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Prevalence of Biofilm Associated Genes in Different Isolates of *Staphylococcus aureus*

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ABSTRACT

Background: *Staphylococcus aureus* is the most important etiological agent of biofilm associated-infections. It is one of the Gram-positive pathogens causing a wide range of nosocomial infections. Genes involved in biofilm formation is a defensive mechanism of this pathogen to combat the host immune response and remain stable in hostile environment. The aim of this study was to investigate prevalence of biofilm associated genes (BAGs).

Methods: Eighty samples of *Staphylococcus aureus* isolates from human infections were collected. Thirteen BAGs including *rbf, sigB, sasG, icaA, sarA, icaR, icaD, clfA, clfB, fib, fnbpB, bap* and *fnbpA* were amplified by PCR assay.

Results: The prevalence of genes were as follows: *sigB* (93.7%) and *sarA* (90%) were the most prevalent BAGs followed by *rbf* (83.7%), *fib* (80%), *sasG* (78.7%), *icaR* (78.7%), *clfB* (78.7%), *clfA* (78.7%), *fnbpA* (73.7%), *icaD* (66.2%), *icaA* (50%), *fnbpB* (22.5%). However, *bap* was not detected in any isolate.

Conclusion: This study showed that the sensitivity and specificity of PCR is high in the identification of biofilm associated genes in *S. aureus*. However, researchers need new molecular methods to improve understanding of the exact role of the genes involved in biofilm formation.

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Introduction

Staphylococci are Gram positive, non-motile, non-spore forming, facultative anaerobes, occurring as cocci in clusters, and are classified in two main groups, coagulase-positive and coagulase-negative (1).

Staphylococcus aureus is a major human pathogen that causes a wide range of clinical infections (2). Approximately 30% of the human population is colonized with *S. aureus* (3). Simultaneously, it is a leading cause of bacteremia and infective endocarditis (IE) as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections, that appear to be caused by biofilm-associated *S. aureus* (2). It is also one of the most common pathogens responsible for contagious bovine mastitis (4).

S. aureus can live in a wide variety of environments. It also has an inherent ability to form biofilms on biotic and a-biotic surfaces. The biofilms protect the cells not only from host immune response but also from antimicrobial agents (5-6).

Biofilms are sessile microbial communities embedded in a self-produced extracellular polymeric matrix (6-7). Two steps appear to be involved in this process: (i) attachment of the bacterial cells to a surface (early adherence) and (ii) growth-dependent accumulation of bacteria in multilayered cell clusters (intercellular adhesion) (8).

Identification of genes involved in biofilm formation is needed to understand the molecular basis of strain variation and the pathogenic mechanisms implicated in chronic staphylococcal infections (8).

The *fnbpA*, f*nbpB* (encoding fibronectin binding proteins A and B), *clfA* and *clfB* (encoding clumping factors A and B) can promote adhesion of *S. aureus* cells to a variety of molecules and surfaces and they have been implicated in cell-cell adhesion (9).

The fibronectin-binding proteins, especially *fnbpB*, can mediate the attachment to tissue or synthetic surfaces of medical devices coated with plasma proteins, such as fibronectin (10).

The *bap* gene (biofilm associated protein) is a newly identified gene that encodes the biofilm-associated protein, BAP, which is involved in biofilm formation in *S. aureus*. Prevalence of this gene among *S. aureus* isolates is very low (11). The *bap* gene has only been found in bovine mastitis isolates and its expression could enhance the intra-mammary adherence and biofilm formation (4).

The expression of Bap decreased cell invasion and increased bacterial persistence in lactating mice mammary glands. Bap promotes adhesion but inhibits the entry of *S. aureus* into epithelial cells. So far, the *bap* gene has never been found in *S. aureus* human isolates (11).

The *sarA* (staphylococcal accessory regulator A) is involved in production of a staphylococcal surface protein called Bap by utilization of its associated gene (*bap*) (4). Very recently, it has been shown that a mutation in the *ica* genes of a clinical *S. aureus* isolate has little effect on biofilm formation, whereas a mutation in *sarA* leads to a biofilm-negative phenotype (12).

Cell aggregation and biofilm accumulation are mediated by the products of a gene locus composing of the genes *icaADB* and *C*, which encode the essential proteins for the production of polysaccharide intercellular adhesion (PIA) and capsular polysaccharide/adhesion (PS/A) in *Staphylococcus* spp. PIA has been described in various reports to play a crucial role in biofilm formation and virulence (12).

The *sasG* is involved in the accumulation phase of biofilm, a process that requires a physiological concentration of Zn^{2+} (13).

To investigate molecular basis of *Staphylococcus* spp. variation and pathogenesis mechanism of chronic infection caused by *S. aureus*, the study of biofilm associated genes is necessary (14). Therefore, the present study was designed to investigate distribution of various biofilm associated genes in *S. aureus* isolates from different sources.

Materials and Methods

Staphylococcus aureus isolates

In total, 80 samples of *Staphylococcus aureus* isolates from human infections referred to the Microbiology Laboratory of the Medical Research Center of Namazi Hospital of Shiraz University of Medical Sciences were collected. The isolates were identified using phenotypic tests such as catalase and coagulase production, biochemistry tests and then molecular confirmation via *nuc* gene detection. The identified strains were stored at 80°C in Luria-Bertani (LB) broth.

DNA extraction

Rose method was used for extracting DNA (15). The frozen suspension of *S. aureus* was revived in nutrient agar medium for 24 h. Then, 3 to 5 colonies of the isolate were moved to a 1.5 mL microtube. About 25 µl of 0.05 normal NaOH was added to the microtube. After 30 min, 25 µl 1M TrisHCl was injected to the suspension of microtube. The final pH was 7.5. About 450 µl distilled water was added quickly and the final volume was 500 µl (15).

PCR assay

Biofilm-associated genes (BAGs) of *S. aureus* isolates (*rbf*, *sigB*, *sarA*, *icaA*, *sasG*, *icaR*, *icaD*, *clfA*, *fib*, *clfB*, *fnbpB*, *bap* and *fnbpA*) were detected by polymerase chain reaction (PCR). Nucleotide sequences of primers and expected sizes of PCR products are listed in table 1. The reaction mixture consisted of 2 µl of buffer, 2 µl of $MgCl_2$, 1 µl of dNTP, 0.2 µl of Taq, 1 µl of each forward and reverse primers, 14.8 µl of distilled water and 3 µl of DNA template.

Initial denaturation step was at 95°C for 8 min, followed by 30 cycles of denaturation for 30 s at 95°C, annealing at different temperatures (table 2 according to different genes) for 30 s, an extension for 30 s at 72°C, and final extension for 10 min at 72°C. The protocol was the same for all 13 genes except the annealing temperatures. The sizes of PCR products were analyzed by electrophoresis on 1.5% (wt/vol) agarose gels stained with safe stain, and visualized under ultraviolet illuminator.

Result

BAGs in S. aureus isolates

The distribution of different biofilm associated genes (BAGs) in 80 isolates of *S. aureus* was investigated. In overall, the *sigB* was the most prevalent genes (93.7%), followed by *sarA* (90%), *rbf* (83.7%), *fib* (80%), *sasG* (78.7%), *icaR* (78.7%), *clfB* (78.7%), *clfA* (78.7%), *fnbpA* (73.7%), *icaD* (66.2%), *icaA* (50%), *fnbpB* (22.5%). The results are shown in figures 1 and 2. However, *bap* was not amplified in any isolates.

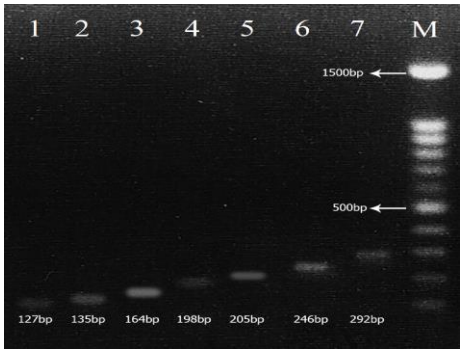

Figure 1. The results of single PCR for detection of genes involve in biofilm formation in *S. aureus*. 1: *fnbpA*; 2: *sarA*; 3: *rbf*; 4: *icaD*; 5: *clfB*; 6: *icaR*; 7: *clfA*; M: Marker 100bp.

Table 1. The primers used for PCR amplifications in this study.

Primers	OligoNucleotide Sequences (5'-3')	Annealing Temperature (°C)	Product Size (bp)	References
nuc	ATATGTATGGCAATCGTTTCAAT-GTAAATGCACTTGCTTCAGGAC	56	395	4
rbf	ACGCGTTGCCAAGATGGCATAGTCTT-AGCCTAATTCCGCAAACCAATCGCTA	62	164	16
sigB	GTTCAAGTTGGTATGGTTGGTT-GTCATAATGGTCATCTTGTTGC	56	395	4
sarA	TTTTTTTACGTTGTTGTGCATTAACA-CATTTAAACTACAAACAACCACAAGTTG	56	135	17
icaA	CCTAACTAACGAAAGGTAG-AAGATATAGCGATAAGTGC	56	1315	18
sasG	CGGATCCGGTGTGACAATCAGTATGAC-CGGAATTCGCGACATTTATGTGGATACAC	55	937	19
icaR	CAATAATCTAATACGCCTGAG-AGTAGCGAATACACTTCATCT	54	246	20
icaD	ATGGTCAAGCCCAGACAGG-CGTGTTTTCAACATTTAATGCAA	56	198	20
clfA	ATTGGCGTGGCTTCAGTGCT-CGTTTCTTCCGTAGTTGCATTTG	55	292	19
fib	CTACAACTACAATTGCCGTCAACAG-GCTCTTGTAAGACCATTTTCTTCAC	56	404	21
clfB	ACATCAGTAATAGTAGGGGGCAAC-TTCGCACTGTTTGTGTTTGCAC	55	205	19
fnbpB	GTAACAGCTAATGGTCGAATTGATACT-CAAGTTCGATAGGAGTACTATGTTC	55	524	19
bap	CCCTATATCGAAGGTGTAGAATTG-GCTGTTGAAGTTAATACTGTACCTGC	60	971	14
fnbpA	CATAAATTGGGAGCAGCATCA-ATCAGCAGCTGAATTCCCATT	55	127	19

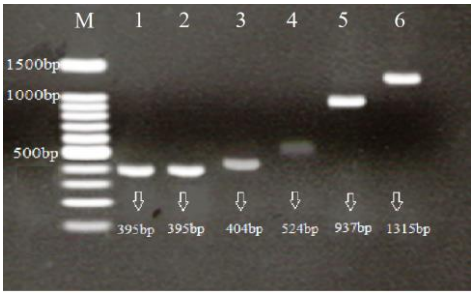

Figure 2. The results of single PCR for detection of genes involve in biofilm formation in *S. aureus*. M: Marker 100bp, 1: *nuc*; 2: *sigB*; 3: *fib*; 4: *fnbpB*; 5: *sasG*; 6: *icaA*.

Discussion

Several serious diseases are caused by biofilm-associated *Staphylococcus aureus*, infections in which the accessory gene regulator (agr) quorum-sensing system is thought to play an important role (2).

Biofilms are sessile microbial communities embedded in a self-produced extracellular polymeric matrix (22). There is increasing awareness that biofilms have a special clinical relevance. Biofilm-associated bacteria show an innate resistance to antibiotics. As biofilm infections are difficult to treat, prevention of

biofilm formation is an important strategy in biofilm control. To study biofilm formation, we attempted to identify genes involved in this process.

We proposed PCR method as a rapid and effective method to be used for identifying the prevalence of biofilm associated genes of *S. aureus* isolates.

In the present study, we investigated the prevalence of 13 genes involved in biofilm production of 80 *S. aureus* isolates from human infections. Among 13 BAGs *sigB* and *sarA* were the most prevalent. Concerning *bap*, our findings seem to be consistent with other researchers, which have not reported this gene in any *S. aureus* isolate of human origin (11 and 14).

An alternative sigma factor sigma B (*sigB*), an important component of the stress response of *S. aureus* required for coping with oxidative, alkaline, heat and salt stress, is involved in the regulation of virulence factor expression (23). The activity of *sigB* peaks early during the stationary phase of growth (24). In particular, *sigB* directly or indirectly influences the production of alpha-toxin, various proteases, lipases, clumping factor (*clfA*), coagulase (*coa*), and fibronectin binding protein (*fnbpA*) (23).

The *rbf* (transcriptional regulator) gene in *S. aureus* is considered to be required for biofilm development in critical conditions (25). The prevalence of *rbf* was 83.7%. Several reports supported our study that *rbf* is widespread among *S. aureus* strains (4 and 25).

In this study the prevalence of *icaR*, *clfB* and *clfA* was the same, and it was 78.7%.

The least prevalent gene was *fnbpB* and it was 22.5%. He et al, 2014 reported lower (19.6%) occurrence of *fnbpB* gene in *S. aureus* isolates (4). In one study in China on 102 *S. aureus* isolated from bovine subclinical mastitis, revealed that *rbf* and *SigB* were the most prevalent. However, *bap* and *fnbpA* genes were not detected in any strain (4).

Several reports (11, 26 and 27) from different geographical areas supported our study and reported the absence of *bap* gene in biofilms formed by *S. aureus* isolates.

Conclusion

Biofilm formation by *S. aureus* is a complex mechanism controlled by multiple genes. The modulations between these genes will offer an important basis and means for the control and prevention of *S. aureus* biofilm formation. This study showed that the sensitivity and specificity of PCR is high in the identification of biofilm associated genes in *S. aureus*. The identification of the virulent genes is the first step in the study of bacterial pathogenesis. However, researchers need new molecular methods to improve understanding of the exact role of the genes involved in biofilm formation.

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Ethics approval and consent to participate

Not needed.

Conflict of interest

The authors declare that they have no competing interests.

References

1. Dehkordi AA, Tajbakhsh E, Tajbakhsh F, et al. Molecular typing of *Staphylococcus aureus* strains from Iranian raw milk and dairy products by coagulase gene polymorphisms. *Adv Stud Biol* 2015; **7**(4):169-77.
2. arwood JM, Bartels DJ, Volper EM, et al. Quorum sensing in *Staphylococcus aureus* biofilms. *J Bacteriol* 2004; **186**(6):1838-50.
3. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus*

aureus infections. *Lancet Infect Dis* 2005; **5**(12): 751-62.
4. He JZ, Wang AQ, Liu G, et al. Biofilm formation and biofilm-associated genes assay of *Staphylococcus aureus* isolated from bovine subclinical mastitis in China. *Pak Vet J* 2014; **34**(4):508-13.
5. Speziale P, Pietrocola G, Foster TJ, et al. Protein-based biofilm matrices in staphylococci. *Front Cell Infect Microbiol* 2014; **10**(4):171.
6. Donlan RM, and Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* 2002; **15**(2):167-93.
7. Ferreira AA, Tette PAS, Mendonça RCS, et al. Detection of exopolysaccharide production and biofilm-related genes in Staphylococcus spp. isolated from a poultry processing plant. *Food Sci Technol* 2014; **34**(4): 710-6.
8. Middleton JR. *Staphylococcus aureus* mastitis: Have we learned anything in the last 50 years. In Proceedings of the National Mastitis Council Regional Meeting 2013; 1-8.
9. Namvar AE, Asghari B, Ezzatifar F, et al. Detection of the intercellular adhesion gene cluster (*ica*) in clinical *Staphylococcus aureus* isolates. *GMS Hyg Infect Control*, 2013; **8**(1):Doc03.
10. Vergara-Irigaray M, Valle J, Merino N, et al. Relevant role of fibronectin-binding proteins in *Staphylococcus aureus* biofilm-associated foreign-body infections. *Infect Immun* 2009; **77**(9):3978-91.
11. Vautor E, Abadie G, Pont A, et al. Evaluation of the presence of the *bap* gene in *Staphylococcus aureus* isolates recovered from human and animals species. *Vet Microbiol* 2008; **127**(3-4):407-11.
12. Beenken KE, Dunman PM, McAleese F, et al. Global gene expression in *Staphylococcus aureus* biofilms. *J Bacteriol 2004*; **186**(14):4665-84.
13. Geoghegan JA, Corrigan RM, Gruszka DT, et al. Role of surface protein *SasG* in biofilm formation by *Staphylococcus aureus*. *J Bacteriol* 2010; **192**(21):5663-73.
14. Cucarella C, Tormo MÁ, Úbeda C, et al. Role of biofilm-associated protein *bap* in the pathogenesis of bovine *Staphylococcus aureus*. *Infect Immun* 2004; **72**(4):2177-85.
15. Osmundson TW, Eyre CA, Hayden KM, et al. Back to basics: an evaluation of NaOH and alternative rapid DNA extraction protocols for DNA barcoding, genotyping, and disease diagnostics from fungal and oomycete samples. *Mol Ecol Resour* 2013; **13**(1):66-74.
16. Cue D, Lei MG, Luong TT, et al. *Rbf* promotes biofilm formation by *Staphylococcus aureus* via repression of *icaR*, a negative regulator of *icaADBC*. *J Bacteriol* 2009; **191**(20):6363-73.
17. Rode TM, Langsrud S, Holck A, et al. Different patterns of biofilm formation in *Staphylococcus aureus* under food-related stress conditions. *Int J Food Microbiol* 2007; **116**(3):372-83.
18. Vasudevan P, Nair MKM, Annamalai T, et al. Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. *Vet Microbiol* 2003; **92**(1-2): 179-85.
19. Li L, Yang HJ, Liu DC, et al. Biofilm formation and analysis of associated genes involved in *Staphylococcus* isolates from bovine mastitis. *Sci Agricult Sinica* 2011; **44**(1):160-6.
20. Chaieb K, Abbassi MS, Touati A, et al. Molecular characterization of *Staphylococcus epidermidis* isolated from biomaterials in a dialysis service. *Ann Microbiol* 2005; **55**(4): 307-12.
21. Tristan A, Ying L, Bes M, et al. Use of multiplex PCR to identify *Staphylococcus aureus* adhesions involved in human hematogenous infections. *J Clin Microbiol* 2003; **41**(9):4465-7.
22. Lister JL and Horswill AR. *Staphylococcus aureus* biofilms: recent developments in biofilm dispersal. *Front Cell Infect Microbiol*

2014; **4**:178.
23. Mitchell G, Fugère A, Gaudreau KP, et al. *SigB* is a dominant regulator of virulence in *Staphylococcus aureus* small-colony variants. *PLoS One* 2013; **8**(5):e65018.
24. Senn MM, Giachino P, Homerova D, et al. Molecular analysis and organization of the σB operon in *Staphylococcus aureus*. *J Bacteriol* 2005; **187**(23): 8006-19.
25. Lim Y, Jana M, Luong TT, et al. Control of glucose-and NaCl-induced biofilm formation by *rbf* in *Staphylococcus aureus*. *J Bacteriol* 2004; **186**(3):722-29.
26. Melchior MB, Vaarkamp H, Fink-Gremmels J, et al. Biofilms: a role in recurrent mastitis infections? *Vet J* 2006; **171**(3):398-407.
27. Seo YS, Lee DY, Rayamahji N, et al. Biofilm-forming associated genotypic and phenotypic characteristics of *Staphylococcus* spp. isolated from animals and air. *Res Vet Sci* 2008; **85**(3):433-38.