# Virulence Factors in Staphylococci Isolated From Nasal Cavities of Footballers

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# Virulence Factors in Staphylococci Isolated From Nasal Cavities of Footballers

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## ABSTRACT

**Aim:** This study aimed to investigate the rate of Panton-Valentine Leukocidin producing *Staphylococcus aureus* and methicillin (*mecA*) and slime *(icaA/icaD)* genes in staphylococcal strains isolated from nasal cavities of footballers.

**Materials and Methods:** Nasal swab samples were taken from each footballers and a healthy control group for the isolation of staphylococcal strains. The polymerase chain reaction technique was used to determine Panton-Valentine Leukocidin, *mecA* and *icaA/icaD* genes in staphylococcal isolates.

**Results:** Among 91 *S. aureus* strains, the presence of *mecA* gene was detected as 9.9%. This ratio was 17.9% (27 of 151) among the coagulase-negative staphylococci. A significant difference was found between coagulase-negative staphylococci and *S. aureus* isolates regarding the presence of *mecA* gene ($P < 0.001$). As for the genes of the slime, *icaA/icaD* genes were detected in 198 of 242 (81.8%) strains. The occurrence of slime genes was 91.2% and 89.4% among the *S. aureus* coagulase and negative staphylococci, respectively ($P > 0.05$). There was a statistically significant difference between the frequency of the *mecA* and slime genes when compared with the healthy control group and the football players ($P < 0.01$). Of 91 isolates, 22 were found to be methicillin resistant by the oxacillin disc diffusion method, whereas the remaining (220) were methicillin susceptible. Methicillin resistance was detected as 14.9% by the polymerase chain reaction method, whereas it was found as 9.1% by phenotypic methods.

**Conclusions:** Early and accurate diagnosis of virulent staphylococcal strains is crucial because the virulent coagulase-negative and coagulase-positive staphylococcal strains in the nasal floras of footballers may be major potential sources of superficial and deep tissue infections.

**Key Indexing Terms:** Footballer; Paton-Valentine leukocidin; *mecA*; Slime; Staphylococci. **[Am J Med Sci 2016;351(3):279–285.]**

## INTRODUCTION

Sports injuries are common in physical contact sports such as football. Skin and soft tissue infections are common among the sports injuries. These infections may be bacterial, viral or fungal in nature. Some bacteria (especially *Staphylococcus aureus*) on the surface of the skin are the most common cause of skin and soft tissue infections.[1,2]

Bacterial skin infections in high–physical contact sports, such as football, can be a serious concern for the health of athletes. Staphylococci are the most frequent causes of skin infection. Any open lesion, like a scratch or abrasion that can occur during sports injuries may turn into a source of serious staphylococcal infections for football players. When there is a crack, abrasion or incision in the skin, some bacteria that may normally be found in the nose and on the skin can enter the body, causing infection. Superficial skin infections can be very important because these kinds of bacterial infections may lead to more serious systemic infections such as deep tissue infections. *S. aureus* is one of the most dangerous human pathogens among the staphylococci in terms of pathogenicity. Various virulence factors of *S. aureus* are known to play an important role in the bacterial pathogenesis.[3,4]

Generally, *S. aureus* colonizes the host tissue surface and then releases bacterial toxins into the bloodstream; which can cause a range of illnesses such as sepsis, endocarditis, pneumonia, enteritis, osteomyelitis, abscess, impetigo and cellulitis. So far, several studies have been conducted about infections caused by *S. aureus* in athletes. In a study conducted by Stacey et al,[5] in a rugby football team, it was determined that *S. aureus* nasal carriage was identified as causes of *S. aureus* infection. In another study, carried out in a university wrestling team, infective endocarditis caused by *S. aureus* was identified in a 21-year-old collegiate wrestler.[6]

Methicillin resistance, slime production Panton-Valentine Leukocidin (PVL) genes are considered to be major virulence factors of *S. aureus*. It is a significant virulence factor associated with skin and soft tissue infections and necrotizing pneumonia. PVL is an exotoxin which kills leukocytes by creating pores in the leukocyte cell membrane.[7] Besides this, the development of methicillin resistance in staphylococcal strains is quite important. Methicillin resistance causes significant morbidity and mortality, because it makes the infections caused by methicillin-resistant *Staphylococcus aureus*

(MRSA) very difficult to treat.[3,4] Furthermore, slime production in staphylococci has been reported to play an important role in developing antibiotic resistance and that slime producing strains are more resistant to antimicrobials. Also, slime production plays an important role in bacterial invasion.[8]

This study investigates the rate of PVL-producing *S. aureus*, the frequency of methicillin resistance gene (*mecA*) responsible for methicillin resistance and the presence of slime genes (*icaA* and *icaD*) responsible for biofilm production in staphylococcal strains isolated from nasal cavities of footballers.

## MATERIALS AND METHODS

This study was carried out in the Department of Microbiology and Clinical Microbiology, Medical Faculty of Mustafa Kemal University, Hatay. A total of 242 footballers were included in the study. The group was included of 242 male athletes (age range: 18-38 year; mean age: 25.9 ± 8.4 year). The control group was included of 114 healthy male volunteers who were selected randomly (age range: 18–37 years; mean age: 27.1 ± 5.2 years) (Table 1). Informed consent was obtained from all players and volunteers. Furthermore, the study protocol was approved by the local ethics committee.

The control group consisted of 114 healthy individuals and none of them was diabetic patients. The control group, matched according to their gender and age, were recruited from the same regions. There was no history of antibiotic use in either the control group or the study group. The subjects have no history of skin or other infections. Also, individuals who had pets were not included in the study for both patient and control groups. None of the healthy subjects and athletes was intravenous drug users. None of the athletes and healthy subjects had a recent history of hospitalization. Furthermore, smoking rates were similar among the athletes (15.7%) and the healthy control groups (17.5%).

## NASAL SWAB COLLECTION

Subjects who had received antibiotics within the last week were excluded from the study. Nasal swab samples were taken from each subject using 2 sterile cotton swabs. The samples were obtained by rotating the swabs gently for (2-5) clockwise and counterclockwise turn in each nostril of the nose. At least 2 nasal swab samples were taken from the subjects. Because nasal *S. aureus* carriage is defined as at least 2 consecutive *S. aureus* isolates in 1 week.

Nasal swab samples obtained from subjects were transported to the laboratory using Stuart transport media. They were brought immediately to the microbiology laboratory for the bacterial evaluations. And then, the samples were inoculated onto 5% sheep blood agar plates (Difco, USA). The plates were incubated at 37°C for 48 hours. All staphylococcal isolates were identified through conventional microbiological techniques.[9]

## OXACILLIN DISC DIFFUSION TEST

Oxacillin disc susceptibility testing was performed on all staphylococcal isolates according to Clinical and Laboratory Standards Institute recommendations using oxacillin disc (μg) (Oxoid, UK). An oxacillin disc was incubated on Mueller-Hinton agar (Oxoid, UK) plates without NaCl supplementation. Subsequently, the plates were incubated for 24 hours at 35°C. The diameter of the zone was interpreted according to the Clinical and Laboratory Standards Institute criteria.[10] *S. aureus* American Type Culture Collection 29213 and *S. aureus* American Type Culture Collection (43300) were chosen as the negative and positive control strains, respectively.

## PCR AMPLIFICATION

Deoxyribonucleic acid (DNA) extraction and DNA amplification procedures for *mecA* and *icaA/icaD* genes were performed as in previous study.[11,12] This study had primer sequences for *mecA* and *icaA/icaD* genes

**TABLE 1.** Primer sequences and predicted sizes used in the multiplex PCRs for *icaA*, *icaD, mecA*, *coa* and *luk PVL*.

| Gene | Primer | Oligonucleotide sequence (5′-3′) | Size of amplified product (bp) | References |
|---|---|---|---|---|
| *icaA* | *icaA-1*<br>*icaA-2* | 5′-CGA GAC CAA GAT TCA ATA AG-3′<br>5′-AAA GAA AAC CAC TCA CAT CAGT-3′ | 1315 | Duran et al[11] and Vasudevan et al[12] |
| *icaD* | *icaD-1*<br>*icaD-2* | 5′-ATCATTAGGTAAAATGTCTGCACATGATCCA-3′<br>5′-GCATCAASTGTATTGGATAGCCAAAAGC-3′ | 381 | Duran et al[11] and Vasudevan et al[12] |
| *mecA* | *mecA-1*<br>*mecA-2* | ACTGCTATCCACCCTCAAAC<br>CTGGTGAAGTTGTAATCTGG | 163 | Duran et al[11] and Vasudevan et al[12] |
| *coa* | *coa* 1<br>*coa* 2 | 5′-CGA GAC CAA GAT TCA ATA AG-3′<br>5′-AAA GAA AAC CAC TCA CAT CAGT-3′ | 900 | Lari et al[13] |
| *luk PVL* | *luk PVL*-1<br>*luk PVL*-2 | 5′-ATCATTAGGTAAAATGTCTGCACATGATCCA-3′<br>5′-GCATCAASTGTATTGGATAGCCAAAAGC-3′ | 433 | Lari et al[13] |

**TABLE 2.** Distribution of nasal staphylococci among footballers and healthy control group.

| Microorganims | Footballers | | Control group | |
|---|---|---|---|---|
| | Number of isolates | Percentage | Number of isolates | Percentage |
| *S. aureus* | 91 | 37.6 | 19 | 16.7 |
| MRSA | 9 | 9.9 | 0 | 0 |
| MSSA | 82 | 90.1 | 19 | 100 |
| CoNS | 151 | 62.4 | 95 | 83.3 |
| MRCoNS | 27 | 17.9 | 2 | 2.1 |
| MSCoNS | 124 | 82.1 | 93 | 97.9 |

CoNS, coagulase-negative staphylococci; MR, methicillin resistant; MS, methicillin sensitive; MSSA, methicillin-sensitive *S. aureus*.

designated in accordance with the study of Duran et al[11] (Table 1).[12]

The oligonucleotide primers for the *PVL* gene were selected from the research of Lari et al.[13] Also, the *coa* gene primers were selected as the internal control primers for *S. aureus* isolates (Table 1).

The polymerase chain reaction (PCR) amplification was performed in a 25 μL final volume. The PCR was performed under the following parameters: the reaction mixture consisted of 2.5 μL of ×10 reaction buffer without $MgCl_2$ (Promega Corp); 200 μM of each deoxynucleoside triphospate (AB Gene, UK), 1.5 μL (10 mM) $MgCl_2$; 0.5 μM of primers for *coa* and *luk PVL* and approximately 10 ng of template DNA, and brought up to a 25 μL final volume with distilled water. Reactions were hot started for 5 minutes at 94°C and placed on ice, and 1 U of Taq polymerase (Fermentas, USA) was added. Reaction mixtures were subjected to 35 PCR cycles (30 seconds at 94°C, 30 seconds at 55°C and 45 seconds at 72°C). A final elongation step at 72°C for 10 minutes was also applied in a thermal cycler (Bioder/Thermal Blocks xp cycler, Tokyo Japan).

After the amplification of the *mecA*, *icaA/icaD* and *coa* and *luk PV* genes, 10 μL volumes of PCR products were mixed with 3 μL of loading buffer (10%, w/v, ficoll 400; 10 mmol/L Tris-HCl, pH 7.5; 50 mmol/L ethylenediaminetetraacetic acid; 0.25% bromophenol blue). The products were analyzed in a 2% (w/v) agarose gel in 1× TAE buffer (40 mmol/L Tris-acetate, 1 mmol/L ethylenediaminetetraacetic acid). Ethidium bromide (0.5 μg/mL TAE)-stained DNA amplicons were visualized using a gel imaging system (Wealtec, Dolphin-View, USA).

## STATISTICAL ANALYSIS

Statistical evaluations of all data were done using a chi-square test. Statistical analyses were performed by the Statistical Package for Social Sciences (SPSS for Windows V. 18.0, Chicago, USA) for Windows software.

## RESULTS

The *coa* gene was detected in staphylococcal strains isolated from 91 athletes. So, *S. aureus* strains were isolated in 91 of 242 (37.6%) footballers and 151 (62.4%) of them were coagulase-negative staphylococci (Table 2). When *Staphylococcus epidermidis* and *S. aureus* were grown in coculture, *S. epidermidis* growth was ignored. Only *S. aureus* isolates were included in this study. Among 91 *S. aureus* strains, the presence of *mecA* gene was detected as 9.9%. This ratio was 17.9% (27 of 151) among the coagulase-negative staphylococci. A significant difference was found between coagulase-negative staphylococci and *S. aureus* isolates in terms of the presence of *mecA* gene ($P < 0.001$) (Figure 3).

The *icaA* and *icaD* were found in 198 of 242 (81.8%) strains. The occurrence of slime genes was 91.2% (83 of 91) and 89.4% (135/151) among the *S. aureus* and coagulase-negative staphylococci, respectively (Figure 1). No significant difference was found between *S. epidermidis* and *S. aureus* isolates in terms of the presence of slime genes ($P > 0.05$) (Figure 4).

Among the samples taken from athletes, 91 (37.6%) *S. aureus* isolates were identified. Among these *S. aureus* isolates, 22 (24.2%) were *PVL* gene-containing isolates (Figure 2). *S. aureus* nasal carriage (the *coa* gene positivity rate) was detected in 19 of 114 (16.7%) subjects in the control group, in which, whereas methicillin resistance was not detected in any *S. aureus* strains and 2 (2.1%) of 95 coagulase-negative staphylococcal strains were found to be methicillin resistant. In the control group, it was found that only 1 isolate revealed the presence of *PVL* gene among the *S. aureus* strains. The occurrence of *icaA/icaD* was found as positive in 13 of 19 (68.4%) *S. aureus* strains and its ratio was 72.6% (69 of 95 strains) coagulase-negative staphylococci ($P > 0.05$).

The athletes and the control group subjects were compared in terms of *S. aureus* nasal carriage and methicillin resistance. Nasal carriage and methicillin resistance in football players were found to be statistically and significantly higher than the control group subjects ($P < 0.001$). Similarly, in these 2 groups, there was a statistically significant difference in terms of *PVL* and slime genes ($P < 0.001$).

## DISCUSSION

*S. aureus* is an opportunistic pathogen, which causes serious infections. *S. aureus* is the most frequently isolated

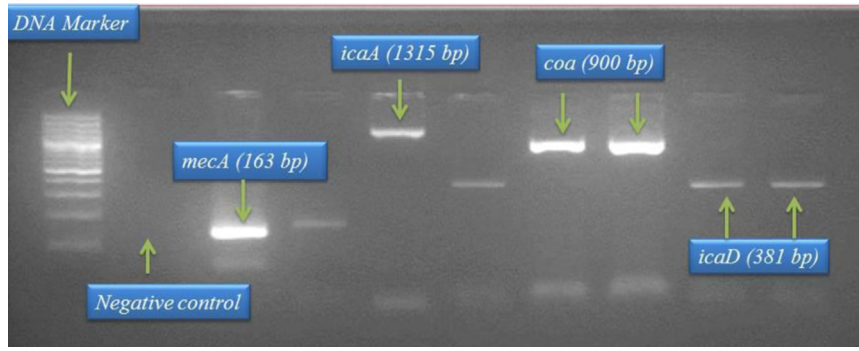



**FIGURE 1.** The PCR products by agarose gel electrophoresis for the *mecA* (163 bp), *coa* (900 bp), *icaA* (1315 bp) and *icaD* (381 bp). DNA molecular size marker (100 bp ladder).

pathogen that causes disease in humans. It is mainly transmitted among people through close contact[14-16] and the main cause of skin and soft tissue infections such as abscesses or cellulitis because this microorganism usually colonizes on the host tissue. Bacterium can easily colonize on open skin wounds after injury. When there is a cut in the skin during a sport competition, *S. aureus* in the nose and on the skin can enter the body and grow there. This eventually leads to a skin infection. Therefore, it is important to know the prevalence of *S. aureus* nasal carriage in athletes.[3,17,18]

During long training periods, football players may need to share items and areas such as towels, bathrooms, razors and personal belongings such as clothing or equipment, putting them at a greater risk for serious infections. Generally, football players live in crowded or shared living quarters with strangers during the football season. They are at greater risk for skin and soft tissue infections because of the fact that football is a game of requiring the athletes to be very close to each other and touch is common. One of the most common causes of these infections is *S. aureus. S. aureus* especially MRSA, is a microorganism that can be easily transmitted through contact and is the most common way for the transmission of this infection to make its way from 1 footballer to another. To date, the true incidence of community-associated methicillin-resistant *S. aureus* (CA-MRSA) in Turkey, as in many countries around the world, among people who share communal living areas is unknown. The outbreaks of community-acquired MRSA infection have been described in various groups with direct physical contact such as football players. In various studies, CA-MRSA has been reported to have caused outbreaks among college and professional teams.[19,20]

The resistance to various antimicrobial agents especially methicillin has become increasingly important with each passing day. Recently, community-acquired MRSA infections have been reported to be on the increase with CA-MRSA infections becoming more common in athletes.[21] The first CA-MRSA case was reported in a wrestling team in 1993.[22]

The main resistance mechanism to beta-lactam antibiotics in staphylococci is related with the carrying of the *mecA* gene.[23] Strains carrying *mecA* gene in the chromosome exhibit intrinsic resistance to beta-lactam class of antibiotics. Although *S. aureus* nasal carriage was found as 37.6% in this study, this rate of *mecA* gene positivity (methicillin resistance) was detected as 14.9% in all staphylococcal strains.

A study conducted by William et al in 2004, aimed to determine the number of athletes carrying *S. aureus* before and after a session of indoor training. According to their findings, it has been reported that *S. aureus* nasal carriage had increased in the nose after training in football players.[24] Our findings showed that high carriage of *S. aureus* might be a serious contaminant factor for football players. We think that this high rate of nasal

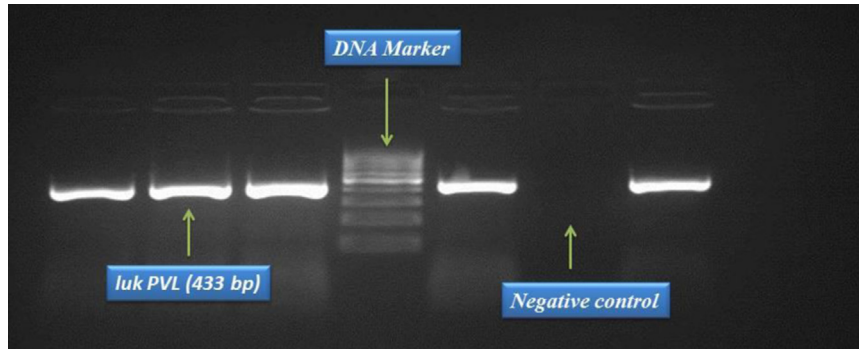
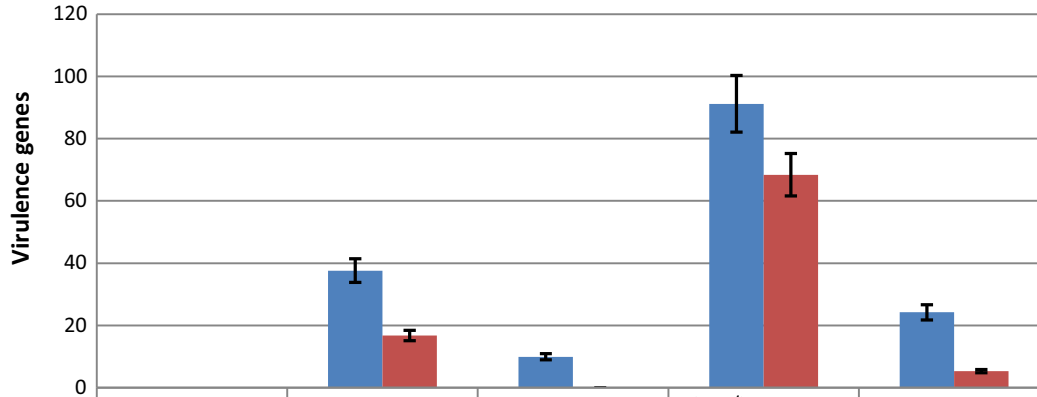


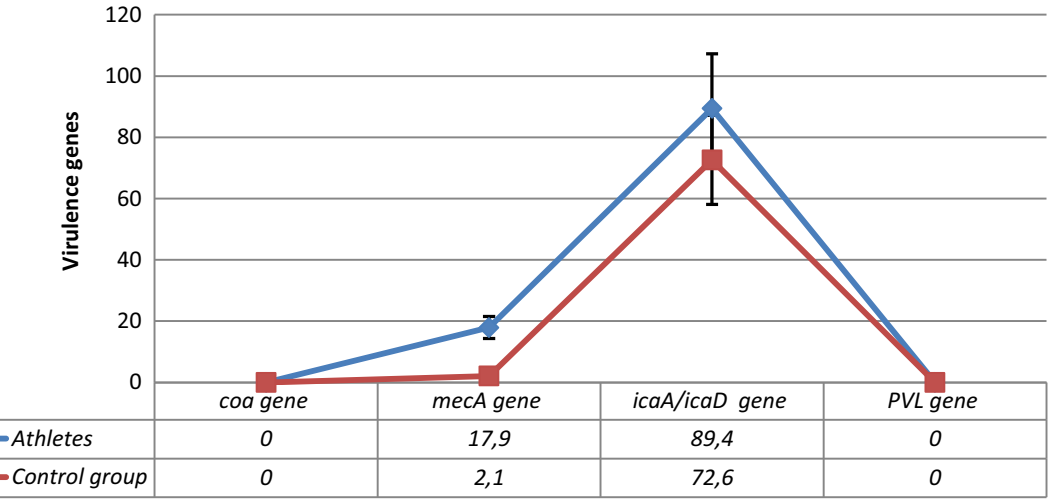

**FIGURE 2.** The PCR products by agarose gel electrophoresis for the *luk PVL* (433 bp. DNA molecular size marker (100 bp ladder).




| Virulence genes | Athletes, n=242 *S.aureus*, n=91 | Control group, n=114 *S.aureus*, n=19 | *p values* |
|---|---|---|---|
| *coa* gene | 37.6% (91/242) | 16.7% (19/114) | $p<.01$ |
| *mecA* gene | 9.9%. (9/91) | 0 | $p<.05$ |
| *icaA/icaD* genes | 91.2% (83/91) | 68.4% (13/19) | $p>.05$ |
| *PVL* gene | 24.2% (22/91) | 5.3% (1/19) | $p<.01$ |

*CoNS: Coagulase negative staphylococci, coa: coagulase, icaA/icaD gene: Slime genes, PVL: Panton-Valentine Leukocidin.*

**FIGURE 3.** Virulence genes distributions in *S. aureus* isolated from athletes and control group.




| Virulence genes | Athletes, n=242 ***CoNS***, n=151 | Control group, n=114 ***CoNS***, n=95 | *p value* |
|---|---|---|---|
| *coa* gene | *ND | ND | - |
| *mecA* gene | 17.9% (27/151) | 2.1% (2/95) | $p<.05$ |
| *icaA/icaD* genes | 89.4% (135/151) | 72.6% (69/95) | $p>.05$ |
| *PVL gene* | ND | ND | - |

**ND: Not determined.*

*CoNS: Coagulase negative staphylococci, coa: coagulase, icaA/icaD gene: Slime genes, PVL: Panton-Valentine Leukocidin.*

**FIGURE 4.** Virulence genes distributions in *CoNS* isolated from athletes and control group. CoNS, coagulase-negative staphylococci.

carriage in football players can be an important source of infection for themselves.

A study conducted in 2010 by Creech et al was aimed at determining the frequency and clinical importance of MRSA in collegiate athletes. In that study, *S. aureus* nasal carriage was found to have a high ratio in athletes. Moreover, the methicillin-resistant *S. aureus* nasal colonization rate was found to vary significantly through the athletic season (4–23%), peaking during times of highest athletic activity.[25] In our study, MRSA carriage was significantly higher in football players, too. Our findings were consistent with study results of Creech et al.

*S. aureus* can be easily transmitted from person to person by direct contact with infected skin. *S. aureus* transmission can also be transmitted during indirect contact via environmental surfaces such as towels, razors and benches. Direct-contact athletes such as football players are at a serious risk of acquiring *S. aureus* infections.[3] In a study Bowers et al conducted in 2008; the methicillin-resistant *S. aureus* infections were investigated in collegiate football players. It was determined that methicillin-resistant *S. aureus* was found in 6.7% of the 491 collegiate football players. In that study, MRSA infection was found to be very common in football players.[3] In our study, MRSA carriage was found significantly higher than the reported rates of Bowers et al. We believe that it can be because of lack of attention to personal hygiene and because of the low level hygienic tools and athletic equipment.

Community-acquired MRSA has been reported to be causing outbreaks among healthy sport participants. Outbreaks of CA-MRSA have been reported to occur among athletes, especially those in contact sports such as football. In a study carried out among a collegiate football team in 2006, complicated skin and soft tissue infections were diagnosed in players. It was reported that outbreaks of CA-MRSA in sports teams can lead to very serious consequences.[26] Frequency of MRSA in our study was found to be quite high. This high rate of MRSA carriage can be a significant risk for the health of athletes. Even, high MRSA carriage among athletes can lead to serious outbreaks.

A high rate of the carriage of *S. aureus* in the present study has been found (37.6%). In the study, the existence of *mecA* gene in *S. aureus* and coagulase-negative staphylococci has been determined as 9.9% and 17.9%, respectively. In total, *mecA* gene positivity was 14.9% in all staphylococcal isolates. A significant difference was found between coagulase-negative staphylococci and *S. aureus* isolates in terms of the presence of *mecA* gene ($P < 0.001$). The results of resistance to methicillin were obviously higher than those reported previously by most of the studies.[3,25]

The pathogenicity of this microorganism which is the major cause of morbidity and mortality in humans has been attributed to various virulence factors. Slime production and *PVL* locus encoding for *PVL* toxin in *S. aureus* are being recognized among the most important virulence factors.[11,27]

The prevalence of nasal carriage of MRSA among both amateur and professional football teams in Turkey has not been determined to date. To the authors' knowledge, this study is the first in Turkey to evaluate the nasal carriage and the various virulence factors such as methicilline resistance, slime and *PVL* gene in *S. aureus* isolates among football players.

No previous study has investigated the rate of nasal *S. aureus* carriage among football players in Turkey. Although various studies have been conducted on this subject throughout the world, no study about the presence of virulence factors such as *PVL* in nasal *S. aureus* isolates has been found.

*S. aureus* infections especially methicillin-resistant *S. aureus* infections have been showing up in athletes such as football players. Players' physical contact shared facilities and equipment, and poor hygienic conditions of athletes are among the primary risk factors for *S. aureus* transmission.[28]

In a study carried out Lear et al, a total of 190 athletes from 6 local high school football teams were included in their prospective observational study to determine nasal colonization rate. In that study, 23.2% (44/190) of the athletes included in the study had been found to be nasal *S. aureus* carriers.[14]

Generally, community-acquired *S. aureus* strains have been reported to be *PVL*-positive strains.[29] In the present study, a total of 242 nasal swab samples were taken from amateur football players, and 91 (37.6%) athletes were determined as nasal carriers of *S. aureus*. The rates of *PVL* genes among the studied *S. aureus* isolates in the patient and control group were 24.2% (22/91) and 5.3% (1/19), respectively. The *PVL* positivity rates were found to be considerably higher in community-acquired *S. aureus* strains isolated from football players.

Furthermore, 1 of the most common virulence factors of staphylococci is slime or biofilm production. Slime layer protects bacteria from phagocytosis and degranulation. It also impedes chemotaxis, lymphocyte activity opsonophagocytosis and reduces neutrophil cell phagocytosis. Slime production is reported to contribute to enhanced infectivity of staphylococcal infections. Slime production is also stated to play a major role in the development of antibiotic resistance.[8,30-33]

In this study, the presence of slime genes (*icaA*/*icaD*) in both coagulase-negative 89.4% (135/151) and positive strains 91.2% (83/91) among the football players was significantly higher than those of the control group ($P < 0.001$). These results were consistent with the previous study carried out with staphylococci isolated from nasal samples of patients with orthopedic implants in Turkey.[11] In a research carried out in 2002, the presence of *icaA* and *icaD* genes was investigated in staphylococci. In that study, the incidence of *icaA* and *icaD* genes was found in 57.5% of 113 *S. epidermidis* strains isolated from biomaterial-associated infections.[34]

In our study, the presence of slime gene rates found to be higher than those reported previously by most of the studies.[32,34] We think that this may be because of inadequate sanitary conditions and poor hygiene applications in team sports.

## CONCLUSIONS

Early and accurate diagnosis of virulent staphylococcal strains is crucial because the virulent coagulase-negative and coagulase-positive staphylococcal strains in the nasal floras of football players may be major potential sources of superficial and deep tissue infections.

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