3. Results

3.1. MRSA distribution

Among 157 HCWs enrolled in the study, 38 (25.48%) were colonized with *S. aureus*. Of the 38 nasal carriers of *S. aureus*, 21 (55.26%) and 17 (44.73%) carried MSSA and MRSA respectively (13.37% and 10.82% of all HCWs, respectively). These MRSA isolates were confirmed as community-acquired MRSA (CA-MRSA) based on the lack of features suggesting hospital-related acquisition (for example history of hospitalization, dialysis, surgery, residence in a long-term care facility within the past year, presence of indwelling catheter, previous detection of MRSA [10].

3.2. Molecular typing

3.2.1. SCCmec typing

Results of SCCmec typing by using of multiplex PCR strategy indicated two different SCCmec types as follow: 70.59% type IV, 29.41% type V (Fig. 1).

3.2.2. The spa typing

MRSA isolates comprised 11 spa types. The predominant spa type was t186. The remaining spa types (t416, t3085, t8507, t320, t11282, t022, t8138, t690 and t1209) were scattered homogeneously among MRSA isolates.

3.2.3. Multilocus sequence typing (MLST)

MLST results revealed 11 different sequence type (ST) for MRSA isolates (ST88, ST39, ST2235, ST80, ST398, ST825, ST2, ST859, ST22 and ST24). Cluster analysis by eBURST v3 showed the CA-MRSA strains belonged to two major clonal complexes (CC), CC88 (ST88, ST825, ST859) and CC30 (ST39, ST2, ST24) and five singletons. The five non-CC88 non-CC30 STs, ST2235, ST80, ST81, ST398 and ST22 belonged to global MLST clonal-complexes CC8, CC80, CC398, CC99 and CC22 respectively. MRSA clone ST88-MRSA-IV was dominant among the isolates. Molecular characteristics of MRSA isolates are summarized in Table 1.

3.2.4. Distribution of pvl genes

PCR assay for detection of pvl genes showed that nine (52.94%) MRSA isolates were PVL-positive.

3.3. Antibiotic susceptibility pattern

Details of the antimicrobial susceptibilities for MRSA isolates are provided in Table 1. All of the MRSA tested (100%) were susceptible to teicoplanin, linezolid, fusidic acid. Resistance rate to some non-β-lactam antibiotics such as tetracycline (76.47%), gentamicin (76.47%) and ciprofloxacin (58.82%) was high.

4. Discussion

The present study was conducted to determine the molecular
characteristics of CA-MRSA isolated from healthy HCWs without any history of MRSA acquisition risk factor. Today, CA MRSA colonization recognized as an important source of MRSA and its prevalence is 1.7–2% [11].

The prevalence of CA-MRSA in our study was 10.82%, which was higher than the 1% reported by Japoni-nejad et al. from central Iran [12] and consistent with previous reports of CA-MRSA prevalence by Moghadami et al. [10].

Previous studies showed that ST30 and ST59 are prevalent in Asian countries and may spread between countries. The most popular clones of CA-MRSA are ST59-MRSA-SCCmeC type IV-SPA type t437 in Taiwan, Hong Kong, Vietnam, and Sri Lanka; ST30-IV-PVL in the Philippines; and ST72-SCCmeC type IV-SPA type t324 in Korea [13] ST30-IV-PVL-positive and ST80-IV-PVL-positive CA-MRSA clones reported as the major clones in Kuwait [14]. But there was not any ST59 and ST30 among our MRSA strains which is not in consistent with these studies.

MLST categorized 17 MRSA isolates into 11 STs including ST88, ST29, ST2235, ST80, ST183, ST398, ST252, ST2, ST859, ST72, and ST24. In the current study, the clone characterized as ST88-MRSA-IV was identified as the predominant clone, ST22-MRSA-IV (EMRSA-15) is a pandemic clone and first reported in the UK [15] as a HA-MRSA and then as CA-MRSA in Ireland [16] which may shows the transmission of MRSA strains from the community to hospitals and vice versa. ST8 (USA300) is a CA-MRSA clone which is prevalent in the USA and currently is reported from Japan [17] and Korea [18]. ST80 is a European clone but it is some reports from Asian countries as well [19,20]. The rare reports of these two clones (ST8 and ST80) among Asian countries may due to the lack of enough investigations about CA-MRSA in this continent. We detected ST8 (USA300) and ST80 (European clone) in our study which is not in consistent with previous report from central Iran [12].

Nowadays, there are 5 major PVL-positive CAMRSA clones are universally distributed among which ST1, ST59 and ST80 were reported from Asia [21]. In present study, 9 isolates were positive for pvl gene and belonged to five STs: ST80, ST285, ST88, ST22, and ST859.

In current study SCCmeC type IV was presented in 70.59% of CA-MRSA strains and the remaining strains carried SCCmeC type V (29.41%). The predominance of SCCmeC type IV among colonizers was reported previously [22].

We detected two CA-MRSA belonged to t690 ST859, SCCmeC type IV; which were pvl positive. Previous study in Iran on school children reported ST859, SCCmeC type IV with spa type t325 [14], and in another study this clone (ST859) was described as PVL-negative HA-MRSA [23].

In summary, this study provides information on the molecular epidemiology of community-acquired S. aureus among Iranian HCWs. There are a variety of CA-MRSA clones in Iran. Compared to previous CA-MRSA clones reported from Iran, which showed only five STs (ST29, ST14, ST22 and ST859) were accounted for nasal colonization of healthy individuals, our study revealed more heterogeneous CA-MRSA lineages. Detection of PVL-positive CA-MRSA clones may pose a new threat in terms of pathogenicity and epidemiology of MRSA in Iran. According to our finding, all of CA-MRSA isolates were remained sensitive to quinupristin-dalfopristin, linezolid, tigecycline, teicoplanin and fusidic acid.

Conflict of interest

All authors confirm that there are no financial and personal relationships with other people or organizations that could inappropriately influence (bias) this study.

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