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# Investigation of Toxin Profiles of Methicillin Resistant and Sensitive *Staphylococcus aureus* Strains Isolated from Various Clinical Specimens

## Çeşitli Klinik Örneklerden İzole Edilen Metisiline Dirençli ve Duyarlı *Staphylococcus aureus* Suşlarının Toksin Profillerinin Araştırılması

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**ABSTRACT**

**Aim:** This study aimed to investigate the superantigenic (SAg) toxin, exfoliative toxin (ET), hemolysin (HLY), leukotoxin (LUK) genes and accessory gene regulator (*agr*) types in *Staphylococcus aureus* isolates from various clinical materials.
**Material and Methods:** A total of 190 *S. aureus* isolates were investigated for the presence of toxin genes, *mec*A gene and *agr* types using by polymerase chain reaction (PCR).
**Results:** *mec*A gene was detected in 87 (45.8%) isolates. Of the 190 *S. aureus* isolates examined, 83.7% (n=159) were found to be positive for SAg genes. The *seg* (41.1%) was determined to be the most common toxin gene, followed by *sei* (38.9%), *selo* (38.9%), *selm* (28.4%), *sea* (%25.8), and *tst* (18.4%) genes, respectively. Seventy one different SAg toxin profiles were identified. Type I νSaβ encoding *seg*, *sei*, *selm*, *seln* and *selo* was the most common mobile genetic element (MGE), which was detected in 37 isolates (19.5%). The *hla*, *hlb*, *hld*, *hlg* and *hlg2* genes were detected in 92.6% (n=176), 1.6% (n=3), 98.9% (n=188), 1.1% (n=2) and 31.6% (n=60) of the isolates, respectively. The *pvl* gene was detected in 12.6% (n=11) of methicillin resistant *S. aureus* (MRSA) and 14.6% (n=15) of methicillin sensitive *S. aureus* (MSSA), respectively (p=0.701). While none of the isolates carried *luk*M gene, 67% (n=69) of MSSA and 69% (n=60) of MRSA isolates were found to be positive for *luk*ED gene (p=0.519).
**Conclusion:** High occurrence and diversity of toxin genes among *S. aureus* isolates could be explained by horizontal transmission of toxin genes through MGEs.
**Keywords:** *Staphylococcus aureus*; methicillin resistance; toxin; gene.

**ÖZ**

**Amaç:** Bu çalışmada çeşitli klinik materyallerden elde edilen *Staphylococcus aureus* izolatlarında süperantijenik (SAg) toksin, eksfoliatif toksin (ET), hemolizin (HLY) ve lökotoksin (LUK) genleri ve aksesuar gen regülatör (*agr*) tiplerinin araştırılması amaçlandı.
**Gereç ve Yöntemler:** Toplam 190 *S. aureus* izolatında toksin genleri, *mec*A geni ve *agr* tipleri polimeraz zincir reaksiyonu (PZR) kullanılarak incelendi.
**Bulgular:** *mec*A geni 87 (%45,8) izolatta tespit edildi. İncelenen 190 *S. aureus* izolatının %83,7'si (n=159) SAg genleri yönünden pozitif bulundu. En yaygın toksin geni *seg* (%41,1) olarak belirlenirken, bunu sırasıyla *sei* (%38,9), *selo* (%38,9), *selm* (%28,4), *sea* (%25,8) ve *tst* (%18,4) genleri izledi. Yetmiş bir farklı SAg toksin profili belirlendi. *sei*, *seg*, *selm*, *seln* ve *selo* genlerini taşıyan tip I νSaβ 37 (%19,5) izolatta tespit edilerek en yaygın mobil genetik element (MGE) olarak belirlendi. *hla*, *hlb*, *hld*, *hlg* ve *hlg2* genleri izolatların sırasıyla %92,6 (n=176), %1,6 (n=3), %98,9 (n=188), %1,1 (n=2) ve %31,6'sında (n=60) tespit edildi. *pvl* geni sırasıyla, metisilin dirençli *S. aureus* (methicillin resistant *S. aureus*, MRSA) izolatlarının %12,6'sında (n=11) ve metisilin duyarlı *S. aureus* (methicillin sensitive *S. aureus*, MSSA) izolatlarının ise %14,6'sında (n=15) saptandı (p=0,701). İzolatların hiçbiri *luk*M geni taşımazken, MSSA izolatlarının %67'si (n=69) ve MRSA izolatlarının %69'u (n=60) *luk*ED geni için pozitif bulundu (p=0,519).
**Sonuç:** *S. aureus* izolatları arasında toksin genlerinin yüksek oranda bulunması ve çeşitliliği, toksin genlerinin MGE'ler aracılığıyla horizontal transferi ile açıklanabilir.
**Anahtar kelimeler:** *Staphylococcus aureus*; metisilin direnci; toksin; gen.

## INTRODUCTION

*Staphylococcus aureus* is a versatile pathogen capable of causing a wide range of infections (1). This feature of the agent is due to its genome plasticity of acquiring and incorporating genetic materials from other bacterial species that may have antimicrobial resistance and virulence. Thus, outcome of the infections caused by *S. aureus* strains are closely related with their resistance and virulence properties (2). *S. aureus* has the ability to produce several virulence factors such as superantigens (SAgs), hemolysins (HLYs), leukotoxins (LUKs), and exfoliative toxins (ETs). Among the exotoxins secreted by *S. aureus*, staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1) have SAg activity. These toxins are important virulence factors that contribute a variety of pathological conditions, including pneumonia, soft tissue infections, toxic shock syndrome, and infective endocarditis (2,3). Moreover, it has been recently reported that SAgs particularly SEs play a prominent role in the development of asthma of hospitalized patients via induction of IgE (4). So far, *S. aureus* strains are reported to secrete at least 26 or more types of SEs and staphylococcal enterotoxin-like toxins (SEls). SAgs have the ability to stimulate synthesis of cytokines by binding concurrently to MHC-II complex and Vβ element of TCR (5).

*S. aureus* is also capable of producing several pore forming toxins (PFTs) that target leukocytes. The *S. aureus* isolates associated with human infections can produce up to five bi-component leukocidins, also known PFTs: Panton-Valentine Leukocidin (PVL), gamma-hemolysin AB and CB (HlgAB and HlgCB), Leukocidin ED (LukED), and Leukocidin AB (LukAB). These toxins protect *S. aureus* from being killed by phagocytes of the host (6).

Exfoliative toxins (ETs), also known as epidermolytic toxins, are highly specific serine proteases secreted by *S. aureus* that are responsible for the clinical manifestation of staphylococcal scalded skin syndrome (SSSS) (7). So far, four types of ET have been reported as exfoliative toxin A/B/C/D (ETA, ETB, ETC, and ETD). Of these, while ETA and ETB were the most common types in humans, ETC was only detected in isolates from horse infections. In 2002, ETD was only defined in clinical *S. aureus* isolates in humans (8).

Among the wide variety of Staphylococcal cytolytic exotoxins produced, HLYs are the most prominent and well-characterized ones which play an important role in the pathogenesis of staphylococcal infections. HLYs α, β, γ, and δ cause pore formation on various cell membranes including immune cells that lead leakage of cellular molecules and metabolites outside of the cell in favor of the survival of the pathogen and progression of the disease (9).

The accessory gene regulator (*agr*) system is one of the main regulatory and control factors involved in the control of pathogenesis of *S. aureus* by regulating virulence factors, biofilm formation and resistance. *S. aureus* is divided into 4 different *agr* groups (*agr* I, *agr* II, *agr* III, and *agr* IV) based on mutations and polymorphisms of *agr*C and *agr*D genes (10). It was stated that prevalence of *agr* types vary according to geographical areas (11). Moreover, a link between *agr* types and certain diseases manifestations and clinical outcome has been reported (12). Jarraud et al. (13) suggested that *agr* type III is more prevalent in TSST-1 producing isolates and *agr* type IV is more prevalent in ET producing isolates.

The main aim of the current study was to investigate the prevalence of SAg (SE, TSST-1), ET, HLY, and LUK genes in methicillin resistant *S. aureus* (MRSA) and methicillin sensitive *S. aureus* (MSSA) isolates.

## MATERIAL AND METHODS

### Ethical Considerations

This study was approved by the non-interventional clinical researches ethics committee of Hatay Mustafa Kemal University (05.09.2019, 14/2019).

### *S. aureus* Isolates

All *S. aureus* strains isolated from different clinical materials submitted to the Microbiology Laboratory of the Hatay Mustafa Kemal University Hospital from January to September 2020 were included in the study. Suspected colonies were identified by hemolysis properties, Gram staining, catalase, coagulase and other biochemical tests (14). Identification of the strains and antibiotic susceptibilities of these isolates were determined with the Vitek 2 compact system (bioMe´rieux, France) and evaluated according to the breakpoints of the European Committee for Antimicrobial Susceptibility Tests (EUCAST) (15). They were confirmed by polymerase chain reaction (PCR) using primers specific to the *nuc* gene (16).

### DNA Isolation

DNA isolation from the isolates was performed using a commercial extraction kit (InstaGeneTM Matrix, Bio-Rad, France). The resulting template DNAs were stored at -20 °C until use.

### Detection of *mec*A Gene

The *mec*A gene in phenotypically methicillin resistant isolates were investigated as previously described by Choi et al. (17).

### Detection of Toxin Genes by PCR

The genes encoding SEs (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *selj*, *selk*, *sell*, *selm*, *seln*, *selo*, *selp*, *selq*, and *selr*), TSST-1 (*tst*), ET (*eta*, *etb*), leukocidins (*pvl*, *luk*ED, and *luk*M) and HLYs (*hla*, *hlb*, *hld*, *hlg*) were examined as previously described by Omoe et al. (18), Jarraud et al. (13), Mehrotra et al. (19) and Lina et al. (20), respectively.

### *agr* Typing

Determination of the *agr* types of *S. aureus* isolates was performed using multiplex PCR method as previously described by Gilot et al. (21).

### Statistical Analysis

The statistical analyses was carried out using SPSS v.16 (SPSS Inc., Chicago, IL, USA). The frequencies of the variables was presented as numbers and percentages. The Pearson chi-square test, Fisher's exact test, and Fisher-Freeman-Halton test were used to compare categorical variables where appropriate. $p<0.05$ was considered as statistically significant.

## RESULTS

### PCR results for *mec*A

All *S. aureus* isolates were positive for *nuc* gene. Based on PCR amplification of *mec*A gene, 87 isolates were identified as MRSA and 103 as MSSA. The distribution of

MRSA isolates according to the clinical materials is given in Table 1. No statistically significant difference was found in terms of the type of clinical materials from which the MRSA strains were isolated (p=0.577).

**Distribution of SAg and ET Genes in MSSA and MRSA Isolates**

Distribution of SAg toxins and ET genes detected in MSSA and MRSA isolates is given in Table 2. Thirty-one (16.3%) isolates did not have any SAg's toxin genes examined. The most common SE genes were *seg* (41.1%), *sei* (38.9%), *selo* (38.9%), *selm* (28.4%), and *sea* (25.8%). While *eta* was observed in 9 (4.7%) isolates (6 MSSA and 3 MRSA isolates), *etb* was carried by seven MSSA and one MRSA isolates. SAg toxin genotypes and their relationship with MGEs are shown in Table 3.

**Distribution of Hemolysin Genes**

Nearly all isolates at least carried one of the HLY genes examined, except one MSSA isolate. There was no statistically significant difference between MSSA and MRSA isolates in terms of distribution of HLY genes (p=0.309). The distribution of HLY genes is given in Table 4.

**Distribution of Leukocidin Genes**

While all isolates were negative for *luk*M gene, the *luk*ED gene was detected in 67% (n=69) of the MSSA isolates and in 69% (n=60) of the MRSA isolates (p=0.519). As seen in Table 5, the *pvl* gene was detected in 14.6% (n=15) of the MSSA isolates and in 12.6% (n=11) of the MRSA isolates (p=0.701).

**Distribution of *agr* Types**

As seen in Table 6, *agr* type I (56.8%) was the most common type among the isolates, followed by *agr* type III (22.1%) and *agr* type II (16.8%). Distribution of *agr* types between MSSA and MRSA isolates was statistically significant (p=0.001).

**Table 1.** The distribution of *S. aureus* isolates according to the clinical materials

| Clinical Material | MSSA | MRSA | p | Total |
|---|---|---|---|---|
| Throat swab | 2 (1.9) | 2 (2.3) | | 4 (2.1) |
| Nasal Swab | 4 (3.9) | 8 (9.2) | | 12 (6.3) |
| Wound | 27 (26.2) | 19 (21.8) | | 46 (24.2) |
| Blood | 30 (29.1) | 27 (31.0) | | 57 (30.0) |
| Urine | 32 (31.1) | 21 (24.1) | 0.609 | 53 (27.9) |
| Sputum | 3 (2.9) | 4 (4.6) | | 7 (3.7) |
| Cerebrospinal fluid | 2 (1.9) | 5 (5.7) | | 7 (3.7) |
| Vaginal Discharge | 2 (1.9) | 1 (1.1) | | 3 (1.6) |
| Tracheal Aspirate | 1 (1.0) | 0 (0.0) | | 1 (0.5) |
| **Total** | **103** | **87** | | **190** |

MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

**Table 2.** Distribution of SAg toxin and ET genes detected in MSSA and MRSA isolates

| SAg | MSSA (n=103) | MRSA (n=87) | p | Total |
|---|---|---|---|---|
| *sea* | 25 (24.3) | 24 (27.6) | 0.603 | 49 (25.8) |
| *seb* | 5 (4.9) | 2 (2.3) | 0.456 | 7 (3.7) |
| *sec* | 3 (2.9) | 1 (1.1) | 0.626 | 4 (2.1) |
| *sed* | 9 (8.7) | 6 (6.9) | 0.639 | 15 (7.9) |
| *selq* | 13 (12.6) | 13 (14.9) | 0.643 | 26 (13.7) |
| *selo* | 40 (38.8) | 34 (39.1) | 0,972 | 74 (38.9) |
| *selm* | 32 (31.1) | 22 (25.3) | 0,378 | 54 (28.4) |
| *selr* | 10 (9.7) | 6 (6.9) | 0.487 | 16 (8.4) |
| *selk* | 16 (15.5) | 10 (11.5) | 0.420 | 26 (13.7) |
| *seln* | 17 (16.5) | 10 (11.5) | 0.324 | 27 (14.2) |
| *sell* | 6 (5.8) | 6 (6.9) | 0.762 | 12 (6.3) |
| *seg* | 48 (46.6) | 30 (34.5) | 0.091 | 78 (41.1) |
| *sei* | 44 (42.7) | 30 (34.5) | 0.246 | 74 (38.9) |
| *seh* | 6 (5.8) | 20 (23) | **0.001** | 26 (13.7) |
| *selj* | 8 (7.8) | 6 (6.9) | 0.819 | 14 (7.4) |
| *selp* | 5 (4.9) | 4 (4.6) | 0.999 | 9 (4.7) |
| *tst* | 14 (13.6) | 21 (24.1) | 0.062 | 35 (18.4) |
| *eta* | 6 (5.8) | 3 (3.4) | 0.512 | 9 (4.7) |
| *etb* | 7 (6.8) | 1 (1.1) | 0.072 | 8 (4.2) |

SAg: superantigen, ET: exfoliative toxin, MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

## DISCUSSION

The pathogenicity of *S. aureus* has been attributed to their ability to evade both innate and acquired immune mechanisms of the host (22). Apart from virulence repertoire of *S. aureus*, which plays an important role in the pathogenesis, the emergence and increasing prevalence of MRSA in healthcare institutions is one of the most important challenges encountered in the treatment of *S. aureus* infections. Methicillin resistance is one of important resistance mechanisms observed in *S. aureus* isolates. Based on *mec*A PCR results, a higher prevalence (55.8%) of MRSA was detected. In Iran, Motamedifar et al. (23) reported a similar prevalence rate (57.7%). In previous studies carried out in Turkey, prevalence of MRSA was reported as 17.9% by Özel et al. (24), 22.9% by Tanrıverdi Çaycı et al. (25), 24% by Arıcı and Aksaray (26), 12.5% in hospital acquired (HA)- *S. aureus* isolates and 43% in community acquired (CA)- *S. aureus* isolates by Duman et al. (27), 30.8% by Kılıç et al. (28), and 44% by Şahin et al. (29). In contrast, Karahan et al. (30) reported higher a prevalence rate in HA- *S. aureus* isolates (83.9%) and CA- *S. aureus* isolates (91.9%). Compared to most of the MRSA rates mentioned above, the high rate of MRSA detected in this study can be explained by the nosocomial infections or widespread use of beta-lactams in the region. It has been suggested that *S. aureus* isolates expressing PVL cause severe skin and soft tissue infections, necrotizing fasciitis and life-threatening infections such as hemorrhagic pneumonia (31). In previous studies conducted in Turkey, it was reported that the prevalence rates of *pvl* gene varied between 1.9-5.4% in MSSA isolates and 1.5-9.5% in MRSA isolates (28,30,32). Another study conducted by Duman et al. (27), showed that *pvl* gene was detected in 15% (6/88) of the CA- *S. aureus* isolates and in 3% (6/177) of the HA- *S. aureus* isolates.

In this study, *eta* and *etb* genes were detected in 4.7% and 4.2% of the isolates, respectively. Although isolation rates were higher in MSSA isolates (1.7% for *eta* and 6.7% for *etb*) compared to MRSA isolates (2.9% for *eta* and 1.1% for *etb*), this was not statistically significant. In previous studies, absence or low prevalence rates of ET genes has been reported. Xie et al. (33) reported the prevalence of *eta*, *etb* and *etd* genes in 108 clinical *S. aureus* isolates as 1.9%, 0% and 8.3%, respectively. Jiménez et al. (34) could

**Table 3.** SAg toxin genotypes identified in MSSA and MRSA isolates and their relationship with MGE

| SAg Toxin | MSSA | MRSA | Total | Possible MGE |
|---|---|---|---|---|
| *sea* | 5 | 4 | 9 | φSa3mu |
| *sea, tst* | - | 1 | 1 | φSa3mu+*tst* |
| *sea, selq* | - | 1 | 1 | φSa3mu+*selq* |
| *sea, sed* | 1 | 1 | 2 | φSa3mu+*sed* |
| *sea, selo* | - | 1 | 1 | φSa3mu+*selo* |
| *sea, seg, sei* | 2 | 1 | 3 | φSa3mu+*seg+sei* |
| *sea, selk, selq* | 2 | - | 2 | φSa3mw+*selk+selq* |
| *sea, selm, selo* | 1 | 1 | 2 | φSa3mu+*selm+selo* |
| *sea, seg, selm, selo* | 2 | - | 2 | φSa3mu+*seg+selm+selo* |
| *sea, seg, sei, tst* | 1 | - | 1 | φ**S**a3mu+*seg+sei+tst* |
| *sea, selm, selo, selr* | - | 1 | 1 | φSa3mu+*selm+selo+selr* |
| *sea, selo, tst* | - | 1 | 1 | φSa3mu+*selo+tst* |
| *sea, seb, seg, sei, seh, tst* | 1 | - | 1 | φSa3mu+*seb+seg+sei+seh+tst* |
| *sea, seg, sei, selm, seln, selo* | 1 | 1 | 2 | φSa3mu, Tip I νSaβ |
| *sea, seg, sei, seh, tst* | 2 | - | 2 | φSa3mu+*seg+sei+seh+tst* |
| *sea, seg, sei, selm, seln, selo* | 1 | - | 1 | φSa3mu, Tip I νSaβ |
| *sea, sed, selj, seln, selk, selr* | - | 1 | 1 | φSa3mu, pIB485 |
| *sea, sec, seg, sei, sell, selm, selo* | - | 1 | 1 | φSa3mu, Tip II νSa3, Tip I νSaβ |
| *sea, seg, sei, sell, selm, selo, selr, tst* | - | 1 | 1 | φSa3mu, Tip I νSaβ |
| *sea, seg, sei, selm, seln, selo, tst* | 1 | - | 1 | φSa3mu, Tip I νSaβ+*tst* |
| *sea, sed, selj, selk, selq, selr* | 4 | - | 4 | φSa3mw, pIB485 |
| *sea, seh, selk, selq* | - | 2 | 2 | φSa3mw+*seh* |
| *sea, seg, sei, selk, selq, tst* | - | 2 | 2 | φSa3mw+*seg+sei+tst* |
| *sea, seg, sei, selk, selq* | - | 2 | 2 | φSa3mw+*seg+sei* |
| *seb* | 3 | - | 3 | ND |
| *seb, seg, sei, selk, selm, selo, selq, tst* | 1 | - | 1 | Tip I νSaβ, νSa1 (SaPI3)+*tst* |
| *seb, seg, sei, selk, selq* | - | 1 | 1 | νSa1 (SaPI3)+*seg+sei* |
| *sec, seg, sei, selm, seln, selo, selr* | 2 | - | 2 | Tip I νSaβ+*sec+selr* |
| *sec, seg, sei, sell, selm, selo, selp* | 1 | - | 1 | Tip II νSa3, Tip I νSaβ, φSa3n |
| *sed, selj, selr* | 1 | - | 1 | pIB485 |
| *sed, seg, sei, seln, tst* | - | 2 | 2 | ND |
| *sed, seg, sei, selm, seln, selo* | 2 | - | 2 | Tip I νSaβ+*sed* |
| *sed, seg, sei, selm, seln, selo, tst* | - | 1 | 1 | Tip I νSaβ+*sed+tst* |
| *sed, seg, sei, selj, selm, selo, selr* | - | 1 | 1 | Tip I νSaβ, pIB485 |
| *sed, seg, sei, selk, seln* | 1 | - | 1 | ND |
| *sed, selm, seln, selo, tst* | - | 1 | 1 | ND |
| *seg, sei* | 4 | 3 | 7 | ND |
| *seg, sei, tst* | 4 | - | 4 | ND |
| *seg, sei, selk, selr* | 2 | - | 2 | ND |
| *seg, sei, selm, seln, selo* | 3 | 1 | 4 | Tip I νSaβ |
| *seg, sei, selj, selm, seln, selo, tst* | 2 | 1 | 3 | Tip I νSaβ+*selj+tst* |
| *seg, sei, selj, selm, selq, selo, tst* | - | 2 | 2 | Tip I νSaβ+*selj+tst* |
| *seg, sei, selm, selo, selq* | - | 1 | 1 | Tip I νSaβ |
| *seg, sei, selo, selm* | 9 | 3 | 12 | Tip I νSaβ |
| *seg, sei, selp, seln, selk* | 1 | - | 1 | φSa3n |
| *seg, sei, seh, selm, selo* | 1 | - | 1 | Tip I νSaβ |
| *seg, sei, selm, seln, selo, tst* | 1 | - | 1 | Tip I νSaβ |
| *seg, sei, sell, selm, selo, selr, tst* | - | 1 | 1 | Tip I νSaβ+*sell+selr+tst* |
| *seg, sei, selp, sell, selm, selr, selo* | - | 1 | 1 | Tip I νSaβ, φSa3n |
| *seg, sei, selk, seln, tst* | - | 2 | 2 | ND |
| *seg, sei, sell, selm, selo, selr* | 1 | 1 | 2 | Tip I νSaβ |
| *seg, sei, selj, selm, selo, tst* | 1 | 2 | 3 | Tip I νSaβ+*selj+tst* |
| *seg, sell, selm, selo, selr* | 1 | - | 1 | ND |
| *seh* | 1 | 10 | 11 | ND |
| *seh, sell* | - | 2 | 2 | ND |
| *seh, selm, selo* | - | 2 | 2 | ND |
| *seh, selq, tst* | - | 1 | 1 | ND |
| *seh, selo, selk, tst* | - | 1 | 1 | ND |
| *seh, selm, selo, tst* | - | 1 | 1 | ND |
| *selo* | 2 | 2 | 4 | ND |
| *selm, selo* | 1 | 1 | 2 | ND |
| *selm, selo, tst* | 2 | 1 | 3 | ND |
| *selp* | 1 | 1 | 2 | φSa3n |
| *selp, selq* | - | 1 | 1 | φSa3n, *selq* |
| *selq* | 1 | - | 1 | ND |
| *selq, selk* | 3 | - | 3 | ND |
| *selm, selo, selq* | 2 | 0 | 2 | ND |
| *selk, selm, selo, selq, tst* | - | 1 | 1 | ND |
| *selk, selq* | 3 | - | 3 | ND |
| *tst* | 1 | - | 1 | ND |
| *sell* | 1 | - | 1 | ND |
| Negative | 17 | 14 | 31 | |

SAg: superantigen, MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*, MGE: mobile genetic element, ND: Since combination of SAg genes was incomplete, any association was not established between suspected MGE and toxin profile

**Table 4.** Hemolysin gene combinations determined in MSSA and MRSA isolates

| Hemolysin | MSSA (n=103) | MRSA (n=87) | p | Total (n=190) |
|---|---|---|---|---|
| *hly*A | 1 (1.0) | 0 (0.0) | | 1 (0.5) |
| *hly*D | 8 (7.8) | 4 (4.6) | | 12 (6.3) |
| *hly*A-*hly*D | 59 (57.3) | 52 (59.8) | | 111 (58.4) |
| *hly*A-*hly*D-*hly*G2 | 32 (31.1) | 27 (31.0) | | 59 (31.1) |
| *hly*A-*hly*D-*hly*B | 0 (0.0) | 2 (2.3) | 0.309 | 2 (1.1) |
| *hly*A-*hly*D-*hly*G | 2 (1.9) | 0 (0.0) | | 2 (1.1) |
| *hly*D-*hly*G2 | 0 (0.0) | 1 (1.1) | | 1 (0.5) |
| *hly*A-*hly*D-*hly*B-*hly*G2 | 1 (1.0) | 0 (0.0) | | 1 (0.5) |
| Negative | 0 (0.0) | 1 (1.1) | | 1 (0.5) |

*hly*: Hemolysin, MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

**Table 5.** Distribution of *pvl* gene among the MSSA and MRSA isolates

| *pvl* | MSSA (n=103) | MRSA (n=87) | p | Total (n=190) |
|---|---|---|---|---|
| Negative | 88 (85.4) | 76 (87.4) | 0.701 | 164 (86.3) |
| Positive | 15 (14.6) | 11 (12.6) | | 26 (13.7) |

MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

**Table 6.** Distribution of *agr* types according to MSSA and MRSA isolates

| *agr* Type | MSSA (n=103) | MRSA (n=87) | p | Total (n=190) |
|---|---|---|---|---|
| *agr* type I | 53 (51.5) | 55 (63.2) | | 108 (56.8) |
| *agr* type II* | 27 (26.2) | 5 (5.7) | | 32 (16.8) |
| *agr* type III* | 17 (16.5) | 25 (28.7) | **0.001** | 42 (22.1) |
| *agr* type I-II | 4 (3.9) | 1 (1.1) | | 5 (2.6) |
| *agr* type I-III | 1 (1.0) | 1 (1.1) | | 2 (1.1) |
| *agr* type II-III | 1 (1.0) | 0 (0.0) | | 1 (0.5) |

*agr*: accessory gene regulator, MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*, *: statistically significant differences for these two *agr* types

not detect the *eta* and *etb* genes in MSSA (n=30) isolates, but detected the *eta* gene in only one the MRSA (n=30) isolate. de Souza et al. (35) did not find the *eta* and *etb* genes in any of MRSA isolates, but detected the *eta* gene in only 2.3% (3/130) of the MSSA isolates. Similarly, Nhan et al. (36) reported a low prevalence rate for *eta* (1/1186) among clinical *S. aureus* isolates. In contrast, high prevalence rates of ET genes among *S. aureus* isolates was reported by Demir et al. (37), who found that 20% (n=24) of the isolates were positive for the *eta* and *etb* genes.

It has been noted that the prevalence of *tst* gene among MRSA isolates has increased in recent years. Similarly, in this study, the frequency of *tst* gene was found to be higher in MRSA isolates in comparison to MSSA isolates (24.1% vs 13.6%, p=0.062). Hu et al. (38) reported higher prevalence rate and found the *tst* gene in 85.6% (101/118) of MRSA isolates and 10% (14/140) of MSSA isolates. However, a contradictory result was reported by Motamedifar et al. (23), who detected the *tst* gene in 18.1% (36/199) of MSSA isolates and 11.6% (17/146) of MRSA isolates.

The presence of SE genes among MRSA and MSSA isolates was found to be abundant and diverse as 83.7% (n=159) of the isolates (Table 2, Table 3). Xie et al. (33) found that 85.2% (92/108) of the *S. aureus* isolates were positive for SE genes and *sea* (44.4%), *selk* (42.6%) and *selq* (40.7%) as the most common SE genes. The researchers also reported *sea-selk-selq* (41.3%, 38/92) as the most common SE combination. In another study, Demir et al. (37) detected that 69 of 120 (57.5%) *S. aureus* isolates had one or more SE genes, with a dominance of *seg* and *sei* (49/120, 40.8%) genes. Hu et al. (38) investigated MRSA and MSSA isolates for classical and newly described SE genes, and found that 78% (92/118) of the MRSA isolates and 75.7% (89/140) of MSSA isolates were positive for SE genes. The authors also noted that *sea* (45%) and *sec* (39%) genes were as the most common SE genes and *sec-seg-sei-sell-selm-seln-selo-tst* as the dominant genotype (52/118, 44.1%) among MRSA isolates; on the other hand, *sea* (42%), *selk* (38%) and *sec* (35%) genes were as the most common SE genes and *seg-sei-sell-selm-seln-selo* as dominant genotype (18/140, 12.9%) among MSSA isolates. Tekeli et al. (39) investigated 100 MRSA isolates from the blood cultures of hospitalized patients for the SE genes, and detected the SE genes in 86% of the isolates and distribution of SE as *sea* (77%), *sea-seg* (4%), *sea-seg-sec* (1%) and *seg-sei* (2%).

Of the exotoxins synthesized by *S. aureus* isolates, HLYs are among the toxins that play important roles in the pathogenesis of staphylococcal infections. These toxins exert a lethal effect on different host cell populations, including immune cells, and have the ability to help the spread of bacteria within the host body (40). Previous studies have revealed that HLY genes are widely distributed among MSSA and MRSA isolates. In this study, nearly all isolates were found to be positive for one or more investigated HLY genes. Similar observations have also been reported in previous studies (41-44).

*S. aureus* is one of notorious bacteria that has the ability to produce PFTs targeting leukocytes (6). The *luk*ED gene, located in mobile staphylococcal pathogenicity island called vSAβ (45), was detected in 67.9% (129/190) of the isolates, of which 69 (67%) were MSSA and 60 (69%) were MRSA. However, none of the isolates was positive for *luk*M gene. Comparable prevalence rate was also reported by Havaei et al. (46), who detected *luk*ED in 73.8% (110/149) of the isolates. On the other hand, He et al. (47) detected *luk*ED in 81.4% (144/177) of the isolates, but *luk*M was not detectable in any isolates.

In this study, most of the isolates (56.8%) belonged to *agr* type I, followed by *agr* type III (22.1%) and *agr* type II (16.8%), respectively. The *agr* type IV was not detected in any of the isolates. Similarly, Peerayeh et al. (48) reported that most of the *S. aureus* isolates belonged to *agr* type I (55.1%), followed by *agr* type II (16.9%), *agr* type III (16.5%) and *agr* type IV (9.4%), respectively. Similar observations were also reported by Shopsin et al. (49) and van Leeuwen et al. (50), who reported that 42.1% and 71% of the isolates were belonged to *agr* type I, respectively. In contrast, Tekeli et al. (39) reported a higher rate (91%) of *agr* type I in 100 MRSA strains isolated from blood cultures. In this study, the authors did not detect *agr* type IV among the isolates. The absence of *agr* type IV has also been reported in previous studies (49-52). The differences observed in the distribution of *agr* types among *S. aureus* isolates could be explained by ecological and geographical

differences. Interestingly, some isolates carried more than one *agr* type in this study. A similar observation was also reported by Yoon et al. (53), who detected co-presence of *agr* type I-II and *agr* type I-III in 4.4% and 0.6% of the isolates, respectively. Ji et al. (54) explained this phenomenon with significant sequence changes in the domain encoding the *agr*D signal peptide.

Jarraud et al. (13) reported that there is a relationship between *agr* groups and infection types, and suggested that the strains belong to *agr* type IV are mostly associated with generalized exfoliative and suppurative infections, whereas the strains belonged to *agr* type I and II are associated with endocarditis cases. Moreover, Ji et al. (54) reported that TSST-1 producing isolates were associated with *agr* type III. In this study, ET and TSST-1 producing isolates were mainly found to belong to *agr* types I and II. The SAg toxin genes have been reported to be largely associated with MGE, such as pathogenicity islands, prophages, SCC*mec* element and plasmids (55). Seventy one SAg toxin gene profile determined in the study suggested possible presence of MGEs encoding SAg toxins. Type I νSaβ (in 24 MSSA and 13 MRSA) and φSa3mu (in 18 MSSA and 16 MRSA) were identified as the most common MGEs, followed by φSa3mw (in 4 MSSA and 7 MRSA), φSa3n (in 3 MSSA and 3 MRSA), pIB485 (in 5 MSSA and 1 MRSA) and Type II νSa3 (in 1 MSSA and 1 MRSA). Of the determined MGEs, φSa3mu encodes *sea*; type I νSaβ *seg*, *sei*, *selm*, *seln* and *selo*; type II νSa3 *sec*, *sell*; νSa1 (SaPI3) *seb*, *selk*, *selq*; type I νSa4 *sec*, *sell*, *tst*; φSa3mw *sea*, *selk*, *selq*; φSa3n *selp*; pIB485 *sed*, *selj*, *selr* and SaPI1 *seb*, *selk*, *selq* genes. In this study, MGEs were detected at a higher frequency in MRSA isolates. Similarly, while Hu et al. (37) found type I νSaβ and type I νSa4 in 83.1% and 77.1% of the MRSA isolates, and also these MGEs were found in 25% and 2.1% of MSSA isolates.

The clinical outcome of *S. aureus* infections is influenced not only by the presence of antimicrobial resistance, but also virulence factors. The acquisition of antibiotic resistance genes in *S. aureus* isolates has been reported to cause changes in the expression of virulence genes depending on the fitness cost associated with the expression of resistance genes leading to a decrease in toxin expression (34). Therefore, it is not surprising in this respect that virulence genes were detected at a higher rate in MSSA isolates than MRSA isolates.

In this study, we aimed to search only the *mec*A gene, not the *mec*C gene among the fenotipitic resistant isolates. Therefore, this situation can be considered as a limitation of the study.

## CONCLUSION

The results of this study showed that there is a high genetic diversity in terms of toxin genes among the clinical *S. aureus* isolates, and that the presence and combination of toxin genes are not limited to MSSA or MRSA, indicating frequent transfer of toxin gene-containing MGEs among *S. aureus* populations. In addition, the novel SE gene combinations observed herein suggest the existence of variants or novel types of MGEs. Comparative molecular studies involving large MSSA and MRSA populations are needed to understand how native MSSA and MRSA populations arise and interact.

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