Detection of Methicillin Resistance and Various Virulence Factors in \textit{Staphylococcus aureus} Strains Isolated from Nasal Carriers

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**Background:** \textit{Staphylococcus aureus} can be found as a commensal on skin and nasal flora or it may cause local and invasive infections. \textit{S. aureus} has a large number of virulence factors.

**Aims:** To investigate the methicillin resistance and frequency of various virulence factors in \textit{S. aureus} nasal isolates.

**Study Design:** Descriptive study.

**Methods:** Nasal samples collected from university students were cultured in media. \textit{S. aureus} was identified by conventional methods and the Staphyloslide latex test (Becton Dickinson, Sparks, USA). Antibiotic susceptibility tests were conducted, and the methicillin resistance was determined. The \textit{mecA}, \textit{nuc}, \textit{pvl} and staphylococcal toxin genes were examined by polymerase chain reaction (PCR).

**Results:** \textit{S. aureus} was isolated in 104 of 600 (17.3\%) nasal samples. In total, 101 (97.1\%) \textit{S. aureus} isolates were methicillin-sensitive and the remaining 3 (2.9\%) were methicillin-resistant. Furthermore, all but five isolates carried at least one staphylococcal enterotoxin gene, with \textit{seg} being predominant. The \textit{tst} and \textit{eta} genes were determined in 29 (27.9\%), and 3 (2.9\%) isolates, respectively. None of the \textit{S. aureus} isolates harbored \textit{see}, \textit{etb}, and \textit{pvl} genes.

**Conclusion:** A moderate rate of \textit{S. aureus} carriage and low frequency of MRSA were detected in healthy students. \textit{S. aureus} isolates had a high prevalence of staphylococcal enterotoxin genes and the \textit{tst} gene. In this study, a large number of virulence factors were examined in \textit{S. aureus} nasal isolates, and the data obtained from this study can be used for monitoring the prevalence of virulence genes in \textit{S. aureus} strains isolated from nasal carriers.

**Keywords:** Methicillin resistance, \textit{Staphylococcus aureus}, virulence factors

\textit{Staphylococcus aureus} can be found as a commensal on the skin and nasal flora, and may cause local, severe, and invasive infections, such as bacteremia or pneumonia (1). The anterior nostrils represent the most common area for colonization of staphylococci; in fact, longitudinal studies have shown that ~50\% of individuals are \textit{S. aureus} nasal carriers (2,3). Curiously, nasal colonization has been identified as a major risk factor for the development of community-acquired and nosocomial \textit{S. aureus} infections (4,5).

The capacity of \textit{S. aureus} to acquire antibiotic resistance genes is important. Methicillin-resistant \textit{S. aureus} (MRSA) isolates have been subsequently reported in hospital and community settings worldwide. The increasing prevalence of MRSA in the hospital acquired (HA-MRSA) and the community acquired (CA-MRSA) is threatening. By definition, all MRSA (carrying \textit{mecA} gene) species are resistant to beta-lactam antibiotics. In addition, MRSA isolates can obtain other resistance determinants. Nevertheless, nowadays, \textit{S. aureus} colonizer strains are mostly methicillin-susceptible ones (MSSA) (3). Although some studies including healthy people have shown that the prevalence of \textit{S. aureus} and the
S. aureus has numerous cell-associated and secreted virulence factors that promote cellular adhesion, invasion, bacterial reproduction, and a deficiency of immune responses. Some of the virulence factors include Panton-Valentine leukocidin (PVL), toxic shock syndrome toxin 1 (TSST-1), hemolysins, exfoliative toxins (ETs), and staphylococcal enterotoxins (SEs) (10). PVL is a toxin that is usually related to complicated skin and soft tissue infections, diffuse cellulitis, necrotizing pneumonia, and osteomyelitis (11). Several toxins such as TSST-1 and SEs belong to the superantigen (SAg) family. More than 20 SAgs have been identified in S. aureus strains, and a minimum of 80% of clinical strains harbor at least one. SEs cause staphylococcal food poisoning, whereas TSST-1 and ETs are responsible for toxic shock syndrome (TSS) and staphylococcal scalded-skin syndrome (SSSS), respectively (12).

This study aims to investigate the methicillin resistance and rates of the TSST gene ( tst), Panton-Valentine leukocidin gene ( pvl), exfoliative toxin genes ( eta and eth), and enterotoxin genes ( sea, seb, sec, sed, see, seg, seh, sei, and sej) in S. aureus nasal isolates from healthy students in our university.

MATERIALS AND METHODS

Specimen collection and bacteriological methods

University students without any disease excluding medical school and health science students were included in the study. The students were informed about the procedure and verbal informed consents were obtained. The samples were taken from both nostrils of students using a swab. The swabs were inoculated in a tryptic soy broth and incubated at 37°C for 18-24 hours. Then, 10 μL of broth was inoculated onto Columbia agar added 5% sheep blood and mannitol salt agar (Becton Dickinson, Sparks, USA) using a sterile pipette. The media were incubated at 37°C for 18-24 hours. All colonies similar to S. aureus were identified by conventional methods (Gram staining, catalase test) and Staphyloslide latex test (Becton Dickinson, Sparks, USA). Only one of the strains isolated from both agars was included in this study.

Susceptibility tests

Antibiotic susceptibility tests were conducted, and methicillin resistance was detected by the Kirby-Bauer disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI). The D-test was carried out to detect the inducible clindamycin resistance (13). The following antibiotics were tested: penicillin (10 U), oxacillin (1 μg), cefoxitin (30 μg), erythromycin (15 μg), clindamycin (2 μg), gentamicin (10 μg), tetracycline (30 μg), ciprofloxacin (5 μg), moxifloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25-23.75 μg), linezolid (30 μg), and mupirocin (200 μg). The vancomycin susceptibility was investigated by an E-test strip (AB Biodisk, Solna, Sweden). S. aureus ATCC 25923 was used as a quality control strain.

Molecular methods

DNA extraction was performed with a commercial DNA isolation kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s recommendations. The presence of mecA (staphylococci methicillin resistance gene), nuc (S. aureus thermonuclease gene used to confirm S. aureus), and pvl was examined by modification using multiplex polymerase chain reaction (PCR) with specific primers (14-16). The PCR reaction mix (50 μL) included 1 μL of DNA, 5 μL of 10X PCR buffer, 25 mM MgCl₂, 10 μM dNTPs, 1 μL of each primer (50 pmol/mL) and 5U Taq DNA polymerase. For multiplex PCR, the amplification was performed under the following conditions: 94°C for 10 min, followed by 35 cycles of 94°C for 90 s, 49°C for 90 s, and 72°C for 90 s, with a final elongation of 72°C for 10 min. The staphylococcal toxin genes were investigated by PCR as previously described (17,18). The amplification was carried out in the LightCycler 2.0 thermocycler (Roche Applied Science, Germany). All PCR amplification products were separated on 2% agarose gel and visualized by staining with ethidium bromide using a UV light transilluminator.

The S. aureus strains ATCC 49775 (mecA negative, pvl positive) and ATCC 25923 (mecA negative, pvl positive, sea positive), and N315 (mecA positive, tst positive) were used as control strains for PCR.

RESULTS

S. aureus was isolated in 104 of the 600 (17.3%) nasal samples assessed. All isolates were found to be nuc-positive. Of these, 101 isolates (97.1%) were MSSA, and the remaining 3 isolates (2.9%) were MRSA according to both PCR and antibiotic susceptibility tests results. The rate of MRSA carriage was 0.5%. Except for five of the isolates, all others (95.2%) were positive for at least one staphylococcal enterotoxin gene. The seg gene was detected in 93 (89.4%) isolates, followed by sei in 68 (65.4%), sec in 55 (52.9%), seh in 37 (35.6%), sej in 23 (22.1%), sea in 14 (13.5%), seb in 9 (8.7%) and sed in 5 (4.8%). The tst and eta genes were determined in 29 (27.9%) and 3 (2.9%) isolates, respectively. None of the S. aureus iso-
lates harbored see, etb, and pvl genes. In this study, the most common combination was determined to be seg plus sei and seg plus sec in 65.4% and 49% of all isolates, respectively. Only 7 isolates encoded a single gene (Table 1).

All S. aureus strains were susceptible to vancomycin, trimethoprim/sulfamethoxazole, and linezolid. The susceptibilities of S. aureus strains for other antibiotics were 98% for gentamicin, ciprofloxacin, and moxifloxacin, 96% for tetracycline and mupirocin, 88% for erythromycin, and 20% for penicillin. The prevalence of inducible clindamycin resistance was 3%.

### DISCUSSION

*S. aureus* is a major human pathogen with a high virulence that causes both hospital-acquired and community-acquired staphylococcal infections. *S. aureus* causes skin and soft tissue infections of varying severity, from uncomplicated abscesses to life-threatening infections such as bacteremia and sepsis. *S. aureus* nasal carriage has been considered as a risk factor for the occurrence of human infections (19). In some studies, the *S. aureus* nasal carriage was detected at a frequency as high as 30% (3,20). In a study from Turkey, the carriage rate of *S. aureus* in Turkish elementary school children was 24.7% (21). The rate of these bacteria differs according to the population surveyed. We investigated the carriage of *S. aureus* in university students, representing young adults. Medical school and health science students were excluded because they are in contact with patients. A moderate rate of *S. aureus* nasal carriage (17.3%) has been detected in healthy university students.

The treatment of MRSA infections is becoming increasingly difficult because MRSA strains are resistant to beta-lactam antibiotics, and can acquire other resistance determinants. Especially in the community, the frequency of infections caused by MRSA has increased in the last decade (22). It is believed that the prevalence of MRSA carriage increases in a healthy community and, therefore, the surveillance of MSSA and MRSA in the nasal carriage has been investigated in healthy individuals. The rates determined in other studies that analyzed healthy adult and children were usually less than 1% (7,21,23). In accordance with previous studies, the prevalence of MRSA nasal carriers is very low (0.5%) in our study.

The capacity of *S. aureus* antibiotic resistance is also important. In this study, all strains were susceptible to trimethoprim-sulfamethoxazole, vancomycin, and linezolid. The susceptibilities of *S. aureus* strains for other antibiotics were 98% for gentamicin, ciprofloxacin and levofloxacin, 96% for tetracycline and mupirocin, 88% for erythromycin, and 20% for penicillin. The prevalence of inducible clindamycin resistance was 3%. The higher resistance to erythromycin could be clarified by the common use of macrolides in empirical treatment.

*S. aureus* is a pathogen with a wide range of virulence factors. The severity of *S. aureus* infections may be related to the production of some of these toxins. Exotoxins provide tissue destruction and escape from the host immune response. Toxins such as α-hemolysin or PVL cause cytolysis of leukocytes and erythrocytes (24). PVL is a virulence marker that is often identified in CA-MRSA strains associated with necrotizing pneumonia and severe skin and soft tissue infections. The *lukS-lukF* genes encoding the PVL toxin are located in a phage, and can be transferred among *S. aureus* (25). The studies conducted in our country have revealed that PVL is spread

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**TABLE 1.** The enterotoxin genes and combinations detected in the 104 *S. aureus* strains
at different rates between MSSA, CA-MRSA and HA-MRSA isolates without discrimination (26). All *S. aureus* strains were *pvl*-negative in our study. It was interpreted that these strains had been isolated from the carriers and not infectious agents.

Superantigens activate T-lymphocytes and macrophages lead to the extreme release of inflammatory cytokines, resulting in septic shock. At least 20 serologically distinct staphylococcal superantigens have been described, including SEs A through V and TSST-1. These bacterial toxins are pyrogenic and related to food poisoning and TSS. SEA and SED are the first and second most common staphylococcal toxins associated with food poisoning worldwide (27). According to these data, SEA was the most common toxin (40.1%) in hospital- and community-acquired *S. aureus* isolates in a study from Turkey (28). In the present study, the *seg* gene was the most common (89.4%). In relation to the standard enterotoxin genes, the *sec* gene (52.9%) was found to be the most frequent gene, followed by *sea* (13.5%). None of the *S. aureus* isolates harbored *see*. It was only determined in less than 1% of the samples in various studies (29-31).

Enterotoxigenic *S. aureus* strains with a combination of different SE genes can also promote the incidence and severity of *S. aureus* infections. In this study, the most common combinations detected were *seg* plus *sei* and *seg* plus *sec* in 65.4% and 49% of all isolates, respectively. Only 7 isolates encoded a single gene.

*S. aureus* isolates producing TSST-1 has been most commonly isolated from patients with important clinical symptoms. The *tst* gene encoding the TSST-1 was detected in 29 (27.9%) isolates in our study at similar rates to another study including healthy people (32).

*S. aureus* strains harboring ETs can cause SSSS and impetigo. Although there are differences in the prevalence between countries, approximately 5% of *S. aureus* human isolates produce ETs (25,29). However, these genes showed significantly higher rates in nasal and clinical MSSA strains in the study (30). In our study, 3 (2.9%) strains carried the *eta* gene, encoding the exfoliative toxin A; however, all isolated *S. aureus* strains were negative for the exfoliative toxin B gene. In a study conducted in our country, these genes were not detected in healthy controls but *etb* was determined in 18 (58.1%) patients with psoriasis, suggesting a potential relationship (33).

In conclusion, a moderate rate of *S. aureus* carriage and very low frequency of MRSA were detected in healthy students. *S. aureus* nasal isolates showed a very high prevalence of staphylococcal enterotoxin genes and the *tst* gene. Most *S. aureus* isolates were susceptible to antimicrobial agents. In this study, a large number of virulence factors were examined in *S. aureus* nasal isolates, and the data obtained from this study can be used for monitoring the prevalence of virulence genes in *S. aureus* strains isolated from nasal carriers.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Selçuk University Faculty of Medicine.

**Informed Consent:** The students were informed about the procedure and verbal informed consents were obtained.

**Peer-review:** Externally peer-reviewed.


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**Conflict of Interest:** No conflict of interest was declared by the authors.

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**REFERENCES**


