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Detection of genes responsible for virulence factors in *Staphylococcus aureus* isolated from Tikrit hospital

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ABSTRACT

Staphylococcus aureus is a non-motile, non-spore-forming, Gram-positive bacterium that is found commensally in approximately 20%–30% of the world's population. This study aimed to identify three of the most prominent genes responsible for biofilm formation: *icaA*, *fnbB*, and *Bap*. Clinical swabs were collected from Tikrit Teaching Hospital from corridors, wards, and operating rooms between January 2024 and April 2024. The swabs were cultured on blood agar and mannitol, incubated for 24 hours at 37°C, and then confirmed by biochemical tests. The *icaA*, *fnbB*, and *Bap* genes were detected using polymerase chain reaction (PCR). The results showed that the *fnbB* gene was the most prevalent in the *Staphylococcus aureus* isolates (100%), followed by the *icaA* gene (80%), while the *Bap* gene was not detected in the studied isolates. Genes responsible for virulence, pathogenicity, and antibiotic resistance were more prevalent in hospital-associated *Staphylococcus aureus* isolates. The absence of the *bap* gene suggests a lack of biofilm formation among these strains. To improve clinical infection and treatment success, genes associated with biofilm formation and the virulence pathway should be targeted.

Keywords: *Staphylococcus aureus*, *bap*, *icaA*, PCR.

Introduction

Staphylococcus aureus (*S. aureus*) is a prevalent Gram-positive bacterium present in various settings and people. *S. aureus* is a non-spore-forming and non-motile Gram-positive bacteria that resides commensal in around 20%-30% of the humans' populations. *Staphylococcus aureus* is an human opportunistic pathogen responsible for both nosocomial and community-acquired infections. It is accountable for various infection based on the infection site and the virulence of the infecting strain [1]. The ability of *S. aureus* to generate a biofilm is a crucial mechanism of its survival and pathogenicity within the host, as the extracellular matrix of the biofilm aggregates the bacterial cells, hence improving their survival prospects within the host [2]. *S. aureus* can cause recurrent infections throughout chance due to its hemolytic ability, resulting from the production of the enzyme hemolysin. It produces coagulase, a characteristic that identifies *Staphylococcus aureus* isolates. This bacterium is positive for catalase and negative for oxidase. *S. aureus* produces enzymes and toxins, including protease, lipase, nuclease, hyaluronidase, hemolysin-beta, and collagenase, which promote bacterial growth and penetration into host tissues, ultimately increasing wound infection. Early

detection and appropriate wound care are crucial because the spread of *Staphylococcus aureus* to soft tissues and bone can significantly contribute to amputation [3].

The primary components of staphylococcal biofilms include polysaccharides, including poly N-acetyl glucosamine (PIA/PNAG), bacterial proteins found on the cell surface and those released, as well as extracellular DNA. PIA/PNAG is synthesized by the products of four genes, *icaADBC*, that are encoded in a single operon[4]. Regulation of *icaADBC* is complex and numerous regulatory factors have been implicated in control of *icaADBC*. Fibronectin-binding proteins contribute to the initial stage of biofilm formation, which is the initial attachment of bacterial cells to a host surface [5]. This binding provides an essential starting point for bacterial cell aggregation and biofilm formation. The *fnbB* genes also called fibronectin-binding proteins. The *fnbB* genes encode surface proteins known as fibronectin-binding proteins (FnbpA and FnbpB). These proteins belong to the MSCRAMM family of virulence factors that recognize matrix adhesin molecules[6]. Bap is a surface protein with multiple functions that has been linked to initial attachment and formation of biofilm in some strains of *Staphylococcus* spp., particularly those isolates from infections[7]. Bap is believed to promote intercellular adhesion and the formation of adhesive surface layers[8]. It is noted that the presence of the *bap* gene is not common in all *S. aureus* strains, as is the case with the *ica* genes, but when present, it can play an important role in a strain's ability to form biofilms[9]. In this study, three of the most prominent genes responsible for biofilm formation were identified: the *icaA*, *fnbB*, and *Bap*.

Methods

Bacterial diagnosis

Swabs were taken from the Tikrit Teaching Hospital's corridors, halls, and operating rooms over a period of approximately 3 months, starting from the beginning of January 2024. The swabs were cultured on blood and mannitol agar and incubated for 24 hours in 37 °C. The identification was confirmed by biochemical tests.

Bacterial DNA Extraction

DNA extraction from bacteria is an important step in detecting biofilm-forming virulence factors. Commercial kits from MacroGen are used to extract genomic DNA from bacteria, such as the Axen™ Genomic DNA Extraction Kit. The extraction was carried out depending on instruction provided in the kit.

Primers used in the study

The primers were ordered from the Korean company MacroGen, and the primer sequences were determined according to what was stated in Article (10,11,12,13). The table below shows the details of the primers.

Primer name and sequences			PCR product	Annealing T _m
<i>icaA</i>	F	TCTCTTGCAGGAGCAATCAA	188	56
	R	TCAGGCACTAACATCCAGCA		
<i>fnbB</i>	F	ACGCTCAAGGCGACGGCAAAG	197	58
	R	ACGCTCAAGGCGACGGCAAAG		
<i>Bap</i>	F	CCCTATATCGAAGGTGTAGAATTG	971	58
	R	GCTGTTGAAGTTAATACTGTACCTGC		

Polymerase Chain Reaction (PCR) Conditions

The process involved amplifying and multiplying the gene fragments to be detected using the PCR technique, according to the cycle program below, programmed on a BIO-RAD T100™ thermocycler, as follows:

Initial Denaturation: five minutes at 95°C. Denaturation: thirty seconds at 95°C. Annealing: thirty seconds at the appropriate temperature for each primer, as shown in the table above. After denaturation, the extension phase: 30 seconds at 72°C. The process concludes with a final extension phase: five minutes at 72°C.

Gel Electrophoresis

After the polymerase chain reaction (PCR) was completed, Cleave electrophoresis was performed, as shown in the figure below, and the migration products were then imaged. The steps included the following:

1. Preparation and dissolution of a 2% agarose gel. After cooling, Safe Green dye was added to the gel.
2. Loading the polymerase products into the gel wells, along with the gene marker.
3. Electrophoresis: The gel was electrophoresed to separate the DNA fragments according to the current and voltage programmed in the migration device.
4. The gel was imaged by using a Karl KOLB radiograph after the separation were completed,

Results

The results in figure (1) showed that *fnbB* gene were more prevalent in *S. aureus* isolates with 100% percentage, followed by *icaA* gene (80%), while the *Bap* gene was not occurred in studied isolates.

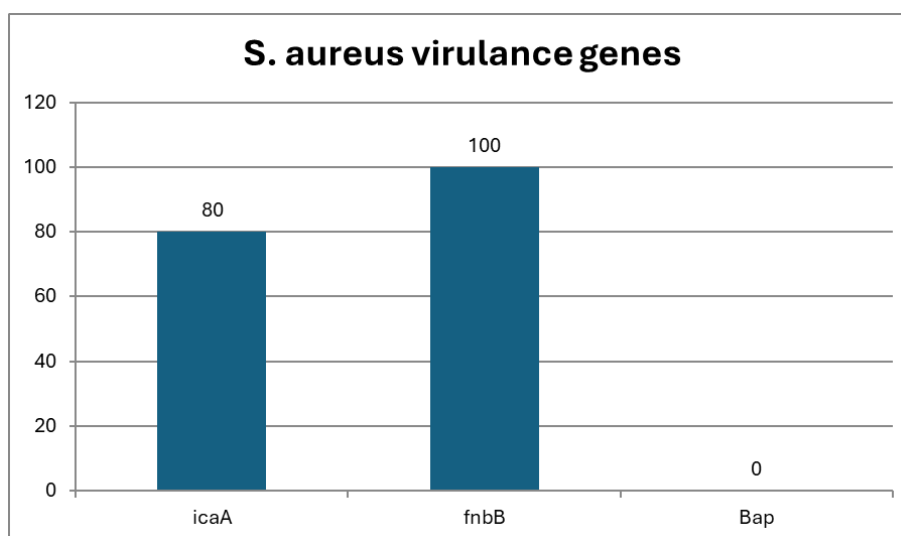


Fig. 1. Percentages of genes related with biofilm in *S. aureus*

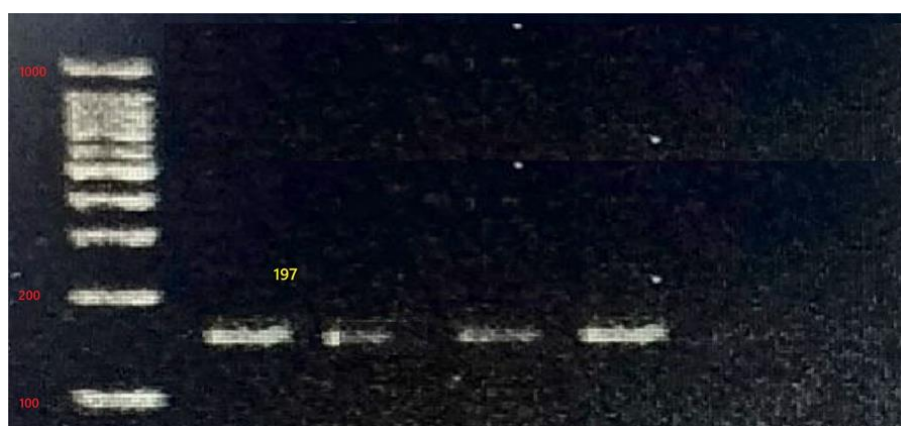


Fig. 2. The *fnbB* gene band on an agarose gel at 197bp.

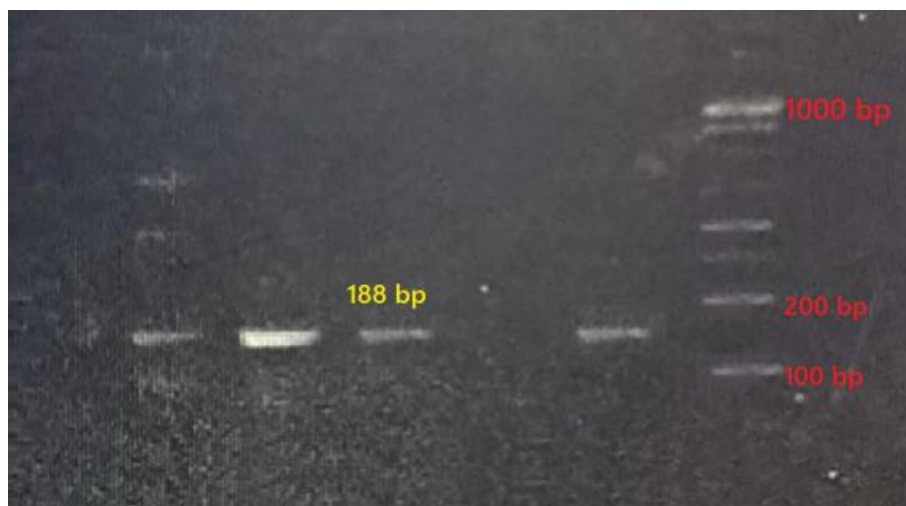


Fig. 3. The *icaA* gene band on an agarose gel at 188 bp.

Discussion

S. aureus was responsible of majority of hospital-acquired infections in which their biofilm formation and antibiotic resistance poses a significant challenge in the treatment of these infections [14].

icaA gene which was a part of *ica* gene cluster, was essential for the synthesis of PIA. The high presence of *icaA* in isolates suggests the capacity of bacteria for biofilm forming [15]. Biofilm makes the infections difficult to treat by protecting bacteria from antibiotics and the host immunity. The presence of the *fnbB* gene enables bacteria's adherence to host cells and tissue matrix. These genes is related with the increased antibiotics resistance in *S. aureus* isolates [16].

Recent investigates concur that *ica* genes, especially *icaA*, are pivotal regulators of PIA synthesis, the principal component of the biofilm matrix in *S. aureus*. This convergence substantiates the veracity of these findings. For example, O'Neill et al. [17] demonstrated that *icaA*-positive *S. aureus* strains typically posed a higher capacity to build biofilms compared to *icaA*-negative strains, resulting to enhanced resistance to many antibiotics, including methicillin and vancomycin [17]. Another study showed the prevalence of *icaADBC* in *S. aureus*, as all of eighty *S. aureus* isolates were positive for *icaADBC* with oligonucleotide specific for *icaA* gene of *S. aureus* [18].

Nashev et al., [19] discovered that *S. aureus* isolates exhibit positivity for the *fnbA* and *fnbB* genes. Out of the 44 isolates that were tested, 36.7% had *fnbB* genes. The present investigation found a large percentages of the *fnb* genes among the *S. aureus* isolates, suggesting that the ongoing occurrence of isolates containing these virulence genes may increased the risk for recurrent invasive infection in carrier. Another study revealed that the *fnbB* gene in *S. aureus* is different among strains. this suggest that the relationship between pathogenesis and a virulence factor is not likely to be a the same for all infection. This substantiates the need to broaden the investigation of staphylococcal pathogenesis from controlled laboratory settings to heterogeneous populations of clinically relevant isolates [3].

Arciola et al., [20] conducted a study on samples that were isolated from orthopedic illnesses. The results of the investigation showed that the percentage of the *fnbA* genes was 98%, while the percentage of the *fnbB* genes was 99%. It has been found that the *clfA* and *fnbA* genes have an influence on the pathogenicity of *S. aureus* in ear infections, with the frequency of these genes being 32% and 76%, respectively [21].

Conclusion

The study investigates the predominance of genes that related with biofilm formation in staphylococcus. To improve clinical infection and successful treatment, the genes associated with biofilm and virulence pathway should be targeted.

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