Detection (Icaa, Icab, Icac ,Icad) Genes of Staphylococcus Aureus Isolated from Diabetic Wound Infections

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Abstract:
Staphylococcus aureus' ability to develop biofilm and adhere to surfaces helps it be more resistant to antibiotics. The goal of this study is to look at the frequency of many genes that are involved in development (icaABCD genes) using PCR, diabetic wound infections using PCR, and biofilm formation using the microtiter plate method. Out of 164 swab samples obtained from diabetic wound infections, 48 were positive for S. aureus utilizing identification morphological and biochemical tests as well as diagnosis by the VITEK2 system. The findings revealed that (48) 100% of isolates were positive for (icaA, icaB, icaD) genes and (56.25%) for the icaC gene, indicating that isolates of S. aureus had varied biofilm formation abilities, with the presence of biofilm-related genes clearly showing the roles for ica genes as biofilm producer markers.
Keywords: icaABCD genes, S. aureus, diabetic wound infections, PCR, biofilm.

1. Introductions

Diabetes mellitus is a chronic health issue that has a major impact on a proportion of the human population. Surface infections in diabetic wounds result in the production of biofilms on the wound's surface, hindering normal physiological wound healing. Bacterial colonization in a wound due to pathogen contamination can result in localized infection, sepsis, systemic infection, and multi-organ failure (Kalita et al., 2018). Because of *S. aureus*' ability to develop biofilm and adhere to surfaces, it is more resistant to medicines (Idrees et al., 2021). Biofilm production is mediated by polysaccharide intracellular adhesion (PIA), which is encoded by the ica operon (icaABCD genes) (Vestby et al.,2020). The creation of polysaccharide intercellular adhesion is reliant on the expression of the intercellular adhesion (IcaADBC) operon, which encodes three membrane proteins (icaA, icaD, and icaC) in addition to one extracellular protein (icaB) (Mohammed *et al.*,2018). *S. aureus* clinical isolates have varying abilities to create biofilms, which may be due to variances in biofilm-related gene expression, genetic make-up, and physiological circumstances (Neopane et al., 2018).

2. Materials and Methods

Samples were taken from 164 diabetic wound infection patients for identification of *S.aureus* using standard techniques and detection of the ica gene using PCR. Specimens were cultivated on blood agar and mannitol salt agar and then examined using conventional microbiological techniques such as macroscopic and microscopic inspection, Gram stain, biochemical tests, and the VITEK-2 GP system (Hussein et al.,2019). Tissue culture plate biofilm test (microtiter plate technique) according to (Smith et al. ,2008) [1].
Molecular Analyses: DNA was "extracted from broth samples using genomic DNA" and purified using a "purification kit" (Promega, USA). For (ica gene) isolates, PCR was used to identify primers described in Table 1 .Primers for detecting (icaA, B, C, and D) genes were designed by (NCBI) Bioneer, Korea[2].

Table 1: Primers designed for the (ica genes).

Primer	Sequence	Bp size	Ref.
icaA F	TGATCCAAAACTTGGTGCAG	306bp	(NCBI) Bioneer Korea
icaA R	TCTGGAACCAACATCCAACA		
icaB F	TGCAGATGACGATCCACCTA	378 bp	
icaB R	TTTTCTTCCCCAACATGACC		
icaC F	CGGCATGATATTGCGTGAAT	566 bp	
icaC R	TGAAAACTGAAAAGCTGACTGA		
icaD F	ATGGTCAAGCCCAGACAGAG	235 bp	
icaD R	CGAAAATGCCCATAGTTTCAA		

Table 2: PCR amplification program for detecting (icaA,B,C,D gene).

Steps	Temperature and Time	Cycles
Pre-denaturation	94 °C/ 5min	1 cycle
Denaturation	94 °C/ 30sec	34cycles
Annealing A,B,C,D	54 °C/ 30sec	
Extension	72 °C/ 1 min	
Final extension	72 °C/ 10 min	1 cycle

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Table 3: The PCR reaction mixture constituent.	
Component	25 µl reaction volume
Master mix	4µl
Template DNA	12.5µl
Forward primer (10 pmole,ul)	2µl
Reverse primer (10pmole,ul)	2µl
Free nuclease distilled water	4.5µl

3. Results and Discussions

The results of S.aureus biofilm formation showed that 43/48 (89.58%) of isolates producing biofilm were grouped based on the OD630 of the bacterial biofilm: (18.75%) were weak biofilm formation, (39.58%) were moderate, (31.25%) were strong biofilm formation, and (10.42%) were not produced, table 4 and figure 1[3].

Table 4: Biofilm formation outcomes using plate technique based on OD value.		
Percentage%	Mean (%)	Biofilm -forming ability
18.75 %	9	Weak
39.58 %	19	Moderate
31.25 %	15	Strong
10.42 %	5	Non produced
100%	48	Total

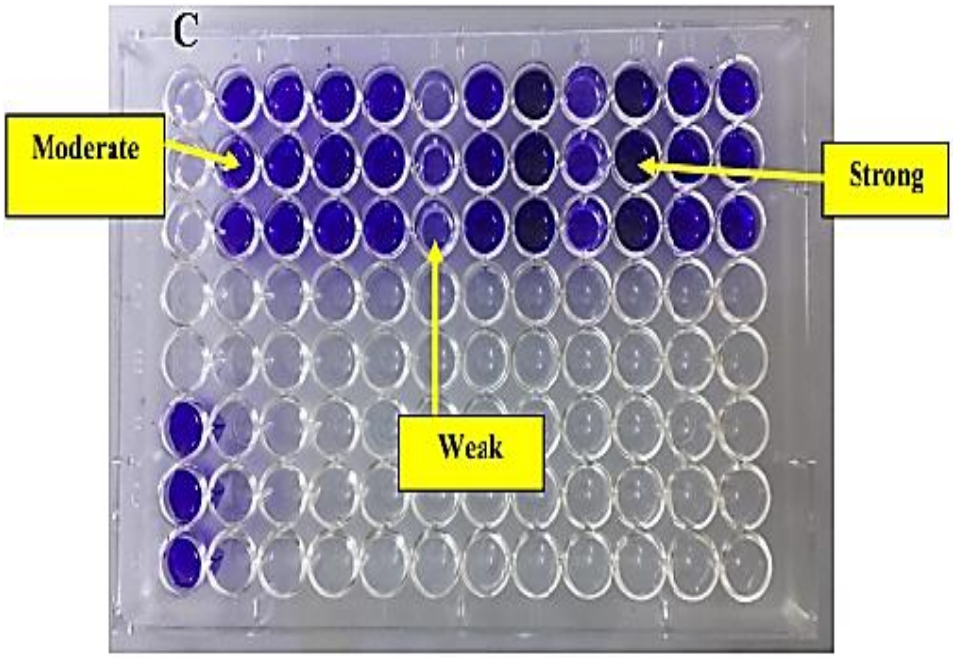

Figure 1: BHIB microtiter plate biofilm development, C: control.

a. The current study found that 43/48 (89.58%) of isolates produced biofilm, which agreed with the findings of Jahanshahi et al. (2018) in Iran, which found that biofilm formation among MRSA isolates was 84.7%, with results showing 17.3%, 41.3%, and 20% formation for weak, moderate, and strong biofilm developers, respectively. Biofilm development has been linked to antimicrobial resistance and is thought to be a virulence factor. This is crucial to the chronic infection's persistence. According to published research, the prevalence of biofilm development among S. aureus can range from 43–88% (Luther et al., 2018)[4].
b. The results of biofilm gene detection revealed that (48) 100% of isolates were positive for (icaA, icaB, icaD) genes and (56.25%) for the icaC gene, indicating that clinical S. aureus isolates displayed varying abilities to produce biofilm, with the presence of the related genes indicating the roles for ica genes as biofilm producer markers (table 5 and figures 2,3,4,5).

\Table 5: The prevalence of (*icaA, B, C, D*) genes in *S. aureus* isolates.			
Genes	No. and % of +ve isolates%	No. and % of -ve isolates%	Total (%)
icaA	48 (100%)	0%	100%
icaB	48 (100%)	0%	100%
icaC	27 (56.25%)	21 (43.75%)	100%
icaD	48 (100%)	0%	100%

In previous research (Ghasemian et al, 2015), the occurrence of icaA, B, C, and D was 73%, 63.7%, 73%, and 73%, respectively. Disparities in the frequency of icaADBC genes in investigations might be related to epidemiological differences as well as the time periods during which these samples were collected. Another study from Iran, Mirzaee et al. (2015), found that the frequency of icaA, icaB, icaC, and icaD was (63.7%), (63.7%), (90.1%), and (100%), respectively, and strains had distinct potential for biofilm formation. This might be due to a variety of reasons, including physiological and structural circumstances, rather than changes in biofilm-related genes. S. aureus biofilm formation is a two-step process that begins with attachment and continues with maturity. Several physiological variables influence these processes, which necessitate step-specific components and ambient conditions (Mirzaee et al., 2015). Another study by (Goudarz et al., 2019) found that the most common biofilm forming gene was icaD (77.3%), followed by icaA (76%), icaB (57.3%), and icaC (50.7%), suggesting that biofilm development may not happen under in vitro conditions despite the existence of the ica genes, showing that isolates with greater adhesion and ica producing genes were accurately related with biofilm formation and strain identification (Goudarz et al., 2019). These findings confirm the significance of ica genes as biofilm producer indicators and biofilm formation determinants in S. aureus, especially in MRSA isolates.

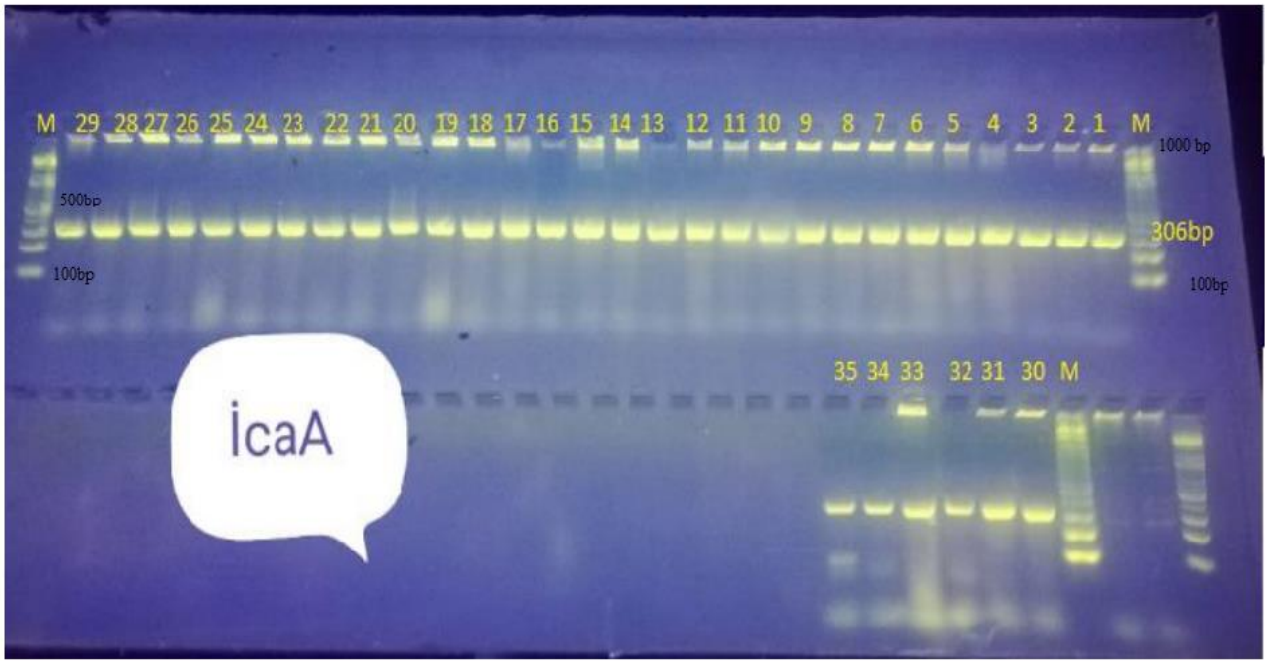

Figure (2) : S. aureus biofilm gene (icaA) is produced by agarose gel electrophoresis of PCR amplification. Amplification products of 306 bp are expected (lanes1-35: samples, lane M: marker 1000bp, 1%agrose gel, and 90 volts for 45 minutes in TBE buffers).

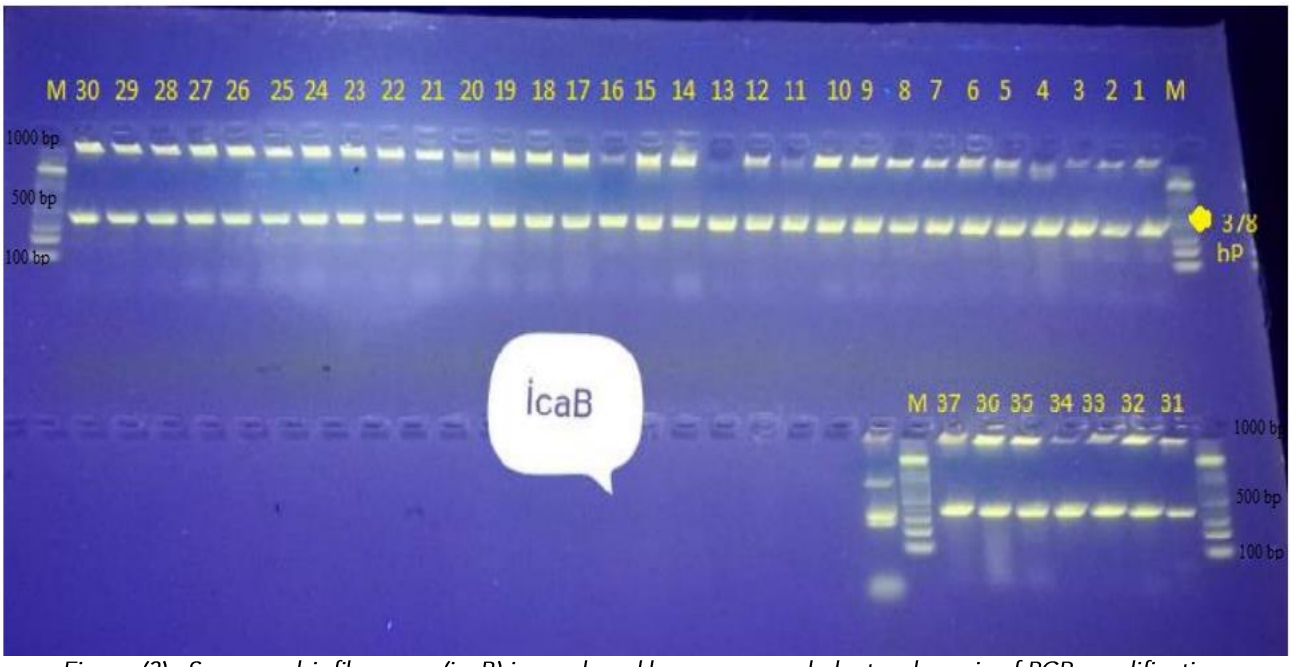

Figure (3) : S. aureus biofilm gene (icaB) is produced by agarose gel electrophoresis of PCR amplification. Amplification products of 378bp are expected (lanes1-37: samples, lane M: marker 1000bp, 1%agrose gel, and 90 volts for 45 minutes in TBE buffers).

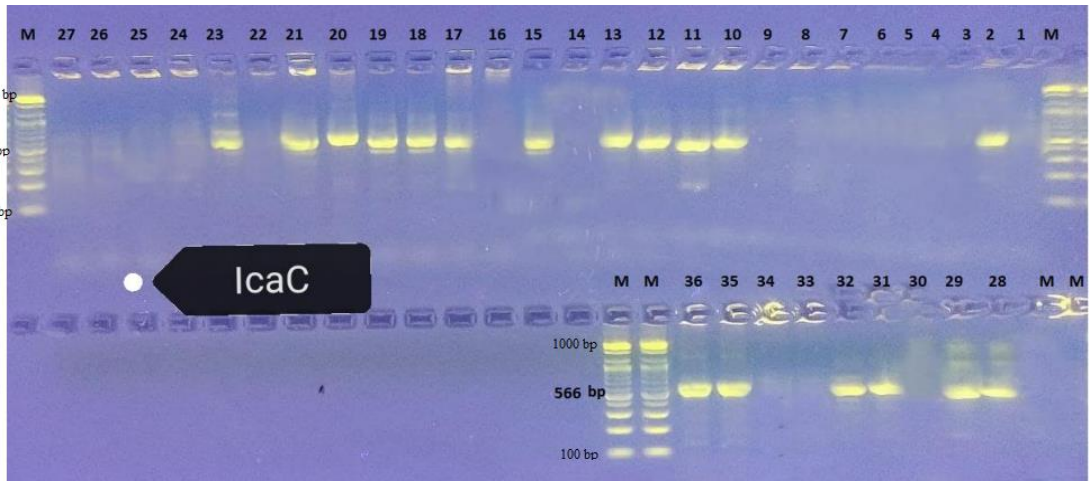

Figure (4) : S. aureus biofilm gene (icaC) is produced by agarose gel electrophoresis of PCR amplification. Expected amplification products are 566 bp (lanes1-36:samples, lane M: marker 1000 bp, 1% agarose gel, and 90 volts for 45 min. in TBE buffers.

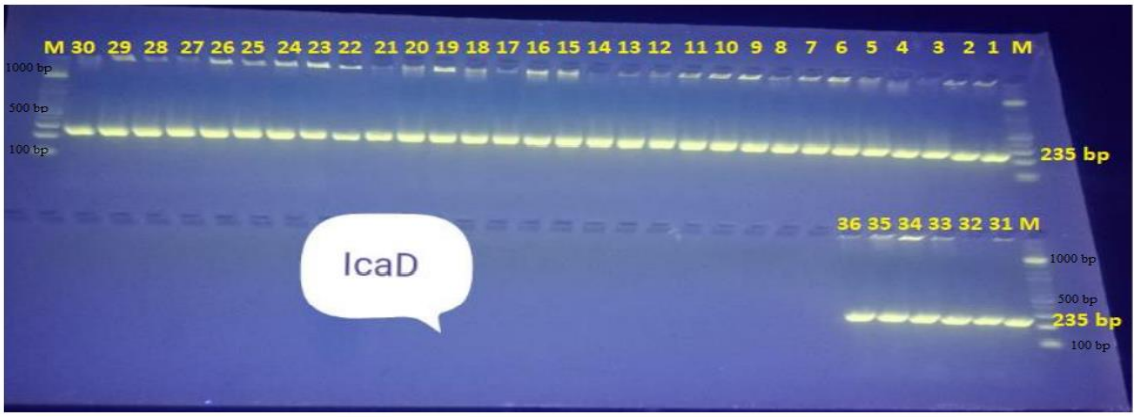

Figure (5) : Agarose gel electrophoresis of PCR amplification produces biofilm gene (icaD) S. aureus. Expected amplification products are 235bp (lanes1-36:samples, lane M: marker 1000bp, 1%agrose gel, and 90 volts for 45 min. in TBE buffers.

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