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PCR investigation of Panton-Valentine leukocidin, enterotoxin, exfoliative toxin, and *agr* genes in *Staphylococcus aureus* strains isolated from psoriasis patients*

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Background/aim: *Staphylococcus aureus* colonization is a determiner of disease activation in psoriasis patients. Here we evaluate the presence of genes encoding Panton-Valentine leukocidin (PVL), enterotoxins, TSST-1, exfoliative toxins, and the accessory gene regulatory locus by polymerase chain reaction (PCR) in *S. aureus* isolates obtained from healthy and diseased skin regions and anterior nares of psoriasis patients and healthy controls.

Materials and methods: The presence of PVL and toxin genes was investigated, and *agr* typing was performed by PCR.

Results: Eighteen of the isolated strains carried the *sei*, 1 carried the *seb-sec*, and 1 carried the *seg* enterotoxin gene. Eight of the strains carrying enterotoxin genes were isolated from nasal swabs, 6 from diseased skin swabs, and 4 from healthy skin swabs. None of the strains isolated from the control group carried the *agr* locus. On the other hand, 11 of the *S. aureus* strains isolated from the patients carried type 1, 7 carried type 1 + 3, 4 carried type 2, 4 carried type 3, and 1 carried type 1 + 2 *agr* loci.

Conclusion: Enterotoxin production and the carried accessory gene regulatory locus may be important in the aggravation of psoriasis.

Key words: Polymerase chain reaction, *Staphylococcus aureus*, toxin genes, *agr* typing

1. Introduction

Psoriasis is a chronic inflammatory disorder involving the skin. Genetic, environmental, and immunological factors play important roles in the development of the disease. Its incidence may change in different parts of the world depending on environmental, ethnic, and geographic differences. Both sexes are affected equally by the disease, and patients are usually diagnosed between 15 and 30 years of age. Although it can be seen in almost all races, it is rare in Asia and Africa. It has an overall prevalence of 2%–3% in the general population (1,2). Its prevalence in children is reported to range from 0% (Taiwan) to 2.1% (Italy), and in adults from 0.91% (United States) to 8.5% (Norway). In the United States, the annual incidence estimate in children is 40.8/100,000, while in adults the annual incidence varies from 78.9/100,000 (United States) to 230/100,000 (Italy) (1). The prevalence in the Turkish population is reported as 1.3% (2).

Bacteria are known to play an important role in the development and chronicity of chronic inflammatory diseases such as atopic dermatitis and psoriasis. The relationship between bacterial colonization or infection of the skin and the development of inflammatory skin diseases is well described in studies reporting the relapse of guttate psoriasis following streptococcal pharyngitis and the development of atopic dermatitis following *Staphylococcus aureus* colonization of the skin (3,4).

S. aureus colonizes the anterior nares of 20%–40% of the healthy adult population. It can also colonize the perineum, perianal region, axilla, gastrointestinal tract, and skin folds. Trauma, burns, diabetes, and immune suppression can lead to opportunistic infections with this colonizing pathogen (5).

S. aureus can be the cause of a wide spectrum of infectious diseases due to its many virulence factors, which facilitate its spread in host tissues and its escape

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from the host's immune responses. Bacterial enzymes and toxins, such as capsular polysaccharide, plasma coagulase, extracellular matrix components, protein A, fibronectin binding factor, microbial surface component recognizing adhesive matrix molecules, and extracellular proteins (i.e. toxins), play important roles in the development and occurrence of staphylococcal infections (6,7) . Adherence factors (adhesins) promote the colonization of *S. aureus* on host cell surfaces (8–11).

S. aureus produces exotoxins with cytolytic activities. These cytolytic toxins (α-, β-, and γ-hemolysins, leukocidin, and Panton-Valentine leukocidin [PVL]) lead to the formation of pores on target cell membranes. The cytoplasmic content of the target cell leaks and the cell lyses. PVL is a bicomponent cytolysin (LukF-PV and LukS-PV) and is cytotoxic for erythrocytes and leukocytes, just like *γ*-hemolysin and leukocidin, which show high affinity for leukocytes. *α*-Hemolysin is especially responsible for osmotic cytolysis of human thrombocytes and monocytes (12–16).

S. aureus secretes toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins A-I (SEA, SEB, SECN, SED, SEE, SEG, SEH and SEI), and exfoliative toxins A and B (ETA and ETB). Among these, TSST-1 and staphylococcal enterotoxins belong to the group of pyrogenic toxin superantigens (17,18).

Superantigens (SAgs) are toxins that play a role in T-cell proliferation. These toxins cause TSST-1, food poisoning (enterotoxins), and staphylococcal scalded skin syndrome (ETA and ETB) (19). The issue of whether exfoliative toxins have any mitogenic activity on T lymphocytes as SAgs is still controversial (20).

S. aureus has some special proteins that may have an effect on innate and acquired immunity. Staphylococcal complement inhibitor, chemotaxis-inhibitory protein of *S. aureus*, staphylokinase, extracellular fibrinogen-binding protein, extracellular adherence protein, and formyl peptide receptor like-1 inhibitory protein are some of these special proteins (21–27).

In this study, in order to contribute to the previous studies on whether or not *S. aureus* colonization is a determiner of disease activation in psoriasis patients, we evaluated the presence of genes encoding PVL, enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*), TSST-1 (*tst*), exfoliative toxins (*eta*, *etb*), and accessory gene regulatory locus (*agr*) by polymerase chain reaction (PCR) in *S. aureus* isolates obtained from healthy and diseased skin regions and anterior nares of psoriasis patients and healthy controls.

2. Materials and methods

2.1. Patients

Diseased skin, healthy skin (cubital volar region), and nasal swabs were obtained from 61 psoriasis patients who attended the dermatology polyclinic of the Kırıkkale University School of Medicine. The control group consisted of 48 healthy volunteers with no personal or family history of psoriasis or other inflammatory skin disorders. Nasal and cubital volar skin swabs were obtained from the control group. Ethics committee approval was received for this study from the Ethics Committee of the Kırıkkale University Medical Faculty (Approval Number: 2010/004), and informed consent was obtained from all study and control subjects.

2.2. Culture

All swabs were cultured on 5% sheep blood agar in the Medical Microbiology Department Laboratory of the Kırıkkale University School of Medicine. *Staphylococcus* identification was made by conventional microbiological methods (Gram staining, catalase, and coagulase tests). Methicillin resistance of the isolates was determined by a Kirby-Bauer disk diffusion test performed by using 1 μg oxacillin and 30 μg cefoxitin disks (Bioanalyse, Turkey) according to the Clinical and Laboratory Standards Institute instructions (28). Methicillin-resistant *S. aureus* (MRSA) ATCC 43300 and methicillin-sensitive *S. aureus* (MSSA) ATCC 25923 control strains were used as standard quality controls for susceptibility testing in the Medical Microbiology Department Laboratory of the Başkent University School of Medicine. All the strains were stored in brain-heart infusion broth containing 20% glycerol at –80 °C until molecular testing was performed.

2.3. Molecular analyses

Molecular analyses of the strains were performed in the Molecular Microbiology Diagnostics and Research Laboratory of the Ankara University School of Medicine, Department of Medical Microbiology. All the strains were subcultured on nutrient agar plates, and DNA was extracted by boiling. Briefly, 2 loopfuls of pure culture were suspended in 500 μL of sterile distilled water and boiled at 95 °C for 10 min. The suspension was centrifuged at 3500 × *g* for 5 min, and the supernatant was used for PCR analysis. The presence of PVL, enterotoxin and TSST-1 (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *tst*), and exfoliative toxin (*eta*, *etb*) genes was investigated, and *agr* typing was performed by PCR as described in the literature (12,13,29,30). The primer sets used for molecular analysis are given in Table 1.

2.4. Statistical analysis

Cochran Q and two-proportion z-tests were performed for the statistical analysis of the results using the SPSS 17.0 (SPSS Inc., Chicago IL, USA). $P < 0.05$ was considered as statistically significant.

3. Results

3.1. Culture results

Of the 61 psoriasis patients, 26 (42.6%) were found to carry *S. aureus* on their diseased and/or healthy skin and/or anterior nares. A total of 56 *S. aureus* strains were

Table 1. The primers used for molecular analysis of *S. aureus* strains.

Gene	Primer (5′-3′)	Product size (bp)	Reference
sea	GCAGGGAACAGCTTTAGGC GTTCTGTAGAAGTATGAAACACG	520	
seb-sec	ATGTAATTTTGATATTCGCAGTG TGCAGGCATCATATCATACCA	643	
sec	CTTGTATGTATGGAGGAATAACAA TGC AGG CAT CAT ATC ATA CCA	283	
sed	GTGGTGAAATAGATAGGACTGC ATATGAAGGTGCTCTGTGG	384	
see	TACCAATTAACTTGTGGATAGAC CTCTTTGCACCTTACCGC	170	
seg	CGTCTCCACCTGTTGAAGG CCAAGTGATTGTCTATTGTCG	327	(30)
seh	CAACTGCTGATTTAGCTCAG GTCGAATGAGTAATCTCTAGG	360	
sei	CAACTCGAATTTTCAACAGGTAC CAGGCAGTCCATCTCCTG	465	
sej	CATCAGAACTGTTGTTCCGCTAG CTGAATTTTACCATCAAAGGTAC	142	
tst	GCTTGCGACAACTGCTACAG TGGATCCGTCATTCATTGTTAA	559	
16S rRNA	GTAGGTGGCAAGCGTTATCC CGC ACA TCA GC GTC AG	228	
eta	GCAGGTGTTGATTTAGCATT AGATGTCCCTATTTTTGCTG	93	(30)
etb	ACAAGCAAAAGAATACAGCG GTTTTTGGCTGCTTCTCTTG	226	
PVL	ATCATTAGGTAAAATGTCTGGACATGATCCA GCA TCA AGT GTA TTG GAT AGC AAA AGC	433	(12,13)
agr1	GTCACAAGTACTATAAGCTGCGAT	441	
agr2	TATTACTAATTGAAAAGTGGCCATAGC	575	
agr3	GTAATGTAATAGCTTGTATAATAATACCCAG	323	(29)
agr4	CGATAATGCCGTAATACCCG	659	
agr-PanR	ATGCACATGGTGCACATGC		

isolated from swab cultures of these 26 patients. Twenty-four (43%) of the strains were isolated from nasal cultures, 20 (36%) from diseased skin swabs, and 12 (21%) from healthy skin swabs.

In the control group, only 4 (8.3%) nasal swabs were positive for *S. aureus* colonization. All of the isolated *S. aureus* strains were methicillin-susceptible.

Nasal *S. aureus* carriage rate was statistically significantly higher in the patient group when compared to the control group (36% versus 8.3%, $P < 0.001$). None of the healthy skin cultures yielded *S. aureus* growth in the control group. *S. aureus* carriage rates on diseased and healthy skin of psoriasis patients were statistically significantly higher than in the control group (21% versus 0%, $P < 0.001$, and 36% versus 0%, $P < 0.001$).

In the patient group, culture positivity of diseased skin correlated with nasal culture positivity (16 patients carried *S. aureus* both in the nares and on diseased skin, $P = 0.453$). Healthy skin cultures yielded less *S. aureus* positivity when compared to nasal ($P < 0.001$) and diseased skin ($P = 0.001953$) cultures.

3.2. Results of molecular analyses

None of the *S. aureus* strains isolated from the study and control groups carried PVL or exfoliative toxin genes. None of the strains isolated from the control group carried toxin genes. In the patient group, 18 (32.1%) of the isolated strains carried the *sei*, 1 (1.8%) carried the *seb-sec*, and 1 (1.8%) carried the *seg* enterotoxin gene (Figure 1).

Eight of the strains carrying enterotoxin genes were isolated from nasal swabs, 6 from diseased skin swabs, and 4 from healthy skin swabs.

The differences of toxin genes among isolation sites were statistically insignificant ($P = 0.135$).

None of the strains isolated from the control group carried the *agr* locus. On the other hand, 11 (19.7%) of the *S. aureus* strains isolated from the patients carried type 1, 7 (12.5%) carried type 1 + 3, 4 (7.1%) carried type 2, 4 (7.1%) carried type 3, and 1 (1.8%) carried type 1 + 2 *agr* loci (Figure 2). Twelve of these strains were isolated from nasal swabs, 10 from diseased skin swabs, and 5 from healthy skin swabs. The *agr* locus was carried at a significantly higher rate in *S.*

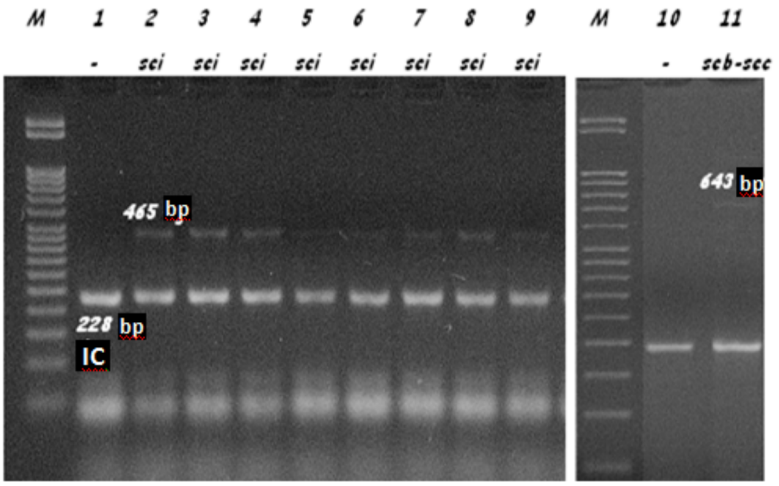

Figure 1. Enterotoxin genes identified by PCR. M represents 50-bp molecular weight marker (Fermentas, Lithuania), IC represents internal control (228-bp product of *S. aureus* 16S rRNA gene).

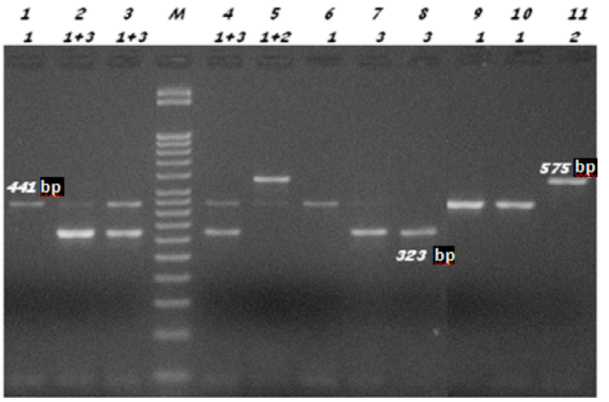

Figure 2. *agr* typing of the strains. M represents 50-bp molecular weight marker (Fermentas, Lithuania).

aureus strains isolated from nasal swabs and diseased skin swabs than the strains isolated from healthy skin swabs ($P < 0.05$). The distributions of toxin genes and *agr* types are given in Table 2 and Figures 3 and 4.

Table 2. Distribution of *S. aureus* strains isolated from patients according to their sampling sites, identified enterotoxin genes, and *agr* types.

		Genes identified					
Patient no.	Sampling site	*agr* types			Enterotoxin genes		
		1	2	3	*sei*	*seb-sec*	*seg*
1	Nasal				+		
2	Nasal				+		
3	Nasal						
4	Diseased skin						
	Healthy skin			+			
5	Nasal	+					
	Diseased skin	+					
6	Nasal	+		+			
	Diseased skin	+		+			
	Healthy skin			+			
7	Nasal						
	Diseased skin						
	Healthy skin						
8	Nasal				+		
	Diseased skin				+		
	Healthy skin				+		
9	Nasal				+		
	Diseased skin				+		
	Healthy skin				+		
10	Nasal				+		
	Diseased skin				+		
	Healthy skin				+		
11	Nasal						
	Diseased skin						
12	Nasal						
	Diseased skin				+		
	Healthy skin				+		
13	Nasal				+		
	Diseased skin	+			+		
14	Nasal						
15	Nasal				+		
	Diseased skin				+		
16	Nasal	+					
	Diseased skin	+					
	Healthy skin	+					
17	Nasal	+		+			
	Diseased skin	+		+			
	Healthy skin	+		+			
18	Nasal	+					
	Healthy skin	+	+				
19	Nasal	+		+			
	Diseased skin	+					
20	Nasal			+			
	Diseased skin	+					
	Healthy skin	+					
21	Nasal			+			
22	Nasal		+				
	Diseased skin		+				
23	Nasal	+			+		
	Diseased skin	+		+			
24	Nasal						
	Diseased skin						
	Healthy skin						
25	Diseased skin						
26	Nasal		+			+	
	Diseased skin		+				+

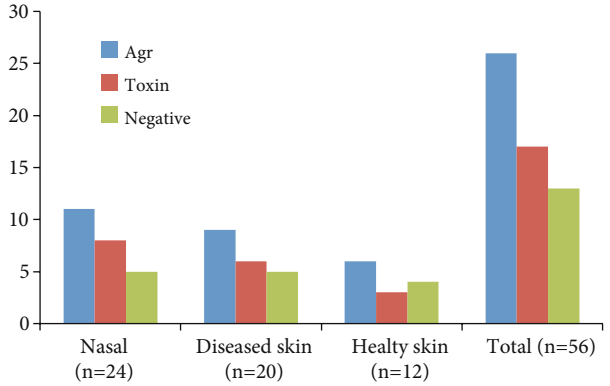

Figure 3. Toxin and *agr* locus presence among the strains isolated from psoriasis patients according to their sampling sites.

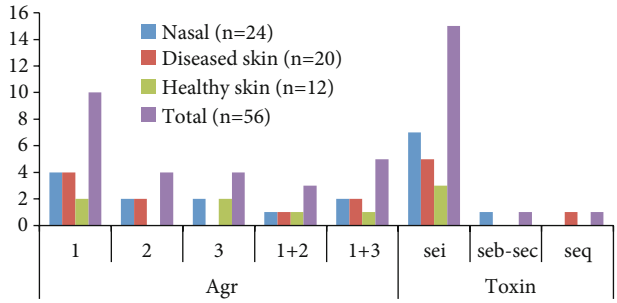

Figure 4. Enterotoxin and *agr* type distribution among *S. aureus* strains according to their sampling sites.

4. Discussion

Psoriasis is a chronic inflammatory disease affecting many components of the immune system. It is characterized by epidermal hyperproliferation and inflammation. Its pathogenesis involves complicated relationships among many cell types, cytokines, chemokines, and skin-derived chemical mediators. The interaction between these components leads to dysregulation in the immune system (31).

Bacterial products and SAgs such as staphylococcal enterotoxins, TSST-1, exfoliative toxins, streptococcal pyrogenic exotoxins, mycoplasma arthritis supernatant, chemicals, UV light, and trauma may take part in the development or aggravation of inflammatory skin diseases (3,4). Toxins and enzymes secreted by the bacteria have an important role in staphylococcal infections. Among these, enterotoxins are encoded by *sea-sej* genes, TSST-1 by the *tst* gene, and exfoliative toxins A and B by the *eta* and *etb* genes (32–34).

Another important virulence factor of *S. aureus* is PVL. It is responsible for pore formation on the membranes of polymorphonuclear leukocytes, leading to cell lysis (12). PVL-secreting strains are responsible for severe skin and soft tissue infections and necrotizing pneumonia (35). None of our patients were colonized with PVL-positive or MRSA strains, both of which may be considered as a benefit for our patients.

Expression of virulence proteins in *S. aureus* is under the control of RNA III, which is a small RNA molecule regulating the expression of *S. aureus* genes for exoproteins and cell membrane proteins. It is the intracellular effector of the quorum sensing system. Secreted proteases are under the control of the *agr* gene (36). In our study, 26 (46.42%) of the 56 *S. aureus* strains carried the *agr* locus, suggesting that secreted proteases may play a role in the aggravation of psoriasis.

The relationship between bacterial SAgs and skin diseases is shown in guttate psoriasis, atopic dermatitis, and cutaneous lupus erythematosus. The mechanism of how SAgs lead to inflammation is not known extensively. SAg-mediated T-cell activation may be involved (35).

In the study of Balcı et al. (37), 64% of the diseased and 14% of healthy skin cultures obtained from psoriasis patients were found to be positive for *S. aureus*. They also found a significant relationship between toxin production of the strains isolated from skin lesions and disease grades (37). These results support the findings of previous studies (38,39). Tomi et al. (39) showed that 36% of the *S. aureus* strains isolated from skin lesions of psoriasis patients secreted toxins. In the same study, psoriasis patients who carried toxin-negative and toxin-positive *S. aureus* strains were compared, and disease grades of the patients with toxin-positive strains were found to be higher. These results show that there is a relationship between toxin-positive *S. aureus* colonization and psoriasis activation (39). On the other hand, Sayama et al. (40) could only demonstrate the presence of enterotoxin (*seb*) and *tst-1* in 5 of the 100 *S. aureus* strains isolated from diseased skin swabs of psoriasis patients, and they concluded that SAgs do not have a role in the development of psoriasis (40).

In our study, we observed that patients who carry *S. aureus* on psoriasis lesions are more likely to also carry *S. aureus* nasally. Healthy skin culture positivity was significantly less prevalent in the patient group. If nasal carriage is regarded as the primary focus, psoriasis lesions can be considered as more prone to the development of *S. aureus* colonization when compared to healthy skin. Another question requiring explanation is the mechanism underlying the high frequency of nasal *S. aureus* carriage among psoriasis patients.

The results of our study demonstrated the presence of toxin genes in 20 (35.71%) of the 56 MSSA strains. Among diseased skin isolates (n = 7), 6 carried the *sei* and

1 the *seg* gene. Four of the 11 healthy skin isolates carried the *sei* gene, and of the 9 nasal isolates, 8 carried the *sei* gene and 1 the *seb-sec* genes. There was no difference in toxin production of isolates according to their sampling sites. On the other hand, the 4 MSSA isolates obtained from the control group did not carry toxin genes or the *agr* locus. These findings suggest that enterotoxins may be important in the aggravation of psoriasis as suggested in previous studies (39,41). Although these previous studies emphasized the high prevalence of enterotoxin genes in *S. aureus* strains isolated from psoriasis patients and correlated their presence with disease severity, they did not investigate the prevalence of enterotoxin I, which was the main enterotoxin gene found in our study. Whether or not this finding has important correlations with disease activation or severity needs further investigation.

Although there was no correlation between *agr* positivity and isolation sites of *S. aureus* strains, this study showed that *S. aureus* strains isolated from psoriasis patients established a high prevalence for the presence of the *agr* gene locus, which is responsible for the secretion of proteases that facilitate the aggregation of the infecting strain on to the skin. Thus, the high rate of *agr* positive *S. aureus* colonization in psoriasis patients may be a provocateur factor for disease activation attacks.

As a result, not only *S. aureus* colonization but also the toxin positivity and *agr* gene presence may be important for disease activation in psoriasis patients.

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