



Molecular Detection and Expression of the *blaZ* Gene in Clinical *Staphylococcus aureus* Isolated from Hospital Patients

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Abstract. This study aimed to investigate the presence and expression of the *blaZ* gene in clinical *Staphylococcus aureus* isolates obtained from patients attending hospitals in Iraq. A total of 140 clinical specimens were collected, including wound swabs (71), vaginal swabs (35), urine samples (20), and sputum samples (14). Bacterial isolation was performed using Mannitol Salt Agar (MSA), followed by morphological and biochemical identification and confirmation using the VITEK-2 Compact system. Among the collected specimens, 25 *S. aureus* isolates were identified. β -Lactamase production was assessed using the Nitrocefin disk test, with 20 isolates showing positive results. These β -lactamase-positive isolates were selected for molecular analysis. PCR was performed for detection of the *blaZ*, *mecA* and 16S rRNA genes, and the *blaZ* gene was detected in all 20 examined isolates. The *mecA* gene was also detected in all selected isolates. The expression of *blaZ* was subsequently analyzed by real-time quantitative PCR using 16S rRNA as the reference gene. The results revealed considerable variation in *blaZ* expression among the examined isolates, with fold-change values ranging from 0.001953 to 128 and an overall mean of 17.90. Overall, the study demonstrated the presence of *blaZ* among the selected β -lactamase-positive clinical *S. aureus* isolates and showed considerable variation in its expression between isolates. These findings provide molecular characterization of *blaZ* expression among the clinical isolates examined in this study.

Keywords: *Staphylococcus aureus*; *blaZ*; β -Lactamase; Antimicrobial resistance; *mecA*; 16S rRNA.

1. Introduction

Staphylococcus aureus is an important Gram-positive bacterial pathogen associated with a wide range of human infections, including skin and soft-tissue infections as well as more severe diseases such as bacteremia, pneumonia, endocarditis and bone and joint infections. It can colonize humans without causing disease but may cause infection when it gains access to damaged tissues or sites where host defenses are compromised (Cheung et al., 2021). The ability of *S. aureus* to adapt to different environments and acquire antimicrobial resistance has contributed to its continued clinical importance in both community and healthcare settings. Resistant *S. aureus* is of particular concern because infections caused by resistant strains may be more difficult to treat and may increase the burden on healthcare systems (WHO, 2024; Touaitia et al., 2025). Antimicrobial resistance (AMR) is a major challenge to the effective treatment of bacterial infections. *S. aureus* can develop resistance through several mechanisms, including alteration of antimicrobial targets, changes in permeability, active efflux and enzymatic inactivation of antimicrobial agents. Among these mechanisms, enzymatic inactivation is particularly important for β -lactam antibiotics. β -lactamase enzymes hydrolyze the β -lactam ring of susceptible antibiotics, reducing or eliminating their antibacterial activity. Consequently, β -lactamase production represents an important mechanism contributing to β -lactam resistance in clinical *S. aureus* isolates (An et al., 2024; Brdová et al., 2024). In *S. aureus*, the *blaZ* gene is the principal genetic determinant encoding the staphylococcal β -lactamase enzyme. Expression of *blaZ* is regulated by the *blaI*-*blaR1* regulatory system, in which *BlaR1* functions as a β -lactam sensor and *BlaI* acts as a transcriptional repressor. Exposure to β -lactam antibiotics can activate this regulatory pathway, resulting in derepression of *blaZ* and subsequent

production of β -lactamase (Cong et al., 2020). Therefore, investigation of *blaZ* is important for understanding the genetic basis of β -lactam resistance and the mechanisms that influence its expression in *S. aureus* (Brdová et al., 2024).

Phenotypic and molecular methods provide complementary information for investigating β -lactam resistance. Antimicrobial susceptibility testing determines the susceptibility or resistance of bacterial isolates to selected antibiotics, while the Nitrocefin disk test provides a rapid phenotypic method for detecting β -lactamase activity. At the molecular level, polymerase chain reaction (PCR) can be used to detect specific resistance-associated genes such as *blaZ*. Molecular identification using the 16S rRNA gene can also support confirmation of bacterial identity because this gene contains conserved and variable regions that are useful for bacterial characterization (Drevinek et al., 2023; López-Aladid et al., 2023). Although PCR detection of *blaZ* demonstrates the presence of the resistance-associated gene, it does not necessarily indicate that the gene is expressed at the same level in all isolates. Differences in gene regulation and bacterial physiological conditions may influence the transcriptional activity of resistance determinants. Therefore, assessment of gene expression can provide additional information beyond simple gene detection. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) enables quantitative assessment of specific RNA transcripts and is therefore useful for investigating the expression of bacterial resistance-associated genes (Bong et al., 2024).

The relationship between the presence of *blaZ*, its expression and the corresponding phenotypic characteristics is particularly relevant in clinical isolates method (Vang et al., 2025). An isolate may carry the *blaZ* gene but show differences in β -lactamase activity or antimicrobial susceptibility depending on the level of gene expression and other regulatory factors. Thus, combining phenotypic testing with molecular detection and expression analysis can provide a more complete characterization of β -lactam resistance than using a single method s (Leistner et al., 2022). This integrated approach can help clarify whether the detection of *blaZ* is associated with phenotypic β -lactamase production and differences in antimicrobial susceptibility among clinical *S. aureus* isolates. The clinical significance of antimicrobial-resistant *S. aureus* also extends to public health because resistant infections can limit therapeutic options, increase treatment complexity and contribute to the persistence and transmission of resistant organisms in healthcare environments (Birlutiu et al., 2026). The World Health Organization has identified methicillin-resistant *S. aureus* as a high-priority bacterial pathogen, emphasizing the importance of continued research and surveillance of antimicrobial resistance. Therefore, studying resistance-associated determinants such as *blaZ* in clinical isolates contributes not only to understanding the molecular mechanisms of resistance but also to the broader monitoring and control of AMR in healthcare settings (WHO, 2024).

Accordingly, the present study aimed to investigate β -lactam resistance among clinical *Staphylococcus aureus* isolates, with particular focus on β -lactamase production and the role of the *blaZ* gene in this resistance mechanism. The study evaluated antimicrobial susceptibility patterns, assessed β -lactamase production using the Nitrocefin disk test, detected the *blaZ* and *mecA* genes using PCR and investigated *blaZ* expression in selected isolates using RT-qPCR to characterize the involvement of *blaZ* in β -lactamase-mediated resistance.

2. Materials and Methods

2.1 Sample Collection and Bacterial Identification

A total of 140 clinical specimens were collected from patients attending Tikrit General Hospital, Salah Al-Din Governorate and Medical City Hospital, Baghdad, Iraq, during the period from January 1 to April 16, 2026. The patients ranged in age from 10 to 50 years. The collected specimens included 71 wound swabs (50.71%), 35 vaginal swabs (25.00%), 20 urine samples (14.29%) and 14 sputum samples (10.00%). Clinical specimens were collected according to the patients' clinical presentation and the physician's request for microbiological investigation. Vaginal swabs were collected from patients presenting with clinical signs and symptoms of vaginal infection, while sputum specimens were collected from patients presenting with clinical signs and symptoms of respiratory tract infection. Clinical specimens were directly inoculated onto Blood Agar (HiMedia, India) and incubated aerobically at 37°C for 18–24 h. Plates showing no growth after the initial incubation were re-incubated for an additional 48 h. Suspected colonies were subcultured onto fresh Blood Agar to obtain pure cultures.

Initial identification of suspected *Staphylococcus aureus* isolates was performed based on colony morphology, Gram staining, catalase activity, coagulase production and mannitol fermentation on Mannitol Salt Agar (Oxoid, England). The isolates were subsequently subjected to automated identification using the VITEK 2 system (bioMérieux, France). Bacterial suspensions were prepared at a turbidity of approximately 0.50–0.63 McFarland and inoculated into Gram-Positive identification cards according to the manufacturer’s recommended procedure. The cards were incubated at approximately $35.5 \pm 1^\circ\text{C}$ and automatically analyzed by the VITEK 2 system. Amplification of the bacterial 16S rRNA gene was additionally performed as a molecular assay for bacterial identification, as shown in Table 1 (Drevinek et al., 2023).

Table 1: Primers used for PCR and RT-qPCR.

Primer	Primer sequence (5-3)	Product size (bp)	Reference
<i>blaZ</i>	F AAAGAAATCGGTGGAATCAAAA 22	248	Gene Pharma (Shanghai, China).
	R TCTTACCGAAAGCAGCAGGT 20		
<i>mecA</i>	F CAAAATGGAGCTTGGGAGTC 20	475	Gene Pharma (Shanghai, China).
	R ATCATCGAAACTTGGCAAATG 21		
16s rRNA	F CCAGACTCCTACGGGAGGCAG 21	210	Gene Pharma (Shanghai, China).
	R CGTATTACCGCGGCTGCTG 19		

Phenotypic Characterization and Antimicrobial Susceptibility

β -Lactamase production was evaluated using a nitrocefin-based chromogenic test. Fresh bacterial colonies obtained after 18–24 h of incubation were tested, and development of a yellow-to-pink/red color was considered indicative of β -lactamase activity. Phenotypic detection of β -lactamase activity was included to complement molecular detection of the *blaZ* gene, as the presence of a resistance gene does not necessarily indicate equivalent phenotypic enzyme activity. *BlaZ* is a major β -lactamase-associated determinant of penicillin resistance in *S. aureus* (Lade & Kim, 2023; Brdová et al., 2024).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Fifteen antimicrobial agents were tested using commercial antibiotic discs (Bioanalyse, Turkey): oxacillin (30 $\mu\text{g}/\text{disc}$), methicillin (30 $\mu\text{g}/\text{disc}$), ampicillin (10 units/disc), gentamicin (10 $\mu\text{g}/\text{disc}$), erythromycin (15 $\mu\text{g}/\text{disc}$), tetracycline (30 $\mu\text{g}/\text{disc}$), doxycycline (30 $\mu\text{g}/\text{disc}$), clindamycin (20 $\mu\text{g}/\text{disc}$), chloramphenicol (30 $\mu\text{g}/\text{disc}$), vancomycin (30 $\mu\text{g}/\text{disc}$), levofloxacin (5 $\mu\text{g}/\text{disc}$), amoxicillin, azithromycin, ceftriaxone (30 $\mu\text{g}/\text{disc}$) and norfloxacin (30 $\mu\text{g}/\text{disc}$). The disk diffusion procedure was performed in accordance with CLSI M02 recommendations, and susceptibility categorization was based on the applicable Clinical and Laboratory Standards Institute (CLSI) M100 breakpoint criteria (CLSI, 2024; CLSI, 2026).

A bacterial suspension equivalent to 0.5 McFarland was prepared from a fresh culture and uniformly inoculated onto the agar surface using a sterile swab. Antibiotic disks were then placed on the inoculated plates and the plates were incubated aerobically at 37°C for 24 h. The inhibition zones were measured in millimeters and interpreted as susceptible, intermediate or resistant according to the applicable Clinical and Laboratory Standards Institute criteria. The antimicrobial agents tested included Ampicillin, Doxycycline, Clindamycin, Chloramphenicol, Vancomycin, Gentamicin, Erythromycin and Tetracycline. The disk diffusion procedure followed the standardized recommendations of CLSI M02, while interpretation was based on the current CLSI M100 breakpoints (CLSI, 2024, 2026). Antimicrobial susceptibility testing was included because antimicrobial resistance in *S. aureus* remains an important clinical concern, particularly in healthcare-associated infections. Recent reviews have emphasized the

diversity of molecular and phenotypic resistance mechanisms in *S. aureus* and the continuing need for standardized susceptibility testing and surveillance (Silva-Santana, 2025; Brdová *et al.*, 2024; Hussein *et al.*, 2024).

DNA Extraction and PCR Detection

Genomic DNA was extracted from 20 selected *S. aureus* isolates. Bacterial cultures were grown in nutrient broth at 37°C for 24 h. Approximately 2 mL of each culture was transferred into a microcentrifuge tube and centrifuged at 14,000 rpm for 5 min. The supernatant was discarded and the bacterial pellet was processed for genomic DNA extraction using the Geneaid genomic DNA extraction procedure (Taiwan). The pellet was treated with 500 µL of TE buffer followed by centrifugation under the same conditions. Subsequently, 500 µL of 2× CTAB solution was added, mixed thoroughly and incubated at 65°C for 30 min with intermittent mixing. Then, 100 µL of 20% SDS and 300 µL of phenol:chloroform:isoamyl alcohol were added. Following centrifugation, the aqueous phase was transferred to a new tube and the extraction step was repeated with an equal volume of phenol:chloroform:isoamyl alcohol. DNA was precipitated using two volumes of absolute ethanol at –20°C, washed with 500 µL ethanol and centrifuged. The DNA pellet was air-dried and dissolved in 100 µL of distilled water before storage at –20°C.

DNA concentration and purity were assessed using a NanoDrop spectrophotometer by measuring absorbance at 260 and 280 nm. DNA quality was evaluated using the A260/A280 ratio before PCR amplification. Conventional PCR was performed to detect the *blaZ*, *mecA* and 16S rRNA genes. The *blaZ* gene was selected as the principal molecular target because *BlaZ*-mediated β-lactamase production represents an important mechanism of β-lactam resistance in *S. aureus* (Lade & Kim, 2023; Brdová *et al.*, 2024). The 16S rRNA gene was included as a bacterial molecular identification target. Recent clinical microbiology literature supports the use of 16S rRNA-based molecular approaches for bacterial identification (Drevinec *et al.*, 2023).

PCR reactions were prepared in a final volume of 25 µL containing 12.5 µL of Master Mix, 5 µL of DNA template, 1 µL of each forward and reverse primer and 5.5 µL of nuclease-free water. Amplification was performed with an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 45 s and extension at 72°C for 45 s, followed by a final extension at 72°C for 6 min. PCR products were separated by electrophoresis on 1% agarose gel and visualized using a UV transilluminator.

RNA Extraction, cDNA Synthesis and RT-qPCR

RNA was extracted from 20 selected *S. aureus* isolates for analysis of *blaZ* gene expression. The 20 isolates were selected based on positive β-lactamase activity detected by the Nitrocefin disk test and were subsequently subjected to molecular analysis. Bacterial cultures were grown in nutrient broth at 37°C for 24 h and centrifuged at 13,000 rpm for 2 min. The bacterial pellet was treated with 200 µL of lysozyme solution for 5 min, followed by the addition of 600 µL of TransZol. The mixture was incubated for 10 min, after which 200 µL of chloroform was added. Samples were centrifuged at 12,000 rpm for 10 min at room temperature. The upper aqueous phase was carefully transferred to an RNA purification column and processed using the appropriate washing buffers. Following the washing steps, an additional dry centrifugation was performed to remove residual ethanol. RNA was eluted in 100 µL of RNA dissolving solution and stored at –20°C until further analysis.

The extracted RNA was used for complementary DNA (cDNA) synthesis using RT-PCR Master Mix (Tinzyme, China). Each 20 µL reaction contained 10 µL of total RNA, 4 µL of 5× RT-PCR Master Mix and 6 µL of RNase-free water. Reverse transcription was performed at 25°C for 10 min, followed by 42°C for 15 min and 85°C for 5 s. The resulting cDNA was used as the template for subsequent real-time PCR analysis. The RT-qPCR workflow was performed in accordance with established methodological recommendations (Bong *et al.*, 2024; Bustin *et al.*, 2025).

Lyophilized primers were briefly centrifuged and dissolved in nuclease-free water. Working primer solutions were prepared at a concentration of 10 pmol/µL. RT-qPCR was performed using UltraSYBR Mixture with Low ROX, 2× (Tinzyme, China). Each reaction was prepared in a final volume of 25 µL containing 12.5 µL of 2× UltraSYBR mixture, 0.5 µL of forward primer, 0.5 µL of reverse primer, 2 µL of cDNA template and 9.5 µL of nuclease-free

water. Amplification was performed using a Real-Time PCR Detection System (Applied Biosystems, USA) (Bustin et al., 2025; Bong et al., 2024).

The thermal profile consisted of an initial denaturation step at 95°C for 10 min, followed by 35–40 amplification cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. The *blaZ* gene was analyzed as the target gene, whereas the 16S rRNA gene was used as the reference gene for normalization. The selection and reporting of RT-qPCR conditions were guided by current recommendations emphasizing assay design, sample handling, reference-gene selection and transparent reporting of qPCR experiments (Bustin et al., 2025; Bong et al., 2024).

Relative *blaZ* expression was determined using the comparative cycle threshold (Ct) method. The Ct values obtained for *blaZ* were normalized to those of the 16S rRNA reference gene by calculating ΔCt ($\Delta Ct = Ct_{\text{target}} - Ct_{\text{reference}}$). The ΔCt value of each tested isolate was compared with that of the designated control sample to obtain $\Delta\Delta Ct$ ($\Delta\Delta Ct = \Delta Ct_{\text{target}} - \Delta Ct_{\text{control}}$). Relative *blaZ* expression was calculated as fold change using the equation $2^{-\Delta\Delta Ct}$ according to Livak and Schmittgen (2001). The analysis and reporting of relative gene expression were conducted with consideration of current qPCR recommendations (Bustin et al., 2025).

Statistical Analysis

Statistical analysis was performed using SPSS software version 26. Quantitative data were expressed as mean $\pm$ standard deviation (SD). A P-value <0.05 was considered statistically significant.

3. Results and Discussion

Isolation and Identification of *Staphylococcus aureus*

A total of 140 clinical samples were examined. Bacterial growth was observed on Mannitol Salt Agar (MSA) in 126 samples (90%), while 14 samples (10%) showed no bacterial growth. Among the growing isolates, 31 (22.14%) were mannitol fermenters and 95 (67.86%) were non-fermenters, as shown in Table (2). The presumptive *Staphylococcus aureus* isolates were identified based on their cultural characteristics and conventional biochemical tests, followed by confirmation using the VITEK-2 Compact system.

Table 2: Number and percentage of isolates on Mannitol Salt Agar

Non-mannitol fermentation on results	Mannitol fermentation results	+ve	-ve	total
95	31	126	14	140
67.86%	%22.14	%90	%10	

The samples were distributed into four age groups, the highest proportion was recorded among individuals aged 41–50 years (30%), followed by those aged 31–40 years (28.12%), 21–30 years (25%) and 10–20 years (16.87%), as shown in Table (3). The higher representation of the older age groups may be related to differences in underlying health conditions, healthcare exposure and antimicrobial use, which can influence susceptibility to *S. aureus* infections. Age-related variation in the occurrence and antimicrobial resistance of *S. aureus* has also been reported in clinical populations (Tong et al., 2015; An et al., 2024).

Table 3: Distribution of samples according to age group.

Age group	Number	Percentage
10-20 years	22	%16.87
21-30 years	35	25%
31- 40 years	40	%28.12

41-50 years	43	%30.0
Total	140	%100

Distribution of Samples According to Sex

Of the 140 examined samples, 83 (59.29%) were obtained from males and 57 (40.71%) from females. Thus, males represented a higher proportion of the examined samples. This difference may be related to biological, behavioral, occupational, and environmental factors that can influence exposure to bacterial infections. However, other studies have reported higher rates among females, indicating that sex-related differences may vary according to the infection site and study population.

Distribution of Samples According to Isolation Source

The distribution of the examined clinical samples according to isolation source showed that wound swabs accounted for the highest proportion, with 71 samples (50.71%), followed by vaginal swabs with 35 samples (25.00%), urine samples with 20 samples (14.29%) and sputum samples with 14 samples (10.00%). The higher proportion of wound specimens may be related to the susceptibility of damaged skin and soft tissues to bacterial colonization and infection by *S. aureus*, which is a common pathogen associated with skin and wound infections (Cheung et al., 2021; Touaitia et al., 2025).

Cultural, Microscopic, Biochemical Identification of *Staphylococcus aureus*.

Cultural examination showed that the suspected *S. aureus* isolates grew on Mannitol Salt Agar and produced yellow colonies with a corresponding yellow color change in the medium due to mannitol fermentation. The colonies were generally circular and medium-sized. Microscopic examination revealed Gram-positive cocci arranged predominantly in grape-like clusters, which is characteristic of the genus *Staphylococcus*. Based on the cultural and microscopic characteristics, 31 isolates were considered presumptive *S. aureus*. The 31 suspected *S. aureus* isolates were subjected to conventional biochemical tests. All isolates were Gram-positive and positive for catalase, coagulase, and mannitol fermentation, with a positivity rate of 100% for each test.

The combined cultural, microscopic, and biochemical characteristics supported the identification of the isolates as *S. aureus*. Catalase positivity distinguishes *Staphylococcus* from catalase-negative genera such as *Streptococcus*, while coagulase positivity is an important characteristic used to differentiate *S. aureus* from most coagulase-negative staphylococci (Cheung et al., 2021).

No Individual Identification Tests

The Gram stain showed purple, Gram-positive cocci arranged mainly in clusters. The catalase test produced visible bubbles following the addition of hydrogen peroxide, indicating a positive reaction. Both slide and tube coagulase tests produced positive reactions in all 31 isolates. Mannitol fermentation resulted in a yellow color change of the MSA medium, confirming the characteristic fermentation reaction.

The results of these tests were consistent with the expected phenotypic characteristics of *S. aureus* and therefore provided reliable preliminary identification before further automated and molecular confirmation.

Detection of β -Lactamase Production in *Staphylococcus aureus* Isolates Using the Nitrocefin Disk Test.

β -Lactamase production was detected in 25 *Staphylococcus aureus* isolates using the Nitrocefin disk test. The results showed that 20 isolates (80%) were positive, as indicated by a color change from yellow to red within 1–5 min. These 20 β -lactamase-positive isolates were subsequently selected for molecular analysis. The present result was comparable with that reported by Kilani et al. (2024), who detected β -lactamase production in 77.5% of *S. aureus* isolates using a Nitrocefin-based assay. Onalapo et al. (2016) reported β -lactamase production in 64% of *S. aureus* isolates, whereas

Oliveira et al. (2016) reported a lower rate of 48%. Such differences may be related to variations in bacterial sources, geographical areas and study conditions.

Antimicrobial Susceptibility Testing.

The antimicrobial susceptibility of 20 *S. aureus* isolates was determined against 15 antibiotics using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to CLSI (2024) guidelines (Fig. 1). The antimicrobial susceptibility testing revealed variable resistance patterns among the tested isolates. The highest resistance rate was observed for Ampicillin (100%), followed by Ceftriaxone (95%), Methicillin (95%) Levofloxacin (85%), Azithromycin (75%), Oxacillin (60%), Norfloxacin (45%), Erythromycin (35%), Amoxicillin (30%), Gentamicin (20%), Tetracycline (10%), Clindamycin (5%), Chloramphenicol (5%), Vancomycin (5%) and Doxycycline (5%).

Intermediate susceptibility was observed for Tetracycline (20%), Norfloxacin (5%), Amoxicillin (5%), Azithromycin (5%) and Vancomycin (5%). The highest susceptibility rates were observed for Doxycycline (95%), Clindamycin (95%), Chloramphenicol (95%), Erythromycin (65%), Gentamicin (80%), Oxacillin (40%) and Methicillin (5%). The susceptibility rates for Tetracycline, Norfloxacin, Amoxicillin, Azithromycin and Vancomycin were 70%, 50%, 65%, 20% and 90%, respectively, while the lowest susceptibility rate was recorded for Ampicillin (0%).

The antimicrobial susceptibility pattern observed in the present study showed both similarities and differences compared with previous reports. The relatively high susceptibility to Doxycycline, Gentamicin and Vancomycin observed in the present study is comparable to findings reported by Birlutiu et al. (2026), who reported low resistance to Doxycycline (9.50%), Gentamicin (with 92.9–100% susceptibility) and Vancomycin (2.92% resistance) among clinical *S. aureus* isolates. However, the present study showed lower resistance to Clindamycin and Tetracycline than that reported in their study, indicating variation in antimicrobial susceptibility patterns between clinical populations.

In contrast, An et al. (2024) reported substantially higher resistance rates to Azithromycin (82.28%), Erythromycin (82.82%) and Clindamycin (82.32%) compared with the resistance rates observed in the present study (75%, 35% and 5%, respectively). Their study also reported higher resistance to Tetracycline (67.06%) and Gentamicin (25.87%) than the corresponding rates in the present study (10% and 20%, respectively). These differences may reflect variation in geographical location, patient populations, specimen types, hospital settings and temporal patterns of antimicrobial use and resistance. The complete resistance to Ampicillin is consistent with Odetokun et al. (2023) and may be associated with β -lactamase production, which contributes to the inactivation of β -lactam antibiotics (Aarris et al., 2025). The resistance observed to Oxacillin and Methicillin indicates reduced activity of these antibiotics against some of the studied isolates. The high susceptibility to Doxycycline, Clindamycin, Chloramphenicol and Vancomycin indicates that these antibiotics retained relatively good activity against the isolates examined. Overall, the findings demonstrate considerable resistance to some β -lactam antibiotics, particularly Ampicillin, while several other antibiotics maintained high activity against the studied *S. aureus* isolates. From a public health perspective, the observed resistance pattern highlights the importance of continuous antimicrobial susceptibility surveillance and antimicrobial stewardship in healthcare settings. The complete resistance to Ampicillin may limit the usefulness of this commonly used β -lactam agent against the clinical *S. aureus* isolates examined in the present study (Table 4).

Table 4: Results of the antimicrobial susceptibility test.

%	Sensitive No.	%	Intermediate No.	%	Resistant No.	Symbol	Antibacterial	No.
65	13	–	–	35	7	E	Erythromycin	1
70	14	20	4	10	2	ET	Tetracycline	2
95	19	–	–	5	1	DO	Doxycycline	3
95	19	–	–	5	1	DA	Clindamycin	4
80	16	–	–	20	4	CN	Gentamicin	5
95	19	–	–	5	1	C	Chloramphenicol	6
40	8	–	–	60	12	OX	Oxacillin	7
5	1	–	–	95	19	ME	Methicillin	8
0	0	–	–	100	20	AMP	Ampicillin	9
50	10	5	1	45	9	NO	Norfloxacin	10
15	3	–	–	85	17	LFX	Levofloxacin	11
65	13	5	1	30	6	AMX	Amoxicillin	12

5	1	–	–	95	19	CTR	Ceftriaxone	13
20	4	5	1	75	15	AZM	Azithromycin	14
90	18	5	1	5	1	VA	Vancomycin	15

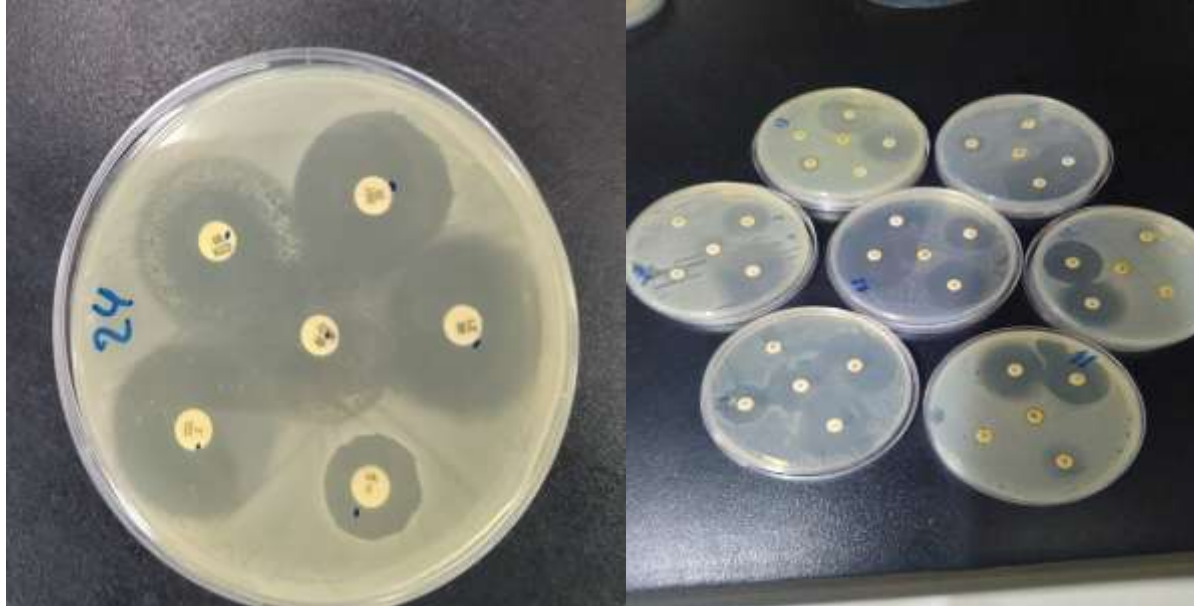


Figure 1: Selected results of the antimicrobial susceptibility test for *Staphylococcus aureus*.
PCR Amplification of the 16S rRNA Gene

The 16S rRNA gene was amplified by conventional PCR to confirm the molecular identification of *S. aureus* (Fig 2). Specific primers were used to amplify a 210-bp fragment. Agarose gel electrophoresis showed clear bands at the expected size in the examined isolates, confirming successful amplification and supporting the identification of the bacterial isolates.

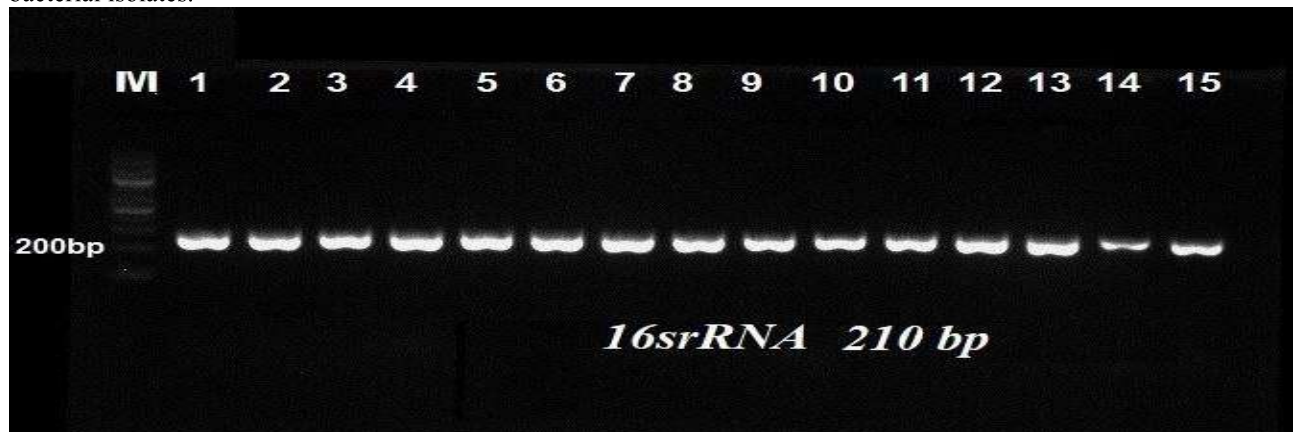


Figure 2: PCR amplification of the 16S rRNA gene, 210-bp product.

Amplification of the *mecA* Gene

The *mecA* gene was detected by PCR in 20 selected clinical *Staphylococcus aureus* isolates. A specific 475-bp PCR product was obtained in all 20 tested isolates, giving a detection rate of 100% (20/20). This result indicates the presence of the *mecA* gene among the isolates selected for molecular analysis. The *mecA* gene encodes penicillin-binding protein 2a (PBP2a), which has low affinity for β -lactam antibiotics and is associated with methicillin resistance (Lade & Kim, 2023). As shown in Figure (3), electrophoresis on 1.5% agarose gel at 100 V for 60 min revealed clear bands at approximately 475 bp, confirming amplification of the *mecA* gene in the tested isolates.

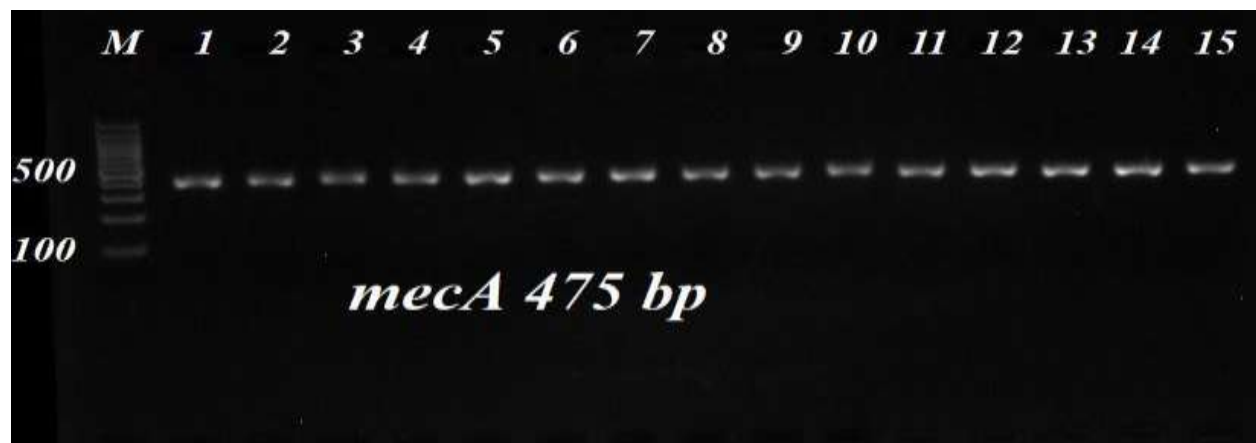


Figure 3: PCR amplification of the *mecA* gene, 475-bp product.

PCR Amplification of the *blaZ* Gene

The *blaZ* gene was detected by conventional PCR in selected *S. aureus* isolates (Fig 4). The primers amplified a specific fragment of 248 bp. Clear bands corresponding to the expected size were observed on agarose gel electrophoresis, indicating the presence of the *blaZ* gene in all examined isolates. The presence of this gene confirms the carriage of *blaZ* in the examined isolates and indicates their genetic potential for β -lactamase production (Aarris et al., 2025).



Figure 4: PCR amplification of the *blaZ* gene, 248-bp product.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from 20 selected *S. aureus* isolates using the TransZol™ Up Plus RNA Kit according to the manufacturer's instructions. The RNA was subsequently converted into complementary DNA (cDNA) using a reverse-transcription Master Mix. The resulting cDNA was used as the template for RT-qPCR analysis.

Real-Time qPCR Analysis of *blaZ* Gene Expression

The expression levels of the *blaZ* gene were determined in 20 selected clinical *S. aureus* isolates using RT-qPCR and the comparative Ct method ($2^{-\Delta\Delta Ct}$). Five replicates were performed for each isolate, and the reported fold-change value represents the mean of the five replicates. The results showed marked variation in *blaZ* expression among the examined isolates, with fold-change values ranging from 0.001953 to 128 and an overall mean of 17.90. Some isolates exhibited very low expression levels, whereas others showed markedly higher expression levels (Table 5).

Table 5: Relative expression (fold-change) of the *blaZ* gene in isolates.

Fold change (Mean $\pm$ SD)	Sample
0.000195 $\pm$ 0.001953 ^a	1
0.05 $\pm$ 0.50 ^b	2
1.60 $\pm$ 16.00 ^d	3
3.20 $\pm$ 32.00 ^f	4
6.40 $\pm$ 64.00 ^g	5
0.05 $\pm$ 0.50 ^b	6
0.000781 $\pm$ 0.007813 ^a	7
0.40 $\pm$ 4.00 ^c	8
6.40 $\pm$ 64.00 ^g	9
0.80 $\pm$ 8.00 ^d	10
1.60 $\pm$ 16.00 ^e	11
0.025 $\pm$ 0.25 ^b	12
0.20 $\pm$ 2.00 ^c	13
0.40 $\pm$ 4.00 ^c	14
0.20 $\pm$ 2.00 ^c	15
0.05 $\pm$ 0.50 ^b	16
0.001563 $\pm$ 0.015625 ^a	17
1.60 $\pm$ 16.00 ^e	18
0.025 $\pm$ 0.25 ^b	19
12.80 $\pm$ 128.00 ^h	20
17.90 $\pm$ 32.90	Mean

Different superscript letters indicate statistically significant differences among the fold-change expression levels of the examined *S. aureus* isolates ($P < 0.05$).

The present study demonstrated considerable variation in relative *blaZ* expression among the examined clinical *Staphylococcus aureus* isolates, with fold-change values ranging from 0.001953 to 128. Values below 1 indicate lower *blaZ* expression relative to the designated control, whereas values above 1 indicate higher relative expression. This wide range suggests substantial isolate-to-isolate variation in *blaZ* transcription. The overall mean fold-change of 17.90 represents the arithmetic mean of the relative expression values and should not be interpreted as a uniform 17.90-fold increase in *blaZ* expression across all isolates. Such variation may reflect differences in the genetic and regulatory characteristics of the *blaZ* system among individual isolates. These findings are consistent with Aarris et al. (2025), who reported substantial genetic variation among *blaZ* variants in clinical *S. aureus* isolates. Similarly, Lee et al. (2025) demonstrated that variation in the regulatory mechanisms of *blaZ* can influence β -lactamase expression. In contrast, a recent study of *blaZ*-positive *S. aureus* isolates under cefazolin exposure reported no consistent increase in *blaZ* expression under the tested conditions, suggesting that expression may also depend on the bacterial strain and experimental conditions. Therefore, the variation observed in the present study may reflect isolate-specific genetic and regulatory characteristics of the *blaZ* system.

The melting curve (Melt Curve) of the *blaZ* gene amplification product was analyzed to verify the specificity and quality of the Real-Time qPCR reaction. The curve showed a distinct main melting peak at approximately 81–82°C, supporting the presence of a specific *blaZ* amplification product and the absence of evident nonspecific amplification products, as shown in Figure (5).

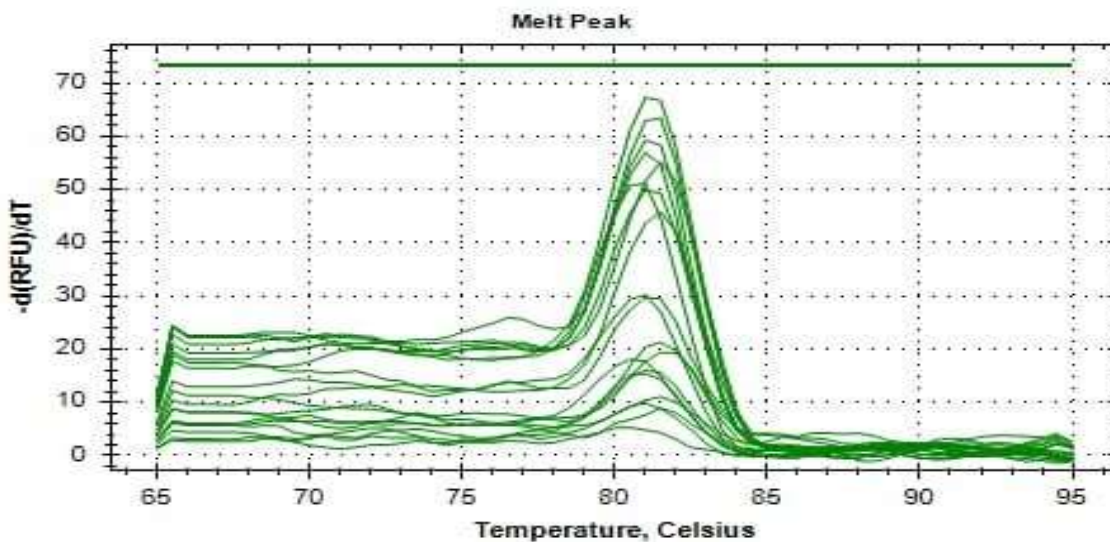


Figure 5: Melt curve analysis of the *blaZ* gene amplification produce using RT-qPCR.

Overall, the variation in *blaZ* expression observed among the clinical *S. aureus* isolates highlights the differences in *blaZ* expression between isolates and supports the importance of examining this gene in clinical isolates.

Conclusions

The present study demonstrated variable antimicrobial susceptibility patterns among clinical *Staphylococcus aureus* isolates, with complete resistance to ampicillin and high susceptibility to several of the tested antibiotics. Molecular analysis detected the *blaZ* gene in the selected β -lactamase-positive isolates, while real-time quantitative PCR revealed considerable variation in *blaZ* expression among the examined isolates. The observed differences in expression may reflect isolate-specific genetic and regulatory characteristics of the *blaZ* system. Overall, these findings characterize antimicrobial resistance patterns and *blaZ* expression among the clinical *S. aureus* isolates examined in this study.

References

- Aarris, M., Hertz, F. B., Nielsen, K. L., Sato, A., Johansen, H. K., Westh, H., Kemp, M., Ellermann-Eriksen, S., Løbner-Olesen, A., Frimodt-Møller, N., & Charbon, G. (2025). Genetic variation in the *blaZ* gene leading to the BORSA phenotype in *Staphylococcus aureus*. *Antibiotics*, 14(5), 449. <https://doi.org/10.3390/antibiotics14050449>
- 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., Kien, H. T., Le, M. N., Viet, N. H., Nguyen, D. H., Pham, N. V., Thang, T. B., Tien, T. V., & Hoang, L. H. (2024). Antimicrobial resistance patterns of *Staphylococcus aureus* isolated at a general hospital in Vietnam between 2014 and 2021. *Infection and Drug Resistance*, 17, 259–273. <https://doi.org/10.2147/IDR.S437920>
- Birlutiu, V., Ciprian Ion, O., & Birlutiu, R.-M. (2026). Susceptibility of *Staphylococcus aureus* strains isolated from blood cultures between 2024 and 2025: A single-center study. *Antibiotics*, 15(7), 695. <https://doi.org/10.3390/antibiotics15070695>
- Bong, D., Sohn, J., & Lee, S.-J. V. (2024). Brief guide to RT-qPCR. *Molecules and Cells*, 47(12), 100141.
- Brdová, D., Ruml, T., & Viktorová, J. (2024). Mechanism of staphylococcal resistance to clinically relevant antibiotics. *Drug Resistance Updates*, 77, 101147.
- Bustin, S. A., Ruijter, J. M., van den Hoff, M. J. B., Kubista, M., Pfaffl, M. W., Shipley, G. L., Tran, N., Rödiger, S., Untergasser, A., Mueller, R., Nolan, T., Milavec, M., Burns, M. J., Huggett, J. F., Vandesompele, J., & Wittwer, C. T. (2025). MIQE 2.0: Revision of the Minimum Information for Publication of Quantitative Real-Time PCR Experiments Guidelines. *Clinical Chemistry*, 71(6), 634–651. <https://doi.org/10.1093/clinchem/hvaf043>
- Bush, K., & Bradford, P. A. (2020). Epidemiology of β -lactamase-producing pathogens. *Clinical Microbiology Reviews*, 33(2), e00047-19. <https://doi.org/10.1128/CMR.00047-19>

- Cheung, G. Y. C., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*, 12(1), 547–569.
- Clinical and Laboratory Standards Institute. (2024a). Performance standards for antimicrobial disk susceptibility tests (14th ed.; CLSI standard M02). Clinical and Laboratory Standards Institute.
- Clinical and Laboratory Standards Institute. (2024b). Performance standards for antimicrobial susceptibility testing (34th ed.; CLSI supplement M100). Clinical and Laboratory Standards Institute.
- Clinical and Laboratory Standards Institute. (2026). Performance standards for antimicrobial susceptibility testing (36th ed.; CLSI supplement M100). Clinical and Laboratory Standards Institute.
- Cong, Y., Yang, S., & Rao, X. (2020). Vancomycin-resistant *Staphylococcus aureus* infections: A review of case updating and clinical features. *Journal of Advanced Research*, 21, 169–176. <https://doi.org/10.1016/j.jare.2019.10.005>
- Drevinek, P., Hollweck, R., Lorenz, M. G., Lustig, M., & Bjarnsholt, T. (2023). Direct 16S/18S rRNA gene PCR followed by Sanger sequencing as a clinical diagnostic tool for detection of bacterial and fungal infections: A systematic review and meta-analysis. *Journal of Clinical Microbiology*, 61(9), e00338-23. <https://doi.org/10.1128/jcm.00338-23>
- Hussein, N. R., Hameed, M. A., & Resho, Q. N. (2024). The trends of *Staphylococcus aureus* antibiotics resistance in Iraq: A narrative review. *BioMed Target Journal*, 2(2), 21–30. <https://doi.org/10.59786/bmtj.222>
- Jasim, A. Y., Samanje, J., & Rasheed, Q. A. (2025). Prevalence of extended-spectrum β -lactamase-producing genes among MRSA isolates from clinical samples in Baghdad city. *Medical Journal of Babylon*, 22(2), 473–478. <https://doi.org/10.4103/mjbl.mjbl.1661.23>
- Kilani, A. M., Alabi, E. D., & Adeleke, O. E. (2024). Coexistence of the *blaZ* gene and selected virulence determinants in multidrug-resistant *Staphylococcus aureus*: Insights from three Nigerian tertiary hospitals. *BMC Infectious Diseases*, 24, 1269. <https://doi.org/10.1186/s12879-024-10171-z>
- Lade, H., & Kim, J.-S. (2023). Molecular determinants of β -lactam resistance in methicillin-resistant *Staphylococcus aureus* (MRSA): An updated review. *Antibiotics*, 12(9), 1362. <https://doi.org/10.3390/antibiotics12091362>
- Lee, S., Park, S., Kim, J. N., Lee, S. O., Lee, J. E., & Lee, S. H. (2025). Polymorphisms in the type A *blaZ* gene as determinants of the cefazolin inoculum effect in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, 69(1), e01106-24.
- Leistner, R., Hanitsch, L. G., Krüger, R., Lindner, A. K., Stegemann, M. S., & Nurjadi, D. (2022). Skin infections due to Panton-Valentine leukocidin-producing *S. aureus*. *Deutsches Ärzteblatt International*, 119(45), 775–784. <https://doi.org/10.3238/arztebl.m2022.0308>
- Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻ $\Delta\Delta$ CT method. *Methods*, 25(4), 402–408. <https://doi.org/10.1006/meth.2001.1262>
- López-Aladid, R., Fernández-Barat, L., Alcaraz-Serrano, V., Bueno-Freire, L., Vázquez, N., Pastor-Ibáñez, R., Palomeque, A., Oscanoa, P., & Torres, A. (2023). Determining the most accurate 16S rRNA hypervariable region for taxonomic identification from respiratory samples. *Scientific Reports*, 13, 3974. <https://doi.org/10.1038/s41598-023-30764-z>
- Rocha, G. D., Nogueira, J. F., Dos Santos, M. V. G., Boaventura, J. A., Soares, R. A. N., de Simoni Gouveia, J. J., da Costa, M. M., & Gouveia, G. V. (2022). Impact of polymorphisms in *blaZ*, *blaR1* and *blaI* genes and their relationship with β -lactam resistance in *S. aureus* strains isolated from bovine mastitis. *Microbial Pathogenesis*, 165, 105453.
- Silva-Santana, G. (2025). *Staphylococcus aureus*: Dynamics of pathogenicity and antimicrobial-resistance in hospital and community environments—Comprehensive overview. *Research in Microbiology*, 176(3–4), 104267.
- Touaitia, R., Mairi, A., Ibrahim, N. A., Basher, N. S., Idres, T., & Touati, A. (2025). *Staphylococcus aureus*: A review of the pathogenesis and virulence mechanisms. *Antibiotics*, 14(5), 470. <https://doi.org/10.3390/antibiotics14050470>
- Vang, T. M., Holzkecht, B. J., Nilsson, S. S., Hoppe, M., Pedersen, M., Krogfelt, K. A., & Eriksen, H. B. (2025). *Staphylococcus aureus* genotypic penicillin susceptibility using a *blaZ* qPCR directly on blood cultures shows high concordance with phenotype. *European Journal of Clinical Microbiology & Infectious Diseases*, 44(12), 3129–3133. <https://doi.org/10.1007/s10096-025-05272-9>
- Vestergaard, M., Frees, D., & Ingmer, H. (2019). Antibiotic resistance and the MRSA problem. *Microbiology Spectrum*, 7(2). <https://doi.org/10.1128/microbiolspec.GPP3-0057-2018>
- World Health Organization. (2024). WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. World Health Organization. <https://doi.org/10.2471/B09179>