JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Toxin production and drug resistance profiles of pediatric methicillin-resistant *Staphylococcus aureus* isolates in Tehran

Mozhgan Esmaeili Benvidi[1*], Hamidreza Houri[1*], Zohreh Ghalavand[1], Bahram Nikmanesh[2], Hadi Azimi[3], Roghayeh Samadi[1], Narges Nodeh Farahani[4], Gita Eslami[1]

[1] *Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[2] *Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran*
[3] *Department of English Language Teaching, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran*
[4] *Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran*

* Both authors contributed equally to this work.

Abstract

Introduction: *Staphylococcus aureus* is known to be a major cause of skin and soft tissue infections, pneumonia and invasive diseases. In this study, attempts were made to examine the prevalence of *tsst-1, eta, etb,* and *luk-PV* genes among methicillin-resistant *S. aureus* (MRSA) isolated from children in Tehran.

Methodology: In the present cross-sectional study, a total of 100 MRSA were isolated from children who were referred to a pediatric hospital during 11-month period of September 2014 to August 2015. Isolates were identified using biochemical tests and then, using PCR, the isolates were tested for the presence of *mecA*, *tsst-1, eta, etb, and luk-PV* genes. Susceptibility of isolates to cefoxitin, penicillin, erythromycin, clindamycin, gentamicin, rifampin, minocycline, co-trimoxazole, linezolid, and vancomycin were evaluated using standard methods.

Results: It was found that the MRSA isolates had the greatest resistance to clindamycin (72%) and erythromycin (59%), while the lowest rates of resistance were observed to be related to minocycline (6%) and rifampin (12%). All of isolates were sensitive to vancomycin and linezolid. The *mecA* gene was detected in all the isolates. Moreover, *luk-PV* and *tsst-1* were detected in 18% and 17% of the isolates, respectively. None of the isolates harbored *eta* and *etb* genes.

Conclusions: Our data provide specifications about the toxin production status of *S. aureus* isolates from pediatric children. The current study showed increased resistance to different antibiotics in *S. aureus* isolates. Therefore, to prevent multi-resistance to other antibiotic classes, it is essential to withhold prescriptions and stop unessential use of available antibiotics.

Key words: Methicillin-resistant *Staphylococcus aureus*; toxic shock syndrome toxin-1; Panton-Valentine leukocidin; staphylococcal exfoliative toxin; pediatrics.

J Infect Dev Ctries 2017; 11(10):759-765. doi:10.3855/jidc.9360

(Received 28 August 2016 – Accepted 21 February 2017)

Introduction

Although *Staphylococcus aureus* normally colonizes the nares and skin in up to 80% of the population, it poses as one of the most important bacterial pathogens that cause a wide variety of diseases in humans. It has long been considered as not only the leading cause of community-acquired skin and soft tissue infections, but also one of the most important organisms causing life-threatening nosocomial infections, including necrotizing pneumonia, bacteremia, and various surgical wound infections [1,2]. Meanwhile, neonates and young children are more susceptible to serious complications of *S. aureus*, particularly skin infections [3].

Pathogenicity of *S. aureus* is mainly related to the repertoire virulence factors including toxins, invasive capacity, adhesins, exoenzymes, and immune-modulating proteins it produces as well as a variety of resistance mechanisms to many existing antibiotics. The most important virulence factors with this pathogen are numerous exotoxins such as hemolysins (Hla, Hlb, Hld, and Hlg), Panton-Valentine leukocidin (PVL), toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEs), and exfoliative toxin A and B (ETA and ETB) [4,5]. However, it should be

pointed out that most of these factors are not always expressed and their expression occurs in specified strains and under certain conditions. For instance, *S. aureus* strains that produce PVL are usually associated with skin and soft-tissue infections [6].

Pervasive resistance among clinical isolates of *S. aureus* strictly complicates management of its infections. In the recent decades, the global concerns about increasing methicillin-resistant *S. aureus* (MRSA) infections in both community and nosocomial settings have attracted significant attentions due to their therapeutic importance. MRSA strains are widespread in the hospital environment, and have recently caused a worldwide epidemic of community-acquired MRSA (CA-MRSA) infections [7,8]. In addition, several authors have reported that MRSA strains have become not only resistant to beta-lactam antibiotics, but they have also acquired additional resistance to non-beta-lactam antibiotics, especially aminoglycosides [9]. Moreover, the presence of resistance gene to methicillin may affect toxin production [6].

Given that the severity and rapid expansion of MRSA strains in the pediatric population is considered as an important health issue, the current study was conducted to characterize the virulence properties and factors of pediatric clinical isolates of MRSA in Tehran, Iran.

Methodology

Bacterial isolation and identification

The present cross-sectional study was carried out on children who had any obvious staphylococcal infection, and also those that were asymptomatic carriers referred to Children's Medical Center Hospital, from September 2014 to August 2015 in Tehran. In this study, asymptomatic carriers are defined as those subjects who had an *S. aureus*-positive nasal culture on the day of admission. Standard conventional biochemical tests for identification of the isolates were performed on colonies from primary cultures. For this purpose, all suspected colonies of *S. aureus* were examined using Gram staining and further identified using catalase, haemolysis, oxidase, coagulase, DNase, and mannitol fermentation tests. The isolates were stored at -70 °C in trypticase soy broth containing 10% glycerol for later analysis.

Phenotypic identification of MRSA

Methicillin resistance of *S. aureus* isolates was screened using disk diffusion test with a cefoxitin disk (30 µg/disk) (Mast Co., Germany) on Mueller-Hinton agar (Merck Co., Germany). According to the Centers for Disease Control and Prevention (CDC), the definition of CA-MRSA strains is any MRSA strain isolated from a patient within 48 to 72 hours of hospital admission, as long as the patient lacks health care-associated MRSA risk factors like surgery, haemodialysis, or hospitalization during the previous year. Accordingly, hospital-acquired MRSA (HA-MRSA) is defined as a MRSA strain isolated from a patient with established risk factors for MRSA infections, including recent surgery, recent hospitalization, residence in a long-term-care facility, or injecting-drug use [10,11].

Antimicrobial susceptibility test

The antibiotic susceptibility test was performed using the standardized Kirby-Bauer disc-diffusion technique on Mueller-Hinton agar (Merck Co., Darmstadt, Germany). Commercially available disks of the antimicrobial drugs (Mast Co., Darmstadt, Germany), that have been used most frequently for the treatment of *S. aureus* infections, were selected and tested as recommended by the Clinical and Laboratory Standards Institute (CLSI) [12]. Accordingly, susceptibility of the isolates to following antibiotics: penicillin G (10 units/disk), trimethoprim/sulfamethoxazole (1.25/23.75 µg/disk), clindamycin (10 µg/disk), erythromycin (15 µg/disk), gentamicin (10 µg/disk), rifampin (5 µg/disk), minocycline (30 µg/disk), linezolid (30 µg/disk) and vancomycin (30 µg/disk) were examined. The susceptibility to vancomycin was confirmed through determining of minimum inhibitory concentration (MIC) using E-test (Liofilchem Co., Roseto, Italy). *S. aureus* ATCC 25923 was used as a quality control reference strain. In this study, a multi-drug resistant (MDR) *S. aureus* strain was considered as a single isolate resistant (intermediate or complete) to three or more unique antimicrobial classes [13].

Preparation of whole-cell DNA for PCR

The isolates were recovered from storage via subculture on blood agar plates and incubated overnight at 37°C. Bacterial cells were suspended in 250 µl of phosphate-buffered saline (PBS) and then DNA extraction was done using whole genome DNA extraction kit (Cinnaclon Co., Tehran, Iran), according to the manufacturer's instructions. Evaluation of concentration and purity of extracted DNA was measured using Nano drop (DeNovix Inc., Wilmington, USA). Eluted DNA was stored at -20°C for later analysis.

Molecular assay for detection of mecA gene

The presence of the *mecA* gene encoding penicillin-binding protein 2a (PBP2a) of *S. aureus* isolates were confirmed using a PCR assay, as previously described by Seni J. *et al.* [14]. Briefly, amplification of the *mecA* gene was performed using the specific primers (forward primer 5'-GTAGAAATGACTGAACGTCCGATAA-3' and Reverse primer 5'-CCAATTCCACATTGTTTCGCTCTAA-3') yielding a PCR product of 307 bp. PCR reaction was performed in a total volume of 25 µL containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 µL DNA template (3 µg/µL), 10 pmol of each primer, and 5 U Taq polymerase (Cinnaclon Co., Iran). Amplification of *mecA* gene was performed via the following program: initial denaturation at 94 °C for 2 min and 35 cycles of 1 min at 94 °C, 1 min at 55°C and 1 min at 72 °C. The final extension was 5 min at 72 °C. The PCR products were analysed using agarose gel electrophoresis on 1.8% NuSieve agarose gel in 1X TAE buffer at 120 V for 60 minutes. The expected amplicon fragments were extracted and purified from agarose gel using agarose gel DNA extraction kit (Roche Co., Mannheim, Germany) and then were sequenced using Bioneer sequencing methods (Bioneer Inc., Daejeon, South Korea).

Molecular assay for detection of toxin genes

The presence of the virulence genes encoding toxins, including PVL (*luk-PV*), TSST-1 (*tsst-1*), and exfoliative toxin A and B (*eta* and *etb*) were detected via separate monoplex PCR reactions, using specific primers, as shown in Table 1. PCR reactions were carried out in a final volume of 25 µL containing 1X PCR reaction buffer,10 pmol of each primer, 1 µL DNA template (3 µg/µL), 1.5 mM $MgCl_2$, 0.2 mM dNTP mixture, and 5 U of Taq DNA polymerase (Cinnaclon, Tehran, Iran). The thermal cycling conditions included an initial denaturation step at 94 °C for 5 minutes followed by 30 cycles of amplification comprising of three steps: 2 minutes' denaturation at 94 °C; one minute annealing at 55 °C for *luk-PV*, 46 °C for *tsst-1*, 44 °C for *eta* and 55°C for *etb*; and then 1 minute extension at 72°C. The final extension step was at 72°C for 5 minutes. The PCR products were analysed using Tris/Borate/EDTA (TBE) buffered agarose gel (1.5%) electrophoresis at 120 V for 45 minutes. The expected amplicon fragments were purified and sequenced as mentioned above.

Statistical analysis

SPSS version 22.0 (IBM Corp., Armonk, USA) was applied for statistical analysis. Chi-square test was used for the comparison between the prevalence of the toxins according to infection site. A $p\ value < 0.05$ was considered to be statistically significant.

Results

Patients and bacterial identification

In the present study, 343 clinical isolates of *S. aureus* were obtained from children during an 11-month period. The patient's age range was from infancy (1 day) to 15 years old. The median age of the patients was 44±5 months.

Among 343 *S. aureus* isolates, 100 (22.5%) were detected as MRSA strains by a phenotypic method. The distributions of *mecA* gene among MRSA isolates were 100% and its sequence is available on NCBI (GenBank accession number KU380333). Of these, 47 isolates (47%) were collected from outpatient or those referred to emergency department and classified as CA-MRSA; with the remainder from different wards, including pediatric intensive care unit (PICU) (10%), respiratory (10%), surgical (10%), infection (8%), gastroenterology (5%), cardiology (5%), and other wards (5%), which were considered as HA-MRSA. In total, 54% of all MRSA isolates were obtained from nasal of asymptomatic carriers, and the remaining 46% were recovered from clinical samples of infected children, including wound (13%), blood (11%), sputum (7%), abscess (4%), ear discharges (3%), urine (2%), eye discharges (2%), catheter (2%), and synovial fluid (2%).

Table 1. Primers sequences used in this study for the detection of genes encoding toxins in clinical isolates of methicillin-resistant *S. aureus*.

PCR target		Sequences(5'-3')	References	Products size (bp)
luk-PV	F	CTCTAGCCGATGTCGCTCAA	Lina G *et al.* (32)	433
	R	ATACCTGAGGCTCGCCACTG		
tsst-1	F	TTATCGTAAGCCCTTTTGTTG	Designated in the present study.	398
	R	TAAAGGTAGTTCTATTGGAGTAGG		
eta	F	GGAGAGTATGAAGTCAAAG	Designated in the present study.	358
	R	GATGCTCTCTATCAAGATG		
etb	F	AAAAGGGGAATCAGCGGGAG	Designated in the present study.	339
	R	TCGTTCCCCAAAGTGTCTCC		

bp: base pairs; *luk-PV:* Panton-Valentine leucocidin gene; *tsst-1*: toxic shock syndrome toxin gene; *eta*: exfoliative toxin A gene; *etb*: exfoliative toxin B gene; F: forward primer; R: reverse primer.

Antibiotic resistance profile

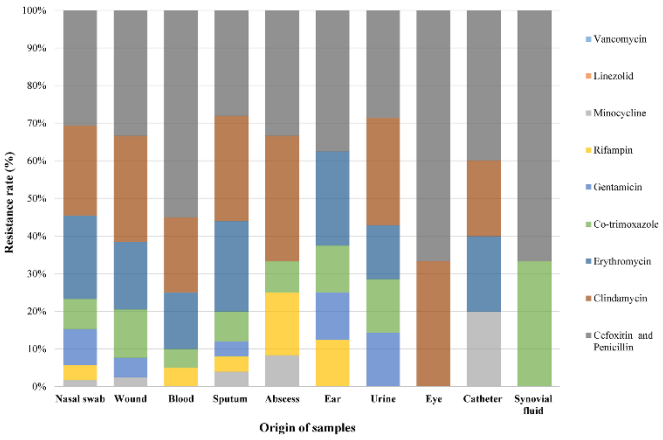
The antibiotics susceptibility results showed that the most active antibiotic against tested MRSA isolates were linezolid and vancomycin (100% as the susceptibility rate), followed by minocycline (94%), rifampin (88%), gentamicin (78%), sulfamethoxazole/trimethoprim (74%). High resistance rates were detected for penicillin (100%), clindamycin (72%), and erythromycin (59%). There was no significant difference in the antibiotic resistance of the strains according to their origin (Figure 1). In the present study, 73% of isolates were classified as MDR *S. aureus* strains. Moreover, our results indicated that 69.5% of clindamycin, 74.5% of erythromycin and 73% of sulfamethoxazole/trimethoprim resistant strains were HA-MRSA isolates.

Toxin profiles

The MRSA isolates were also tested for the presence of PVL (*luk-PV*), TSST-1 (*tsst-1*), and exfoliative toxin A and B (*eta* and *etb*). The *luk-PV* gene (18%) (GenBank accession numbers KU664618 and KU664619) was the most prevalent virulence gene among the MRSA isolates, followed by *tsst-1* gene (17%) (GenBank accession number KU380334).

None of the MRSA isolates carried *eta* and *etb* genes. The percentage of isolates harboring each of the virulence genes is shown in Table 2. The relationships between antibiotic resistance and virulence genes are indicated in Table 3.

Figure 1. Prevalence of antibiotic resistance among methicillin-resistant *S. aureus* isolates according to the sample origin.



Discussion

In the present study, we described the drug resistance profiles and prevalence of *luk-PV*, *tsst-1*, *eta* and *etb* virulence genes among MRSA isolated from both carrier and infected children in Tehran, Iran. MRSA is one of the most common causes of community- and hospital-acquired localized and systemic infections. Approximately, all clinical isolates of MRSA produce a range of more than 30 various extracellular proteins and exoenzymes, most of which

Table 2. Distribution of *luk-PV* and *tsst-1* genes among methicillin-resistant *S. aureus* isolates according to the origin of samples.

Origin (number)	% of isolates harboring *luk-PV* (number)	% of isolates harboring *tsst-1* (number)
Nasal swab (n = 54)	11.1% (n = 6)	11.1% (n = 6)
Wound (n = 13)	30.7% (n = 4)	7.7% (n = 1)
Blood (n = 11)	36.3% (n = 4)	18.1% (n = 2)
Sputum (n = 7)	28.6% (n = 2)	28.6% (n = 2)
Abscess (n = 4)	0% (n = 0)	25% (n = 1)
Ear (n = 3)	0% (n = 0)	33.3% (n = 1)
Urine (n = 2)	0% (n = 0)	100% (n = 2)
Eye (n = 2)	0% (n = 0)	50% (n = 1)
Catheter (n = 2)	50% (n = 1)	50% (n = 1)
Synovial fluid (n = 2)	0% (n = 0)	50% (n = 1)

luk-PV: Panton-Valentine leucocidin gene*; tsst-1*: toxic shock syndrome toxin gene; A *p-value* < *0.05* was considered statistically significant. Only the relationship between the urine isolates and presence of the *tsst-1* was significant (*p value* = 0.02).

Table 3. Characterization of methicillin-resistant *S. aureus* isolates through the determination of antibiotic resistance profiles and prevalence of virulence factors.

Resistance pattern	Total number (%)	*luk-PV* –positive isolates number (%)	*tsst-1* -positive isolates number (%)
Resistant to 7-8 antibiotics	9 (9%)	1 (5.5%)	3 (17.6%)
Resistant to 5-6 antibiotics	28 (28%)	3 (16.7%)	5 (29.5%)
Resistant to 3- 4 antibiotics	36 (36%)	8 (44.4%)	3 (17.6%)
Resistant to 2 antibiotics	27 (27%)	6 (33.3%)	6 (35.3%)

luk-PV: Panton-Valentine leucocidin gene*; tsst-1*: toxic shock syndrome toxin gene.

contribute to staphylococcal pathogenesis. Of note, a vast majority of these factors play a direct role in pediatric morbidity and mortality [15]. Although our study revealed a relatively low incidence (22.5%) of MRSA among infants and children in the community and hospital setting, overall a higher incidence of MRSA infections have previously been reported in Iran [16-18]. However, lower distribution of MRSA (5.1%) was observed in the study reported by Mashouf *et al.* [19]. The observed differences can be explained by the fact that the other studies were conducted on all age groups or healthy children, while in the present study, we focused on pediatric patients.

Since a large proportion of patients (47%) enrolled in the study were outpatients, there is reason to assume about half of the MRSA isolates were considered as CA-MRSA. CA-MRSA can have the following sources: patients who were discharged from hospital and carry MRSA, employees of nursing homes with MRSA, outpatients that were colonized by MRSA, and MRSA arising *de novo* in the community [20]. The first three sources comprise MRSA strains that spread from health care settings, which are considered to indicate a limited number of diverse genotypes, disseminate clonally, and express resistance to multiple antimicrobial agents [21]. In contrast, *de novo* MRSA isolates are assumed to evolve from methicillin-susceptible *S. aureus* (MSSA) through acquisition of Staphylococcal Cassette Chromosome mec (SCC*mec*) elements containing a *mec* gene [22]. In the present survey, considerable prevalence of nasal MRSA carriage has been found among staphylococcal carriers. Epidemiological investigation on pediatric nasal carriage have evaluated small numbers of patients, but it can often propose that nasal carriage may be common in children [23,24]. Although the vast majority of pediatric nasal carriage of CA-MRSA are self-limited within a one-year period, some of them are at the risk of recurrent skin and soft tissue infections and therefore decolonization of these cases is recommended.

Antibiotic resistance among pathogenic bacteria became an important public health problem in Iran, similar to that in the other parts of the world. Limitation to the use of beta lactam agents in MRSA infections required use of other types of antimicrobial agents for the treatment of these infections, so survey of susceptibilities of MRSA isolates for antibiotics other than beta lactams became very vital. Inexpensive oral antibiotics commonly suggested for the treatment of MRSA infections include tetracyclines, erythromycin, clindamycin, trimethoprim/sulfamethoxazole, and rifampin [8]. In the present survey, linezolid, vancomycin, minocycline, rifampin, gentamicin, and trimethoprim/sulfamethoxazole showed a good antibacterial activity against the tested MRSA isolates compared with the other antimicrobial agents mentioned. Vancomycin, for the moment, remains the first-line intravenous agent for severe MRSA infections, both CA-MRSA and HA-MRSA, and in the present study, we observed no resistance to vancomycin. The same results have been obtained in the studies reported by Mazloomi *et al.* and Ghasemian *et al.*, individually [25,26]. In addition, a vast majority of the MRSA isolates (73%) showed a MDR pattern. In accordance with our study, high rates of MDR *S. aureus* isolates were identified in the studies conducted by Koosha *et al.* and Rezaei *et al*. in Iran [27,28]. In developing countries, unreasonable administration of antimicrobial agents, especially beta-lactam antibiotics, could be responsible for the development of the MDR bacteria. In light of these findings, Iranian physicians and clinicians are more concerned than ever with the increasing spread of clinical isolates of MDR *S. aureus*.

In the present study, it was found that less than one-fifth of MRSA isolates, obtained from pediatric patients, harbored *luk-PV* (18%) and *tsst-1* (17%) virulence genes, whereas no isolate had *eta* or *etb*. However, high detection frequencies of *eta* (11.3%) and *etb* (9%) were reported in Sabouni study [29] and almost the same incidence was reported in other studies [6,30,31]. This may be sensible because none of the MRSA strains in the current survey was isolated from children with skin diseases including staphylococcal scalded skin syndrome (SSSS), impetigo, or blisters. Generally, PVL has been associated with *S. aureus* soft tissue infections and severe necrotizing pneumonia, with the mortality rate for the latter being reported to be about 75% [32]. In an investigation by Lina *et al*., it was indicated that 50%-93% of *S. aureus* isolates responsible for cellulites, furunculosis, or cutaneous abscess and 85% of those responsible for community-acquired pneumonia harbored the *luk-PV* gene, as compared with none of those causing diseases, such as urinary tract infection, infective endocarditis, enterocolitis, nosocomial pneumonia, or toxic-shock syndrome [33]. In the present study, *luk-PV* positive isolates were strongly associated with blood, respiratory secretion and wound cultures. The *luk-PV* gene was found in 5% of blood and 3% of pharynx samples. Overall, the prevalence of the *luk-PV* gene has been estimated to be 2-35% among MRSA isolates in various types of nosocomial infections [34,35]. In the study by Dormanesh *et al.,* high *luk-PV* gene distribution (63.5%) among MRSA isolated from

pediatric nosocomial infections was found [36]. Studies carried out in different countries have shown the same distribution, for instance, Esan *et al.* in Nigeria and Shallcross *et al.* in the United Kingdom reported that 16 % and 11.3% of MRSA isolates harbored *luk-PV* gene, respectively. Although the association between *luk-PV* gene and CA-MRSA strains is a subject of debate, to date, PVL has emerged as a significant virulence factor of CA-MRSA isolates making it a putative marker of these strains [37]. In a study conducted by D'Souza *et al.* in Mumbai between 2006 and 2009, it was found that none of the HA-MRSA isolates carried the *luk-PV* gene [38]. In contrast, in the present study, only 25% of *luk-PV* positive strains were isolated from outpatients and others were isolated from patients who were hospitalized for more than 48 hours. Thus, the association of CA-MRSA isolates and *luk-PV* gene could not be verified in the current study. The present study also showed that the prevalence of *tsst-1* genes is high in eye and urine samples.

Conclusion

To the extent of our knowledge, the present study is one of the few studies that demonstrates the prevalence of virulent determinants of MRSA isolated from pediatric patients. In the current study, MRSA isolates were typically associated with both hospital and community acquired infections. In addition, the results show that the virulence gene content of MRSA isolated from pediatric patients varies widely. Therefore, efficient and accurate epidemiological typing methods such as pulsed-field gel electrophoresis (PFGE) and *spa* typing are suggested for surveillance and limiting the occurrence and spread of epidemic clones within and between hospitals and to community settings. Moreover, to prevent the spread of MRSA strains in health care settings, the decision was made to implement MRSA measures derived from those in use in many hospitals. The measures involved the frequent usage of nasal mupirocin and chlorhexidine showers for patients who are at risk for MRSA transmission or infection.

Acknowledgements

We would to thank the members of Department of Microbiology of Shahid Beheshti University of Medical Sciences.

Authors' contributions

GE and ZG conceived and designed the experiment. MEB and HH performed the experiments BN and RS analysed the data. HH and HA wrote the paper. GE and ZG edited the paper. NNF encouraged the research.

References

1. Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, Cai M, Hansel NN, Perl T, Ticehurst JR, Carroll K, Thomas DL, Nuermberger E, Bartlett JG (2005) Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. Clin Infect Dis 40:100-107.
2. Kluytmans J, van Belkum A, Verbrugh H (1997) Nasal carriage of *Staphylococcus aureus:* epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 10:505–520.
3. Shi D, Higuchi W, Takano T, Saito K, Ozaki K, Takano M, Nitahara Y, Yamamoto T (2011) Bullous impetigo in children with methicillin-resistant *Staphylococcus aureus* alone or in combination with methicillin-susceptible *S. aureus*: Analysis of genetic characteristics, including assessment of exfoliative toxin gene carriage. J Clin Microbiol 49:1972-1974.
4. Arvidson S, Tegmark K (2001) Regulation of virulence determinants in *Staphylococcus aureus*. Int J Med Microbiol 291:159-170.
5. Dinges MM, Orwin PM, Schlievert PM (2000) Exotoxins of *Staphylococcus aureus*. Clin Microbiol Rev 13:16-34.
6. Sina H, Ahoyo TA, Moussaoui W, Keller D, Bankolé HS, Barogui Y, Stienstra Y, Kotchoni SO, Prévost G, Baba-Moussa L (2013) Variability of antibiotic susceptibility and toxin production of *Staphylococcus aureus* strains isolated from skin, soft tissue, and bone related infections. BMC Microbiol 13:188.
7. Mahdiyoun SM, Kazemian H, Ahanjan M, Houri H, Goudarzi M (2016) Frequency of aminoglycoside-resistance genes in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from hospitalized patients. Jundishapur J Microbiol 9:e35052.
8. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF (2010) Community-associated methicillin-resistant *Staphylococcus aureus*. Lancet 375:1557–1568.
9. Schmitz FJ, Fluit AC, Gondolf M, Beyrau R, Lindenlauf E, Verhoef J, Heinz HP, Jones ME (1999) The prevalence of aminoglycoside resistance and corresponding resistance genes in clinical isolates of staphylococci from 19 European hospitals. J Antimicrob Chemother 43:253–259.
10. Sanford MD, Widmer AF, Bale MJ, Jones RN, Wenzel RP (1994) Efficient detection and long-term persistence of the carriage of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 19:1123-1128.
11. Dhawan B, Rao C, Udo EE, Gadepalli R, Vishnubhatla S, Kapil A (2015) Dissemination of methicillin-resistant *Staphylococcus aureus* SCC*mec* type IV and SCC*mec* type V epidemic clones in a tertiary hospital: challenge to infection control. Epidemiol Infect 143:343-353.
12. Clinical and Laboratory Standards Institute (2015). Performance standards for antimicrobial susceptibility testing, Approved Standard M100-S25.
13. Biedenbach DJ, Bouchillon SK, Hackel M, Miller LA, Scangarella-Oman NE, Jakielaszek C and Sahm DF (2016) *In Vitro* activity of Gepotidacin, a novel triazaacenaphthylene bacterial topoisomerase inhibitor, against a broad spectrum of bacterial pathogens. Antimicrob Agents Chemother 60:1918-1923.
14. Seni J, Bwanga F, Najjuka CF, Makobore P, Okee M, Mshana SE, Kidenya BR, Joloba ML, Kateete DP (2013) Molecular characterization of *Staphylococcus aureus* from patients with

surgical site infections at Mulago hospital in Kampala, Uganda. PLoS ONE 8:e66153.
15. Ladhani S, Joannou CL, Lochrie DP, Evans RW, Poston SM (1999) Clinical, microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded-skin syndrome. Clin Microbiol Rev 12:224–242.
16. Ohadian Moghadam S, Pourmand MR, Davoodabadi A (2015) The detection of mupirocin resistance and nasal carriage of methicillin resistant *Staphylococcus aureus* among healthcare workers at university hospitals of Tehran, Iran. Iran J Public Health 44:361-368.
17. Erami M, Soltani B, Taghavi Ardakani A, Moravveji A, Haji Rezaei M, Soltani S, Moniri R (2014) Nasal carriage and resistance pattern of multidrug resistant *Staphylococcus aureus* among healthy children in Kashan, Iran. Iran Red Crescent Med J 16: e21346.
18. Sahebnasagh R, Saderi H, Owlia P (2014) The prevalence of resistance to methicillin in *Staphylococcus aureus* strains isolated from patients by PCR method for detection of *mecA* and *nuc* genes. Iran J Public Health 43:84-92.
19. Mashouf RY, Hosseini SM, Mousavi SM, Arabestani MR (2015) Prevalence of enterotoxin genes and antibacterial susceptibility pattern of *Staphylococcus aureus* strains isolated from animal originated foods in west of Iran. Oman Med J 30:283–290.
20. Cookson BD (2000) Methicillin-resistant *Staphylococcus aureus* in the community: New battlefronts, or are the battles lost? Infect Control Hosp Epidemiol 21:398-403.
21. Salmenlinna S, Lyytikäinen O, Vuopio-Varkila J (2002) Community-acquired methicillin-resistant *Staphylococcus aureus*, Finland. Emerg Infect Dis 8:602–607.
22. Wielders CL, Vriens MR, Brisse S, de Graaf-Miltenburg LAM, Troelstra A, Fleer A, Schmitz FJ, Verhoef J, Fluit AC (2001) Evidence for in-vivo transfer of *mecA* DNA between strains of *Staphylococcus aureus* . Lancet 357:1674-1675.
23. Nakamura MM, McAdam AJ, Sandora TJ, Moreira KR, Lee GM (2010) Higher prevalence of pharyngeal than nasal *Staphylococcus aureus* carriage in pediatric intensive care units. J Clin Microbiol 48: 2957–2959.
24. Vlack S, Cox L, Peleg AY, Canuto C, Stewart C, Conlon A, Stephens A, Giffard P, Huygens F, Mollinger A, Vohra R, McCarthy JS (2006) Carriage of methicillin-resistant *Staphylococcus aureus* in a Queensland Indigenous community. Med J Aust 184: 556–559.
25. Mazloomi Nobandegani N, Mahmoudi S, Pourakbari B, Sadeghi RH, Najafi Sani M, Farahmand F, Motamed F, Nabavizadeh Rafsanjani R, Mamishi S (2016) Antimicrobial susceptibility of microorganisms isolated from sputum culture of patients with cystic fibrosis: Methicillin-resistant *Staphylococcus aureus* as a serious concern. Microb Pathog 100: 201-204.
26. Ghasemian A, Najar Peerayeh S, Bakhshi B, Mirzaee M (2015) The microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) genes among clinical isolates of *Staphylococcus aureus* from hospitalized children. Iran J Pathol 10: 258-264.
27. Koosha RZ, Fooladi AAI, Hosseini HM, Aghdam EM (2014) Prevalence of *exfoliative toxin* A and B genes in *Staphylococcus aureus* isolated from clinical specimens. J Infect Public Health 7: 177-185.
28. Rezaei M, Moniri R, Mousavi SGA (2015) Molecular analysis and susceptibility pattern of meticillin-resistant *Staphylococcus aureus* strains in emergency department patients and related risk factors in Iran. J Hosp Infect 89:186-191.
29. Sabouni F, Mahmoudi S, Bahador A, Pourakbari B, Sadeghi RH, Ashtiani MT, Nikmanesh B, Mamishi S (2014) Virulence factors of *Staphylococcus aureus* isolates in an Iranian referral children's hospital. Osong Public Health Res Perspect. 5: 96-100.
30. Roetzer A, Haller G, Beyerly J, Geier CB, Wolf HM, Gruener CS, Model N, Eibl MM (2016) Genotypic and phenotypic analysis of clinical isolates of *Staphylococcus aureus* revealed production patterns and hemolytic potentials unlinked to gene profiles and source. BMC Microbiol 16:13.
31. Diawara I, Bekhti K, Elhabchi D, Saile R, Elmdaghri N, Timinouni M, Elazhari M (2014) *Staphylococcus aureus* nasal carriage in hemodialysis centers of Fez, Morocco. Iran J Microbiol 6:175-183.
32. Miller LG, Perdreau-Remington F, Rieg G, Mehdi S, Perlroth J, Bayer AS, Tang AW, Phung TO, Spellberg B. (2005) Necrotizing fasciitis caused by community-associate methicillin-resistant *Staphylococcus aureus* in Los Angeles. N Engl J Med 352:1445-1453.
33. Lina G, Piémont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V, Vandenesch F, Etienne J (1999) Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29:1128-1132.
34. Zorgani A, Franka RA, Zaidi MM, Alshweref UM, Elgmati M (2010) Tends in nosocomial bloodstream infections in a burn intensive care unit: an eight-year survey. Ann Burns Fire Disasters 30:88-94.
35. Melles DC, Gorkink RF, Boelens HA, Snijders SV, Peeters JK, Moorhouse MJ, van der Spek PJ, van Leeuwen WB, Simons G, Verbrugh HA, van Belkum A (2004) Natural population dynamics and expansion of pathogenic clones of *Staphylococcus aureus*. J Clin Invest 114: 1732-1740.
36. Dormanesh B, Siroosbakhat S, Khodaverdi Darian E, Afsharkhas L (2015) Methicillin-resistant *Staphylococcus aureus* isolated from various types of hospital infections in pediatrics: Panton-Valentine leukocidin, staphylococcal chromosomal cassette mec *SCCmec* phenotypes and antibiotic resistance properties. Jundishapur J Microbiol 8: e11341.
37. Shrestha B, Singh W, Raj VS, Pokhrel BM, Mohapatra TM (2014) High prevalence of Panton-Valentine leukocidin (PVL) genes in nosocomial-acquired *Staphylococcus aureus* isolated from tertiary care hospitals in Nepal. BioMed Res Int 2014:790350.
38. D'Souza N, Rodrigues C, Mehta A (2010) Molecular characterization of methicillin-resistant *Staphylococcus aureus* with emergence of epidemic clones of sequence type (ST) 22 and ST 772 in Mumbai, India. J Clin Microbiol 48:1806–1811.

Corresponding author
Dr Gita Eslami
Department of Microbiology, Shahid Beheshti University of Medical Sciences, Koodakyar St., Tabnak Blv., Yaman Av., Chamran highway, Tehran, Iran.
P. Box: 19857-17443
Phone No: +98 21 23872556
E-mail: g_eslami@yahoo.com

Conflict of interests: No conflict of interests is declared.