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Prevalence, Antimicrobial Resistance Patterns and Molecular Detection of Methicillin- and Vancomycin-Resistant Staphylococcus aureus in a Tertiary Hospital in North-Eastern Nigeria

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Abstract

Staphylococcus aureus is a major human pathogen accountable for a wide range of infections and increasing antimicrobial resistance. This study assessed the prevalence, antibiotic resistance patterns, and molecular detection of methicillin- and vancomycin-resistant Staphylococcus aureus among patients attending the Federal Teaching Hospital, Gombe, Nigeria. A total of 340 clinical specimens were processed using standard microbiological techniques. Identification was achieved through cultural and biochemical methods, while antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Methicillin and vancomycin resistance were phenotypically detected using cefoxitin and vancomycin discs, respectively. Molecular confirmation was carried out by PCR detection of the mecA gene in all MRSA isolates and the vanA gene in selected vancomycin-resistant isolates. Of the 340 samples analyzed, 185 (54.4%) were identified as S. aureus. Methicillin resistance was observed in 9 (4.9%) isolates, vancomycin resistance in 10 (5.4%), while 4 (2.2%) isolates exhibited resistance to both antibiotics. Multidrug resistance was detected in 15 (8.1%) isolates. Gentamicin demonstrated the highest antimicrobial activity, whereas resistance was more frequent against tetracycline, clindamycin, erythromycin, and penicillin. Molecular analysis confirmed the presence of the mecA and vanA genes. Although the prevalence of MRSA and VRSA was relatively low, the detection of these resistant strains reflects ongoing circulation within the study setting and underscores the importance of continuous antimicrobial resistance surveillance and strengthened antibiotic stewardship in tertiary healthcare facilities.

Keywords: Staphylococcus aureus, MRSA, VRSA, mecA, vanA, Antibiotic resistance

INTRODUCTION

Staphylococcus aureus is a common bacterial organism and one of the most significant human pathogens worldwide. It frequently exists as a commensal, colonizing the skin and anterior nares of approximately one-third of healthy individuals; however, under appropriate conditions, it can invade host tissues and cause a wide spectrum of infections ranging from mild skin and soft tissue infections to severe, life-threatening conditions such as pneumonia, septicemia, and endocarditis (Diso *et al.*, 2018). Historically, infections caused by S. *aureus* were susceptible to many commonly used antimicrobial agents; however, the emergence and spread of antimicrobial resistance have adversely affected treatment outcomes.

Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a major global public health concern due to its resistance to ß-lactam antibiotics and its association with both hospital-acquired and community-associated infections. Methicillin resistance in S. *aureus* is mainly mediated by the *mecA* gene, which encodes an altered penicillin-binding protein (PBP2a) with reduced affinity for ß-lactam antibiotics, allowing cell wall synthesis to continue despite antibiotic exposure. The *mecA* gene is carried on the staphylococcal cassette chromosome *mec* (SCCmec), a mobile genetic element that facilitates horizontal gene transfer and contributes to the widespread dissemination of MRSA strains.

Globally, the prevalence of MRSA varies widely, with systematic analyses reporting rates exceeding 20% in many regions and reaching much higher levels in certain healthcare settings, particularly in low- and middle-income countries (Adeiza, *et al.*, 2024). In Africa, pooled data from a recent systematic review and meta-analysis demonstrate that MRSA carriage and infection constitute a substantial public

health challenge, with notable prevalence across different populations and healthcare environments (Azzam, *et al.*, 2025). These variations are influenced by differences in antibiotic use, infection control practices, and surveillance capacity.

Vancomycin, a glycopeptide antibiotic, remains a cornerstone in the treatment of MRSA infections. However, increasing reliance on vancomycin has raised concerns regarding the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA). A comprehensive global systematic review and meta-analysis reported that although VRSA remains less prevalent than MRSA, its occurrence has been documented across multiple regions, with an estimated prevalence of approximately 2.5% in Africa, underscoring its clinical and epidemiological relevance (Shariati, *et al.*, 2020). Furthermore, regional meta-analyses within Africa have revealed higher VRSA prevalence in specific settings, highlighting substantial heterogeneity in resistance patterns (Belete, 2023).

Despite reports of methicillin- and vancomycin-resistant Staphylococcus aureus from various parts of Nigeria, data from north-eastern Nigeria remain limited. A recent nationwide meta-analysis by Ezeh et al. (2023) *revealed a marked geographical imbalance in available studies, with only 3 of 98 studies originating from the North-East. Notably, these few studies were largely restricted to urban, community-based nasal carriage surveys, providing limited insight into hospital-based infections. Consequently, the epidemiology and antimicrobial resistance patterns of methicillin- and vancomycin-resistant Staphylococcus aureus within tertiary healthcare settings in north-eastern Nigeria remain insufficiently characterized. This study therefore, aimed to determine the prevalence, antimicrobial susceptibility profiles, and resistance characteristics of methicillin- and vancomycin-resistant Staphylococcus aureus isolates obtained from patients attending the Federal Teaching Hospital, Gombe, North-Eastern Nigeria.*

MATERIALS AND METHODS

Study design and location

The study was hospital-based, cross-sectional, observational study conducted at Federal Teaching Hospital Gombe (FTHG), Gombe State, Nigeria.

Inclusion and exclusion criteria

Both in and out patients (male, female, children and adult) were included in the study. However, Patients that declined participation were excluded.

Ethical consideration and consent

Ethical approval for this study was obtained from the Federal Teaching Hospital, Gombe, Nigeria (Ref.no.-NHREC/25/10/2013). Written informed consent was obtained from all participants prior to sample collection.

Sample Collection

A total of 340 clinical specimens comprising wound swabs, nasal swabs, ear swabs, urine, and sputum were collected from patients attending the Federal Teaching Hospital, Gombe, following standard procedures (Ariom *et al.*, 2017). Swab samples were collected using sterile swab sticks, while urine and sputum samples were collected in sterile specimen containers. All samples were properly labelled and transported immediately to the laboratory for analysis.

Isolation and Identification of *Staphylococcus aureus*

Sample processing and bacterial isolation were performed as described by Adeiza *et al.* (2020). Specimens were aseptically inoculated onto mannitol salt agar and incubated at 37 °C for 24 h. Presumptive *S. aureus* isolates were identified based on colony morphology, Gram staining, and standard biochemical tests, including catalase and coagulase tests (Anyanwu and John, 2013). Confirmed isolates were preserved on nutrient agar slants for further analysis.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby–Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI, 2024) guidelines. Bacterial suspensions were adjusted to 0.5 McFarland standard and inoculated onto Mueller-Hinton agar plates. The antibiotics tested included penicillin G (10 µg), tetracycline (30 µg), gentamicin (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), and clindamycin (2 µg). Plates were incubated at 37 °C for 24 h, and inhibition zones were interpreted using CLSI criteria. Isolates resistant to three or more classes of antibiotics were classified as multidrug-resistant.

Phenotypic Detection of MRSA and VRSA

Methicillin resistance was detected using the cefoxitin disc (30 µg) on Mueller-Hinton agar enhanced with 5% NaCl, following CLSI (2024) recommendations. Vancomycin resistance was determined using the vancomycin disc (30 µg).

Molecular Characterization of Resistant Isolates

Genomic DNA was extracted from confirmed MRSA and VRSA isolates using the Qiagen DNA extraction kit, following the manufacturer's instructions. All nine phenotypically confirmed MRSA isolates were analyzed by PCR to confirm resistance genes. For VRSA, 10 phenotypically resistant isolates were identified; six representative isolates were selected for molecular analysis based on their phenotypic similarity and source diversity. This approach provides a reliable confirmation of vancomycin resistance while efficiently using molecular testing resources. Polymerase chain reaction (PCR) was performed to detect the *mecA* gene in MRSA isolates and the *vanA* gene in VRSA isolates. PCR amplification was carried out in a 25 µL reaction mixture containing 2.0µl DNA template, 4.0 µl of 10 x Buffer (Bioline), 0.5 µl of 50mM $MgCl_2$ (Bioline), 3.0 µl of mM dNTPs (Bioline), and 0.2 µl *Taq* DNA polymerase (Bioline), 1.0 µl DMSO (dimethyl sulfoxide), 1.0 µl of each primer and 12.3 µl of Nuclease-free water (Invitrogen Corporation). Thermal cycling conditions for *mecA* included initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. Amplification of the *vanA* gene involved initial denaturation at 94 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR products were resolved on 1% agarose gel electrophoresis stained with ethidium bromide and visualized under ultraviolet illumination using a gel documentation system. Primer sequences and expected amplicon sizes were as previously described by Umar *et al.* (2023) and Khanal *et al.* (2023).

Primers used were as follows:

Primer	Primer Sequence (5^{l}-3^{l})	Product size (bp)	Reference
*mec*A F	AAAATCGATGGTAAAGGTTGGC	533	Umar *et al.*, 2023
*mec*A R	AGTTCTGCAGTACCGGATTTGC		
*van*A F	AATAGCGCGGACGAATTGGAC	125	Khanal *et al.*, 2023
*van*A R	AACGCGGCACTGTTTCCCAA		

Data analysis and result presentation

Data were analyzed using SPSS software version 21.0 (IBM Corp., USA). Results were expressed as frequencies and percentages and presented in figures and tables. The Chi-square test was used to assess statistical significance, with $p < 0.05$ considered statistically significant.

RESULTS

Table 1 presents the distribution of *Staphylococcus aureus* isolated from different clinical samples analyzed in this study. Out of the 340 clinical specimens examined, *S. aureus* was isolated from 185 samples, giving an overall prevalence of 54.4%. *The distribution of Staphylococcus aureus isolates varied across different clinical specimens. Urine samples yielded the highest number of isolates (85; 25.0%), followed by sputum (40; 11.8%) and wound swabs (35; 10.3%). Lower isolation rates were observed in nasal swabs (4.4%) and ear swabs (2.9%). Chi-square analysis showed a statistically significant association between clinical sample type and S. aureus isolation ($x^2 = 20.11$, $df = 4$, $p = 0.0005$).*

The distribution of Staphylococcus aureus isolates among in-patients and out-patients is presented in Table 2. *Of the 185 isolates, 73 (39.5%) were recovered from in-patients, while 112 (60.5%) were obtained from out-patients. Urine samples yielded the highest number of isolates in both in-patients and out-patients. Chi-square analysis showed no statistically significant association between patient status and distribution of Staphylococcus aureus across clinical specimen types ($x^2 = 2.36$, $df = 4$, $p = 0.67$).*

Table 3 summarizes the antibiotic susceptibility patterns of *S. aureus* isolates recovered in this study. High levels of susceptibility were observed for most of the antibiotics tested.

Gentamicin showed the highest activity, with 97.3% of isolates being susceptible, followed by cefoxitin (95.1%) and vancomycin (94.6%). Ciprofloxacin (91.9%) and penicillin (91.4%) also demonstrated high susceptibility rates. Higher resistance rates were observed against tetracycline and clindamycin (each 13.5%) and erythromycin (11.4%).

Table 1: Distribution of *Staphylococcus aureus* Identified from Different Clinical Samples

Type of Clinical Sample	No. of Samples Collected	No. of *S. aureus* isolated (%)
Urine	180	85(25.0)
Wound swab	60	35(10.3)
Sputum	50	40(11.8)
Ear swab	15	10(2.9)
Nasal swab	35	15(4.4)
TOTAL	340	185(54.4)

x^2 = 20.11, df = 4, p = 0.0005

Table 2: Prevalence of *Staphylococcus aureus* in Inpatient Versus Outpatient clinical populations

Sampling Site	Inpatients n (%)	Outpatients n (%)
Urine	35 (18.9)	50 (27.0)
Sputum	18 (9.7)	22 (11.9)
Wound swab	10 (5.4)	25 (13.5)
Ear swab	4 (2.2)	6 (3.2)
Nasal swab	6 (3.2)	9 (4.9)
Total	**73 (39.5)**	**112 (60.5)**
Statistical test		
x^2	**2.36**	
df	**4**	
p	**0.67**	

Table 3: Antibiotic Susceptibility Profile of *Staphylococcus aureus* Isolates from Federal Teaching Hospital, Gombe, Nigeria

Antibiotic	Potency (ug)	No. of *S. aureus* (%)	
		Resistant (R)	Sensitive (S)
Penicillin (P)	10	16(8.6)	169(91.4)
Ciprofloxacin (CIP)	5	15(8.1)	170(91.9)
Erythromycin (E)	15	21(11.4)	164(88.6)
Gentamicin (GM)	30	5(2.7)	180(97.3)
Tetracycline (TE)	30	25(13.5)	160(86.5)
Clindamycin (DA)	2	25(13.5)	160(86.5)
Cefoxitin (CF)	30	9(4.9)	176(95.1)
Vancomycin (VA)	30	10(5.4)	175(94.6)

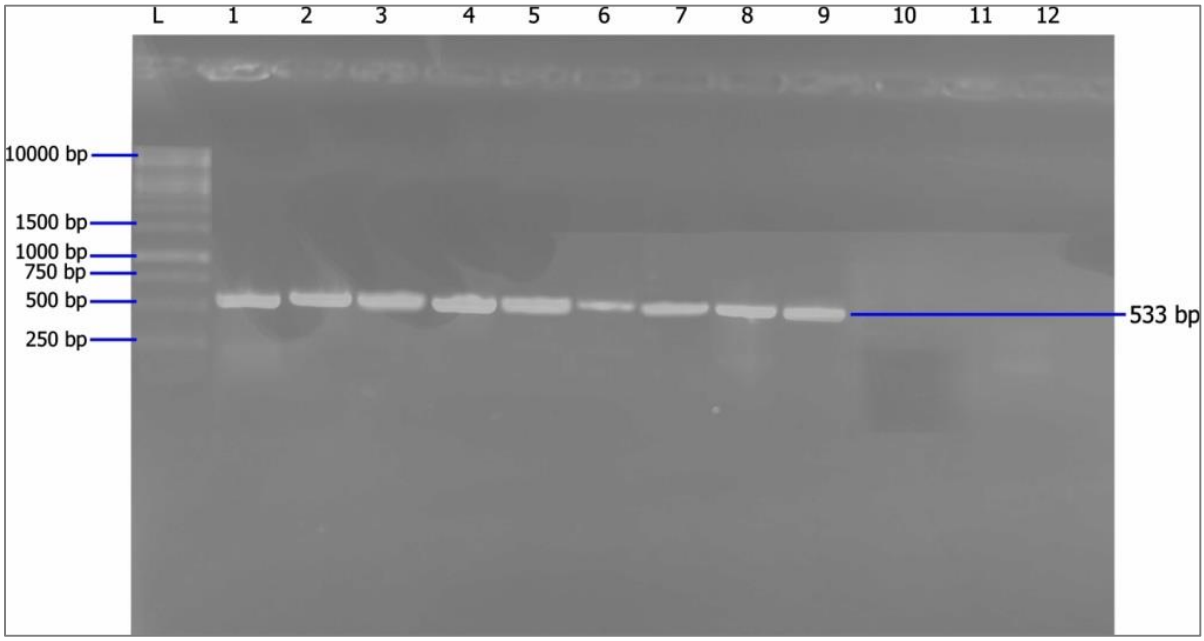

Fig 1: PCR for detection of mecAgene from methicillin resistant *S. aureus* isolates
Legend: lane L: 10kbp ladder; Lane 1 to 9 postive to *mec*A gene for Methicillin-resistan *S. aureus*

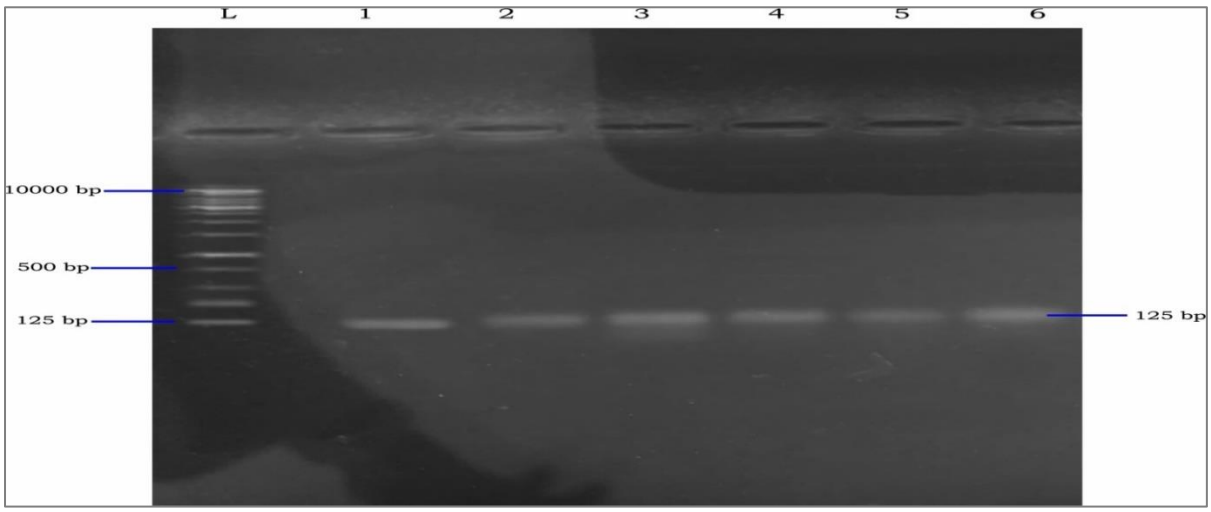

Fig 2: PCR for detection of vanAgene from vancomycin resistant *S. aureus* isolates (Legend: lane L: 10kbp ladder; Lane 1 to 6 postive to *van*A gene for Vancomycin-resistan *S. aureus*)

Figure 1 shows the agarose gel electrophoresis results of PCR amplification of the *mecA* gene among MRSA isolates. Bands corresponding to the expected size of 533 bp were observed, confirming the presence of the *mecA* gene and validating phenotypic detection of methicillin resistance. Figure 2 presents PCR amplification of the *vanA* gene, with bands detected at 125 bp, confirming vancomycin resistance at the molecular level.

DISCUSSION

The overall prevalence of *Staphylococcus aureus* in this study was 54.4%, indicating that the organism remains a common cause of infection among patients at the Federal Teaching Hospital, Gombe. *It is important to note that this prevalence represents the rate of Staphylococcus aureus isolation from submitted clinical specimens and does not differenciate between confirmed infection and colonization, particularly for clinical sample types such as urine and nasal swabs.* The proportions of methicillin-resistant (MRSA) and vancomycin-resistant (VRSA) isolates, however, were relatively low. These findings suggest that, although resistant strains are present, their circulation within the hospital has not yet reached high levels. The prevalence observed in this study is higher than previous reports by Aminu *et al.,* (2017) (44.6%), Tsige *et al.,* (2020) (32.3%), and Kejela and Dekosa (2022) (32.8%). Such differences may reflect variations in geographical location, sample size, sample types, patient populations, laboratory methods, and infection control practices. Futhermore, increased hospital attendance and inappropriate antibiotic use in the study area may have contributed to the higher prevalence recorded.

The observed distribution of *S. aureus* isolates, with a higher proportion among out-patients than in-patients, is consistent with reports by Adeiza *et al.,* (2020) and Tsige *et al.,* (2020). This pattern may reflect increased community exposure, self-medication, and frequent healthcare visits without admission. Nevertheless, the relatively low prevalence of resistant strains among these isolates indicates that community-associated transmission of resistant *S. aureus* remains limited in the study area at the time of investigation.

The antibiotic susceptibility pattern showed gentamicin as the most effective agent against *S. aureus*, consistent with findings by Ariom *et al.,* (2017), Bamigboye *et al.,* (2018), and Onanuga *et al.,* (2021). Its high efficacy may be attributed to its parenteral route of administration and relatively controlled use, which limit misuse and resistance development. In contrast, higher resistance was observed against tetracycline, clindamycin, and erythromycin, likely due to their widespread availability and frequent empirical use. Overall, cefoxitin, vancomycin, ciprofloxacin, penicillin, and gentamicin were largely effective against the majority of isolates, indicating that commonly used antibiotics remain reliable for treating *S. aureus* infections in this setting. The generally favorable susceptibility profile also aligns with the relatively low prevalence of multidrug-resistant isolates observed in this study.

An interesting finding in this study was the relatively low level of resistance to penicillin among Staphylococcus aureus isolates, which contrasts with global reports describing widespread penicillin resistance in this species.

This observation should be interpreted cautiously. Possible explanations may include local epidemiological characteristics, variations in empirical prescribing practices, or differences in selective pressure within the study setting. In addition, methodological factors related to disc diffusion testing and isolate selection may have influenced the observed resistance rates. Therefore, while the findings suggest preserved activity of penicillin in this setting, further studies using expanded sample sizes and complementary susceptibility methods are warranted to confirm this pattern.

Molecular analysis confirmed the presence of the *mecA* and *vanA* genes, validating the phenotypic detection of methicillin and vancomycin resistance in this study. These findings align with reports by Karasin *et al.,* (2021), Alghizzi *et al.,* (2021), and Agbo *et al.,* (2024), though they differ from Khanal *et al.,* (2023), who detected *mecA* without *vanA*. Such discrepancies may reflect geographical variation, antibiotic use patterns, and selective pressures in healthcare settings. Although the genes were detected in only a small proportion of isolates, their presence is clinically significant, highlighting the need for ongoing surveillance to detect emerging resistance trends early and prevent potential escalation within the hospital environment.

We acknowledge that vancomycin resistance in *Staphylococcus aureus* was initially determined using the disk diffusion method, which is not the gold standard according to CLSI guidelines, as MIC-based methods are recommended for accurate detection of VRSA. However, to ensure the reliability of our results, molecular testing was performed to confirm the presence of vancomycin resistance genes in the isolates. This dual approach phenotypic screening followed by molecular confirmation strengthens the validity of our VRSA detection and mitigates the limitations of using disk diffusion alone. We also recommend that future studies incorporate MIC testing for confirmation of VRSA to further enhance the accuracy of resistance determination.

The findings of this study have important clinical and public health implications. Although the prevalence of MRSA and VRSA was relatively low, the detection of these resistant strains in a tertiary healthcare setting suggests the need for sustained infection prevention and control measures to prevent their potential spread. The generally favourable susceptibility profile observed supports the continued use of commonly prescribed antibiotics; however, ongoing antimicrobial stewardship is essential to preserve their effectiveness. This study has some limitations, including its single-centre design, the moderate sample size, and the inability to distinguish between infection and colonization. In addition, vancomycin resistance was initially assessed using disc diffusion rather than MIC-based methods. Despite these limitations, the study provides valuable baseline data for north-eastern Nigeria and underscores the importance of continuous antimicrobial resistance surveillance to inform clinical practice and policy.

CONCLUSION

This study demonstrated that *Staphylococcus aureus* was isolated from 54.4% of clinical samples obtained from patients attending the Federal Teaching Hospital, Gombe, with urine, wound swabs, and sputum being the major sources of isolation. This finding reflects the frequency of *S. aureus* isolation from submitted clinical samples rather than confirmed infection, given that some specimen types may represent colonization. Although a higher occurrence of isolates was observed among out-patients compared with in-patients, this difference was not statistically significant.

Antibiotic susceptibility testing showed high sensitivity of the isolates to gentamicin, cefoxitin, and vancomycin, while higher resistance rates were observed against tetracycline, clindamycin and erythromycin. Multidrug resistance was detected in 15 (8.1%) of the isolates. Phenotypic and molecular analyses further confirmed the presence of methicillin- and vancomycin-resistant *Staphylococcus aureus* through the detection of the *mecA* and *vanA* genes.

Overall, the findings indicate that while multidrug-resistant *S. aureus* strains are present within the hospital setting, their prevalence remains relatively low. These results suggest the need for continuous antimicrobial resistance surveillance, sustained infection prevention and control practices, and strengthened antibiotic stewardship programmes to prevent the emergence and spread of resistant *Staphylococcus aureus* strains in tertiary healthcare facilities.

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