



JHB



SPBH

Original Article**Determine some of the virulent factors for isolates of *Staphylococcus aureus* by using a gene (icaA)**Ali Salam Ali Al-Karaawi¹ Yousif Sahib Fakher²¹College of Dentistry, University of Alkafeel, Najaf, Iraq alisalam@alkafeel.edu.iq²College of Dentistry, University of Alkafeel, Najaf, Iraq. yousif.alasdy@alkafeel.edu.iqCorresponding Author E-mail: alisalam@alkafeel.edu.iqDOI: <https://doi.org/10.71428/JHB.2026.0116>**Abstract**

Identifying the *icaA* gene in *S. aureus* isolates and assessing biofilm development using the PCR method were the goals of this investigation. The study included 20 *S. aureus* isolates sourced from patients with wound infections and other instances. The catalase, motility, coagulase, and growth on mannitol. We used salt agar tests to find *S. aureus*. We used the disc diffusion method to evaluate all *S. aureus* isolates for antibiotic resistance against four β -lactam drugs. Using the PCR approach, scientists found the *icaA* gene in *S. aureus* isolates. 90% of the isolates were *icaA* positive, while 10% were *icaA* negative. The analysis revealed that 60% of the *S. aureus* isolates had positive biofilm formation, whereas 40% displayed negative biofilm formation.

Keywords: *S. aureus*, *icaA* gene, antibiotic resistance against four β -lactam drugs, biofilm formation.

1. Introduction

Staphylococcus aureus poses a significant global public health threat due to its ability to produce severe, sometimes fatal infections in both community and hospital environments, and its increasing resistance to existing antibiotic treatments [1]. According to [2], 28% of the golden-hued, gram-positive *Staphylococcus aureus* bacteria exhibit resistance to methicillin and all known β -lactam antibiotics. According to [3], twenty percent of individuals exhibit a permanent *S. aureus* infection, sixty percent are intermittent carriers, and twenty percent are non-carriers.

Potential virulence factors of *Staphylococcus aureus* include surface proteins that facilitate host tissue colonization, invasins that promote bacterial dissemination within tissues (e.g., leukocidin, kinases, and hyaluronidase), surface components that inhibit phagocytic engulfment (such as the

capsule and Protein A), and biochemical characteristics that enhance bacterial survival within phagocytes (including the synthesis of carotenoids and catalase). persist inside phagocytes (synthesizing carotenoids and catalase, for instance) [4].

2. Specimens Collection

For this study, 300 clinical samples were gathered between the months of August 2023 and February 2024. The samples came from people who had attended the Central Laboratory Hospitals, such as Najaf, Al-Hakim General, and Al-Furat Al-Awsat. The age range of the participants was 1 to 60 years. Patients with prostatitis (40 samples), urinary tract infections (80 samples), vaginal infections (50 samples), and purulent infections, specifically surgical wound infections (60 samples) and burn wound infections (70 samples), were included in the research. The transport medium was used to move a

swab that had been taken from the afflicted region to the lab. The swab included information on the patients' age, sex, and kind of infection. Samples were taken from people who had not taken antibiotics for a week before the sample collection. Urine samples were taken midstream and placed in sterile, wide-mouthed jars. After that, the aperture was cultivated on mannitol and salt agar plates with a ring.

Method

Isolate of Bacteria (Routine Test) *S. aureus* primary identification Separate:

When *Staphylococcus aureus* germs are stained violet (purple), they show up in the Gram stain test as gram-positive bacteria. Beta hemolysis in bacteria is often detected using the blood agar test.

Mannitol contains the *Staphylococcus aureus* bacterium. Test for salt agar: If the pink media around the bacterial growth has become yellow. has produced acid by fermenting the mannitol. As acid lowers the medium's pH, the phenol becomes yellow. Mannitol fermentation will go well if the bacteria are yellow.

Catalase test. By distinguishing between *Staphylococci* and *Streptococci*, the test enables an accurate categorization according to the presence or absence of the catalase enzyme. This enzyme is present in *staphylococci* but absent in *streptococci*. When it comes to differentiating and identifying bacteria, motility testing is quite helpful. The bacteria *Staphylococcus aureus* are not able to move. [5].

Staphylococcus aureus Isolate Identification Using the VITEK-2 System

The VITEK 2 System, the next advancement in bacterial diagnostics, incorporates a novel colorimetric technique. Protocol: In accordance with the manufacturer's protocol (Biomérieux), [6, 7] executed the following actions:

1. A colony was introduced via a loop subsequent to the addition of 3 milliliters of standard saline to a test tube.

The colonization was calibrated to the McFarland standard solution (1.5×10^8 cells/ml) after the test tube was placed into a dense check instrument.

3. A barcode is utilized to enter a sample detection number into the software program once the inoculum has been implanted in the container.

4. Upon scanning the barcode on the VITEK 2 card formula, the card was attached to the sample's identity.

5. Following the completion of the cards, the container was moved to the incubator system for reading after being placed within the filler element.

The apparatus carried out the following action: it controls the temperature during incubation. Test results are continually monitored and sent to the gadget for assessment by the visual interpretation of the cards. The system automatically disposed of the cards in a residual bin at the end of the evaluation period.

3. Molecular Study

Molecular analysis of the virulence gene (*icaA*). The PCR method was performed.

Extraction of DNA

Mannitol salt agar and brain heart infusion agar were used to cultivate the *S. aureus* isolates. Following the manufacturer's instructions, the genomics DNA Extraction Kit (Favorgen® Genomic DNA Mini Kit) was used to extract DNA in the following manner:

1. Fill the 1.5 ml tube for microcentrifuge (that is not included) with the required number of bacterial cells (minimum of 1×10^9) and centrifuge for 1 minute at 18,000 x g. It is necessary to discard the residue that remains.
2. 200 microliters of lysozyme solution (20 mg/ml lysozyme, 20 mM Tris-HCl, 2 mM EDTA, 1% Triton X-100, pH 8.0) were used and then stirred to resuspend the precipitate.

3. Allow it to stand for 10 minutes at room temperature during the incubation period, then turn the tube over every 2 to 3 minutes.
4. Vortex the sample for five seconds after adding 200 μ l of FABG buffer.
5. The sample lysate should turn transparent after 10 minutes of incubation at 70°C. Throughout the incubation period, flip the tube every three minutes.
6. Fill the sample with 200 μ L of 96–100% ethanol, then vortex for 10 seconds. After that, put an FABG column in a collecting tube, pour the sample mixture into the centrifuge and the column for 1 min at 18000 x grams
7. Fill the FABG Column with 400 μ l of W1 Buffer, then centrifuge at 18000 x g for 30 seconds. Return the FABG column to the collecting tube after removing the flow-through.
8. Fill the FABG column with 600 μ l of buffer for washing, then centrifuge at 18000 x grams for 30 seconds. Return the FABG column to the collecting tube after removing the flow-through.
9. Centrifuge the column for an additional three minutes at 18000 x g to facilitate drying.
10. The desiccated FABG column should thereafter be transferred to a tube microcentrifuge 1.5 ml.
11. 100 μ L of hot TE solution or the solvent solution was added to the central membrane of the FABG column.
12. The FAGB column was incubated for 10 minutes at 37°C.
13. Centrifuged to elute the DNA for one minute at a maximum speed of 18000 x g.
14. The DNA fragment was stored at -20 degrees Celsius.

Table 1: PCR Program Status

| Gene | Temperatures (degrees Celsius) / Time | | | | | Quantity of cycles |
|------|---------------------------------------|------------------------|-----------|-----------|-------------------|--------------------|
| | First Denatured | Condition of one cycle | | | Ultimate Extended | |
| | | Denatured | Annealed | Extended | | |
| icaA | 95/5 min | 95/30 sec | 55/30 sec | 72/30 sec | 72/5 min | 35 cycles |

4. Results and Discussion:

Biofilm Production

It is well known that the pathogenicity of *S. aureus* is associated with a number of virulence factors, including toxins and enzymes. The ability of the MRSA and MSSA isolates to produce biofilm was investigated using the Congo red agar method. As seen in Figure 1.

This method, first introduced by [8], uses a properly prepared solid medium to identify the formation of a bacterial slime layer. 48 out of 80 isolates (60%) formed a resilient mucosal layer, as shown by the

generation of colony black exhibiting a dry crystalline structure, whereas 32 out of 80 isolates (40%) did not generate a slime layer [9]. This result aligns with the findings of [10], who discovered that 43% of MRSA tested negative, but 57% were able to form a slime layer.

The results corroborated the conclusions of researchers [11] who assert that CRA studies are a reliable method for evaluating biofilm formation [12] and revealed that the CRA test is an effective means of determining slime and biofilm creation capabilities.

The study's resilient mucosal layer development in the MRSA isolate aligned with many investigations that validated MRSA's propensity for slime layer development. Bacteria may synthesize glycocalyx, an extracellular substance comprised of polysaccharides, polypeptides, or a combination of both. The slime layer forms in this way. This layer protects the bacterial cell against desiccation and nutritional depletion. When the glycocalyx is weakly attached to the cell wall, it becomes irregular, leading to the formation of the slime layer. The glycocalyx, composed of materials intimately associated with the cell wall, is referred to as a capsule [13]. The slime layer forms biofilms, which are thin, living membranes, by aggregating bacterial cells. These biofilms serve as a barrier that diminishes the efficacy of antibiotics on bacterial cells, hence imparting resistance [14].

Bacterial colonization of external surfaces is significantly influenced by slime layer generation, which is the primary component of biofilm development [15]. The CRA approach, which was used in this investigation, is one of many established techniques for studying slime layer development.

The PCR study results for *icaA* showed that 18 out of 20 (90%) *S. aureus* isolates tested positive, as shown in Figure 2.

Furthermore, our findings demonstrated the crucial function of *ica* genes as virulence indicators in *staphylococcal* infections by showing that the gene (*icaA*) was present in all strains that produced biofilms [16].

The synthesis of various microbial surface components that recognize adhesive matrix molecules initiates the first attachment, marking the first phase of *Staphylococcus aureus* biofilm formation [17]. Before evolving into structurally dynamic biofilms, *Staphylococcus aureus* first adheres to one another during the last phases of adhesion. The biofilm matrix forms multi-layer structures by action of polysaccharide intercellular adhesion (PIA), consisting of β -1,6-linked N-acetylgalactosamine (PNAG) [18,19]. It is encoded by the four primary genes *icaA*, *icaD*, *icaB*, and *icaC*. Additionally, the gene *icaR* functions as a regulator [20,21]. The proteins ICAA, ICAD, ICAB, and ICAC are encoded by these specific genes. The synthesis of mucus is enabled by the coexpression of the *icaA* and *icaD* genes. Strains possessing the *icaADBC* cluster have shown the ability to form biofilms [22].

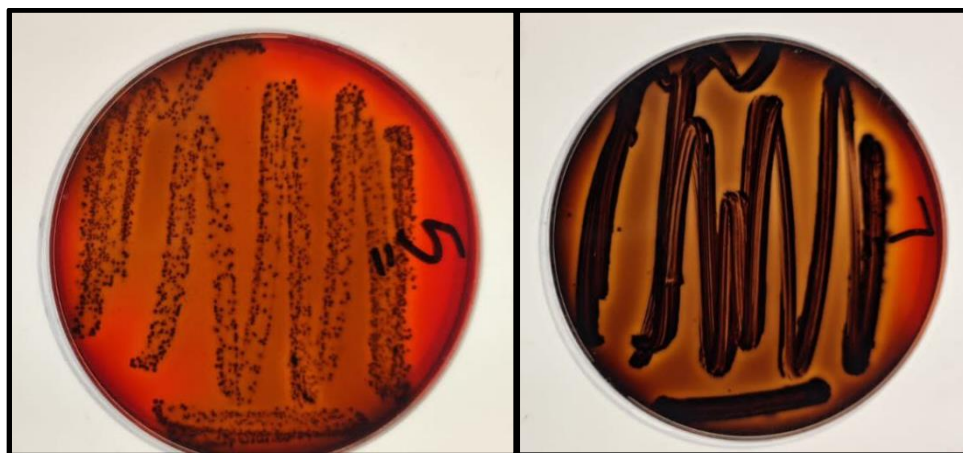


Figure (A)

Figure (B)

Figure 1: Figure (A) Biofilm formation of *Staphylococcus aureus* by the Congo red agar method (isolate No. 5), Figure (B) (isolate 6)

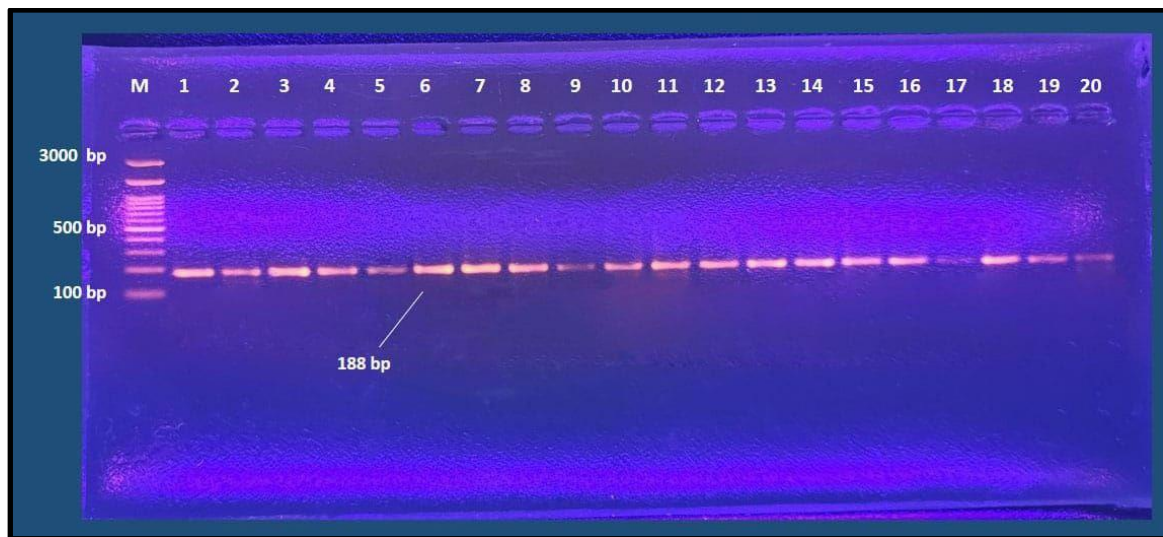


Figure 2: In 1.5% agarose gel electrophoresis, the *icaA* gene's PCR product was injected into each well with 5 μ L of the PCR product, voltage (80 V), and duration (45 minutes). Lane M: PCR product (positive case band 188 bp) and DNA ladder (100 bp-3000 bp).

5. Conclusions

The research for this study led to the following conclusions:

The highest rate of *S. aureus* isolation during our study occurred in urinary tract infections. and many more microorganisms. The *Staphylococcus aureus* bacteria were the most prevalent kind.

This research also discovered that burn wound infections had the greatest prevalence of *S. aureus* isolation.

60% of the *S. aureus* isolates showed an obvious potential to generate biofilm.

We observed that most bacterial isolates had a very high level of expression of the *icaA* gene (90%). This gene is one of the virulence factor genes.

Conflict of interest: NIL

Funding: NIL

6. References

- [1] Brea D.; Duval, A. M.; Sarah W. S. and William M. S.(2010). Altered Growth, Pigmentation, and Antimicrobial Susceptibility Properties of *Staphylococcus aureus* Due to Loss of the Major Cold Shock Gene *cspB* Antimicrob. Agents Chemother. 54 (6): 2283-2290
- [2] Zetola, N.; Francis, J.S.; Nuemberger, E.L. and Bishai, W.R. (2005). Community acquired methicillin- resistant *Staphylococcus aureus*: an emerging threat. *Lancet Infect. Dis.*, 9: 978-984.
- [3] VonEiff, C.; Becker, K.; Machka, K. Stammer, H. and Peters, G. (2001). Nasal carriage as a source of *Staphylococcus aureus* bacteremia. *N. Engl. J. Med.*, 344: 11-16.
- [4] Alhameedawi, A.; Sadeghizadeh, M. and Al-Rubaii, B. A. (2025). Nanocurcumin: An Innovative Strategy to Combat Virulence and Methicillin Resistance in *Staphylococcus aureus* Isolated from Burn Wounds. *Iranian Journal of Medical Sciences*, published 2025. DOI: 10.30476/ijms.2025.105799.3976.
- [5] Al-Khafaji, A. N. (2018). Isolation and Identification of Methicillin Resistance *Staphylococcus aureus* and Detection their Ability to the Production of Virulence Factors.

Journal of University of Babylon for Pure and Applied Sciences, 26(8), 100-111.

- [6] Lee, J.Y.H.; Monk, I.R.; Gonçalves da Silva, A.; et al. (2018). Global spread of three multidrug-resistant lineages of *Staphylococcus epidermidis*. *Nat Microbiol* 3, 1175-1185.
- [7] Lal, U. A. P.; Ambhore, N.; Raut, S.; Mantri, R.; Malak, N. and Sharma, P. (2024). Identification of Inducible Clindamycin Resistance in *Staphylococcus aureus* using Automated Vitek-2 Compact System and D test. *Journal of Medical Science and Health*, 10(2), 136-141.
- [8] Freeman, D.J.; Falkiner, F.R.; and Keane, C.T.(1989). New method for detecting slime production by coagulase negative staphylococci. *J. Clin. Pathol.* 42:872-874.
- [9] Khan, F.; Shukla, I.; Rizvi, M.; Mansoor, T. and Sharma, S.C. (2011). Detection of biofilm formation in *Staphylococcus aureus*. Does it have a role in treatment of MRSA infection. *Trends in Med. Res. Academic J.*6(2).116-123.
- [10] Silva, V.; Pereira, J. E.; Maltez, L.; Poeta, P. and Igrejas, G. (2022). Biofilm Formation, Virulence, and Antimicrobial Resistance of *Staphylococcus aureus* from Different Sources. *Pathogens*, 11(10), 1069. DOI: 10.3390/pathogens11101069.
- [11] Arciola, C.R.A.; Campoccia, D.; Baldassarri, L.; et al. (2006): Detection of biofilm development in *Staphylococcus epidermidis* from implant infections. Comparison of a PCR approach that detects *ica* genes with two traditional phenotypic methods. *J Biomed Mater Res A* 76: 425–430.
- [12] Drożdż, K.; Ochońska, D.; Ścibik, Ł.; Gołda-Cępa, M.; Biegun, K. and Brzywczy-Włoch, M. (2022). The Frequency of Occurrence of Resistance and Genes Involved in the Process of Adhesion and Accumulation of Biofilm in *Staphylococcus aureus* Strains Isolated from Tracheostomy Tubes. *Microorganisms*, 10(6), 1210. DOI: 10.3390/microorganisms10061210.
- [13] Kirisits, M. J.; Margolis, J. J.; Purevdorj-Gage, B. L.; Vaughan, B.; Chopp, D. L.; Stoodley, P. and Parsek, M. R. (2007). Influence of the hydrodynamic environment on quorum sensing in *Pseudomonas aeruginosa* biofilms..
- [14] Sharan, M.; Vijay, D.; Dhaka, P.; Bedi, J. S. and Gill, J. P. S. (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, published July 2024. DOI: 10.1186/s13213-024-01768-5.
- [15] O'GARA, J. P., and Humphreys, H. (2001). *Staphylococcus epidermidis* biofilms: importance and implications. *Journal of Medical Microbiology*, 50(7), 582-587.
- [16] Mousavi, S. M.; Babashahi, E. and Khaledi, A. (2024). Evaluation of *icaA* and *icaD* Genes Involved in Biofilm Formation in *Staphylococcus aureus* Isolates from Clinical Sources Using Reverse Transcriptase PCR. PMC/NCBI, Collection date 2024 Dec. Received 2023 Dec 19; Accepted 2024 Apr 27. PMC12207945.
- [17] Otto, M. (2014). *Staphylococcus aureus* toxins. *Current opinion in microbiology*, 17, 32-37.
- [18] Periasamy, S.; Joo, H. S.; Duong, A. C.; Bach, T. H. L.; Tan, V. Y.; Chatterjee, S. S.; and Otto, M. (2012). How *Staphylococcus aureus* biofilms develop their characteristic structure. *Proceedings of the National Academy of Sciences*, 109(4), 1281-1286.
- [19] McKenney, D.; Ubner, J.H.; Muller, E.; Wang, Y.; Goldmann, D. A., and Pier, G.B. (1998). The *ica* locus of *Staphylococcus epidermidis* encodes production of the capsular

polysaccharide/adhesion. *Infect. Immun.* 66, 4711–4720.

- [20] Atshan, S. S.; Nor Shamsudin, M.; Sekawi, Z.; Lung, L. T. T.; Hamat, R. A.; Karunanidhi, A.; and Pei Pei, C. (2012). Prevalence of adhesion and regulation of biofilm-related genes in different clones of *Staphylococcus aureus*. *Journal of Biomedicine and Biotechnology*.
- [21] Banneshin, K.; Poudineh, M.; Alibabaei, R. H.; Amiri, M. R. J.; Fateminasab, Z. S.; Ghorbani, Z.; Maleki, R. and Khaledi, A. (2024). Prevalence of IcaADBC genes, and correlation with biofilms and antibiotic resistance in *S. aureus*: a systematic review and meta-analysis. *Germs*, 14(4), 387. DOI: 10.18683/germs.2024.1448.
- [22] Singh, A. K.; Prakash, P.; Singh, R.; Gupta, N.; Bhushan, G. and Tripathi, M. (2024). Investigation of Biofilm Formation and Presence of IcaA/D Genes in Clinical *Staphylococcus* Isolates. *African Journal of Biomedical Research*, 27(3s), 5175-5182.