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ORIGINAL ARTICLE

A comparative characterization of nasal and clinical isolates of *Staphylococcus aureus* from west of Iran

Gholamreza Goudarzi[1,2], Yaser Hasanvand[1,2], Faranak Rezaei[1,2], Somayeh Delfani[1,2*]

[1]*Department of Microbiology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran*

[2]*Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran*

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ABSTRACT

Background and Objectives: Recently, the rise of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from hospital healthcare workers (HCWs) and various infectious samples has become one of the main concerns in hospital settings. Therefore, epidemiological studies are necessary to monitor antibiotic resistance patterns in each region and to study the pathogenesis of this strain to control infections.

Materials and Methods: In this cross-sectional study, a total of 100 *S. aureus* isolates, including 50 isolates obtained from the anterior nares of healthcare workers, as well as 50 other isolates cultured from the various clinical specimens from the referral hospitals in Khorramabad (West of Iran) were tested. All isolates were examined to determine antibiotic resistance pattern, and the presence of staphylococcal enterotoxin A (*sea*), staphylococcal enterotoxin B (*seb*) and *mecA* genes.

Results: The *mecA* gene was found among 36% (18/50) of the clinical *S. aureus* isolates (CSIs) and 14% (7/50) of nasal *S. aureus* isolates (NSIs), with statistically significant difference (X2 = 6.53; p = 0.011). The difference between the frequency rate of *sea* gene among MRSA strains isolated from clinical specimens (46.6%, 7/15) was significant compared to strains isolated from nostrils (14.3%, 1/7) (X2 = 3.85; p = 0.049).

Conclusion: The frequency of *mecA*, *sea*, and *seb* genes among the clinical samples was more than strains isolated from the nostrils of healthcare personnel.

Keywords: *MecA*; Methicillin resistance; Staphylococcal enterotoxins; *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus is a pathogen and opportunistic bacterium which can colonizes the skin and nasal cavity of many people, causes local and even systemic diseases such as bacteremia, osteomyelitis, and pneumonia (1, 2). There has been a dramatic increase in the occurrence of *S. aureus* infections, especially methicillin-resistant *S. aureus* (MRSA), which is resistant to several classes of antibiotics, as well as disinfectants (3-5).

The *mecA* gene is mainly responsible for the methicillin resistance phenotype among MRSA isolates (6). Reviews have shown that the mean frequency of MRSA in Iran has been reported at 43%, and Lorestan province is ranked amongst the provinces with the high prevalence rates (30-70%) of MRSA (6). Nostrils are the primary ecological niche for MRSA and the association between nasal carriage of MRSA and infectious diseases has been proven in recent years (7, 8). The colonization of MRSA strains in the nares of hospital health care workers (HCW) is considered

*Corresponding author: Somayeh Delfani, Ph.D, Department of Microbiology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran; Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran. Tel: +98-6633120133 Fax: +98-6633120133 Email: somayehdelfani@gmail.com

to be the main source of transmission of these resistant clones to patients (9).

Previous studies in Iran revealed the prevalence of *S. aureus* and MRSA strains among medical personnel was 22.7 and 32.8%, respectively (10). Approximately 15 to 80% of isolated staphylococci from different resources are enterotoxigenic (11). Up to now, 21 staphylococcal enterotoxins (SEs) or staphylococcal enterotoxin-like (SEl) proteins have been identified (12, 13), the most prevalent being staphylococcal enterotoxin A (SEA) and B (SEB) (14). The SEs and SEl proteins share common characteristics: they are small molecules with similar three-dimensional structures, heat-stable and have super antigenic properties, which are implicated in food poisoning (11, 15). The most SEs and SEl genes are located on mobile genetic elements such as plasmids, which could be horizontally transferred between staphylococcal strains. While serologic methods are not sufficient to identify the different types of these toxins, molecular techniques such as PCR and real-time PCR have been developed to detect SE and SEl genes (11, 16).

Regarding the importance of *mecA* and enterotoxin genes in the pathogenesis of *S. aureus*, data about strains (nasal and clinical) isolated from Lorestan province hospitals has not been documented. This study aimed to determine the frequency of staphylococcal enterotoxin A (*sea*), staphylococcal enterotoxin B (*seb*) and the *mecA* genes among *S. aureus* isolated from the anterior nares of healthcare workers (HCWs), as well as various clinical specimens.

MATERIALS AND METHODS

Bacterial isolates. During this cross-sectional study, a total sample size of 100 *S. aureus* strains originated from nasal and clinical sources were investigated. Fifty of the tested strains were included from our previous study (July 2011 to January 2012) on nasal *S. aureus* isolates (NSIs) obtained from anterior nares samples of HCWs employed in the four referral hospitals in Khorramabad (West of Iran) (17). The remaining fifty strains were clinical *S. aureus* isolates (CSIs) collected from culturing of various clinical specimens (October 2015 to February 2016). The isolates were identified by the following methods: Gram-staining and positive reactions for catalase, coagulase, DNase, and mannitol fermentation on mannitol salt agar (yellow colonies) (18). The isolates which were identified as *S. aureus*, were stored at -70ºC in TSB medium containing 15% glycerol for further investigations. This study was approved by the Ethical Committee of Lorestan University of Medical Sciences, Khorramabad, Iran. (IR.LUMS.REC.1397.060).

Antimicrobial susceptibility testing. To determine the antibiotic resistance profile of *S. aureus* isolates, the disc diffusion method was used, according to CLSI guidelines (19) by applying the following antibiotic disks (Rosco, Denmark): penicillin G (10U), cefoxitin (30 µg), tetracycline (30 µg), rifampin (5 µg), trimethoprim / sulfamethoxazole (23.75 / 1.25), erythromycin (15 µg) clindamycin (2 µg) and ciprofloxacin (5 µg).

PCR amplification of *mecA*, *sea*, and *seb* gene. DNA of all *S. aureus* isolates was purified using an AccuPrep® DNA Extraction Kit (Bioneer, Korea) according to the manufacturer's protocol and with some modifications. First, a fresh culture of strains was prepared in Luria Bertani broth (LB; Merck, Germany). Then, 1 ml of each bacterial suspension was centrifuged for 5 minutes at 3000 rpm. The supernatant was discarded, and 10 µl of Lysostaphin enzyme solution (100 µg/ml in sterile deionized water; Sigma) was added to the harvested bacteria (17). After incubation for 1 hour at 37°C, DNA extraction was continued according to the kit instruction. Specific primers designed by Mehrotra et al. (Table 1) were used to detect the *sea*, *seb* and *mecA* genes. PCR for the amplification of the *sea* and *seb* genes was performed as a multiplex assay as follows: 2.5 µl PCR buffer (10×), 200ng DNA as a template, 1.2 unit of *Taq* DNA Polymerase, 20pml of each primer, 0.4mM dNTP, 1.5mM $MgCl_2$ and distilled water was added up to 25 µl final volume. The *mecA* gene was amplified in a sep-

Table 1. Primers used for the amplification of staphylococcal enterotoxin A (*sea*), B (*seb*) and *mecA* genes.

Gene	Primer sequence (5' to 3')	Product size (bp)
sea	F: GGTTATCAATGTGCGGGTGG	102
	R: CGGCACTTTTTTCTCTTCGG	
seb	F: GTATGGTGGTGTAACTGAGC	164
	R: CCAAATAGTGACGAGTTAGG	
mecA	F: ACTGCTATCCACCCTCAAAC	163
	R: CTGGTGAAGTTGTAATCTGG	

arate reaction, including all of the above mentioned conditions, except for 20pmol of *mecA* primers, 0.2 mM dNTP and 1 unit of *Taq* Polymerase. The PCR program was set up as mentioned in Table 2 in the Thermocycler (Bio-Rad, My cycler, US) (20). The amplified DNA fragments were visualized in 1.5% agarose gel electrophoresis. A *Staphylococcus aureus* strain carrying both *sea* and *seb* genes and another containing the *mecA* gene were prepared as positive controls by Dr. Jalil Fallah Mehrabadi (Malek Ashtar University, Tehran).

Table 2. Temperature program to replicate *seb, sea* and *mecA* genes.

Step	Temperature	Time
Initial denaturation	94°C	5 min
Denaturation	94°C	1 min
Annealing	57°C	1 min
Extension	72°C	1 min
	35 Cycle	
Final extension	72°C	8 min

Statistical analysis. Descriptive data were analyzed by SPSS software version 19. The Chi-Square (X^2) test was used to compare the resistances and also the frequency of enterotoxins among CSIs and NSIs. *P* values of <0.05 were considered statistically significant.

RESULTS

Microbial isolates and antibiotic susceptibility. A total of 100 *S. aureus* isolates, including 50 CSIs, as well as 50 NSIs were investigated. All isolates (100%) showed resistance to penicillin. Resistance rate to cefoxitin (MRSA), erythromycin, rifampin, clindamycin, trimethoprim/sulfamethoxazole (Co-trimoxazole), tetracycline, and ciprofloxacin were found 22%, 22%, 7%, 22%, 14%, 30% and 15%, respectively. Also, the intermediate susceptibility phenotypes were observed for erythromycin (7%), clindamycin (6%), ciprofloxacin (5%), tetracycline (3%), cefoxitin, and trimethoprim/sulfamethoxazole (1%). The pattern of microbial susceptibility of isolates, based on the site of sampling is listed in Table 3.

Frequency of enterotoxin A and B using multiplex PCR. PCR results revealed that among the 100 isolates evaluated for the presence of enterotoxin A and B genes, 36 (36%) carried at least one of the enterotoxin genes. The frequency of sea and seb genes were 35% and 8% among all samples, respectively. Twenty-eight isolates carried only the *sea* gene, one isolate contained the *seb* genes, and 7 isolates simultaneously harbored both genes (Fig. 1). The prevalence of the enterotoxin genes based on the origin of samples (CSIs or NSIs), is illustrated in Fig. 2. The difference in the relative frequency of enterotoxin genes among CSIs and NSIs was not statistically significant (x2 = 1.286, p = 0.733).

Frequency of *mecA* gene and enterotoxins among MRSA isolates. All 100 isolates were assessed to detect the *mecA* gene as a source of resistance to methicillin. The *mecA* gene was positive in 25% (25/100) of total isolates, in which 36% (18/50) CSIs and 14% (7/50) NSIs harbored this gene (Fig. 3). This difference was statistically significant (X2 = 6.53; p = 0.011). 22 isolates with the cefoxitin-resistant phenotype (MRSA) were positive for the *mecA* gene in the PCR test. Interestingly, 3 clinical isolates in the disk diffusion method were susceptible to cefoxitin, while carrying the *mecA* gene.

Of the 22 MRSA isolates, 8 isolates were positive for at least one of the tested enterotoxin genes. The prevalence of *sea* and *seb* genes among MRSA isolates was 36.3% (8/22) and 9% (2/22), respectively. However, out of the 78 isolates with susceptible phenotype for cefoxitin (MSSA), 27 (34.6%) isolates were positive for at least one of the enterotoxin genes, with a frequency of 34.6% and 69.7% for *sea* and *seb* genes, respectively. The occurrence of *mecA* and enterotoxin genes, based on the cefoxitin susceptibility and the origin of isolates is shown in Table 4. While the difference between the frequency rate of *sea* gene among MRSA strains isolated from clinical specimens (46.6%, 7/15) was significant compared to strains isolated from nostril (14.3%, 1/7) (X2 = 3.85; p = 0.049), the difference in a total number of *sea* and *seb* genes among CSIs and NSIs was not statistically significant (X^2 = 2.163; p = 0.141).

DISCUSSION

Although the overall difference of antimicrobial resistance pattern between strains isolated from clinical samples and nares of hospital employees was not

Table 3. Antibiotic susceptibility pattern of clinical isolated *S. aureus* (CSIs) and nasal isolated *S. aureus* (NSIs).

Antibiotic	CSIS n=50 (%)			NSIS n=50 (%)			22= MRSA Resistant No. (%)	
	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible	CSIs n=15	NSIs n=7
Cotrimoxazole	(18) 9	0	(82) 41	(10) 5	(2) 1	(88) 44	(6.46) 7	(1.57) 4
Rifampin	(8) 4	0	(92) 46	(6) 3	0	(94) 47	(6.26) 4	(8.42) 3
Cefoxitin	c(30) 15	(2)1	(68) 34	(14) 7	0	(86) 43	(100) 15	(100) 7
Erythromycin	(30) 15	(2)1	(68) 34	(14) 7	(12) 6	(74) 37	(80) 12	(1.57) 4
Ciprofloxacin	(18) 9	(4)2	(78) 39	(12) 6	(6) 3	(82) 41	(3.53) 8	(1.57) 4
Tetracycline	(38) 19	(2)1	(60) 30	(22) 11	(4) 2	(74) 37	(6.86) 13	(8.42) 3
Clindamycin	(28) 14	0	(72) 36	(16) 8	(12) 6	(72) 36	(80) 12	(4.71) 5
Penicillin G	(100) 50	0	0	(100) 50	0	0	(100) 15	(100) 7

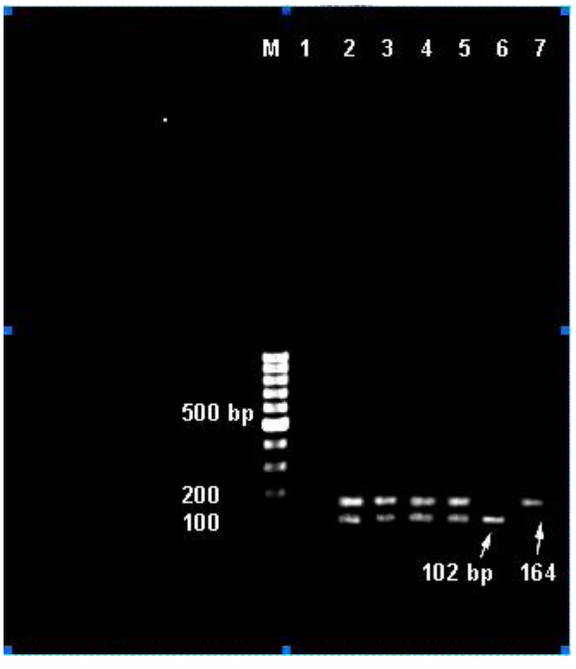

Fig. 1. Agarose gel electrophoresis of multiplex PCR products for enterotoxin A and B genes (*sea, seb*). Lane 1, a strain negative for *sea* and *seb* genes; Lane 2, positive control strain containing both the enterotoxin genes; Lanes 3-5, isolates contain *sea* and *seb* genes, simultaneously; Lane 6, *sea* gene positive strain (102 bp); Lane 7, *seb* gene positive strain (164 bp); lane M, 100 bp DNA size marker.

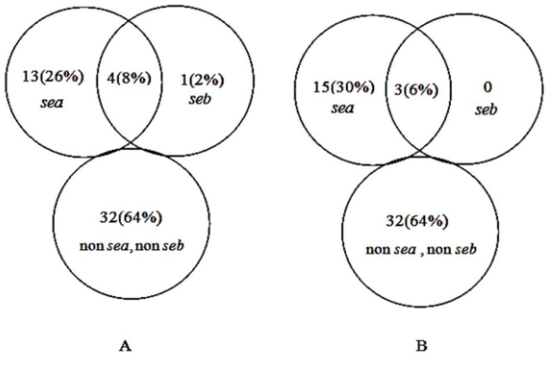

Fig. 2. The frequency of enterotoxin A (*sea*) and B (*seb*) genes among 100 isolates of *Staphylococcus aureus*. A, Frequency of the *sea* and *seb* genes among 50 NSIs; B, Frequency of the *sea* and *seb* genes among 50 CSIs (x2 = 1.286, p = 0.733).

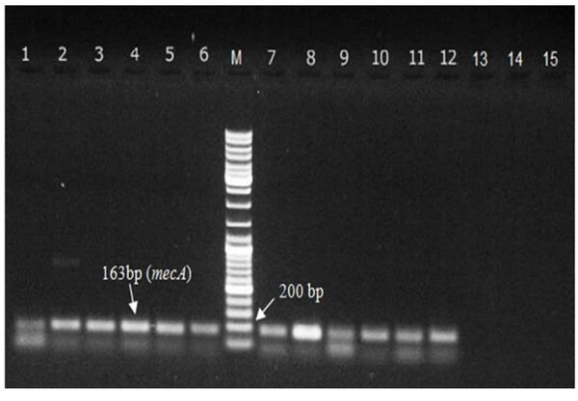

Fig. 3. Agarose gel electrophoresis of PCR products generated from *mecA* gene amplification. Lanes 1-10, *mecA* gene positive isolates; Lanes 13-15 *mecA* gene negative isolates; Lanes 11 and 12, positive control strains; Lane M, 100 bp DNA size marker.

statistically significant, our results revealed that the prevalence of antibiotic resistance was higher among CSIs. Resistance to cefoxitin (MRSA) was statistically significant between both group samples (p = 0.047). In the other words, the frequency of MRSA isolates from CSIs was approximately 2 times that of NSIs. It is obvious that due to the increased use of an-

Table 4. The frequency rates of *mecA* gene, enterotoxins and cefoxitin susceptibility pattern among *Staphylococcus aureus* isolates.

Source of isolate	No. of isolates tested by disk diffusion with CFX[¶]	PCR for *mecA*		PCR for enterotoxin genes[£]		
		No. of positive	No. of negative	*sea*[¥]	*seb*	*sea* + *seb*
Clinical n=50	15 resistant (MRSA)	15	0	6	0	1
	35 susceptible	3	32	9	0	2
Nasal n=50	7 resistant (MRSA)	7	0	0	0	1
	43 susceptible	0	43	13	1	3
Total	100	25	75	28	1	7

[¶]CFX, cefoxitin (30 µg)

[¥] *sea* gene was more frequent in clinical than nasal strains ($X^2 = 3.85$; p= 0.049)

[£]X2 = 2.163; p= 0.141

tibiotics in hospital settings, the selective pressure on hospital-acquired infectious agents such as *S. aureus* is higher than the acquired infectious agents from the community (21). Methicillin-resistant staphylococci have been reported in the UK since 1961 (22), and today its spread in community and health care settings is considered a global concern and is associated with significant mortality and morbidity. These resistant strains have been isolated from various CSIs as well as from NSIs (23-25). Based on the CLSI recommendations, the disk diffusion method using cefoxitin disk (30 mg) has a higher sensitivity to detect MRSA phenotypes (19, 26). The results of our study showed that 14% and 36% of *S. aureus* isolated from NSIs and CSIs were methicillin-resistant (MRSA), respectively.

In the Middle East, nasal carriers for MRSA have been reported to be between 0%-13.2% (23). Mamishi et al. in Tehran reported that the prevalence of MRSA isolated from NSIs and CSIs were 14.9% and 48%, respectively (27). Also, in the study by Wongboot et al. 47% (70/149) of the CSIs and 3% (1/37) of the NSIs were MRSA(28). According to the CLSI guidelines, the criterion for resistance to methicillin is a MIC greater than 4 mg/l for oxacillin or the existence of the *mecA* gene (29). Therefore, in the current study, all isolates were evaluated for the presence of the *mecA* gene by the PCR method. The results of the frequency for the *mecA* gene in various studies such as Rajabiani et al. in Tehran (36.3%), and Hoseini Alfatemi et al. in Shiraz (42.3%) were in agreement with our results (36% of the CSIs and 14% of the NSIs) (30, 31). The occurrence of the *mecA* gene among CSIs compared with NSIs was statistically significant. Selective pressure from the widespread use of β-lactams in hospital systems, as well as compliance with hospital-acquired infectious disease control programs by staff, can explain such discrepancy. MRSA phenotypes are mainly created by the acquiring of the *mecA* gene, and in fewer cases by PBP4 and the *ica* gene (32). In this study, 3 clinical isolates were susceptible to cefoxitin (MSSA) but were *mecA* carriers. In previous studies, similar results have been reported for the discrepancy between phenotype and genotype of MRSA strains (33, 34). For the characterizing of such isolates, Hososaka et al. classified oxacillin susceptible and *mecA*-positive phenotypes (OS-MRSA) as new types of MRSA, which have distinct characteristics from MRSA acquired from the community or hospital (29).

Our PCR results revealed that 30%, 0%, and 6% of the CSIs and 26%, 2% and, 8% NSIs were carriers for the *sea*, *seb*, and *sea+seb*, respectively. These results are consistent with those of the study by, Wongboot et al. (2013), which stated that of 149 isolates of *Staphylococcus aureus* obtained from the clinical samples, 45%, 11%, and 5% were carriers of the *sea*, *seb*, and *sea+seb* genes, respectively. Besides, it is reported that *S. aureus* isolated from nares, carried 38%, 8%, and 5%, the above genes, respectively (28). Despite our results, Peck et al. (2006), found the most common enterotoxin gene in *S. aureus* taken from blood samples was *seg* gene (41/70, 58.6%), while for nasal isolates was *sei* (67/95, 70.5%) (35). The results of our study demonstrated that 36.3% and 34.6% of isolates of MRSA and MSSA harbored at least one of the tested enterotoxin genes, respectively; but this difference wasn't significant. These results are not consistent with those of the study by Wongboot et al. (2013), in which the prevalence of enterotoxin genes

among MRSA was higher than MSSA (28). It should be noted that the differences between toxin profiles among various samples and studies could be due to the existence of genes of staphylococcal enterotoxins on various genetic elements, such as bacteriophage, plasmid, and pathogenic islands. It is evident that the difference in the genetic content could affect the prevalence of these genes. On the other hand, the distribution of the genes of enterotoxin A and B varies in different genetic clones. Cha et al. proved that the main genetic clones containing the sea gene were ST59, ST30, ST1 (35, 36).

Therefore, it is not to be expected that our tested isolates would have different clones in terms of genetic background, which requires more complementary experiments. In addition, *Staphylococcus aureus* toxins profile could be completely variable based on the geographical location (the spread of specific clones), origins of sample (clinical, nasal), and even the site of isolation (blood, ulcer, etc.). As far as the authors know, this is the first study to evaluate the frequency of the *mecA* gene and enterotoxins of *S. aureus* in two different clinical and nasal populations in Khorramabad city. However, the limited sample size, lack of additional examinations such as the minimum inhibitory concentration (MIC) for oxacillin, the molecular typing, and the determination of the clonality of isolates were the limitations of this study.

In summary, the results of this study proved that the frequency of the *mecA* gene in clinical specimens is more common than those isolates from nares of clinical workers in selected hospitals in Khorramabad, and the gene is found in about one-third of the samples. It is assumed, selective pressure of antibiotics on clinical strains significantly increases the frequency of genetic exchanges between bacteria in hospital settings. It is obvious, in addition to transferring of resistance-encoding genes, some of the virulence-associated genes may also be transmitted. Therefore, the screening of hospital staff for *S. aureus* carriage, training of patients and medical staff, adherence to the principles of hospital infections controlling, and hygienic conditions in contact with physicians or tools among personnel and patients, particularly the careful observance of food hygiene principles in cooking and handling can help to prevent and reduce the *S. aureus* infections and intoxications. One of the limitations in the current experiment was the small sample size, Therefore studies should be carried out using more samples to confirm the results.

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REFERENCES

1. Boncompain CA, Suárez CA, Morbidoni HR. *Staphylococcus aureus* nasal carriage in health care workers: first report from a major public hospital in Argentina. *Rev Argent Microbiol* 2017;49:125-131.
2. Dağı HT, Fındık D, Demirel G, Arslan U. Detection of methicillin resistance and various virulence factors in *Staphylococcus aureus* strains isolated from nasal carriers. *Balkan Med J* 2015;32:171-175.
3. Bitrus AA, Zunita Z, Bejo SK, Othman S, Nadzir NA. *In vitro* transfer of methicillin resistance determinants *mecA* from methicillin resistant *Staphylococcus aureus* (MRSA) to methicillin susceptible *Staphylococcus aureus* (MSSA). *BMC Microbio*l 2017;17:83.
4. Assis LM, Nedeljković M, Dessen A. New strategies for targeting and treatment of multi-drug resistant *Staphylococcus aureus. Drug Resist Updat* 2017;31:1-14.
5. Opacic D, Lepsanovic Z, Sbutega-Milosevic G. Distribution of disinfectant resistance genes qacA/B in clinical isolates of meticillin-resistant and -susceptible *Staphylococcus aureus* in one Belgrade hospital. *J Hosp Infect* 2010;76:266-267.
6. Dadashi M, Nasiri MJ, Fallah F, Owlia P, Hajikhani B, Emaneini M, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) in Iran: A systematic review and meta-analysis. *J Glob Antimicrob Resist* 2017;12:96-103.
7. Xia J, Gao J, Kokudo N, Hasegawa K, Tang W. Methicillin-resistant *Staphylococcus aureus* antibiotic resistance and virulence. *Biosci Trends* 2013;7:113-121.
8. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* 2005;5:751-762.
9. Hawkins G, Stewart S, Blatchford O, Reilly J. Should healthcare workers be screened routinely for meticillin-resistant *Staphylococcus aureus*? A review of the evidence. *J Hosp Infect* 2011;77:285-289.
10. Emaneini M, Jabalameli F, Rahdar H, Leeuwen WBV, Beigverdi R. Nasal carriage rate of methicillin resistant *Staphylococcus aureus* among Iranian healthcare workers: A systematic review and meta-analysis. *Rev Soc Bras Med Trop* 2017;50:590-597.
11. Lawrynowicz-Paciorek M, Kochman M, Piekarska K, Grochowska A, Windyga B. The distribution of enterotoxin and enterotoxin-like genes in *Staphylococcus*

aureus strains isolated from nasal carriers and food samples. *Int J Food Microbiol* 2007;117:319-323.
12. Omoe K, Hu DL, Ono HK, Shimizu S, Takahashi-Omoe H, Nakane A, et al. Emetic potentials of newly identified staphylococcal enterotoxin-like toxins. *Infect Immun* 2013;81:3627-3631.
13. Sergelidis D, Angelidis AS. Methicillin-resistant *Staphylococcus aureus*: A controversial food-borne pathogen. *Lett Appl Microbiol* 2017;64:409-418.
14. Pinchuk IV, Beswick EJ, Reyes VE. Staphylococcal enterotoxins. Toxins (Basel) 2010;2:2177-2197.
15. Ortega E, Abriouel H, Lucas R, Gálvez A. Multiple roles of *Staphylococcus aureus* enterotoxins: pathogenicity, superantigenic activity, and correlation to antibiotic resistance. *Toxins (Basel)* 2010;2:2117-2131.
16. Klotz M, Opper S, Heeg K, Zimmermann S. Detection of *Staphylococcus aureus* enterotoxins A to D by Real-Time fluorescence PCR assay. *J Clin Microbiol* 2003;41:4683-4687.
17. Goudarzi G, Tahmasbi F, Anbari K, Ghafarzadeh M. Distribution of genes encoding resistance to macrolides among staphylococci isolated from the nasal cavity of hospital employees in Khorramabad, Iran. *Iran Red Crescent Med J* 2016;18(2):e25701.
18. Mahon C, Lehman D, Manuselis G (2011). Textbook of diagnostic microbiology. 5th ed. Maryland Heights: Saunders Elsevier Company.
19. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 25th ed. CLSI supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
20. Mehrotra M, Wang G, Johnson WM. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J Clin Microbiol* 2000;38:1032-1035.
21. Fey PD, Said-Salim B, Rupp ME, Hinrichs SH, Boxrud DJ, Davis CC, et al. Comparative molecular analysis of community-or hospital-acquired methicillin-resistant *Staphylococcus aureus. Antimicrob Agents Chemother* 2003;47:196-203.
22. Harkins CP, Pichon B, Doumith M, Parkhill J, Westh H, Tomasz A, et al. Methicillin-resistant *Staphylococcus aureus* emerged long before the introduction of methicillin into clinical practice. *Genome Biol* 2017;18:130.
23. Aqel AA, Alzoubi HM, Vickers A, Pichon B, Kearns AM. Molecular epidemiology of nasal isolates of methicillin-resistant *Staphylococcus aureus* from Jordan. *J Infect Public Health* 2015;8:90-97.
24. Dibah S, Arzanlou M, Jannati E, Shapouri R. Prevalence and antimicrobial resistance pattern of methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated from clinical specimens in Ardabil, Iran. *Iran J Microbiol* 2014;6:163-168.
25. Hussein NR, Assafi MS, Ijaz T. Methicillin-resistant *Staphylococcus aureus* nasal colonisation amongst healthcare workers in Kurdistan region, Iraq. *J Glob Antimicrob Resist* 2017;9:78-81.
26. Farahani A, Mohajeri P, Gholamine B, Rezaei M, Abbasi H. Comparison of different phenotypic and genotypic methods for the detection of methicillin-resistant *Staphylococcus aureus. N Am J Med Sci* 2013;5:637-640.
27. Mamishi S, Mahmoudi S, Sadeghi RH, Movahedi Z, Hadipour R, Pourakbar B. Genotyping of *Staphylococcus aureus* strains among healthcare workers and patients in the tertiary referral children's medical hospital in Tehran, Iran. *Br J Biomed Sci* 2012;69:173-177.
28. Wongboot W, Chomvarin C, Engchanil C, Chaimanee P. Multiplex PCR for detection of superantigenic toxin genes in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* isolated from patients and carriers of a hospital in northeast Thailand. *Southeast Asian J Trop Med Public Health* 2013;44:660-671.
29. Hososaka Y, Hanaki H, Endo H, Suzuki Y, Nagasawa Z, Otsuka Y, et al. Characterization of oxacillin-susceptible *mecA*-positive *Staphylococcus aureus:* A new type of MRSA. *J Infect Chemother* 2007;13:79-86.
30. Rajabiani A, Kamrani F, Boroumand MA, Saffar H. *mec-A*-mediated resistance in *Staphylococcus aureus* in a referral hospital, Tehran, Iran. *Jundishapur J Microbiol* 2014;7(4):e9181.
31. Hoseini Alfatemi SM, Motamedifar M, Hadi N, Sedigh Ebrahim Saraie H. Analysis of virulence genes among methicillin resistant *Staphylococcus aureus* (MRSA) strains. *Jundishapur J Microbiol* 2014;7(6):e10741.
32. Zhang K, McClure JA, Elsayed S, Conly JM. Novel staphylococcal cassette chromosome mec type, tentatively designated type VIII, harboring class A mec and type 4 ccr gene complexes in a Canadian epidemic strain of methicillin-resistant *Staphylococcus aureus. Antimicrob Agents Chemother* 2009;53:531-540.
33. Yadegar A, Sattari M, Mozafari NA, Goudarzi GR. Prevalence of the genes encoding aminoglycoside-modifying enzymes and methicillin resistance among clinical isolates of *Staphylococcus aureus* in Tehran, Iran. *Microb Drug Resist* 2009;15:109-113.
34. Mistry H, Sharma P, Mahato S, Saravanan R, Kumar PA, Bhandari V. Prevalence and characterization of oxacillin susceptible *mecA*-positive clinical isolates of *Staphylococcus aureus* causing Bovine Mastitis in India. *PLoS One* 2016;11(9):e0162256.
35. Peck KR, Baek JY, Song JH, Ko KS. Comparison of genotypes and enterotoxin genes between *Staphylococcus aureus* isolates from blood and nasal colonizers in a Korean hospital. *J Korean Med Sci* 2009;24:585-591.
36. Cha JO, Lee JK, Jung YH, Yoo JI, Park YK, Kim BS, et al. Molecular analysis of *Staphylococcus aureus* isolates associated with staphylococcal food poisoning in south Korea. *J Appl Microbiol* 2006;101:864-871.