

Detection of Intercellular Adhesion Genes (*icaA* and *icaD*) in *Staphylococcus aureus* Clinical Isolates in Zabol- Iran

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Abstract

Background: The nosocomial infections that cause the establishment of biofilms on the embedded biomedical surfaces are the leading cause of sepsis and are often related to colonization of implants by *Staphylococcus epidermidis*.

Materials and Methods: A total of 40 clinical *S. aureus* isolates were collected from Zabol, Iran. The ability of these strains to form biofilm was determined by microliter tissue culture plates. All clinical isolates were tested for the presence of *icaA* and *icaD* genes by Polymerase Chain Reaction (PCR).

Results: The outcome of this study showed that from 40 isolates of *Staphylococcus aureus*, 12 (30%) of them were contained *icaA* gene and 8 (20%) isolates were positive for *icaD* gene and five (12.5%) isolates were contained both genes.

Conclusion: The capability of *S. aureus* clinical isolates to form biofilm is different/diverse. This may be caused by the differences in the containing of biofilm- related genes, genetic make-up and physiological situation.

Keywords: Biofilm, *Staphylococcus aureus*; *icaA*; *icaD*; Zabol

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Introduction

Biofilm formation is a conservative mode of growth that permits microorganisms to survive in hostile environments. Unwelcome biofilm formation leads to biofouling of heat alteration systems and pelagian structures, microbial aroused corrosion of metal sheets, deterioration of dental surfaces, impurity of household outputs including food and pharmaceuticals as well as the infection of short- and long-time biomedical implants and devices (1, 2, 3). Staphylococci are known as the most repetitive causes of biofilm-associated infections. This exclusive status among biofilm-associated pathogens is due to the fact that staphylococci are frequent mess bacteria on the human body skin and mucous surfaces (and those of many other lagomorphs. So, Staphylococci are among the most likely germs to infect any medical device that penetrates those surfaces, like when being inserted during surgery (4). For a long time, research on the molecular basis of biofilm

constitution was constructed on gram-negative pathogens, predominantly *Pseudomonas aeruginosa*, which is more easily available to molecular genetic research. More recently, advances in staphylococcal molecular biology have permitted researchers to define the molecular basis of biofilm constitution in staphylococci. In a staphylococcus in vitro habitation biofilm model, four separable metabolic states were identified: aerobically growing cells, fermentation, dormant cells (including very slow growing cells and persists (or dead cells. The cells exposed to the upside air oxygen-rich space and lower liquid-nutrient-rich interface were metabolically active (5). Although heterogeneity of biofilm protein expression was demonstrated with multiple cell wall-associated proteins. Expression was indicated to turn in cell bunches throughout the biofilm, and in one case, differential expression was observed on a cell-cell basis (6). The importance of biofilm output for the

epidemic of *S. aureus* was confirmed by a number of clinical and animal studies (7). Cell density and biofilm density were mediated by the outputs of a gene locus composing of the genes *icaADB* and *C*, that coded the necessary proteins for the production of polysaccharide intercellular stick (PIA) and capsular polysaccharide/adhesion (PS/A) in *Staphylococcus* spp. (8, 1). It was displayed that the *ica*-encoded genes are responsible for the biosynthesis of the PIA that includes N-acetylglucosamine as an original constituent and in the accumulation phase of biofilm configuration, playing a categorical role in invasiveness of *S. aureus* (9). Different researches have illustrated the fundamental role of the *ica* gene as virulence factors in staphylococcal infections (10). The aim of the present study was to investigate a variety of *Staphylococcus aureus* strains.

Materials and methods

Forty isolates from 300 patients were collected from a hospital in Zabol. The isolates were collected quarterly from infected males. Ten microliters (10 µl) of each sample were cultured on blood agar. Separable gram- and catalase-positive cocci were further tested for biochemical specification. Following the biochemical test, *Staphylococcal* isolates were determined to gain species-specific gene expansion (16S rDNA, *icaA*, *icaD*). The primers used for PCR are shown in Table 1.

Table 1. List of primers used in this study

Primers	Sequences	Product size
<i>icaA</i> -F	5'-ACACTTGCTGGCGCAGTCCA-3'	188
<i>icaA</i> -R	5'-TCTGGAACCAACATCCAACA-3'	
<i>icaD</i> -F	5'-ATGGTCAACCCAGACAGAG-3'	198
<i>icaD</i> -R	5'-AGTATTTTCAATGTTTAAAGCAA-3'	

The reaction mixture of PCR (25 µl), included 2 µl of dNTP (200 µl, 2.5 µl of 10 X Taq buffer containing 15 mM MgCl₂, 1 µl of every oligonucleotide onward and each primer (10 pm/µl), 0.35 µl of Taq DNA Polymerase (3 u/µl), 1 µl DNA (30 mg/µl) and distilled water (17.2 µl). All tests were repeated at least three times. Statistical analyses were performed using SPSS and Excel 2010.

Table 2. The prevalence of *icaA* and *icaD*.

Gene	<i>icaA</i>	<i>icaD</i>	<i>icaA+icaD</i>
No. (%)	12 (30%)	8 (20%)	5 (12.5%)

Results

The results of this study showed that 12 (30%) *S. aureus* isolates carried *icaA* gene, while 8 (20%) of the isolates were contained *icaD* gene. However, five

(12.5%) isolates were positive for both *icaA* and *icaD* genes. (Table 2). Also, the results of PCR were shown in figure (1).

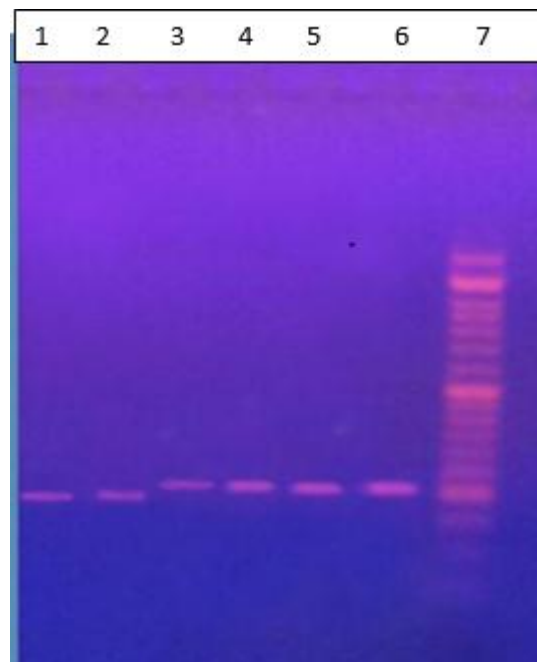


Figure 1. Gel electrophoresis of PCR products. Line 1 and 2, *icaA*; Line 3-6, *icaD*; Line 7, 100 bp DNA marker.

Discussion

Staphylococcus aureus has long been distinguished as the leading cause of body skin and soft tissue infections including abscesses, atopic dermatitis, carbuncles, cellulitis, furuncles, folliculitis, impetigo, pemphigus, and psoriasis. In particular, necrotizing soft-tissue infections may be rapidly fatal because of the toxin-induced circulatory collapse. It has been shown that the biofilm producer strains of this bacterium are the cause of additional “chronic polymer-associated” infection. Cramton et al (1999) demonstrated the presence of *icaA* gene in *S. aureus* strains (11), Arciola et al (2001) have been explained 23 *S. aureus* isolated from 14 patients with catheter-associated infections that were able to form a thick layer biofilm based on the presence of *icaA* and *icaD* genes (9). Even though *icaA* is required to encode N-acetylglucosaminyl transmission, co-expression of *icaD* can increase the capsular polysaccharide phenotypes (12), and signal important part of the *icaD* locus as a virulence element in the pathogenesis of *S. aureus* separated from catheters (13, 14). The study of Eftekhari and Dadaei (2011), indicated that 53.3% of the isolates had the potential to constitute biofilm by colony morphology of which, 75% carried the *ica* operon. Infirm biofilm output was seen in the Media Transfer Protocol assay by 57.8%, of which

53.8% harbored the *ica* operon. However, about 70% of biofilm non-producer isolates contained the *ica* operon (15). The research of Mirzaee et al (2014), showed that twelve (38.7%) isolates were strong biofilm producers. Their results indicated that 18 (80.6%) *S. aureus* isolates harbored *icaD* gene, whereas the prevalence of *icaA*, *icaB* and *icaC* were 51.6%, 45.1% and 77.4%, respectively (16). A higher rate of biofilm formation was reported by Gad et al (2009) where 83.3% of *S. aureus* isolated from urinary catheterized patients were the biofilm producer by the Media Transfer Protocol assay (17). The inconsistency across various studies might be due to heterogeneity in the origins of bacteria such as genetic characterization, source of isolation and environmental conditions.

Ciftci et al (2009) isolated 59 strains from mastitis of which 16 isolates were positive for *icaA* and 38 isolates were contained *icaD* gene. Iorio et al (2011) in another study showed that from 47 *S. aureus* isolated from the blood samples, 40 isolates carried *icaA* gene (18, 19, 20). The emergence of resistant strains with the ability to form biofilm in the hospital environment can be considered as a serious threat for patients, especially in immunocompromised persons who are undergoing aggressive treatments such as the use of mechanical ventilator. This phenomenon can result in increased morbidity and mortality in the hospital environment. On the other hand, bacteria in biofilms are able to transfer different mechanisms of drug resistance. The development of resistance among bacteria causes infection and is a serious threat to patients who are hospitalized in health care institutions. Given the importance of *S. aureus* in medical centers and the ability of this bacteria to cause numerous infections, preventing biofilm formation on medical instruments is in a high priority.

Conclusion

The biofilm formation ability of one isolate in the lack of *icaAD* genes highlights the significance of major genetic researches on *ica* independent biofilm formation mechanisms and other *ica* genes.

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Author contributions

All authors of this article have the same contribution to perform this project.

Conflict of Interest

The authors declare no conflict of interest.

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