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ORIGINAL RESEARCH ARTICLE

Phenotypic and Molecular Profiling of Multidrug-Resistant *Staphylococcus aureus* Isolated from Labour Rooms in Selected Hospitals of Katsina State, Nigeria

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ABSTRACT

Fluoroquinolone antibiotics are widely used in managing infections caused by multidrug-resistant (MDR) *Staphylococcus aureus*. However, the emergence and spread of resistance to these agents pose serious public health threats, particularly in healthcare environments across Katsina State, Nigeria. This study investigated the presence of selected quinolone resistance genes in MDR *S. aureus* isolates obtained from labour room environments in four hospitals within the state. A total of 240 environmental swab samples were collected and analyzed for the presence of *S. aureus*. Isolates were identified using standard biochemical methods, and their antibiotic susceptibility profiles were determined via the disk diffusion method. MDR status was defined as resistance to three or more antibiotic classes. Species confirmation was performed using PCR targeting the *nuc* gene, followed by detection of quinolone resistance genes (*qnrA*, *qnrD*, and *parC*) in the confirmed MDR isolates using PCR. Twenty-eight *S. aureus* isolates were recovered. High levels of resistance were observed to amoxicillin-clavulanate (100%), cefoxitin (100%), erythromycin (54.5%), and ciprofloxacin (54.5%). Fourteen isolates (50%) were identified as MDR. Among the five selected MDR isolates, PCR confirmed all as *S. aureus*, with 80% harbouring *qnrD*, 40% harbouring *qnrA*, and 20% harbouring *parC*. These findings highlight a significant burden of MDR *S. aureus* and associated resistance genes in labour room environments, underscoring the urgent need for strengthened infection prevention practices and responsible antibiotic use to mitigate further dissemination within maternity healthcare settings.

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INTRODUCTION

Multidrug-resistant *Staphylococcus aureus* (MDR-*S. aureus*) has emerged as a critically significant pathogen in healthcare environments, undermining effective antimicrobial treatment and significantly contributing to healthcare-associated infections (HAIs) globally (Tigabu and Getaneh, 2021). The organism's capacity to resist various antibiotic classes prolongs hospital stays, escalates treatment expenses, and heightens maternal and neonatal morbidity and mortality, an urgent issue in low-resource settings like Nigeria, where limited infection prevention and control (IPC) infrastructure and unregulated antibiotic usage exacerbate resistance dynamics (Ajekiigbe *et al.*, 2025). Systematic reviews and meta-analyses have revealed widespread resistance patterns among *S. aureus* across Nigeria, with consistently high resistance to commonly used antibiotics and increasingly restricted clinical treatment options (Ahmed *et al.*, 2024).

Although the epidemiology of MDR-*Staphylococcus aureus* has been investigated in various clinical and environmental contexts within Nigeria, research has largely focused on general patient populations and non-specific hospital settings, including tertiary hospital emergency departments and fomites in public areas (Salehi *et al.*, 2025). Although the epidemiology of MDR-*Staphylococcus aureus* has been investigated in various clinical and environmental contexts within Nigeria, research has largely focused on general patient populations and non-specific hospital settings, including tertiary hospital emergency departments and fomites in public areas (Salehi *et al.*, 2025). These investigations offer valuable baseline information but do not account for the unique infection dynamics in labour rooms, where obstetric procedures, invasive monitoring, high patient turnover, and frequent staff–patient interactions generate distinct ecological pressures that may heighten the risk of pathogen transmission along maternal–neonatal pathways (Garvey, 2024). Indeed, while environmental contamination with MDR organisms has been observed in neonatal intensive care units and other high-risk wards in Nigeria, the specific connection between environmental reservoirs in labour rooms and obstetric infection pathways remains inadequately characterized. This conceptual void is further exacerbated by the lack of comparative

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environmental risk profiling across hospital wards (Long *et al.,* 2024).

Resistance to quinolones via *qnr* and *parC* genes, a class of broad-spectrum antibiotics widely used in obstetrics and gynecology has become increasingly prevalent due to the overuse and misuse of these agents in both clinical and community settings (Kareem *et al*., 2021; Mahapatra *et al*., 2022). These resistance mechanisms significantly diminish therapeutic options, especially in resource-limited healthcare systems.

Phenotypic and molecular profiling of resistant *S. aureus* strains provides critical insights into the local epidemiology of antimicrobial resistance. This approach aids in identifying specific resistance patterns, informing empirical therapy, and supporting infection control strategies. In Nigeria, limited data exist on the antimicrobial resistance profiles of *S. aureus* isolates from labour and delivery wards, particularly with respect to quinolones (Medugu *et al*., 2021). Given the heightened risk of maternal and neonatal infections in labour rooms, understanding the distribution of MDR *S. aureus* in such environments is vital for guiding effective prevention and control measures.

Despite increasing reports of multidrug-resistant bacterial infections in Nigerian hospitals, comprehensive data on phenotypic resistance traits of *S. aureus* within maternity settings remain scarce (Johnson, 2021). Labour rooms, often under rigorous use with limited sterilization protocols (Gall *et al.,* 2020), may act as reservoirs for MDR pathogens, facilitating nosocomial transmission to mothers and neonates. The lack of routine surveillance and resistance profiling hampers early detection and control of these pathogens. Consequently, there is an urgent need for systematic studies on the antimicrobial resistance characteristics of *S. aureus* in labour room environments within Katsina State and similar resource-constrained regions.

This study aims to molecularly characterize multidrug-resistant *S. aureus* isolates from labour room settings in selected hospitals in Katsina State, Nigeria, with a specific focus on quinolone resistance. The findings will contribute to a better understanding of local resistance trends and provide evidence-based recommendations for antimicrobial stewardship and infection control in maternal healthcare. Moreover, this study will help inform policy and practice to curb the spread of MDR pathogens in critical hospital environments.

MATERIALS AND METHODS

Study Design

This study was conducted across four selected healthcare facilities (i.e., General Hospital (GH), Comprehensive Hospital (CH), Federal Teaching Hospital Katsina (FTH), and Turai Children Hospital (TCH) in Katsina State, Nigeria, using a purposive sampling technique. These hospitals were chosen for their high patient throughput and key roles in maternal and neonatal healthcare delivery within the region. A cross-sectional descriptive study design was adopted to investigate the presence and characteristics of multi-drug resistant (MDR) *S. aureus* isolates from labour room environments, including surfaces and instruments. The study spanned a period of six months, from October to April, 2025.

Sample Collection

A total of 240 samples were collected using sterile swab sticks from various surfaces that include: delivery beds, walls, floors, trolleys, scissors, curtains, mopping sticks, door handles, dripping hangers, drawers, forceps, fridge, fan regulators, infant radiant warmer, light switch, medication drawer, nasal tubes, wheel chairs, toilet seat, oxygen cylinder, sinks and weighing machine using sterile swab sticks moistened with normal saline within the labour rooms of the four hospitals. Afterwards, samples were transported in ice packs to the Department of Microbiology laboratory at the Federal University Dutsin-Ma for further processing. Samples collected were immediately streaked onto sterile Manitol Salt Agar (MSA) in petri dishes. Plates were, thereafter, incubated at 37 °C for 24 hours. Colonies showing morphologic characteristic of yellow pigmentation for *S. aureus* on MSA were selected, re-streaked and subcultured onto Nutrient Agar (NA) to obtain pure isolates. Preliminary identification was performed through Gram staining, and results were confirmed by a series of biochemical tests, including catalase and coagulase tests, according to standard protocols.

Antibiotic Susceptibility Profile

Antibiotic susceptibility testing of the isolates was carried out using the Kirby-Bauer disc diffusion method in accordance with CLSI (2022) guidelines. Antibiotics tested included representatives from major classes: β-lactams (amoxicillin-clavulanic acid), fluoroquinolones (ciprofloxacin), carbapenems (imipenem), aminoglycosides (gentamicin), macrolides (erythromycin), and cephalosporins (cefoxitin, ceftazidime, cefuroxime). Bacterial suspensions were standardized to a 0.5 McFarland standard and uniformly inoculated onto Mueller-Hinton Agar (MHA) plates before the addition of antibiotic discs. After 24 hours of incubation at 37°C, zones of inhibition were measured and interpreted using CLSI (2022) breakpoints. Multidrug resistance was defined as resistance to three or more antibiotic classes.

DNA extraction and molecular confirmation of *S. aureus*

Molecular confirmation of *Staphylococcus aureus* was performed by DNA extraction and PCR amplification. Genomic DNA was extracted using the AccuPrep® Genomic DNA Extraction Kit according to the

manufacturer's protocol (Sloan *et al.*, 2021; Medugu *et al.*, 2021). Pure colonies of *S. aureus* were suspended in phosphate-buffered saline (PBS), lysed, and subjected to ethanol precipitation. After passing through adsorption columns, the DNA was washed, eluted, and stored at 20°C until use. Detection of antibiotic-resistant genes (*qnrA*, *qnrD* plasmid-mediated quinolone resistance), and *parC* (chromosomal) in subsequent PCR assays was performed using multiplex PCR (Mahapatra *et al.*, 2022; Badamasi and Salisu, 2025; Salisu *et al.*, 2025, 2024).

Table 1. Primers used for the Detection of Antibiotic Resistance Genes in *Staphylococcus aureus*

Bacteria	Target Genes	Primer Sequence (5'→3')	Product Sizes (bp)	Annealing Temp (°C)	Final Extension	References
S. aureus	*nuc*	F: GCGATTGATGGTGATACGGTT R: AGCCAAGCCTTGACGAACTAAAGC	279	55°C	72°C for 5 minutes	Jauro *et al.*, 2022
S. aureus	*qnrA*	F: ATTTCTCACGCCAGGATTTG R: GATCGGCAAAGGTTGGTCA	516	56°C	72°C for 5 minutes	Mahapatra *et al.*, 2022
S. aureus	*qnrD*	F: AGGTGTAGCATGTATGGAAAAGC R: ACATTGGGGCATTAGGCGTT	691	58°C	72°C for 5 minutes	Mahapatra *et al.*, 2022
S. aureus	*parC*	F: CTATGCGATGTCAGAGCTGG R: TAACAGCAGCTCGGCGTATT	270	60°C	72°C for 5 minutes	Mohamed *et al.*, 2024

Table 2. Distribution of *Staphylococcus aureus* isolates from different sites at selected Hospitals, Katsina State, Nigeria

SITES	Comprehensive Hospital	General Hospital	Turai Children's Hospital	Federal Teaching Hospital	Total Occurrence of Isolate (%)
Bed	-	4(57.14)	2(28.57)	1(14.28)	7(24.14)
Curtains	-	-	1(100)	-	1(3.44)
Chair	1(33.33)	-	1(33.33)	1(33.33)	3(10.35)
Door Handles	-	-	-	-	0(0.00)
Dripping Hanger	1(50.00)	-	-	1(50.00)	2(6.90)
Drawer	-	-	1(100)	-	1(3.44)
Floor	-	2(40.00)	1(20.00)	1(20.00)	4(14.29)
Forceps	-	-	-	-	0(0.00)
Fridge	-	-	-	1(100)	1(3.44)
Fan Regulator	-	-		-	0(0.00)
Infant Radiant Warmer	-	-	-	-	0(0.00)
Light Switch	-	-	-	-	0(0.00)
Medication Drawer	1(100)	-	-	-	1(3.44)
Mopping Stick	1(50.00)	-	-	1(50.00)	2(6.90)
Nasal Tube	-	-	-	-	0(0.00)
Oxygen Cylinder	-	-	1(100)	-	1(3.44)
Sink	-	-	1(100)	-	1(3.44)
Scissors	-	-	1(100)	-	1(3.44)
Toilet Seat	-	-	-	-	0(0.00)
Tap	-	-	-	-	0(0.00)
Table	1(100)	-	-	-	1(3.44)
Trays	-	-	-	-	0(0.00)
Wheel Chair	-	-	-	-	0(0.00)
Weighing Machine	-	-	1(50.00)	1(50.00)	2(6.90)
Wall	-	-	-	-	0(0.00)
TOTAL	5(100)	6(100)	10(100)	7(100)	28(100)

Identification of *S. aureus* by PCR amplification of *nuc* genes

The extracted DNA was used in the amplification of the *Staphylococcus* thermonuclease gene, *nuc* (*S. aureus* specific primer), for the identification of the Gram-positive isolates. A duplex PCR was carried out in a 15 µL final volume reaction containing 7.5µL of mastermix, 1 µL of *nuc* forward and reverse primers. 5µL of PCR-grade water and 1µL of DNA. The reaction was carried out using the following PCR conditions: initial denaturation at 94℃ for 3 minutes, followed by 30 cycles of denaturation at 94℃ for 30 seconds, annealing at 51℃ for 30 seconds and elongation at 72℃ for 60 seconds. This was followed by a final elongation at 72℃ for 7 minutes. *S. aureus* ATCC 43300 was used as a positive control (Jouhar *et al.*, 2020).

Table 3. Regression analysis of *S. aureus* Distribution between hospitals in Katsina metropolis and Dutsin-Ma LGA to identify predictors of MDR occurrence

		Comprehensive Hospital Dutsin-Ma	General Hospital	Turai Children's Hospital	Federal Teaching Hospital
Pearson Correlation	Comprehensive Hospital Dutsin-Ma	1.000	.036	-.207	-.097
	General Hospital	.036	1.000	-.124	-.081
	Turai Children's Hospital	-.207	-.124	1.000	-.222
	Federal Teaching Hospital	-.097	-.081	-.222	1.000
Sig. (1-tailed)	Comprehensive Hospital	.	.458	.270	.389
	General Hospital	.458	.	.359	.407
	Turai Children's Hospital	.270	.359	.	.256
	Federal Teaching Hospital	.389	.407	.256	.

KEYS: LGA= Local Government Area, MDR= Multi-drug resistance

Detection of Antibiotic Resistance Gene (*qnrA, qnrD* and *parC* gene) among the *Staphylococcus spp*

The antibiotic resistance genes encoding quinolone resistance (*qnrA, qnrD* and *parC*) were tested for the isolates by PCR. The gene was detected in a PCR reaction containing 7.5μL mastermix, 1μL each of the *qnrA, qnrD* and *parC* forward and reverse primers (Table 1), 4.5μL of PCR-grade water and 1μL of the DNA template. The reaction was carried out in a cycler using the following conditions: initial denaturation at 94℃ for 3 minutes, 30 cycles of denaturation at 94℃ for 30 seconds, annealing at 55℃ for 30 seconds and elongation at 72℃ for 45 seconds. A final elongation at 72℃ for 7 minutes was also carried out, this was carried out using a 15 microlitre final reaction (Merza and Jubrael 2015).

Ethical Statement

Ethical approval was obtained from the Katsina State Ministry of Health with reference number MOH/ADM/SUB/1152/1/918 to ensure that the study adheres to ethical guidelines for research involving human samples.

RESULTS

Distribution of S. aureus Isolates from different sites at Various Hospital

Table 2 reveals the environmental prevalence of *S. aureus* across different hospital equipment and surfaces in four hospitals. A total of 28 *S. aureus* isolates were recovered, with the highest occurrence from Turai Children Hospital (10 isolates, 34.48%), followed by General Hospital (6 isolates, 20.69%), Federal Teaching Hospital (7 isolates, 24.14%), and Comprehensive Hospital (5 isolates, 17.24%). Beds were the most contaminated site (7 isolates, 24.14%), especially in the General Hospital, where they accounted for 57.14% of the bed-related isolates. Floors were the second most contaminated, with 5 isolates (14.29%), notably from General and Turai Children's Hospitals. Certain highly touched sites, such as door handles, forceps, fan regulators, light switches, and taps, yielded no isolates. Single occurrences were observed in several equipment pieces, such as the oxygen cylinder, fridge, scissors, and medication drawer, all indicating sporadic but high contamination.

Table 3 presents the regression analysis evaluating the distribution of *Staphylococcus aureus* isolates across selected hospitals in the Katsina metropolis and the Dutsin-Ma Local Government Area (LGA), aiming to identify potential predictors of multidrug resistance (MDR). Pearson correlation coefficients were used to assess the strength and direction of associations among hospitals, while one-tailed significance tests evaluated the statistical significance of these relationships. The correlation coefficients reveal weak associations across all pairs of hospitals, ranging from −0.222 to 0.036. The relationship between Comprehensive Hospital, Dutsin-Ma, and General Hospital exhibited a very weak positive correlation (r = 0.036), indicating minimal similarity in the distribution patterns of *S. aureus* between these facilities. Conversely, negative correlations were observed between Comprehensive Hospital and Turai Children Hospital (r = −0.207), Comprehensive Hospital and the Federal Teaching Hospital (r = −0.097), and General Hospital and Turai Children Hospital (r = −0.124). The most substantial negative association was found between Turai Children Hospital and the Federal Teaching Hospital (r = −0.222). Notably, none of the observed correlations were statistically significant, as all one-tailed p-values exceeded the conventional threshold of 0.05 (p = 0.256–0.458).

Antibiotic Susceptibility Profile for *Staphylococcus aureus* isolated from various Hospitals from Katsina State, Nigeria

The antibiotic susceptibility profiles (Table 4) of *S. aureus* isolates from four hospitals in Katsina State (Table 4) reveal considerable variation (CLSI, 2022) in resistance and susceptibility patterns across the sampled locations, as shown by the Chi-square outcome (χ^2 = 59.093). Imipenem exhibited the highest and most consistent efficacy, with 100% susceptibility across three hospitals and 83.33% in General Hospital Dutsin Ma.

Table 4. **Antibiotic susceptibility profiles for *Staphylococcus aureus* isolated from selected Hospitals**

Antibiotics	General Hospital Dutsin Ma (n=6)			Comprehensive Healthcare centre (n=5)			Federal Teaching Hospital (n=7)			Turai Children and Maternity Hospital (n=11)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ciprofloxicin	5 (83.33)	0 (0.00)	1(16.67)	3 (60.00)	1 (20)	1(20.00)	6(85.71)	0(0.00)	1(14.29)	4(36.3)	1(9.09)	6(54.55)
Erythromycin	4 (66.67)	0 (0.00)	2 (33.3)	1 (20.00)	0 (0.0)	4(80.00)	6(85.71)	0(0.00)	1(14.29)	5(45.4)	0(0.00)	6(54.55)
Imipenem	5 (83.33)	0 (0.00)	1(16.67)	5 (100.00)	0(0.00)	0(0.00)	7(100.0)	0(0.00)	0(0.00)	11(100)	0(0.00)	0(0.00)
Amoxicillin- clavanuate	3 (50.00)	0 (0.00)	3 (50.00)	1(20.00)	0(0.00)	4(80)	4(57.14)	0(0.00)	3(42.86)	0(0.00)	0(0.00)	11(100)
Gentamicin	6 (100)	0 (0.00)	0 (0.00)	5 (100.00)	0(0.00)	0(0.00)	5(71.43)	0(0.00)	2(28.57)	10(90.91)	0(0.00)	1(9.09)
Cefoxitin	3 (50.00)	0 (0.00)	3 (50.00)	1 (20.00)	0(0.00)	4(80.00)	4(57.14)	0(0.00)	3(42.86)	0(0.00)	0(0.0)	11(100)

KEYS: S= Susceptible, I= Intermediate, R= Resistant, (χ^2 = 59.093)

Gentamicin also demonstrated strong activity, particularly in Dutsin-Ma and the Comprehensive Healthcare Centre (100% susceptibility), though moderate resistance (28.57%) was observed at the Federal Teaching Hospital.

Conversely, amoxicillin-clavulanate and cefoxitin showed alarmingly high resistance levels, particularly at Turai Children and Maternity Hospital, where all *S. aureus* isolates (100%) were resistant to both drugs, indicating widespread β-lactam resistance. Ciprofloxacin and erythromycin showed variable activity: Ciprofloxacin was most effective among *Staphylococcus* isolates at the Federal Teaching Hospital (85.71% susceptibility) but showed reduced efficacy among those from Turai (only 36.3% susceptible), while erythromycin resistance was notably high among isolates from the Comprehensive Healthcare Centre (80%) and Turai (54.55%).

Prevalence of Multi-drug resistant *S. aureus* from various Hospitals in Katsina State, Nigeria

The results presented in Table 5 reveal the prevalence and distribution of multidrug-resistant (*S. aureus*) (MDR *S. aureus*) isolates across four hospitals in Katsina State, Nigeria. Out of a total of 240 samples examined, 28(82.35%) were positive for *S. aureus*, and 14 of these isolates (50.00%) were multidrug-resistant. The highest prevalence of *S. aureus* was observed in Turai Hospital with 6 (42.86%) MDR strains. Although the Comprehensive had fewer MDR strains of S. aureus isolates, 2 (14.29%) exhibited MDR. General and FTHK hospitals each recorded 3 (21.43%) MDR *S. aureus* isolates, indicating a significant level of resistance. These findings highlight a concerning spread of MDR *S. aureus* in healthcare environments, particularly in Turai hospitals, underscoring the need for robust infection control and antibiotic stewardship programs.

Resistant Phenotypes of *Staphylococcus aureus* Isolates Grouped by Hospital

Table 6 illustrates the distribution of resistant phenotypes among multidrug-resistant (MDR) *Staphylococcus aureus* isolates categorized by hospital, highlighting the number of antibiotics to which each isolate was resistant, its specific MDR pattern, the total number of isolates, and the associated Multiple Antibiotic Resistance (MAR) indices.

At Turai Children and Maternity Hospital, three MDR *S. aureus* isolates were found to be resistant to four antibiotics, displaying a consistent resistance pattern that included ciprofloxacin (CIP), erythromycin (ERY), amoxicillin–clavulanate (AUG), and cefoxitin (FOX). These isolates recorded a MAR index of 0.7. In the Federal Teaching Hospital Katsina, three unique MDR phenotypes were identified among the isolates. Two isolates each showed resistance to three antibiotics, with resistance patterns of ERY–AUG–FOX and AUG–GEN–FOX, both linked to a MAR index of 0.5. Additionally, a single isolate exhibited resistance to four antibiotics (CIP–AUG–GEN–FOX), which

corresponded to a higher MAR index of 0.7. At General Hospital Dutsin-Ma, MDR isolates demonstrated resistance to 3 or 4 antibiotics. Two isolates were resistant to the ERY–AUG–FOX pattern (MAR index = 0.5), while one isolate displayed a four-drug resistance pattern (CIP–GEN–AUG–FOX; MAR index = 0.7). Likewise, isolates from the Comprehensive Health Centre in Dutsin-Ma revealed two MDR phenotypes. One isolate was resistant to three antibiotics (ERY–AUG–FOX) with a MAR index of 0.5, while another isolate was resistant to four antibiotics (CIP–GEN–AUG–FOX), yielding a MAR index of 0.7.

Table 5. Prevalence of Multidrug-resistant *Staphylococcus aureus* from selected Hospitals in Katsina State, Nigeria (n=28)

HOSPITALS	No. of Samples examined	No of MDR *S. aureus*
GENERAL	60	3 (21.43%)
COMPREHENSIVE	60	2 (14.29%)
FTHK	60	3 (21.43%)
TURAI	60	6 (42.86%)
TOTAL	**240**	**14 (50%)**

Table 6: Resistant phenotypes of MDR *Staphylococcus aureus* isolates grouped by Hospital

Hospital	No. of Antibiotics	MDR Pattern	No. of Isolates	MAR Index (%)
TCMH	4	CIP, ERY, AUG, FOX	3	0.7
FTHK				
	3	ERY, AUG, FOX	1	0.5
	3	AUG, GEN, FOX	1	0.5
	4	CIP, AUG, GEN, FOX	1	0.7
GHD				
	3	ERY, AUG, FOX	2	0.5
	4	CIP, GEN, AUG, FOX	1	0.7
CHCD				
	3	ERY, AUG, FOX	1	0.5
	4	CIP, GEN, AUG, FOX	1	0.7

KEYS: IMI= Imipenem, GEN= Gentamycin, FOX= Cefoxitin, ERY= Erythromycin, CIP= Ciprofloxacin, AUG= Amoxillin-Clavalanate, MAR= Multiple Antibiotic Resistant; TCMH: Turai Children and Maternity Hospital; FTHK: Federal Teaching Hospital Katsina; GHD: General Hospital Dutsin-Ma; CHCD: Comprehensive Health Centre, Dutsin-Ma

Table 7: Molecular Confirmation of selected MDR isolates and detection of antibiotics resistance genes among *Staphylococcus aureus from* selected hospitals environment in Katsina State, Nigeria

Isolate IDs	Source	*Nuc*	*qnrA*	*qnrD*	*ParC*
06	Bed	+	+	+	+
14	Weighing Balance	+	-	+	+
20	Sink	+	+	+	+
28	Floor	+	-	+	+
56	Bed	+	-	-	+
Total (%)		**5 (100)**	**2 (40)**	**4 (80)**	**1(100)**

Codes: *nuc*, thermonuclease gene, *qnrA and qnrB*: quinolone resistance gene, *parC* = quinolone resistance gene + = presence, - = absence

Molecular Confirmation of identities of selected MDR isolates and detection of selected antibiotics resistance genes among *S. aureus from* selected hospitals environment in Katsina State, Nigeria

The molecular confirmation of selected multidrug-resistant (MDR) *S. aureus* isolates from hospital environments in Katsina State (Table 7) revealed that all five isolates (100%) tested positive for the *nuc* gene, confirming their identity as *S. aureus*. Among the quinolone resistance genes screened, *parC* was the most prevalent, detected in 100% of the isolates, followed by *qnrD* (80%), while *qnrA* was present in only two isolates (40%). Notably, one isolate (ID 56) lacked both *qnrA* and *qnrD*, while isolates 14 and 28 lacked *qnrA,* suggesting the involvement of chromosomal quinolone resistance mechanisms in some strains.

Fig. 1 Agarose gel electrophoresis showing molecular identification of *Staphylococcus aureus* isolates through amplification of the *nuc* gene (276 bp) using conventional PCR. Lane M represents the 100 bp DNA molecular weight marker, with visible bands ranging from 100 bp to 1500 bp. Amplified products of approximately 276 bp were observed in all tested isolates (Lanes 06, 14, 20, 28, and 56), confirming the presence of the *nuc* gene, a species-specific marker for *S. aureus*. The positive control (+ve) showed a clear band at the expected 276 bp position, validating the PCR assay. No amplification was detected in the negative control (–ve), demonstrating the absence

of contamination. These results confirm that all tested isolates were molecularly identified as *Staphylococcus aureus* based on the amplification of the *nuc* gene as it is in Figure 1.

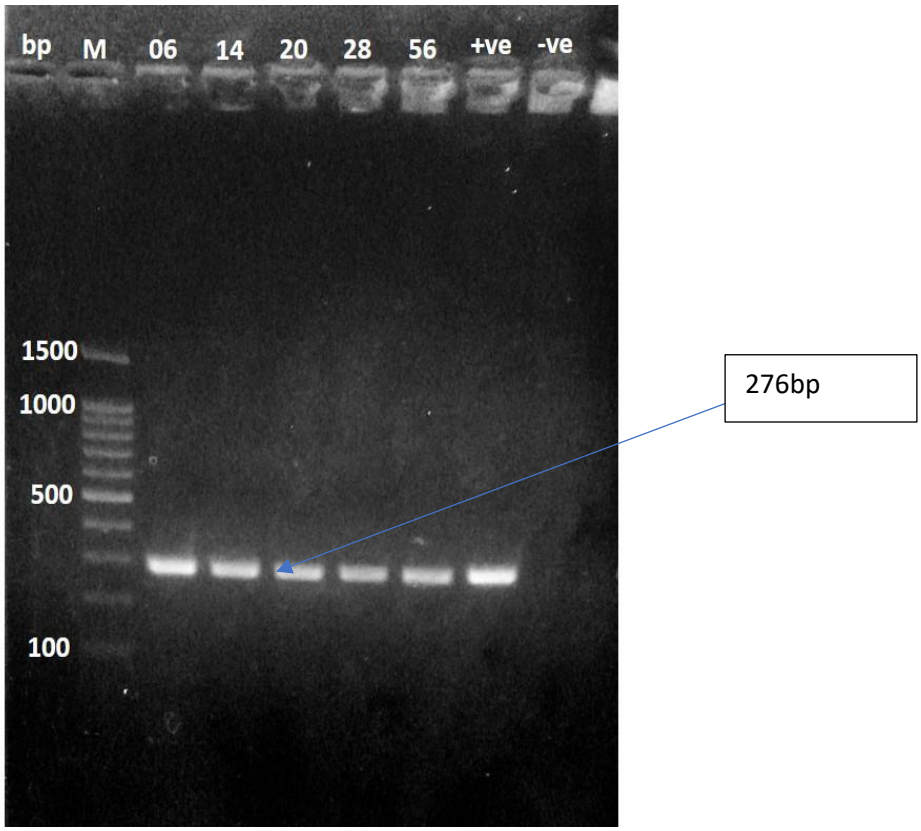

Fig.1: Gel-electrophoresis (Molecular Identification of *Staphylococcus aureus*) using Conventional PCR by amplifying the *nuc* Gene at 276bp

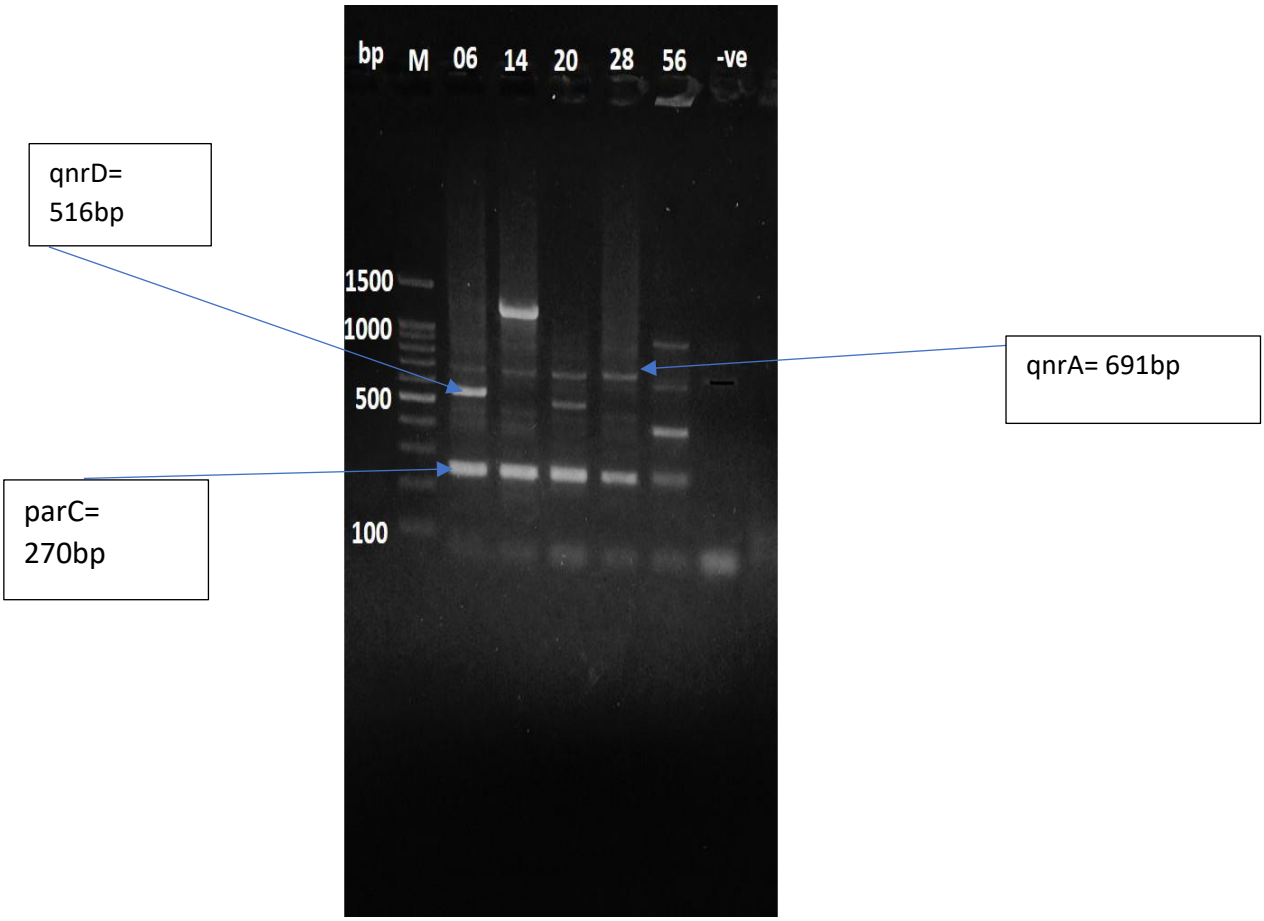

Fig. 2: Gel electrophoresis of amplified *parC*; 270bp *qnrA*; 516bp and *qnrD;* 691bp genes using PCR

Gel electrophoresis (Fig. 2) showing PCR amplification of *parC* (270 bp), *qnrA* (516 bp), and *qnrD* (691 bp) genes from *Staphylococcus aureus* isolates. Lane M represents the 100 bp molecular weight marker. Amplified products of the expected sizes were observed in several isolates: Lane 06 showed multiple amplicons, including bands consistent with the *qnrD* gene (~691 bp). Lane 14 showed a clear, intense band corresponding to the qnrA gene (~516 bp). Lanes 20 and 28 yielded bands within the expected size ranges for *parC* (~270 bp) and *qnrA* (~516 bp), although with some faint non-specific fragments. Lane 56 also exhibited a visible amplification product around ~270 bp, consistent with *parC*. No amplification was observed in the negative control (Lane –ve), confirming the absence of contamination. The presence of these amplicons indicates the detection of quinolone resistance-associated genes in the tested *S. aureus* isolates.

DISCUSSION

The observed environmental prevalence of *S. aureus* (Table 2) across hospital surfaces in this study aligns with previous reports that emphasize the persistence of *S. aureus* on inanimate hospital surfaces, particularly high-contact areas. The highest contamination found on hospital beds in this study corroborates findings by Otter and Protano *et al.* (2019), who noted that hospital beds and linens serve as frequent reservoirs for nosocomial pathogens due to continuous patient contact and inconsistent disinfection. The relatively high prevalence of *S. aureus* at Turai Children Hospital may reflect differences in infection control practices, similar to the study by Thakur and Rao (2024), which reported inter-facility variability in *S. aureus* contamination linked to hygiene protocols and patient turnover rates. Interestingly, the absence of *S. aureus* on door handles, light switches, and taps contrasts with reports by Hor *et al.* (2017), who identified these as common contamination points, suggesting more effective surface hygiene or less frequent contact in the studied hospitals. The sporadic presence of the bacterium on items such as scissors, oxygen cylinders, and drawers further supports Chirca's (2019) findings, which emphasized the role of overlooked surfaces in contributing to the overall microbial burden in hospital environments.

The high susceptibility to imipenem (Table 4) in this study aligns with previous reports by Iwalokun *et al.* (2019), who noted that carbapenems remain effective against multidrug-resistant *S. aureus* in Nigerian healthcare settings due to their restricted use and strong bactericidal activity. Gentamicin's effectiveness, particularly in two hospitals, is consistent with the findings of Oche *et al.* (2019), who reported low aminoglycoside resistance among *S. aureus* isolates from clinical and environmental sources in North-Western Nigeria. The possible reasons might be that gentamicin is usually difficult to abuse in Nigeria because it's not administered orally but intravenously and intramuscularly. Therefore, it's not sold as over the counter drugs for oral use. However, the widespread resistance to amoxicillin-clavulanate and cefoxitin, especially the 100% resistance observed at Turai Children and Maternity Hospital, strongly suggests the presence of high-level resistant *S. aureus,* corroborating studies by Richter *et al.* (2022), who documented similar β-lactam resistance trends and highlighted poor antibiotic stewardship as a key factor. The variable efficacy of ciprofloxacin and erythromycin mirrors the fluctuating resistance patterns reported by Shariati *et al.* (2022), suggesting localized antibiotic pressure and inconsistent prescribing practices.

The observed prevalence of *S. aureus* and the proportion of MDR strains (Table 5) across hospitals in Katsina State align with the findings of Ezeh *et al.* (2023), who reported a comparable MDR *S. aureus* prevalence of 50.4% among clinical isolates in southwestern Nigeria, underscoring the widespread nature of antimicrobial resistance in healthcare settings. The high MDR rate at the General Hospital mirrors the findings of Onyedibe *et al.* (2020), who reported elevated resistance in tertiary health facilities, potentially due to greater antibiotic exposure and poor infection control practices. The relatively lower MDR rates in Comprehensive and FTHK hospitals may reflect more antimicrobial policies that are effective or lower patient turnover, as Melariri *et al.* (2024) found in their study of hospital-acquired infections in sub-Saharan Africa.

The observed MDR patterns (Table 6) among *S. aureus* isolates in this study align with global and regional reports highlighting the rising prevalence of antibiotic-resistant strains in hospital environments, particularly in maternity and pediatric units. The high resistance to β-lactam antibiotics such as amoxicillin-clavulanate (AUG) and cefoxitin (FOX) corroborates findings by Uzoma *et al.* (2025), who reported widespread β-lactam resistance among *S. aureus* isolates in southwestern Nigeria, largely due to β-lactamase production. The concentration of extensively drug-resistant strains at Turai Children and Maternity Hospital may be linked to antibiotic overuse or inadequate infection control, consistent with the findings of Onyedibe *et al.* (2020), who emphasized the role of hospital-specific antimicrobial policies in shaping local resistance profiles.

The detection of the *nuc* gene (Table 7) in all *S. aureus* isolates aligns with previous findings, affirming its reliability as a molecular marker for species confirmation (Kadhum *et al.*, 2024). The high prevalence of *parC* among the isolates indicated that *parC* is present in all isolates, although studies have reported high but not universal rates of QRDR mutations in *parC* (often in combination with gyrA mutations) among fluoroquinolone-resistant *S. aureus* strains (de Oliveira *et al.,* 2019). This elevated occurrence in Katsina may reflect regional differences in antibiotic usage and infection control practices. The detection of *qnrA* (Fig. 2) in isolates is consistent with the findings of Kumari *et al.* (2020), who reported *qnrA* in 35–50% of clinical *S. aureus* isolates in India, suggesting a moderate but concerning level of plasmid-mediated resistance. The co-expression of multiple resistance genes, underscores the risk of horizontal gene transfer, which has been widely reported as a driver of multidrug

resistance in hospital-associated *S. aureus* (Ioannou *et al.*, 2022).

CONCLUSION

The findings from this study provide critical insights into the burden and complexity of Staphylococcus aureus contamination in maternity hospitals in Katsina State, Nigeria. The relatively high prevalence of isolates from high-contact surfaces such as beds and floors underscores the potential for environmental transmission of pathogenic and multidrug-resistant strains. Alarmingly, over 50% of the isolates were multidrug-resistant, with especially high resistance rates in General Hospital and Turai Hospital, posing serious treatment challenges for maternal and neonatal care. The consistent resistance to β-lactam antibiotics, particularly amoxicillin-clavulanate and cefoxitin, raises concerns over the spread of resistant *S. aureus*. Molecular analysis further confirmed the presence of plasmid-mediated quinolone resistance genes (*qnrA* and *qnrD*) and chromosomal resistance gene (*parC*), suggesting both horizontal and vertical mechanisms of resistance propagation. The co-existence of multiple resistance determinants in several isolates suggests the potential for widespread dissemination in clinical settings. These findings underscore the urgent need for routine environmental surveillance, stricter hygiene protocols, and rational antibiotic-use policies in hospitals. Implementing targeted infection prevention and antimicrobial stewardship programs will be essential to mitigate the risk of healthcare-associated infections and limit the emergence of untreatable *S. aureus* strains in Nigeria's healthcare system.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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