Journal of Pioneering Medical Sciences
Received: September 13, 2025 | Accepted: December 02, 2025 | Published: August 05, 2026
Volume 15, Issue 07, Pages 77-84

DOI https://doi.org/10.47310/jpms2026150711

Molecular Detection of *blaZ* genes of *Staphylococcus aureus* Isolated from Urinary Tract Infection

Ashwaq Muthanna Wahib[1*], Weam Ahmed Alwan[2], Noor Ghassan Kadom[3], Wurood Khalid Mohammed[4], Adawia Fadhil Abbas Alzubaidi[5] and Khitam Juma'a Dawood Salman[6]

[1-6]Department of Biology, College of Education for Pure Sciences, University of Diyala, Iraq

*Corresponding author: Ashwaq Muthanna Wahib (e-mail: bioe.ashwaq.mothana@uodiyala.edu.iq).

Abstract **Background:** Bacterial diseases play a major role in urinary tract infections and *Staphylococcus aureus* (SA) is one of the most common causes of UTIs in humans. Infections caused by *Staph. aureus* can develop into invasive, potentially life-threatening infections due to disruption of the body's defense mechanisms, such as bacteremia. **Objectives:** The present study aimed to evaluate the correlation between phenotypic beta-lactamase production and PCR-based detection of the blaZ gene among urinary *Staphylococcus aureus* isolates in Diyala province. **Methods:** One hundred samples were collected from sources of urinary tract infections and cultured on blood agar, nutrient agar and mannitol agar. Bacterial isolates were identified using biochemical tests and molecular detection of beta-lactamase using beta-lactamase-specific *blaZ* primers. **Results** Out of 100 urine samples collected, 40 isolates of *Staphylococcus aureus* were identified. The isolates in our study showed 40 (100%) production of hemolysin, 32 (80%) production of urease and 28 (70%) production of biofilms. They also produced 62.5% of gen of beta-lactamase *blaZ*. Molecular detection of the gene responsible for beta-lactamase production revealed its presence in 100% of bacterial isolates that phenotypically expressed beta-lactamase. **Conclusion:** Variations in the production of virulence factors, including hemolysin, urease, cell membrane factor and beta-lactamase enzymes, were observed in the bacterial isolates. The bacteria demonstrated resistance to antibiotics, particularly amoxicillin. The presence of the gene responsible for beta-lactamase production was also detected in the isolates, contributing to the bacterial resistance to antibiotics.

Key Words *Staphylococcus aureus*, Beta-Lactamase Enzyme, Biofilm, Urease, Virulence Factors, Urinary Tract Infection

INTRODUCTION

Certain bacteria cause urinary tract infections, including *Staphylococcus aureus*. These bacteria are among the most common infections in the community, second only to respiratory tract infections Although Gram-negative bacteria are the major cause of urinary tract infections, *Staphylococcus aureus* has emerged as an important opportunistic uropathogen associated with complicated infections, catheterization and increasing antimicrobial resistance [1]. *Staphylococcus aureus* is a clinically important Gram-positive opportunistic pathogen capable of causing a wide range of human infections, including urinary tract infections, particularly in complicated or healthcare-associated cases [2]. *Staphylococcus aureus* produces several virulence factors, including enzymes and surface-associated structures, that contribute to its ability to adhere to host tissues, evade immune responses and establish persistent infections. Among these, biofilm formation plays a particularly important role in facilitating colonization and persistence in clinical infections such as urinary tract infections [3]. Hemolysins produced by *Staphylococcus aureus* contribute to tissue damage and enhance bacterial invasiveness, thereby facilitating the progression of infection [4]. *Staphylococcus aureus* is a clinically significant pathogen responsible for a wide range of human infections, including both localized and systemic diseases. Its clinical importance is particularly associated with its ability to cause persistent and difficult-to-treat infections [5]. A key virulence factor of *Staphylococcus aureus* is its ability to form biofilms, which enhance bacterial survival by protecting cells from antimicrobial agents and host immune responses, thereby contributing to persistent and recurrent infections, including urinary tract infections, particularly in complicated cases [6]. The clinical management of *Staphylococcus aureus* infections is complicated by its ability to develop antimicrobial resistance. The emergence

and spread of Methicillin-Resistant *Staphylococcus aureus* (MRSA) have significantly limited therapeutic options and contributed to increased clinical burden worldwide [7]. Understanding the genetic determinants of antimicrobial resistance, particularly beta-lactamase-mediated mechanisms, is essential for improving diagnostic accuracy and guiding appropriate antimicrobial therapy in *Staphylococcus aureus* infections [8]. In particular, β-lactam resistance in *Staphylococcus aureus* remains a significant clinical concern due to its impact on the effectiveness of commonly used antibiotics [9]. Following the introduction of penicillin, *Staphylococcus aureus* rapidly developed resistance, highlighting its remarkable ability to adapt to antimicrobial pressure [10]. However, resistance in *Staphylococcus aureus* emerged shortly after the introduction of penicillin, with early reports indicating the development of penicillin-resistant strains [11,12]. The study aimed to detect the present study aimed to evaluate the correlation between phenotypic beta-lactamase production and PCR-based detection of the *blaZ* gene among urinary *Staphylococcus aureus* isolates in Diyala province. Due to the increasing prevalence of beta-lactam resistance among *Staphylococcus aureus* isolates and the limited molecular data available in Diyala province, this study aimed to detect the *blaZ* gene in *S. aureus* isolated from urinary tract infection patients using PCR technique.

METHODS

Specimen Collection and Transportation

Staphylococcus aureus was isolated from clinical specimens of Urinary Tract Infections (UTIs) from patients attending Baqubah Teaching Hospital and Al-Batoul Hospital in Baqubah and patients attending external health laboratories in Baqubah, Diyala, Iraq, during the period from June 1, 2025 to August 25, 2025. One hundred samples were collected from UTI cases and transported in a cork-lined box containing ice to the Microbiology Unit, Agriculture Laboratory, Midstream clean-catch urine samples were collected using sterile containers under aseptic conditions. Samples were transported to the laboratory within 1-2 hours of collection and processed immediately upon arrival or stored at appropriate conditions to minimize contamination. The study included 100 urine samples collected from patients clinically suspected of having Urinary Tract Infections (UTIs) based on hospital diagnosis and presenting symptoms such as dysuria, increased urinary frequency and other relevant clinical indicators. Both catheterized and non-catheterized patients were included in the study. Repeated samples from the same patient were excluded to avoid duplication bias. Only single, unique patient samples were considered for analysis. The selection of samples was based on routine hospital attendance without further restriction, provided that clinical suspicion of UTI was present.

Morphological Examination

Diagnosis of *Staphylococcus aureus*: All bacterial isolates recovered from the 100 urine samples were subjected to Gram staining. Different bacterial species were identified among the isolates. Only Gram-positive cocci arranged predominantly in clusters were presumptively identified as *Staphylococcus* spp. Among the recovered isolates, 40 fulfilled these criteria and were selected for further identification and characterization.

Identification Procedure

Clinical urine samples were cultured on selective and differential media for primary bacterial isolation. Samples were inoculated onto nutrient agar and mannitol salt agar and incubated aerobically at 37°C for 24 h. After incubation, colony morphology, including color and texture, was examined. Different bacterial species were recovered from the cultured samples. Colonies suspected to belong to *Staphylococcus* spp. were further evaluated by Gram staining and standard biochemical tests, including catalase and coagulase tests. Confirmatory identification of isolates was subsequently performed using the VITEK system [13].

Biochemical Identification Tests

Standard microbiological procedures were used for the identification of *Staphylococcus aureus* using biochemical tests. Primary identification was based on key biochemical characteristics, including positive catalase and coagulase reactions. Additional phenotypic characteristics, including hemolysin production and urease activity, were evaluated among confirmed isolates to assess strain-level variation and potential virulence-associated traits. *S. aureus* isolates also commonly ferment glucose, mannitol and maltose and may exhibit gelatin hydrolysis and beta-hemolysis on blood agar [14].

Antibiotic Susceptibility Testing

In vitro antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute [19] guidelines. Three antibiotics commonly used in the treatment of urinary tract infections were selected for testing, including amoxicillin, gentamicin and tetracycline. All *Staphylococcus aureus* isolates were tested against these antibiotics and the results were interpreted based on CLSI breakpoints as shown in Table 1.

Detection Biofilm Formation Assay

Biofilm formation was assessed using the Tube Method (TM), a qualitative assay based on direct visual observation of biofilm formation [16]. A fresh bacterial colony was inoculated into 10 mL of Brain Heart Infusion Broth (BHIB) and incubated at 37°C for 24 h. Following incubation, the contents of the tubes were discarded and the tubes were gently washed with buffered saline to remove non-adherent cells. The tubes were then dried and stained with 0.1% crystal violet. Excess stain was removed and the tubes were rinsed with deionized water. Biofilm formation was visually evaluated based on the presence of a stained adherent layer on the inner walls and bottom of the tubes. Formation of a visible dark-stained film was considered a positive result for biofilm production.

Table 1: Antibiotics Used in Susceptibility Testing and Their Inhibitory Diameters

Antibiotics	The symbol	Antibiotic Concentration\mg	Standard diameters		
			R	I	S
Amoxicillin	AUG	20			
Gentamicin	GM	10	≥12	13-14	≤15
Tetracycline	TE	30	≥11	12-14	≤15

Table 2: Components of the Presto Mini gDNA Bacteria Kit

The Components	Volume
G· Buffer	30 mL
GT Buffer	30 mL
GB Buffer	40 mL
BufferW1	45 mL
Wash Buffer (Add ethanol)	25 mL (100 mL)
Lysozyme	110 mg
Proteinase K(Add ddH_2O)	11 mg (1.1 mL)
Elution Buffer	30 mL
GD Columns	100
2ml collection tube	200

Table 3: Primers Used in PCR Reactions

Bacteria	Name of primer	5 to 3)) Sequence	volume (bp)	Source
Staphylococcus aureus	*blaZ*	F: AAG AGA TTT GCC TAT GCT TC R: GCT TGA CCA CTT TTA TCA GC	559	Dehbashi *et al.* [20]

Phenotypic Detection of β-Lactamase Production

β-lactamase production was detected using the filter paper method as previously described [17]. Isolates showing resistance to one or more β-lactam antibiotics were selected for testing. Filter paper strips (5×1 cm) were prepared and soaked in a 1% amoxicillin solution, then allowed to dry at room temperature. The tested bacterial isolates were inoculated onto the prepared strips using a sterile loop and spread over an area of approximately 5 cm^2. The inoculated filter papers were placed in Petri dishes and incubated at 37°C for 30 min. After incubation, the filter papers were immersed in iodine solution. A positive result indicating β-lactamase production was recorded when the inoculated area became colorless within 10 min. In contrast, persistence of the purple color was considered a negative result, indicating the absence of detectable β-lactamase production.

Molecular Detection of the Virulence Factor β-Lactamase of *Staphylococcus aureus*

After visually identifying the ability of bacterial isolates to produce specific virulence factors, the isolates with the highest rates of beta-lactam enzyme production were selected for molecular detection of the genes encoding this enzyme using the *blaz* primer for beta-lactamase detection.

Extraction of Genomic DNA from Bacteria

Genomic DNA was extracted from bacterial isolates using the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan) according to the manufacturer's instructions. The extraction procedure was performed as described in the kit protocol and is summarized in Table 2.

Primers Used in PCR Reaction

The primer prepared by Macrogen shown in Table 3 was used.

Characterisation of DNA

Measuring the concentration and purity of DNA using the Nano Drop device was done.

Agarose Gel Electrophoresis of DNA

Eight microliters of each DNA sample were mixed with two microliters of bromophenol blue staining. Each sample mixture was then placed on a 1% agarose gel. The power supply was turned on and the apparatus was primed. After electrophoresis, the gel was removed from the gel plate in the electrophoresis apparatus and examined in a dark room after being exposed to 260 nm ultraviolet light. The gel was imaged using gel documents. The molecular size of the DNA fragments was estimated by comparing the band position and thickness with a standard size guide (100 base pairs). The molecular weight of the DNA was estimated based on the distance the particles traveled in the gel [20].

Polymerase Chain Reaction

A master mix reaction mixture (19 µL of nuclease-free water, 2 µL of premix solution, 1 µL of each forward primer at a concentration of 10 pmol/µL and 1 µL of each reverse primer at a concentration of 10 pmol/µL) was prepared according to the number of bacterial samples being prepared. The master mix reaction mixture was then placed in 0.2 mL Eppendorf tubes, with a volume of 23 µL per sample. 2 µL of DNA was then added to each tube, until the final volume was 25 µL. It was then placed in a thermocycler using the appropriate program as shown in Table 4. The primers prepared by Macrogen were used and re-dissolved according to the manufacturer's instructions, after which a crude solution of each primer at a concentration of 100 pmol/µL was obtained. Also, a premix solution prepared by Pioneer, consisting of PCR buffer, NTPS deoxynucleotide solution and Taq DNA polymerase, was used.

Table 4: Thermopolymer Program for the PCR Reaction Mixture for the Primers Used

Brimer	Denatur.-1	Denatur.-2	Annealing	Exten.-1	Exten.-2
blaZ	95 c° /5 sec	94 c° /30 sec	55 c° /30 sec	72 c° /60 sec	72 c° /5 sec
	1 Cycle	35Cycle			1Cycle

Statistical Analysis

All experiments were performed in triplicate to ensure reproducibility. Data were analyzed using descriptive statistics and presented as frequencies and percentages. Graphs and tables were prepared using Microsoft Excel (64-bit edition) and Origin software (64-bit version).

RESULTS

Diagnosis of *Staphylococcus aureus*

Initial isolation results on mannitol salt agar medium showed that out of 100 samples collected from patients with urinary tract infections, 40 samples showed positive growth of *Staphylococcus aureus*, representing 40% of the total. The samples were collected from sources of urinary tract infections and were of different ages, including females and males, ranging in age from one to 50 years. The number of infected females was 29 samples, representing 72.5%, while the number of infected males was 11 samples, representing 27.5%. The samples were collected under the supervision of a specialist physician.

Phenotyping Screening of Virulence Factors

The isolated bacteria exhibited the ability to produce several virulence-associated factors, including β-hemolysin, biofilm formation and urease enzyme activity, as presented in Table 5. These phenotypic traits may contribute to the pathogenic potential of the isolates.

Urease Production

Staphylococcus aureus isolates showed that 32 isolates (80%) were urease-positive, while 8 isolates (20%) were urease-negative. Urease production was indicated by a color change of the medium from yellow to pink, as shown in Figure 1.

Hemolysins Production

All *Staphylococcus aureus* isolates (100%) demonstrated hemolysin production, as indicated by clear zones around the bacterial colonies. Among the isolates, 30 (75%) showed complete β-hemolysis, while 10 (25%) exhibited partial α-hemolysis, as shown in Figure 2.

Biofilm Formation

Among the 40 tested *Staphylococcus aureus* isolates, 28 isolates (70%) demonstrated the ability to form biofilms, whereas 12 isolates (30%) showed no detectable biofilm-forming ability. These findings indicate a high prevalence of biofilm production among the examined isolates, as shown in Figure 3.

Enzyme Beta-Lactamase Production

The ability of *Staphylococcus aureus* isolates to produce β-lactamase was clearly demonstrated. Out of the 40 tested isolates, 25 (62.5%) were β-lactamase producers, whereas 15 isolates (37.5%) were non-producers.

Figure 1: The Production of Urease Enzyme by *Staphylococcus aureus*

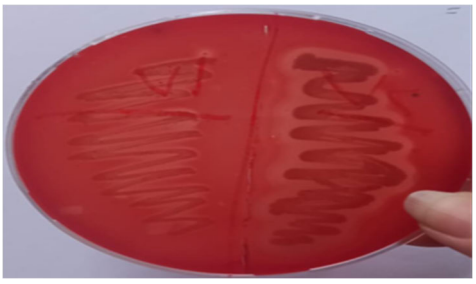

Figure 2: The Production of the Hemolysin Enzyme by *Staphylococcus aureus*

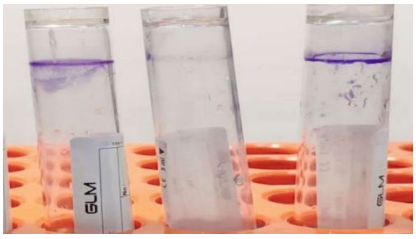

Figure 3: The Production of Biofilm Enzyme by *Staphylococcus aureus*

***Staphylococcus aureus* Sensitivity**

The sensitivity of *Staphylococcus aureus* bacteria to three antibiotics was tested. The results showed that *Staphylococcus aureus* isolates recorded the highest resistance rate to amoxicillin (95%) and the highest sensitivity (5%), followed by gentamicin (90%) and tetracycline (77.5%) (Table 6, Figure 4).

Polymerase Chain Reaction

PCR Detection of *blaZ* Genes in *Staphylococcus aureus*:

The blaZ-specific primer was used to detect the presence of the *blaZ* gene, which encodes the β-lactamase enzyme in *Staphylococcus aureus*. PCR products were separated by agarose gel electrophoresis and visualized under UV light. A

distinct amplification band of approximately 559 bp, corresponding to the expected size of the *blaZ* gene fragment, was observed in the *blaZ* gene was detected in 6 out of 8 isolates (Figure 5).

PCR analysis using *blaZ*-specific primers revealed the presence of the *blaZ* gene in the examined *Staphylococcus aureus* isolates. Agarose gel electrophoresis showed a distinct amplification product of approximately 559 bp, corresponding to the expected size of the *blaZ* gene fragment (Figure 5). Lane M contained the 100 bp DNA ladder, while lanes 1-8 represented the tested isolates. The appearance of the 559 bp band in the positive isolates confirmed the successful amplification and presence of the *blaZ* gene, indicating their potential ability to produce β-lactamase enzyme and contribute to β-lactam antibiotic resistance.

Table 5: Rate of Production of Virulence Factors by *Staphylococcus aureus*

	Staphylococcus aureus (40 out of 100)	
Virulence factors	No.	%
β.hem	40	100
Bio.	28	70
Ure.	32	80

Table 6: The Percentage of the Sensitivity of *Staphylococcus aureus* is Under Study Towards Antibiotics

	Staphylococcus aureus (40)		
Antibiotics	S%	I%	R%
Amoxicillin	5	0	95
Gentamicin	10	0	90
Tetracycline	22.5	0	77.5

Figure 4: Shows Sensitivity of *Staphylococcus aureus* to Antibiotics

DISCUSSION

The detection of the *blaZ* gene among the examined *Staphylococcus aureus* isolates indicates their ability to produce β-lactamase enzymes that hydrolyze the β-lactam ring of penicillins, thereby reducing the effectiveness of several β-lactam antibiotics. Although *blaZ* is commonly associated with resistance to penicillin and amoxicillin, its clinical significance extends to treatment selection and antimicrobial stewardship. The presence of *blaZ* suggests that β-lactamase-sensitive antibiotics may be ineffective against these isolates and highlights the need for susceptibility-guided therapy. Moreover, *blaZ* represents only one component of the antimicrobial resistance profile of *S. aureus*. Other resistance determinants, particularly the mecA gene, confer resistance through a different mechanism involving the production of the altered penicillin-binding protein PBP2a, which is characteristic of methicillin-resistant *S. aureus* (MRSA). Therefore, the detection of *blaZ* provides valuable information regarding β-lactamase-mediated resistance but comprehensive molecular characterization including mecA screening would offer a more complete understanding of the resistance patterns present in the studied isolates.

The results of the current study were consistent with those of Ahmed *et al.* [21], who indicated that the infection rate among females was higher than that of males, at 51.53%. The difference in infection rates between males and females may be due to the unequal distribution of samples between females and males, as more female samples were taken than males. The difference may also be due to the quantity and quality of the natural flora in the bodies of the different sexes, in addition to differences in sample collection methods. It may also be due to females' exposure to repeated pregnancy and childbirth, hormonal and physical activity, psychological conditions such as anxiety and stress, health

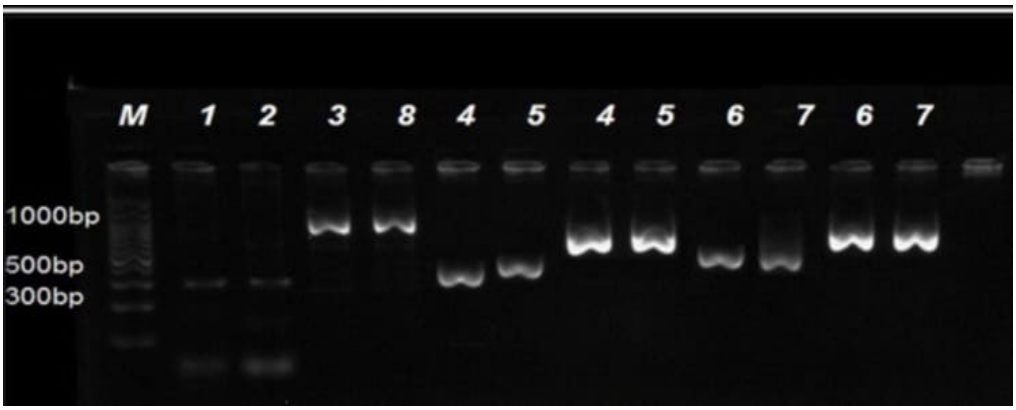

Figure 5: Results of Electrophoresis on 2% Agarose Gel *blaZ* (559 bp)

status, poor personal hygiene and nutritional quality. All of these factors contribute to the higher number of infected females than males. In addition to the proportion of physiological differences between the sexes, the effect of age on infection rates is also taken into account.

The process of exchanging plasmids in bacteria to receive antibiotics and the changing local microbial environment, also sheds light on the biological activities of bacteria [22].

The results of our study were consistent with those of Hussien and Makhrmash [23] and Obaid [24], both of them demonstrated, through separate local studies, that *Staphylococcus aureus* is capable of producing this agent at 100% efficiency. However, the results differed from those of Al-Masoudi [25], in his study, *Staphylococcus aureus* isolated from various clinical infections showed a hemolysin production rate of 53.33%, a result that is not similar to our research results.

While the result was consistent with the result of the study of researcher Kai *et al.* [26], who proved through his study the ability of *Staphylococcal* bacteria isolates isolated from urinary tract infections to produce the urease enzyme, as he showed the intense urease activity through the high expression of urease genes in the bacteria.

The enzyme urease works to create a suitable environment for bacterial survival at the site of infection by changing the pH and neutralizing the toxic effect of urea after converting it to carbon dioxide and ammonia (NH3). This increases the pH and thus promotes bacterial growth [27]. The production of ammonia in the medium raises the pH, which in turn leads to the precipitation of naturally soluble calcium and magnesium ions. These salt crystals can develop into large crystals, causing bladder and kidney stones, thus impeding the effectiveness of antibiotic treatment, as bacteria are resistant to antibiotics [28].

The current results are consistent with the results of Al-Hayali [29], as his isolates (13, representing 65%) were biofilm-producing *Staphylococcus aureus*. However, this result differs from the results of Al-Masoodi [25]. The percentage of isolates capable of producing biofilm factor was 100% and it is also different from the results of Bissong and Ateba [30], who explained through the study they conducted that the percentage of biofilm production reached 90.9% and this result does not match the result of our study.

In a study by Khalaf *et al.* [31] to detect the beta-lactam gene in *Staphylococcus aureus* bacteria causing urinary tract infections in Diyala Governorate, this gene was found in 100% of the isolates producing the beta-lactamase enzyme. Snoussi *et al.* [32]. In their study to detect this gene, they reported its presence in 87.5% of isolates producing this enzyme. These results are similar to those of our current study.

The detection of alternative genes, such as *mecA* and *mecC*, which are involved in β-lactamase enzyme production, accounts for the ability of certain bacterial isolates to synthesize this enzyme even in the absence of the *blaZ* gene [32]. In addition, some isolates lose the ability to produce this enzyme after long-term storage, especially since this gene is carried on a plasmid. This is the same thing we observed during repeated phenotypic detection, as upon first detection, some isolates showed apparent production of the beta-lactamase enzyme. After we preserved them until genetic detection and after we activated the preserved isolates and repeated the phenotypic detection test for the beta-lactamase enzyme, some isolates lost this ability to produce.

The high level of resistance to amoxicillin observed among the studied isolates suggests that empirical use of this antibiotic for the treatment of *Staphylococcus aureus*-associated urinary tract infections in the study area should be approached with caution. However, given the limited number of antimicrobial agents and molecular resistance markers investigated, further studies involving broader resistance profiling are required before definitive clinical recommendations can be made.

CONCLUSIONS

In conclusion, the examined *Staphylococcus aureus* isolates exhibited variable expression of important virulence factors, including hemolysin, urease, cell membrane factor and β-lactamase, highlighting their pathogenic potential. Antimicrobial susceptibility testing revealed resistance to several antibiotics, with the highest resistance observed against amoxicillin, indicating the growing challenge of antibiotic-resistant *S. aureus* strains. Molecular analysis confirmed the presence of the *blaZ* gene in all phenotypically β-lactamase-producing isolates, demonstrating a strong correlation between phenotypic resistance and the underlying genetic determinant. The detection of *blaZ* emphasizes the role of β-lactamase-mediated resistance in reducing the efficacy of penicillin and other β-lactamase-sensitive antibiotics and underscores the importance of molecular diagnostics for the accurate identification of resistant isolates. The integration of phenotypic and genotypic approaches provides a more comprehensive understanding of resistance mechanisms and supports informed antibiotic selection and effective infection management. These findings contribute to ongoing efforts to monitor antimicrobial resistance, improve public health outcomes and support Sustainable Development Goal 3 (Good Health and Well-being) through the early detection and control of antibiotic-resistant bacterial pathogens.

Limitations

This study has several limitations that should be considered when interpreting the findings. First, the study was conducted on a relatively limited number of isolates collected from a single geographical area (Diyala Province), which may restrict the generalizability of the results. Second, the antimicrobial susceptibility analysis included a limited panel of antibiotics and therefore may not fully represent the resistance profile of the examined isolates. Third, molecular characterization was restricted to the detection of the *blaZ* gene, while other important resistance determinants, such as *mecA* and additional resistance-associated genes, were not

investigated. Furthermore, the study relied mainly on descriptive analyses and more advanced statistical approaches could provide stronger evidence regarding the relationships between virulence factors, antimicrobial resistance and molecular findings. Future studies involving larger sample sizes, broader antimicrobial testing and expanded molecular analyses are recommended to provide a more comprehensive understanding of antimicrobial resistance in *Staphylococcus aureus*.

Future Research Directions

Future studies should include a broader molecular investigation of antimicrobial resistance in *Staphylococcus aureus*, particularly through the detection of additional resistance genes such as *mecA* and other clinically relevant genetic determinants. Expanding the antimicrobial susceptibility panel and examining genotype-phenotype correlations would provide a more comprehensive understanding of resistance mechanisms. The application of advanced molecular techniques, including multiplex PCR and DNA sequencing, would further improve the accuracy and depth of resistance characterization. In addition, multicenter studies involving different Iraqi provinces are recommended to generate more representative epidemiological data and support national antimicrobial resistance surveillance programs.

Ethical Approval

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Prior to sample collection, verbal informed consent was obtained from all participants. Urine samples were collected solely for research purposes and all personal information was kept confidential. No identifying information was included in the laboratory records or data analysis, ensuring participant anonymity and privacy throughout the study.

REFERENCES

[1] Al-Dulaimi, A.M. and M.N. Maaroof. *Determining the Effectiveness of Saccharomyces Cerevisiae Extract Against Bacterial Species Isolated from Different Infection Sources*. 2024. University of Tikrit, PhD Dissertation.

[2] Getaneh, A. "*Staphylococcus aureus*, ESKAPE Bacteria Challenging Current Health Care and Community Settings: A Literature Review." *Clinical Laboratory*, vol. 67, 2021.

[3] Ana, G.A. *et al.* "*Staphylococcus aureus* Exotoxins and Their Detection in the Dairy Industry and Mastitis." *Toxins*, vol. 12, no. 537, 2020.

[4] Imanishi, I. *et al.* "Exfoliative Toxin E, a New *Staphylococcus aureus* Virulence Factor with Host-Specific Activity." *Scientific Reports*, vol. 9, no. 1, 2019.

[5] Taylor, T.A. and C.G. Unakal. "*Staphylococcus aureus* Infection." *StatPearls*, StatPearls Publishing, February 2025, http://www.ncbi.nlm.nih.gov/books/NBK441868/.

[6] Peng, Q. *et al.* "A Review of Biofilm Formation of *Staphylococcus aureus* and Its Regulation Mechanism." *Antibiotics*, vol. 12, no. 12, 2022.

[7] Siddiqui, A.H. and J. Koirala. "Methicillin-Resistant *Staphylococcus aureus*." *StatPearls*, StatPearls Publishing, 2023. http://www.ncbi.nlm.nih.gov/books/NBK482221/.

[8] Rajput, P. *et al.* "Evaluation of Antibiotic Resistance Mechanisms in Gram-Positive Bacteria." *Antibiotics*, vol. 13, 2024.

[9] Wang, D. *et al.* "Virulence Factors in Biofilm Formation and Therapeutic Strategies for *Staphylococcus aureus*: A Review." *Animal Diseases and Zoonoses*, 2025, https://www.sciencedirect.com/science/article/pii/S2950248924000130.

[10] Risser, F. *et al.* "Adhesive Virulence Factors of *Staphylococcus aureus* Resist Digestion by Coagulation Proteases Thrombin and Plasmin." *ACS Bio and Med Chem Au*, vol. 2, 2022, pp. 586-599.

[11] Bhattacharya, M. and A.R. Horswill. "The Role of Human Extracellular Matrix Proteins in Defining *Staphylococcus aureus* Biofilm Infections." *FEMS Microbiology Reviews*, vol. 48, 2024.

[12] Speziale, P. and G. Pietrocola. "The Multivalent Role of Fibronectin-Binding Proteins A and B (FnBPA and FnBPB) of *Staphylococcus aureus* in Host Infections." *Frontiers in Microbiology*, vol. 11, 2020.

[13] McPherson, R.A. and M.R. Pincus. *Clinical Diagnosis and Management by Laboratory Methods*. 23rd Ed., Elsevier Inc., 2017.

[14] Ponnusamy, P. and R. Nagappan. "Extended Spectrum Beta-Lactamase, Biofilm-Producing Uropathogenic Pathogens and Their Antibiotic Susceptibility Patterns from Urinary Tract Infection - An Overview." *International Journal of Microbiology Research*, January 2013, pp. 1836-1842.

[15] Procop, G.W. *et al. Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. Jones and Bartlett Learning, July 2020.

[16] Tula, M.Y. *et al.* "Screening for Some Virulent Factors Among Bacterial Isolates from Surfaces of Hospital Fomites and Hands of Healthcare Workers." *Cellular, Molecular and Biomedical Reports*, vol. 3, no. 1, 2023, pp. 9-16.

[17] Otu, J.U. *et al.* "Carriage of Plasmid-Mediated β-Lactamase Genes of *Staphylococcus aureus* Isolates from Outpatients and Inpatients of UCTH, Calabar, Cross River State, Nigeria." *International Journal of Current Research in Medical Sciences*, vol. 7, no. 2, 2021, pp. 5-18.

[18] Morello, J.A. *et al. Laboratory Manual and Workbook in Microbiology*. 8th Ed., McGraw-Hill Higher Education, 2006.

[19] CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 32nd ed., CLSI document M100, Clinical and Laboratory Standards Institute, 2022.

[20] Dehbashi, S. *et al.* "Regulation of Virulence and β-Lactamase Gene Expression in *Staphylococcus aureus* Isolates: Cooperation of Two-Component Systems in Bloodstream Superbugs." *BMC Microbiology*, vol. 21, no. 1, 2021, pp. 1-12.

[21] Ahmed, M.A. *et al.* "Bacterial Species Isolated from Patients with Sepsis and Their Resistance to Some Commonly Used Antibiotics." *University of Kirkuk*, 2019.

[22] Makia, R.S. *et al.* "Biofilm Production as a Virulence Factor in Uropathogenic Bacteria and Yeasts." *Journal of Biotechnology Research Center*, January 2013, pp. 29-34.

[23] Hussien, H.M. and J.H. Makhrmash. "The Role of Hemolysine in Pathogenesis of *Staphylococcus aureus S. haemolyticus* Causing Urinary Tract Infections in Pregnant and Non-Pregnant Women." *Annals of Forest Research*, vol. 66, no. 1, 2023, pp. 4431-4443.

[24] Obaid, H.A.E. "Investigation of Some Physiological, Biochemical Characteristics and Multiple Antibiotic Resistance of *Staphylococcus aureus* Isolated from Wound Samples." *Journal of Advanced Education and Sciences*, vol. 3, no. 3, 2023, pp. 9-16.

[25] Al-Masoodi, A.K.F. *Phenotyping Analysis of Tetracycline Resistance Staphylococcus aureus Isolated from Different Clinical in Baqubah City*. 2020. University of Diyala, Master's Thesis.

[26] Kai, X. *et al.* "*Staphylococcus aureus* ST1 Promotes Persistent Urinary Tract Infection by Highly Expressing the Urease." *Frontiers in Microbiology*, February 2023.

[27] Nile, H.R. *et al.* "Virulence Factors of Pseudomonas aeruginosa Isolated from Wound and Burn Infections." *International Journal of Current Research in Biosciences and Plant Biology*, vol. 2, no. 6, 2015, pp. 153-162.

[28] Al-Fahham, H.R.A. and K.R. Kareem. "Molecular Study of Urease ureR Gene of Proteus mirabilis Isolated from Urinary Tract Infections, Najaf, Iraq." 2022.

[29] Al-Hayali, S.A. *A Genetic and Bacterial Comparison Between Streptococcus* spp. and *Staphylococcus* spp. *Resistance to Anti-Macrolide from Various Clinical Injuries*. 2014. Diyala University, Master's Thesis.

[30] Bissong, M.E.A. and C.N. Ateba. "Genotypic and Phenotypic Evaluation of Biofilm Production and Antimicrobial Resistance in *Staphylococcus aureus* Isolated from Milk, North West Province, South Africa." *Antibiotics*, vol. 9, no. 4, 2020.

[31] Khalaf, A. *et al.* "Detection of Genes ermB, mecA, blaZ and msrA in Uropathogenic *Staphylococcus aureus* Isolates Between the Gram-Positive Bacteria that Cause Urinary Tract Infections." *Iranian Journal of War and Public Health*, vol. 14, no. 1, 2022, pp. 99-104.

[32] Snoussi, M. *et al.* "Antibiotic Susceptibility Profiling of Human Pathogenic *Staphylococcus aureus* Strains Using Whole Genome Sequencing and Genome-Scale Annotation Approaches." *Microorganisms*, vol. 11, no. 5, 2023.

[33] Siddique, A.B. *et al.* "Preparation and Characterisation of Essential Oil from Lavandula spica Plant and Its Antimicrobial Activity Against *Pseudomonas aeruginosa* and *Staphylococcus aureus*." *Microbial Pathogenesis*, vol. 198, January 2025.