Genomic diversity and antimicrobial susceptibility profiling of nasal carriage *Staphylococcus aureus* isolated from pediatric ward in Western Iran

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**Abstract**

Nasal carriage of *Staphylococcus aureus* (*S. aureus*) probably causes the transmission of infection between individuals in hospital and community. This study aimed to evaluate the molecular epidemiology and antibiotic resistance pattern of nasal carriage *S. aureus* in pediatric ward patients and personnel. A total of 122 Nasal samples were taken from 28 personnel and 94 hospitalized patients in the pediatric ward. Minimum Inhibitory Concentration (MIC) to vancomycin and cefoxitin was determined by Agar dilution method strips. All *S. aureus* isolates were analyzed by pulsed-field gel electrophoresis (PFGE). A total of 41 *S. aureus* were isolated from the patients, 16 isolates (39.09%) were hospital-associated *S. aureus* (HA-SA) and 25 (60.97%) were community-associated *S. aureus* (CA-SA); also, 13 *S. aureus* isolates were obtained from the personnel. Based on MIC results, all of *S. aureus* isolates were susceptible to vancomycin, and in 41 patient isolates, 13 isolates (31.7%) were resistant to cefoxitin (MRSA). Of 13 *S. aureus* isolates of the personnel, 3 (23%) isolates were MRSA. Totally 11 common clones and 13 single clones were obtained. In conclusion the prevalence of CA-SA in the ward was higher than that of HA-SA. In the strains obtained from a hospital ward, there was a high epidemiology, genotypic diversity in the studied ward. However, horizontal transfer of *S. aureus* was observed between patients and between personnel and patients, which indicated the risk of transmission of resistant strains in the hospital wards.

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1. **Introduction**

*Staphylococcus aureus* is a Gram-positive, catalase-positive, aerobic and non-aerobic, halophilous, and immobile bacterium, that can be found in both commensal and pathogen organism in the human. It can cause both community- and
hospital-acquired infections (van Bijnen et al., 2014; Mozaffari Nejad et al., 2014).

The anterior nares are the main ecological niches for colonization of \textit{S. aureus}. Approximately 20\% of individuals are persistently nasally colonized with \textit{S. aureus}. Nasally, colonized individuals are associated with increased infection risk with their colonizing isolates (Von Eiff et al., 2001). \textit{S. aureus} is one of the most important pathogenic bacteria causing chronic wounds such as diabetic foot ulcers, venous leg ulcers, and pressure ulcers (Dowd et al., 2008). Moreover, \textit{S. aureus} can cause a range of infectious diseases from mild skin and soft tissue infections to more severe infections, such as bacteremia, sepsis, and osteomyelitis (Jenkins et al., 2015). The frequency of nasal carriage among hospital employees in Iran has been reported to be in the range 12.7–45\% (Rahimi-Alang et al., 2011) and the \textit{S. aureus} nasal carriage rate in the hospitals of central cities of Iran is about 20.7–25.2\% (Fard-Mousavi et al., 2015).

MRSA is a global public health problem. The MRSA infections can be found in both hospitals and the community. Generally, hospital-associated MRSA (HA-MRSA) infects hospitalized individuals with predisposing risk factors. On the other hand, community-associated MRSA (CA-MRSA) infects healthy individuals without any previous healthcare contact. CA-MRSA causes a paradigm shift in the management of staphylococcal infections in countries in which CA-MRSA is highly endemic (Khokhlova et al., 2015).

Pulsed-field gel electrophoresis (PFGE) is a common technique and a useful molecular epidemiological tool for the study of genetic diversity in \textit{S. aureus} and numerous other pathogenic bacteria. PFGE remains an overall gold standard for assessing epidemiological inters relationship of pathogens. The remarkable longevity of PFGE usefulness is in part due to its ability to provide visual genomic comparisons, representing more than 90\% of the bacterial chromosome (Sen Gupta et al., 2014). The main objective of this study was to investigate the relationship between \textit{S. aureus} genotypes isolated of personnel and patients in pediatric ward. Moreover, the prevalence of VRSA and MRSA isolates in the nasal carriage of hospital ward was also studied.

2. Material and methods

2.1. Bacterial collection

A total of 122 nasal samples were taken by cotton sterile swab inoculated in sterile saline. The swab trolled five times in both anterior nares of 28 personnel that commute among the rooms pediatric ward and have direct contact with hospitalized children and 94 hospitalized patients in all patients of rooms in the pediatric ward of Imam Reza hospital in Kermanshah (Iran) from November 2013 to December 2014.

Patient samples were taken on the first hours of hospitalization. Positive samples in these step were considered as CA-SA. Sampling from patients with negative culture results was repeated every 48 h (Lim et al., 2010; Saderi et al., 2008). Swabs were sub-cultured on Mannitol Salt Agar medium (Merck, Germany) and incubated at 37°C for 24–48 h. Yellow colonies were sub-cultured on a blood agar plate (Merck, Germany) distinguished by gram staining, DNase, catalase, and coagulase tests (Pereira and Riboli, 2014).

2.2. Antimicrobial susceptibility test

MIC to vancomycin and cefoxitin was determined by E-test strips (liofilchem, Italy). According to the manufacturer’s instructions and CLSI 2014 guideline, isolates with cefoxitin MIC values <4 µg/mL and ≥ 8 µg/mL were considered as susceptible and resistant, respectively. Also, isolates were susceptible to vancomycin when the MIC value was ≤2 µg/mL, and considered as resistance when MIC value was ≥16 µg/mL (Mohajeri et al., 2014a,b; Safari et al., 2014).

2.3. Molecular typing

All \textit{S. aureus} isolates were analyzed by CHEF Mapper PFGE in accordance with the protocols previously described by Bosch et al. (2010). With little modifications, we used ATCC 25923 as an external reference. For each test sample, a pure culture was done into the brain–heart infusion broth (Merck, Germany), then the subculture of isolates were incubated for 24 h at 37°C with gentle shaking. Regulated absorbance of culture to attain Abs = 0.9–1.1 at 610 nm (Spectrophotometer, JENWAY Genova, UK). 400 µl of cell suspension was centrifuged at 12,000 rpm for 5 min, the supernatant was removed and obtained pellet resuspend in 300 µl normal saline, then was centrifuged again and re-suspended in 160 µl EC buffer containing 40 microliter lysostaphin (Sigma #L7386) (1 mg/ml in 20 mM sodium acetate, pH 4.5). So, 200 µl of 2\% low melting point agarose (Merck, Germany) was added and plugs were solidified at room temperature. The plugs were transferred into 1 ml of lysis buffer I (EC buffer and lysozyme 10 mg/ml) and incubated overnight at 37°C in shaker water bath. Lysis buffer I was discarded and replaced with 1 ml of lysis buffer II (0.5 mM EDTA pH = 8, 1% Sarkosyl, proteinase K 2.5 mg/ml) and then incubated overnight in shaker water bath at 52°C. The supernatant was discarded and the plugs were washed with 5 ml distilled water, and then incubated in the shaker water bath at 37°C for 30 min in three times. The restriction digestions of all isolates were performed with 40 U \textit{SmaI} (Thermo, USA) according to the manufacturer's instructions. Prepared running gel 1% with low electroendosmosis agarose (Roche, USA) added into 100 ml of TBE 0.5X and running buffer (1 L of 0.5X TBE with pH 8). The plugs were fixed in running gel wells. The wells were sealed with the same agarose. DNA separation was done in pulsed-field electrophoresis system (Chef Mapper; Bio-Rad Laboratories, Hercules, CA, USA) was conducted using two states program by set run parameters including: temperature 14°C; voltage 6 V/cm; switch angle, 120°; switch ramp 5 s and final switch ramp is 40 s for 22 h. The Lambda Ladder PFGE Marker (NEB: N0340, US) were used as the molecular size marker. 14 bands were found in the molecular size markers which were between 48.5 and 679 kb. The gel was stained by 0.5 mg/ml ethidium bromide solution for 30 min. photographed from patterns by UV gel Doc (BIO-RAD, USA) (Fig. 1). Analysis of banding patterns was done with the Molecular Analyzed Gel compare II version 6.5 software (Applied Maths, St Martens-Latem, and Belgium). Dendrogram for all of strains were obtained for the detection of pulsotypes correlations,
and also pulsotypes were identified at a cut-off value of 80% and isolates with more than 80% similarity in genotype were classified in clones which are considered as subtypes according to the study conducted by Tenover et al. (Tenover et al., 1995).

2.4. Statistical analysis

The statistical analysis of data was performed using SPSS (version 2.4. Statistical analysis

3. Results

From 122 swab samples, 94 were taken of which 41 (43.9% Female, 56% Male) cases were S. aureus isolates; 16 (39.09%) of them were Hospital-acquired S. aureus (HA-SA), and 25 (60.97%) were community-acquired S. aureus (CA-SA) (Table 1), and 28 personnel samples were taken and S. aureus was isolated from 13 samples of them (76.92% female, 23.07% male) from pediatric ward in Imam Reza Hospital in Kermanshah (Iran). The mean age of patients and personnel were 4.41 ± 3.54 and 31.23 ± 6.49 years, respectively. There was no significant correlation between patient age and HA-SA (P < 0.905).

Based on the MIC results, all of them (100%) were susceptible to vancomycin (VRSA), and from 41 patient isolates, 13 isolates (31.7%) were resistant to cefoxitin (MRSA) that one of these MRSA isolates (2.43%) had MIC 98 µg/mL, from 13 S.aureus isolates of the personnel 3(23%) isolates were MRSA. There was no significant correlation between MIC isolates and age (P < 0.593).

From 54 S. aureus isolates in this survey, 11 common clones (pulsotypes) and 13 single clones were obtained. Types contained I, II, III, IV, V, VI, VII, VIII, IX, X, and XI (Figs. 1 and 2). Type I is the most common member, which contained two sub-types, sub I and sub II with 9 members (16.66%). In this type, 5 members obtained from patients and 4 members obtained from personnel. In type I, two isolates from patients’ had full similarity in genotype which one of them is HA-SA and another is CA-SA, both isolates were resistant to the cefoxitin. Moreover, in this type one isolate that obtained from patients and one of personnel isolate were completely similar. That patient isolate was HA-SA and it was resistant to cefoxitin (Fig. 1). In two cases of isolates patient, in types III and VI likewise, similar patients’ isolates type III include one HA-SA and other was CA-SA. From similar patients’ isolates types VI both were CA-SA and one of them was cefoxitin resistant. Also in type XI, a case with more complete similarity between personnel (26 N) and patient (36P) isolate was observed and patients strain was resistant to cefoxitin. More resistance was detected in type I, which contains 7 (43.75%) strains and was resistant to cefoxitin. The distribution of resistance in other pulsotypes were type II, IV, VI, VII, VIII and XI; each of them contained one resistant strain (6.25%), and three strains resistant (18.75%). There are also in single clones (Table 2).

4. Discussion

According to the literature, Methicillin-Resistant S. aureus (MRSA) has been identified as a serious pathogen related to the bacteremia and hospital association infection (Shokravi et al., 2015). Also, vancomycin was used for the treatment of MRSA infections. But due to the increase in the utilization of vancomycin, susceptibility to this antibiotic was reduced in the last decade (Appelbaum, 2007). Nasal colonization of S. aureus increased the risk of nosocomial infection in the community and hospitalized patients had high rates of mortality and morbidity in the world (Eko et al., 2015). Previous studies in Iran have reported a high rate of S. aureus infections (Askari et al., 2012). Results of the present study showed that the carriage rate of S. aureus in pediatric patients in the pediatric ward was 41.62%, and MRSA rate of these patients was 31.7%; also, S. aureus carriage rates in the personnel was 42.85%, MRSA rates was 23.1%. One study in Iran reported that the carriage rate of S. aureus isolates was 26.3% and 35.9% of them were MRSA (Erami et al., 2014). Also, another research showed that the rate of S. aureus carriers in infectious ward were 39.8% of which 59% were MRSA (Naderi Nasab et al., 2014). These results are consistent with the finding of the present study. In the survey in Iran identified that, nasal colonization for S. aureus from the personnel in 5 hospitals was 27% in which 32% were MRSA (Rastegar Lari et al., 2011). The diversity in the results was presumably caused by differences in sampling organs including hand or nose.

**Table 1** Demographic details for Hospital-associated S. aureus community-associated S. aureus.

| Staphylococcus aureus | Sex | Total (%) |
|-----------------------|-----|-----------|
|                       | Male (%) | Female (%) |       |
| **HA-SA** | 10 (43.47%) | 6 (33.33%) | 16 (39%) |
| **CA-SA** | 13 (56.52%) | 12 (66.66%) | 25 (60.97) |
| **Total** | 23 (100%) | 18 (100%) | 41 (100%) |

* HA-SA: hospital-associated S. aureus, CA-SA: community-associated S. aureus.
Also, TAN Shan et al. concluded that there was 39% nasal colonization among hospitalized children and their rates of MRSA were 32% (Tan et al., 2015), this study’s result matches the results of our study.

Figure 2  PFGE dendrogram of *Staphylococcus aureus* isolates. The vertical black line shows the 80% cut-off.
Due to the diversity in the studied wards and conditions of hospitalization, types of taking antibiotics, history of antibiotic therapy, and chronological changes during the colonization, *S. aureus* carrier rate and MRSA in our study were higher than those in other similar studies (Kenner et al., 2003).

In this survey, nasal carriage prevalence of HA-SA in patients was 39% and prevalence of CA-SA was 60.97%. Moreover, our results showed that most patient isolates were community acquired *S. aureus*. From CA-SA isolates; 29.62% were resistant to cefoxitin, and all of them were susceptible to vancomycin. As much as 25% of HA-SA patient’s isolates were resistant to cefoxitin. Resistance to cefoxitin encoded by *meCA* gene, set up in specific location of chromosome, and can be transmitted from bacteria to other bacterial species (Demir et al., 2016). Infection by MRSA was a major problem for health systems worldwide. Compared to the 2010 study, which was conducted at the Iraqi hospital, resistance to cefoxitin was more than 2.5 times compared to our study (72%) (Mustafa Mohammed, 2011). In a similar study, the nasal isolates could be transferred by hands of health personnel to patients or from patients to the personnel in the ward. Chen et al. showed that health care workers were faced with increased risk of nasal *S. aureus* carriage (Chen et al., 2015). Like other studies, there was a high conformity between two pairs of isolates in PFGE colons. This result indicated that these isolates were transmitted among patients or could be spread from hospital to patients. Therefore, hospital was considered as a source of these isolates.

In the present study, in the two pairs of identical isolates, complete genotype similarities were found between personnel and patients in the pediatric ward. Moreover, both the patient isolates were hospital-associated *S. aureus*. These results conducted that those isolates were transmitted through the healthcare personnel to patients or from patients to the personnel in the ward. Chen et al. showed that health care workers were at risk for secondary diseases such as, bacteremia associated with venous catheters, cellulitis and skin ulcers. Therefore, patients who are immunosuppressive, or those using corticosteroids medications, and patients with liver disease are at greater risk (Naber, 2009).

The nasal isolates could be transferred by hands of healthcare personnel to the patients. The transformation of *S. aureus* isolates through the healthy system could increase the risk of colonization and developing infections in patients (de Lencastre et al., 1994). As a result, hospitalized patients are at risk for secondary diseases such as, bacteremia associated with venous catheters, cellulitis and skin ulcers. Therefore, patients who are immunosuppressive, or those using corticosteroids medications, and patients with liver disease are at greater risk (Naber, 2009).

Another important result in the present study indicated that complete similarities were not found among the personnel isolates in the studied wards. Furthermore, isolates through personnel did not transfer to another in the ward.

### 5. Conclusion

In this study we described the nasal carriage rate of *S. aureus* and MRSA in a ward of a teaching hospital, which has a high prevalence of nosocomial infections. Results indicated that the
prevalence of CA-SA in the studied ward was higher than that of HA-SA. Moreover, in the strains obtained from a hospital ward, there was a high epidemiology, genotypic diversity in the studied ward. However, horizontal transfer of S. aureus was observed between patients and between personnel and patients. Therefore, it can be found that there is the risk of transmission of resistant strains in the hospital wards.

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