

Prevalence of Virulence-Related Determinants in Clinical Isolates of *Staphylococcus epidermidis*

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Abstract

Background: *Staphylococcus epidermidis*, a member of the human flora, is recognized as an opportunistic pathogen and cause of nosocomial infections. *Staphylococcus epidermidis* surface components are able to establish bacteria on the host surface, and cause infection.

Objectives: The frequency of *icaA*, *IS256*, *aap*, *fbe* and *bhp* in clinical isolates of *S. epidermidis* were investigated in this study.

Materials and Methods: Fifty-nine *S. epidermidis* isolates were collected from blood (50), wound (1), urine (4) and tracheal (4) samples (Tehran, Iran). *Staphylococcus epidermidis* isolates were identified with conventional bacteriological tests. Virulence-associated genes were detected by specific polymerase chain reactions (PCRs).

Results: Of the 59 *S. epidermidis*, *fbe* was found in 89.8%, while *aap* and *bhp* were observed in 64.4% and 15.3% of the samples, respectively. Coexistence of *aap* and *fbe* was found in 32 isolates, while coexistence of *bhp* and *fbe* was observed in five isolates. Two isolates were negative for the investigated genes.

Conclusions: Prevalence of *fbe* and *aap* was significantly different from similar studies, yet frequency of *bhp* was in accordance with other studies. Prevalence of *icaA* and *IS256* was not significantly different from some studies while a significant difference was observed when results were compared with some other studies.

Keywords: Virulence Factors, Staphylococcal Infections, *Staphylococcus epidermidis*

1. Background

Staphylococcus epidermidis is the most common bacteria isolated from the human skin and is the most persistent bacteria in some body parts. *Staphylococcus epidermidis*, which is part of the human flora, is also recognized as an opportunistic pathogen and causes nosocomial infections associated with medical devices (1). More information about bacterial physiology not only under infectious conditions but also when *S. epidermidis* is in a symbiosis condition could help evaluate the therapeutic strategies in the case of infection. It has been proposed that *S. epidermidis* acts as a probiotic and prevents the colonization of pathogens such as *S. aureus* (2, 3). *Staphylococcus epidermidis* causes chronic infections associated with medical devices, such as intravascular catheter or prosthetic joint infections, vascular graft infection, surgical site infection, central nervous system shunt infection, and cardiac device infection (4).

Fbe acts as an adhesin, which can facilitate the interaction with fibrinogen. The expression of *fbe* is poor in vitro

and increased in the presence of serum. In the presence of anti-*fbe* antibody, adherence of *S. epidermidis* to fibrinogen was blocked and infection was attenuated in animal models (5, 6). The *ica* operon, includes *icaA*, *icaB*, *icaC* and *icaD* genes and encodes polysaccharide intercellular adhesin (PIA), a microbial surface component-recognizing adhesive matrix molecule (MSCRAMM) of *S. epidermidis* that affects biofilm formation and is crucial for bacterial adhesion (7). The *IS256* is a mobile genetic element present in the genome of *S. epidermidis* at several copy numbers. It is believed that *IS256* is associated with virulence determinant genes, contributes to genetic adaptation, and is active in invasive strains during infection. Expression of *ica* operon may be affected by *IS256*. *IS256* increases the production of PIA, which plays an important role in biofilm formation and immune system invasion (4, 8).

Accumulation-associated protein (*aap*) is a 140-KD protein essential for *S. epidermidis* accumulation on the surfaces by formation of *Aap*-based fibril-like structures, which can potentially underlie the formation of biofilm (9,

10). Biofilm-associated protein (Bap), a 239-KD surface protein, is found only in some *S. aureus* strains. Its equivalent protein in *S. epidermidis* is called Bap homologue protein (Bhp). Bap homologue protein plays an important role in bacterial adhesion and colonization and catalyzes biofilm formation (11).

2. Objectives

The aim of this study was to determine the prevalence of *icaA*, *IS256*, *aap*, *fbe* and *bhp* in clinical isolates of *S. epidermidis*.

3. Materials and Methods

3.1. Bacterial Isolates

This study had a cross-sectional design. Overall, 59 *S. epidermidis* strains, isolated from blood (n = 50), urine (n = 4), tracheal (n = 4) and wound (n = 1) samples, were obtained from patients at intensive care units of various hospitals of Tehran, Iran.

3.2. *Staphylococcus epidermidis* Identification

For *S. epidermidis* identification, the sample was cultured on sheep blood agar (Merck, Germany), overnight under aerobic conditions at 37°C; Staphylococci should produce white, smooth, medium colonies on sheep blood agar. Gram stain was performed for suspected colonies, after gram-positive cocci observation. Catalase test was performed and catalase positive colonies were cultured on mannitol salt agar (Merck, Germany), overnight under aerobic conditions at 37°C. Staphylococci can grow on mannitol salt agar while *S. epidermidis* and some other Staphylococci are not able to ferment mannitol, thus the medium remained red. Tube coagulase test with rabbit plasma was performed for mannitol negative colonies. *Staphylococcus epidermidis* and some other Staphylococci are coagulase negative and could not produce clot in the tube. Final identification of *S. epidermidis* was performed by the ability of urease production and carbohydrate fermentation. *Staphylococcus epidermidis* are able to produce urease, thus the medium turned red. It is also unable to ferment D-trehalose, thus the medium turned red, while it is able to ferment D-mannose and D-maltose, thus the medium turned yellow (12, 13).

3.3. DNA Extraction

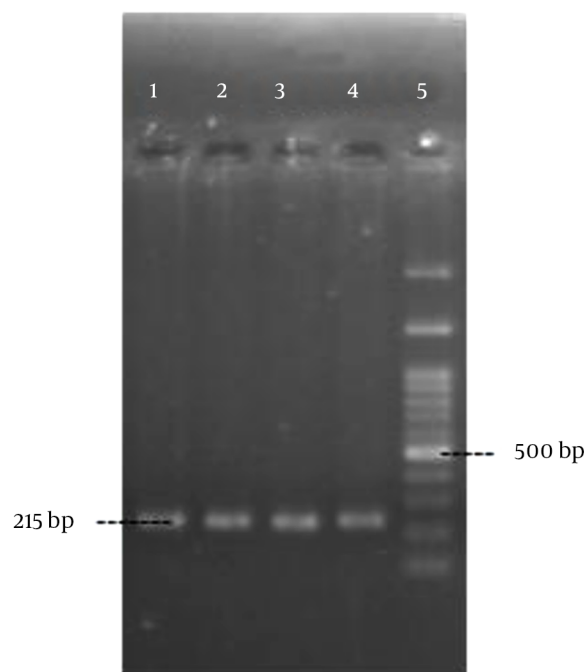
DNA of each *S. epidermidis* isolate was extracted from 1 mL of overnight bacterial culture. Extraction was performed by adding TE [10 mM Tris, 1 mM EDTA (pH = 8)] buffer and lysostaphin, boiling at 95°C for five minutes,

then cooling at 0°C for five minutes, followed by centrifugation at 10000 rpm for five minutes. The supernatant was used as the template DNA in PCR reactions. DNA was measured using a BioPhotometer (Eppendorf, Germany) to determine the concentration and purity.

3.4. Detection of Virulence Determinants

Polymerase chain reaction was performed as described by Rohde et al. (14) with the specific primers (Pishgam biotech, Iran) shown in Table 1. Amplification was performed in a MJ mini Gradient thermal cycler PTC-1148, USA. The amplified products were visualized by UV light after electrophoresis on 1% agarose gel (Figures 1 - 5). A positive control and negative control (reaction mixture without DNA) were included in each PCR run.

Figure 1. Polymerase Chain Reaction for *icaA*



1 - 3, Positive clinical strains; 4, positive control (215 bp); 5, marker (100 bp).

3.5. Statistical Analysis

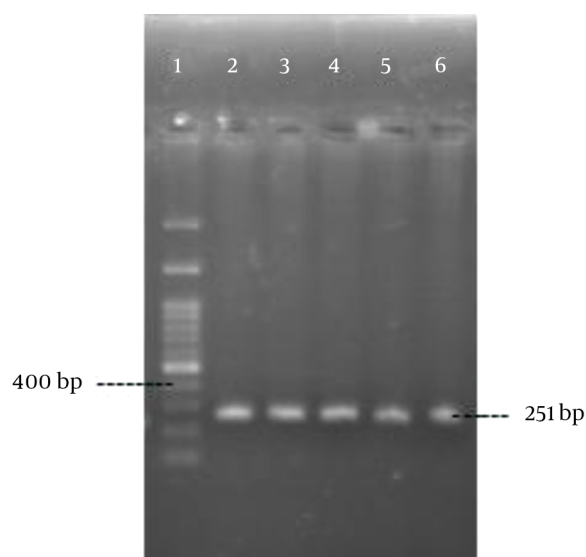
Confidence interval test was used to assess the statistical significance with confidence level of 95% ($\alpha = 0.05$).

4. Results

The most frequent virulence determinant in this study was *fbe* [89.8% (95% CI: 97.5 - 82.1)] and the least frequent

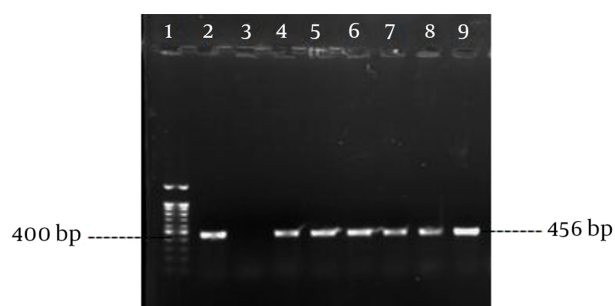
Table 1. Primers and Polymerase Chain Reaction Conditions for Detection of Virulence Determinants

Gene	Primer (5' → 3')	PCR Condition	Expected Size, bp
<i>icaA</i>		5 min, 95°C; 30 cycles of: 30 s, 94°C; 30 s, 52°C; 50 s, 72°C; final extension, 4 min, 72°C	215
	Forward	TCGATGCGATTGTCAAACAT	
	Reverse	CTGTTTCATGGAAACTC	
<i>IS256</i>		5 min, 95°C; 30 cycles of: 30 s, 94°C; 30 s, 52°C; 50 s, 72°C; final extension, 4 min, 72°C	251
	Forward	CCAGTTCATTGGGTTTATAGC	
	Reverse	CATATACATGGCAAGCTCTAGG	
<i>fbe</i>		5 min, 95°C; 30 cycles of: 30 s, 94°C; 30 s, 60°C; 50 s, 72°C; final extension, 4 min, 72°C	273
	Forward	CTACAAGTTCAGGTCAAGGACAAGG	
	Reverse	GCGTCGGCGTATATCCTCAG	
<i>aap</i>		5 min, 95°C; 30 cycles of: 30 s, 94°C; 30 s, 60°C; 50 s, 72°C; final extension, 4 min, 72°C	465
	Forward	AAACGGTGGTATCTTACGTGAA	
	Reverse	CAATGTTGCACCATCTAAATCAGCT	
<i>bhp</i>		5 min, 95°C; 30 cycles of: 30 s, 94°C; 30 s, 61°C; 90 s, 72°C; final extension, 4 min, 72°C	1585
	Forward	ATGGTATTAGCAAGCTCTCAGCTGG	
	Reverse	AGGGTTTCCATCTGGATCCG	

Figure 2. Polymerase Chain Reaction for *IS256*

1, Marker (100 bp); 2, positive control (251 bp); 3-6, positive clinical strains.

virulence determinant was *bhp* [15.3% (95% CI: 24.5 - 6.1)]. The frequency of *IS256*, *icaA* and *aap* were 72.9% (95% CI: 84.2 - 61.6), 55.9% (95% CI: 68.6 - 43.3) and 64.4% (95% CI: 73.4 - 55.4), respectively. Coexistence of *icaA* and *IS256* was observed in 44.1% (95%CI: 56.8 - 31.4) of the isolates. Coexistence of *aap* and *icaA* was seen in 39% (95% CI: 51.4 - 26.6) of

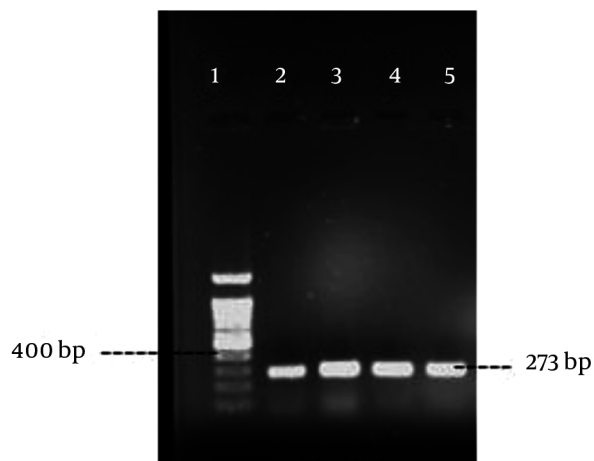
Figure 3. Polymerase Chain Reaction for *aap*

1, Marker (100 bp); 2, positive control (465 bp); 3, negative control; 4-9, positive clinical strains.

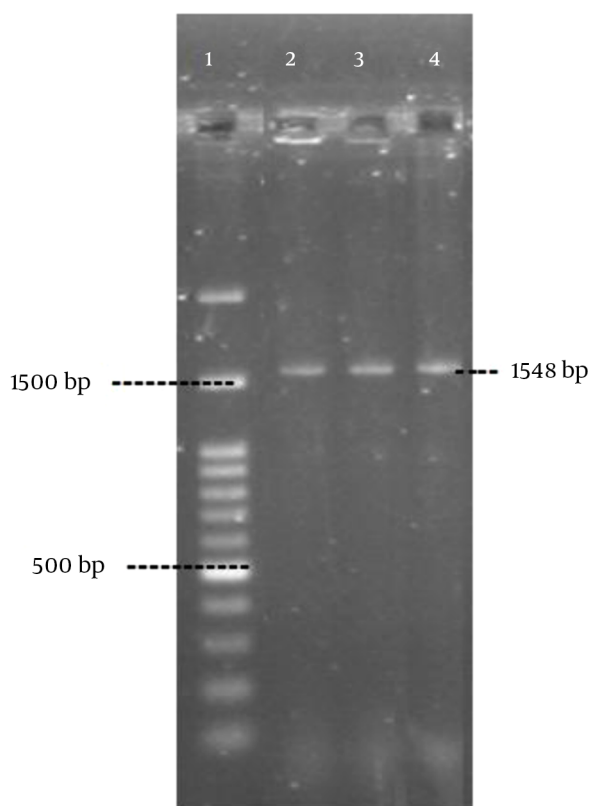
the isolates. In six isolates [10.1% (95% CI: 17.8 - 2.4)] only one of the investigated virulence determinants was observed, while in three isolates [5% (95% CI: 10.5 - 0)] all of the investigated virulence determinant was observed.

5. Discussion

Staphylococcus epidermidis, which was previously regarded only as part of the human flora, is now considered as an infectious agent that causes infections associated with medical devices. These infections may be transmitted from the patient's skin to the hospital staff during device insertion. The adhesion ability of *S. epidermidis* is

Figure 4. Polymerase Chain Reaction for *fbe*

1, Marker (100 bp); 2, *S. epidermidis* strain k28 (positive control, 273 bp); 3 - 5, positive clinical strains.

Figure 5. Polymerase Chain Reaction for *bhp*

1, Marker (100 bp); 2, positive control (1548 bp); 3 and 4, positive clinical strains.

one of the most important mechanisms through which it causes infections (4, 15). Polysaccharide intercellular adhesin, which is produced by *ica* operon, catalyzes cell-cell adhesion and biofilm formation (16). Thus, *ica* operon, producing PIA and adhesion to host protein, coated medical devices are the two main determinants of *S. epidermidis* invasiveness (8, 17). *Fbe*, an adhesion protein present in *S. epidermidis* and *S. aureus*, facilitates binding to fibrinogen-coated device surfaces, which is an essential mechanism for bacterial persistence in or on the human body that leads to infections (18). Thus, *ica* operon and *IS256* are the two determinants of *S. epidermidis* invasiveness, which play an important role in biofilm formation and immune system invasion (4, 8). It is believed that *aap* is one of the most important factors for PIA-independent biofilm formation in *S. epidermidis* (9). The increased expression of *aap* may lead to biofilm formation (19, 20). Furthermore, *Bhp* plays an important role in bacterial accumulation and adhesion and catalyzes biofilm formation (11, 21).

In our study, frequency of *icaA* was found to be 55.9%. Diamond-Hernandez et al. (22) reported that the frequency of *icaA* was 51.1% while Koskela et al. (8) reported that this frequency was 50%. Our results are not significantly different from those of these studies. Frequency of *icaA* was reported as 73% by Okee et al. (23), 88.6% by Gad et al. (16), and 88.6% by Rohde et al. (14). The frequency observed in our study was significantly lower than that reported in these studies. In our study, frequency of *IS256* was found to be 72.9%, which is not significantly different from 81% reported by Koskela et al. (8); however, it is significantly lower than 93.8% reported by Rohde et al. (14) and 100% reported by Okee et al. (23) and significantly more than 61.1% reported by Mekni et al. (24). *Fbe* was found in 89.8% of the isolates, which is significantly less than 100% reported by Rohde et al. (14), and does not show significant difference with 50.8% reported by Mekni et al. (24). Overall, 15.3% of investigated isolates in this study harbored *bhp* which is not significantly different from 10% reported by Rohde et al. (14), 11.1% reported by Okee et al. (23), and 18.8 reported by Mekni et al. (24). In our study, the frequency of *aap* was found to be 64.4%, which is significantly higher than 17% reported by Okee et al. (23) yet significantly lower than 93.8% reported by Rohde et al. (14) and Pourmand et al. (19). Coexistence of *aap* and *icaA* was detected in 39% of the isolates in our study, which is not significantly different from 37.1% reported by Liduma et al. (25). Coexistence of *IS256* and *icaA* was detected in 44.1% of the isolates in our study, which is significantly lower than 58% reported by Koskela et al. (8).

In conclusion, prevalence of *fbe* and *aap* observed in our study was significantly different from that reported by other similar studies; however, the frequency of *bhp* was in accordance with that reported by other studies. Preva-

lence of *icaA* and *IS256* was not significantly different from that reported by some studies yet was significantly different from other studies.

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Footnotes

Authors' Contribution: All authors participated in the research design and contributed to different parts of the research.

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