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High prevalence of SCC mec-associated Phenol-soluble modulin gene in clinical isolates of methicillin-resistant *Staphylococcus aureus*

M.R. Khorasani[1], B. Zamanzad[2], S. Rostami[3], A. Gholipour[1]

Key words: Biofilms, Methicillin-Resistant Staphylococcus aureus, Panton-Valentine leukocidin, Phenol-soluble modulin

Parole chiave: Biofilm, Staphylococcus aureus meticillina-resistente, leucocidina Panton-Valentine, modulina fenol-solubile

Abstract

Introduction. *We aimed to determine the distribution of Phenol-soluble modulin-mec (psm-mec) gene and its relationship with biofilm formation in clinical methicillin-resistant S. aureus (MRSA).*

Methods. *In a descriptive study, a total of 94 cefoxitin-resistant S. aureus isolates were collected from patients and tested for antibiotic susceptibility testing, multiplex polymerase chain reaction (MPCR) for detection of mecA and pvl genes, PCR for detection of psm-mec gene and SCCmec typing of psm-mec and pvl-positive isolates. Furthermore, isolates were tested by microtiter plate method for biofilm formation assay.*

Results. *Multiplex PCR for detection of mecA and pvl genes was performed for all cefoxitin-resistant isolates. The mecA gene was found in 92 (97.9%) isolates but none of the isolates carried the pvl gene. Sixty-five (69.1%) isolates harbored psm-mec genes and 95.4% of these isolates belong to SCCmec type III. Statistical analysis showed a significant difference between the presence or absence of psm-mec gene and biofilm production (P<0.001).*

Conclusion. *In this study, more than half of the MRSA strains harbored psm-mec gene and almost one-fifth of them produced strong biofilm. Since the strains with strong biofilm formation have more antibiotic resistance and cause the long-lasting infection, for the suitable treatment of hospitalized patients with this kind of MRSA strains, we should be paid more attention to these strains.*

Introduction

Staphylococcus aureus has been recognized as a famous bacterium in human infectious diseases. According to increasing rate of methicillin-resistant *S. aureus* (MRSA) strains isolation in health care facilities and community, which are now multidrug resistant, therapy has become difficult (1). In addition to the multi-drug resistance phenomena, *S. aureus* expresses an extraordinary collection of virulence factors that allows it to cause severe invasive diseases (2). Virulence factors encoded in two ways: i) on the core genome and ii) acquired by mobile genetic elements (MGEs)

[1] *Department of Microbiology, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran*

[2] *Cellular and Molecular Research Center, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran*

[3] *Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

such as plasmids, phages and transposons (3). Panton-Valentine leukocidine (PVL) is one of the well-known virulence factors that encoded by the prophage-encoded adjacent *lukS* and *lukF* genes. There is an epidemiological association between presences of these genes and community-associated MRSA (CA-MRSA) (4). On the other, since it is believed that the virulence of CA-MRSA is higher than that of the hospital-associated MRSA (HA-MRSA) (4, 5), being and spreading these strains in health care setting, could be harmful for hospitalized patients. Therefor detection of *pvl* gene in clinical isolates of MRSA is a marker for detection of CA-MRSA.

Phenol-soluble modulins (PSMs) are another type of virulence factors in *S. aureus*, which is encoded in core genome (6). These virulence factors are a family of small amphipatic, α-helical peptides that have cytolitic activities and play an important role in *S. aureus* pathogenesis (7). In the *S. aureus* core genome, *agr* locus regulates the expression of various virulence genes and *agrA* gene acts as a positive regulator for PSMs (8). Despite of all PSMs, recently, a specific type of PSM was described (9), which encodes on the Staphylococcal Cassette Chromosome *mec* (SCC*mec*), a type of mobile elements that carried the *mecA* gene responsible for resistance to methicillin (6). The *psm-mec* gene located within *mec* gene complex class A of SCC*mec* clusters (SCC*mec* type II, III and VIII) (9). This novel PSM, PSM-mec, is the first toxin of *S. aureus* that its gene localized on a MGE with an antibiotic resistance gene (10). Transcription of the *psm-mec* gene negatively regulates the *agrA* in *agr* locus, resulting in decreased PSMs production but translation of this gene stimulates biofilm formation (6). These functions may be contributed to decreasing the additional damage to host and surviving the bacteria in the host environment (3).

Since the *psm-mec* gene is present within the mobile element and its presence could be increase the biofilm formation, and the presence of *pvl* gene could be a sign of CA-MRSA attendance, in this study we aimed to screen clinical MRSA isolates for the presence of *psm-mec* and *pvl* genes and biofilm formation. Furthermore, we determined the SCC*mec* type of *psm-mec* and *pvl* positive isolates.

Methods

Bacterial isolates

In a descriptive study approved by the Ethics Committee of Shahrekord University of Medical sciences (Research project number: 1853), a total of 94 non-replicated Cefoxitin-resistant *S. aureus* isolates were collected between March and December 2015 at one of the Teaching Hospitals of Shahrekord University of Medical sciences, Iran. These isolates originated from routine diagnostic tasks and different specimens.

The following data were collected from patients that bacteria were isolated from them: age, sex, type of specimen and the ward of hospitalization. After transporting the isolates to Isfahan Infectious Diseases and Tropical Medicine Research Center laboratory, the isolates were identified as *S. aureus* by conventional methods (Gram staining, catalase test, slide and tube coagulase tests, mannitol fermentation and production of DNase enzyme).

Antimicrobial susceptibility testing

Antibiotic susceptibility testing of the isolates was performed by using Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standard Institute guideline (CLSI, 2015). The tested antimicrobial agents were as follows: penicillin (10U), cefoxitin (30μg), gentamicin (10μg), Trimethoprim-sulfamethoxazole (1.25/23.75μg), erythromycin (15μg), ciprofloxacin (5μg), rifampin (5μg), tetracycline (30 μg), clindamycin (2 μg), linezolid (30μg)

and chloramphenicol (30µg) (MAST, Group Ltd, Merseyside, UK).The minimal inhibitory concentration (MIC) of vancomycin was determined by E-Test (Liofilchem, Italy) according to CLSI guideline. *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were included as quality control strains for disk diffusion and MIC tests; respectively (CLSI, 2015).

Molecular assays

DNA extraction - DNA extraction was performed by boiling method with some modification (11). An aliquot of 1 mL of the culture grown overnight at 37°C in 5 mL of BHI (Brain Heart Infusion, Merck, Germany) was centrifuged for 5 min at 3,000 × g. The supernatant was discarded, the pellet was washed three times in 1 mL of Phosphate-buffered saline, pH 7.8 and the cells were resuspended in 100 µl of TE buffer. The suspension was boiled for 10 minutes at 100°C and immediately placed in -20˚C for 10 minutes. After centrifugation, bacterial suspensions at 13000× g for 2 minutes at 4°C, the supernatant was collected and used as DNA template for PCR reaction.

Identification of mecA and pvl genes - For identification of mecA and pvl genes in MRSA isolates, which were screened by resistance to cefoxitin disk, multiplex polymerase chain reaction (MPCR) was performed (12). Two sets of primers, synthesized by Bioneer, Inc. (Seoul, South Korea) were used to amplify the selected genes that are shown in Table 1. MPCR mixture consisted of 1X reaction buffer (50 mM KCl, 10 mM Tris-HCl [pH 9.0]), 1.5 mM $MgCl_2$, 200 µM concentration of each of the four deoxyribonucleoside triphosphates (dNTPs) (SinaClon BioScience Co. Tehran, Iran), 0.08 and 0.24 µM primers specific for the pvl and mecA; respectively, 5 U of *Taq* DNA-polymerase (SinaClon BioScience Co. Tehran, Iran) and 50 ng DNA. The final volume of the MPCR was 25 µL. Amplification was done in a T100™ Thermal Cycler (Bio-Rad Laboratory, Inc. USA). After an initial denaturation step for 3 min at 94°C, 10 cycles of amplification were performed as follows: 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s and then 25 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 45 s. The reaction was completed with a final extension at 72°C for 5 min. Amplified products were analyzed by 1.5% agarose gel electrophoresis containing ethidium bromide (0.5 µg/mL) and visualized on an ultraviolet illumination.

Identification of psm-mec gene - Since the psm-mec gene is located in the mobile genetic elements SCCmec type II, III and VIII, we designed new primers, refers as psm-mec-F and psm-mec-R in table 1, according to SCCmec type III DNA sequence of S. aureus strain: OC3 (Accession number: AB983237.1) using Primer 3 software (13). The PCR product size of psm-mec gene amplification was 335 bp. After synthesize the primer (Bioneer, Inc. Seol, South Korea), PCR reaction was performed in a 25 µl mixture reaction consisted of: 1X reaction buffer (50 mM KCl, 10 mM Tris-HCl [pH 9.0]), 2 mM $MgCl_2$, 200 µM concentration of each of the four deoxyribonucleoside triphosphates (dNTPs), 5 U of *Taq* DNA-polymerase (SinaClon BioScience Co. Tehran, Iran), 0.4 µM specific primers and 50 ng DNA. After an initial denaturation step for 3 min at 94°C, 35 cycles of amplification were performed as follows: 94°C for 30 s, 51°C for 30 s, and 72°C for 45 s. The final extension carried out at 72°C for 5 min. Amplified products were analyzed by 1.5% agarose gel electrophoresis containing ethidium bromide (0.5 µg/mL) and visualized on an ultraviolet illumination.

SCCmec Typing - For identification of SCC*mec* types of *psm-mec* and *pvl* positive isolates, multiplex PCR was performed according to the established method by Boye et al (14). In this method five type (I-IV) of SCCmec types were determined. Primer sets used for multiplex SCCmec PCR are shown in Table 1.

Table 1 - Oligonucleotide Primers used in this study.

Genes	Name of Primers	Oligonucleotide sequences	Size of the amplified products	References
mecA	*mec*A-F	5'-GTAGAAATGACTGAACGTCCGATGA-3'	310bp	12
	*mec*A-R	5'- CCAATTCCACATTGTTTCGGTCTAA-3'		
pvl	*Pvl* S/F-F	5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3'	433bp	12
	Pvl S/F-R	5'-GCATCAACTGTATTGGATAGCAAAAGC-3'		
Psm-mec	*psm-mec*-F	5'-CAATTCACTTGTCTTAAACTTTGTAGAAAAAGAAG-3'	335bp	This study
	psm-mec-R	5'-TATTTTATTTTCCATATTGCCTACCCCATAAG-3'		
SCCmec Typing	β-F	5'-ATTGCCTTGATAATAGCCYTCT-3'	937bp	14
	α3-R	5'-TAAAGGCATCAATGCACAAACACT-3'		
	CCrC-F	5'-CGTCTATTACAAGATGTTAAGGATAAT-3'	518bp	14
	CCrC-R	5'-CCTTTATAGACTGGATTATTCAAAATAT-3'		
	1272-F1	5'-GCCACTCATAACATATGGAA-3'	415bp	14
	1272-R1	5'-CATCCGAGTGAAACCCAAA-3'		
	5RmecA	5'-TATACCAAACCCGACAACTAC-3'	359bp	14
	5E431	5'-CGGCTACAGTGATAACATCC-3'		

Biofilm formation assay

The microtiter plate method was used for MRSA biofilm formation assay as previously described (15). Briefly, 200 µl of each bacterial suspension grown in trypticase soy broth (TSB) supplemented with 1% glucose was poured into the three wells of a sterile flat-bottomed 96-well polystyrene tissue culture plate and incubated 24 hours at 37°C. Trypticase soy broth (TSB) supplemented with 1% glucose without bacteria was used as negative control. Wells were washed three times with sterile phosphate-buffered saline (PBS; pH 7.2). After that, the fixation step was done by 99% methanol. Then, plates were stained with 2% crystal violet and after washing plates were air-dried. The dye bound to the adherent cells was resolubilized with ethanol (95%). Finally, the optical density (OD) of each well was measured at 570 nm, and average OD value of negative controls and samples was calculated. The cut-off OD (ODc) was defined as three standard deviations above the mean OD of the negative control. All strains were classified into the following categories: non-adherent ($OD \leq ODc$), weakly ($ODc < OD \leq 2\times ODc$), moderately ($2\times ODc < OD \leq 4\times ODc$) or strongly ($4\times ODc < OD$) adherent, based upon the OD. *Staphylococcus epidermidis* ATCC 35984 was used as the biofilm producer control strain.

Statistical Analysis

Statistical Package for the Social Sciences Version 19.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Fisher's exact test or Chi-squared test was performed for analysis of categorical data. A *P*-Value of <0.05 was considered statistically significant.

Results

Sixty nine percent of patients that bacteria were isolated from them were male and mean age of them was 57.75±17.545 years. The most prevalent sample types were wound samples (39.7%) and the most isolates were referred from emergency room (Table 2). The results of antimicrobial susceptibility testing are shown in table 3. All of the isolates were susceptible to vancomycin by E-test (Vancomycin MIC ≤2) and resistant to cefoxitin. Multiplex PCR for detection of *mecA* and *pvl* genes was performed for all cefoxitin-resistant isolates.

Table 2 - The sources of MRSA clinical isolates

Source	% of isolates
Wards	
Emergency Room	30.8
Surgery	21.2
ICU	19.2
Medicine	17.3
Others	11.5
Sample type	
Wound	39.7
Respiratory tract	24.1
Blood	10.4
Urine	6.9
Others	19

The *mecA* gene was found in 92/94 (97.9%) isolates but none of the isolates carried the *pvl* gene. According to PCR results, 65/94 (69.1%) isolates carried *psm-mec* genes. Since the positive control for detection of *psm-mec* was not available, PCR reaction was performed for a number of MRSA isolates and first PCR product that showed 335 bp band on agarose gel was used for sequencing (Genbank Accession number:KU720302). The two *mecA*-negative isolates were *psm-mec*-negative. SCC*mec* analysis determined 62/65 (95.4%) of *psm-mec* positive isolates as SCC*mec* type III and 3/65 (4.6%) of the *psm-mec* positive isolates were non-type able. There were no differentiation between the antibiotic resistance patterns of type able and non-type able isolates. Biofilm formation assay was done for all of the MRSA isolates. Statistical analysis showed significant difference between the presence or absence of *psm-mec* gene and biofilm production ($P<0.001$). The majority of MRSA *psm-mec* positive strains (No=26, 40%) were moderate biofilm producers and on the contrary, in 15 (51.7%) *psm-mec* negative isolates were not seen biofilm formation (Table 4).

Table 3 - The results of antimicrobial susceptibility testing in MRSA clinical isolates

Antibiotics	Sensitive No. (%)	Intermediate No. (%)	ResistanT No. (%)
Penicillin	0 (0)	0 (0)	94 (100)
Cefoxitin	0 (0)	0 (0)	94 (100)
Gentamicin	24 (25.5)	0 (0)	70 (74.5)
Erythromycin	13 (13.8)	1 (1.1)	80 (85.1)
Tetracycline	14 (14.9)	73 (77.7)	7 (7.4)
Ciprofloxacin	22 (23.4)	2 (2.1)	70 (74.5)
Clindamycin	22 (23.4)	0 (0)	72 (76.6)
Trimetoprim-sulfametoxazole	60 (63.8)	1 (1.1)	33 (35.1)
Chloramphenicol	89 (94.7)	1 (1.1)	4 (4.3)
Rifampin	42 (44.7)	0 (0)	52 (55.3)
Linezolid	86 (91.5)	0 (0)	8 (8.5)

Table 4 - The results of psm-mec gene PCR according to biofilm formation

		Biofilm formation No. (%)				P-value
		No adherent	Weekly	Moderately	Strongly	
psm-mec gene	Positive	4 (6.2)	18 (27.7)	26 (40)	17 (26.2)	<0.001
	Negative	15 (21.7)	8 (27.6)	6 (20.7)	0 (0)	

Discussion

CA-MRSA strains produce a high level of toxins such as PVL. This toxin is often associated with skin and soft tissue infections in different animal models but the exact molecular mechanism of PVL pathogenesis is not completely understood in humans (2). In this study, we did not find *pvl*-positive MRSA isolate. However, in other studies, prevalence of PVL in health care facilities was reported differently. Ohadian moghadam *et al*. found that out of 56 cutaneous samples from hospitalized patients, 14.3% (n=8) were *pvl*-positive and of this number 75 %(n=6) were MRSA (15). In another study from Iran, 7.23% of MRSA strains isolates from burn patients were identified as *pvl*-positive strains (16). In a study conducted by Aoyagi et al., none of the MRSA strains isolated from blood cultures carried the *pvl* gene (5). These differences may be due to variety in sample type. Although, recent studies has shown that there is no evidence based on strong correlation between presence of PVL and specific infection types (4, 17). However, in some other studies was demonstrated that *pvl*- positive isolates associated with an aggressive clinical phenotype (18). Although, in our study most of the MRSA strains were isolated from wound samples but we could not find *pvl*-positive isolates.

Since the PSM-mec toxin gene is located on an antibiotic resistance element, this accompaniment simultaneously facilitates the spread of antibiotic resistance and virulence among the *S. aureus* population (10). In present study, 69.1% isolates were *psm-mec* positive strain. More than half of the MRSA strain in this study were *psm-mec* positive and these strains are a threat to public health. Unfortunately, there is not enough study about the distribution of *psm-mec* gene in MRSA strains isolated from different clinical samples. In one study by Aoyagi *et al*., 52 patients with MRSA bacteremia investigated and found 51(98.1%) *psm-mec* positive MRSA strains(5). Most of the *psm-mec* positive isolates in this study showed SCC*mec* type III (95.4%). As mentioned before, *psm-mec* gene located on the class A of SCC*mec* element (10) and our findings confirmed this subject. However, 3 of *psm-mec* positive isolates were non-type able and probably they were other types of SCCmec type that not concluded in used SCCmec typing method. This point was one of the our study limitation.

In the present study biofilm formation was investigated in all MRSA strains. There was a significant difference between the *psm-mec*-positive and -negative strains. Furthermore, strong biofilm formation was detected in 20.7% *psm-mec* positive strains. In contrast, none of the *psm-mec*-negative strains produced strong biofilm. In addition, 49.3% *psm-mec*-negative strains were considered to be none or week biofilm producer. These findings confirmed the relation between the *psm-mec* presence and biofilm formation. As mentioned before, *psm-mec* translation and transcription products have different function. The *psm-mec* RNA acts as an inhibitor for exotoxin production, while, PSM-mec protein have a stimulating effect on the biofilm formation (3, 6). On

the other hand, the difference in the genetic backgrounds of the MRSA strains, for example mutation in the promoters of *psm-mec* could affect the ratio of the biofilm formation (6). Aoyagi *et al*. reported that mutated *psm-mec* promoter MRSA formed significantly less biofilm than intact *psm-mec* MRSA and the *psm-mec* negative MRSA also showed decreased biofilm formation(5). In this study, some of the *psm-mec* positive MRSA strains (33.9%) formed week or none biofilm. These kinds of strains should be investigated more from the point of the probability of mutation in *psm-mec* promoter.

The presence of *psm-mec* in HA-MRSA leads to increasing the biofilm formation and may be beneficial for HA-MRSA to establish long lasting infection such as catheter-related infections. Detection of such strains could be useful to patient treatment strategies and epidemiological studies.

Our study has some limitation, such as being a single-center study with small sample and lack of funds for more experiments such as molecular typing. However, since the study on distribution and function of *psm-mec* gene in clinical MRSA is very low, further studies, like our study, are required.

In our study, the presence of two virulence factor genes was investigated. The *pvl* gene, one of the probable indicators for CA-MRSA, was not detected. However, more than half of MRSA strains harbored *psm-mec* gene and almost one fifth of them produced strong biofilm. For the suitable treatment of hospitalized patients with MRSA infections should be paid special attention to these strains.

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Conflict of interest
None declared

Riassunto

Elevata prevalenza del gene SCC mec-associated PSM in isolamenti clinici di MRSA

Premessa. Abbiamo inteso determinare la distribuzione del gene "Phenol-soluble Modulin-mec" o "psm-mec" ed il rapporto di questo con la formazione di biofilm da parte di ceppi clinicamente isolati di *S aureus* resistenti alla meticillina.

Metodi. Nell'ambito di uno studio descrittivo, 94 ceppi di *S. aureus* resistenti alla cefoxitina furono isolati da pazienti e sottoposti al saggio per la sensibilità agli antibiotici, alla MPCR per l'eventuale presenza dei geni *mecA* e *pvl*, a PCR per la ricerca del gene *psm-me* ed alla tipizzazione SCC*mec* per identificare i ceppi *psm-mec* e *pvl* positivi. Inoltre, i ceppi isolati sono stati sottoposti alla microtitolazione su piastra per verificare l'eventuale produzione di biofilm.

Risultati. il test Multiplex PCR è stato eseguito su tutti i ceppi resistenti alla cefoxitina per l'identificazione dei geni *mecA* e *pvl*. In 92 (97.9%) ceppi è stato trovato il gene *mecA*, mentre da nessuno è stato isolato il gene *pvl*. Sessantacinque ceppi (69.1%) ospitavano i geni *psm-mec* ed il 95.4% di questi apparteneva al tipo III dello SCC*mec*. L'analisi statistica ha evidenziato una differenza significativa tra la presenza/assenza dei geni *psm-mec* e la produzione di biofilm (P<0.001).

Conclusioni. nel presente studio oltre la metà dei ceppi di MRSA ospitavano il gene *psm-mef* ed almeno un quinto di essi mostrava un'intensa capacità produttiva di biofilm. Dato che i ceppi che producono biofilm mostrano una maggiore antibiotico-resistenza e causano infeezioni di più lunga durata, dobbiamo porre molta attenzione clinica proprio a questa categoria di *S. aureus* meticillina-resistenti produttori di biofilm.

References

1. Foster TJ. The Staphylococcus aureus "superbug". J Clin Invest 2004; **114**: 1693-6.
2. Graves SF, Kobayashi SD, DeLeo FR. Community-associated methicillin-resistant *Staphylococcus aureus* immune evasion and virulence. J Mol Med (Berl) 2010; **88**: 109-14.
3. Ikuo M, Nagano G, Saito Y, et al. Inhibition of exotoxin production by mobile genetic element SCCmec-encoded psm-mec RNA is conserved in staphylococcal species. PLoS One 2014; **9**: e100260.
4. Otto M. Community-associated MRSA: what makes them special? Int J Med Microbiol 2013; **303**: 324-30.

5. Aoyagi T, Kaito C, Sekimizu K, et al. Impact of psm-mec in the mobile genetic element on the clinical characteristics and outcome of SCCmec-II methicillin-resistant *Staphylococcus aureus* bacteraemia in Japan. Clin Microbiol Infect 2014; **20**: 912-9.
6. Kaito C, Saito Y, Nagano G, et al. Transcription and translation products of the cytolysin gene psm-mec on the mobile genetic element SCCmec regulate *Staphylococcus aureus* virulence. PLoS Pathog 2011; **7**: e1001267.
7. Joo HS, Cheung GY, Otto M. Antimicrobial activity of community-associated methicillin-resistant *Staphylococcus aureus* is caused by phenol-soluble modulin derivatives. J Biol Chem 2011; **286**: 8933-40.
8. Novick RP. Autoinduction and signal transduction in the regulation of staphylococcal virulence. Mol Microbiol 2003; **48**: 1429-49.
9. Queck SY, Khan BA, Wang R, et al. Mobile genetic element-encoded cytolysin connects virulence to methicillin resistance in MRSA. PLoS Pathog 2009; **5**: e1000533.
10. Chatterjee SS, Chen L, Joo HS, et al. Distribution and regulation of the mobile genetic element-encoded phenol-soluble modulin PSM-mec in methicillin-resistant *Staphylococcus aureus*. PLoS One 2011; **6**: e28781.
11. Moosavian M, Shoja S, Rostami S, et al. Inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus* due to erm genes, Iran. Iran J Microbiol 2014; **6**: 421-7.
12. McClure J-A, Conly JM, Elsayed S, et al. Multiplex PCR assay to facilitate identification of the recently described staphylococcal cassette chromosome mec type VIII. Mol Cell Probes 2010; **24**: 229-32.
13. Untergasser A, Nijveen H, Rao X, et al. Primer3Plus, an enhanced web interface to Primer3. Nucleic Acids Res 2007; **35**: W71-4.
14. Boye K, Bartels MD, Andersen IS, et al. A new multiplex PCR for easy screening of methicillin-resistant *Staphylococcus aureus* SCCmec types I–V. Clin Microbiol Infect 2007; **13**: 725-7.
15. Ohadian Moghadam S, Pourmand MR, Aminharati F. Biofilm formation and antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* isolated from burn patients, Iran. J Infect Dev Ctries 2014; **8**: 1511-7.
16. Khosravi AD, Hoveizavi H, Farshadzadeh Z. The prevalence of genes encoding leukocidins in *Staphylococcus aureus* strains resistant and sensitive to methicillin isolated from burn patients in Taleghani Hospital, Ahvaz, Iran. Burns 2012; **38**: 247-51.
17. McClure JA, Conly JM, Lau V, et al. Novel multiplex PCR assay for detection of the staphylococcal virulence marker Panton-Valentine leukocidin genes and simultaneous discrimination of methicillin-susceptible from -resistant staphylococci. J Clin Microbiol 2006; **44**: 1141-4.
18. Hewagama S, Spelman T, Woolley M, et al. The Epidemiology of *Staphylococcus aureus* and Panton-Valentine Leucocidin (pvl) in Central Australia, 2006-2010. BMC Infect Di 2016; **16**: 382.

Corresponding author: Dr. Soodabeh Rostami, Nosocomial Infection Research Center, Isfahan University of Medical Sciences, Alzahra Hospital, Comprehensive laboratory, Infectious Diseases and Tropical Medicine Research Center Isfahan, Soffeh Street, Iran
e-mail: srostami1876@gmail.com