

## Original Article



# Staphylococcus Aureus Isolated from Surgical Site Infections: Evaluation of Biofilm and Biofilm-Related Genes

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## Abstract

**Introduction:** *Staphylococcus aureus* is the most common bacterium causing surgical site infections (SSIs). The biofilm formation of *S. aureus* leads to increased survival and bacterial persistence. This study aimed to evaluate the frequency of biofilm formation and the presence of *icaD*, *icaA*, *fnbA*, *clfA*, and *cna* genes in *S. aureus* from SSIs isolated from patients in two hospitals in southwest Iran.

**Methods:** This cross-sectional study included 77 *S. aureus* isolates from SSIs. The ability of *S. aureus* for biofilm formation was assessed by the Congo Red Agar method. The prevalence of *icaD*, *icaA*, *clfA*, *fnbA*, *cna*, and *mecA* genes was determined by PCR. Data were analyzed using SPSS software (version 20) through chi-square test and Fisher's exact tests.

**Results:** Biofilm formation was observed in 55 isolates (71.4%), including 30 (54.5%) strong and 25 (45.5%) weak biofilm producers' isolates. The ability to form biofilm was higher among MRSA isolates (83.7%) compared to MSSA isolates (50%). The frequency of the *icaD*, *fnbA*, *clfA*, *cna*, and *icaA* genes was 62.3%, 42.9%, 28.6%, 20.8%, and 19.5%, respectively. There was a significant association between the presence of *fnbA* (P=0.000), *clfA* (P=0.000), *icaD* (P=0.02) genes and biofilm formation; however, it was not observed for *icaA* (P=0.054), and *cna* (P=0.132) genes.

**Conclusions:** Our findings reinforce the role of Biofilm-related genes (*fnbA*, *clfA*, *icaD*) and biofilm formation. Given the role of genes in biofilm formation, we can pursue the development of scientific solutions to control biofilms, which ultimately lead to improved public health.

**Key words:** Polymerase Chain Reaction, *Staphylococcus aureus*, Surgical Wound Infection

## Introduction

The Centers for Disease Control and Prevention defines surgical site infections (SSIs) as infections that occur at the incision site within 30 days of surgery. SSI is the third most common cause of nosocomial infections. Moreover, it is one of the most common problems for patients undergoing surgery. Surgical wound infection leads to an increase in the length of hospital stay, leading to an increased rate of mortality. SSIs are usually caused by exogenous and/or endogenous microorganisms that enter the wound during surgery (primary infection) or after surgery (secondary infection) (1). Among organisms, *S. aureus* is currently the most common organism isolated from surgical wound

infection, which causes 37% of SSIs in the community (2,3). *S. aureus* is a common human bacterium and an opportunistic pathogen that causes a wide range of nosocomial and community-acquired infections (4,5). These include some skin infections, postoperative infections, wound infections, soft tissue infections, pneumonia, toxic shock syndrome, sepsis, infective endocarditis, as well as bone and joint infections (6-8). Nosocomial pathogens can infect patients by forming biofilms on foreign implanted objects in the body (9). More than 65% of Nosocomial infections are caused by the infecting organisms that have the ability to produce biofilms (10). The most common microorganism isolated from SSIs is *S. aureus* (11). Biofilm



formation increases the severity of *S. aureus*-related infections and also leads to increased resistance to antimicrobial drugs (12). Biofilm formation by organisms is a potential factor of delay in the healing of surgical wounds. Various factors contribute to the formation of *S. aureus* biofilms. These include the *icaADBC* operon, which encodes enzymes required for polysaccharide intercellular adhesin (PIA) synthesis (13), and adhesion-related proteins known as *MSCRAMMs*, such as collagen-binding protein (*cna*), fibronectin-binding proteins (*fnbA* and *fnbB*), laminin-binding protein (*Eno*), fibrinogen-binding protein (*clfA*), biofilm-associated protein (*Bap*), and elastin-binding protein (*EbpS*), all of which facilitate bacterial adhesion (14–16). The primary objectives of this study were to assess biofilm formation in *S. aureus* isolates using the Congo Red Method and to determine the frequency of biofilm-associated genes, including *icaA*, *icaD*, *clfA*, *fnbA*, and *cna*, in strains isolated from surgical site infections in patients admitted to two hospitals in Yasuj, southwest Iran.

## Methods

This cross-sectional study was performed on 77 isolates of *S. aureus* isolated from patients with SSI who were hospitalized at Imam Sajjad and Shahid Beheshti Hospitals, Yasuj, Iran, between January and December 2019. The bacteria studied in this study are taken from a research project with a code of ethics (IR.YUMS.REC.1397.124), approved by the Student Research Committee of the University of Medical Sciences of Yasuj.

### Detection of Biofilm Formation by Congo Red Method

In order to prepare this medium, first, Brain Heart Infusion Broth medium was prepared along with agar, and Concord reagent was prepared separately in the form of an aqueous solution. Then the two solutions were autoclaved. After autoclaving, the sucrose was filtered from the medium and added to the medium. Biofilm formation by the Congo Red Method was performed according to Arciola et al.'s method (17). A positive result was indicated by black colonies. Weak biofilm producer isolates remained pink, and occasional darkening at the centers of colonies was also observed. Bright red colonies were considered negative.

### PCR assay

According to Cayci et al.'s method, DNA extraction was performed by the boiling method

(18). Detection of *methicillin-resistant S. aureus* (*MRSA*) was based on the *mecA* gene. PCR was performed for the detection of *icaD*, *icaA*, *fnbA*, *clfA*, and *cna* genes using primers displayed in Table 1. The PCR mixture was prepared separately for each gene in a total volume of 25  $\mu$ L, including 12.5  $\mu$ L of Master Mix (Amplicon, Denmark), 2  $\mu$ L of each primer, 5  $\mu$ L of bacterial DNA, and 3.5  $\mu$ L of sterile distilled water. After amplification, 10  $\mu$ L of the PCR products were electrophoresed (Major Science MP300, Taiwan) on 1.5% agarose gel (Pishgam, Iran) at 90 V for 45 minutes. The PCR products were stained with Gel Stain (Pishgam, Iran). They were then visualized by Gel Documentation (Major Science, Taiwan).

### Statistical analyses

The data were analyzed using SPSS software (version 20) through the Chi-square and Fisher's exact tests.  $P < 0.05$  were regarded as statistically significant.

## Results

Of the 77 *S. aureus* isolates, according to the *mecA* gene, 63.6% and 36.4% isolates were *MRSA* and *MSSA*, respectively. Biofilm formation was noted in 55 (71.4%) isolates, including 30 (54.5%) strong and 25 (45.5%) weak biofilm producers' isolates. In total, 65 (84.4%) *S. aureus* isolates were positive for at least one of the studied biofilm-related genes (*icaD*, *icaA*, *fnbA*, *clfA*, and *cna*). None of the genes involved in biofilm formation were observed in 12 isolates. Of the 22 isolates that were not able to form biofilms, 12 isolates were positive for at least one of the biofilm-related genes. Frequency of biofilm-related genes in biofilm-positive isolates and biofilm-negative isolates. In this study, the prevalence of *icaD*, *fnbA*, *clfA*, *cna*, and *icaA* genes was (48/77, 62.3%), (33/77, 42.9%), (22/77, 28.6%), (16/77, 20.8%), and (15/77, 19.5%), respectively. The significant association was observed in phenotypic production of biofilm, and the presence of *fnbA*, *clfA*, and *icaD* genes in *S. aureus* isolates ( $P < 0.05$ ); however, this connection was not observed for *icaA* and *can* ( $P > 0.05$ ) genes (Table 2).

The frequency of biofilm formation among *MRSA* isolates (83.7%) was higher than that among *MSSA* isolates (50%). The statistical difference between *MRSA* and *MSSA* regarding the frequency of the *clfA*, *fnbA*, *cna*, *icaA*, and *icaD* genes was not significant (Table 3).

**Table 1.** The primers used in this study

Target Gene	Primer Sequence (5' → 3')	Amplicon Length, bp	Reference	PCR program
<i>cna</i>	F- AAAGCGTTGCCTAGTGGAGAC R- AGTGCCTTCCCAAACCTTTT	192	(19)	94°C – 5 Minutes 94°C - 30 Seconds 30 Seconds at 54°C 72°C - 1 Minutes 32X 72°C - 10 Minutes annealing: 60°C - 30 Seconds
<i>clfA</i>	F- CCGGATCCGTAGCTGCAGATGCACC R- GCTCTAGATCACTCATCAGGTTGTTCA GG	1000		annealing: 30 Seconds at 52°C annealing: 30 Seconds at 49°C annealing: 30 Seconds at 49°C According to the reference
<i>fnbA</i>	F- GATACAAACCCAGGTGGTGG R- TGTGCTTGACCATGCTCTTC	191		
<i>icaD</i>	F- AAACGTAAGAGAGGTGG R- GGCAATATGATCAAGATAC	381	(20)	
<i>icaA</i>	F- CCTAACTAACGAAAGGTAG R- AAGATATAGCGATAAGTGC	1351		
<i>mecA</i>	F- GTGAAGATATACCAAGTGATT R- ATGCGCTATAGATTGAAAGGAT	147	(21)	

**Table 2.** The relationship between the strength of biofilm formation and the presence of biofilm-related genes

Genes	<i>icaA</i>		<i>icaD</i>		<i>clfA</i>		<i>fnbA</i>		<i>Can</i>	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
<b>Positive (n= 55)</b>	14 (25.5%)	41 (74.5%)	39 (70.9%)	16 (29.1%)	22 (40%)	33 (60%)	31 (56.4%)	24 (43.6%)	14 (25.5%)	41 (74.5%)
<b>Negative (n= 22)</b>	1 (4.5%)	21 (95.5%)	9 (40.9%)	13 (59.1%)	0 (0%)	22 (100%)	2 (9.1%)	20 (90.9%)	2 (9.1%)	20 (90.9%)
<b>P-value</b>	0.054*		0.02**		0.000*		0.000**		0.132*	

\* Fisher's exact tests

\*\* Pearson Chi-square

**Table 3.** The relationship of MRSA and MSSA with the presence of biofilm-related genes

Genes	MRSA (n= 49)	MSSA (n= 28)	P-value
<i>icaA</i>	Positive	12 (24.5 %)	0.142**
	Negative	37 (75.5 %)	
<i>icaD</i>	Positive	29 (59.2 %)	0.45**
	Negative	20 (40.8 %)	
<i>clfA</i>	Positive	17 (34.7 %)	0.116**
	Negative	32 (65.3 %)	
<i>fnbA</i>	Positive	23 (46.9 %)	0.338**
	Negative	26 (53.1 %)	
<i>cna</i>	Positive	11 (22.4 %)	0.633**
	Negative	38 (77.6 %)	

\*\* Pearson Chi-square

**Table 4.** Patterns observed from gene

Related genes patterns	Number in pattern	MRSA / MSSA	Biofilm + / Biofilm -
<i>icaA</i>	3	2 / 1	2 / 1
<i>icaA – icaD</i>	4	3 / 1	4 / 0
<i>icaA – fnbA</i>	2	2 / 0	2 / 0
<i>icaA – icaD – fnbA</i>	3	3 / 0	3 / 0
<i>icaA – icaD – clfA</i>	3	2 / 1	3 / 0
<i>icaA – icaD – fnbA – clfA</i>	2	2 / 0	2 / 0
<i>icaD – fnbA – clfA – can</i>	3	2 / 1	3 / 0
<i>icaD</i>	10	4 / 6	3 / 7
<i>icaD – fnbA</i>	11	5 / 6	10 / 1
<i>icaD – clfA</i>	2	2 / 0	2 / 0
<i>icaD – can</i>	4	2 / 2	3 / 1
<i>icaD – fnbA – clfA</i>	5	3 / 2	5 / 0
<i>icaD – clfA – can</i>	3	3 / 0	3 / 0
<i>fnbA – clfA – can</i>	2	2 / 0	2 / 0
<i>fnbA</i>	3	2 / 1	2 / 1
<i>fnbA – clfA</i>	2	2 / 0	2 / 0
<i>fnbA – can</i>	2	1 / 1	2 / 0
<i>clfA</i>	1	0 / 1	1 / 0
<i>clfA – cna</i>	1	1 / 0	1 / 0
<i>Can</i>	1	0 / 1	0 / 1
without genes	12	7 / 5	2 / 0

According to the results of this study, 20 different patterns were observed for the distribution of studied genes in the isolates. None of the isolates had all genes simultaneously. Two genes were observed in 28 isolates, three genes in 16 isolates, and 4 genes in 5 isolates simultaneously (Table 4).

## Discussion

Biofilm-producing *S. aureus* isolates cause bacterial resistance to antibiotics and host immune response, and cause chronic infections (22). Biofilm formation was observed in 71.4% of studied isolates, which was similar to the studies of Yousefi and Goudarzi et al. (23,24). Moreover, the ability to produce biofilm was higher in MRSA isolates,

Overall, the results of this study showed that the highest rate of gene isolation was related to *icaD* and *fnbA*, in 64.9% and 45.5% of *S. aureus* isolates, respectively. In terms of gene prevalence, their frequency rate was more than that of the study by Nourbakhsh et al. (27). and lower than that of the study by Demir et al. (28). The *icaA* gene was

compared to MSSA isolates, consistent with the findings of a study performed by Goodarzi et al. (24). In a study by Zalipour et al., the rate of biofilm formation in *S. aureus* isolates was 54.4% by the Congo Red Method (25). Karki et al. showed the ability to produce biofilm in more than 90% among *S. aureus* isolates (26). Differences in the biofilm properties of isolates among various studies might be due to the origins of bacteria, the geographical area, and genetic characterization. Several studies have shown that biofilm formation ability in *S. aureus* isolates is associated with the presence of *ica* locus (especially *icaA* and *icaD*) and *MSCRAMMs* proteins (14,16).

detected in 19.5% of the isolates, which is close to the results of a study by Keikhaie et al. (29). In the study of Azmi et al., the rate of gene *clfA* isolation was higher than that of our study (30). The lowest frequency was related to the *cna* gene, which is similar to Garcia et al. (31). In the present study, there was a significant association between the

attendance of *fnbA*, *clfA*, *icaD* genes and biofilm formation in *S. aureus* isolates; however, this connection was not observed for *icaA* and the *cna* gene. According to the results of this study, the frequency of the *icaA*, *icaD*, *fnbA*, *clfA*, and *cna* genes in *MRSA* and *MSSA* isolates was lower than that of the study by Ghasemian et al., and the statistical difference between *MRSA* and *MSSA* regarding the frequency of the genes (*clfA*, *fnbA*, *cna*, *icaD*, *icaA*) was not significant (32).

*S. aureus* is one of the bacteria that is of particular importance in nosocomial infections, especially surgical site infections. Biofilm formation can lead to increased pathogenicity, protection of microorganisms against the host's defense system and antibacterial agents, and the lack of early recovery of such patients. Therefore, it is necessary to observe the hygienic standards and proper sterilization of treatment tools to prevent the formation of biofilms in these infections.

### Conclusions

The results of the present study showed that the most common genes associated with biofilm were *icaD* and *fnbA*. There was a significant association between the presence of genes and biofilm formation in *S. aureus* isolates, but this connection was not observed for the *cna* gene.

### Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the University of Medical Sciences of Yasuj, Iran (Ethics Code: IR.YUMS.REC.1397.124). Written informed consent was obtained from all participants before their enrollment in the study.

### Consent for Publication

Not applicable.

### Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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### Authors' Contribution

ZSN contributed to the study design, supervised data acquisition procedures, and drafted the initial manuscript. FM conceived and designed the study, supervised the entire research process, interpreted the results, and was the primary contributor to the writing and revision of the final manuscript. The authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

### Conflict of Interest

All authors declared that there are no conflicts of interest to report.

### Declaration of Generative Artificial Intelligence (AI) in Scientific Writing

Artificial intelligence was not used at any stage of this study.

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