Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue 2, 483-492

Molecular Detection of Biofilm related Genes in *Staphylococcus aureus*Isolated from UTI Patients in Baghdad City

[1]Shaymaa Fouad Rasheed Al-Khazraji, [2]Rasmiya Abd Aburesha

[1,2] Biology Department, College of Science, University of, Baghdad, Iraq

Received: February 20, 2025 / Accepted: May 20, 2025 / Published: November 16, 2025

Abstract : The increase in the prevalence of resistance to antibiotics among pathogenic bacteria is a severe danger to public health predicated to cause almost five million fatalities. *Staphylococcus aureus* is one of the most efficient pathogens that can form biofilm. The infection resulting from this bacterium's biofilm is considered a serious problem, as it is difficult to treat it with traditional antibiotics.The current study was conducted to estimate the prevalence of some biofilm-mediated genes of *S. aureus* collected from urinary tract infections, to detect their ability to construct biofilms, and to determine their resistance to antibiotics.The *S. aureus* isolates were obtained from patients with urinary tract infections in different local hospitals in Baghdad city. The antibiotic susceptibility pattern toward twenty antibiotics and quantitative assays for biofilm construction was performed for all bacterial isolates. Moreover, a multiplex polymerase chain reaction (PCR) was adopted to detect the prevalence of six targeted genes *(fib, eno, sdrC, bap, clfA and clfB).*The results of the antibiotic susceptibility pattern indicated that most isolates exhibited resistance to Benzylpenicillin, oxacillin, Erythromycin, Piperacillin/Tazobactam and Tetracycline. In contrast, all isolates were susceptible to Gentamicin, Tigecycline and Linezolid. The multidrug resistance characteristics appeared in all isolates under the study that were resistant to at least three or more distinct classes of antibiotics. Furthermore, the result revealed that most isolates produced strong and moderate biofilms, 42.42% and 48.48%, respectively; meanwhile, 6.06% and 3.03% of the isolates were formerly weak, non-biofilms. The presence of the *fib* gene was detected in 90.9% of the isolates, while the *eno* and *sdrC* genes were observed in all the isolates 100%. In contrast, the *bap* gene did not appear in any of the isolates 0%. In addition, the prevalence of *clfA* and *clfB* in isolates under investigation was 90.9% and 87%, respectively. In conclusion, the ability to develop biofilms is an efficient strategy that may contribute to preventing antimicrobial agents from overcoming *S. aureus*, as all isolates are multidrug-resistant and have a high percentage of strong biofilm producers. In addition, the high prevalence of some biofilm-associated genes highlights their crucial role in biofilm development in these pathogenic bacteria. It provides an insight into the relation between biofilm formation and multidrug resistance to different classes of antibiotics.

Keyword: Biofilm, PCR,*S. aureus* ,Urinary tract infection

Corresponding author: (Email : sh_frbio@yahoo.com**)**

Introduction

Infection of the urinary tract is one of the most frequent infectious diseasesin humans, both in hospital and community settings. Its global incidence is expected to be 250 million cases each year(1) The gram-positive pathogen *Staphylococcus aureus* results in relatively unusual urinary tract infectionsin the general population, with a rate ranging from 0.5% to 6%,. However, it can be distributed in specific groups of people, for instance,

elderly catheterized patients or people infected with *S. aureus*bacteremia (2). Itis frequently present without symptoms in different parts of the host's body. Itcan adapt to different hosts and environmental conditions and produce various types of diseases(3). It produces multiple virulence factors that contribute to its ability to cause severe illnesses. These factors are categorizedin the form of secreted exotoxins and cell-surface-associated virulence determinants(4). In addition, the biofilm construction by *S. aureus* represents a significant virulence factor, which is facilitated mainly by the genes involved in intercellular adhesion and acts as one of the most efficient protective strategies of this opportunistic bacteria (5,6). *S. aureus*is one of the most effective biofilm producer pathogens;hence, this property enables it to attach itself to tissues of the host and medical devicesand stay there for a long period of time (7,8). The biofilm prevents antibiotics from reaching the *S. aureus*, and it can evade the host immune system's destruction and develop into persistent cells. *S. aureus* can express different genes that play a crucial role in biofilm construction, such as the *fib, eno,sdrC, bap, clfA,and clfB*genes,which encode the fibrinogen binding protein.The *eno* gene encodes the laminin-binding protein that has a role of attaching the cells to a solid surface, while the *sdrC* gene encodesa serine-aspartate repeat protein, whose role is cell-to-cell attachment andattachment of cells to solid surfaces(9).The *bap* gene encodesa big protein that promotes both primaryattachment to the inert surface and intracellular adhesion(10).The clumping factor genes *clfA* and *clfBare*intracellular adherence code cell wall-anchored proteins that bind to the surface fibrinogen of the host .The colonizationof *S. aureus*is facilitated bythe attachment of clumping factors *AB*,biofilm construction and pathogenicity, bybinding the solublefibrinogen(11).Owing to a little research on the existence of some biofilm-related genes in the clinical isolates of*S. aureus*, this study was conducted to detect, phenotypically, thecapacity of *S. aureus*isolates to develop a biofilm and,at the same time, estimate the existence of some biofilm-associated genes among the *S. aureus* isolates.

Methodology

Collection of specimens

During four months, from October 2023 to January 2024, 250 clinical specimens (urine) were collected at the local hospital of Baghdad City-Iraq (Alyarmok Teaching Hospital, Baghdad Teaching Hospital, and Ghazgical al Hariri Surgical Specialties Hospital). The current study comprises urine specimens obtained from patients of different ages and genders who visit the hospital with suspected infection during the specimen collection period.

Isolation and Growth Conditions of *S. aureus*

The isolation and identification of *S. aureus* isolates were done based on the morphological characteristics of the isolates and the results of the biochemical tests. The samples were cultured directly on different media types, such as mannitol salt agar and blood agar, for morphological identification. The biochemical tests were utilized to examine the ability of the isolates to produce catalase, oxidase, and coagulase enzymes (12). Finally, the identification of the *S. aureus* isolates in the urine samples was confirmed by using the VITEK 2 compact system.

Antibiotic Sensitivity Test

A Vitek 2 compact system (BioMerieux/ France) with a Gram-positive sensitivity card (AST) was adopted to analyze and evaluate the antibiotic susceptibility profile of all the isolates under investigation, against 20 different antibiotics.

Biofilm Formation Assay

The biofilm development assay was carried out for all isolates under investigation, using the colorimetric plate technique. The assay was performed in triplicate for each isolate. In summary, a volume of 0.5 ml from 18 hours of *S. aureus* growth, with no 0.5 McFarland turbidity, was added to sterile Tryptic soy broth containing 1% Glucose (TSBG). Following that 1: 100 dilutions were prepared and a volume with 150 µl was poured into the wells of the plate. Negative control was applied by loading 150 µl of TSBG, without *S aureus.* growth. The plate was incubated for 24 hours at 37°C. After that the broth in the wells was discarded and the plate was carefully washed by applying 150 µl of phosphate buffer saline (PBS) to remove all planktonic cells. The adherent *S. aureus* cells were fixed by methanol and stained with 150µl of crystal violet (0.1%). The plate was put on filter paper in an inverted position for 60 minutes for air drying. A volume of 0.1 ml of 96% ethanol was added, for 30 minutes to solubilize the fixed crystal violet (8). The plate was subjected to a micrplate reader at 590 nm, to estimate the optical density for each control (ODC) and isolate (ODI). The isolates were divided into four main groups: The ones that did not create a biofilm (ODI < ODC), isolates with weak biofilm development (ODI < 2 *ODC), isolates with moderate biofilm development (ODI < 4*ODC) and isolates with strong biofilm development (ODI > 4*ODC)(13).

Molecular Detection of Some Biofilm-Mediated Genes

The isolation and purification of genomic DNA for all isolates under the study were carried out by following the instructions of the ABIOpure extraction Protocol (Promega, USA). The DNA concentration was estimated by adopting the Quantus Fluorometer. The specific primers that were used to confirm the presence of *genes* (*fib, eno, sdrC, bap, clfA and clfB),* their names, sequences, product sizes and references are described in Table 1.The specific primers for *clfA* and, *clfB* genes were designed according to the *S. aureus* genome information available on the NCBI primers designed.The multiplex polymerase chain reaction was adopted to detect the target genes. The PCR reaction component and conditions are illustrated in Table (2). Presence of the interest genes after PCR amplification was performed, using 2% agarose gel with 4 µl of ethidium bromide (10 mg/ml) for electrophoresis.

Table (1) : Spcific primers that were utilised in the current study

Gene Name	Sequence of Primers	Annealing temperature	Size of product	Reference
fib	Forward CGTCAACAGCAGATGCGAGCG Reverse TGCATCAGTTTTCGCTGCTGGTTT	60°C	239	14
eno	Forward TGCCGTAGGTGACGAAGGTGGTT ReverseGCACCGTGTTCGCCTTCGAACT	60°C	195	14
sdrC	Forward AAAAGGCATGATACCAAATCGA Reverse AATTCTCCATTCGTATGTTCTG	53°C	144	15
bap	ForwardCCCTATATCGAAGGTGTAGAATT ReverseGCTGTTGAAGTTAATACTGTACCTGC	55 °C	971	16
clfA	Forward AGTGCGCCTAGAATGAGAGC Reverse TAAGCGGGCATGGTCAAAGT	60°C	389	Designed
clfB	Forward AGCTGTTGCTGAACCGGTAG Reverse TTTAGGTGCCTTTGCTCGGT	60°C	415	Designed

Table (2): The PCR reaction component that was applied in the current study

PCR Component				Volume (µl) for PCR Tubes	
GoTag Green Master Mix (2x)				10	
F primer 10µM				1	
R primer 10µM				1	
Nuclease Free Water				6	
DNA ng/µl				2	
Total volume				**20**	
PCR program					
Initial denaturation	30 cycle			**Final extension**	**Hold**
	Denaturation	**Annealing**	**Extension**		
95°C (5min)	95°C (3 sec)	60,55,53°C(3 sec)	72 °C(3 sec)	72°C (7min)	10°C(10min)

Results

Isolation and identification of *S. aureus*

Thirty-three isolates with a percentage of 13.2% were identified as *S. aureus*, depending on primary identification by chemical test.The result of the VITEK system was compatible with the result of primary identification of isolates, indicating that all thirty-three isolates belonged to the genus *S. aureus* with a 99% probability.

Antibiotic Sensitivity Test

The antibiotic sensitivity patterns revealed that all *S. aureus* isolates under examination resisted at least one of the 20 antibiotics tested. Resistance to Benzylpenicillin and oxacillin was the highest, 97%, followed by Erythromycin, 78%, Piperacillin/ Tazobactam, 75%and Tetracycline, 51%. In contrast, there was low resistance to Fusidic acid, 30%, vancomycin, 24%, Levofloxacin 12%, Tobramycin 6%and Moxifloxacin 3%.Furthermore, 100% of the isolates were susceptible to Gentamicin, linezolid and Tigecycline, while 97% were sensitive to Nitrofurantoin, Rifampicin and sulfamethoxazole, Figure (1). Moreover, all isolates (100%) were multidrug resistant,with resistance to at least three or more distinct classes of antibiotics.

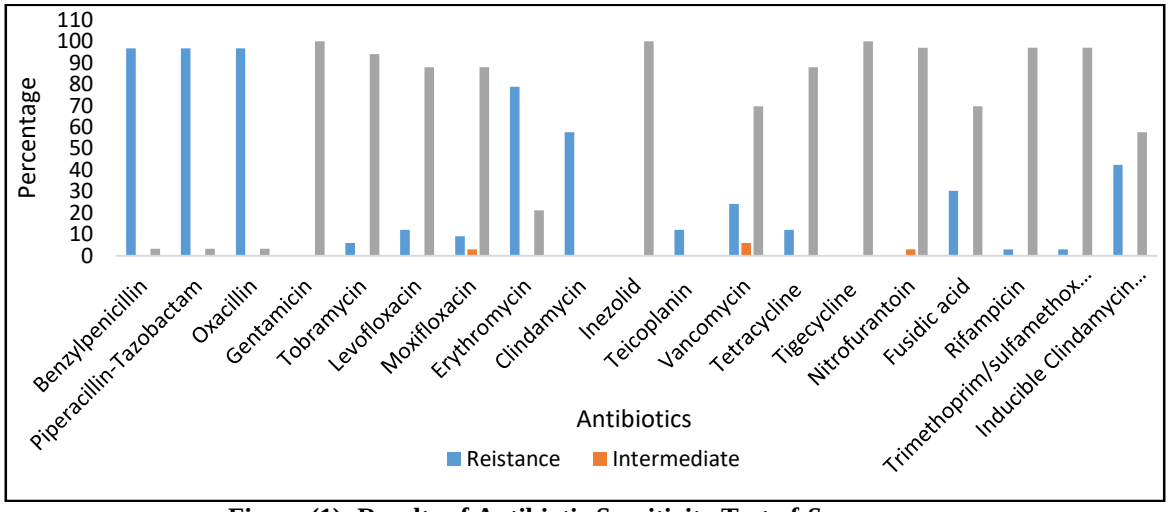

Figure (1): Results of Antibiotic Sensitivity Test of *S. aureus*

Biofilm Formation Assay

The biofilm development assay revealed that 96.96% of *S. aureus* isolates form biofilms on compression, with 3.03% of isolates being non-biofilm producers, with significantly different (p = 0.0001). The capacity of biofilm development for positive isolates shows various levels of biofilm production. Only two isolates (6.06%) were defined as weak biofilm producers, while 48.48% of the isolates were indicated as moderate biofilm producers, followed by 42.42%of isolates, which were noted to be strong biofilm developers. The comparison between strong and weak biofilm producer isolates was checked and it was not statistically significant with a p value of 0.3, as illustrated in Table 3).

Table (3): Results of Biofilm Construction Assay in *S. aureus*isolates

Biofilm Degree	No of isolates	%
Weak producer	2	6.06%
Moderate producer	16	48.48%
Strong producer	14	42.42%
Total	32	96.96%

Detection of the presence of some biofilm-related genes

The existence of a single band in the agarose gel demonstrated the specificity of each primer employed in the experiment, to detect the presence of the target gene. Six genes associated with the biofilm essential for adhesion and proliferation of *S. aureus* cells (*fib, eno, sdrC, bap, clfA, clfB*) were tested using the conventional PCR to amplify it. The result indicated that the prevalence of these genes varied across various *S. aureus* isolates.

As shown in Figures (2,3,4,5,6), the *fib* gene was noted in 30 isolates (90.9%). The prevalence of the *eno* and *sdrC* genes was observed in all isolates at 100%. In contrast, the *bap* gene was not present among isolates 0%. In addition, the prevalence of *clfA* and *clfB* in isolates under investigation was detected in 30 isolates 90.9%and 29 isolates87%, respectively.

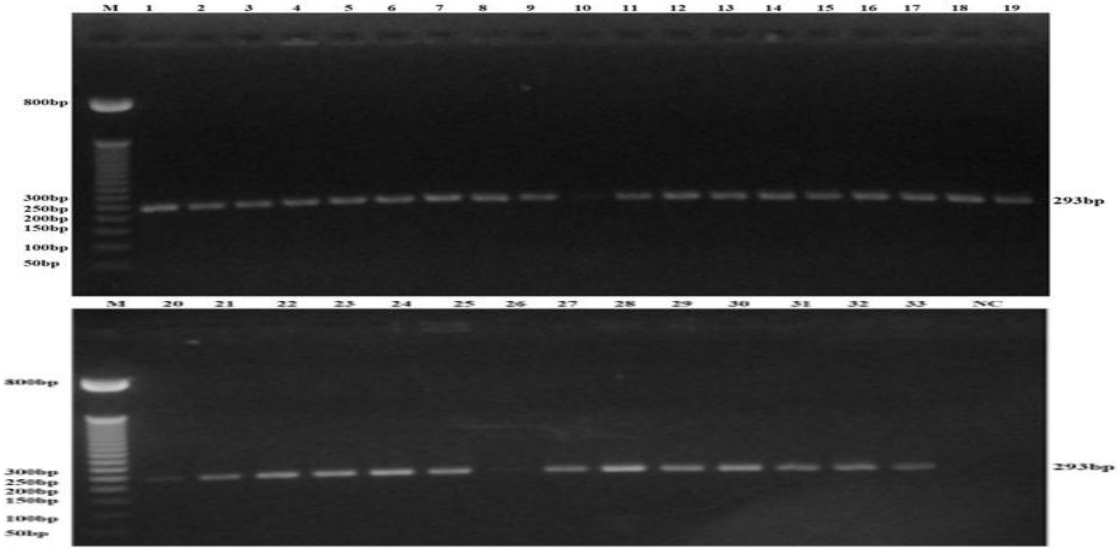

Figure (2): Results of *fib gene* amplification in *S. aureus* isolates which fractionated on gel electrophoresis (2% agarose stained with Eth.Br). M: 100bp ladder marker. lanes1-33 resembles 293 bp PCR products. NC: negative control.

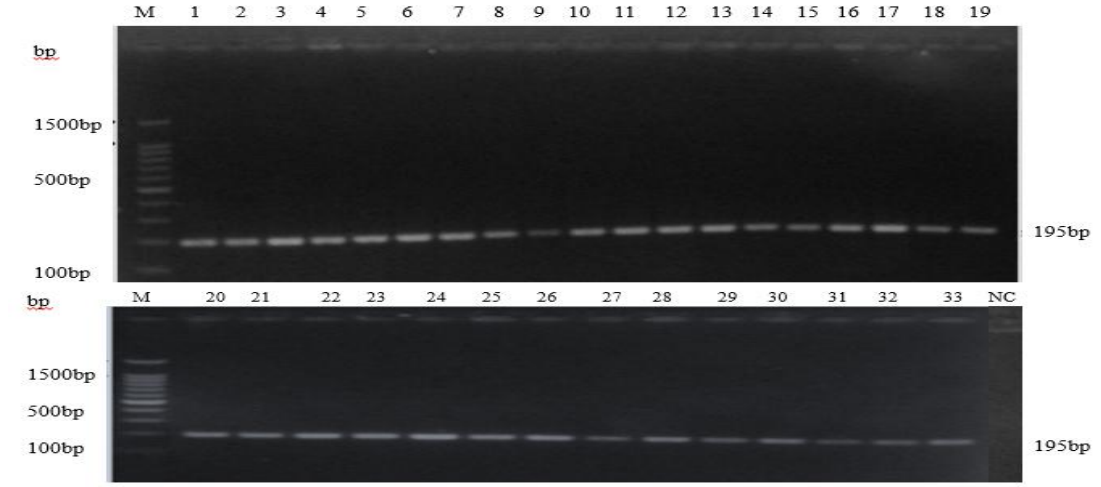

Figure (3):Results of *eno gene* amplification of *S. aureus* isolates which fractionated on gel electrophoresis (2% agarose stained with Eth.Br). M: 100bp ladder marker. lanes1-33 resembles 293 bp PCR products. NC: negative control.

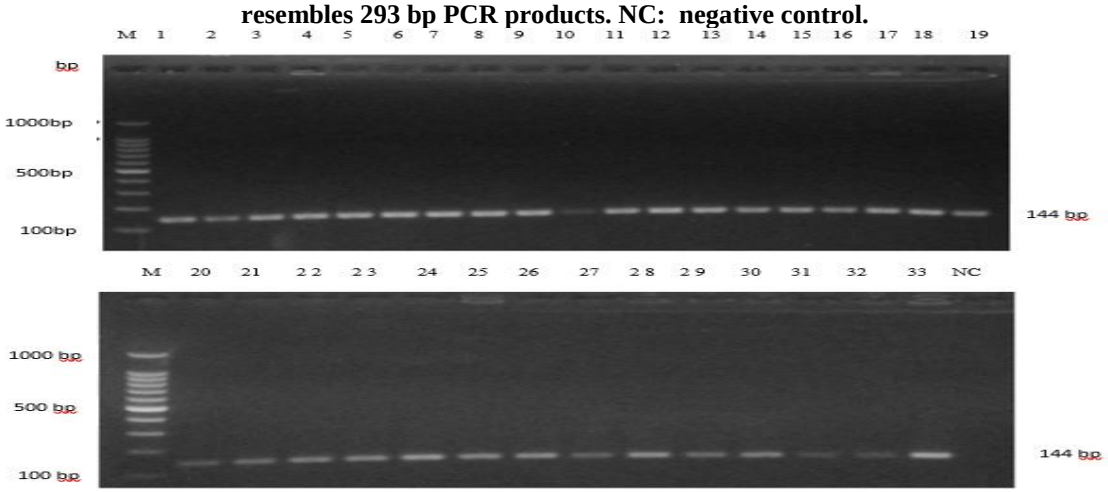

Figure (4): Results of *sdrC gene* amplification of *S. aureus* isolates which fractionated on gel electrophoresis (2% agarose stained with Eth.Br). M: 100 bp ladder marker. lanes1-33 resembles 144 bp PCR products. NC: negative control.

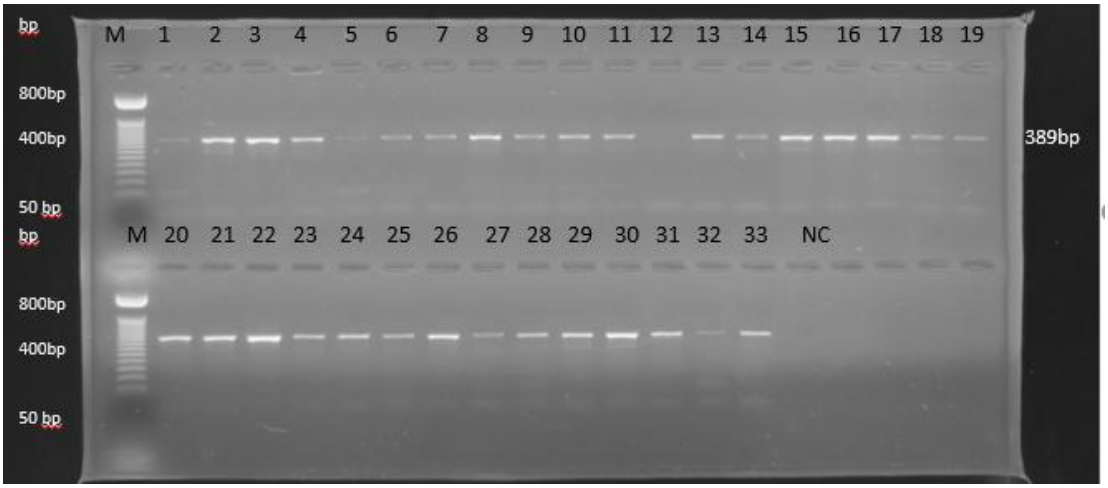

Figure (5): Results of *ClfA gene* amplification of *S. aureus* isolates which fractionated on gel electrophoresis (2% agarose stained with Eth.Br). M: 50 bp ladder marker. lanes1-33 resembles 389 bp PCR products. NC: negative control.

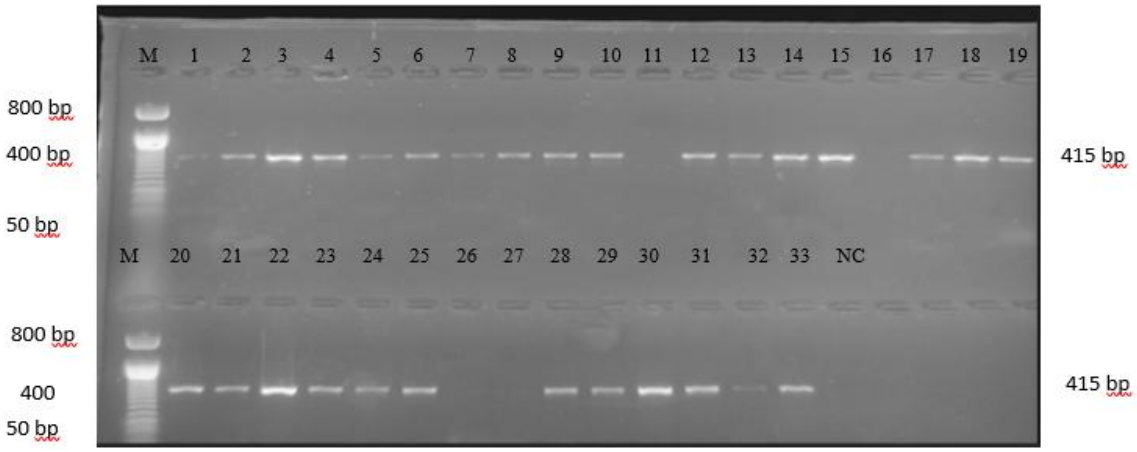

Figure (6): Results of *clfB gene* amplification of *S. aureus* isolates fractionated on gel electrophoresis (2% agarose stained with Eth.Br). M: 50 bp ladder marker. lanes1-33 resembles 415 bp PCR products. NC: negative control.

Statistical Analysis

The p-value was calculated depending on the estimation of the proportion of the data under investigation, using the Chi-squared test, and a *p*-value that was less or equal to 0.05 was considered a significant result.

Discussion

The antibiotic susceptibility tests carried out on the isolates under study revealed a high percentage of resistance, ranging from 97% to 51%, mostly for penicillin, oxacillin and Erythromycin, and this agreed with the studies of Fasiku *et al.* and Adhikari *et al.,* (17, 18),wherein, high resistance was detected,particularly against penicillin and Erythromycin. On the other hand, resistance to antibiotics in *S. aureus* isolates was noted in azithromycin, erythromycin and clindamycin,of around 82% (19, 20). Moderate resistance to vancomycin,of 24%,was detected,while it dropped down to 2% and 0%, as reported by Adhikari *et al.*and Gurung *et al.*, (18, 21). All the isolates exhibited sensitivity to linezolid and gentamicin, which was compatible withGurung *et al* andPokhrel *et al* (21, 22). All the isolates were multidrug resistant, with 100% comparison to 94% and 77% as indicated by Adhikarii *et al.*and An *et al.*,(18, 20), and as announced in our study. The antibiotic resistance in bacteria increased due to various mechanisms of resistance. The reason why resistance for a particular antibiotic was high in the isolates in the current study, in comparison with other isolates in other studies, is that the isolate under investigation possessed this mechanism. In contrast, it was not found in others, owing to a difference inthe sequences of genes among the isolates. For example, mutation could occur on the target site ofan antibiotic, hence,the antibiotic might not be able to bind with a specific target, therefore, resistance is raised in isolates under investigation.The biofilm formation in our investigation indicated that most isolates consisted of 96.96% biofilms,made up mainly of strong, moderate and weak biofilm producers. In comparison, only 3.03% of the isolates were non-forming biofilms.

The result by Aniba *et al.*, (23) indicated that 90% of the isolates were biofilm producers, but only 50% were detected as multidrug resistant. Pokhrel *et al.*(22)and Tang *et al.*,(24)declared that 80% of the isolates were among the moderate and strong biofilm development isolates. However, Wiszniewska *et al.*(25)and Tuon *et*

al.(26), observed that around 20% of the isolates did not produce biofilms.

The reason for producing strong biofilms in the isolates under investigation was due to the fact that the isolates showedhigh virulence factors, especially as all the isolates were collected from patients who attended hospitals. Moreover, biofilm development was considered a key virulence factor handled by the bacteria to defend against strategies for immune attack. This result highlights the potential role of a biofilm as a virulence factor in the isolates under study, and its contribution to antibiotic resistance, especially as the prevalence of multidrug resistance was 100%. In other words, biofilm development might encourage the isolates to persist in their host, by introducing resistance to antimicrobial agents. The matrix's anti-penetration ability, the presence of polysaccharides, antibiotic-modifying enzymes, external DNA and bacteriophages, promote biofilm resistance and antibiotic tolerance(27).

The prevalence of genes associated with biofilm construction was investigated in the current study and the results indicated that all *S. aureus*isolates harbored *fib,eno, sdrC,clfA*and *clfB* genes, with the percentage of 90.9%,100%,100%,90.9% and 87%, respectively. Meanwhile, the *bap* gene was absent in the isolates. The high prevalence of genes under investigation revealed the essential role of these genes in biofilm generation and encouraged the bacteria to overcome the antibiotic effects, as all isolates were multidrug-resistant and former biofilm.

Another study by Chen*et al.*, (28) was similar to the current investigation, which revealedthat the prevalence of the *eno gene was* 97.14% and *sdrC* gene was 94.29%, whereas, the *bap* gene was not found among the isolates. Inaddition, the results by Hadi (29) agreed with our investigation, by which the existence of *clf A* and *clfB* genes was detected to be 100% among the isolates. In contrast, all isolates did not harbor the *bap* genes.However,Aniba *et al.*, (23), revealed that 95% of the harbored the *bap* geneand *clfA*gene in all the uropathogenic isolates.

The results by Wang *et al*., (30), were in consent with our results, by which the existence of the *clfA* and*clfB* genes was 98.7% and 98%among the *S. aureus* . On the otherhand, Contreras *et al.*, declared that 67.2% of the*S. aureus* isolates harboured the *sdrC*and *clfA* genes. The variation in the existence of biofilm-related genes among the *S. aureus* isolates in our study and the previous studies could be due to the difference in sources of isolation and the number of isolates. There were some limitations in our investigation, asexploration for the presence of target genes was carried out only in thirty-three isolates; however, we highly recommend increasing the number of isolates in future studies.

Conclusion

The capability to develop a biofilmis an efficient strategy that may contribute to the prevention of antimicrobial agents from overcoming *S. aureus,* as all isolates are multidrug-resistant and have a high percentage of strong biofilm producers. In addition, the high prevalence of certainbiofilm-associated genes under investigation refer to the crucial role of these genes in biofilm development, in these pathogenic bacteria. It establishes the relation between biofilm development and the appearance of multidrug resistance in different classes of antibiotics among the isolates.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Reference

1. Yang, X.; Chen, H.; Zheng, Y.; Qu, S.; Wang, H.; and Yi, F. (2022). Disease burden and long-term trends of urinary tract infections: a worldwide report. Front Public Health 10: 888205.
2. .Alshomrani, M. K.; Alharbi, A. A.; Alshehri, A. A.; Arshad, M.; and Dolgum, S. (2023). Isolation of *Staphylococcus aureus* urinary tract infections at a community-based healthcare center in Riyadh. *Cureus*, *15*(2).
3. Abdrabaa, M. K.;and Abd Aburesha, R. (2023b). Gene Expression Evaluation of Intracellular Adhesins and Regulatory Genes among Biofilm Producing MRSA Isolates. *Iraqi Journal of Science*, 75-83.
4. .Abdrabaa ,M.K.;and Abd Aburesha R.(2023). Assessment of ica A and D Genes in Biofilm-Producing methicillin-resistant *Staphylococcus aureus* isolates. *Iraqi Journal of Science*. 2696-706.
5. 5.Kaushik, A.;Kest, H.; Sood, M.; Steussy, B. W.; Thieman, C.;and Gupta, S. (2024). Biofilm Producing Methicillin-Resistant *Staphylococcus aureus* (MRSA) Infections in Humans: Clinical Implications and Management. *Pathogens*, *13*(1), 76.
6. AbdulRazzaq, B. A.; Shami, A. M.;and Ghaima, K. K. (2022). Detection of vanAand vanB genes Among VancomycinResistant *Staphylococcus aureus* Isolatedfrom Clinical Samples in BaghdadHospitals. *Iraqi Journal of Biotechnology*,21(1).
7. Peng, Q.; Tang, X.;Dong, W.; Sun, N.;and Yuan, W. (2022). A review of biofilm formation of *Staphylococcus aureus* and its regulation mechanism. *Antibiotics*, *12*(1), 12.
8. .Monaam, Z. A. (2022). Effect of Chitosanon Biofilm Formation of Multi-DrugResistant *Pseudomonas aeruginosa*and Staphylococcus *aureus*. *Iraqi Journal ofBiotechnology*, 21(2).
9. Feuillie, C.;Formosa-Dague, C.; Hays, L. M.; Vervaeck, O.; Derclaye, S.; Brennan, M. P.; Foster, T. J.; Geoghegan, J. A.;and Dufrêne, Y. F. (2017). Molecular interactions and inhibition of the staphylococcal biofilm-forming protein *SdrC*. *Proc National Academy* of *Sciences U S A*, *114*(14), 3738-3743.
10. Achek, R.; Hotzel ,H.; Nabi, I.; Kechida, S.; Mami, D.; Didouh, N.(2020) . Phenotypic and molecular detection of biofilm formation in *Staphylococcus aureus* isolated from different sources in Algeria. *Pathogens*. ;9(2):153.
11. Idrees, M.; Sawant, S.;Karodia, N.;and Rahman, A. (2021). *Staphylococcus aureus* biofilm: Morphology, genetics, pathogenesis and treatment strategies. *International Journal of Environmental Research and Public Health*, *18*(14), 7602.
12. James, C.; Natalie, S.; Microbiology. A laboratory manual. Pearson Education; 2014.
13. Al-Khazraji, S..FR. (2023).Study on *tssC1* Gene Mediating Biofilm Antibiotics Resistance of *Pseudomonas aeruginosa*.*Iraqi Journal of Science*. :4356-65.
14. Atshan, S. S.; Hamat, R. A.; Coolen,.M. J. L .; Dykes,G.; Sekawi,Z.; Mullins, B. J.;*et al.*(2021).The Role of Subinhibitory Concentrations of Daptomycin and Tigecycline in Modulating Virulence in *Staphylococcus aureusAntibiotics (Basel)*, vol. 10, no. 1.10010039.
15. Ajayi,C. Åberg,E. Askarian,F.. Sollid, J. U. E. Johannessen, M and Hanssen,A. M. 2018.Genetic variability in the sdrD gene in Staphylococcus aureus from healthy nasal carriers," *BMC Microbiology*, vol. 18, no. 1, 34.
16. Vasil, M.; Farkasova, Z.; Elečko, J.; Illek, J.;and Zigo, F. (2017). Comparison of biofilm formation by *Staphylococcus aureus* and *Staphylococcus epidermidis* strains isolated from sheep milkusing three diagnostic methods. *Polish journal of veterinary sciences.*
17. Fasiku, O. S.; Omotosho, O. O.;Ajulo, S. O.; Adesanwo, E. E.; Oni, O. D.; Awoyele, A. A.;*et al.* (2023). Occurrence and Antibiotic Sensitivity Patterns of Methicillin-Resistant and Methicillin-Sensitive *Staphylococcus aureus* in Pigs in Ibadan, Nigeria. *Medical Sciences Forum.*
18. Adhikari, P.; Basyal ,D.; Rai ,J.R.; Bharati, L.; Budthapa,A.; Gharti, K.P.;*et al.* Prevalence, antimicrobial susceptibility pattern and multidrug resistance of methicillin-resistant *Staphylococcus aureus* isolated from clinical samples at a tertiary care teaching hospital: an observational, cross-sectional study from the Himalayan country, Nepal. *British medical journal*. 2023;13(5):e067384.

19. Carrel, M.; Smith, M., Shi, Q.; Hasegawa, S.; Clore, G. S.; Perencevich, E. N.;and Goto, M. (2024). Antimicrobial Resistance Patterns of Outpatient *Staphylococcus aureus* Isolates. *American Medical Associationjournal* *7*(6), e2417199-e2417199.
20. An, N. V.; Hai, L. H. l.; Luong, V. H.; Vinh, N. T. H.; Hoa, P. Q.; Hung, L. V.; Son, N. T.; Hong, L. T.;Hung, D. V.;and Kien, H. T. (2024). Antimicrobial resistance patterns of *Staphylococcus aureus* isolated at a General Hospital in Vietnam between 2014 and 2021. *Infection and Drug Resistance*, 259-273.
21. Gurung, R.; Maharjan, P.;and Chhetri, G.(2020). Antibiotic resistance pattern of *Staphylococcus aureus* with reference to MRSA isolates from pediatric patients. *Future science OA*. 6 (4): FSO464.
22. Pokhrel, S.; Sharma, N.; Aryal, S.; Khadka, R.; Thapa, T. B.; Pandey, P.;*et al*. (2024). Detection of Biofilm Production and Antibiotic Susceptibility Pattern among Clinically Isolated *Staphylococcus aureus.Journal of Pathogens*,(1), 2342468.
23. Aniba, R.; Dihmane, A.; Raqraq, H.; Ressmi, A.; Nayme, K.; Timinouni, M.;*et al*(2024). Characterization of biofilm formation in uropathogenic *Staphylococcus aureus* and their association with antibiotic resistance. *The Microbe*, *2*, 100029.
24. Tang, Y.; Xiao, N.; Zou, J.; Mei, Y.; Yuan, Y.; Wang, M.;*et al*(2024). Antibiotic resistance, biofilm formation, and molecular epidemiology of *Staphylococcus aureus* in a tertiary hospital in Xiangyang, Ch&ina. *Brazilian Journal of Microbiology*, 1-11.
25. Wiszniewska, A.; Szteyn, J.; Sołtysiuk, M.; Kowalik, J.; Modzelewska-Kapituła, M. (2024). Potential for Biofilm Formation and Antibiotic Resistance of Staphylococci Isolated from Bulk Tank Milk Samples. *Applied Sciences*, *14*(7), 3027.
26. Tuon, F. F.; Suss, P. H.; Telles, J. P.; Dantas, L. R.; Borges, N. H.;and Ribeiro, V. S. T. (2023). Antimicrobial Treatment of *Staphylococcus aureus* Biofilms. *Antibiotics (Basel)*, *12*(1).
27. Mirghani, R.;Saba, T.; Khaliq, H.; Mitchell, J.; Do, L.; Chambi, L.;*et al*(2022). Biofilms: Formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiology*, *8*(3), 239-277.
28. Chen, Q.; Xie, S.; Lou, X.;Cheng, S.; Liu, X.; Zheng, W.;*et al.* (2020). Biofilm formation and prevalence of adhesion genes among *Staphylococcus aureus* isolates from different food sources. *Microbiology*. 9: e00946.
29. Hayfa, H. (2024). Detection of Biofilm Operon, Some Virulence Factors, and Antibiotics Susceptibility of *S. aureus* Isolated from Patients in Holly Karbala City. *Iraqi journal of biotechnology*, *23*(2).
30. Wang,X.; Wu, H. ;Xiong, J.; Yang, G. X.; Hu,J. F.; Zhu, Q.;*et al*.(2024).*Staphylococcus aureus* biofilm: Formulation, regulatory, and emerging natural products-derived therapeutics," *Biofilm*, vol. 7. 100175.