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ORIGINAL ARTICLE

Prevalence and Antimicrobial Resistance Patterns of *Staphylococcus aureus* Isolated from Clinical Samples in Raipur City

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ABSTRACT

Introduction: *Staphylococcus aureus* is a commensal organism residing on human skin and mucosal surfaces as part of the normal flora. However, it is an opportunistic pathogen capable of causing infections when enters human body. The emergence of multidrug-resistant *S. aureus,* particularly methicillin-resistant strains (MRSA), has become a significant public health challenge.

Aim: This study aimed to determine the prevalence and antimicrobial resistance patterns of *S. aureus* isolated from various body fluids.

Methods: The bacterial samples were procured from Dr. Micro Lab between July 2024 and September 2024. *S. aureus* isolates were identified based on microscopic examination and biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar.

Results: Out of over 160 bacterial isolates, 70 were identified as *S. aureus.* Among these, 29 isolates (40%) were recovered from pus and wound infections. Methicillin-resistant *S. aureus* (MRSA) accounted for 33 (49%) of the *S. aureus* isolates. Resistance patterns showed that Linezolid exhibited no resistance, while Nitrofurantoin demonstrated the highest resistance rates.

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Conclusion: The study highlights a significant prevalence of *S. aureus* infections, particularly in females aged 21–30 years, with a higher frequency of isolates obtained from urine and pus infections. The findings show the growing resistance of *S. aureus* to commonly used antibiotics such as Penicillin and Erythromycin, raising concerns about treatment efficacy. However, Linezolid's lack of resistance suggests it remains a reliable therapeutic option for managing MRSA infections.

KEYWORDS

• *S. Aureus* • MRSA • MSSA • Antibiotic Resistivity • Human Body Fluids

INTRODUCTION

Antibiotic resistance is one of the major increasing global health issues.[1] *Staphylococcus aureus* is the Gram-positive bacterium[2] which normally reside on human skin as commensalism but become pathogenic when enters inside body and cause several diseases ranging from minor skin infections to life threating diseases such as sepsis.[3] Over time, *S. aureus* has developed resistance to several antibiotics including penicillin, methicillin, and vancomycin, posing significant challenges to healthcare systems worldwide.[4]

The rise of multidrug-resistant *S. aureus* has further exacerbated the problem.[5] Notably, the emergence of methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) has led to complications in treatment, increased mortality rates, and higher healthcare costs.[6]

The rapid emergence of antibiotic resistance in clinical isolates is largely attributed to the horizontal transfer of resistance genes. Bacterial plasmids frequently harbour these genes, facilitating their transfer through conjugation, transformation, or transduction.[7]

Previous research has shown that, In India, studies have revealed a concerning increase in MRSA prevalence, with isolation rates rising from 41% in 2008–2009 to 45% in recent years. Among *S. aureus* isolates from wound infections, 78.7% were *S. aureus*, of which 26.6% were identified as MRSA, and 4% were VRSA.[8]

While numerous studies have explored the global prevalence and resistance patterns of *S. aureus*, data specific to certain regions, particularly small cities, remain scarce. This study aims to assess the prevalence and resistance patterns of *S. aureus* in hospital patients in Raipur, Chhattisgarh. By analysing antibiotic susceptibility profiles, this research seeks to inform better treatment strategies and raise awareness among the local population.

MATERIALS & METHODS

Study period

This descriptive observational study was conducted over a three-month period, from July 2024 to September 2024, at Dr. Micro Lab and Research Centre, Raipur.

Sample collection

Samples were collected from diverse hospitals and clinics across Raipur.[9] The specimens included pus, sputum, urine, blood, and other body fluids. Only patients not undergoing any medication and referred by clinicians were included in the study. All samples were collected in sterile, leak-proof containers with appropriate labelling.

Isolation of *S. aureus*

Specimens were processed within 2–3 hours of collection. Blood samples were collected in Brain Heart Infusion broth, incubated at 37°C for up to 72 hours prior to isolation. Blood, pus, urine, sputum, semen, and vaginal swab samples were directly inoculated onto Nutrient Agar Media, Blood Agar, MacConkey Agar, and Mannitol Salt Agar.[9]

Morphological characterization

Colonies formed on the culture media were picked and subjected to Gram staining. Only Gram-positive cocci were processed further for the identification of Staphylococcus aureus.[9]

Biochemical test

Biochemical tests, including the catalase test, were performed to distinguish Staphylococcus

species from *Streptococcus* species. Briefly, one loop full of bacterial colony was picked by the inoculation loop and placed in slide. Few amounts of hydrogen peroxide solution were added and observed the bubble formation.[10]

The coagulase test was employed to differentiate *Staphylococcus aureus* from coagulase-negative *Staphylococcus* species. A drop of plasma mixed with saline was added to the colony, and clot formation was observed.[11]

Antibacterial test

All *Staphylococcus aureus* isolates were subjected to Antimicrobial Susceptibility Testing (AST) using the Kirby-Bauer disk diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute.[12] The overnight culture of *S. aureus* was inoculated in nutrient broth and allowed for the 3-4 hours to growth. The growth was assessed at 620 nm to ensure the optical density between 0.08-0.13 which is equivalent to 1 x 108 CFU/ml. The cotton swab was loaded with inoculum and extra moisture of cotton swab was reduced by pressing the swab on tube wall. The inoculum was spread on sterile Mueller Hinton Agar plate. The following 10 antibiotics were transferred to plate: cefoxitin (30µg), penicillin (10µg), erythromycin (15µg), ciprofloxacin (5µg), cotrimoxazole (25µg), tetracycline (30µg), gentamicin (10µg), nitrofurantoin (300 nitrofurantoin), linezolid (30 µg), and rifampin (5 µg). The zone of inhibition around each antibiotic disk was measured to determine the susceptibility or resistance of the isolates.

RESULTS

Isolation of *Staphylococcus aureus*

A total of 200 clinical samples, including pus, sputum, urine, blood, and other body fluids, were processed during the study. Out of these, the bacterial growth was observed in 160 samples (80%), while the remaining 40 samples (20%) showed no detectable growth under the conditions employed. Among the 160 samples with bacterial growth, Staphylococcus aureus was identified in 70 samples (43.75%) as confirmed after growth in blood agar media, gram staining, catalase and coagulase test. The bacteria had haemolytic activity and it was coagulase positive (figure 1). The remaining 90 samples with bacterial growth included other organisms were not the focus of this study.

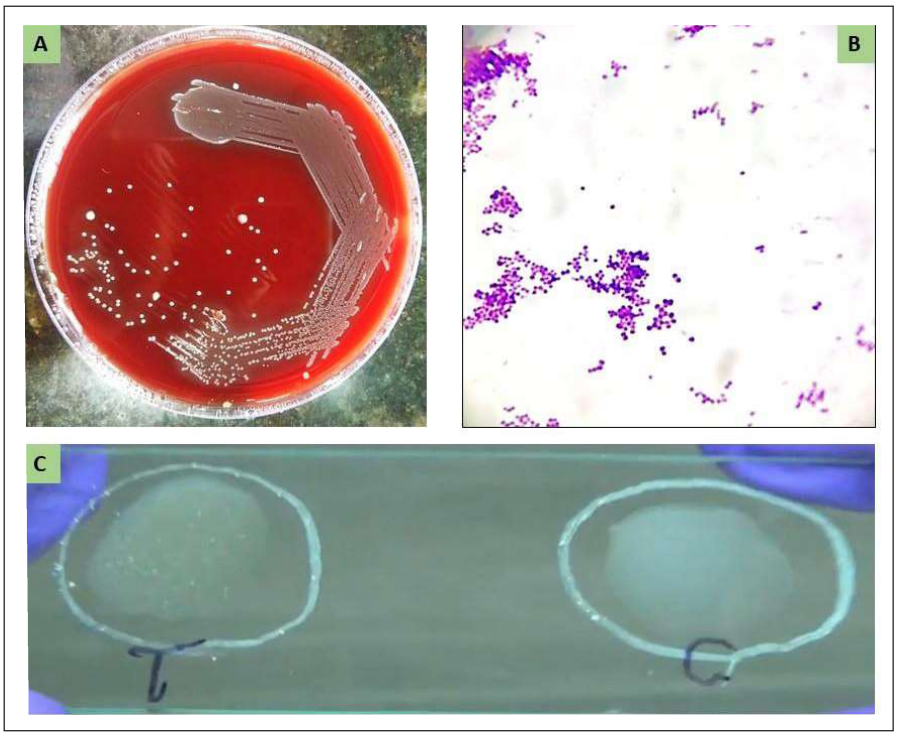

Figure 1: The images show growth characters, cell wall type and biochemical test of *S. aureus*. **(A)** is showing β-hemolysis in blood agar media; **(B)** shows Gram positive cocci seen under microscope; **(C)** indicates positive coagulase test demonstrated by clot formation (T indicate test and C is for control)

The study comprised 70 participants, in which 40 of the participants were female (57.14%), while 30 were male (42.85%) as shown in figure 2. This distribution suggests a major predominance of female participants in the sample. This may be due to the hormonal imbalance, menstruation, pregnancy, unhygienic conditions of rural females and slum area.

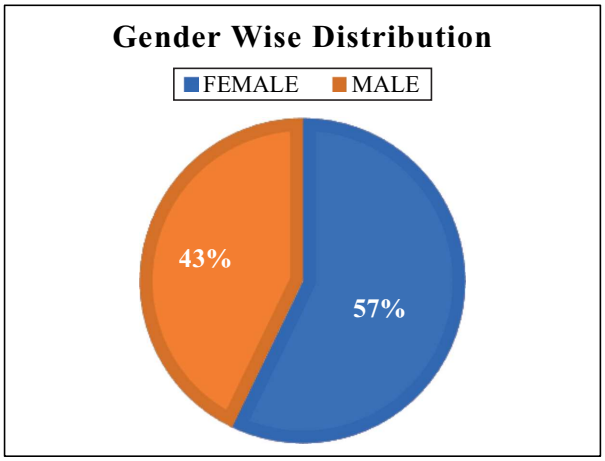

Figure 2: Gender distribution among sample population

Among the 70 *Staphylococcus* samples analysed, 33 (49%) were identified as Methicillin-Resistant *Staphylococcus aureus* (MRSA), while the remaining 37 (51%) were classified as Methicillin-Sensitive *Staphylococcus aureus* (MSSA) as shown in figure 3. In this study we found more *S. aureus* isolates in urine sample (50%), followed by pus (41%) as shown in figure 4.

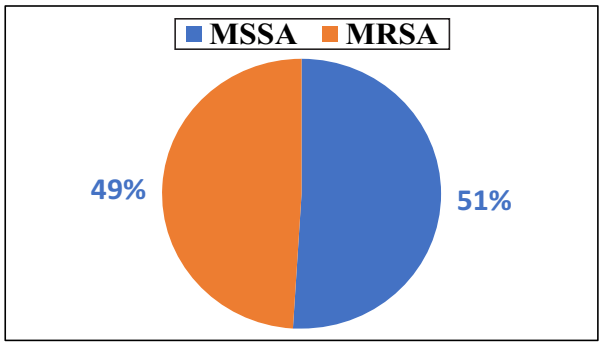

Figure 3: The distribution of methicillin sensitive Staphylococcus aureus (MSSA) and methicillin resistant Staphylococcus aureus (MRSA)

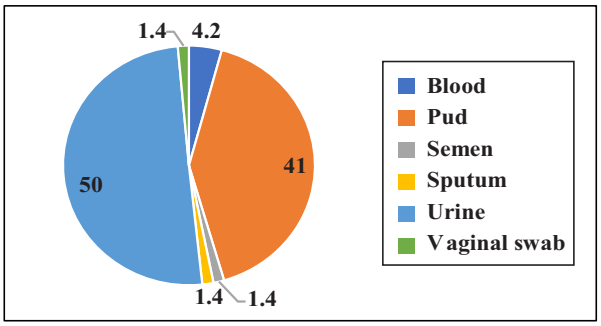

Figure 4: Specimen distribution in sample population

Females in the age group of 21–30 years exhibited the highest susceptibility to these infections compared to other age groups. In contrast, males demonstrated a peak in susceptibility within the 51–60 age group (figure 5). The 21–30 age group demonstrated the highest susceptibility to urinary tract infections (UTIs). In contrast, the 31–40 age group exhibited a higher propensity for pus infections compared to the 21–30 age group (table 1).

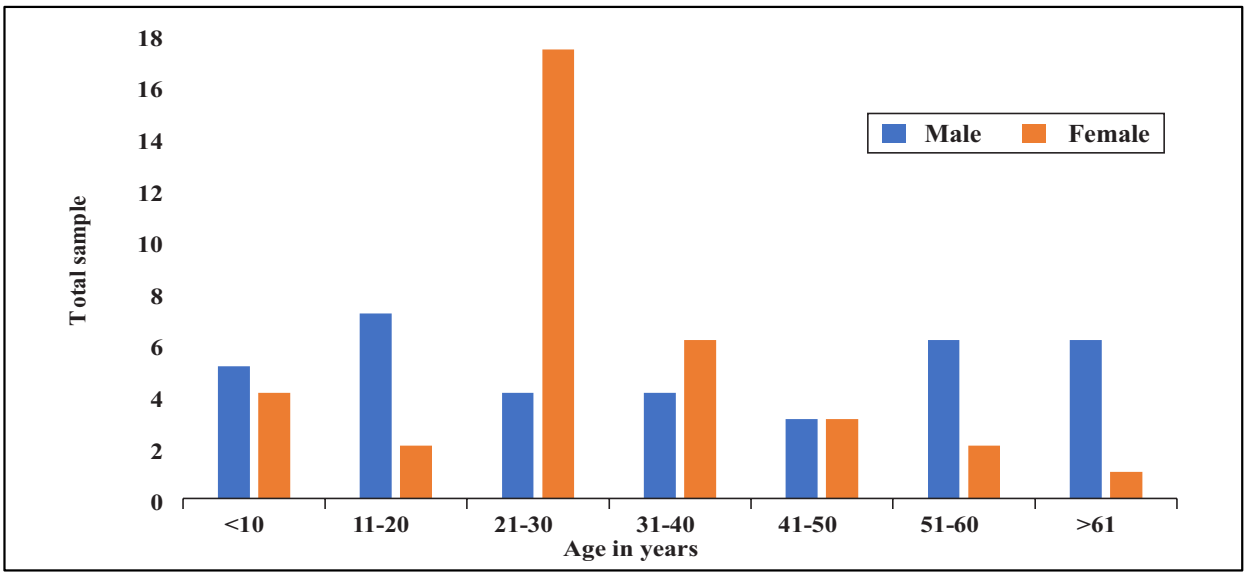

Figure 5: Age Distribution in the Sample Population infected with *S. aureus*

Table 1: Distribution of specimens from different age groups in the sample population

Age in years	Specimen						Total
	Blood	Pus	Semem	Sputum	Urine	Vaginal Swab	
<10	0	3	0	0	6	0	9
11-20	1	4	0	0	4	0	9
21-30	2	5	1	0	12	1	21
31-40	0	6	0	0	4	0	10
41-50	0	3	0	1	2	0	6
51-60	0	5	0	0	3	0	8
>61	0	3	0	0	4	0	7
Total	**3**	**29**	**1**	**1**	**35**	**1**	**70**

Linezolid is the best antibiotic as it shows no resistance towards *S. aureus* while nitrofurantoin and penicillin show the highest sensitivity. Pus, urine, and blood samples exhibited resistance to penicillin, cefoxitin, erythromycin, ciprofloxacin. (Figure 6 & table 2).

The 50% bacterium isolates were resistant against penicillin, indicating significant limitations in its effectiveness. The 38.57% isolates were resistant against Cefoxitin, reflecting the presence of methicillin-resistant strains (MRSA). 45.71% isolates were resistant against Erythromycin and Ciprofloxacin, each showing moderate resistance levels. 7.14% isolates were resistant against Gentamicin, suggesting it remains a reliable treatment option. All the bacterial isolates were sensitive for Linezolid where no resistant observed, making it a highly effective antibiotic. 12.85% isolates were resistant against Tetracycline, and 15.71% isolates were resistant against Co-trimoxazole. Both showed relatively low resistance. 51.42% isolates were resistant against Nitrofurantoin, with the highest resistance rate observed, especially concerning in urinary tract infections. 2.85% isolates were resistant against Rifampin, with minimal resistance, demonstrating its potential as an effective therapy.

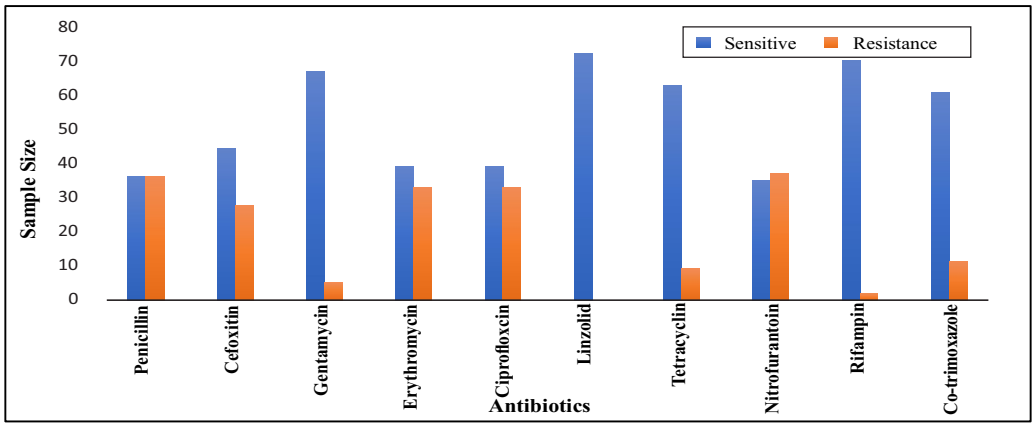

Figure 6: Antibiogram of *S. aureus* against various antibiotics

Table 2: Resistance frequency against antibiotics in Staphylococcus aureus isolates of infected patients

Specimen	Blood		Pus		Semen		Sputum		Urine		Vaginal swab	
	S	R	S	R	S	R	S	R	S	R	S	R
Penicillin	1	2	16	13	0	1	1	0	17	18	0	1
Cefoxitin	1	2	20	9	1	0	1	0	20	15	0	1
Gentamicin	3	0	26	3	1	0	1	0	33	2	1	0
Erythromycin	2	1	20	9	1	0	0	1	15	20	0	1
Ciprofloxacin	2	1	20	9	1	0	0	1	15	20	0	1
Linezolid	3	0	29	0	1	0	1	0	35	0	1	0
Tetracycline	3	0	23	6	1	0	1	0	32	3	1	0
Nitrofurantoin	3	0	3	0	1	0	1	0	25	10	1	0
Rifampin	3	0	28	1	1	0	1	0	34	1	1	0
Co-trimoxazole	2	1	27	2	1	0	1	0	27	8	1	0

DISCUSSION

Staphylococcus species were detected in 40 (57.14%) females and 30 (42.85%) males out of the 70 samples (fig 2). Research by Alahmadi *et al.*[13] shows that Staphylococcus was more commonly seen in females (7.1%) compared to males (6.3%). According to Kika *et al.*[14], males accounted for 59.8% and females for 40.2%. Since the *S. aureus* is a part of skin normal flora and it is opportunistic pathogen.[3] Since, females are more likely to do household works, so more prone to get cuts and wounds on hand. The bacterium can enter via this cuts and wounds.

In the study of Sapkota *et al.*[15] *Staphylococcus* spp. was most found in pus (55.64%), followed by sputum (3.75%), urine (2.26%), wound (23.31%), and blood (11.28). Mehta et al.16 reported that in Lucknow, urine culture showed the highest number of *Staphylococcus* isolates (43.71%), followed by pus (24%), blood (4.29%), and sputum (11.14%). In Mangalore, urine sample also showed the highest number of isolates at 42.8%, followed by pus (27.07%), sputum (29.2%), and blood sample (22.22%). In our study (figure 4), the highest prevalence of *Staphylococcus* was found in urine (48.7%), followed by pus (40.3%), blood (4.2%), semen (1.4%), sputum (1.4%) and vaginal swab (1.4%). It is a well-known fact that *Staphylococcus* is strongly associated with pyogenic infections. Our study results are consistent with this fact and reaffirm previous findings.

Our study shows that 33 samples (49%) were resistant to methicillin (MRSA), while 37 samples (51%) were sensitive to methicillin (MSSA). In the study by Adhikari *et al.*[17] MRSA was found in Nepal was 26.4% of cases, while MSSA accounted for 73.6%. From the study of Kika *et al.*, (2020)[14] the prevalence of MRSA in Albania was 20.5%, and MSSA accounted for 79.5%. The study of Yao *et al.*[18] revealed that in Southwest China, MRSA was found in 32.75% of cases, while MSSA made up 67.25% of the total. It is evident from our study that drug resistance in *Staphylococcus* is on the rise. We need good Antimicrobial Stewardship in hospitals and even in clinics.

According to our research, individuals in the 21-30 age group were more susceptible to urinary tract infections than those in the 0-10 age group. Additionally, the 31-40 age group was more prone to pus-related infections compared to the 21-30 age group, while the 51-60 age group showed similar susceptibility to pus infections. No relevant research was found to support these specific findings.

In our study, females in the age group of 21-30 are more prone to *Staphylococcus* infections, while males in the 51-60 age group are more susceptible than those in other age groups. According to the study of Soe *et al.*[19] males in the age group of 15-44 are more prone to infections, while females above the age of 15 are more susceptible. The research of Chukwueze *et al.*[20] states that individuals in the 25-29 age group are more likely to be susceptible to Staphylococcus infections. In the study by Adhikari *et al.*[17] the 15-24 age group accounted for 15.6% of *Staphylococcus* isolates. Females aged 21-30 are often in their childbearing years and are frequently admitted to hospitals for delivery, which may explain the higher incidence of *Staphylococcus* infections in this age group. On the other hand, males in the 51-60 age group are more commonly affected, possibly due to factors like declining immunity, higher rates of diabetes, and hypertension in later years.

Our study indicates the higher resistance pattern for Staphylococcus as Nitrofurantoin 51.42%, because activation of nitrofurantoin requires the presence of functional nitro-reductase enzymes, primarily NfsA and NfsB. Mutations in the nfsA or nfsB genes, or the loss of enzymatic activity, impede the bio reduction of nitrofurantoin into its active form.[21] As a result, the drug fails to exert its bactericidal effect, allowing the organism to survive and leading to the development of resistance.

Linezolid shows 0% sensitivity because linezolid binds to the 50S ribosomal subunit at the peptidyl transferase centre, inhibiting protein synthesis.[22] In linezolid-sensitive Staphylococcus aureus strains, this binding remains effective due to the absence of mutations at the target site and the lack of resistance genes such as *cfr or optrA*.[23]

Rifampin shows less 2.85% sensitivity as it targets the β-subunit of bacterial RNA polymerase, which is encoded by the rpoB gene. In rifampin-sensitive strains, the absence of mutations in the rpoB gene allows the antibiotic to bind effectively, thereby inhibiting transcription (Mandell *et al.*, 2019).[24] Additionally, the lack of rifampin resistance genes, such as arr, which encode ADP-ribosyl transferases capable of inactivating rifampin,

contributes to the susceptibility of the bacteria. In the absence of these resistance mechanisms, rifampin remains active and exerts its antibacterial effect.[25]

According to Gurung *et al.*[26] the resistance pattern for staphylococcus is penicillin 75%, cefoxitin 75%, erythromycin 46.2%, ciprofloxacin 61.5%, gentamicin 7.7%, tetracycline 40.4%, co-trimoxazole 40%. Sammi and Qassim[27] resistance pattern for Staphylococcus is penicillin 100%, cefoxitin 100%, erythromycin 91.1%, ciprofloxacin 26.4%, gentamicin 85.2%, linezolid 5.8%, tetracycline 64.7%, rifampin 5.8%. From the study of Kot *et al.*[28,] in 2015, the resistance pattern for Staphylococcus was: penicillin 100%, erythromycin 72.2%, ciprofloxacin 83.3%, gentamicin 16.6%, linezolid 0%, tetracycline 22.2%, and co-trimoxazole 5.5. From the study of An *et al.*[29] in 2021, resistance pattern for staphylococcus is cefoxitin 72.11%, erythromycin 80%, ciprofloxacin 31.03%, gentamicin 24.83%, linezolid 0%, tetracycline 61.69%, nitrofurantoin 0%, rifampin 9.74%, co-trimoxazole 34.93%.

Our study shows that pus samples exhibited resistance to penicillin, cefoxitin, gentamicin, erythromycin, ciprofloxacin, and tetracycline. Urine samples displayed resistance to penicillin, cefoxitin, erythromycin, ciprofloxacin, nitrofurantoin, and co-trimoxazole. Blood samples showed resistance to penicillin, cefoxitin, erythromycin, and ciprofloxacin. This low to high resistance pattern in our study may be due to these being mostly OPD cases whereas studies mostly conducted to find out resistance in Staphylococcus are in indoor patients hence we got this type of result in antibiogram.

The research of Alahmadi *et al.*[13] states that multidrug-resistant (MDR) phenotypes were observed in bloodstream infections against tetracycline, penicillin, tobramycin, oxacillin, fusidic acid, and gentamicin. Additionally, MRSA in urinary tract infections (UTI) was found to be resistant to moxifloxacin, penicillin, levofloxacin, oxacillin, and fusidic acid.

In overall study, it has been found that prevalence of *S. aureus* infection was more prominent in females. The isolates from pus and urine samples were more resistant against different antibiotics. Since the linezolid was effective against all the isolates, it may a good choice to prevent *S. aureus* infection. The future study will be directed to assess the genes and proteins in resistant bacterial samples.

CONCLUSION

The antimicrobial resistance patterns revealed significant concerns, especially with penicillin and nitrofurantoin. However, antibiotics like linezolid and gentamicin remain highly effective. The findings underscore the importance of continuous surveillance and tailored antibiotic therapies based on resistance profiles. Future studies should focus on investigating the factors contributing to gender-specific susceptibilities and the growing resistance, particularly in rural areas where data is scarce. Further exploration into novel therapeutic options and strategies to combat rising resistance will be essential in managing *Staphylococcus* infections effectively.

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Statement of Declaration

Conflict of interest: The authors declare that they have no conflict of interest.

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Ethical issue: There are no use of human or any animal in this study.

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