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Original Article

16S RRNA-BASED IDENTIFICATION AND ANTIBIOTIC RESISTANCE PROFILING OF *Staphylococcus aureus* ISOLATED FROM WOMEN WITH URINARY TRACT INFECTIONS FROM KURDISTAN REGION OF IRAQ

Ali A. Aziz[1, *], and Aqeel I. Gheni[1]

[1]Department of Medical Microbiology, Faculty of Science and Health, Koya University, Koya 44023, Kurdistan Region, Iraq.

*Corresponding author. E-mail: ali.azad@koyauniversity.org (Tel: +964-7507865501)

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ABSTRACT

Among women, urinary tract infections (UTIs) remains a significant health issue, with *Staphylococcus aureus* is increasingly identified as a prominent yet often overlooked causal agent. This work examined the presence of *S. aureus* in female UTI patients and evaluated their antibiotic resistance patterns. The total sample consisted of 167 individuals, with ages ranging from 17 to 56 years (standard deviation = 8.38). Samples were collected from women living in Koysinjaq, Erbil, and Sulaymaniyah cities in the Kurdistan Region of Iraq. Among 29 isolates identified using phenotypic methods, 24 were confirmed by 16S rRNA sequencing, closely linked to *S. aureus* strain S33 R. High degrees of resistance to ciprofloxacin (87.5%), erythromycin (83.3%), and cefoxitin (54.2%) were found in antibiotic sensitivity testing. By contrast, every isolate turned out to be sensitive to vancomycin. Moreover, in assessing vancomycin sensitivity, minimum inhibitory concentration (MIC) testing proved more accurate than the disc diffusion technique. The study highlights the high prevalence of *MRSA* and multidrug-resistant *Staphylococcus aureus* in urinary tract infections, confirming 16S rRNA gene sequencing as an effective diagnostic tool and emphasizing the clinical significance of resistance patterns in guiding appropriate infection management.

KEYWORDS: *Staphylococcus aureus*, Urinary tract infection, 16S rRNA, Antibiotic resistance, MRSA

1. INTRODUCTION

Urinary tract infections (UTIs) are a common health issue among women, and their susceptibility is increased by anatomical and physiological factors like a shorter urethra and its proximity to the rectum (Álvarez *et al*., 2017). Urinary tract infections brought on by *S. aureus* are generally quite uncommon. Nonetheless, indwelling catheters and other urinary tract devices raise the risk of *S. aureus* transmission, and in some people, *S. aureus* causes ascending urinary tract colonization and infection (Selim *et al.*, 2022).

Staphylococcus aureus is a commensal gram-positive bacterium initially identified in the 1880s. *Staphylococcus aureus* induces several infections in both community and hospital settings and colonizes multiple regions of the body. Asymptomatic human carriers function as the principal natural reservoirs, with the predominant colonization site being in the anterior region of the nasal mucosa (Hussein *et al.*, 2025). The pathogenicity of *S. aureus* strains depends in great part on virulence genes including enzymes, toxins, adhesion proteins, cell surface proteins. One of the primary causes of hospital and community-acquired infections, which can lead to severe outcomes and potentially fatal illnesses, is *S. aureus* (Nandhini *et al.*, 2022).

Methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) are two subtypes of *S. aureus* (Guo *et al.*, 2020). Due to bacterial evolution and antibiotic misuse, *S. aureus* drug resistance has steadily increased in recent decades, and MRSA infection rates have risen globally (Abdulkareem *et al.*, 2020). The bacteria that is resistant to methicillin is called methicillin-resistant *Staphylococcus aureus* (MRSA), and it becomes a superbug because of its ability to tolerate the antibiotics and drugs that are most frequently used to treat severe and minor infections (Nandhini *et al.*, 2022, Abduljabar & Naqid, 2023). According to a prior study conducted from August 2021 to March 2022 at Zakho General Hospital in Zakho City, Kurdistan region, Iraq. *S. aureus* was found and molecularly confirmed in 12.6% of the total samples. According to the mecA gene, these isolates of *S. aureus* had a significant prevalence of MRSA (75.7%) (Hami & Ibrahim, 2023).

Rapid site identification, culture and susceptibility testing, evidence-based therapy, and suitable preventative measures are all necessary for effective MRSA infection management (Nandhini *et al.*, 2022). 16S rRNA sequencing can accurately identify bacteria, even those that are hard to grow, using standard methods. This makes it easier to diagnose infections, which helps doctors better understand infections and choose the best treatments. Because of this, it is now an essential tool for

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managing microbiology and infectious diseases (Zhang *et al.*, 2023).

The purpose of this study is to consider the clinical importance of *S. aureus* in UTIs and the challenges posed by drug resistance, and to employ 16S rRNA gene sequencing to precisely identify *S. aureus* isolates from women with UTIs and assess their antibiotic resistance profiles. Our understanding of the epidemiology of *S. aureus* infections will be enhanced by the findings, which will also direct the creation of effective treatment strategies.

2. MATERIALS AND METHODS

Study Design:

This laboratory-based cross-sectional study was conducted from 1st September to 5th October 2024 and investigated the presence of *Staphylococcus aureus* in 167 urine samples collected from women with urinary tract infections in the Kurdistan Region, Iraq. Samples were collected from Koya, Erbil and Sulaymaniyah cities from both hospital and private laboratories. Identification involved microscopy, culture, 16S rRNA PCR/sequencing, and antibiotic susceptibility testing via Kirby-Bauer and E-test. Data were analysed using SPSS v25 and GraphPad Prism 8.

Sample Collection:

Sterile disposable containers were used to collect clean-catch midstream urine samples (4–5 ml) from 167 female patients exhibiting symptoms of urinary tract infections. All samples were accurately labeled and immediately transported to the microbiology laboratory for quick microscopic analysis and subsequent processing for bacterial culture and identification.

Inclusion and Exclusion Criteria:

Women aged 17 years and older presenting with symptoms suggestive of urinary tract infections such as dysuria, urgency, frequency, or lower abdominal pain was included in the study. Exclusion criteria comprised male patients, females under the age of 17, asymptomatic individuals, and those with a history of chronic urinary conditions or recent antibiotic use. Inclusion was based solely on clinical presentation, without the need for prior laboratory confirmation at the time of sample collection.

Microscopic Urine Examination:

After centrifuging each 4–5 ml urine sample for 5 minutes at 3000 RPM, the supernatant was removed, and the pellet was carefully resuspended. After being put on a glass slide and covered with a coverslip, a drop of the pellet was viewed at 40× magnification. WBC levels (pyuria) in urine were measured in accordance with clinical urinalysis guidelines and classified into four groups: normal (≤5/HPF), mild (6–10/HPF), moderate (11–50/HPF), and severe (>50/HPF) (Kuo *et al.*, 2020). This pyuria grading aids in distinguishing between infectious and non-infectious findings, particularly in samples that can contain crystals or structures resembling yeast.

Bacterial Isolation and Identification:

All samples were cultured on both blood agar and macConkey agar for 24h at 37º C. Blood agar was used for growing both gram-positive and gram-negative bacteria, but macConkey agar was used only for growing gram-negative bacteria. After growth gram stain, catalase test, and coagulase test were performed for beta-hemolytic colonies that were grown on blood agar only. Finally, beta-hemolytic colonies with catalase positive test were cultured on Mannitol salt agar, which is a selective medium for the growth of *Staphylococcal* species and differentiating *Staphylococcus aureus* from other species.

Antibiotic Susceptibility testing:

After the identification of *S. aureus* phenotypically, antibiotic susceptibility testing was performed to determine sensitivity profiling for all isolates, Kirby-Bauer disc diffusion method was performed. At first, the bacterial suspension was prepared and adjusted to the 0.5 McFarland standard, then cultured on Mueller-Hinton agar by spread method (McFarland, 1907). Nine antibiotics, each from a class of antibiotics, were tested, including Cefoxitin (30µg), Ceftriaxone (10µg), Meropenem (10µg), Erythromycin (10µg), Ciprofloxacin (10µg), Tetracycline (10µg), Trimethoprim-Sulfamethoxazole (25µg), Azithromycin (10µg) and Vancomycin (30µg).

Also the minimum inhibitory concentration (MIC) of Vancomycin was determined using the Epsilometer test (E-test), as the disc diffusion method is not standardized for Vancomycin susceptibility testing according to CLSI guidelines (CLSI, 2020). A Vancomycin solution was prepared with methanol and applied to sterile rectangular filter paper discs at concentrations ranging from 1 to 16 µg/mL. These discs were then placed in a gradient pattern onto Mueller-Hinton agar plates previously inoculated with bacterial cultures. After incubation at 35° C for 18–24 hours, the MIC was established by detecting the lowest Vancomycin concentration that inhibited visible bacterial growth, as shown by the intersection of the bacterial inhibition zone with the discs (Kowalska-Krochmal & Dudek-Wicher, 2021). The antibiotic sensitivity profile was established in accordance with the Clinical and Laboratory Standards Institute (CLSI), and the results were interpreted accordingly.

DNA Extraction:

DNA extraction from the 29 isolates was performed using the GeneSand Genomic DNA Extraction Kit (GeneSand, South Korea), according to the manufacturer's instructions. Briefly, Genomic DNA was extracted from 2 mL of an overnight bacterial culture by centrifuging at 10,000 rpm for 1 minute. The pellet was lysed using GB1 buffer, lysozyme, Proteinase K, and GB2 buffer. After incubation at 70° C for 10 minutes, 200 µL of ethanol was added, and the mixture was transferred to a spin column. Following washes with GW1 and GW2 buffers, DNA was eluted with TE buffer and quantified using NanoDrop.

DNA Integrity and PCR Application of 16S rRNA Gene:

The quality of the extracted DNA and PCR product was checked using a Nanodrop spectrophotometer to determine the concentration and purity of the extracted DNA and gel electrophoresis was performed by running 100 ng on 0.7% agarose gel for 1.5 h at 90 V using 1X TBE buffer. PCR was used to amplify 16s rRNA gene (~1513bp). The total volume of PCR was 35µl consisting of 17.5µl of 2X Add Taq PCR mix (GeneSand), 10 pmol (1µL) of each forward (16s F-AGAGTTTGATCCTGGCTCAG) and reverse (16s R-GGTTACCTTGTTACGACTT) primer (Zhou *et al.*, 2024). The extracted DNA was added as required according to the concentration of the DNA sample. The volume was completed by adding nuclease-free water to a final volume of 35µl. A BIO-RAD thermal cycler was used for PCR reactions following the manufacturer's recommendation (GeneSand master mix kit). PCR amplification began with an initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 94 °C for 25 s, annealing at 56 °C for 25 s, and extension at 72 °C for 15 s. A final extension at 72 °C for 5 min was performed, and products were held at 4 °C until analysis. The PCR amplicons were sent to South Korea (Macrogen Inc.).

Data Analysis:

The data were assessed using IBM SPSS Statistics, version 25, and GraphPad Prism 8. Chi-square was used to find any relation between categorical variables for urine examination data; also, frequency distribution was obtained for all data. A p-value of less than 0.05 was considered statistically significant. For sequences, SnapGene was used for obtaining the V3 and V4 regions of 16S rRNA, and BLAST was used for determining the bacterial identity. The 16S rRNA consists of 9 variables; V3 and

V4 showed the highest classification accuracy in bacterial diversity studies (Zhang *et al.*, 2023).

3. RESULT

Demographic Characteristics:

The age of participants indicated that, among 167 individuals, ages ranged from 17 to 56 years, with a mean age of 29.37 years (SD = 8.38). 167 samples were collected: 41 (24.6%) from age 22 or below 22 years old, 46 (27.5%) from 23-28 years old, and 80 (47.9%) samples from age 29 or above 29 years old. Most of the samples were collected from Shahid Dr. Khalid Hospital (44.9%), followed by Rizgary Hospital and Koya Private Labs (15.6%). We collected fewer samples from Shar Hospital (9.6%), Sulaymaniyah Private Lab (8.4%), and Erbil Private Labs (6%).

Table 1: Age frequency distribution among samples.

Age group	Frequency (%)
22 ≥ Years	41 (24.6%)
23 - 28 Years	46 (27.5%)
29 ≤ Years	80 (47.9%)
Total	167 (100%)

Table 2: Frequency of urine samples collected from different hospitals and private laboratories.

Location	Frequency (%)
Shahid Dr. Khalid Hospital	75 (44.9%)
Rizgary Hospital	26 (15.6%)
Shar Hospital	16 (9.6%)
Koya Private Labs	26 (15.6%)
Erbil Private Labs	10 (6%)
Sulimanyah Private Labs	14 (8.4%)
Total	167 (100%)

Microscopic Urine Examination:

Results indicate a strong association between raised WBC levels and an increase in bacterial severity. Most cases of normal WBCs had either normal bacteria or mild levels, whilst moderate to severe WBCs had bacteria of corresponding moderate to severe levels. It seems that there is a strong association between bacterial Load and WBC levels, p-value <0.001 (Table 3) (Figure 1). Neutrophils were increased among WBCs during bacterial UTI. The elevation of WBC level in some cases may be due to the presence of yeast infection, also in typical cases without infection symptoms, primarily due to crystal formation.

Table 3: Association between bacterial Load and white blood cell (WBC) levels.

WBC	Bacteria					
	Normal	Mild	Moderate	Severe	Total	p-value
Normal	32 (60.4%)	21 (39.6%)	0 (0%)	0 (0%)	53 (100%)	<0.001
Mild	1 (2.2%)	22 (48.9%)	22 (48.9%)	0 (0%)	45 (100 %)	<0.001
Moderate	0 (0%)	2 (3.2%)	55 (87.3%)	6 (9.5%)	63 (100%)	<0.001
Severe	0 (0%)	0 (0%)	2 (33.3%)	4 (66.7%)	6 (100%)	<0.001
Total	33 (19.8%)	45 (26.9%)	79 (47.3%)	10 (6.0%)	167 (100%)	
p-value	<0.001	<0.001	<0.001	<0.001		

The p-value was determined using the Chi-square test
A p-value of less than 0.05 was considered statistically significant

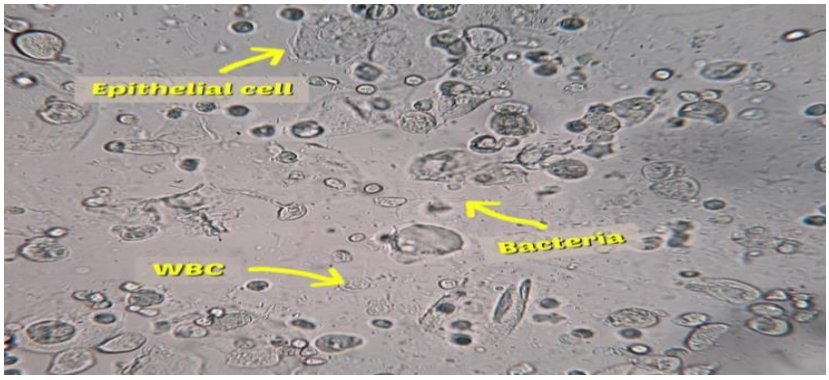

Figure 1: Microscopic image of a urine sample observed under 40× magnification, showing white blood cells and bacterial presence.

Bacterial Identification:

Identification of *Staphylococcus aureus* phenotypically includes 29 isolates because of β-hemolytic activity on blood agar (Figure 2.A), gram-positive cluster shaped cocci, positive catalase test, positive coagulase test for 24 isolates and negative for 5 isolates and agar turning yellow of mannitol salt agar (MSA) with yellow colony formation for all isolates (Figure 2.B). By these standard tests, *S. aureus* differentiates from other gram-positive bacteria with β-hemolytic activity like *Streptococcus agalactaie* and *Streptococcus pyogenes* (Table 4).

Table 4: Standard biochemical tests for identification of *Staphylococcus aureus*

Biochemicals	Features
Gram Stain	Positive
Morphology	Cluster-shaped cocci
Hemolytic activity	Beta Hemolytic
Catalase	Positive
Coagulase test	Positive for 24 isolates and negative for 5 isolates
Activity on Mannitol Salt Agar	Yellow colonies with yellow background for all 29 isolates

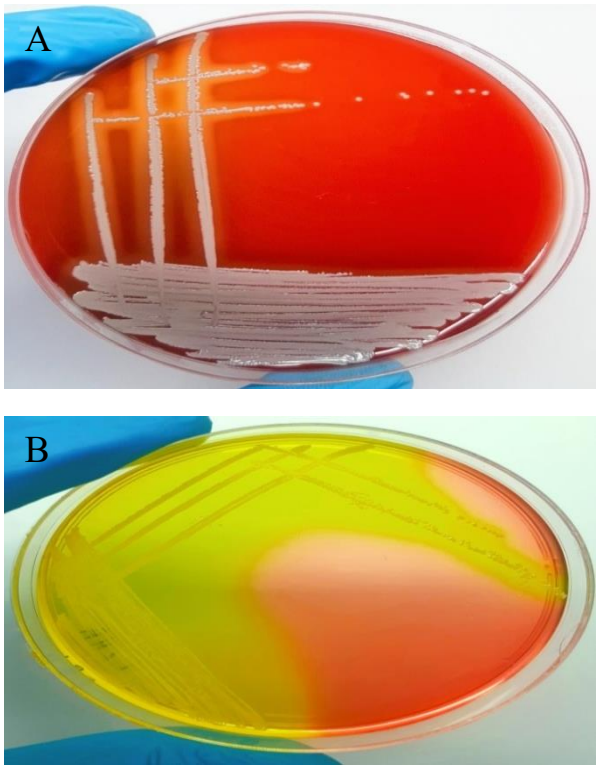

Figure 2: Bacterial Growth on (A) Blood agar that are white colonies with completely hemolysis zone (beta hemolysis), (B) Mannitol salt agar that are yellow colonies with yellow background.

Antibiotic Susceptibility Test:

The antibiotic susceptibility results for *Staphylococcus aureus* isolates demonstrated that 100% of the isolates were susceptible to vancomycin. Meropenem exhibited a high sensitivity of 75%. The antibiotics trimethoprim-sulfamethoxazole and ceftriaxone displayed moderate sensitivity, with rates of 54.2% and 50%, respectively. Conversely, significant antibiotic resistance was identified against ciprofloxacin (87.5%), erythromycin (83.3%), and tetracycline (66.7%), which represented the highest levels of resistance observed. Furthermore, cefoxitin resistance was detected in 54.2% of the isolates, confirming the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Gentamicin demonstrated moderate sensitivity, with a rate of 37.5% (Table 5). Among 24 *Staphylococcus aureus* isolates, the E-test showed all were sensitive to vancomycin, while disc diffusion results showed most were intermediate (87.5%), with few sensitive (8.3%) or resistant (4.2%) (Table 6).

Table 5: Antibiotic susceptibility testing of *Staphylococcus aureus* isolates was performed using the disc diffusion method 8 of the antibiotics and E-test for vancomycin.

Antibiotic disc	Sensitive	Intermediate	Resistance
Cefoxitin 30µg	6 (25%)	5 (20.8%)	13 (54.2%)
Ceftriaxone 10µg	12 (50%)	5 (20.8%)	7 (29.2%)
Meropenem 10µg	18 (75%)	4 (16.7%)	2 (8.3%)
Ciprofloxacin 10µg	2 (8.3%)	1 (4.2%)	21 (87.5%)
Erythromycin 10µg	3 (12.5%)	1 (4.2%)	20 (83.3%)
Vancomycin 30µg	24 (100%)	0 (0%)	0 (0%)
Gentamycin 10µg	9 (37.5%)	5 (20.8%)	10 (41.7%)
Trimethoprim Sulfamethoxazole 25µg	13 (54.2%)	2 (8.3%)	9 (37.5%)
Tetracycline 10µg	3 (12.5%)	5 (20.8%)	16 (66.7%)

Table 6: Vancomycin sensitivity by using two methods; (1) disc diffusion and (2) Epsilometer test.

Vancomycin Sensitivity	Sensitive	Intermediate	Resistance
Disc Diffusion (Inhibition zone)	2 (8.3%)	21 (87.5%)	1 (4.2%)
E-test (MIC)	24 (100%)	0 (0%)	0 (0%)

DNA Integrity and PCR Application of 16S rRNA gene:

Genomic DNA extraction was successfully performed with high quality, and the concentrations of extracted DNA ranged from 98.5 ng/µl to 374 ng/µl, and the purity was 1.40 in NanoDrop results. The successful result of the 16S rRNA following gel electrophoresis confirms that the extracted DNA was of good quality and free from issues (Figure 3). All isolates displayed bands at approximately ~1513 bp, which corresponds to the full length of the 16s rRNA gene in *S. aureus*. This matches the expected product size based on the primers used, which are exactly 1513 bp.

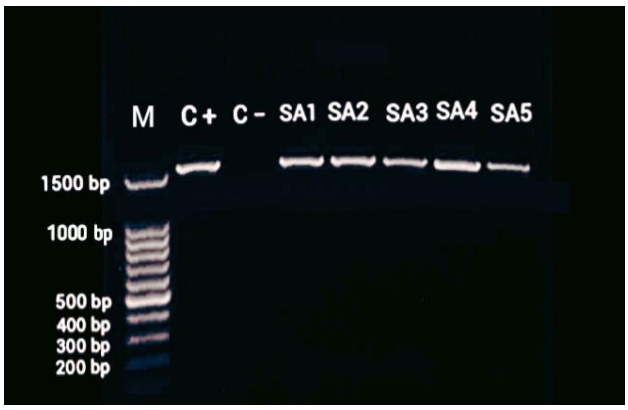

Figure 3: Agarose gel electrophoresis of representative PCR products of (16s rRNA gene): panels 1& 2 represented control positive & negative, respectively. The first PCR product was sequenced and confirmed, then used as a positive control. A negative control (without DNA) was included to detect contamination; any band appearance indicated contamination in one or more components. While panels (3-7) represented 16S rRNA gene that gave (~1500 bp), panel M contained 100 bp DNA marker by using 1X TBE/Agarose gel 0.7%.

16S rRNA Gene Sequencing:

V3–V4 regions from 29 *Staphylococcus aureus* isolates were trimmed using SnapGene and aligned against a reference sequence (strain S33 R). This targeted approach ensured consistency for comparative analysis, and the trimmed regions were subjected to BLAST analysis against the NCBI database to identify closely matching strains and assess sequence identity.
We compared 29 *Staphylococcus aureus* isolate 16S rRNA gene sequences to the NCBI BLAST database. Of these, 24 isolates (82.76%) were identified as *Staphylococcus aureus* with similarities ranging from 89.02% to 100%, while 5 of them were *Staphylococcus haemolyticus* with similarities of 97.21% and above. Six isolates (SA3, SA8, SA25, SA29, SA1, SA22) showed 100% identity with *Staphylococcus aureus* strain S33 R, confirming complete sequence homology. Four isolates (SA2, SA6, SA10, and SA16) showed lower identity values between 89.02% and 93.81%, indicating sequence divergence. Isolate SA25 matched *S. aureus* strain NBRC 100910 with 100% identity, SA15 matched strain ATCC 12600 at 95.56%, and SA11 matched strain GHA12 at 96.42%. Query coverage was 100% for most hits, except for SA10 (88%) matched with strain MVF-7 and SA7 (99%), indicating generally high-quality alignments (Table 7).

Table 7: V3 and V4 sequence of isolates with top hit match identity and query value from BLAST in NCBI.

V3&V4 Sequence	Top Hit Match in BLAST	Identity	Query value
SA1	*Staphylococcus aureus strain S33 R*	99.66%	100%
SA2	*Staphylococcus aureus strain S33 R*	92.78%	100%
SA3	*Staphylococcus aureus strain S33 R*	100%	100%
SA4	*Staphylococcus aureus strain S33 R*	98.63%	100%
SA5	*Staphylococcus haemolyticus strain SM 131*	100%	100%
SA6	*Staphylococcus aureus strain S33 R*	93.81%	100%
SA7	*Staphylococcus aureus strain S33 R*	93.43%	99%
SA8	*Staphylococcus aureus strain S33 R*	100%	100%
SA9	*Staphylococcus haemolyticus strain SM 131*	100%	100%
SA10	*Staphylococcus aureus strain MVF-7*	89.02%	88%
SA11	*Staphylococcus aureus strain GHA12*	96.42%	100%
SA12	*Staphylococcus aureus strain S33 R*	97.95%	100%
SA13	*Staphylococcus aureus strain S33 R*	99.01%	100%
SA14	*Staphylococcus aureus strain S33 R*	95.27%	100%
SA15	*Staphylococcus aureus strain ATCC 12600*	95.56%	100%
SA16	*Staphylococcus aureus strain S33 R*	90.34%	100%
SA17	*Staphylococcus haemolyticus strain SM 131*	99.66%	100%
SA18	*Staphylococcus aureus strain S33 R*	98.63%	100%
SA19	*Staphylococcus haemolyticus strain SM 131*	97.21%	100%
SA20	*Staphylococcus aureus strain S33 R*	98.63%	100%
SA21	*Staphylococcus aureus strain S33 R*	98.97%	100%
SA22	*Staphylococcus aureus strain S33 R*	99.66%	100%
SA23	*Staphylococcus haemolyticus strain SM 131*	100%	100%
SA24	*Staphylococcus aureus strain S33 R*	95.89%	100%
SA25	*Staphylococcus aureus strain NBRC 100910*	100%	100%
SA26	*Staphylococcus aureus strain S33 R*	95.20%	100%
SA27	*Staphylococcus aureus strain S33 R*	98.06%	100%
SA28	*Staphylococcus aureus strain S33 R*	99.66%	100%
SA29	*Staphylococcus aureus strain S33 R*	100%	100%

Phylogenetic Tree:

A Neighbor-Joining phylogenetic tree was constructed using 16S rRNA gene sequences from clinical *Staphylococcus aureus* isolates (e.g., SA1–SA29) alongside reference strains (e.g., ATCC 12600, NBRC 100910) and an outgroup (*Klebsiella pneumoniae* DSM 30104). The tree reveals strong evolutionary clustering among most isolates, with bootstrap values ≥93% for major nodes (Figure 4). High genetic similarity suggests close evolutionary relationships, supporting accurate identification and classification of the clinical isolates.

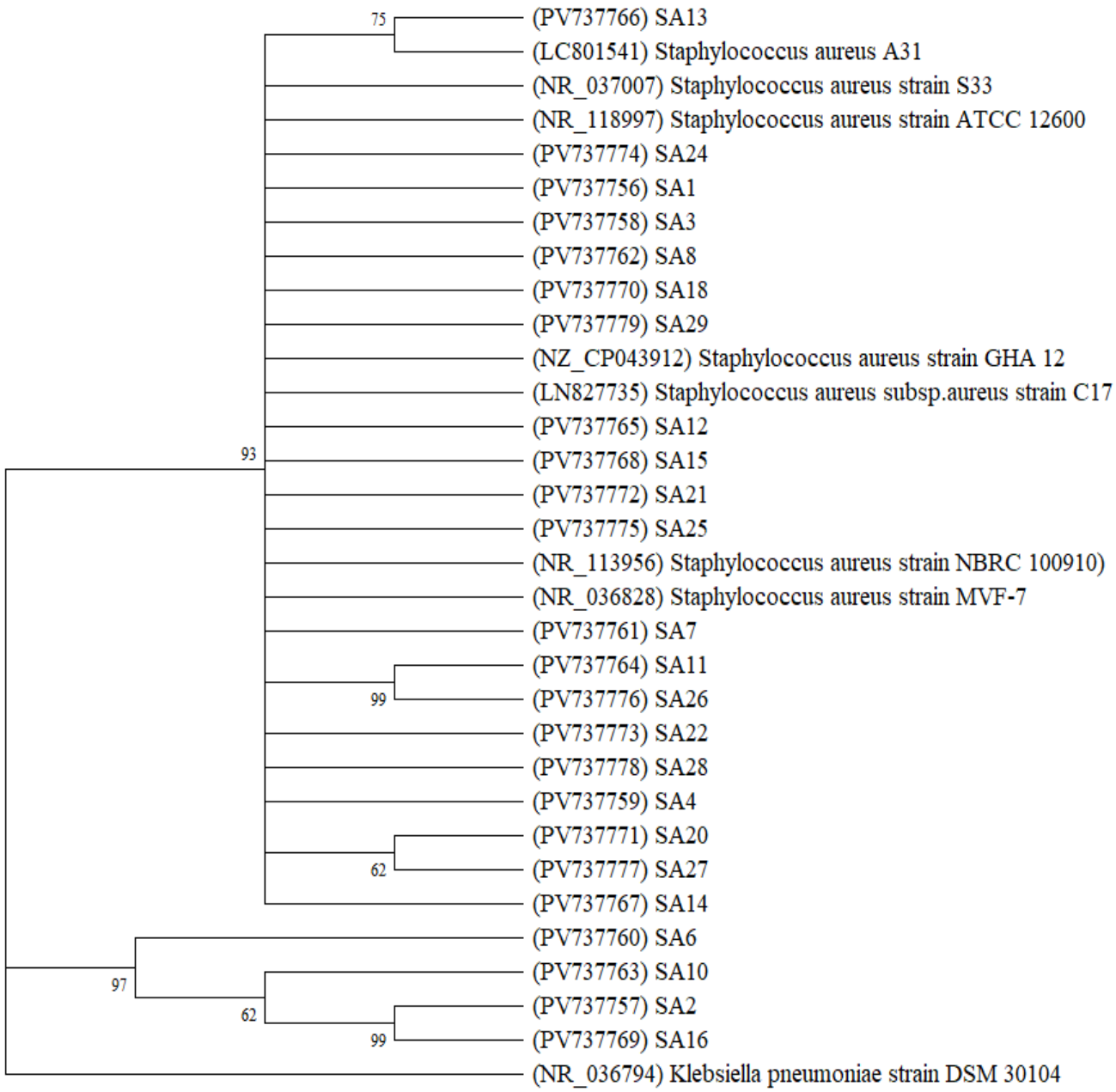

Figure 4: Neighbor Joining phylogenetic tree based on 16S rRNA sequences of *Staphylococcus aureus* isolates and reference strains. Bootstrap values ≥60% are shown at branch points. *Klebsiella pneumoniae* was used as an outgroup.

4. DISCUSSION

The present study highlights the clinical relevance and diagnostic value of accurately identifying *Staphylococcus aureus* in urinary tract infections (UTIs) among women, using both phenotypic methods and 16S rRNA gene sequencing, which may improve early detection and guide appropriate antimicrobial therapy. Phenotypic methods identified 29 *S. aureus* isolates, while 16S rRNA sequencing confirmed only 24, indicating that molecular methods offer greater specificity and reliability in confirming bacterial identification (Church *et al.*, 2020, Farhood & Hamim, 2024). The presence of 100% identity in several isolates confirms the method's accuracy, while the identification of divergent sequences suggests the emergence of novel or variant strains, warranting deeper genomic analysis (Ghazi *et al.*, 2022; Vashisht *et al.*, 2023).

Using phenotypic methods, it was found that 54.2% of the *Staphylococcus aureus* isolates were cefoxitin-resistant, indicating a high prevalence of MRSA (Abdulkareem *et al.*, 2020; Abduljabar & Naqid, 2022, 2023). Related regional information from Kurdistan-Iraq highlights the extent of resistant *S. aureus* in UTI cases (Hami & Ibrahim, 2023). This increasing resistance is due to excessive use of antibiotics and insufficient monitoring and control of infections (Tacconelli *et al.*, 2018; Chokshi *et al.*, 2019).

The organism was susceptible to vancomycin, which suggests it is the drug of choice, especially for MRSA infections (Yu *et al.*, 2025). Meropenem also showed potential efficacy, which fits with its broad-spectrum activity (Raza *et al.*, 2021). The high level of resistance documented to ciprofloxacin, erythromycin, and tetracycline may render these agents unreliable for use as empiric therapy (Nandhini *et al.*, 2022; Tuon *et al.*, 2023). It is alarming to observe resistance to ciprofloxacin,

a commonly prescribed antibiotic in outpatient settings (Mintz *et al.*, 2023).

Importantly, this study revealed inconsistencies in vancomycin sensitivity results between disc diffusion and E-test methods. While the disc diffusion showed unreliable intermediate results, the E-test confirmed complete sensitivity. This supports prior literature indicating that broth microdilution or E-test should be preferred over disc methods for glycopeptides like vancomycin due to their poor diffusion properties (Hossain, 2024, CLSI, 2020).

Microscopic urine examination showed a strong association between elevated WBC counts and bacterial counts, reinforcing its utility as a quick diagnostic indicator (Gbinigie *et al.*, 2019). The most common WBCs in urine during UTIs are neutrophils, which move quickly to infection sites, release antimicrobial enzymes, and result in pyuria (Krivošíková *et al.*, 2023). Furthermore, this reinforces the importance of accurately distinguishing non-bacterial causes, such as crystals or fungal elements, to avoid misdiagnosis of UTI symptoms (Liu *et al.*, 2021).

Strengths of the current study include its clinical relevance in investigating the role of *Staphylococcus aureus* in UTIs within a specific regional context, using both phenotypic and molecular methods, and the methodological value of comparing disc diffusion with MIC-based E-test for vancomycin susceptibility. Additionally, the documentation of high MRSA prevalence and its resistance patterns offers valuable insights for guiding empirical therapy decisions and regional antibiotic stewardship efforts. The study is limited to *S. aureus* isolates and includes only women patients.

CONCLUSION

This study identified *Staphylococcus aureus* in urinary tract infections among women using 16S rRNA gene sequencing, confirming its diagnostic accuracy. More than 50% of the isolates were methicillin-resistant (MRSA) and highly resistant to other antibiotics like ciprofloxacin and erythromycin. Vancomycin showed complete effectiveness. The findings show that routine molecular diagnostics, targeted antibiotic treatment, and constant monitoring are necessary for the management of urine infections due to *S. aureus* and address the worsening problem of antimicrobial resistance in the clinical setting.

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Ethical Statement

The study was approved by the Ethical Committee of the Faculty of Science and Health at Koya University (Erbil, Kurdistan Region, Iraq) under reference number 017, issued on 17 February 2025.

Author Contributions:

A.A.A. and A.I.G. contributed equally to the conceptualization and design of the study and the collection of the sample. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest:

The authors declare no conflict of interest.

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