TÜBİTAK

Original Article

Turk J Med Sci
2011; 41 (3): 543-547
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1005-837

An investigation of genes coding fibronectin binding proteins in *Staphylococcus aureus* isolated from carriers aged between 6 and 14 years*

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Aim: Nasal carriage rates for *Staphylococcus aureus* have been reported to vary between 18% and 50% in different populations and it represents a risk factor for invasive infections. The aim of the present study was to investigate the presence of genes associated with fibronectin binding proteins mediating the adhesion of *S. aureus* to human epithelial cells.

Materials and methods: Fifty strains isolated from nasal swab specimens of children were included. Specimens were inoculated on mannitol salt agar plates and after 24-48 h of incubation at 37 °C the isolates were identified according to their biochemical properties. The presence of fnbA and fnbB genes encoding fibronectin binding protein A and B was investigated via PCR. *S. aureus* NCTC8325 was used as the reference strain.

Results: All isolates were identified as *S. aureus* according to their cultural properties and the positive tube coagulase test results. Of the 50 *S. aureus* strains, 14 (28%) were positive for fnbA and 5 (10%) for fnbB.

Conclusion: The presence of fnbA and fnbB in our study population was lower than in previous studies performed in nasal carriers. We concluded that this difference might have resulted from the lower age of the study population and geographical diversities.

Key words: *Staphylococcus aureus*, carriers, virulence factors, *fnbA, fnbB*

Altı ondört yaş arasındaki taşıyıcılardan soyutlanan *Staphylococcus aureus* kökenlerinde fibronectin bağlayan proteinleri kodlayan genlerin araştırılması

Amaç: *Staphylococcus aureus*'un burun taşıyıcılığı değişik topluluklarda % 18 ile % 50 oranında bildirilmektedir ve bu durum invazif infeksiyonlar için risk oluşturmaktadır. Bu çalışmanın amacı, *S. aureus*'un insan epiteline bağlanmasını sağlayan fibronectin bağlayan proteinlerle ilişkili genleri araştırmaktı.

Yöntem ve gereç: Çocukların burun sürüntülerinden üreyen 50 köken çalışmaya alındı. Örnekler mannitol salt agara ekildi ve 37 °C'de 24-48 saatlik inkübasyondan sonra kökenler biyokimyasal özelliklerine göre tanımlandı. Fibronectin bağlayan protein A ve B'yi kodlayan fnbA ve fnbB genleri PCR yöntemiyle araştırıldı. Kontrol kökeni olarak *S. aureus* NCTC8325 kullanıldı.

Bulgular: Bütün kökenler kültür özellikleri ve tüp koagülaz testinin pozitifliği ile *S. aureus* olarak tanımlandı. Elli *S. aureus* kökeninin 14'ünde (% 28) fnbA ve 5'inde (% 10) fnbB geni saptandı.

Sonuç: Çalışma grubumuzda fnbA ve fnbB saptanma oranı, burun taşıyıcılarında yapılmış önceki çalışmalardan düşüktür. Bu düşük oran konusunda çalışma grubundaki taşıyıcıların yaşlarının küçük olmasının yanında coğrafik değişikliklerin de etkili olabileceği yorumu yapılabilir.

Anahtar sözcükler: *Staphylococcus aureus*, taşıyıcı, virülans faktörleri, *fnbA, fnb*B

Received: 24.05.2010 – Accepted: 01.09.2010

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* The study was supported by the TÜBİTAK (The Scientific and Technological Research Council of Turkey) project numbered 107S434 (SBAG HD 270) and some of the data were presented at the 19th European Congress of Clinical Microbiology and Infectious Diseases.

Introduction

Staphylococcus aureus is a pathogen capable of causing a variety of infections, most prominently soft tissue and bone infections, and is also known to cause carriage (1,2). Its primary site of carriage is the skin and it is mostly observed in the nose, axillary region, and the rectum. Nasal carriage rates in adults have been reported to vary between 18% and 50% and it has also been stated that the carriage is persistent in about 20% to 35% and intermittent in about 30% to 70% of the affected individuals (1). In another study that we performed on an outpatient population in our hospital, nasal carriage rates of *S. aureus* among children and adults were 19.1% and 6.5%, respectively (3). Nasal *S. aureus* carriage may particularly constitute a risk factor for invasive infections, particularly in populations such as hemodialysis patients and immunosuppressive patients (1,2). If these strains possess the virulence factors required for invasive infections, they can cause infections in the carriers and the individuals in their social surroundings (4). The relation between nasal carriage and infection was first shown in 1931 by Danbolt, who conducted studies on furuncles (5). Eighty percent of skin infections such as furuncle and impetigo are seen in nasal carriers. Most of the studies on the mechanism and possible risks of nasal carriage have been conducted in western countries and it has been stated that the data obtained in those studies are far from reflecting the worldwide clinical picture.

The most important stage in the occurrence of colonization and infection is the binding of the bacterium to the cells and the extracellular matrix. The staphylococcal surface adhesins named as "microbial surface components recognizing adhesive matrix molecules" (MSCRAMMs) enable bacteria to bind to the fibronectin, fibrinogen, and collagen of the host. In staphylococci, binding to proteins such as fibrinogen, elastin, and fibronectin is mediated by adhesins, which are named FNBPA and FNBPB and are under the control of *fnbA* and *fnbB* genes (1,6). Fibronectin binding proteins (FNBP) and collagen binding proteins are involved in the epithelial binding (2,7,8). Fibronectin binding proteins were investigated in both methicillin susceptible and resistant *S. aureus* strains isolated from patients with staphylococcal infections such as osteomyelitis and skin and soft tissue infections. Presence of the *fnbA* gene was reported as approximately 100% and for the *fnbB* gene this proportion was reported to be between 0% and 98% in clinical isolates (6,8,9). The aim of the present study was to investigate the genes that regulate the production of the fibronectin binding proteins in the *S. aureus* strains isolated from healthy volunteers.

Methods

Subjects:

For the detection of carriage, 1397 students were chosen by simple randomized sampling from the schools located in our town. Information about the study and procedure was given to the student's parents and permission was obtained from the Ministry of National Education. Ethical approval was obtained from the Ethical Committee of Dokuz Eylül University School of Medicine.

Data on age, gender, underlying diseases, hospitalization history, immunosuppressive disorders such as HIV infection, and antibiotic treatment in the previous 6 months were recorded and subjects with a history of hospitalization or antibiotic treatment or those suspected of having upper respiratory tract and staphylococcal skin and soft tissue infection were not included in the study.

Isolation and identification of bacteria

After informed consent was obtained from the families of the volunteers aged between 6 and 14 years, swab specimens were taken from the nose and axillary region. Samples were cultured in Mannitol-Salt Agar media (Salubris, İstanbul, Turkey) and incubated at 35 °C for 1 night. Preparations were obtained from the colonies that turned yellow on the media and Gram staining was performed. Catalase and coagulase tests were performed on the bacteria, which appeared as gram-positive cocci, and those positive were named *Staphylococcus aureus*. The strains obtained were stored in a deep-freeze at -20 °C until the molecular investigation.

Polymerase Chain Reaction (PCR)

The bacteria stored in the deep-freeze were subcultured on 5% sheep blood agar (Difco Laboratories Sparks, MO, USA). A concentrated bacteria suspension was prepared in 0.5 mL of distilled water using the staphylococcus colonies grown as pure culture. Phenol/chloroform extraction was used for DNA isolation (10,11).

Fifty *S. aureus* strains isolated from child carriers were initially tested for presence of the *sau* gene, an *S. aureus* specific sequence (12). Subsequently, the *fnbA* and *fnbB* genes were investigated to detect FNBPs. Two microliters were removed from the isolated DNA sample and a 25 µL reaction mixture was prepared. The reaction mixture consisted of primers (*sau, fnb A,* or *fnb B*), dNTP, Taq polymerase, and buffer solution. X174 Hinf I DNA marker (Promega) and PCR Marker, 50-2000 bp (Sigma) were used as DNA markers. The sample was duplicated using a thermal cycler. *S. aureus* NCTC 8325 (positive control) and *S. aureus* ATCC 25923 (negative control) were used as reference strains.

Details about the primers and PCR programs used in the study are presented in the Table.

By using agarose (BIO-RAD, 161-3101), 2% agarose gel solution was prepared in 1 × TBE buffer. Then 2 µL of loading buffer was mixed with 10 µL of PCR product and the concentration was loaded into the wells of the gel. After the electrophoresis tank was filled with 1 × TBE buffer, electrophoresis was performed at 100 to 120 V for 30 to 45 min and the results were obtained by the examination of the gel under ultraviolet light.

Results

Of the 1397 students 668 (47.8%) were female and 729 (52.2%) male. Mean age was 10.30 ± 2.27 years (6-14 years). *S. aureus* was determined in 346 (24.8%) students but none of them were resistant to oxacillin. For molecular investigation, 50 methicillin sensitive *S. aureus* strains isolated from 50 stable carrier children without risk factors for *S. aureus* carriage mentioned in the methods section were chosen.

The *sau* gene, which is a *S. aureus* specific sequence, was detected in all studied strains. *fnbA* and *fnbB* genes were detected in 14 (28%) and 5 (10%) of the strains, respectively. One strain (2%) was positive for both *fnbA* and *fnbB* while neither was present in 33 strains (66%). Both *fnbA* and *fnbB* were detected in the *S. aureus* NCTC 8325 strain, which was used as the reference strain. The gel images of the positive strains are presented in the Figure.

Discussion

Staphylococcus aureus is a pathogen capable of causing numerous community and hospital acquired infections and results in asymptomatic carriage on the skin and most commonly in the nostrils (4). As for community acquired infections, it is the leading pathogen in osteomyelitis and nongonococcal septic arthritis. Some 85% of the hematogenous osteomyelitis cases developing in the absence of penetrating wounds or surgical intervention are seen among children (7). Host tissue colonization is an important factor in staphylococcal infections.

Table. The primers and PCR programs used.

Primer	Oligonucleotide sequence (5'-3')	Product (bp)	PCR Cycle
sau[9]	AATCTTTGTCGGTACACGATATTCTTCACG CGTAATGAGATTTCAGTAGATAATACAACA	107	30 × (95 °C 30 s, 60 °C 30 s, 72 °C 60 s)
fnbA[1]	GCGGAGATCAAAGACAA CCATCTATAGCTGTGTGG	1279	30 × (95 °C 30 s, 50 °C 30 s, 72 °C 60 s)
fnbB[1]	GGAGAAGGAATTAAGGCG GCCGTCGCCTTGAGCGT	812	30 × (95 °C 30 s, 55 °C 30 s, 72 °C 60 s)

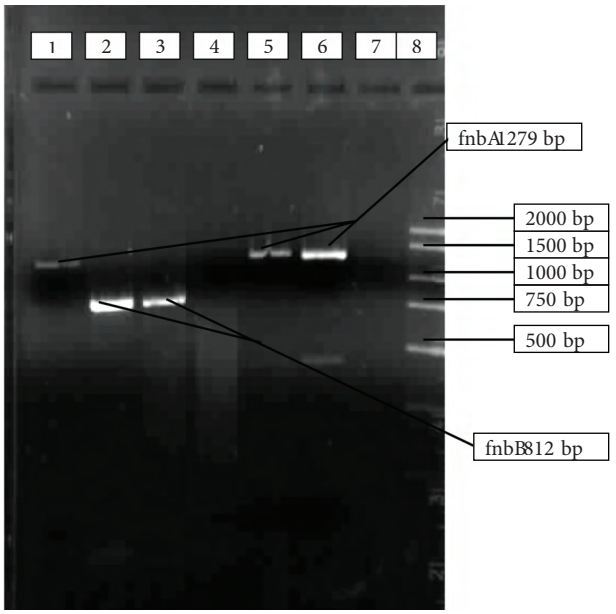

Figure. Gel images of the strains encoding fibronectin-binding proteins.

1. *S. aureus* NCTC 8325 (fnb A positive), 2. *S. aureus* NCTC 8325 (fnb B positive), 3. Study strain (fnb B positive), 4. fnb B negative control strain, 5. Study strain (fnb A positive), 6. Study strain (fnb A positive), 7. fnb A negative control strain, 8. Marker

Bacteria express MSCRAMMs, which promote colonization of host tissue and contribute to infection (6,13). The aim of the present study was to investigate the virulence factors considered to be associated with musculoskeletal and skin/soft tissue infections of the *S. aureus* strains, which cause nasal carriage in children.

In the present study, the rates of *fnbA* and *fnbB* genes in the 50 *S. aureus* strains isolated from child carriers were 28% and 10%, respectively. Only one strain (2%) was positive for both genes. In their study conducted on strains isolated from children with musculoskeletal infections, Martinez-Aguilar et al. determined the rates of *fnbA* and *fnbB* genes in methicillin-susceptible *S. aureus* and methicillin-resistant *S. aureus* strains as 64% and 90%, respectively (8). The factors responsible for the adhesion have been investigated in a small number of studies and the rates of *fnbA* and *fnbB* genes were reported as 100% and 40%, respectively, in a study including 16 healthy carriers (1). Although the rates determined in that study are much higher than those of the present study, the study's sample size was smaller than ours and the authors included 4 patients along with the healthy carriers and calculated the overall PCR results collectively. Moreover, the results of that study could not be accurately compared with those of ours since the age group of the patients was not noted by the authors. In a study conducted by Mishaan et al. in the USA, the authors studied community-acquired, methicillin-resistant *S. aureus* infections and showed that infections were caused by microorganisms belonging to 3 different clones. They detected *fnbA* in all strains while the rate of *fnbB* was 99%, 43%, and 0% in the 3 clones, respectively (14). One study reported the rates of *fnbA* and *fnbB* in *S. aureus* strains isolated as causative agents of orthopedic infections as 99% and 98%, respectively (14). In another study focusing on strains isolated from soft tissue infections, the rate of *fnbA* was reported as 76.1% (15).

As a result, in the present study, the genes encoding the virulence factors that play a role in the pathogeneses of community acquired musculoskeletal and skin/soft tissue infections were not detected at high levels in the *S. aureus* strains isolated from child carriers. There are a small number of similar studies involving strains isolated from carriers. On the other hand, the studies conducted on infection pathogens have reported discrepant rates of the genes investigated in the present study. The lower rates of fibronectin-binding proteins in our study than those previously reported in the literature may be associated with the fact that the microorganisms used in the study were not infection pathogens and with the young study group or the regional characteristics of the strains, which are commonly found in the general population. Our review of the English literature showed that, although there are many studies on the subject in the field of veterinary medicine, studies involving human subjects and investigating the virulence factors of strains isolated from carriers are few. This shows that serious studies on this subject are needed (16,17). To our knowledge, and according to our review of the literature, the present study is the

first to include such a large study group consisting wholly of asymptomatic child carriers. The results obtained in this study may constitute a basis for further studies on the virulence of strains in carriers. Moreover, further studies are needed to demonstrate to what extent the strains possessing virulence factors express their gene products in in vitro and in vivo settings.

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