Malaysian Journal of Medical Research, 10(2), 22-32
https://doi.org/10.31674/mjmr.2026.v010i02.003

MJMR
Malaysian Journal of Medical Research

Malaysian Journal of Medical Research

Online ISSN: 2550-1607

www.mjmr.com.my

Original Article

IcaA Gene in Environmental Isolates of Biofilm Producing *Staphylococcus aureus*

Ismaal Jmia Abas

Department of Biology, College of Education in Qurina, University of Basrah. Basrah, Iraq

Corresponding Author's Email: ismail.abbs@uobasrah.edu.iq

Abstract

Background: Biofilm-forming (BF) *Staphylococcus aureus* bacteria are a major environmental and health concern due to their role in antibiotic resistance and chronic infections. The presence of biofilm-associated genes, such as the *IcaA* gene, plays a significant role in biofilm formation and increases its virulence in the environment. **Objectives:** This study aims to isolate and identify biofilm-producing *Staphylococcus aureus* from environmental samples, detect the presence of the *IcaA* gene using polymerase chain reaction, compare different biofilm detection methods, and detect antibiotic resistance and its patterns in the isolates. **Methods:** One hundred and forty environmental samples were collected from various locations, including soil and wastewater. *Staphylococcus aureus* was isolated and identified by culturing on different media and then characterized using biochemical methods. Biofilm formation was detected using Congo Red agar and tube-based methods. The *IcaA* gene was detected molecularly using polymerase chain reaction (PCR). Antibiotic susceptibility testing was performed using disk diffusion. **Results:** A total of 140 environmental samples, 55 isolates exhibited biofilm-forming potential. Thirteen of these isolates were identified as *Staphylococcus aureus*. Biofilm detection showed that 76.92% of the isolates were positive using the Congo Red agar method, and 23.07% were positive using the tube method. Polymerase chain reaction (PCR) results indicated that 4 of the 13 isolates (30.76%) carried the *IcaA* gene. Antibiotic susceptibility testing revealed high resistance to vancomycin, amoxicillin, and oxacillin, while high susceptibility was observed to gentamicin and amikacin. **Conclusion:** Environmental *Staphylococcus aureus* isolates exhibited a marked capacity for biofilm formation and antibiotic resistance. The presence of the *IcaA* gene confirmed the genetic basis for biofilm formation in some isolates. The tube method showed greater agreement with molecular detection compared to the Congo red agar method. Continued monitoring of environmental *Staphylococcus aureus* and biofilm-associated genes is recommended due to their role in antibiotic resistance and public health risks.

Keywords: *Antibiotic Resistant; BF Gene; Congo Red; S. aureus; Virulence Factor*

Introduction

The Biofilm (BF) in *S. aureus* consists of the main materials in addition to the bacteria that are embedded in extracellular polymeric materials (Peng *et al.*, 2022). BF in these bacteria contains polysaccharides, phospholipids, proteins, nucleic acids, and tetroic acid, in addition to other polymeric materials that are dissolved in a high-water content (85–95%) (Nasser *et al.*, 2022)

According to earlier studies, BF-lifestyle is a never-ending cycle, and the five main stages of BF production are as follows: (I) Attachment: Whether on a biotic or abiotic surface, microbes are reversibly adsorbed to the surface through weak contacts (such as Van der Waals forces). (II) Colonization: Via

Received: 3rd March 2026; Revised version received on: 15th March 2026; Accepted: 30st March 2026

greater hydrophilic/hydrophobic interactions mediated by flagella, pili, lipopolysaccharides, exopolysaccharides, collagen-binding adhesive proteins, etc., microorganisms are permanently adhered to the surface. (III) Development: Multilayered cells multiply and accumulate, producing and secreting exopolysacharide EPS (IV) Maturation: BF matures, forming a stable, three-dimensional community with pathways for distributing nutrients and signaling chemicals. (V) Active dispersal: Microbial cells are separated or detached in clumps because of interactions with intrinsic or extrinsic stimuli, and the dispersed cells subsequently colonize other sites (Sauer *et al*., 2022)

In this dynamic process, certain enzymes are engaged in dissolving and reconfiguring BF, leading to active dispersion of BF and subsequent surface recolonization in addition to partial matrix destruction (Wang *et al*., 2023). This result revealed that the development of BFs can lessen the susceptibility of microbes to antibiotics. Second, antibiotics can be transported via multidrug efflux pumps in BFs to minimize toxic buildup (Zhang *et al*., 2024). The intracellular adhesion (*Ica*). cluster (*IcaA*), which encodes the necessary proteins for polysaccharide synthesis intercellular adhesion (PIA), which mediates cell-to-cell adhesion, aids Staphylococcus species in the production of BFs (Pugazhendhi *et al*., 2022). Identification of the Ica locus in *S. aureus* isolates is crucial, and it would enhance the diagnostic process for selecting the most appropriate course of action when combined with the phenotypic identification of BF (Mohammed & Al-Iedani, 2020) This approach aligns with the objective of the current study, in which it aimed to evaluate the ability of *S. aureus* to produce BFs and compare these isolates according to the environment from which they were isolated.

Methodology

Collection of Samples

About 140 environmental samples were collected from 12 sites, the most important includes: water treatment plants, college laboratories, marine and river sediment such as Tigris and Euphrates rivers., sewage, polluted soil in Basrah, Iraqi.

Identification of Bacteria

The bacterium *S. aureus* was diagnosed and identified by microscopy and phenotypic examination, biochemical tests were performed, and the bacteria were grown on Mannitol Salt Agar according to Moraes *et al*. (2021).

BFs Formation Tests

Assay via Congo Red Agar:

The *S. aureus*, which has the ability to form BFs, were detected using the Congo Red Agar method, where the plates were incubated at 37 °C for overnight (Paul *et al*., 2026)

Assay via Tube Method:

The identified bacteria were transferred to sterile glass test tubes containing Tryptic broth medium prepared according to the manufacturer's instructions. One percent glucose was added to the medium, and the dishes were incubated for 24 hours at 37°C. The samples were then drained, and the tubes were washed with phosphate-buffered saline. After drying, 1% crystal violet stain was applied for three minutes. Excess stain was discarded, and the tubes were rinsed with distilled water. The samples were inverted, dried, and the formation of BFs on the tube walls and bottoms was monitored (Taj *et al*., 2012).

Detection of *IcaA* Gene

DNA Extraction

According to the manufacturer's instructions.

By using DNA extraction kit from Presto TM Mini gDNA Bacteria Kit- South Korea, bacterial DNA was extracted (this work was according to manufacturer's instructions)

1. Bacterial cells were collected from an overnight culture by centrifugation at 10,000 rpm for 5 minutes.

2. The cells were then resuspended in TE buffer.
3. Lysozyme enzyme was added, and the sample was incubated at 37°C for 30 minutes for cell wall analysis.
4. Protease lysis solution was added, and the sample was incubated at 56°C for 30 minutes.
5. A phenol-chloroform mixture was added, and the sample was centrifuged to separate the phases.
6. DNA was precipitated using cold ethanol.
7. The DNA sample was washed with 70% ethanol and air-dried.
8. The DNA was resuspended in nuclease-free water and stored at -20°C until polymerase chain reaction (PCR) analysis.

Detection of Adhesion *IcaA* Genes Using PCR

Primer sequences for IcaA gene:

Primers used for detection of the *IcaA* gene selected based on published sequences from previous molecular studies (Arciola *et al*., 2001) and verified using GenBank database to ensure specificity for *Staphylococcus aureus*. Primers synthesized by a commercial oligonucleotide company in Table 1.

Table 1: Shows Primer Sequence

Forward	TCTCTTGCAGGAGCAATCAA	Product size: 814 bp	Arciola *et al*., 2001
Reverse	TCAGGCACTAACATCCAGCA		

PCR Master Mix:

Chromosomal DNA amplification was performed using polymerase chain reaction (PCR) with a master mix prepared by Pioneer (South Korea). The master mix contained Taq DNA polymerase, dNTPs, magnesium chloride ($MgCl_2$), a reaction buffer, a stabilizer, and a tracer. A 25-µL final volume of PCR mix was prepared, containing 12.5 µL of the master mix, 1 µL of forward primer, 1 µL of reverse primer, 5 µL of DNA template, and nuclease-free water to complete the final volume (Kim *et al*., 2025).

PCR Program:

DNA amplification was performed using Polymerase Chain Reaction (PCR) in a thermal cycler with the following program according to (Cheung & Otto, 2023)

1. Initial DNA separation: 95°C for 5 minutes
2. 35 cycles of:
   - DNA separation: 95°C for 30 seconds
   - Annealing: 55°C for 30 seconds
   - Extension: 72°C for 45 seconds
3. Final extension: 72°C for 7 minutes
4. Storage at 4°C

After the reaction was completed, electrophoresis was used to analyze the PCR products on a 1 percent agarose gel in 1X TBE buffer. The PCR products were seen under UV light.

Antibiotic Resistance

The antibiotic susceptibility of these bacteria on Mueller-Hinton agar using the disc diffusion method and adopted antibiotics in the process of detecting antibiotic resistance according to work requirements and in accordance with the specifications of the Institute for Clinical and Laboratory Standards, (Humphries *et al*., 2021) in order to determine the extent of resistance of the studied bacterial strains (Weinstein & Lewis, 2020). The antibiotic use in the study was listed in Table 2.

Table 2: List of Antibiotics in this Study

Antibiotic	Abbreviation	Concentration (µg/disc)	Type (Class)	Reference
Amoxicillin–clavulanic acid	AMC	20/10 µg per disc	Beta-lactam	(Humphries *et al*., 2021)
Oxacillin	OX	10 µg/disc	Beta-lactam	
Amoxicillin	AX	25 µg/disc	Beta-lactam	
Vancomycin	VA	30 µg/disc	Glycopeptide	
Gentamicin	GEN	10 µg/disc	Aminoglycoside	
Amikacin	AK	30 µg/disc	Aminoglycoside	
Ciprofloxacin	CIP	5 µg/disc	Fluoroquinolone	
Azithromycin	AT	15 µg/disc	Macrolide	
Tobramycin	TOB	10 µg/disc	Aminoglycoside	
Ceftazidime	CAZ	30 µg/disc	Cephalosporin	
Trimethoprim-sulfamethoxazole	SXT	1.25/23µg/disc	Sulfonamide	
Polymyxin B	PB	300 Units/disc	Polymyxin	
Tetracycline	TIC	30 µg/disc	Tetracycline	
Doxycycline	DO	10 µg/disc	Tetracycline	

Statistical Analysis

At a significance level of 0.05, all statistical analyses were performed using SPSS software (version 25) based on the Chi-Square test (Levesque, 2005).

Results and Discussion

Environmental Samples Distribution

A total of 140 environmental samples were collected, including soil, wastewater from homes, wastewater from schools, and wastewater from colleges as shown in Table 3.

Table 3: Distribution of Environmental Samples

Sample Source	Number of Samples	Percentage (%)
Soil	33	23.5%
Household wastewater	43	30.7%
School wastewater	29	20.7%
College wastewater	35	25%
Total	140	100%

Isolation and Identification of Bacteria

The samples classified based on Microscopic examine by using Gram stain and colony color and morphology, biochemical tests (Gale & Gale, 2022). The results shown from 140 sample 49 was Gram negative and 91 Gram positive Table 4.

Table 4: Isolation and Identification of Bacteria

Total	Gram negative	Gram positive
140	49	91
Percentage	35%	65%
$\chi^2 = 12.6$, $p < 0.05$		

The results in Table 4 showed that Gram-positive bacteria were more prevalent than Gram-negative bacteria, at 65% and 35% respectively. Statistical analysis using the chi-square test confirmed a significant difference between the two groups ($p < 0.05$), indicating that Gram-positive bacteria were dominant in the environmental samples. The prevalence of Gram-positive bacteria may be attributed to the structural characteristics of their cell wall. This wall contains a thick layer of peptidoglycan, which protects them from environmental stresses such as drought, temperature fluctuations, and nutrient deficiencies. This helps them survive longer in soil and wastewater compared to Gram-negative bacteria (Omidi *et al*., 2021). These results are consistent with studies showing that Gram-positive bacteria are more commonly isolated from soil and wastewater due to their ability to adapt to environmental conditions and their high resistance to stress condition (Ajmal *et al*., 2021).

Biofilm Formation

The results showed from 91-gram positive bacteria 55 isolates ability to form BFs, and the number of *S. aureus* which able to form BFs isolates was 13 Table 5.

Table 5: Biofilm Formation

Total Gram-Positive Bacteria	No. of Isolates Ability to form Biofilm	No. of *S. aureus* which able to form Biofilm
91	55	13
$\chi^2 = 3.97, p < 0.05$		

The results of this study showed that 60.4% of the Gram-positive bacterial isolates were capable of forming biofilms. This indicates that biofilm formation is a common characteristic among Gram-positive bacteria obtained from environmental samples.

Biofilm formation is a crucial virulence factor, particularly in *Staphylococcus aureus*, as it enhances bacterial survival, antibiotic resistance, and the ability to endure harsh environmental conditions. Biofilm-forming bacteria have the ability to adhere to surfaces and protect themselves within an extracellular polymer matrix (Peng *et al*., 2022). The presence of 13 biofilm-producing *Staphylococcus aureus* isolates indicates that this species plays a significant role in biofilm formation among Gram-positive bacteria. This finding is consistent with numerous studies reporting that *Staphylococcus aureus* is one of the most common biofilm-forming bacteria, particularly in environmental and clinical isolates (Kadkhoda *et al*., 2020).

The significant statistical variation observed in this study indicates that biofilm formation is not randomly distributed among isolates and may be related to the characteristics of bacterial genera and species, as well as environmental factors.

Comparison of BF Detection Modalities in *S. aureus*

The results of the current study showed that the rates of BF detection of *S. aureus* bacteria by the CRA method were 76.92%, by the TM method, 23.07%, and the positive and negative result for both methods showed in Figure 1.

The results of the statistical analysis showed a significant difference between the CRA and TM methods ($p < 0.05$), indicating that the Congo red agar method is more accurate and sensitive in detecting biofilm formation in *Staphylococcus aureus* isolates. And these results, were agreed with these results (Khan *et al*., 2011) that the CRA method is easy to use and interpret, while the TM method is more accurate and has high sensitivity and specificity (Oliveira & Cunha, 2010).

Figure 1: Formation of BFs Using Congo Red Method (Positive)

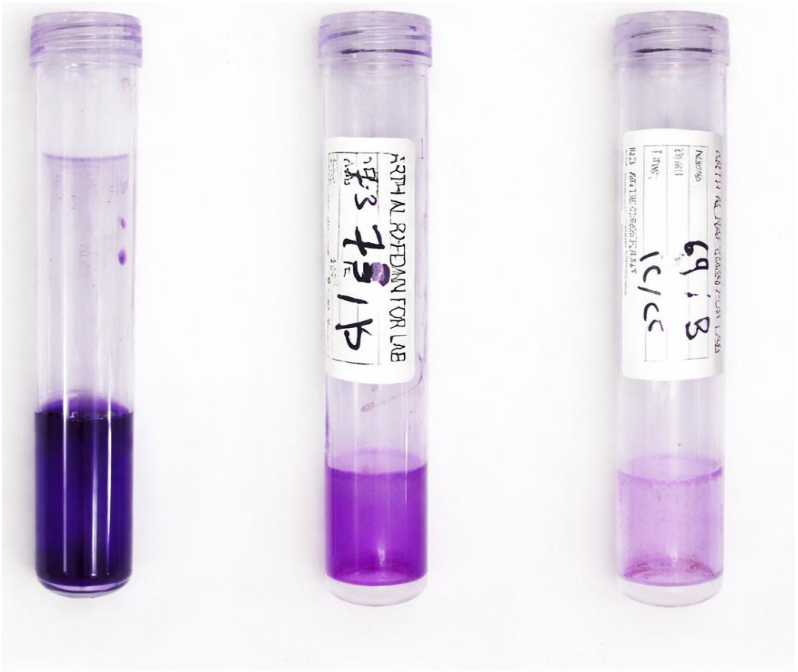


Figure 2: Formation of BFs by the Tube Method (a, Strong; b, Medium; c, Weak)

Antibiotic Resistance in *S. aureus*

The study finds that there are no significant differences ($p > 0.05$) for antibiotic resistance between *S. aureus* isolates (Figure 2). The results of the current study showed that the isolates under study are resistant to Vancomycin (VA), reaching 92.30% Vancomycin is one of the most important broad-spectrum antibiotics, as it has an effective effect on many types of Gram-positive bacteria (Alharbi *et al.*, 2025).

The results of the current study showed a very high resistance rate compared to many previous studies. This indicates that the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) is a serious global public health problem, due to the widespread and inappropriate use of vancomycin in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections (Girijan & Pillai, 2021). The high resistance observed in this study may be attributed to the overuse of antibiotics, horizontal gene transfer, and the bacteria's ability to form biofilms, which increases their antimicrobial resistance (Michaelis & Grohmann, 2023).

The results of the study showed that the resistance of the isolates under study to Amoxillin (AX) and Oxyacillin (OX) reached 100%. The reason for the resistance to these antibiotics may be due to the change in permeability of the outer membrane of the bacteria represented by the systems of flow pumps and the secretion of beta-lactamase enzymes (Hussein *et al.*, 2024).

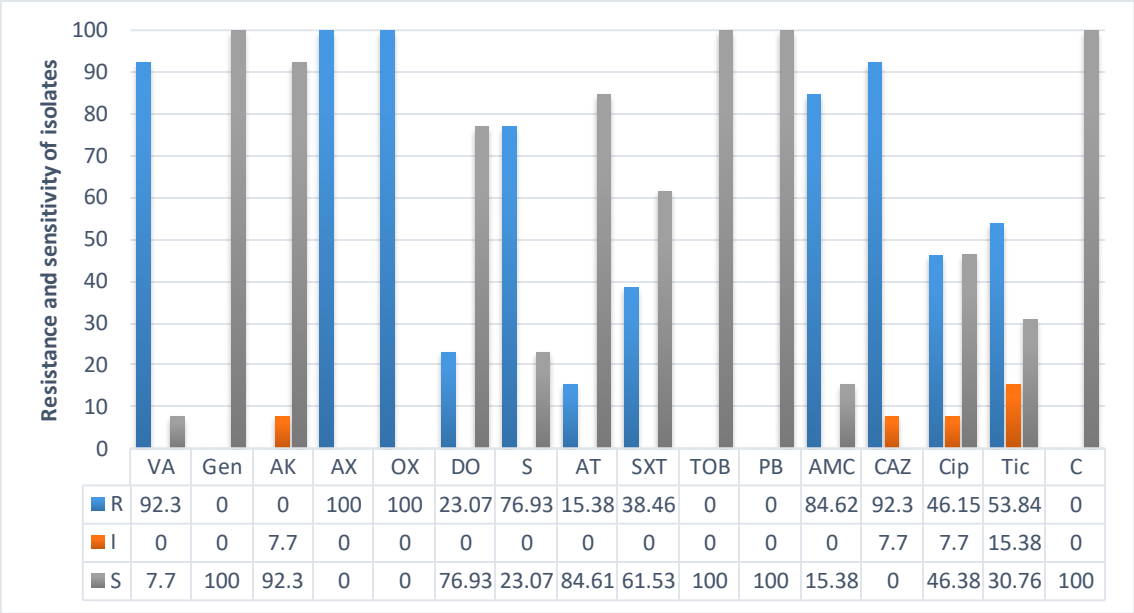
The results of current study also showed that the isolates were sensitive to the Gentamicin antibiotic by 100%, and Amikacin (AK) by 92.30%, These results indicate that aminoglycoside antibiotics retain their efficacy against *Staphylococcus aureus* isolates in the study area. The high sensitivity observed with gentamicin and amikacin may be attributed to their limited use compared to other commonly used antibiotics, thus reducing the development of bacterial resistance (Thy *et al.*, 2023). For the antibiotic Azithromycin the isolates under study show resistance to about 15.38%. It is considered one of the antibacterial growth inhibitors because it inhibits bacterial protein synthesis and is effective against most gram-positive bacteria (Sandman & Iqbal, 2024).

As for the resistance to the Ciprofloxacin (CIP) antibiotic, it was 46.15%, These findings are consistent with several previous studies that have demonstrated the increasing resistance of *Staphylococcus aureus* to fluoroquinolones. These reports have shown that ciprofloxacin resistance rates range from 30% to 70% in different regions (Awayid & Qassim Mohammad, 2022; Afzal *et al.*, 2021; Mohammadi

et al., 2020), depending on the local epidemiological situation and antibiotic use patterns. it may be due to the high sensitivity of this antibiotic, which is a quinolones antibiotic, as it is characterized by its rapid absorption and high penetration into cells and is characterized by its effectiveness in killing bacteria in a short period of time by inhibiting DNA replication (Tang & Zhao, 2023).

The current study showed that the isolates under study exhibited high sensitivity to the antibiotic Gentamicin 100%, Tobramycin and Amikacin, at rates of 100% and 92.3% respectively, observed in this study indicates that aminoglycosides remain highly effective against these isolates, The role of aminoglycosides and their antibacterial effect is achieved by inhibiting protein synthesis through binding to the 30S ribosomal subunit, leading to the killing of bacterial cells (Tang & Zhao, 2023).

The isolates under study showed moderate resistance to tetracycline antibiotics groups, with approximately 50% resistance to Tetracycline and 23.1% to Doxycycline. The presence of tetracycline-resistant pathogens may be attributed to the use of these drugs in treating diseases. Tetracycline resistance is often attributed to the acquisition of new genes, either genes responsible for the energy-based outflow of tetracycline from the cell, or genes responsible for a protein that protects bacterial ribosomes from the effects of tetracycline (Colaco *et al*., 2021).



	VA	Gen	AK	AX	OX	DO	S	AT	SXT	TOB	PB	AMC	CAZ	Cip	Tic	C
R	92.3	0	0	100	100	23.07	76.93	15.38	38.46	0	0	84.62	92.3	46.15	53.84	0
I	0	0	7.7	0	0	0	0	0	0	0	0	0	7.7	7.7	15.38	0
S	7.7	100	92.3	0	0	76.93	23.07	84.61	61.53	100	100	15.38	0	46.38	30.76	100

Figure 3: Diagram of Percentage of Antibiotic Resistance of S. aureus Isolates (R: Resistance, S: Sensitive, I: Intermediate)

PCR Detection of *IcaA* Gene

The results of this study showed that 4 out of 13 isolates (30.7%) carried the *IcaA* gene (Figure 3). The *IcaA* gene is closely related to biofilm formation, as it is part of the *IcaA*DBC operon, which is responsible for the synthesis of intercellular adhesive polysaccharides (PIAs), essential components in biofilm formation and bacterial adhesion (Ahmad *et al*., 2022). The percentage obtained in the current study (30.7%) falls within the range indicated by several previous studies, where the prevalence of the *IcaA* gene varies considerably among *Staphylococcus aureus* isolates. A systematic review and meta-analysis showed that the prevalence of *Ica* operon genes ranges from 28% to 51.5%, and that the average prevalence of the *IcaA* gene is approximately 38.4%, ranging from 3.1% to 80% depending on the sampling source and geographic region (Bamneshin *et al*., 2024). A study conducted on animal feed sources revealed that the percentage of *IagA* gene in *Staphylococcus isolates* was approximately 45%, which is close to the results of the current study (Sharan *et al*., 2024).

On the other hand, some studies have shown very high prevalence rates of the *IcaA* gene, especially among clinical and multidrug-resistant isolates, with prevalence rates reaching 90% and possibly higher

in some MRSA isolates, indicating a strong correlation between biofilm formation, virulence, and antibiotic resistance (Alibegli *et al*., 2025).

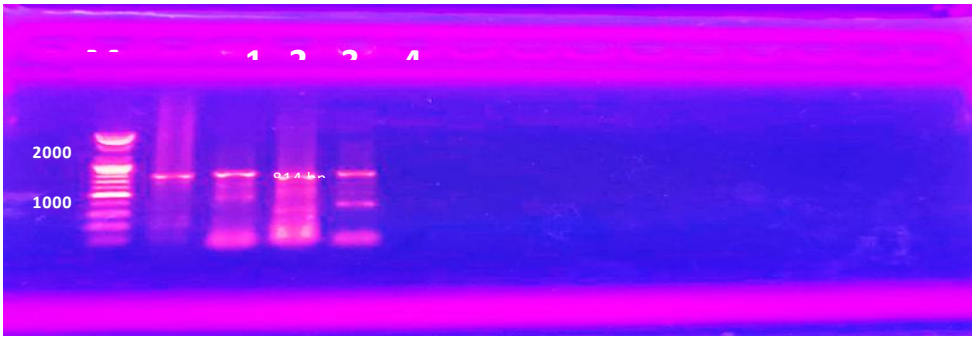


Figure 4: Agarose Gel Electrophoresis of PCR-Amplified IcaA Gene in Staphylococcus aureus Isolates

The Expected Amplicon Size is 814 bp. Lane M Represents the 100 bp DNA Ladder, while Lanes 1–4 Show Positive Amplification of the *IcaA* Gene

The PCR analysis confirmed the presence of the *IcaA* gene in selected *S. aureus* isolates, as evidenced by the clear amplification bands at 814 bp (Figure 4). This finding supports the role of the *IcaA* gene in biofilm formation and highlights its contribution to the virulence and persistence of environmental isolates. The detection of this gene further correlates with the observed biofilm-forming ability and may explain the increased resistance patterns reported in this study.

Limitation

One limitation of this study is the relatively small number of *Staphylococcus aureus* isolates analyzed for molecular detection of the *IcaA* gene, which may limit the generalizability of the findings. Additionally, only a single biofilm-associated gene (*IcaA*) was investigated, whereas other genes involved in biofilm formation were not assessed.

Instruction for AI Assistance Declaration

The author hereby declares that, during the preparation of this manuscript, generative AI tools such as ChatGPT, Microsoft Copilot, and Google Gemini were utilized to assist with language enhancement and grammar correction. Following the use of these tools, the author thoroughly reviewed and revised the content and takes full responsibility for the final version of the manuscript, ensuring its accuracy and adherence to the required academic standards.

Conclusion

The presence of the *IcaA* gene in environmental isolates of BF-producing *S. aureus* highlights the adaptability and significance of this gene in various ecological contexts. Understanding its role is essential for both clinical and environmental perspectives, as it sheds light on antibiotic resistance mechanisms and microbial interactions within natural ecosystems. Further research is warranted to explore the full scope of *IcaA*'s impact on biofilm formation and its implications for public health and environmental science

Conflict of Interest

The authors declare that there are no conflicts of interest related to this study.

Acknowledgement

The author is grateful to Department of Biology, College of Education- Qurna, University of Basrah, Iraq.

References

Afzal, M., Vijay, A. K., Stapleton, F., & Willcox, M. D. (2021). Susceptibility of ocular *Staphylococcus aureus* to antibiotics and multipurpose disinfecting solutions. *Antibiotics, 10*(10), 1203. https://doi.org/10.3390/antibiotics10101203

Ahmad, S., Rahman, H., Qasim, M., Nawab, J., Alzahrani, K. J., Alsharif, K. F., & Alzahrani, F. M. (2022). Staphylococcus epidermidis pathogenesis: Interplay of *IcaA*dbc operon and mscramms in biofilm formation of isolates from pediatric bacteremia in Peshawar, Pakistan. *Medicina, 58*(11), 1510. https://doi.org/10.3390/medicina58111510

Ajmal, A. W., Saroosh, S., Mulk, S., Hassan, M. N., Yasmin, H., Jabeen, Z., ... & Mumtaz, S. (2021). Bacteria isolated from wastewater irrigated agricultural soils adapt to heavy metal toxicity while maintaining their plant growth promoting traits. *Sustainability, 13*(14), 7792. https://doi.org/10.3390/su13147792

Alharbi, O. S., Alhazmi, K. A., Gazzaz, M., Almuhayya, S., Aldehalan, F. A., Sharif, A. T., ... & Ibrahem, K. (2025). A review vancomycin role in Gram Positive biofilm-associated infections: challenges and emerging solutions. *Therapeutics and Clinical Risk Management*, 1569-1578. https://doi.org/10.2147/TCRM.S541780

Alibegli, M., Bay, A., Fazelnejad, A., Ghezelghaye, P. N., Soghondikolaei, H. J., & Goli, H. R. (2025). Contribution of *IcaA*DBC genes in biofilm production ability of *Staphylococcus aureus* clinical isolates collected from hospitalized patients at a burn center in North of Iran. *BMC Microbiology, 25*(1), 302. https://doi.org/10.1186/s12866-025-04018-z

Arciola, C. R., Baldassarri, L., & Montanaro, L. (2001). Presence of *IcaA* and icaD genes and slime production in a collection of staphylococcal strains from catheter-associated infections. *Journal of Clinical Microbiology, 39*(6), 2151–2156. https://doi.org/10.1128/jcm.39.6.2151-2156.2001

Awayid, H. S., & Qassim Mohammad, S. (2022). Prevalence and Antibiotic Resistance Pattern of Methicillin-Resistant *Staphylococcus aureus* Isolated from Iraqi Hospitals. *Archives of Razi Institute, 77*(3). https://doi.org/10.22092/ARI.2022.357391.2031

Bamneshin, K., Poudineh, M., Alibabaei, R. H., Amiri, M. R. J., Fateminasab, Z. S., Ghorbani, Z., Maleki, R., & Khaledi, A. (2024). Prevalence of *IcaA*DBC genes, and correlation with biofilms and antibiotic resistance in *S. aureus*: a systematic review and meta-analysis. *Germs, 14*(4), 387. https://doi.org/10.18683/germs.2024.1448

Cheung, G. Y., & Otto, M. (2023). Virulence mechanisms of staphylococcal animal pathogens. *International Journal of Molecular Sciences, 24*(19), 14587. https://doi.org/10.3390/ijms241914587

Colaco, H. G., Barros, A., Neves-Costa, A., Seixas, E., Pedroso, D., Velho, T., Willmann, K. L., Faisca, P., Grabmann, G., & Yi, H.-S. (2021). Tetracycline antibiotics induce host-dependent disease tolerance to infection. *Immunity, 54*(1), 53–67. https://doi.org/10.1016/j.immuni.2020.09.011

Gale, M. S., & Gale, B. D. S. M. (2022). Diagnosis: fundamental principles and methods. *Cureus, 14*(9). https://doi.org/10.7759/cureus.28730

Girijan, S. K., & Pillai, D. (2021). Identification and characterization of vancomycin-resistant *Staphylococcus aureus* in hospital wastewaters: evidence of horizontal spread of antimicrobial resistance. *Journal of Water and Health, 19*(5), 785-795. https://doi.org/10.2166/wh.2021.117

Humphries, R., Bobenchik, A. M., Hindler, J. A., & Schuetz, A. N. (2021). Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100. *Journal of Clinical Microbiology, 59*(12), 10-1128. https://doi.org/10.1128/jcm.00213-21

Hussein, R. A., Al-Kubaisy, S. H., & Al-Ouqaili, M. T. (2024). The influence of efflux pump, outer membrane permeability and β-lactamase production on the resistance profile of multi,

extensively and pandrug resistant Klebsiella pneumoniae. *Journal of Infection and Public Health, 17*(11), 102544. https://doi.org/10.1016/j.jiph.2024.102544

Kadkhoda, H., Ghalavand, Z., Nikmanesh, B., Kodori, M., Houri, H., Maleki, D. T., Bavandpour, A. K., & Eslami, G. (2020). Characterization of biofilm formation and virulence factors of *Staphylococcus aureus* isolates from paediatric patients in Tehran, Iran. *Iranian Journal of Basic Medical Sciences, 23*(5), 691. https://doi.org/10.22038/ijbms.2020.36299.8644

Khan, F., Shukla, I., Rizvi, M., Mansoor, T., & Sharma, S. C. (2011). Detection of biofilm formation in *Staphylococcus aureus*. Does it have a role in treatment of MRSA infections? *Trends in Medical Research, 6*(2), 116–123. https://doi.org/10.3923/tmr.2011.116.123

Kim, D. H., Joo, M. S., Jang, S. R., Kim, H. J., Min, J. G., & Nam, B. H. (2025). Impact of Antibiotic Exposure on Growth and Biofilms Formation in Aeromonas salmonicida Subspecies Isolated from Atlantic Salmon (Salmo salar). *Microorganisms, 13*(12), 2863. https://doi.org/10.3390/microorganisms13122863

Levesque, R. (2005). SPSS programming and data management: a guide for SPSS and SAS users. Spss. https://dl.acm.org/doi/abs/10.5555/1199139

Michaelis, C., & Grohmann, E. (2023). Horizontal gene transfer of antibiotic resistance genes in biofilms. *Antibiotics, 12*(2), 328. https://doi.org/10.3390/antibiotics12020328

Mohammadi, M., Bahrami, N., Khajavian, M., & Faghri, J. (2020). The Occurrence of Type I, II, and III Integrons in Multi-drug Resistance and Methicillin-Resistant *Staphylococcus aureus* Isolates in Iran: M. Mohammadi *et al*. *Current Microbiology, 77*(8), 1653-1659. https://doi.org/10.1007/s00284-020-01956-x

Mohammed, A. L., & Al-ledani, A. A. (2020). Molecular detection of ica gene and some surface proteins in biofilm producer of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. *Biochemical & Cellular Archives, 20*. https://faculty.uobasrah.edu.iq/uploads/publications/1619436104.pdf

Moraes, G. F. Q., Cordeiro, L. V., & de Andrade Júnior, F. P. (2021). Main laboratory methods used for the isolation and identification of Staphylococcus spp. *Revista Colombiana de Ciencias Químico-Farmacéuticas, 50*(1), 5-28. https://doi.org/10.15446/rcciquifa.v50n1.95444

Nasser, A., Dallal, M. M., Jahanbakhshi, S., Azimi, T., & Nikouei, L. (2022). *Staphylococcus aureus*: biofilm formation and strategies against it. *Current Pharmaceutical Biotechnology, 23*(5), 664-678. https://doi.org/10.2174/1389201022666210708171123

Oliveira, A., & Cunha, M. D. L. R. (2010). Comparison of methods for the detection of biofilm production in coagulase-negative staphylococci. *BMC Research Notes, 3*(1), 260. https://doi.org/10.1186/1756-0500-3-260

Omidi, A. H., Sabati, H., Amini, S., Zonobian, M. A., & Mohammadi, M. R. (2021). *Staphylococcus aureus* in the environment of healthcare centers. *Cellular, Molecular and Biomedical Reports, 1*(4), 147-157. https://doi.org/10.55705/cmbr.2021.403541.1156

Paul, A. J., Chandra, M., Kaur, G., Narang, D., & Gupta, D. K. (2026). Isolation and Characterization of *Staphylococcus aureus* and Its Biofilm Implicated in Bovine Mastitis. *Indian Journal of Microbiology*, 1–13. https://doi.org/10.1007/s12088-026-01542-y

Peng, Q., Tang, X., Dong, W., Sun, N., & Yuan, W. (2022). A review of biofilm formation of *Staphylococcus aureus* and its regulation mechanism. *Antibiotics, 12*(1), 12. https://doi.org/10.3390/antibiotics12010012

Pugazhendhi, A. S., Wei, F., Hughes, M., & Coathup, M. (2022). Bacterial adhesion, virulence, and biofilm formation. In Musculoskeletal Infection (pp. 19-64). *Cham: Springer International Publishing*. https://doi.org/10.1007/978-3-030-83251-3_2

Sandman, Z., & Iqbal, O. A. (2024). Azithromycin. *StatPearls [Internet].*

Sauer, K., Stoodley, P., Goeres, D. M., Hall-Stoodley, L., Burmølle, M., Stewart, P. S., & Bjarnsholt, T. (2022). The biofilm life cycle: expanding the conceptual model of biofilm formation. *Nature Reviews Microbiology, 20*(10), 608-620. https://doi.org/10.1038/s41579-022-00767-0

Sharan, M., Dhaka, P., Bedi, J. S., Mehta, N., & Singh, R. (2024). Assessment of biofilm-forming capacity and multidrug resistance in *Staphylococcus aureus* isolates from animal-source foods: implications for lactic acid bacteria intervention. *Annals of Microbiology, 74*(1), 22. https://doi.org/10.1186/s13213-024-01768-5

Taj, Y., Essa, F., Aziz, F., & Kazmi, S. U. (2012). Study on biofilm-forming properties of clinical isolates of *Staphylococcus aureus*. *The Journal of Infection in Developing Countries, 6*(05), 403–409. https://doi.org/10.3855/jidc.1743

Tang, K., & Zhao, H. (2023). Quinolone antibiotics: resistance and therapy. *Infection and Drug Resistance*, 811–820. https://doi.org/10.2147/IDR.S401663

Thy, M., Timsit, J. F., & de Montmollin, E. (2023). Aminoglycosides for the treatment of severe infection due to resistant gram-negative pathogens. *Antibiotics, 12*(5), 860. https://doi.org/10.3390/antibiotics12050860

Wang, S., Zhao, Y., Breslawec, A. P., Liang, T., Deng, Z., Kuperman, L. L., & Yu, Q. (2023). Strategy to combat biofilms: a focus on biofilm dispersal enzymes. *NPJ Biofilms and Microbiomes, 9*(1), 63. https://doi.org/10.1038/s41522-023-00427-y

Weinstein, M. P., & Lewis, J. S. (2020). The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. *Journal of Clinical Microbiology, 58*(3), 10-1128. https://journals.asm.org/doi/full/10.1128/jcm.01864-19

Zhang, L., Tian, X., Sun, L., Mi, K., Wang, R., Gong, F., & Huang, L. (2024). Bacterial efflux pump inhibitors reduce antibiotic resistance. *Pharmaceutics, 16*(2), 170. https://doi.org/10.3390/pharmaceutics16020170