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### Molecular Characterization of Exotoxin Genes in *Staphylococcus aureus* Recovered From Hospitalized Patients

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#### ABSTRACT

**Background:** *Staphylococcus aureus* is considered as a major cause of skin and soft tissue infections, arthritis, osteomyelitis, infective endocarditis, and pneumoniae through community or nosocomial transmission. In this study, attempts were made to investigate the distribution of some important exotoxin genes, including *hla*, *hly*, *tsst-1*, *eta*, *etb*, and *etd* among methicillin-resistant *S. aureus* (MRSA) isolated from a hospital patients in Tabriz, Iran.

**Methods:** In the present cross-sectional study, a total of 90 *S. aureus* were isolated from children who admitted to a hospital during six-month in 2017. Isolates were identified using biochemical tests and then, using PCR, the isolates were tested for the presence of, *hla*, *hly*, *tsst-1*, *eta*, *etb*, and *etd* genes.

**Results:** It was found that 40% of the *S. aureus* were considered as MRSA strains by biochemical and molecular tests. The results of molecular detection of virulence determinants showed that *eta*, *hla*, *etb*, *tsst-1*, *hly* and *etd* were detected in 86.1%, 80.5%, 30.5%, 27.7%, 22.2%, and 19.4% of isolates, respectively.

**Conclusion:** Our findings clarify characterization of toxin production status of *S. aureus* isolates from patients in Iran. The current study showed that a majority of *S. aureus* isolates harbored *eta* and *hla* virulence gene.

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## Introduction

*Staphylococcus aureus* has been considered as an important human pathogen causing a wide range of infections in both hospital and community settings (1). This ever-present opportunistic pathogen is among the most common causes of skin and soft tissue infections, arthritis, osteomyelitis, infective endocarditis, pneumoniae, as well as nosocomial infections, including various surgical infections and bacteremia (2, 3). As a matter of fact, neonates, infants and young children are more likely to be affected by *S. aureus* infections, notwithstanding whether these infections had their onset in the community or were acquired in the hospital (4).

A characteristic feature of pathogenic *S. aureus* strains is the expression of a broad range of adhesion proteins and virulence factors that play a significant role on every level of host-pathogen interactions during different phases of colonization and infection (5). These virulence factors allow the bacterium to escape the immune responses, attach to the host tissue, and cause severe damage to the host tissues (6, 7). Exotoxins such as hemolysins (*Hla*, *Hlb*, *Hld*, and *Hlg*), panton-valentine leukocidin (PVL), Exfoliative toxin A and B (*EtA* and *EtB*), Staphylococcal Enterotoxins (SEs), Toxic shock syndrome toxin-1 (TSST-1) are the most important virulence factors produced by this pathogen (8). However, it should be pointed out that most of these factors may not exist in all strains of *S. aureus* and their expression occurs in specified strains and under certain conditions. For instance, the *S. aureus* strains that produce PVL produce PVL are associated with community-acquired hemorrhagic and necrotizing pulmonary infections affecting previously healthy children and young adults (9, 10).

In addition to the high pathogenicity of *S. aureus*, the pathogen is well identified for its capacity to acquire resistance to several antibiotics. In the early of 1960s, methicillin-

resistant *S. aureus* (MRSA) strains have emerged as significant pathogens of nosocomial- and community acquired- infections and then ultimately spread worldwide over the next several decades (11, 12). The *mecA* gene is part of the staphylococcal chromosome cassette *mec* (SCC*mec*) which is responsible resistance to methicillin and other beta-lactam antibiotics (13). Several researchers have indicated that MRSA strains have become not only resistant to beta-lactam agents, but they can also acquire additional resistance to other antibiotic classes, especially aminoglycosides (14-16). Moreover, the presence of resistance genes, such as *mecA*, may also affect toxin production among *S. aureus* strains (17).

Considering of the increasing spread of MRSA strains among adults and pediatrics population, the current study was conducted to detect the virulence determinants, including *hla*, *hnb*, *eta*, *etb*, *etd*, and *tsst-1*, among MRSA strains isolated from patients in Tabriz, Iran. *Staphylococcus aureus* has been considered as an important human pathogen causing a wide range of infections in both hospital and community settings (1). This ever-present opportunistic pathogen is among the most common causes of skin and soft tissue infections, arthritis, osteomyelitis, infective endocarditis, pneumoniae, as well as nosocomial infections, including various surgical infections and bacteremia (2, 3). As a matter of fact, neonates, infants and young children are more likely to be affected by *S. aureus* infections, notwithstanding whether these infections had their onset in the community or were acquired in the hospital (4).

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pathogen is among the most common causes of skin and soft tissue infections, arthritis, osteomyelitis, infective endocarditis, pneumoniae, as well as nosocomial infections, including various surgical infections and bacteremia (2, 3). As a matter of fact, neonates, infants and young children are more likely to be affected by *S. aureus* infections, notwithstanding whether these infections had their onset in the community or were acquired in the hospital (4).

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## Materials and Methods

### Bacterial Isolation and Identification

The present study 90 *S. aureus* isolates were obtained from hospitalized patients with clinical signs and symptoms of colonization such as increased fever and white and blood cell counts admitted to hospital, during 6-month Tabriz, Iran. Standard conventional biochemical tests were carried out on colonies from primary cultures in order to identification of the bacterial isolates. Accordingly, all suspected colonies of *S. aureus* were examined using haemolysis, gram staining, catalase, oxidase, DNase, coagulase and mannitol fermentation tests. The confirmed isolates were stored at -70 °C in trypticase soy broth (TSB) (Merck Co., Germany) containing 20% glycerol for later analysis.

### Detection of MRSA Strains

Phenotypic detection of MRSA isolates was performed using disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines with a 30 µg cefoxitin disc (Mast Co., UK) on Mueller–Hinton agar followed by incubation at 30° C for 24 hr. The

isolates that exhibited inhibition zone size ≤19 mm were considered as MRSA strains.

### Molecular Assay for Detection of Virulence Genes

Total DNA of the isolates was extracted via High Pure PCR Template Preparation Kit (Thermo Fisher Co., USA) according to the manufacturer's instructions. The presence of the virulence determinants including *hla*, *hly*, *eta*, *etb*, *etd*, and *tsst-1* were detected by separate PCR reactions, using specific primers (Table 1). All PCR reactions were performed in a final volume of 25 µL containing the 12.5 µL of master mix (Cinnaclon, Tehran, Iran), 8.5 µL of distilled water, 10 pmol of each primer, and 1 µL DNA template (3 µg/µL). The thermal cycling condition of the mixture included an initial denaturation step at 95 °C for 5 minutes followed by 35 cycles of amplification comprising of three steps: denaturation at 94 °C for 1 min; 30 s at different annealing temperatures for each primer set (Table 1), and with primer extension at 72°C for 45 s. The final extension step was done 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.

### Statistical Analyses

Categorical variables were calculated by using SPSS 22.0 statistical software (IBM Corp., Armonk, USA). A statistically significant difference was considered if P value was <0.05.

## Results

### Patients and Bacterial Identification

In this study, 90 clinical isolates of *S. aureus* were collected from adults (18-85 year) and paediatrics that was under 18 years of age during a six-month period. Of these, 17 isolates were

collected (43.5%) from outpatient or those referred to the emergency department, with the remainder from different wards, including infection (23%), internal (20.5%), and pediatric units (13%). In total, 19 (52.77%) clinical isolates of *S. aureus* were recovered from blood, and the remaining were obtained from urine (16%), wound (10.7%), tracheal (10.3%), synovial (5.1%), bronchial (2.6%), and abscess (2.6%). Among 90 *S. aureus* isolates, 36 (40%) were recognized as MRSA strains by phenotypic method. In addition, the distributions of *mecA* gene among MRSA isolates were 100%.

#### Presence of Virulence Determinants

The results of molecular detection of virulence determinants showed that *eta*, *hla*, *etb*, *tsst-1*, *hly* and *etd* were detected in 86.1%, 80.5%, 30.5%, 27.7%, 22.2%, and 19.4% of isolates, respectively. Table 2 illustrates the distribution of the virulence determinants among *S. aureus* isolates from patients in this study.

#### Discussion

The production of exotoxins in clinical isolates of *S. aureus* is the most important factor in the development of severe infection and allows for immune evasion (18). Specific staphylococcal exotoxins seem to be responsible for individual syndromes caused by the bacterium. For example, scalded skin syndrome (SSSS) has been associated with ETA and ETB and toxic shock syndrome with TSST-1 (19). Hence, in order to improve surveillance, control and treatment of *S. aureus* infections among pediatric patients, it is essential to understand the biofilm formation that lead to chronic infections (20). In the current study, we focused on evaluation of virulence gene profiles of clinical isolates of MRSA isolated from patients, which may affect the severity of infection among patients.

Using of phenotypic and molecular assays a relatively high frequency of MRSA strains was identified and is in accordance with the findings reported by Esmaili Benvidi et al. among paediatric patients in Tehran (21). However, the higher prevalence of MRSA infections has been previously reported in Iranian population (22, 23). These variations may detected be attributed to the efficiency of screening methods for MRSA identification. Moreover, the variations in the results can be clarified by the fact that the other researches were conducted on all age groups or healthy carrier persons, especially in pediatrics patients.

In the present study, it was found that a majority of the MRSA isolates, recovered from patients, harbored *eta* and *hla* virulence gene particularly all of pediatrics patients in our study detected *hla* gene. However, in a cross-sectional study, Esmaili Benvidi et al. described that none of the investigated MRSA isolates from pediatric patients harbored *eta* gene in Tehran (21). In addition, the low detection frequency of *eta* (11.3%) were previously reported in a study conducted by Sabouni et al (24). The *eta* and *etb* loci can be detected from *S. aureus* isolated from children with skin diseases including SSSS, impetigo, or blisters (25). Notwithstanding the advancement of antibiotic therapy, SSSS display a significant mortality rate mainly among infants with secondary infections followed by epidermal loss and among adults with underlying diseases (26).

The *etd* gene is located within a 9.0-kb pathogenicity island (chromosomal site encoding virulence-associated factors), has also been found in this study, but was less common (20% of isolates) than other two exfoliative toxins. ETD-producing *S. aureus* are principally isolated from with suppurative skin infection such as cutaneous abscesses or furuncles, and not from SSSS (27). Recently, Mohseni et al. reported a frequency of 54% *S. aureus* harborin etd in Iran

**Table 1.** The sequences, product length, annealing temperature (T<sub>m</sub>) of the oligonucleotide primers used in the study.

Gene target	Sequences (5'-3')	Product (bp)	T <sub>m</sub> (°C)	Reference
<i>hla</i>	CTGATTACTATCCAAGAAATTCGAT CTTTCCAGCCTACTTTTTTATCAGT	210	53	(36)
<i>hlb</i>	GTGCACTTACTGACAATAGTGC GTTGATGAGTAGCTACCTCAGT	310	53	(36)
<i>tsst-1</i>	TTATCGTAAGCCCTTTTGTTG TAAAGGTAGTTCTATTGGAGTAGG	398	46	(21)
<i>eta</i>	TTTGCTTTCTTGATTTGGATTC GATGTGTTTCGGTTTGATTGAC	464	51	(37)
<i>etb</i>	ACGGCTATATACATTCAATT TCCATCGATAATATACCTAA	226	51	(37)
<i>etd</i>	GGGGAGACTATAGCTTCTGGTGATTA TCCAACATGAATACCAACTAECTCT	477	55.5	(37)

**Table 2.** The distribution of exotoxin genes among MRSA isolates from pediatric patients.

No. Percent	virulence genes					
	<i>hla</i>	<i>hlb</i>	<i>eta</i>	<i>etb</i>	<i>etd</i>	<i>tsst-1</i>
10.25%	+	-	+	+	+	-
2.56%	+	+	+	-	-	+
7.69%	+	+	+	-	-	-
2.56%	-	-	-	+	-	+
5.12%	+	-	+	-	-	+
2.56%	+	+	+	-	+	-
10.25%	+	-	+	+	-	-
2.56%	+	+	+	-	+	-
2.56%	+	-	-	-	-	-
2.56%	+	-	-	-	+	+
2.56%	+	-	+	+	-	+
2.56%	+	-	+	-	+	+
2.56%	-	-	+	-	-	+
2.56%	+	+	+	+	-	+
20.51	+	-	+	-	-	-
10.25%	-	-	+	-	-	-
5.12%	+	-	+	-	+	-
2.56%	-	-	-	-	-	-
2.56%	+	+	-	-	-	+

(28). In a study conducted by Abimanyu et al. in India it was reported that none of the *S. aureus* isolates was found to carry *etd* gene but in our study all of our pediatric patients had *etd* gene (29).

In our study we detected that 25.64% of the MRSA isolates were *tsst-1* positive. Similarly, in the study conducted by Arabestani it was reported that *tsst-1* was found in 30.61% of the *S. aureus* isolates (30). However, Esmaili Benvidi et al. in 2017 reported that *tsst-1* was detected in 17% of the staphylococcal isolates from pediatric

patients in Tehran. In addition, Sina et al. previously described that *tsst-1* was detected at a very low rate (1%) of isolated *S. aureus* in Iran (31). The disorders mediated by *tsst-1*, toxic shock syndrome, is a superantigen-mediated illness and characterized by rash, hypotension and multi-organ dysfunction (32). Infants and young children have a greater risk of developing severe toxic shock syndrome than adults. It is believed that this risk is associated with colonization by TSST-1-producing *S. aureus* in this population with insufficient antibody titers

(33). If this illness is not cured quickly in children, the mortality rate can be high (34, 35). It is imported to find spread of MRSA strains and the virulence determinants among adults and pediatrics population but in Izzard Aglua et al. in 2018 reported just detection of MRSA strains and antibiotics patterns (36).

## Conclusion

To the best of our knowledge, the current study is one of the few researches investigating the frequency of exotoxin genes of MRSA isolated from patients in Iran. In the present study, the results showed that the virulence determinants content of MRSA isolated from patients varies widely. Consequently, accurate and powerful epidemiological typing approaches such as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) are recommended for active surveillance of MRSA strains and limiting the spread of epidemic clones within and between hospitals and to community settings. In addition, to avoid the spread of MRSA strains in community and healthcare settings, the decision should be made in conjunction with the infection control staff of the facility and must be clearly documented.

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## Ethics approval and consent to participate

The study was conducted under the ethics approval code IR.SBMU.MSP.REC.1397.631.

## Conflict of interest

No conflict of interests is declared.

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