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Molecular characteristics of methicillin-resistant *Staphylococcus aureus* nasal carriage from hospitalized patients and medical staff in Isfahan, Iran

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Key words: MRSA, Nasal carriage, SCCmec typing, agr group, PVL toxin
Parole chiave: MRSA, colonizzazione nasale, tipizzazione SCCmec, gruppo agr, tossina PVL

Abstract

Objectives. *Nasal carriage of methicillin-resistant Staphylococcus aureus (MRSA) has been accounted as one of the main risk factors for the development of complicated nosocomial infections. The present study aimed to determine nasal carriage rate, antimicrobial susceptibility pattern and molecular characteristics of MRSA isolates.*

Methods. *This cross-sectional study was performed within 6 months period from July 2015 at 3 hospitals of Isfahan, Iran. Totally, 326 nasal samples were collected by cotton sterile swab from the nasal cavity of participants. Standard microbiological methods were used for identification S. aurues and MRSA isolates. Antibiotic susceptibility pattern was determined by the disc diffusion method according to the CLSI recommendation. Determination of SCCmec typing, agr groups, and virulence genes were performed by PCR method.*

Results. *Overall, 23.6% of cases were S. aureus carriers including, 23.4% (25/107) of HCWs and 23.7% (52/219) of patients. The rate of MRSA nasal carriages among patients was found to be 51.9% and 16% in HCWs. The highest levels of resistance among MRSA isolates were against ampicillin (93.5%) and tetracycline (83.4%); while, the most effective antibiotics were vancomycin and co-trimoxazole with 100% and 71%, susceptibility. The presence of hla and pvl genes was detected in 80.6% and 3.2% of MRSA isolates, respectively. SCCmec types I, III, IV and V were found in 16.1%, 25.8%, 25.8%, and 16.1% of isolates, respectively. Moreover, agr group I was the predominant type with 43.3%.*

Conclusion. *Our results showed a high rate of MRSA colonization in hospitalized patients which remains a significant healthcare problem in our region.*

Introduction

Staphylococcus aureus is one of the most important community-associated (CA) and health-care associated (HA) pathogen that is responsible for a wide range of infections including, skin and soft tissue infections, bloodstream infections and pneumonia (1, 2). Nasal carriage of *S. aureus* has been accounted as one of the main risk factors for

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the development of nosocomial infections (2-4). Surveys on *S. aureus* nasal carriage showed variations in the incidence of the nasal carriage according to the studied population and geographic location (5, 6). Nasal carriage rates reach to more than 30% among hospitalized patients and health care workers (HCWs) in some region of Iran (7, 8).

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has been considered as a global public health concern (9). Antibiotic resistance nature of MRSA strains to β-lactams and other antibiotic groups complicated the treatment of related infections (10). Therefore, MRSA infections are associated with longer hospital stays, higher health care costs and higher morbidity and mortality in comparison to methicillin-susceptible *S. aureus* (MSSA) infections (11). HCW seems to be an important source of nosocomial transmission in developing countries (12). The early diagnosis and treatment of MRSA carriage among patients and HCWs can be an essential part of the management and prevention of MRSA spreading in healthcare settings (13).

Applying an appropriate and accurate typing method can be helpful in determining the source of nosocomial infections (14). To date, several molecular methods including pulsed-field gel electrophoresis (PFGE); multilocus sequence typing (MLST), and typing based on polymorphisms of the following genetic loci: the staphylococcal cassette chromosome mecA (SCCmec), the X region encoding protein A (spa) and the accessory gene regulator (agr) have been introduced for typing of *S. aureus* isolates (15). *S. aureus* strains according to their clonality background may carry a number of virulence and adhesion factors such as exotoxins and several cytolytic toxins including Panton-Valentine leukocidin and αhemolysin, which are essential for *S. aureus* pathogenicity (16, 17). Despite the significance of *S. aureus* nasal carriage in a healthcare setting, there is limited local information and comprehensive data focused on MRSA prevalence among HCWs and hospitalized patients. Therefore, the present study aims to determine nasal carriage rate, antimicrobial susceptibility pattern, virulence markers and genetic diversity of MRSA isolates collected in Isfahan hospitals, Iran.

Materials and methods

Study design and setting

This cross-sectional study was performed between July 2015 and December 2015 at 3 teaching hospitals (Al-Zahra, Kashani, and Shariati) affiliated to Isfahan University of Medical Sciences, Iran. The ethics committee of the Isfahan University of Medical Sciences approved this study and written approval consents were obtained from the participants of the study. Demographic data were obtained for each participant.

Bacterial isolation and identification

Nasal swabs from nostrils were collected from hospitalized patients and HCWs who were working in different wards using a sterile cotton swab. The specimens were transported to the laboratory using transport media. The swabs were inoculated on Mannitol Salt Agar (Merck, Germany) and incubated at 37 ˚C for 24 h. *S. aureus* isolates were identified by standard microbiological methods such as Gram staining, Catalase, Coagulase and DNase activities. All *S. aureus* isolates were confirmed by detection of *fem A* gene as described previously (18). The bacterial isolates for long time preservation were transferred into brain heart infusion broth (Merck, Germany) medium containing 20% glycerol and stored at -80°C.

Antimicrobial susceptibility testing

Susceptibility to gentamicin (10μ), ciprofloxacin (5μ), co-trimoxazole

(1.25/23.75μ), rifampin (5μ), tetracycline (30μ) and clindamycin (2μ) (Mast Co. UK) was performed by disk diffusion method and the minimum inhibitory concentration (MIC) of oxacillin and vancomycin (Sigma Chemical, Steinheim, Germany) were performed by agar dilution method in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI) (19). *S. aureus* ATCC 25923 was used as the control for antimicrobial susceptibility testing. The MRSA isolates were screened based on resistance to cefoxitin (30 μg) discs (MAST, UK) by the disc diffusion method according to the CLSI guidelines and confirmed by identification of *mecA* gene (20).

DNA extraction and detection of the virulence genes

Bacterial genomic DNA from each MRSA isolate was extracted using the Phenol-chloroform method as described previously (21). The PCR amplifications were performed to detect *mecA*, *fem A* and two different virulence genes, *pvl and hla* genes as described previously (20). PCR conditions consisted of 5 min initial denaturation at 95 °C, followed by 35 cycles of denaturation (94°C/30 seconds), annealing 30 s at 49 °C for *mecA*, at 60 °C for *fem A*, at 52 °C for *pvl* and *hla* and extension (72 °C/60 seconds), and a final extension at 72 °C for 10 min. PCR products were analyzed using 1.5% agarose gel with KBC loading dye (CinnaGen Co. Iran).

SCCmec typing by multiplex-PCR

SCC*mec* typing was carried out on the *mecA* positive isolates by multiplex-PCR method using previously described method (20), with the following PCR conditions, 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.

Determination of agr group by multiplex-PCR

The *agr* typing of each MRSA isolate was performed using the *agr*-group specific primers and amplification conditions as was described by Shopsin et al. (22). The amplification was carried out for 5 min at 95 °C for denaturation initially, followed by 25 cycles (1 min at 94 °C for denaturation, 1 min at 55 °C for annealing, and 1 min at 72 °C for extension), and 10 min at 72 °C for the final extension.

Statistical analysis

The analysis was performed using SPSS™ software, version 21.0 (IBM Corp., USA). The results are presented as descriptive statistics in terms of relative frequency. Values were expressed as the percentages of the group (categorical variables). Chi–square or Fisher's exact tests were used to determine the significance of differences. A difference was considered statistically significant if the p-value was less than 0.05.

Results

Totally, 326 nasal swabs were taken from personals and patients during the course of study. Of 326 nasal swab samples, 107 (32.8%) and 219 (67.2%) nasal swabs belong to HCWs and patients, respectively. According to phenotypic and genotypic results, 77 (23.6%) cases were *S. aureus* carriers including, 23.4% (25/107) of HCWs and 23.7% (52/219) of patients (Table 1). These positive cases comprised 46 (60%) males and 31 (40%) females, with an age range between 4 to 93 years old. The statistical analysis showed no significant relevance between sex and age of participants with becoming *S. aureus* carriers. The rate of MRSA nasal carriages among patients was found to be 51.9% (27/52) which was significantly higher than HCWs with 16% (4/25) (P <0.002). The MIC of vancomycin

Table 1- Disturbations of *S. aureus* among nasal carriers

Methicillin-resistant	Patients No. (%)	HCWs No. (%)	Male No. (%)	Female No. (%)	Total No. (%)
MRSA	27 (51.9)	4 (16)	19 (41.3)	12 (38.7)	31 (40.3)
MSSA	25 (48.1)	21 (84)	27 (58.7)	19 (61.3)	46 (59.7)
Total	52	25	46	31	77

and oxacillin for MRSA isolates were ≤ 4 and ≥ 16 μg/mL, respectively.

The results of susceptibility testing showed all of the MSSA isolates were sensitive to vancomycin and only 1 (6.2%) isolate was resistant to rifampin. The majority of MSSA isolates were resistant to ampicillin (80%). Our results showed the highest levels of resistance among MRSA isolates against ampicillin 93.5% (29/31) and tetracycline 83.4% (26/31); while, the highest rate of sensitivity was towards co-trimoxazole (71%). All of the MRSA isolates were found to be sensitive to vancomycin by agar dilution method. MDR defined by resistance to at least 3 different antibiotics in MRSA isolates was significantly ($P < 0.001$) higher than MSSA isolates, 77.4% vs. 13%, respectively.

PCR analysis showed the presence of the gene encoding *hla* in 80.6% (25/31) of MRSA isolates and 1 MRSA isolate was found to harbor a *pvl* gene. Totally, of the tested MRSA isolates, 16.1%, 25.8%, 25.8%, and 16.1% harbored SCC*mec* types I, III, IV and V, respectively. Moreover, 16.1% of isolates were classified as untypeable by multiplex PCR. Typing of agr groups showed that a majority of isolates belonged to *agr* group I 43.3% (18/31), followed by *agr* group III 16.1% (5/31), and one isolate belonged to *agr* group II. Meanwhile, 22.5 % (7/31) of MRSA isolates were untypable for *agr* groups. The majority of untypable *agr* group harbored SCC*mec* type III. The detailed characteristics of MRSA isolates are described in Table 2.

Discussion

With regard to the spread of MRSA strains, the local epidemiology of molecular characteristics and antibiotic resistance pattern of *S. aureus* nasal carriers are useful for understanding dynamics of MRSA isolates. We found similar carriage rate of *S. aureus* in HCWs (23.4%) and hospitalized patients (23.7%) in the region. These findings are consistent with those studies, which showed environmental risk factors such as higher frequency of patient contact increased the risk of nasal *S. aureus* carriage in HCWs (23-25). Carriage rate in our HCWs was closest to previous reports from Iranian studies ranging from 10% to 31% (7, 26, 27). Compared to HCWs, *S. aureus* carriage rate was reported slightly higher in Iranian patients ranging from 19.7% to 36.9%, and was closest to our estimated prevalence (8, 28, 29). We found a high carriage rate of MRSA in hospitalized patients compares to HCWs, 51.9% vs. 16%, respectively. This finding was almost similar with previous reports that cited higher prevalence of MRSA colonization among patients compare to HCWs (8, 24, 25, 28). Moreover, the rate of MRSA in our patients was in accordance with the pooled prevalence of MRSA in Iran (52.7%±4.7) (30). However, the observed differences in *S. aureus* and MRSA carriage rate in the country and other parts of the world can be attributed to variations in sample size, identification methods and local infection control polices.

Table 2 - The detailed characteristics of studied MRSA isolates

Isolate	Source	Ward	Resistance pattern [c]	*mecA*	SCC*mec* type	Virulence factor	*agr* group
1	Patient	ICU [b]	GM, AMP, CD, RIF, T, CIP, OXA	+	I	*hla*	1
2	Patient	ICU	GM, AMP, CD, RIF T, TS, CIP, OXA	+	UN	-	3
3	Patient	ICU	AMP, OXA	+	III	*hla*	-
4	Patient	ICU	CD, AMP, RIF, T, CIP, OXA	+	IV	*hla*	1
5	Patient	ICU	GM, AMP, CD, RIF, T, CIP, OXA	+	I	*hla*	1
6	Patient	ICU	GM, AMP, CD, RIF, T, CIP, OXA	+	IV	*hla*	1
7	Patient	ICU	AMP, CIP, OXA	+	UN	*hla*	1
8	Patient	Surgery	GM, AMP, CD, TS, CIP, OXA	+	III	*hla*	2
9	Patient	Surgery	GM, AMP, RIF, T, CIP, OXA	+	III	*hla*	1
10	Patient	Surgery	GM, AMP, CD, T, TS, CIP, OXA	+	III	*hla*	1
11	Patient	Surgery	GM, AMP, CD, T, TS, CIP, OXA	+	UN	*hla*	3
12	Patient	Surgery	GM, AMP, CD, RIF, T, CIP, OXA	+	I	*hla*	1
13	Patient	Surgery	GM, AMP, CD, T, TS, CIP, OXA	+	UN	-	3
14	Patient	Surgery	GM, AMP, CD, RIF, T, CIP, OXA	+	I	*hla*	1
15	Patient	Surgery	GM, AMP, TS, CIP, OXA	+	UN	*hla*	-
16	Patient	Internal wards	GM, AMP, CD, RIF, CIP, OXA	+	III	*hla*	1
17	Patient	Internal wards	AMP,T,OXA	+	IV	*hla*	1
18	Patient	Internal wards	GM,AMP,CD, T,TS,CIP, OXA	+	I	-	-
19	Patient	ICU	GM,AMP,RIF,T,TS,CIP, OXA	+	IV	*hla*	1
20	Patient	ICU	GM,AMP,RIF,T,TS,CIP OXA	+	V	-	1
21	Patient	Internal wards	GM,CD,RIF,T,CIP,OXA	+	V	*hla*	3
22	Patient	Internal wards	GM,AMP,CD,RIF,T,CIP, OXA	+	V	-	1
23	Patient	Internal wards	GM,AMP,CD,RIF,T,CIP, OXA	+	IV	-	-
24	Patient	Internal wards	GM,AMP,CD,RIF,T,CIP, OXA	+	V	*hla*	3
25	Patient	Internal wards	GM,T,OXA	+	III	*hla*	1
26	Patient	Internal wards	GM,AMP,CD, RIP,T,CIP, OXA	+	IV	*hla*	-
27	Patient	Internal wards	GM,AMP,CD,RIF,CIP,T OXA	+	V	*hla*	1
28	HCW [a]	Surgery	AMP,T,OXA	+	IV	*hla+pvl*	1
29	HCW	Surgery	AMP,T,OXA	+	III	*hla*	-
30	HCW	Surgery	AMP,T	+	IV	*hla*	1
31	HCW	Internal wards	GM,AMP,CD,RIF,T,CIP, OXA	+	III	*hla*	-

[a] HCW: healthcare worker; [b] ICU: intensive care unit; [c] GM: Gentamicin, AMP: Ampicillin, CD: Clindamycin, CIP: Ciprofloxacin, RIF: Rifampin, T: Tetracycline, TS: Cotrimoxazole, OXA: Oxacillin; [d] UN: untypeable

In our results, distribution of CA-MRSA and HA-MRSA associated SCC*mec* types were closet to each other, which indicates the spread of MRSA from both community and hospital origins. However, the prevalence of isolates harboring CA-MRSA (types IV and V) was remarkable (48.4%, 13/31), which was in accordance with previous reports, confirming the emergence of CA-MRSA strains in Iranian hospitals (31-33). PVL was epidemiologically linked with CA-MRSA (34), but in our findings, the prevalence of PVL-containing isolates was low and detected in one isolate with SCC*mec* type IV. Previously several authors reported the predominance of PVL-negative strains among the CA-MRSA and stated that PVL is not a critical element in the spreading of CA-MRSA strains (23). To the best of our knowledge, there is no data on the prevalence of *hlyA* among *S. aureus* carriers in Iran. However, the prevalence of *hlyA* (80.6%) in our results was comparable with previous Iranian reports which showed a high rate of MRSA isolates containing alpha-toxin encoding gene obtained from clinical samples (35, 36). Our findings showed that the majority of MRSA isolates belonged to *agr* group I (43.3%). With few exception, a fundamental principle which can be concluded from previous studies is a global predominance of *agr* group 1 in the nasal carriage of *S. aureus* (38).

As the first limitation related to present work, it was better to track the clonal origin of *S. aureus* nasal carriers with more precise typing methods such as MLST of PFGE. Also, lack of continuous sampling for determination of persistent or transient nature of *S. aureus* nasal carriers can be mentioned as one of our limitations.

In summary, results of the present study showed a high rate of MRSA colonization in hospitalized patients which remains a significant healthcare problem in our region. Moreover, the emergence of CA-MRSA isolates in hospital settings require restricted infection control policies. Rational prescription and antibiotic usage can be an important step toward reducing the risk of MRSA colonization in the region.

Conflict of interest

None declared.

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Riassunto

Caratteristiche molecolari di Staphylococcus aureus meticillino-resistente nei portatori nasali da pazienti ospedalizzati ed operatori sanitari ad Isfahan, Iran

Obiettivi. La colonizzazione nasale da *Staphylococcus aureus* meticillino-resistente (MRSA) è stata indicata come uno dei principali fattori di rischio per lo sviluppo di complicate infezioni nosocomiali. Il presente studio era finalizzato a determinare il tasso di colonizzazione nasale, lo spettro di antibiotico resistenza e le caratteristiche molecolari degli isolati di MRSA.

Metodi. Questo studio è stato condotto in 6 mesi dal luglio 2015 in 3 ospedali di Isfahan, Iran. In totale, 326 isolati nasali sono stati raccolti mediante tampone sterile dalle cavità nasali dei partecipanti. Metodi microbiologici standard sono stati adottati per l'identificazione dei ceppi di MRSA. La resistenza antibiotica è stata determinata mediante la tecnica dei dischi per diffusione secondo le raccomandazioni CLSI. La determinazione della tipizzazione di SCCmec, gruppi agr, e geni di virulenza sono stati ottenuti mediante metodo PCR.

Risultati. In generale, il 23,6% dei casi erano portatori di *S. aureus,* incluso il 23,4% (25/107) degli operatori sanitari ed il 23,7% (52/219) dei pazienti. Il tasso di portatori nasali di MRSA tra i pazienti è risultato del 51,9% e del 16% negli operatori sanitari. I livelli più elevati di resistenza negli isolati di MRSA erano nei confronti di penicillina (93,5%) e tetraciclina (83,4%); mentre gli antibiotici più efficaci erano vancomicina e co-trimossazolo con sensibilità del 100% e 71%,. La presenza di geni *hla* e *pvl* è stata evidenziata nel 80,6% e 3.2% degli isolati MRSA, rispettivamente. I tipi SCC*mec* I, III, IV and V sono stati ritrovati bel 16,1%, 25,8%, 25,8%, e 16,1% degli isolati, rispettivamente. Inoltre, il gruppo *agr* I era il tipo predominante con il 43,3%.

Conclusioni. I nostri risultati hanno evidenziato un alto tasso di colonizzazione da MRSA nei pazienti ospedalizzati che rappresenta un serio problema sanitario nella nostra regione.

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