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* *icaA-2*	5′-CGA GAC CAA GAT TCA ATA AG-3′ 5′-AAA GAA AAC CAC TCA CAT CAGT-3′	1315	Duran et al[11] and Vasudevan et al[12]
icaD	*icaD-1* *icaD-2*	5′-ATCATTAGGTAAAATGTCTGCACATGATCCA-3′ 5′-GCATCAASTGTATTGGATAGCCAAAAGC-3′	381	Duran et al[11] and Vasudevan et al[12]
mecA	*mecA-1* *mecA-2*	ACTGCTATCCACCCTCAAAC CTGGTGAAGTTGTAATCTGG	163	Duran et al[11] and Vasudevan et al[12]
coa	*coa* 1 *coa* 2	5′-CGA GAC CAA GAT TCA ATA AG-3′ 5′-AAA GAA AAC CAC TCA CAT CAGT-3′	900	Lari et al[13]
luk PVL	*luk PVL*-1 *luk PVL*-2	5′-ATCATTAGGTAAAATGTCTGCACATGATCCA-3′ 5′-GCATCAASTGTATTGGATAGCCAAAAGC-3′	433	Lari et al[13]