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Identification of the *mecA* Gene and Evaluation of Biofilm Formation and Presence of *IcaB* Gene in *Staphylococcus aureus* Isolated from Eye Infection

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Abstract: *Staphylococcus aureus* is the most frequent ocular infection that irritates the cornea and conjunctivitis. Determining the function of the biofilm gene (*icaB*) in *S. aureus* isolated from eye infection is the goal of the study. Through the identification of the *mecA* gene, the study assessed the presence of the *icaB* gene. Between the beginning of September 2023 and the end of January 2024, 125 patients with a clinical diagnosis of bacterial eye infections were seen in Ibn Al-Haitham Teaching Hospital, Baghdad city. The result of study of 125 swap specimen, 55 (44%) specimen were given positive results for infections caused by pathogenic bacteria and/or fungus, or other species of *staphylococci*, while 70 (56%) specimen were given negative results, which mean the infection caused by other agents. However among 55 positive bacterial isolation swap was 42 isolate (76%) were given positive results for bacterial infections, as (*Pseudomonas*, *Streptococcus* spp, *Aspergillus* spp, *Escherichia* coli, *Proteus* spp and others), while 13 (24%) specimen were *S. aureus*, depending on the morphologic characteristics of this bacterium on the culture media and biochemical tests included manual conventional biochemical tests and automated biochemical tests. Molecular detection was conducted using Polymerase Chain Reaction technique of *icaB* and *mecA* gene with 117bp, and 533bp respectively, conclusion: 100% of isolates had the *icaB* gene, while 69% had the *mecA* gene.

Key words: *Staphylococcus aureus*, eye infection. Keratitis, *MecA*, *icaB*, biofilms, Api staph

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Introduction

In primary care, eye infections are common, with corneal ulcers/keratitis and red eye conjunctivitis being the top five issues. They can be caused by bacterial, viral, fungal, or both. Infections can involve one or both eyes, and can spread in the opposite direction. Conjunctivitis is an inflammation of the conjunctiva, characterized by ocular discharge, discomfort, itching, and a foreign body sensation (1). The ocular surface area is home to a specific collection of bacteria, making it susceptible to infections. Understanding these microbial diversity can help develop new therapeutic techniques for eye diseases. Major ocular pathogen *S. aureus* may infect several areas of the eye, particularly the cornea and inner chambers. Infections affecting these areas are the most risky due to their potential to impair vision or blindness(2). Predisposing factors

include contact lenses, trauma from cataract surgery, or intravitreal injections. *Staphylococcus* clinical isolates have varying biofilm production capacities, which can be identified using phenotypic techniques and PCR. Finding antimicrobial agents through combating bacterial biofilms is a promising strategy.

The ability of this organism to synthesize polysaccharide intercellular adhesion (PIA) was one of the most crucial elements. The four genes in the intracellular adhesion (*ica*) locus are ABCD these genes produce proteins that act as mediators in the PIA synthesis. The enzyme N-acetylglucosamyl transferase is encoded by the *icaA* gene. Although this enzyme is not very active in vitro, co-expression of the *icaD* gene makes it more active(3). The mature PIA and the transmembrane protein are deacetylated by the deacetylase *icaB*. Encoded by *icaC* is the transmembrane protein, which may play a role in the secretion and elongation of the expanding polymer (4). A contributing factor to the increased expression of this operon is the correlation between *icaB* and *IPA*, whereby a higher correlation results in increased biofilm formation (5).

Material and methods

Samples collection

This study includes One hundred and twenty-five cotton swab samples obtained from individuals of various sexes and ages with eye infections. The samples were gathered from the Ibn Al-Haytham Eye Teaching Hospital. Samples were collected from the beginning of September 2023 to the end of January 2024. Age of Patients ages ranged from 1 to 78 years. Out of 125 patients female were 73 (58%) and male patients were 52 (42%). The samples were put in a transport medium and sent to laboratory Ibn Al-Haytham Hospital for transplantation in the laboratory for a period of no more than 24 hours. *S. aureus* was then isolated and identified utilizing bacteriological methods.

Samples collection and *S. aurous* isolation

A cotton swab was used to collect the conjunctival, corneal, and vitreous swabs that are frequently used to identify acute bacterial or viral conjunctivitis (keratoconjunctivitis). Note whether the samples were taken from the left or right eye and label each one as cornea or conjunctiva. To collect and handle these materials, a strictly aseptic method is needed. Although some diseases can be better identified with corneal specimens after corneal scraping, these pathogens are not usually likely to cause an epidemic. Only an ophthalmologist or other qualified practitioner should take corneal scrapings. All samples used to treat eye infections were inoculated into 5% human blood agar and mannitol salt agar plates. Gram staining was used to screen *S. aureus* strains after an 18–24 hour aerobic incubation period at 37°C. Prescott reported on colony growth and structure (golden colonies on nutrient medium, beta hemolytic colonies on blood agar, and yellow discoloration on mannitol salt agar), as well as standard biochemical tests (6).

Coagulase test

The coagulase enzyme is responsible for causing blood plasma to coagulate. Gram-positive, catalase-positive organisms are used in this test to identify the coagulase-positive

S. aureus. After 0.5 ml of human plasma has been produced and incubated at 37°C for humans, 0.5 ml of bacterial suspension is added to ascertain if the coagulase enzyme is bound or free. When coagulase clots form four hours following the plasma injection, the composition of fibrin is regularly examined, and this is considered a positive result for strains of *S. aureus*. The negative outcome is the lack of a clot during a 24-hour incubation period(7).

The Catalase test

Detects the catalase enzyme to distinguish between streptococci (Catalase -ve) and *staphylococci* (Catalase +ve). Bacteria that produce catalase when exposed to hydrogen peroxide showed positive conversion of peroxide to water and oxygen gas. Using a capillary tube filled with hydrogen peroxide solution, a single colony was carefully dipped into the test, and when catalase was present, bubbles in the capillary tube and the release of oxygen gas were visible (8).

API® Staph

Is a standardized system for identifying *Staphylococcus*, Micrococcus bacteria using miniaturized biochemical tests and a specially adapted database. Twenty micro tubes with dehydrated substrates injected with a bacterial suspension in API Staph Medium make up the system. Metabolism causes color changes over the 18–24 hour incubation period, which can occur naturally or be detected by reagent. The Analytical Profile Index is used to identify the responses once they have been read in accordance with the Reading Table. The identification table contains the whole list of bacteria(9).

Biofilm formation test

Staphylococcus aureus ability to produce biofilm on abiotic surfaces was measured. In sterile 96-well polystyrene microtiter plates, A 200 µl aliquot of an overnight brain heart infusion broth (BHI) bacterial culture, matching McFarland standard no. 0.5, was added to each well. The microplates were then all covered and allowed to incubate aerobically at 37°C for the entire day. Three duplicate assays were performed on each isolate. As a control, BHI wells free of bacteria were employed. The contents of each well were aspirated, and biofilms were visualized by cleaning the wells three times with 200 µl of distilled water (18). After adding 200 µl of methanol, the microplates were left to air dry for fifteen minutes. The mixture was added to 200 µl of 0.1% crystal violet solution and allowed to stand at room temperature for five minutes. As was already mentioned, the washing procedure was carried out once more (19). Reference to ensure total drying, after that, the plates were incubated at 37°C for about 30 minutes. Next, 200 µl of100% ethanol was added, and it was allowed to stand for approximately ten minutes. The optical density (OD) of each well was then measured at 630 nm using a microtiter plate reader. Three standard deviations above the negative control mean OD (20) was the definition of the cut-off OD (ODc). Based on their ODc value, the isolates were divided into four groups: non-producer, weak biofilm producer, moderate biofilm producer, and high biofilm producer, as indicated in (Table 1).

Table (1): Level of biofilm in the isolated bacteria.

Average of OD	Biofilm Density
OD <= ODc*	Non – adherent
2ODc> OD> ODc	Weak
4ODc> OD> 2ODc	Moderate
OD > 4 ODc	Strong

Optical density (OD) and cut-off value (ODc) (21).

PCR Test

The isolated genomic DNA was amplified using the PCR method employing *S. aureus* specific 16S rRNA genetic primers as shown in (Table 2). 3 µl of template DNA, 1 µl of sterile nuclease-free water, 2 µl of each primer (forward and reverse), and 10 µl of master mix were added to make a 20 µl PCR mixture. After that, the mixture was gently vortexed. The following were the requirements for amplification: Thirty cycles of denaturation at 95°C for thirty seconds, primer annealing at 50°C for forty-five seconds, and strand extension at 72°C for seven minutes ensued after an initial five minutes of denaturation at 95°C. The products were electrophoresed for 80 minutes (80 volts) on a 1.5 w/v agarose gel in 1x TAE buffer after red safe dye was applied to make the PCR results visible. This was done before the products were analyzed.

Table (2): The primers, sequencings for detection of genes in *S. aureus*.

Primer name	Primer sequence 5′→3′	Amplicon size pb	Reference
***icaB*-F**	CACAGGTCATGTTGGGGAAGAA	117bp	Newly Designed
***icaB*-R**	TGCAAATCGTGGGTATGTGTTTC	117bp	Newly Designed
***mecA*-F**	AAAATCGATGGTAAAGGTTGGC	533bp	(Bühlmann *et al*., 2008)
***mecA*-R**	AGTTCTGGAGTACCGGATTTGC	533bp	(Bühlmann *et al*., 2008).

Results and discussion

This study presents findings from 125 infections of the eyes in all. Ophthalmologists, patients, these criteria are used to patients suspected of having *S. aureus* infection in order to confirm the clinical diagnosis of ocular infection. A total of 125 clinical samples, only 13 (10%) were tested for *S. aureus* specific biochemical and morphology. The remaining 112 (90%) isolates were from other *Staphylococci*, pathogenic bacteria, and fungi as shown in Figure (1). Blood agar medium was used to cultivate the colonies of clinical specimens. The shape of the bacteria was found to be smooth and somewhat round based on the results (10). When these colonies are cultivated on blood agar, they can produce hemolysis (β) as shown in Figure (2). Due to the fermentation of mannitol salt, thirteen clinical isolates grow yellow (golden) colonies. This turns phenol red into golden and makes them resistant to high salt concentrations in MSA (mannitol salt agar) selective medium. These provided the usual morphological traits associated with *S. aureus* (11). It was *S. aureus* specific differential culture mediums that inhibit the growth of other bacteria as shown in Figure (3). Clinical specimens for diagnoses are examined under a microscope. Gram staining reveals the features of *Staphylococci* spp. When they exhibit a Gram positive response, are non-spore-forming, and, when examined under a microscope, form cocci and are grouped in irregular clusters (12).

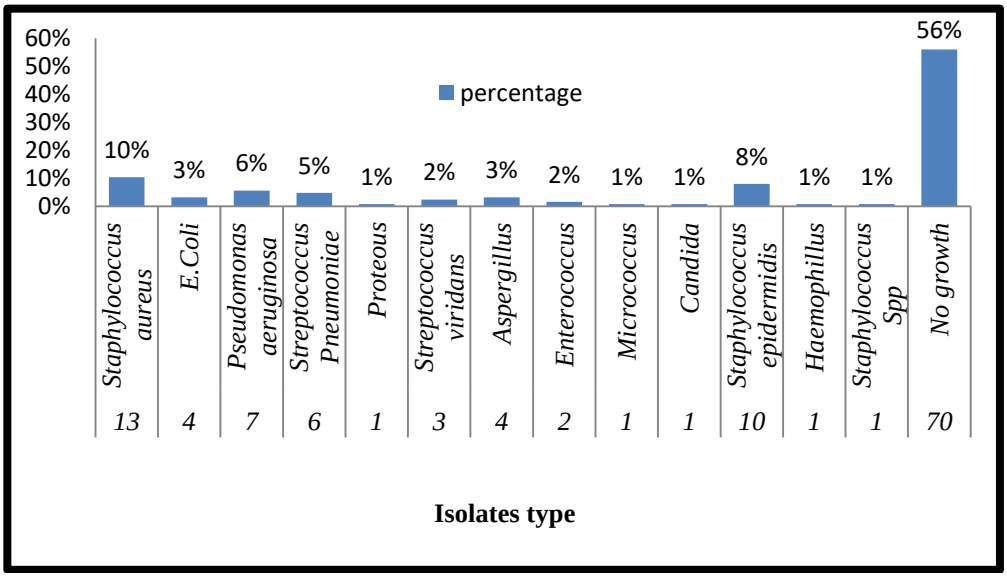

Figure (1): Types of bacteria and fungi that were separated from the eye infection.

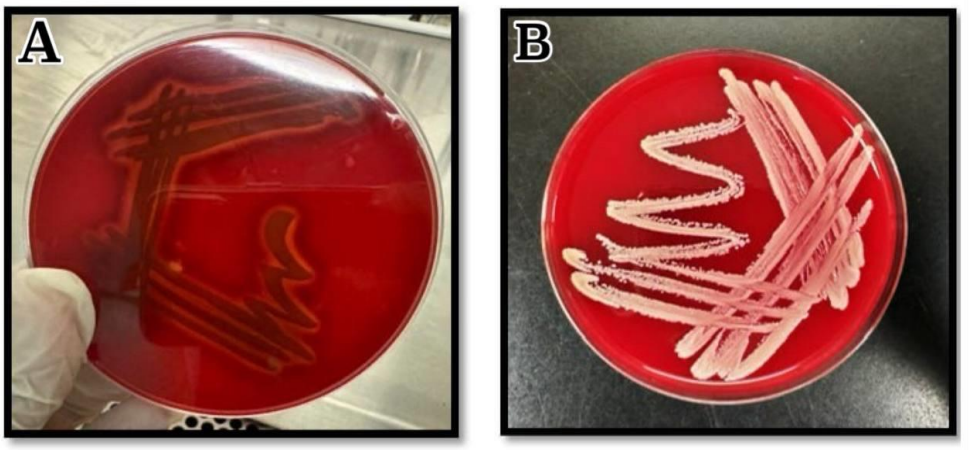

Figure (2): A: β -Hemolysis *S. aureus* on blood agar. B: Colonies of *S. aureus* growing on blood agar for 24 hour at 37 °C.

Figure (3): Cultivation of *S. aureus* colonies for 24 hours at 37 °C on MSA.

Biochemical identification of *S. aureus* isolates

Specimens that tested positive for coagulase and catalase underwent the standard biochemical testing (13). In the oxidase test, though, the result was not favorable. They go through coagulase assays, which demonstrate the bacteria's capacity to manufacture the coagulase enzyme and aid in distinguishing between other *Staphylococcus* species (negative coagulase) and *S. aureus* species (positive coagulase). This is due to the fact that, the bacterial coagulase enzymes combine with human blood prothrombin to generate staphylo-thrombin (clot), which transforms fibrinogen into fibrin. (14). In order to stop the accumulation of dangerous metabolites, the enzyme catalase breaks down hydrogen peroxide (H2O2) into oxygen and water. Positive isolates of *S. aureus* were found (15). The catalase genus is identified from other *Streptococcus* genera by differing them from other Micrococcus species using the Api staph strip (16, 17).

Biofilm formation detection of *S. aureus*

Using microtiter plates, thirteen isolates of *S. aureus* were found to be responsible for biofilm formation. Figure (4) displays the selection criteria for the clinical isolates of No biofilm 6 (46.1%), Weak biofilm 1 (7.8%), Moderate biofilm 2 (15.3%), and Strong biofilm 4 (30.8%) that exhibited high absorption. A prior study by Shaimaa and Hala (21) revealed that out of 9 isolates, only 1 isolate (11%) was a strong producer of biofilm, while 6 isolates (67%) were moderate producers and 2 isolates (22%) were weak producers. Two *S. aureus* isolates formed biofilm on CRA without the presence of any of the genes under investigation, as reported by additional research by Salina *et al*. (22).

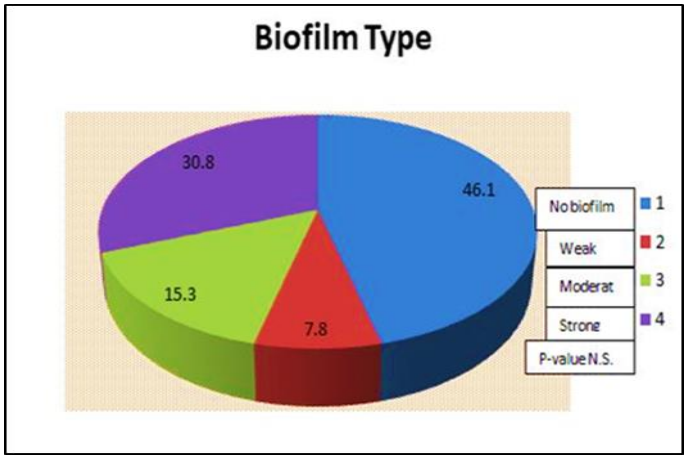

Figure (4): Sample distribution for the study based on the formation of biofilm.

Isolates of *S. aureus* identified by molecular methods

DNA extraction

The Qubit® gDNA Bacteria Kit was utilized to extract DNA. The manufacturing company's detailed technique was followed to harvest DNA from the overnight cultures of the staphylococcal isolates that were carefully chosen.

***Staphylococcus aureus* molecular identification**

A *Staphylococcus aureus* isolate DNA sample was selected for this study

in order to identify the *mecA* gene. Using the ideal PCR conditions and particular primers, an ultraviolet (UV) transilluminator, and agarose gel electrophoresis, the bacterial DNA corresponding to this gene was amplified in a monoplex pattern. The PCR reaction results, as shown in Figure (5), indicated that the *mecA* gene (533 bp) is present in all *S. aureus* isolates (69%, 9/13), with the exception of samples No. 1, 8, 9, and 13, where it was negative (31%, 4/13). This was determined by analyzing the bands on the gel electrophoresis and comparing the molecular weight of the PCR products with a 100 bp DNA ladder.

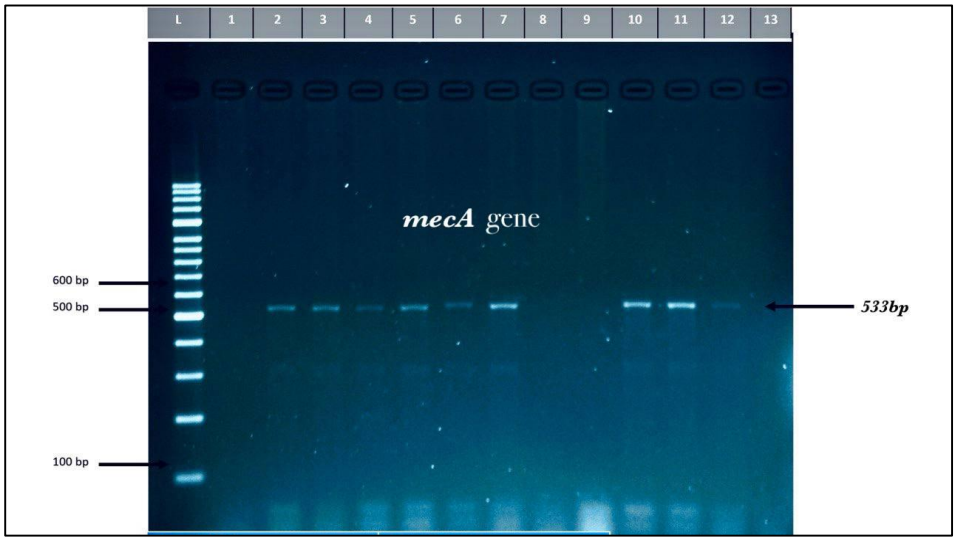

Figure (5): *mecA* gene (533 bp) PCR amplification in *S. aureus* isolates on 1.5% agarose at 90 volts for 60 minutes. Except for N. 1, 8, 9, and 13, all isolates were positive using a 500 bp DNA ladder.

These findings agreed with those of studies by Aytekin *et al*., (23), in which *mecA* gene positivity was found in 315 of the *S. aureus* strains (63.8%), and Felipe *et al.*, (24) 69% of *S. aureus* isolates were identified in the study, and 13.04% of them carried the *mecA* gene, which codes for the penicillin-binding protein and provides methicillin resistance. And in Iraq by Neamah *et al*., (25) illustrated that *mecA* was detected in (90%) of *S. aureus* isolates (23, 24, 25).

Detection of *icaB* gene by PCR technique

The results of the study showed that detect *icaB* gene in 13 isolates as *S. aureus*, by analyzing the bands on gel electrophoresis, the PCR products have been verified. All of the *S. aureus* isolates have the (*icaB*) gene (117 bp) (100%, 13/13) according to the results of the PCR reaction, as shown in Figure(6). The *icaB* gene regulates the creation of slime layers in biofilms.

The result is consistent with according to Shoaib *et al*., (26) showed that the results of biofilm gene detection revealed that (48) 100% for the *icaA* and *icaB* genes, 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. As the intracellular adhesion (*icaB*) gene was found in

100% of the *Staphylococcus aureus* isolates from wound infections carried the icaB gene by Abbas *et al*. (27).

The main infection that affects the eyes, *S. aureus*, can infect several ocular locations, particularly the cornea and inner chambers. With a single band, every primer employed in the investigation demonstrated specificity. Using the PCR technique, the biofilm-related gene was found in *Staphylococcus* strains. The prevalence of S. aureus strains with the genes *icaB* and *mecA* was 100% and 69%, respectively. Using microtiter plates, thirteen isolates of *S. aureus* were found to be responsible for biofilm formation, biofilm-forming was weakly present in only one (7.8%, 1/13) of the isolates of *S. aureus*, whereas moderate biofilm in two (15.3%, 2/13) and strong biofilm in four (30.8%, 4/13) exhibited high absorption, and no biofilm in six isolates of *S. aureus* (46.1%), as shown in Figures (6). The propensity for biofilm development in MRSA strains did not correlate with the type of specimen or the source of the bacteria.

Staphylococcus aureus is a major eye pathogen that can infect various ocular sites, particularly the cornea and inner chambers. These infections can cause visual loss or blindness. Antimicrobial factors protect these sites, but predisposing factors like contact lenses or trauma can weaken defenses. Structural carbohydrates induce an inflammatory response, while secreted proteins mediate tissue damage. Quantitative animal models have provided insights into *S. aureus* virulence and host factors limiting these infections. Biofilms are bacterial aggregations on biotic and abiotic surfaces that create an environment conducive to the growth of microorganisms (28). They have the ability to endure harsh conditions, develop chronic illnesses, intensify drug resistance, promote gene transfer, and activate the pathogenicity-causing genes of *S. aureus*. Bacterial virulence is dependent on the presence of biofilms, which are encoded by intracellular adhesion. The *icaA* gene induces biofilm production, activated by UDP-N-acetyl glucosamine. The *icaD* protein serves as a messenger for other genes, activating enzymes to express the *icaC* and *icaD* genes. When these genes cooperate, biofilm production increases 20 times. The *icaC* gene communicates between the cytoplasmic membrane and the icaD, causing superficial association with intercellular adhesion polysaccharide (IAP). The relationship between *icaB* and IPA increases the expression of this operon, leading to increased biofilm production. Biofilms in hospital environments cause 65% of nosocomial infections, binding bacteria to surfaces and medical devices. Proper use of antibiotics and regular evaluation of resistance and gene expression can reduce these problems. This study aims to evaluate genotypic and phenotypic biofilm formation by *S. aureus* and their association with antimicrobial resistance due to the high prevalence of eye infections caused by *S. aureus* (29).

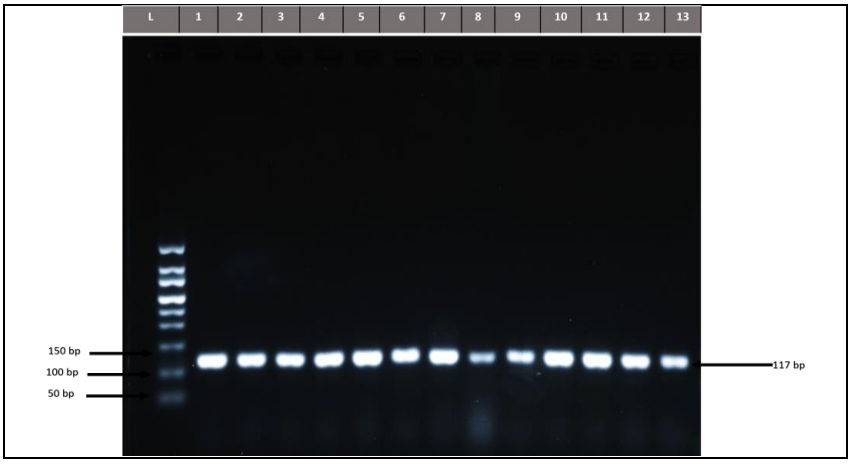

Figure (6): PCR amplification of *icaB* gene (117 bp) in *S. aureus* isolates, on 1.5% agarose at 90 volt for 60 mins. All isolates were positive isolates except N. 9 with a 100 bp DNA ladder.

Conclusion

The study reveals varying biofilm-forming capacities in clinical *S. aureus* isolates, increasing infection risk and antibiotic resistance. This poses a significant challenge in eye care, limiting the use of contact lenses. Future research should focus on anti-biofilm coatings and biofilm-active therapeutics. Additionally, it was found that the *icaB* gene was more strongly associated with strong biofilm formation in the *staphylococci* species group.

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