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Molecular investigation of biofilm genes in Staphylococcus epidermidis

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Abstract:

Biofilms play a major role in antibiotic resistance, making the resulting infections difficult to treat. Biofilms are a key factor in the successful survival of bacteria in complex environments such as those inside the human body, where they can protect them from damaging environmental factors and immune attack. The aim of study was to investigate some of the genes that make the biofilm, determine their sequence. Blood samples were collected from 100 patients undergoing chronic hemodialysis at AL-Diwaniyah General Hospital. Samples were achieved aseptically applying sterile syringes by trained phlebotomists following standard infection control protocols to minimize contamination risks. The results showed that Electrophoresis showed that 10 isolate (66.66%) belonging to the *S. epidermidis* species possessed the atle gene with a molecular weight of 682 base pairs (bp). The aap gene with a molecular weight of 466 base pairs, 9 isolates (60%) of the *S. epidermidis*. Also, it notes that 4 isolates (25.66%) of *S. epidermidis* encoded the bhp gene with a molecular weight of 1083 base pairs, while there are 7 isolates (46.66%) of *S. epidermidis* that possess the icaA gene with a molecular weight of 188 base pairs (bp).

Keywords: *Molecular investigation, biofilm, Staphylococcus epidermidis, PCR*

Introduction:

Gram-negative *Staphylococcus spp.* is an important bacterium that contributes to many urinary and respiratory tract infections, as well as skin and soft tissue infections. These bacteria have become a major cause of urinary tract infections, especially among kidney failure patients who undergo regular dialysis. These bacteria thrive in hospitals and medical centers, where they are likely to be transmitted between patients due to the specific sanitary and environmental conditions in those settings (Sastry and Kulkami 2016; Salman and Ahmed, 2025). Staphylococcus are spherical, Gram-positive bacteria with a diameter ranging from 0.5 to 1.5 µm. Their cells divide in multiple planes, forming bipartite, tetrapartite, or irregular grape-like clusters, hence the name "staphylococcus." They are non-motile, non-sporulating, and non-capsulated, except for a few species (Plata et al., 2009). Staphylococcus has the ability to grow

on a variety of culture media, utilizing carbohydrates and amino acids as carbon and energy sources. They grow in a wide temperature range of 10–40°C and pH of 4.8–9.4. It can grow in media containing 10% NaCl (Adnan et al., 2018).

Biofilm is Gram-negative staphylococci. It forms a complex structure of bacterial cells attached to surfaces, surrounded by a matrix of substances secreted by the bacteria themselves (Jamal et al., 2019). These biofilms play a major role in antibiotic resistance, making the resulting infections difficult to treat. Biofilms are a key factor in the successful survival of bacteria in complex environments such as those inside the human body, where they can protect them from damaging environmental factors and immune attack (Vestby et al., 2020; Abdulrazzaq and Ali, 2025). Biofilms have been shown to make bacteria more capable of adhering to the surfaces of medical devices, such as catheters and dialysis drums, facilitating recurrent and difficult-to-treat infections.

Hemodialysis patients typically have central venous catheters (CVCs), and bloodstream infections associated with vascular access devices are extremely common, with 70% of these cases being associated with CVCs specifically. While arteriovenous catheters (AVFs) and AVGs are the most commonly used in dialysis centers, only 19% of patients commonly use CVCs. When a pathogen enters the bloodstream, it can adhere to the catheter site or embed itself within the fibrin layer. Because catheters are considered inert medical products, the adhesion of microbes to their surfaces leads to the formation of biofilms—organized communities of microorganisms within an external polymeric matrix. Bacterial biofilms that form within the catheter lumen are a major source of catheter-associated infections (CAS) (Bergin et al., 2017; Abbood and Hateet, 2025).

Molecular characterization of biofilms in Gram-negative staphylococci is vital for understanding the mechanism of these bacteria's interaction with human plasma, particularly in patients undergoing hemodialysis (Sujana, 2013). Recent studies indicate that microorganisms may contribute to plasma clotting under certain conditions, such as persistent infections or prolonged use of medical devices (Jiménez et al., 2008). This study relies on advanced molecular techniques such as genetic analysis and chiral interaction to identify the chemical compounds and genes responsible for biofilm formation and their ability to interact with various substances in plasma. Therefore, the aim of study was to study some of the genes that make the biofilm, determine their sequence.

Materials and Methods:

Sample collection and initial culturing:

Blood samples were collected from 100 patients undergoing chronic hemodialysis at AL-Diwaniyah General Hospital between 2024 to March 2025. Samples were achieved aseptically applying sterile syringes by trained phlebotomists following standard infection control protocols to minimize contamination risks.

Each blood sample (8-10 mL) was immediately inoculated into aerobic and anaerobic blood culture bottles (BacT/ALERT® FA Plus and FN Plus, bioMérieux, Marcy-l'Étoile, France) and incubated in the BacT/ALERT® 3D automated microbial diagnosis system (bioMérieux) for up

to 5 days or until a positive signal for microbial growth was detected (Mahmood et al., 2024). This system was selected for its high sensitivity in detecting bloodstream pathogens in hemodialysis patients. Samples yielding positive growth signals were subcultured onto Blood Agar (Asan Pharmaceutical Co., Ltd., Seoul, South Korea) and MacConkey Agar (Becton Dickinson, Sparks, MD, USA) to isolate bacterial colonies.

Plates were incubated aerobically at 37°C for 24–48 hours. Preliminary identification of isolates was based on colony morphology, hemolytic patterns, and Gram staining characteristics. To ensure purity, single colonies were re cultured onto fresh media before further analysis. Negative cultures were re-incubated for an additional 48 hours to rule out slow-growing organisms.

Isolation and identification of coagulase-negative Staphylococci:

Isolates exhibiting Gram-positive cocci in clusters were subjected to a coagulase test to differentiate Staphylococcus species from Micrococcus species, following established protocols (Baker, 1984). The tube coagulase test was carried out applying rabbit plasma, with results read after 4 and 24 hours of incubation at 37°C. Isolates testing negative for coagulase were presumptively identified as coagulase-negative staphylococci (CoNS). Species-level identification was managed applying sugar fermentation tests, containing mannitol, sucrose, and trehalose, as described by Cunha et al. (2004). Additional biochemical tests, like novobiocin susceptibility and urease production, were carried out to distinguish between CoNS species, like S. epidermidis, S. haemolyticus, and S. saprophyticus (Vickers et al., 2007). Affirmed CoNS isolates were preserved for subsequent molecular and phenotypic analyses by suspending pure cultures in nutrient broth supplemented with 20% glycerol and storing at −80°C. This storage method ensured the viability of isolates for downstream experiments, containing antimicrobial susceptibility testing and genetic characterization.

Molecular characterization by 16S rRNA gene amplification:

To affirm the species identity of CoNS isolates, polymerase chain reaction (PCR) amplification of the 16S rRNA gene was carried out. Genomic DNA was extracted from overnight cultures applying a commercial DNA extraction kit (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany) based on the manufacturer's instructions. PCR was managed applying a Gradient Thermocycler (MJ Research, Watertown, MA, USA) with universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R

(5'TACGGYTACCTTGTTACGACTT-3') (Alpha DNA, Montreal, Canada), which target conserved regions of the bacterial 16S rRNA gene (Fredriksson et al., 2024). Each PCR reaction was prepared in a 50 µL volume containing 100 ng of template DNA, 25 µL of 2X Red Taq Master Mix (Ampliqon, Odense, Denmark), 20 pmol of each primer, and nuclease-free water.

The thermal cycling situations included an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 90 seconds, with a final extension at 72°C for 7 minutes. Amplicons (~1,500 bp) were separated on a 2% agarose gel stained with ethidium bromide and visualized

under UV illumination applying a gel documentation system (Bio-Rad, Hercules, CA, USA) to affirm successful amplification. PCR products were purified and sequenced bidirectionally (Sanger sequencing, Macrogen, Seoul, South Korea), and sequences were compared against the NCBI GenBank database applying BLAST to affirm species identity.

Results and Discussion:

Molecular detection of biofilm genes in *S. epidermidis*:

15 *S. epidermidis* isolates were selected, as this species is the most common in the study and also exhibits multiple resistances to antibiotics. These isolates were subjected to molecular analysis to detect the icaA, aap and bhp genes, which encode biofilm. PCR technology was used, and primers specific to the studied genes were used, targeting the specific sequences of the genes under study to detect the isolates possessing these genes. The results of the multiplex chain reaction were transferred onto an agarose gel. After exposure to ultraviolet light, the bands for each gene appeared at the same level as those of the studied bacterial isolates possessing these genes. This was due to the primers binding to complementary sequences in the DNA strand of each gene. Of the genes studied, the molecular weights of the above genes were determined using standard markers (base pairs) shown in lane M. The results showed that the studied isolates exhibited bands and molecular weights specific to each of the genes under study. The letter E was assigned as the symbol for *S. epidermidis* isolates.

Electrophoresis results showed that 10 isolate (66.66%) belonging to the *S. epidermidis* species possessed the atle gene with a molecular weight of 682 base pairs (bp) (Figure 1).

Regarding the aap gene with a molecular weight of 466 base pairs, 9 isolates (60%) of the *S. epidermidis* isolates encoded this gene (Figure 2). As for the fbe gene, 7 isolates (46.66%) of *S.epidermidis* encoded this gene with a molecular weight of 495 base pairs (Figure 3). We note that 4 isolates (25.66%) of *S.epidermidis* isolates encoded the bhp gene with a molecular weight of 1083 base pairs. While Figure 4-5 shows that there are 7 isolates (46.66%) of *S.epidermidis* species that possess the icaA gene with a molecular weight of 188 base pairs (bp).

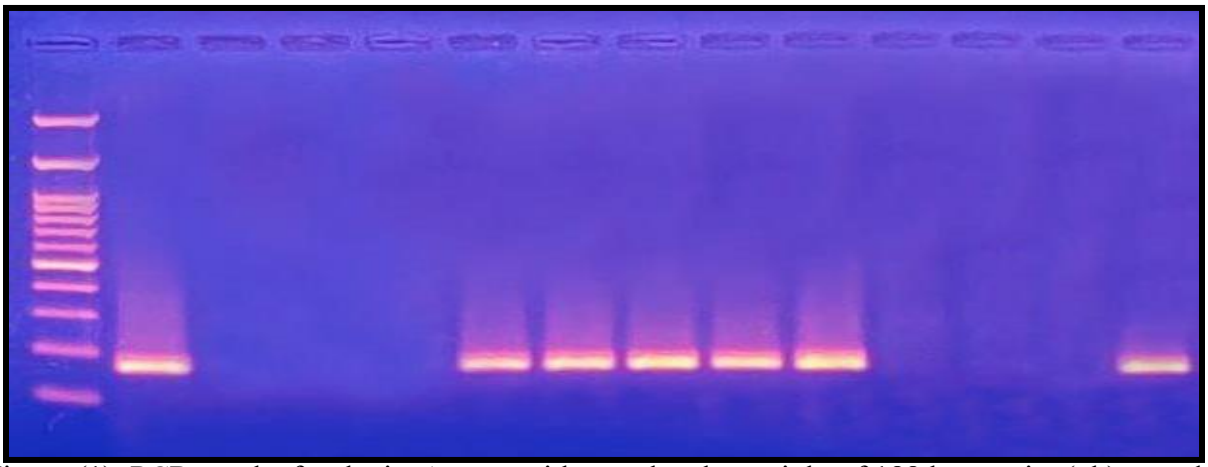

Figure (1): PCR results for the icaA gene with a molecular weight of 188 base pairs (pb) encoded by some *S. epidermidis* isolates. Electrophoresis on an agarose gel at a potential difference of 100 volts for 60 minutes, M = standard marker (DNA Marker)

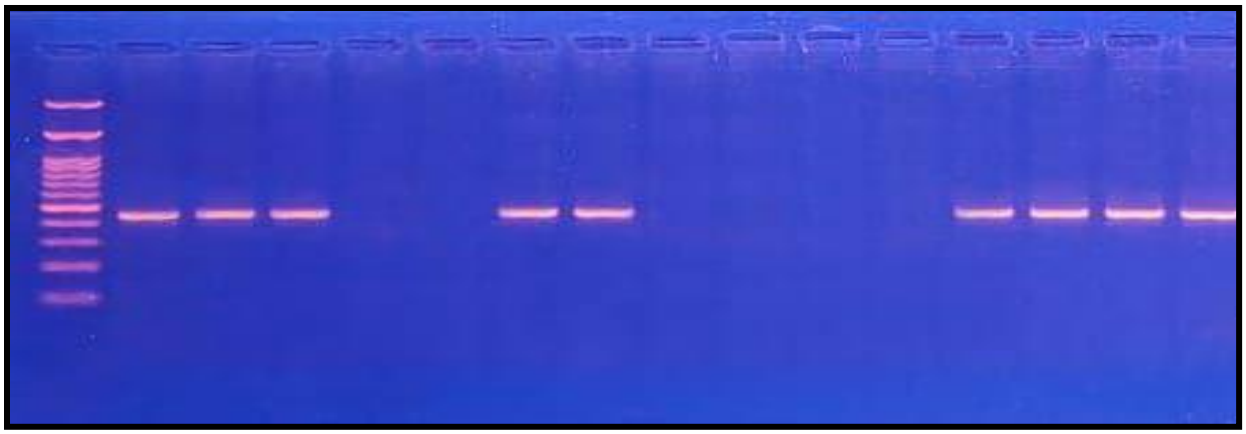

Figure (2): PCR results for the aap gene with a molecular weight of 466 base pairs (pb) encoded by some isolates of *S.epidermidis* (E) bacteria, electrophoresed on an agarose gel at a potential difference of 100 volts for 60 minutes M = standard marker (DNA Marker)

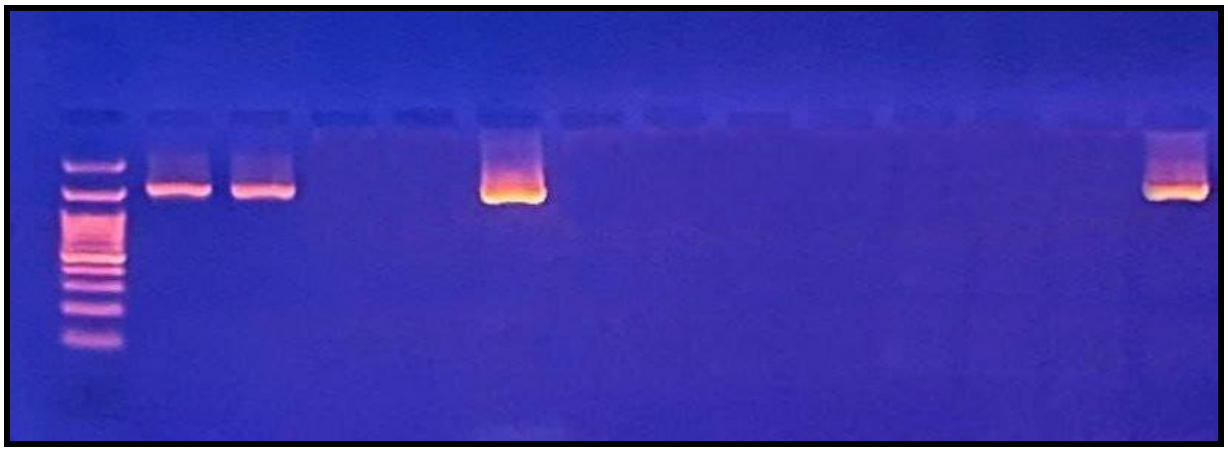

Figure (3): PCR results for the bhp gene with a molecular weight of 1083 base pairs (pb) encoded by *S.epidermidis* (E) electrophoresis on an agarose gel at a potential difference of 100 volts for 60 minutes M = standard marker (DNA Marker)

The results of this study are agreed with Soumya et al. (2017), who found that *S. epidermidis* possessed the fbe, atlEd, and icaA genes.They are also agreed with those of Manandhar et al. (2021), who indicated that *S. epidermidis* bacteria have the ability to produce biofilms, whether isolated from patients or from environmental sources, as they possess the genes encoding these films, such as atlE, aaP, and bap. François et al. (2023) found that all 46 *S. epidermidis* isolates in their study possessed the icaABCD genes. The results of studies vary in terms of the proportions, nature and type of gene, and the method used to investigate biofilm production in coagulase-negative staphylococci (Lu et al., 2022).

Zalewska et al. (2021) noted that *S. epidermidis* is an important pathogen in medical device-associated infections, particularly in hospitals, due to its ability to form biofilms that adhere to both biological and non-biological surfaces. Consequently, with the increasing use of medical devices in patient care, infections caused by these bacteria have become a major clinical concern

in healthcare. Biofilm formation is one of the most important means by which saprophytic or commensal microbes can become opportunistic pathogens, particularly coagulase-negative staphylococci. Through biofilms, these bacteria can adhere to both biological and non-biological surfaces. Similarly, damaged host tissue resulting from prolonged use of antibiotics and medical devices used for treatment facilitates the survival, proliferation, and virulence of these pathogens (François et al., 2023).

Conclusions:

The study concludes that these isolates were subjected to molecular analysis to detect the icaA, aap and bhp genes, which encode biofilm. PCR technology was used, and primers specific to the studied genes were used, targeting the specific sequences of the genes under study to detect the isolates possessing these genes. The results note that 4 isolates of *S.epidermidis* encoded the bhp gene with a molecular weight of 1083 base pairs. While there are 7 isolates of *S.epidermidis* species that possess the icaA gene with a molecular weight of 188 base pairs (bp).

Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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