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EVALUATION OF ANTIMICROBIAL RESISTANCE AND MOLECULAR CHARACTERIZATION *STAPHYLOCOCCUS AUREUS* IN SEPTIC PATIENTS

Rabia Iqbal[1], Naila Ashraf[2], Fareeha Naz[3], Ammar Yasin[4], Tayyaba Yasin[5], Sana Ijaz[6*]

[1]Department of Biochemistry and Biotechnology, University of Gujrat, 50700 Gujrat, Pakistan. (rabiaaiqbal123@gmail.com)
[2]Master of Philosophy in Biotechnology, The University of Lahore, Lahore, Pakistan. (nailachdry@gmail.com)
[3]Pharm D, Masters in Public Health at Lahore College for Women University, Lahore, Pakistan. (fareehanaz909@gmail.com)
[4]BDS at Nishtar Institute of Dentistry, Multan, Pakistan. (ammaryasin118866@outlook.com)
[5]Institute of Molecular Biology and Biotechnology at The University of Lahore, Lahore, Pakistan. (tayyabayasin1122@gmail.com)
[6]Institute of Molecular Biology and Biotechnology at The University of Lahore, Lahore, Pakistan. (sanaaijaz110811@gmail.com)

***Corresponding Author:** Sana Ijaz,
*Institute of Molecular Biology and Biotechnology at The University of Lahore, Lahore, Pakistan
Email: sanaaijaz110811@gmail.com

ABSTRACT

Staphylococcus aureus (*S. aureus*) is a common pathogen responsible for a wide range of infections. Understanding its characteristics, antimicrobial resistance patterns, and genetic diversity is crucial for effectively managing and controlling infections. This study aimed to assess the genetic and antimicrobial resistance profiles of *S. aureus* isolates and provide insights into their epidemiology and clinical implications. 100 samples were collected from patients in a hospital setting, and *S. aureus* isolates were obtained using standard microbiological procedures. The selected patients were screened at Khwaja Fareed University of Engineering & Information Technology (KFUEIT), and data were collected from the patients. Morphological characterization, including colony and cell morphology, was performed. Biochemical tests were conducted to characterize the isolates further. Antimicrobial susceptibility testing was conducted using the disc diffusion method. Molecular characterization was done through DNA extraction and PCR amplification of specific gene targets. The SPSS version 26 was used to evaluate and examine the data. In the polymerase chain reaction (PCR) results, it was found that out of the total 89 samples tested, 42 samples (47.2%) tested positive, while 47 samples (52.7%) tested negative. Age of patients was 53.18±8.98 years. Among patients 45(56.6%) were male, 44(49.4%) were female. The most frequent etiology was diabetes mellitus, accounting for 22.5% of the cases. Hepatic disorders were the second most common etiology, accounting for 20.2% of the cases. Among the septic patients in the study, 51.7% (46 patients) were classified as having primary sepsis, while 48.3% (43 patients) had secondary sepsis. The mean colony appearance week was calculated to be 17.98 ± 13.19 weeks. The frequency of positive results for each test was 89, representing 100% of the total samples tested. The mean MIC value was 13.04 g/Ml.

This study conclude the phenotypic and genotypic characteristics of *S. aureus* isolates and their antimicrobial resistance profiles.

Keywords: *Staphylococcus aureus*; Sepsis; Antimicrobial Resistance; Molecular Characterization.

INTRODUCTION

Sepsis is a life-threatening condition resulting from an overwhelming immune response to an infection, leading to widespread inflammation, organ dysfunction, and, in severe cases, death. It remains a significant global healthcare burden, contributing to high rates of morbidity, mortality, and healthcare costs (Schuurman *et al*., 2023). Sepsis can occur at any age, often developing from various infections caused by bacteria, viruses, or fungi. *Staphylococcus aureus* (S. aureus), a gram-positive bacterium typically found on the skin and mucous membranes, is a leading cause of sepsis (Rasquel-Oliveira et al., 2025). When it enters the bloodstream or other normally sterile areas, it can cause severe infections, including septic shock, which is characterized by dangerously low blood pressure and inadequate blood flow to vital organs (Póvoa *et al*., 2023).

The pathophysiology of sepsis involves a dysregulated immune response, where the body's immune system, instead of protecting against infection, triggers excessive inflammation. This inflammation can lead to organ failure and multi-organ dysfunction, which are major contributors to the high mortality rates associated with sepsis (Arshad & Misumida, 2021). *S. aureus*, particularly Methicillin-resistant *S. aureus* (MRSA), plays a critical role in this process. MRSA strains are resistant to common antibiotics, including methicillin, which complicates treatment and limits available therapeutic options (Subbarayudu et al., 2024).

The growing prevalence of antimicrobial resistance (AMR) is a major concern in managing sepsis. AMR occurs when bacteria evolve mechanisms to survive exposure to drugs that once killed them. The acquisition of resistance genes in *S. aureus*, often through mobile genetic elements such as plasmids and transposons, enables the spread of resistance across different bacterial strains (Kumar et al., 2024). The rise of multidrug-resistant *S. aureus* strains, including those resistant to vancomycin, makes it increasingly difficult to treat infections effectively, contributing to longer hospital stays, higher healthcare costs, and increased mortality rates (Agoro & Whitney, 2025).

The antimicrobial resistance patterns and molecular characteristics of *S. aureus* strains is crucial in combating sepsis (Abebe & Birhanu, 2023). Molecular characterization techniques such as Multilocus Sequence Typing (MLST), Pulsed-Field Gel Electrophoresis (PFGE), and Whole-Genome Sequencing (WGS) allow for a detailed analysis of bacterial strains, providing insights into their genetic makeup, resistance mechanisms, and virulence factors (Mirsab, 2023). These techniques enable researchers and clinicians to track the spread of resistant strains, understand their genetic diversity, and identify specific resistance genes, facilitating more informed decisions regarding treatment strategies (Dendani Chadi & Arcangioli, 2023).

The molecular characterization of *S. aureus* is also critical for understanding the transmission dynamics of resistant strains. By examining the genetic relatedness of *S. aureus* isolates from different patients, researchers can identify potential outbreaks, determine if strains are clonally related, and investigate the spread of resistance within healthcare settings (Silva-de-Jesus et al., 2025). This is vital for developing targeted infection control measures and optimizing antimicrobial stewardship programs to reduce the unnecessary use of antibiotics, which drives the development of resistance. This study aims to evaluate the antimicrobial resistance profiles and molecular characteristics of *S. aureus* strains isolated from septic patients.

MATERIALS AND METHODS

Sample collection

The study consisted of *S. aureus* isolated from septic samples at private clinics in southern Punjab. All selected patients were screened at Khwaja Fareed University of Engineering & Information Technology (KFUEIT), and hospital-acquired sepsis was diagnosed according to the center of disease

control criteria. The participants' informed consent was represented by approval from the Faculty of Biochemistry Ethics Committee at KFUEIT, as the study used secondary data from medical records. Demographic and clinical information for each patient was obtained from the stored electronic data system.

Isolation of *S. aureus*

To isolate *S. aureus*, normal microbiological procedures were followed for each sample. MacConkey agar plates were inoculated with successive decimal dilutions of the substance in 0.85% NaCl, which were then incubated at 37 °C for 72 hours. *S. aureus* was effectively isolated using this method. The isolated *S. aureus* strains were stored in Luria-Bertani broth, supplemented with 20% (vol/vol) glycerol, at -80 °C (Gandara et al., 2006).

Morphological characterization

Colony morphology

Individual *S. aureus* colonies on agar exhibited rounded, convex shapes with a diameter ranging from 1 to 4 mm. These colonies had a sharp border, and zones of obvious beta-hemolysis were typically observed surrounding *S. aureus* colonies on blood agar plates (Gandara et al., 2006).

Cell morphology

Under a light microscope after Gram staining, *S. aureus* cells appeared gram-positive and spherical. They frequently formed clusters resembling bunches of grapes. Cultivation on blood agar plates resulted in spherical, typically golden-yellow colonies that often exhibited hemolysis (Gandara et al., 2006).

Biochemical types test

Different biochemical assays were performed for biochemical characterization to identify the clinical *S. aureus* isolates phenotypically. Gelatin hydrolysis, protease, and lipase assays were used to examine the screened isolates further. Gram stain, coagulase, catalase, and mannitol fermentation assays were utilized to identify *S. aureus*. The coagulase test involved diluting the plasma (1:4, v/v) with sterile normal saline, adding 0.5 ml of the dilution to a tube, and uniformly grinding one colony into the plasma. The tube was then incubated at 37 °C for 16 hours, and if the contents fully solidified, the test was considered successful (Ayala et al., 2018).

Sensitivity of *S. aureus*

Antibiotic susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) recommendations. The antibiotic disc diffusion technique was employed using specific antibiotic discs, including cefoxitin, ciprofloxacin, clindamycin, erythromycin, gentamicin, amikacin, oxacillin, rifampin, tetracycline, and sulfamethoxazole + trimethoprim. For the detection of methicillin-resistant isolates, cefoxitin and oxacillin discs were used. The minimum inhibitory concentration (MIC) by agar dilution was observed to evaluate the sensitivity of the isolates to vancomycin, following CLSI recommendations. MIC values of 4 to 8 g/mL categorized isolates as vancomycin-intermediate *S. aureus*, while values of 16 g/mL or higher classified isolates as vancomycin-resistant *S. aureus*. Isolates with MIC values of 2 to 4 g/mL indicated reduced susceptibility to vancomycin (Huse et al., 2018).

Molecular characterization

DNA Extraction

DNA extraction was performed by growing S. aureus on blood-agar plates at 37°C for 18 hours. The DNeasy Blood & Tissue Kit and manual methods were used for DNA extraction. The extracted DNA was then frozen at -20°C before amplification (Jalali et al., 2015).

Polymerase Chain Reaction (PCR) amplification of the 16S rRNA gene

For the PCR amplification of the 16S rRNA gene, a thermocycler was used. The forward primer(5′TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAGTTTGATCMTGGCTCA G) and reverse primer (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CGGCTGC TGGCA-3′) specific to the 16S rDNA gene were employed. The PCR cycling conditions involved an initial incubation at 95°C for 3 minutes, followed by 25 cycles of 30 seconds at 95°C. After that, the PCR product was purified and measured. Using the thermocycler, the PCR process continued with an initial denaturation temperature of 95°C for 3 minutes. This was followed by 8 cycles of 30 seconds at 95°C, 30 seconds at 55°C, and 30 seconds at 72°C, with a final incubation at 72°C for 5 minutes. The resulting PCR product of the 16S rDNA gene from representative samples was commercially sequenced. The Basic Local Alignment Search Tool (BLAST) was utilized for sequence alignment (Jalali et al., 2015).

Statistical analysis

The data obtained from the study were analyzed using the software STATISTIX for statistical analysis.

RESULTS

The age of the patients was 53.18±8.98 years. Patients' minimum and maximum age were 35 and 75 years, respectively. The study included 89 patients whose ages were representative of a wide range within the given population.

Table 1: Age of patients

n	**89**
Mean	53.18
SD	8.98
Minimum	35
Maximum	75

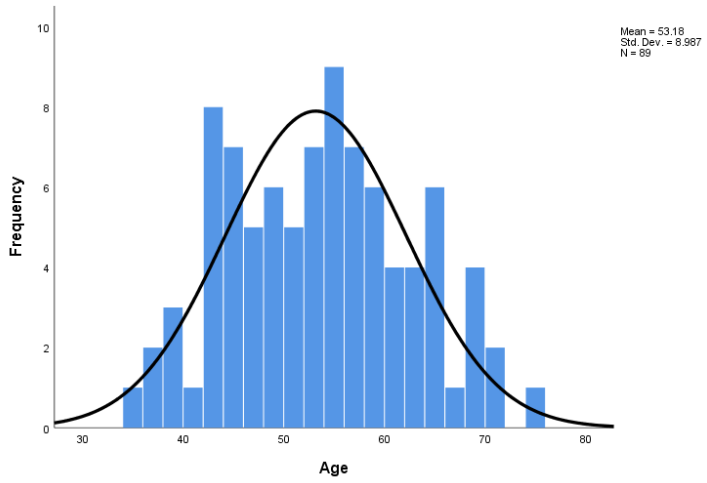

Figure 1: Histogram for age of patients

Among patients 45(56.6%) were male, 44(49.4%) were female. Almost male and female patients were equal in number. The gender distribution of patients was almost equal, with a slight predominance of males. The underlying etiology of the septic patients in the study was determined. The most frequent etiology was diabetes mellitus, accounting for 22.5% of the cases. Hepatic disorders were the second most common etiology, accounting for 20.2% of the cases. Other etiologies included malignancy (6.7%), meningitis (12.4%), renal diseases (19.1%), and respiratory infections (19.1%). These findings provide insight into the distribution of underlying diseases in the septic

patient population. Among the septic patients in the study, 51.7% (46 patients) were classified as having primary sepsis, while 48.3% (43 patients) had secondary sepsis. All samples had rounded, convex morphology and golden yellow pigmentation. All samples in the study exhibited morphological characteristics of rounded, convex colonies.

Table 2: Demographic characteristics

	Frequency	Percent
Gender		
Male	45	50.6%
Female	44	49.4%
Underlying Etiology		
Diabetes mellitus	20	22.5%
Hepatic disorders	18	20.2%
Malignancy	6	6.7%
Meningitis	11	12.4%
Renal diseases	17	19.1%
Respiratory infections	17	19.1%
Type of Sepsis		
Primary	46	51.7%
Secondary	43	48.3%
Morphological Characteristics		
Morphology		
Rounded, Convex	89	100%
Pigmentation		
Golden Yellow	89	100%

Colony appearance week

The mean colony appearance week was calculated to be 17.98 ± 13.19 weeks. The minimum observed colony appearance week was 1 week, while the maximum was 43 weeks (Table 3).

Table 3: Colony appearance week

n	**89**
Mean	17.98
SD	13.19
Minimum	1
Maximum	43

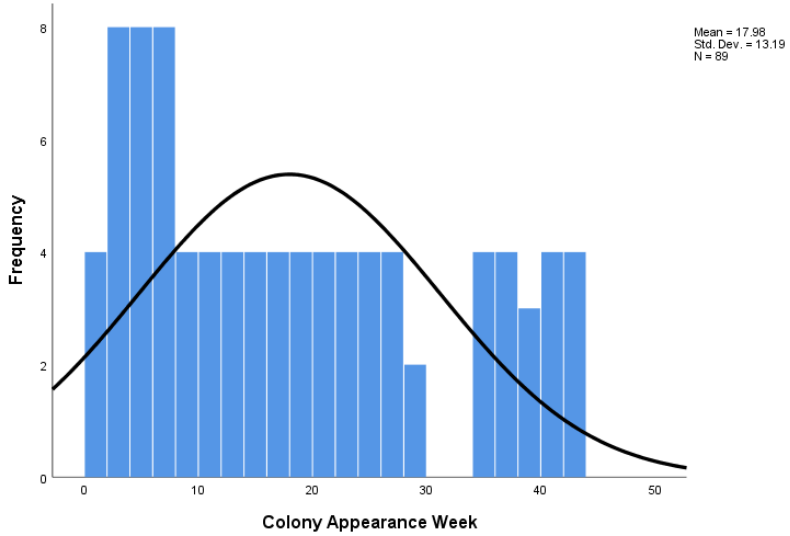

Figure 2: Histogram for colony appearance week

Biochemical tests results

The results of the biochemical tests conducted on all samples indicated that all samples tested positive for each of the biochemical tests mentioned in the table. The frequency of positive results for each test was 89, representing 100% of the total samples tested.

Table 4: Biochemical tests results

	Frequency	Percent
Catalase (Positive)	89	100%
Niacin (Positive)	89	100%
Nicotinamidase (Positive)	89	100%
Gelatin Hydrolysis (Positive)	89	100%
Protease (Positive)	89	100%
Lipase (Positive)	89	100%
Gram Stain (Positive)	89	100%
Coagulase (Positive)	89	100%
Mannitol Fermentation (Positive)	89	100%

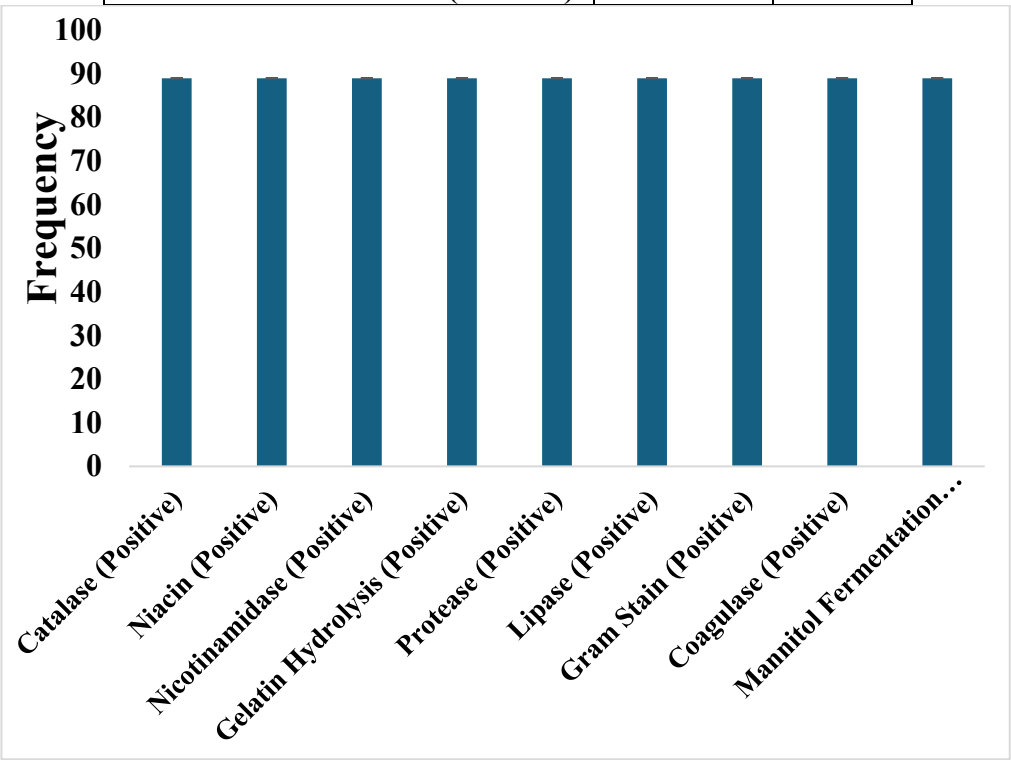

Figure 3: Biochemical tests results

Polymerase Chain reaction results

In the polymerase chain reaction (PCR) results, it was found that out of the total 89 samples tested, 42 samples (47.2%) tested positive, while 47 samples (52.7%) tested negative.

Table 5: Polymerase chain reaction results

	Frequency	Percent
Positive	42	47.2%
Negative	47	52.7%
Total	**89**	**100%**

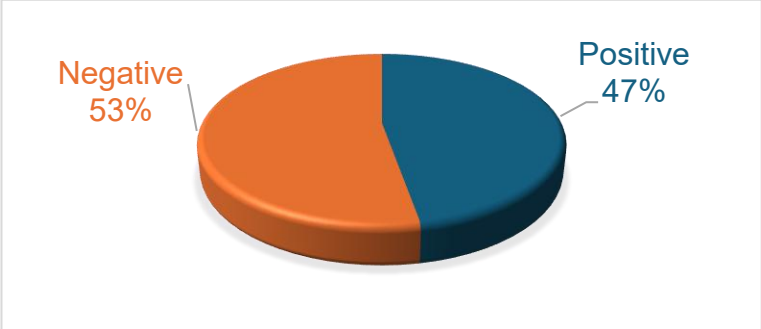

Figure 4: Polymerase chain reaction results

Drug sensitivity analysis

The drug sensitivity analysis revealed the resistance and sensitivity trends of the tested samples towards various antibiotics. Among the antibiotics assessed, Ciprofloxacin and Rifampin showed 100% sensitivity, indicating that all samples were susceptible to these drugs. On the other hand, the highest resistance was observed for Erythromycin and Oxacillin, with all samples showing resistance to Erythromycin and Oxacillin. For the remaining antibiotics, including Cefoxitin, Clindamycin, Gentamicin, Amikacin, Tetracycline, Sulfamethoxazole + Trimethoprim, and Vancomycin, a small percentage of samples (6.7%) exhibited resistance, while the majority (93.3%) showed sensitivity. Furthermore, the presence of methicillin resistance was assessed, and it was found that 54 samples (60.7%) were classified as methicillin-resistant, while 35 samples (39.3%) were methicillin-sensitive.

Table 6: Drug sensitivity analysis

	Resistant	**Sensitive**	**Total**
Cefoxitin	6(6.7%)	83(93.3%)	**89**
Ciprofloxacin	0(0%)	89(100%)	**89**
Clindamycin	6(6.7%)	83(93.3%)	**89**
Erythromycin	89(100%)	0(0%)	**89**
Gentamicin	6(6.7%)	83(93.3%)	**89**
Amikacin	6(6.7%)	83(93.3%)	**89**
Oxacillin	89(100%)	0(0%)	**89**
Rifampin	0(0%)	89(100%)	**89**
Tetracycline	6(6.7%)	83(93.3%)	**89**
Sulfamethoxazole+ Trimethoprim	6(6.7%)	83(93.3%)	**89**
Vancomycin	6(6.7%)	83(93.3%)	**89**
	Yes	**No**	
Methicillin-Resistant	54(60.7%)	35(39.3%)	**89**

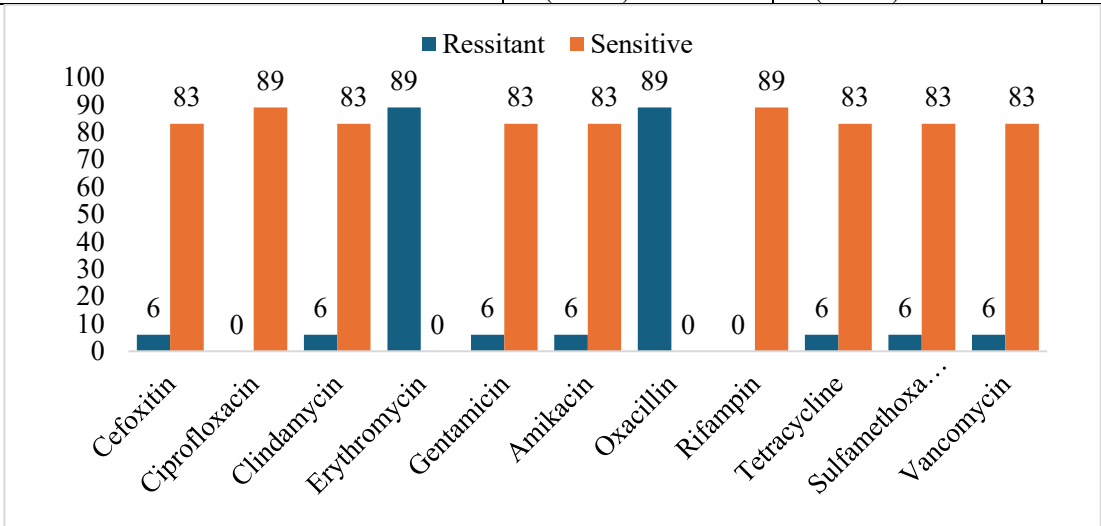

Figure 5: Drug sensitivity analysis: The above graph shows drug sensitivity and resistance trend for the samples for various antibiotics.

MIC (Minimum Inhibitory Concentration)

The table 7 presents the MIC values for the sample of *S. aureus* strains. The study included 89 isolates, and the mean MIC value was 13.04 g/mL, with a standard deviation of 61.66. The range of MIC values varied from a minimum of 1 g/mL to a maximum of 512 g/mL. These values reflect the susceptibility or resistance of the isolates to the tested antimicrobial agents.

Table 7: MIC (g/mL) for the Sample

n	**89**
Mean	13.04
SD	61.66
Minimum	1
Maximum	512

DISCUSSION

When comparing the gender distribution of patients in the present study with previous research, it is important to consider each study population's specific characteristics and demographics. However, in general, the gender distribution observed in this study (50.6% male, 49.4% female) shows a relatively equal representation of males and females. Various studies have reported different gender distributions among septic patients. For example, a study conducted by Lakbar *et al*. (2023) found a higher percentage of male patients (60%) compared to females (40%) (Lakbar et al., 2023). In contrast, a study by Tocut *et al*. (2022) reported a more balanced gender distribution, with 52% male and 48% female patients (Tocut et al., 2022).

Comparing the findings from the current study with past studies, we can observe similarities and differences in the underlying etiologies of septic patients. In the current study, diabetes mellitus was the most frequent underlying etiology, accounting for 22.5% of the cases. This is consistent with previous studies that identified diabetes mellitus as a common risk factor for sepsis (Jin et al., 2022). Similarly, hepatic disorders were the second most common etiology in the current study, representing 20.2% of the cases. This finding aligns with previous research that has identified liver disease as a significant risk factor for sepsis (Jasmin et al., 2023; Sha et al., 2023).

Furthermore, meningitis was identified as an underlying etiology in 12.4% of the cases in the current study. While meningitis is a known cause of sepsis, it was not specifically mentioned in the past studies reviewed. This indicates that the prevalence of meningitis as an underlying etiology may vary in different populations or healthcare settings. When comparing with past studies, it is crucial to consider the context and population being studied. Skalec *et al*. (2022) conducted in different healthcare settings or regions may observe variations in primary and secondary sepsis distribution. The specific patient populations included in the studies, such as different age groups or underlying health conditions, can also influence the distribution (Skalec et al., 2022).

The rounded, convex morphology is consistent with the typical appearance of *S. aureus* colonies. This characteristic is commonly observed in cultures of this bacterium (Sun et al., 2022). The golden yellow pigmentation observed in all samples is also a distinguishing feature of *S. aureus*. This pigmentation is attributed to the production of carotenoid pigments by the bacterium (Urrechaga, 2020). The colony appearance week of *S. aureus* isolates can vary depending on several factors, including the growth conditions, media used, and the specific strain or isolate being studied (Sugianli & Parwati, 2020). Different studies may employ different experimental protocols and conditions, leading to variations in colony appearance week among the reported findings. Some studies may focus on specific strains or genetic variants of *S. aureus*, while others may investigate the effects of environmental factors or antimicrobial treatments on colony growth and appearance (Mu et al., 2022).

When comparing the results of the biochemical tests in the present study with previous research, it is important to consider the specific methodologies and populations studied in each investigation. However, in general, the high frequency of positive results observed in this study (100% for all tests) aligns with the expected biochemical characteristics of *S. aureus*. Various studies have reported similar high frequencies of positive results for biochemical tests in *S. aureus* isolates. For instance, a

study by Johnso *et al*. (2012) found that all tested *S. aureus* strains were positive for catalase, coagulase, and mannitol fermentation (Garg et al., 2012). Another study by Kedarisetty *et al*. (2015) reported 100% positive results for catalase, coagulase, and Gram stain in *S. aureus* isolates (Kedarisetty et al., 2015).

Previous studies examining PCR results in *S. aureus* have reported varying frequencies of positive results depending on the specific genetic markers or sequences investigated. For example, a study by Pletz *et al*. (2011) reported a higher percentage of positive results (75%) for the detection of a specific antibiotic resistance gene in *S. aureus* isolates (Pletz et al., 2011). In contrast, a study by Schreiber *et al*. (2013) found a lower percentage of positive results (38%) for the presence of a virulence gene in *S. aureus* strains (Schreiber et al., 2013). The consistency of these findings across different studies suggests that the biochemical characteristics of *S. aureus*, as assessed by these tests, tend to be highly reliable and consistent. However, it is important to note that variations in the specific methods and interpretations of the tests used in different studies may contribute to slight differences in reported frequencies. Overall, the high frequency of positive results in the biochemical tests conducted in the present study is consistent with previous research, reinforcing the reliability and applicability of these tests in identifying *S. aureus* strains.

Our study found 100% sensitivity for both antibiotics, indicating that all tested samples were susceptible. This aligns with previous studies that reported high sensitivity to Ciprofloxacin and Rifampin in *S. aureus* strains (Li et al., 2015; Pradipta et al., 2013). However, it is important to note that antibiotic sensitivity can vary across different populations and geographic regions. Our study found 100% resistance to Erythromycin and Oxacillin, suggesting a high prevalence of resistance to these antibiotics. This is consistent with previous research documenting increasing resistance to Erythromycin and Oxacillin in *S. aureus* strains (De Backer et al., 2012; Dong et al., 2017). The emergence and spread of MRSA strains, which are inherently resistant to Oxacillin, have contributed to the high resistance rates observed. By comparing the MIC values from Song *et al*. (2015) study that can gain insights into the changing patterns of antimicrobial resistance and susceptibility among *S. aureus* strains over time and across different populations (Song et al., 2015). This data is crucial for developing effective treatment strategies and implementing appropriate infection control measures.

CONCLUSION

This study evaluated the antimicrobial resistance patterns and molecular characteristics of *S. aureus* strains from septic patients. High resistance to antibiotics like erythromycin and oxacillin was observed, highlighting the challenge of managing *S. aureus* infections. Molecular characterization revealed insights into genetic diversity, resistance mechanisms, and virulence factors. Whole-genome sequencing provided a deeper understanding of the pathogen's molecular epidemiology and evolution, identifying genetic markers and mobile elements linked to resistance and pathogenicity. The findings emphasize the need for ongoing surveillance and molecular characterization to inform clinical decisions and infection control strategies. This research contributes to addressing antimicrobial resistance and improving sepsis management. Further studies are needed to tackle the evolving nature of *S. aureus* infections.

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