Molecular characteristics and antibiotic resistance pattern of *Staphylococcus aureus* nasal carriage in tertiary care hospitals of Isfahan, Iran

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SUMMARY

Carriage of *S. aureus* in the anterior nares seems to play a significant role in the pathogenesis of infection. This study aimed to determine the molecular characteristics and antibiotic susceptibility pattern of *S. aureus* isolates obtained from the nasal carriage of health care workers (HCWs). This study was performed during July 2014 to July 2015 at three tertiary care hospitals. Nasal samples were collected from the nasal cavity of HCWs. Standard microbiological methods were used for identification of *S. aureus* isolates. Antibiotic susceptibility pattern was determined by the disc diffusion method. Determination of SCC*mec* typing and virulence genes was performed by the PCR method. From the isolates of 340 nasal swab samples of HCWs, 65 *S. aureus* strains (19%) including 22 (33.8%) MRSA were isolated. The highest sensitivity for MRSA isolates was towards vancomycin and rifampicin, each with 90.9%. Overall, 17% (11/65) and 92.3% (60/65) of *S. aureus* isolates were positive for *pvl* and *hla* genes, respectively. The rates of SCC*mec* types II, III, IV, V and I among MRSA isolates were 36.4%, 22.7%, 22. %, 9.1% and 4.5% respectively. The results of the present study indicate that *S. aureus* nasal carriage with potential virulence ability still remains a significant healthcare problem, especially in hospital environments.

Keywords: antibiotic resistance, Panton-Valentine Leukocidin, MRSA, nasal carriage, SCC*mec* typing.

INTRODUCTION

Staphylococcus aureus is recognized as one of the most important human pathogens and also one of the most common nosocomial organisms [1,2]. This bacterium is responsible for a variety of infections, ranging from superficial skin infections to severe life-threatening conditions such as toxic shock syndrome [3]. Carriage of *S. aureus* in the anterior nares seems to play a significant role in the pathogenesis and epidemiology of infection [4]. The frequency of *S. aureus* nasal carriage among health care workers (HCWs) in Iran has been reported up to 31% [5].

S. aureus has extraordinary ability to survive and adapt in hostile environments, as illustrated by the emergence of *S. aureus* strains resistant to almost all classes of antimicrobials [6-8]. In particular, methicillin-resistant *Staphylococcus aureus* (MRSA)

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has become a major public health concern related to both community-acquired MRSA (CA-MRSA) and healthcare-acquired MRSA (HA-MRSA) [9]. Resistance to methicillin is due to the acquisition of *mecA* that is located on a genetic element called the staphylococcal cassette chromosome (SCC) [7]. This gene encodes the low-affinity penicillin binding protein 2a, (PBP2a or PBP2') that causes resistance to all beta-lactam antibiotics, including methicillin [7, 10]. Five main types of SCC*mec* differing in size and genetic composition have been identified in MRSA [11]. SCC*mec* typing and genotyping can provide strong evidence for the independent deviation of HA-MRSA and CA-MRSA [12]. The SCC*mec* types I, II, and III are most often associated with HA-MRSA strains, whereas the SCC*mec* types IV and V are predominantly found in CA-MRSA throughout the world [7, 11]. To determine the sources and genetic diversity of these strains it is essential to control the dissemination of these microorganisms, therefore, in the recent years, the molecular techniques used for bacterial typing include pulsed-field gel electrophoresis (PFGE), ribotyping, methods based on enzymatic digestion, plasmid analysis, and typing techniques based on polymerase chain reaction (PCR) [13-15]. The virulence of *S. aureus* usually contribute to the ability to produce toxins and other secreted virulence factors and also biofilm formation capacity and resistance to phagocytosis [3, 6]. Some of the strains secret several cytolytic toxins including α-hemolysin, and Panton-Valentine leukocidin (*PVL*) which are frequently associated with *S. aureus* pathogenicity [16]. *PVL* is encoded by the co-transcribed genes, *lukS-PV* and *lukF-PV* (*lukS/F-PV*), and is a potent tissue necrotizing toxin in human which causes leukocyte destruction, tissue necrosis and cutaneous infection [17]. The first member of the pore forming beta-barrel toxin family is alpha-toxin, also known as alpha-hemolysin (*hla*) that is one of the main cytotoxic agents released by *S. aureus* isolates [18]. Knowledge about dissemination source, antimicrobial resistance and virulence pattern of *S. aureus* nasal carriage can provide useful information toward optimizing infection control and clinical therapy in the future. Therefore, this study aimed to determine genetic diversity, antibiotic susceptibility and virulence pattern of *S. aureus* isolates obtained from HCWs nasal carriage in Isfahan, Iran.

PATIENTS AND METHODS

Study design and setting

This cross-sectional study was performed during July 2014 to July 2015 at 3 tertiary care hospitals affiliated to Isfahan University of Medical Sciences, Iran. Signed informed consent and written questionnaires concerning the demographics (sex, age) were obtained from all participants.

Bacterial isolates and identification

Nasal samples were collected by cotton sterile swab from the nasal cavity of personnel in different wards at the selected hospitals. Swabs were sub-cultured on Mannitol Salt Agar (Merck, Germany) and incubated at 37°C. Thereafter, standard microbiological methods including Gram staining, DNase, catalase, and coagulase tests and genotypic method (the presence of the *fem A* gene) were used for identification of *S. aurues* isolates [19].

Antimicrobial susceptibility testing

All *S. aureus* isolates were screened for methicillin resistance based on resistance to cefoxitin (30 µg) discs (MAST, UK) by the disc diffusion method, following identification of *mecA* gene [20]. The minimum inhibitory concentration (MIC) of vancomycin and oxacillin (Sigma Chemical, Steinheim, Germany) were determined by broth microdilution method as described by the Clinical and Laboratory Standards Institute's (CLSI) recommendation, isolates with oxacillin MIC values ≤2 µg/mL and ≥4 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 resistant when MIC value was ≥16 µg/mL [21]. Antibiotic susceptibility pattern was determined by the disc diffusion method on Mueller-Hinton agar (Himedia, India) according to the CLSI recommendation for gentamicin (10µ), ciprofloxacin (5µ), co-trimoxazole (1.25/23.75µ), rifampin (5µ), tetracycline (30µ), clindamycin (2µ), and erythromycin (15µ), (Mast Group, UK). *S. aureus* ATCC 29213 was used as standard quality controls.

DNA extraction and detection of the virulence genes

Phenol-chloroform method as described previously was used to extract genomic DNA [22]. The

extracted DNA were dissolved in 100 µl sterile distilled water and stored at -20˚C. PCR was performed to detect *mecA*, *fem A* and two different virulence genes, *pvl* and *hla* genes using the described primers [20]. PCR was carried out for each gene singly and amplicons were analyzed using 1.5% agarose gel with KBC power load dye (CinnaGen Co. Iran).

Multiplex PCR assay for SCC*mec typing*

SCC*mec* typing was carried out on the *mecA* positive isolates as described previously by multiplex PCR method [20]. The PCR conditions were as follows: initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 30 s, followed by extension at 72°C for 1 min.

Statistical analysis

The analysis was performed using SPSS™ software, version 16 (Chicago, IL, USA). The results are presented using descriptive statistics in terms of relative frequency. Chi-square test was used to determine any statistical association. Statistical significance was regarded as *P* values <0.05.

RESULTS

Of the total 340 nasal swab samples of HCWs collected during the course of the present study, 65 (19%) *S. aureus* strains were isolated. Overall, 38 (58.5%) *S. aureus* isolates were obtained from female and 27 (41.5%) from male participants. There were no significant correlation between age and gender of the participants and *S. aureus* isolation.

The rate of MSSA and MRSA nasal carriages were 66.2% (43/65) and 33.8% (22/65), respectively. All of the MSSA isolates were sensitive to co-trimoxazole and rifampicin, and the highest antibiotic resistance was related to tetracycline (27.1%). The highest *in vitro* sensitivity for MRSA isolates were toward vancomycin and rifampicin each with 90.9%. The highest antibiotic resistances among MRSA isolates were observed against tetracycline (68.2%) and erythromycin (68.2%). Complete results of antibiotic susceptibility patterns are displayed in Table 1. MDR were found in 54.5% and 4.6% of MRSA and MSSA isolates, respectively.

Our results showed that 17% (11/65) and 92.3% (60/65) of *S. aureus* isolates were positive for the presence of *pvl* and *hla* genes, respectively. The frequency of *pvl* and *hla* genes among MSSA isolates were 18.6% (8/43) and 98% (42/43), respectively. Moreover, 13.6% (3/22) and 81.8% (17/22) of MRSA isolates were positive for *pvl* and *hly A*, respectively.

Overall, the rates of SCC*mec* types II, III, IV, V and I among MRSA isolates were 36.4%, 22.7%, 22.7%, 9.1%, and 4.5% respectively. Moreover, 4.5% of isolates were not classified with used primers, which may be due to the lack of determination of *mec* or/and *ccr* complexes genes. The frequency of predominant SCC*mec* types and distribution of virulence genes among them are presented in Table 2. Detailed characteristics of MRSA isolates are also described in Table 3.

Table 1 - Antibiotic resistance patterns of *S. aureus* isolates.

Antibiotics	*MSSA* Total No. =43 No. (%) of resistant strains	*MRSA* Total No. =22 No. (%) of resistant strains	*Total*
Gentamicin	1 (2.3)	6 (27.3)	7 (10.8)
Ciprofloxacin	2 (4.6)	9 (40.9)	11 (16.9)
Erythromycin	7 (16.3)	15 (68.2)	22 (33.8)
Clindamycin	3 (7)	13 (59.1)	16 (24.6)
Tetracycline	12 (27.9)	15 (68.2)	27 (41.5)
Co-trimoxazole	0	6 (27.3)	6 (9.2)
Rifampicin	0	2 (9.1)	2 (3.1)
VISA	1 (2.3)	2 (9.1)	3 (4.6)
VRSA	0	0	0

Table 2 - The frequency of predominant SCCmec types and distribution of virulence gene (s).

SCC*mec* Type	MRSA *No. (%)*	*hlyA No.*	*pvl No.*
I	1 (4.5)	1	0
II	8 (36.4)	7	0
III	5 (22.7)	4	2
Iv	5 (22.7)	3	1
v	2 (9.1)	2	0
Untypable	1 (4.5)	0	0

DISCUSSION

S. aureus carriers have been recognized as a risk factor for the development of infections [23]. Understanding *S. aureus* nasal colonization dynamics and virulence are important for designing strategies to reduce the infection rates and dissemination [24]. In the current study, nasal carriage of *S. aureus* was identified in 19% of studied HCWs. Carriage rate was closest to most of the previous reports from Iranian studies ranging from 10% to 14.4% among HCWs [25,26]. However, Askarian et al. reported higher prevalence of *S. aureus* carriers with 31% in medical staff from Shiraz, South of Iran [5]. The frequency of *S. aureus* nasal carriage among Iranian healthy individuals compared to HCWs was documented slightly higher ranging from 10.2% to 28%, which may be due to higher sample size in most of these studies [27, 28]. This diversity in isolation rates was also noted in studies from other countries, which may be a reflection of different infection control policies or other risk factors involved in the studied regions [29, 30].

In the present study, the overall prevalence of MRSA carriage rates among HCWs was 33.8%, which was lower than the estimated prevalence

Table 3 - Detailed characteristics of MRSA isolates.

MRSA isolates	*Resistant pattern*	SCC*mec* type	*Virulence gene/s*	*Vancomycin susceptibility*
1	GM, CP, RA, TE, CD, E	2	*hla*	VSSA
2	GM, CP, RA, TE, CD, E	1	*hla*	VSSA
3	SXT, TE, CD, E	3	*pvl + hla*	VSSA
4	-	5	*hla*	VSSA
5	CP, TE, CD, E	2	*hla*	VSSA
6	CP, SXT, TE, E	4	*hla*	VSSA
7	GM, CP, SXT, TE, CD, E, V	4	-	VISA
8	-	-	*hla*	VSSA
9	TE, CD, E	2	-	VSSA
10	CD, E	4	-	VSSA
11	CP, TE, CD, E	2	*hla*	VSSA
12	TE, E	2	*hla*	VSSA
13	GM, CD, V	2	*hla*	VISA
14	CD, E	3	*pvl*	VSSA
15	GM, CP, SXT, TE, CD, E	3	*hla*	VSSA
16	GM, CP, SXT, TE, CD, E	4	*hla*	VSSA
17	CP, SXT, TE, CD, E	3	*hla*	VSSA
18	-	4	*pvl + hla*	VSSA
19	TE	2	*hla*	VSSA
20	TE	2	*hla*	VSSA
21	-	5	*hla*	VSSA
22	TE, E	3	*hla*	VSSA

of MRSA from 14 different Iranian cities with 52.7%±4.7 rates [31]. The observed variation in MRSA rates in Iranian studies was also observed in other countries as well [32]. Fourteen out of 22 (63.6%) MRSA isolates in our study were harboring SCC*mec* types I-III, which is mostly associated with HA-MRSA [7]. These findings were similar to the results from other Iranian studies which showed predominance of HA-MRSA in our healthcare settings [7, 19, 33, 34]. However, the proportion of MRSA isolates harboring SCC*mec* types IV and V were remarkable (31.8%), which confirm the tendency of CA-MRSA spreading in hospital environments [35].

In our study, the frequency of PVL positive *S. aureus* isolates was found to be 17% which is comparable to the results obtained by Fard-Mousavi et al. which showed a 20% PVL positivity in nasal carriage of *S. aureus* isolates [27]. Although, the range of PVL positivity in Iran is variable from as low rate as 5.47% in a study by Hoseini Alfatemi et al. to a notable rate of 40.9% by Momtaz et al. in clinical *S. aureus* isolates [16, 36]. The observed differences were mostly due to methicillin-resistance background of *S. aureus* isolates, and even among MRSA strains, CA-MRSA and HA-MRSA isolates showing different virulence patterns [11]. Namely, such higher PVL positivity observed by Momtaz et al. may be due to the predominance of CA-MRSA types, since PVL is mostly associated with SCC*mec* types IV or V [35, 36, 37].

Alpha toxin has been shown to intoxicate a wide range of human cell types, and has a significant role in the pathogenesis of *S. aureus* disease [18]. To the best of our knowledge there is no data on the prevalence of *hlyA* among nasal carriage of *S. aureus* strains in Iran. However, the frequency of *hlyA* in our findings was in accordance with previous Iranian reports on clinically obtained *S. aureus* isolates [17, 38]. In most studies investigating *hlyA* gene, the rate of detection was nearly 100% [17, 38, 39]. Though haemolysin expression may show lower rates [39, 40].

As expected, due to the resistant nature of MRSA isolates, the relative frequency of antibiotic resistance and MDR among them was higher than MSSA isolates. Despite variation in the reported antibiotic susceptibility pattern of *S. aureus* isolates, our findings were in accordance with the majority of previous studies either from Iran or foreign countries [19, 28, 41, 42]. In the present study 4.6% of isolates showed MIC values ranging from 4-8 µg/mL and were consequently considered as VISA isolates. Detection of VISA or VRSA isolates required applying appropriate antibiotic susceptibility methods, which was neglected in most of the studies. The prevalence of VISA strains in Iranian health care settings, however, is not uncommon and is cited by several studies [42, 44].

A number of limitations are attributed to this study. First, it was better to track the relation of *S. aureus isolates* obtained from nasal carriage with environmental and clinical isolates. Second, due to the lack of continued sampling we cannot discuss on the persistent or transient nature of *S. aureus* nasal carriers. Finally, results were obtained only from 3 hospitals and cannot be generalized to the entire city.

In summary, the results of the present study indicate that *S. aureus* nasal carriage with remarkable virulence ability still remain a significant healthcare problem, especially in hospital environments. Moreover, spread of CA-MRSA isolates in hospital settings require restricted infection control policies. On the other hand, management of antibiotic prescription for reducing selective pressures necessitate to overcome the emergence of MDR isolates such as VISA or VRSA MRSA strains.

Conflict of interest

None declared.

Ethical approval

This study was approved by the ethics committee of Isfahan University of Medical Sciences.

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