COMPARISON OF TOXIN GENES IN STAPHYLOCOCCUS AUREUS ISOLATES OBTAINED FROM PATIENTS AND CARRIERS

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ABSTRACT

It is important to have information about virulence characteristics of S. aureus isolates obtained from the nasal cultures of carriers in terms of the capability to cause severe infections. In the present study, S. aureus isolates obtained from carriers and isolates obtained from clinical specimens, and which were determined as causative agents of infection, were compared in terms of the genes of staphylococcal enterotoxin A and B (sea and seb), TSST-1 (tst), and PVL (pvl).

A total of 44 S. aureus strains isolated from clinical specimens and 44 carrier isolates were tested for the genes with polymerase chain reaction method. As the results, the most common gene was sea with a rate of 45.5% (40/88). This gene was found in 34.1% (15/44) of the carrier isolates and in 56.8% (25/44) of the clinical group. Furthermore, the presence of the sea gene was found to be significantly higher in the clinical isolates than in the carriers' (P=0.032). The tst, seb, and pvl genes were positive at rates of 18.2% (16/88), 4.5% (4/88), and 5.7% (5/88), respectively. No significant differences were found between the groups according to positivity rates of the tst, seb, and pvl genes (p>0.05 for each gene)

To our knowledge, differences between carrier isolates and clinical isolates in terms of toxin genes have not been investigated before the present study. The presence of sea at a significantly higher rate in clinical isolates shows the need for advanced molecular analysis on this topic.

***Key words**: Staphylococcus aureus; toxin gene; staphylococcal enterotoxin; PVL; toxic shock syndrome toxin-1; carrier.*

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Introduction

Staphylococcus aureus is an important pathogen which may cause various clinical manifestations, from asymptomatic colonization to fatal community- or hospital-acquired infectious diseases[1,2]. Virulence factors that are found on the surface or that are secreted from the cell play important roles in the pathogenesis of *S. aureus*[1,3]. Membrane-disruptive or cytolytic toxins such as alpha, beta, gamma, delta and Panton-Valentine leukocidin (PVL); exfoliative toxins, as classified as A and B; and enterotoxins as A, B, C, D, E, G, H and I, and toxins like toxic shock syndrome toxin-1 (TSST-1) are among such virulence factors[1-4]. Food poisoning caused by staphylococcal enterotoxins is commonly observed throughout the world[3,4].

TSST-1 leads to toxic shock syndrome, particularly during women's menstrual periods[3,4]. PVL is a toxin that plays a role in causing necrotizing pneumonia, as well as skin and soft tissue infections from staphylococci[1-4].

Studies identified the anterior nares as the primary colonization site for *S. aureus*. Nasal colonization of *S. aureus* is important in workers of both industries of food and health, and periodic scanning of nasal cultures is carried out in these individuals[5,6]. Colonization with *S. aureus* is reported to raise the risk for staphylococcal infection after invasive procedures such as medical or surgical interventions. Asymptomatic colonization is far more common than infection. Transmission can occur by direct contact, and colonization can be either transient or persistent.

Colonization rates are reported between 25-50%, and high rates are observed in health-care workers, injection drug users, patients with insulin-dependent diabetes, patients with dermatologic diseases, patients with long-term catheters[6]. It is important to have information about virulence characteristics in terms of the capability of *S. aureus* isolates obtained from the nasal cultures of carriers to cause severe infections[5]. In the present study, *S. aureus* isolates obtained from carriers and isolates obtained from clinical specimens, and which were determined as causative agents of infection, were compared in terms of the genes expression of staphylococcal enterotoxin A and B (*sea* and *seb*), TSST-1 (*tst*), and PVL (*pvl*).

Materials ans method

This study was approved by the Clinical Researches Ethical Committee of Abant Izzet Baysal University.

Isolates

A total of 44 *S. aureus* strains isolated from clinical specimens as the patient group and 44 strains isolated from nasal cultures of individuals without any symptoms of infection accepted as carriers were included in the study. The isolates were obtained from Medical Microbiology Laboratories of Recep Tayyip Erdogan University Faculty of Medicine (RTEU) (55 isolates), Abant Izzet Baysal University Faculty of Medicine (AIBU) (26 isolates), and Amasya University Serafeddin Sabuncuoglu Training and Research Hospital (AUSS) (7 isolates). Amongst the clinical isolates, 22 strains were isolated from blood cultures and remaining 22 strains were recovered from respiratory tract specimens. The inpatients whose blood cultures revealed *S. aureus* included in our study were followed with a diagnosis of bacteriemia or sepsis. The individuals that we used the isolates of their respiratorial isolates for the study consisted of inpatients (15 patients) and outpatients (7 patients) with diagnosis of community- or hospital-acquired pneumoniae. The carrier group isolates were routinely or periodically obtained from the nasal cultures of individuals working in food industry. Each isolate group consisted of seven methicillin-resistant *S. aureus* (MRSA) and 37 methicillin-susceptible *S. aureus* (MSSA) strains (Table I). The methicillin susceptibility of the isolates was tested using the Kirby-Bauer disk diffusion method, employing a cefoxitin disk (Oxoid, England) according to the criteria of the Clinical and Laboratory Standards Institute (CLSI)[7].

Centers	Carrier isolates			Clinical isolates		
	MRSA[a]	MSSA[b]	Total	MRSA	MSSA	Total
Total	7	37	44	7	37	44

Table I: Distribution of the isolates according to methicillin-resistance.
[a]: Methicillin-resistant Staphylococcus aureus, [b]: Methicillin-susceptible Staphylococcus aureus.

Just one among different isolates from the same patient was used for the study. In addition, to rule out duplicates or the probability of epidemic strains, just one isolate among the strains isolated from the same clinics or isolated within several days of one another was included in the study.

DNA Extraction

DNAs of the isolates were extracted using the commercial GF-1 Bacterial DNA Isolation Kit (Vivantis, Malaysia) according to the recommendations of the manufacturer. The extraction products were stored at -20ºC until used.

Amplification of the toxin genes

The polymerase chain reaction (PCR) method was used for the detection of sea, seb, tst, and pvl genes in the two isolate groups. The primers used are shown in Table II. A PCR mix consisted of 1X PCR buffer (1 µl template DNA, 0.2 mM dNTP, 4 mM MgCl2), 0.5 µl primers (50 pmol/ml) and 0.2 µl Taq DNA polimerase (5 U/µl) was used. Amplification conditions were performed as follows; initial denaturation for 2 minutes at 95°C and 30 cycles as denaturation for 15 seconds at 95°C, annealing for 30 seconds at 55°C, and elongation for 30 seconds at 72°C[8-10]. The bands produced after amplification were evaluated with an ultraviolet monitoring system (KODAK Gel Logic 200 Imaging System, Germany), and differences between carriers and patients groups in terms of presence of the virulence genes were analyzed.

Statistical analysis

Data analysis was performed using SPSS software (ver. 17.0 for Windows; SPSS Inc., Chicago, IL). Descriptive statistics were expressed as num-

bers and percentages. Differences between groups in terms of categorical variables were analyzed using the chi square and Fisher's exact tests. The results were evaluated at a 95% confidence interval and a p value of <0.05 was accepted as significant.

Toxin	Gene region	Primers (5'-3')	Product (bp)	Reference
SEA[a]	*sea*	GCA GGG AAC AGC TTT AGG C GTT CTG TAG AAG TAT GAA ACA	530	(Zecconi et al. 2006)
SEB[b]	*seb*	TGT ATG TAT GGA GGT GTA AC ATA GTG ACG AGT TAG GTA	165	(Sharma et al. 2000)
PVL[c]	*pvl*	TTC ATT TAG ACG CAG CAG GA TTG AAT AGC CGT CCC TTA CG	465	(Zecconi et al. 2006)
TSST-1[d]	*tst*	AAG CCC TTT GTT GCT TGC G ATC GAA CTT TGG CCC ATA CTT T	445	(Becker et al. 1998)

Table II: Primers used for polimerase chain reaction tests.
[a]*Staphylococcal enterotoxin A,* [b]*Staphylococcal enterotoxin B,* [c]*Panton-Valentine leukocidin,* [d]*Toxic shock syndrome toxin-1.*

Results

As the results of PCR tests, the most common gene was *sea* with a rate of 45.5% (40/88). This gene was found in 34.1% (15/44) of the carrier isolates and in 56.8% (25/44) of the clinical group. Furthermore, the presence of the *sea* gene was found to be significantly higher in the clinical isolates than in the carriers' (P=0.032). The tst, seb, and pvl genes were positive at rates of 18.2% (16/88), 4.5% (4/88), and 5.7% (5/88), respectively. No significant differences were found between the groups according to positivity rates of the *tst*, *seb*, and *pvl* genes (p>0.05 for each gene) (Table III).

In addition, no significant differences were found between the groups of blood and respiratorial samples in terms of each gene (p>0.05 for each gene). At least one gene was detected in 54.5% (24/44) of the isolates in the carriers' group, and in 68.2% (30/44) of the ones in the patients; no significant difference was found between the groups in terms of presence of any toxin genes (p=0.189).

Despite the low numbers of MRSA isolates, no significant difference was found between MRSA and MSSA isolates in terms of the presence of each gene (p>0.05 for each gene). In addition, no difference was found between blood and respiratory tract specimens for each gene (p>0.05 for each gene) (Table III).

Toxin genes	Total	Carrier isolates (n=44)	Clinical isolates (n=44)	p
sea[a]	40 (45.5%)	15 (34.1%)	25 (56.8%)	0,032
seb[b]	4 (4.5%)	2 (4.5%)	2 (4.5%)	1,000
tst[c]	16 (18.2%)	9 (20.5%)	7 (15.9%)	0,580
pvl[d]	5 (5.7%)	3 (6.8%)	2 (4.5%)	1,000
Presence of at least one toxin gene	54 (61.4%)	24 (54.5%)	30 (68.2%)	0,189

Table III: Distribution of rates of presence of toxin genes detected by PCR.
[a]*Staphylococcal enterotoxin A,* [b]*Staphylococcal enterotoxin B,* [c]*Panton-Valentine leukocidin,* [d]*Toxic shock syndrome toxin-1.*

No difference was found between the RTEU and AIBU centers in terms of the presence of each gene; AUSS was not included in this analysis because of the low number of isolates (p>0.05 for each gene).

Discussion

Nasal carriers of *S. aureus*, particularly MRSA, play a role in the transmission of the microorganism in the community[5]. It is crucial to know whether individuals, especially food industry workers, are carriers[5]. In addition, it is critical to have information about the differences in the virulences of carrier isolates and clinical isolates of *S. aureus*. In the present study, the virulence factors of some toxin genes were compared between these groups for the first time in the literature.

The roles of extracellular staphylococcal products in the pathogenesis of systemic pathogenesis have not yet been clearly identified. Staphylococcal enterotoxins and TSST-1, as superantigens, continue to attract the attention of researchers. These toxins, in contrast to normal of antigen processes, bind directly to major histocompatibility complex (MHC) class II and cause extreme stimulation of T cells, thereby leading to the production of extreme amounts of interleukin-1, interleukin-2, interferon gamma, and tumor necrotizing factor alpha.

These findings have shown that the mentioned toxins play important role in pathogenesis of sepsis and septic shock[3,11].

In the present study, the rate of presence of at least one toxin gene was found to be 61.3% (54/88). This rate was reported as 63.2% by Yilmaz et al.,[12] 73.4% by Kim et al.,[13] 62.4% Hu et al.,[14] and 61.4% by Demir et al.[15]. The rate of our study is highly close to these reports.

In our study, *sea* was the most common gene (45.5%). The positivity rate of *sea* was found to be 26.4% by Demir et al.[15] and 40.1% (59/147) by Yilmaz et al.[12]; in these two studies, it was reported to be the most common gene. Yilmaz et al.[12] reported that the prevalence of *sea* was found to be significantly higher in hospital-acquired isolates than community-acquired ones. In addition, in that study, *sea* was reported to be detected at a significantly higher rate in MRSA isolates.

However, because the higher consistence of MRSA in hospital isolates in their study would be likely to cause a higher positivity rate of the *sea* gene in hospital isolates, their finding is questionable. Besides, in this study, clonal relatedness was investigated, and when the data were analyzed, it was observed that most of their hospital isolates were epidemic clones. This finding also raises questions about the significance of the higher rate of hospital isolates. In our study, the prevalence of the *sea* gene was found to be significantly higher in clinical isolates than in carriers. According to this finding, the isolates obtained from carriers seem to be less virulent. This result, however, does not identify whether the sea gene was obtained from carrier isolates in the process of causing infection or whether the carrier isolates are less virulent in general. To differentiate this accurately, larger studies with a higher number of isolates and including determination of clonal relatedness need to be done on this topic.

In our study, the frequencies of presence of all toxin genes were found to be similar within the MRSA and MSSA isolates. This finding suggests that the presence of toxin genes and hence levels of virulence are not associated with antimicrobial resistance. Yilmaz et al.[12] reported an association between sea and methicillin resistance, but their consideration was likely inaccurate due to the probability epidemic clones in their study; besides, they stated that no associations were found between resistance and the other toxin genes that were also included in our study.

Larger studies would provide more accurate results concerning this relationship.

PVL is a virulence factor associated with necrotizing pneumonia and soft tissue infections[16]. PVL, a component encoded by phages, is also associated epidemiologically with community-acquired MRSA infections[16,17]. PVL is capable to lyse human myeloid cells in vitro, but its role in community-acquired infections has not been clearly identified yet. It has been reported that PVL is not associated directly with the expression of the other virulence genes[17]. It has also been shown that PVL is mostly isolated from community-acquired infections[3,16]. The *pvl* gene has been detected in low rates in most studies. Oumokhtar et al[5]. detected it in 3.3% of the strains isolated from hemodialysis patients who were also nasal carriers of *S. aureus*. The rate of presence of *pvl* was reported as 1.6% by Holmes et al.[18]; as 2% by Issartel et al.[19]; as 3.8% by Karahan et al.[20]; and as 0% (0/37) by Kirdar et al.[21]. In our study, the rate of *pvl* was found to be 5.7% (5/88), and we could not determine a significant difference between clinical and carrier isolate groups in terms of the presence of *pvl*.

In the present study, the *seb* gene was detected in 4.5% (4/88) of the isolates. Yilmaz et al.[12] reported this rate as 5.4% (8/147). In their study, they reported no difference between community and hospital isolates. We also found no significant difference between clinical and carrier groups in terms of the presence of the *seb* gene. Furthermore, the number of isolates included in our study seems to be too limited to show any significant differences between groups in terms of the presence of rarely found genes such as *seb* and *pvl*, so larger studies need to be done on this topic.

In the present study, we detected the tst gene in 18.2% (16/88) of isolates and we found no significant difference between groups. Yilmaz et al.[12] reported a rate of 11.6% (17/147) with no significant difference between community and hospital isolates. Kim et al.[13] found TSST-1 in 40.8% of their isolates. These findings show no association between the presence of tst and any of situations mentioned, as well as demonstrating that the rate of tst varies in a wide range of S. aureus isolates.

We did not investigate the clonal relationship between the isolates in our study, and this seems to be a limitation of the research. However, to rule out any probable duplications or the use of any epidemic strains, we took care to choose only one isolate per patient or one strain isolated from the same clinic within a few days of one another. In addition, did the same thing with the carrier isolates; however, we did not gather any information about the

workplaces of the individuals, so some of the carrier isolates might be the same clone. However, we consider the probability of this occurrence to be low, and it likely did not affect our results significantly.

We chose the same numbers of blood and respiratory tract isolates to analyze the results more accurately. In addition, we chose these two types of specimens for the study to be sure that the isolates were infectious agents, and we could not found any significant differences in terms of presence rates of the toxin genes.

To our knowledge, differences between carrier isolates and clinical isolates in terms of toxin genes have not been investigated before the present study. The presence of sea at a significantly higher rate in clinical isolates shows the need for advanced molecular analysis on this topic. Future data on this subject will be helpful in terms of treatment and prevention.

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