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Research Article

Biofilm Formation and Detection of the icaA Gene in Staphylococcus Aureus Isolates from Ocular Infections

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Abstract

Background: Staphylococcus aureus is a major opportunistic pathogen implicated in various infections, including ocular infections. One of its key virulence factors is the ability to form biofilms, which are structured bacterial communities embedded in a self-produced extracellular matrix. Biofilm formation enhances bacterial survival under adverse conditions, including antibiotic exposure and host immune defenses, thereby contributing to chronic and recurrent infections. *Aim:* This study aimed to evaluate the biofilm-forming ability of *S. aureus* isolates obtained from ocular infections using the Congo Red Agar (CRA) method and to detect the presence of the biofilm-associated *icaA* gene using polymerase chain reaction (PCR). *Methods:* A total of 35 clinical isolates of *S. aureus* were included in this study. Biofilm formation was assessed phenotypically using the Congo Red Agar (CRA) method. Based on the results, isolates were categorized into strong, moderate, weak, and non-biofilm producers. Additionally, PCR was performed on 10 selected isolates to detect the presence of the *icaA* gene, a key determinant involved in biofilm formation. *Results:* Out of the 35 isolates, 10 (28.6%) exhibited strong biofilm formation, 15 (42.8%) showed moderate formation, 5 (14.3%) demonstrated weak formation, and 5 (14.3%) were non-biofilm producers. Overall, 71.4% of the isolates displayed varying degrees of biofilm production, indicating a high prevalence of this virulence trait. PCR analysis revealed that 7 out of 10 tested isolates (70%) were positive for the *icaA* gene. A correlation was observed between phenotypic biofilm formation and the presence of the *icaA* gene. *Conclusion:* The findings demonstrate a high prevalence of biofilm-forming *S. aureus* isolates in ocular infections and highlight the role of the *icaA* gene in biofilm production. The observed correlation between phenotypic and genotypic methods suggests that molecular detection can serve as a reliable complementary tool. Identification of biofilm-producing strains is essential for improving treatment strategies and reducing the risk of persistent and recurrent infections associated with biofilm-mediated antibiotic resistance.

Keywords

Biofilms, Staphylococcus Aureus, icaA, PCR, Ocular Infection

1. Introduction

Biofilms are structured microbial communities that attach to living or non-living surfaces and are embedded within a self-produced extracellular polymeric substance (EPS). This matrix is mainly composed of polysaccharides, proteins, lipids, and extracellular DNA, which collectively provide structural integrity and protection against environmental and host-related stresses [6]. In recent years, biofilms have become a major focus in clinical microbiology because of their significant

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role in chronic and recurrent infections, especially those associated with implanted medical devices and mucosal tissues [10].

Among gram-positive pathogens, *Staphylococcus aureus* is particularly well known for its capacity to form strong biofilms on both abiotic and biotic surfaces, a characteristic that markedly enhances its virulence and tolerance to antimicrobial agents [17]. Infections caused by biofilm-producing *S. aureus* are often challenging to eradicate and frequently require extended courses of antibiotic therapy or surgical removal of infected tissues. In ocular infections, including conjunctivitis, keratitis, and endophthalmitis, biofilm formation significantly compromises treatment efficacy by limiting antibiotic penetration and shielding bacterial cells from host immune responses [4].

The Congo Red Agar (CRA) method is one of the most commonly applied phenotypic techniques for identifying biofilm production. This medium contains Congo red dye and sucrose, allowing biofilm-producing strains to form black, dry, crystalline colonies as a result of polysaccharide intercellular adhesin (PIA) production, whereas non-biofilm-forming strains typically produce smooth red colonies [3]. Despite its simplicity and low cost, several studies have raised concerns regarding the sensitivity and reproducibility of the CRA method. [13], for example, reported that CRA detected biofilm formation in only 3.8% of *S. aureus* isolates, whereas microtiter plate assays demonstrated substantially higher detection rates.

At the genetic level, biofilm development in *S. aureus* is strongly linked to the intercellular adhesion (ica) operon, particularly the *icaA* gene. The *icaADBC* operon encodes enzymes responsible for the synthesis of PIA, a key component involved in cell-to-cell adhesion and biofilm matrix stabilization [7]. Specifically, the *icaA* gene encodes N-acetylglucosaminyltransferase, which initiates the polymerization of N-acetylglucosamine units required for PIA formation. The expression of *icaA* is modulated by environmental conditions and regulated through global regulatory systems such as the accessory gene regulator (*agr*) and sigma B (σ^B) pathways [2].

Polymerase chain reaction (PCR)-based detection of the *icaA* gene has therefore emerged as an important molecular approach for evaluating the biofilm-forming potential of clinical isolates. Several recent investigations have demonstrated a strong association between the presence of *icaA* and biofilm production in ocular *S. aureus* isolates [12]. reported that approximately 67% of ocular isolates harboring the *icaA* gene exhibited biofilm-forming activity. Nevertheless, biofilm formation has also been documented in certain *S. aureus* strains lacking *icaA*, suggesting the involvement of alternative genes or regulatory mechanisms in biofilm development [16].

Taken together, these observations highlight the importance of integrating both phenotypic and genotypic methods to achieve a more accurate assessment of biofilm production in clinical isolates. Accordingly, the present study aims to investigate the prevalence of biofilm formation among ocular *S. aureus* isolates using the CRA method and to determine the presence of the *icaA* gene by PCR. By correlating phenotypic findings with molecular data, this study seeks to improve the understanding of biofilm-associated pathogenicity in ocular infections and to support the development of more effective diagnostic and therapeutic strategies.

2. Materials and Methods

2.1. Sample Collection

Thirty-five *S. aureus* isolates were collected from patients with confirmed ocular infections. Identification was done using standard biochemical and molecular methods.

2.2. Ethical Management of the Study

The current study was carried out in compliance with guidance issued by the College of Veternary Medicine, University of Al-Qadisiyah. No banned biological materials or genetically modified organisms were included in the report. All *Staphylococcus aureus* isolates included in this study were obtained from patients at the Hospitals and clinics of eye Dept Consultation Once official approvals have been received by the doctor and the patient who allowed samples to be collected.

2.3. Congo Red Agar (CRA) Method

CRA plates were used to evaluate biofilm formation. Isolates were classified into four groups: strong, moderate, weak, and non-biofilm formers based on colony morphology.

2.4. PCR Detection of icaA Gene

Genomic DNA was extracted, and PCR was performed using primers specific for the icaA gene. Amplicons were analyzed by agarose gel electrophoresis.

3. Results

3.1. Biofilm Detection by CRA

The ability of the bacterial isolates to form biofilms was evaluated using the Congo Red Agar (CRA) method. Based on colony morphology, the isolates were classified into four categories as shown in Table 1. Out of the 35 isolates tested, 10 isolates (28.6%) showed a strong ability to form biofilms, 15 isolates (42.8%) demonstrated moderate biofilm-forming ability, 5 isolates (14.3%) exhibited weak biofilm formation, while 5 isolates (14.3%) were non-biofilm producers.

Overall, the results indicate that biofilm formation is a predominant characteristic among the tested isolates, with more

than 70% exhibiting some degree of biofilm-forming capability. Biofilm formation plays a critical role in bacterial virulence and antibiotic resistance, as it enhances bacterial survival by providing protection against environmental stressors, antimicrobial agents, and host immune responses (Figure 1).

Figure 1. *Representative CRA plates showing various biofilm categories.*

Table 1. *Biofilm Formation by CRA.*

Biofilm Category	Number of Isolates	Percentage (%)
Strong	10	28.6
Moderate	15	42.8
Weak	5	14.3
None	5	14.3
Total	35	100

3.2. icaA Gene Detection

The presence of the icaA gene among Staphylococcus aureus isolates was investigated using polymerase chain reaction (PCR). Out of the 10 tested S. aureus isolates, 7 isolates (70%) were positive for the icaA gene, while 3 isolates (30%) were negative, as illustrated in Figure 2. The icaA gene is a key component of the ica operon and is responsible for the synthesis of polysaccharide intercellular adhesin (PIA), which plays an essential role in biofilm formation. The detection of this gene supports the phenotypic findings obtained using the CRA method.

Table 2. *This 10 tested S. aureus isolates, 7 (70%) were positive for the icaA gene.*

icaA	F	GAGGTAAAGCCAACGCACTC	151	(Nourbakhsh and Namvar, 2016)
	R	CCTGTAACCGCACCAAGTTT		

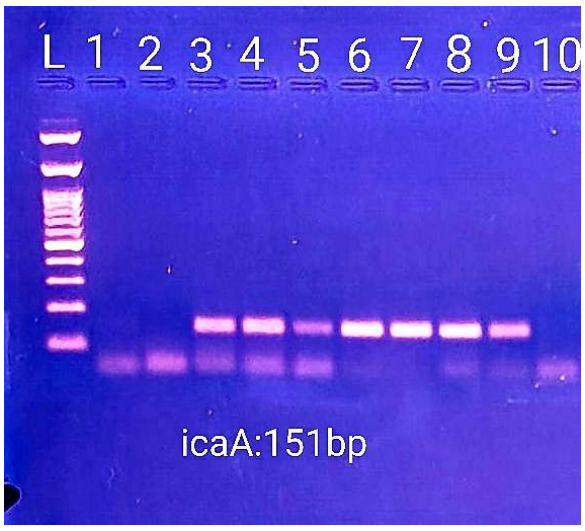

Figure 2. *DNA amplification of a bp for of S. aureus.*

detecting gene icaA using PCR. Lane 1:1 adder, lane 3, 4, 5, 6, 7, 8 and 9 positive results., lane 1: 3000bp marker (Ladder).

4. Discussion

The results of the present study demonstrate a high prevalence of biofilm formation among Staphylococcus aureus isolates recovered from ocular infections. Based on the Congo Red Agar (CRA) assay, 71.4% of the examined isolates exhibited varying degrees of biofilm-producing capacity. Similar prevalence rates have been reported in previous studies, in which biofilm formation among clinical S. aureus isolates ranged from 40% to 80% [1, 18]. These findings further emphasize the importance of biofilm formation as a major virulence factor in ocular infections, as it enhances bacterial persistence and contributes to decreased susceptibility to conventional antimicrobial therapies.

Despite its widespread use as an initial screening method due to its simplicity and low cost, the CRA assay has several well-documented limitations concerning its diagnostic accuracy. [13] reported that CRA exhibits low sensitivity, estimated at approximately 11%, although it retains high specificity (92%). Similarly, [11]. demonstrated that CRA failed to detect biofilm production in a substantial number of isolates previously confirmed as biofilm producers. Collectively, these

findings suggest that exclusive reliance on CRA may underestimate the actual biofilm-forming capacity of clinical isolates and increase the risk of false-negative results.

To address the limitations associated with phenotypic detection methods, molecular techniques such as polymerase chain reaction (PCR) have been increasingly employed to identify genes involved in biofilm formation. In the present study, PCR analysis revealed that 70% of the tested S. aureus isolates harbored the icaA gene. This gene is a key component of the icaADBC operon, which is responsible for the biosynthesis of polysaccharide intercellular adhesin (PIA), a crucial constituent of the biofilm extracellular matrix [7, 17]. The relatively high prevalence of icaA among the isolates examined in this study is consistent with previous reports demonstrating a strong correlation between icaA carriage and phenotypic biofilm production [2, 16].

Nevertheless, some isolates in the present study exhibited biofilm-forming ability despite the absence of the icaA gene, indicating that biofilm formation in S. aureus is not solely dependent on the icaADBC operon. Alternative genetic factors and regulatory systems, including bap, fnbA, and agr, have been implicated in biofilm development and maturation [5, 8]. Moreover, environmental parameters such as nutrient availability, osmolarity, and pH have been shown to influence gene expression and modulate biofilm phenotypes [14]. This multifactorial nature underscores the complexity of biofilm formation in S. aureus.

In conclusion, although the presence of the icaA gene serves as a valuable molecular marker for assessing biofilm-forming potential in S. aureus, it should not be considered the sole determinant. The combined application of phenotypic assays, such as CRA, alongside genotypic methods like PCR, provides a more accurate and comprehensive approach for the identification of biofilm-producing isolates. This integrated strategy is particularly important in clinical settings, where biofilm-associated infections pose substantial diagnostic and therapeutic challenges. Early and precise detection of biofilm-forming pathogens may facilitate optimized antimicrobial management, improve patient outcomes, and limit the spread of antimicrobial resistance [9, 15].

5. Conclusion

This study underscores the high prevalence of biofilm formation among *Staphylococcus aureus* isolates obtained from ocular infections, revealing that a significant proportion of these clinical isolates possess the capacity to form biofilms. Phenotypic detection through the Congo Red Agar (CRA) method demonstrated that over 70% of the isolates exhibited varying degrees of biofilm-forming ability. However, despite its convenience and low cost, CRA showed limitations in sensitivity and reliability, potentially leading to an underestimation of true biofilm prevalence. The molecular detection of the *icaA* gene, a key component of the *icaADBC* operon responsible for the synthesis of polysaccharide intercellular adhesin (PIA), confirmed its presence in 70% of the tested isolates. This finding affirms the strong association between *icaA* and biofilm formation and highlights the gene's diagnostic value as a molecular marker. Nevertheless, the absence of *icaA* in a subset of biofilm-positive isolates suggests that additional genetic determinants and regulatory pathways also contribute to biofilm development. Given the clinical significance of biofilms in antibiotic resistance and treatment failure, especially in ocular infections, integrating molecular diagnostics such as PCR for *icaA* detection into routine microbiological workflows can enhance the accuracy of diagnosis. Such integration will enable clinicians to tailor antimicrobial strategies more effectively, ultimately improving therapeutic outcomes and preventing chronic, biofilm-associated infections.

Abbreviations

CRA	Congo Red Agar
PCR	Polymerase Chain Reaction
EPS	Extracellular Polymeric Substance
PIA	Polysaccharide Intercellular Adhesin

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Author Contributions

Karar Ali Abdulkhuder: Conceptualization, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft

Mahmood Nabeel Awad: Investigation, Validation, Writing – review & editing

Data Availability Statement

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

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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