

Isolation, Molecular Characterization, and Multidrug Resistance Profiling of Extended-spectrum β -lactamase-producing *Escherichia Coli* Found on Healthcare Environmental Surfaces in Katsina State, Nigeria

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ABSTRACT

Multidrug resistance (MDR) in *E. coli* is an escalating public health threat, especially in clinical and maternity environments. A cross-sectional study was conducted across four healthcare facilities: General Hospital Dutsin-Ma, Comprehensive Healthcare Centre (CHC) Dutsin-Ma, Federal Teaching Hospital Katsina (FTHK), and Turai Children and Maternity Hospital (TCMH). A total of 120 swab samples were taken from 24 different environmental surfaces and equipment, including clinical devices, furniture, and wet utilities. Gram staining, biochemical test and cultural techniques were used to identify the isolates using standard methods. Antimicrobial susceptibility testing was carried out using Kirby-Bauer disc diffusion method. The results showed that *E. coli* recovery was found only on the wet utilities at CHC (n=3, 50.0%) and TCMH (n=3, 50.0%), with no bacterial recovery noted at General Hospital or FTHK. In total, sinks accounted for 50.00% (n=3) of the isolated bacteria, followed by toilet seats at 33.33% (n=2) and floors at 16.67% (n=1); clinical tools and direct care equipment showed no bacterial growth. All isolates were confirmed as *E. coli* through the amplification of the uidA gene. Notably, 66.67% (n=4) of the isolates were ESBL producers. Complete resistance to ciprofloxacin, erythromycin, amoxicillin-clavulanate, and cefoxitin was found among the isolates from CHC. All the isolates were susceptible to imipenem. MDR strains screened (n=2, 50.0%) carried both the blaTEM and blaCTX resistance genes. In conclusion, wet utilities, particularly sinks and toilet seats, serve as significant environmental reservoirs for ESBL-producing, MDR *E. coli*. There is need for targeted environmental decontamination strategies focusing on sanitary infrastructure

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INTRODUCTION

Antimicrobial resistance (AMR) among common bacterial infections is a serious challenge to the efficient provision of healthcare around the globe. Particularly, one of the main agents behind nosocomial infections, *Escherichia coli*, have become more resistant to quinolones and β -lactam antibiotics, which are cornerstones in the fight against bacterial infections (WHO, 2022). Given that invasive procedures and potentially unhealthy conditions put vulnerable women and babies at higher risk of infection, this trend is particularly concerning in hospital labour rooms (Feldmann-Jensen *et al.*, 2024).

Labour rooms can act as harbours for resistant bacteria, allowing them to spread through contaminated surfaces, tools, and healthcare personnel's hands. This fact is frequently disregarded in surveillance attempts (Kalpana *et al.*, 2022). Specific measures to minimize infection risks and stop the spread of resistance are limited by the absence of localized data on multi-drug resistant (MDR) *E. coli* and *S. aureus* in Katsina State. In order to improve infection control procedures in labour rooms and inform antibiotic policy, it is crucial to characterize β -lactamase-producing and quinolone-resistant bacteria from these environments.

Mothers and newborn outcomes are greatly impacted by AMR in labour rooms. Infections by resistant strains of *E. coli* frequently cause puerperal sepsis, a leading cause of maternal mortality in Nigeria and other low- and middle-income countries (LMICs), making treatment more difficult and increasing hospital stays (Oladokun *et al.*, 2023). Likewise, newborn sepsis caused by MDR agents raises the risk of both

long-term morbidity and neonatal mortality. In comparison to infections caused by susceptible strains, studies conducted in Nigeria reveal that neonates infected with ESBL-producing *E. coli* experienced worse clinical outcomes and greater rates of treatment failure (Joseph *et al.*, 2023). Since treating resistant infections necessitates the use of more costly antibiotics, longer hospital stays, and more intensive care units, the financial burden on healthcare systems is also significant (Ajekigbe *et al.*, 2025). These effects emphasize how important it is to incorporate MDR management into initiatives for mother and newborn health.

Environmental studies in African hospitals had revealed alarming rates of contamination with MDR bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii*. These organisms were frequently isolated from highly-touched surfaces such as delivery beds, baby cots, sinks, and incubators. Contamination was particularly high in maternity, newborn, and surgical departments (Odoayo *et al.*, 2023). Antibiotic resistance among these pathogens is often due to the acquisition of genes that confer resistance to critical antibiotics such as β -lactams and quinolones. These genes can be carried on mobile genetic elements, allowing bacteria to spread resistance rapidly across hospital environments. In addition, inadequate disinfection, overcrowding, and poor antimicrobial stewardship further facilitate the survival and dissemination of MDR organisms (Valzano *et al.*, 2024). Public health is seriously threatened by the rising incidence of antimicrobial resistance (AMR), particularly among

Escherichia coli and *Staphylococcus aureus*, especially in environments with low resources like Katsina State, Nigeria. Due to the intrusive procedures and frequent contact to biological fluids, labour rooms are high-risk environments that might harbor MDR infections (Elkady *et al.*, 2022). Surveillance information on the bacterial contamination and resistance profiles of isolates in these settings is, however, severely lacking (Doi *et al.*, 2017).

In order to guide empirical therapy and update antibiotic policy in maternal healthcare, it is crucial to understand the mechanisms of resistance, especially β -lactamase synthesis in *E. coli*. Despite the widespread use of β -lactams and quinolones for obstetric infections, growing resistance has resulted in treatment failures, longer hospital admissions, and higher rates of maternal and newborn deaths (WHO, 2022). The lack of regional data frequently forces healthcare professionals to rely on old or universal protocols, which may not be effective against changing resistance trends.

This study intends to close a significant information gap by directly investigating and characterizing resistant strains from labour rooms' environment in some selected hospitals within Katsina State, Nigeria. The results will support regional and national AMR surveillance efforts, encourage antimicrobial stewardship in maternal health settings, and help create targeted interventions for infection prevention and control (IPC). Additionally, the study will offer baseline data required for more research and policy development,

particularly in Nigeria's endeavours to carry out the National Action Plan on AMR (Alabi, 2024).

MATERIALS AND METHODS

Study Area

The study was conducted in Dutsin-Ma and Katsina metropolis, both located in Katsina State, Northwestern Nigeria. Dutsin-Ma ($12^{\circ}26'N$, $07^{\circ}29'E$) covers 552.323 km² with a population of 169,829 as recorded in the 2006 National Census, projected to 174,245 in 2021 using a 2.6% annual growth rate. It is bordered by Kurfi and Charanchi to the north, Kankia to the east, Safana and Dan-Musa to the west, and Matazu to the southeast. The town also hosts the Federal University Dutsin-Ma, established in 2011, which has contributed to increased population influx. Katsina town ($12^{\circ}59'N$, $07^{\circ}36'E$), the state capital, had a 2006 census population of 318,459 (Dauda *et al.*, 2016), projected to over 366,000 in 2021. It is bordered by Batagarawa, Charanchi, and Jibia Local Government Areas (LGAs) (Milaham *et al.*, 2022).

This study was conducted across four selected healthcare facilities in Katsina State, Nigeria: General Hospital Dutsin-Ma, Comprehensive Hospital Dutsin-Ma, Federal Teaching Hospital Katsina (FTHK), and Turai Children Hospital Katsina. These hospitals were chosen based on their high patient throughput and their key roles in maternal and neonatal healthcare delivery within the region.

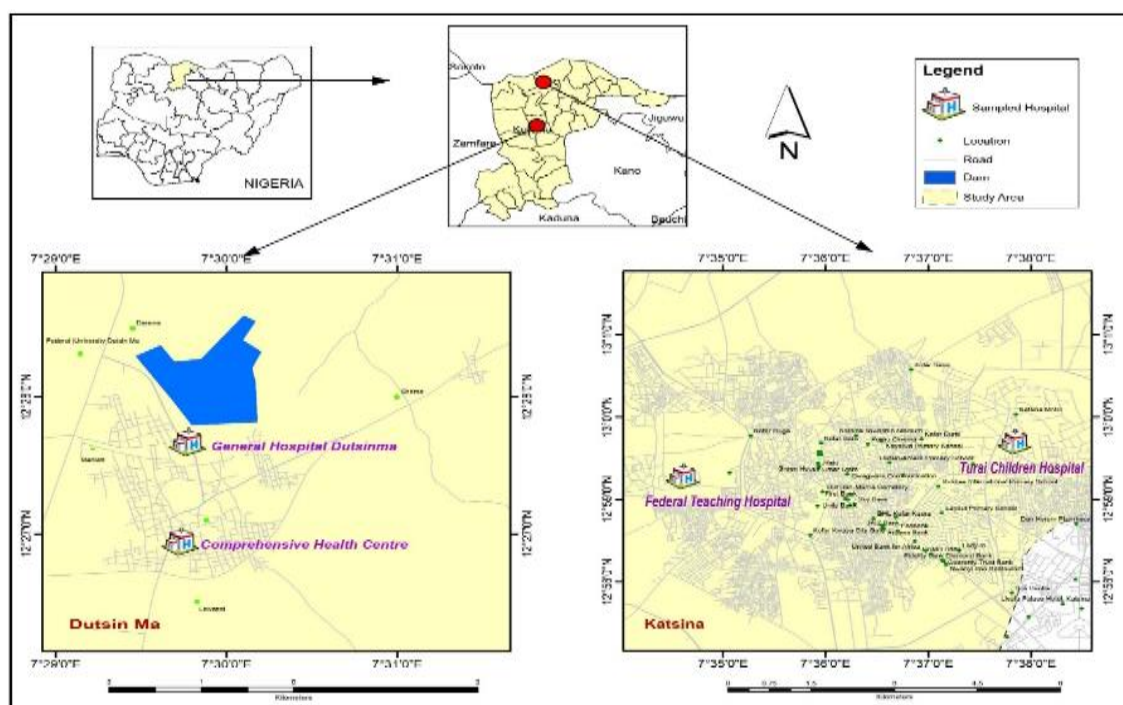


Figure 1: Map showing sampling locations within Katsina State Metropolis (Menegbo & Ojiako, 2018)

Study Design

A cross-sectional descriptive study design was adopted to investigate and characterize quinolone-resistant and β -lactam resistance multidrug-resistant (MDR) *Escherichia coli* from labour rooms' environment that includes: surfaces and instruments of some hospitals within Katsina State, Nigeria (Fagbamila *et al.*, 2016).

Collection of Samples

A total of 120 samples were collected from various surfaces that include: beds, walls, floors, trolleys, scissors, curtains,

mopping sticks, door handles, dripping hangers, drawers, forceps, refrigerators, fan regulators, infant radiant warmers, light switches, medication drawers, nasal tubes, wheelchairs, toilet seats, oxygen cylinders, sinks, and weighing machines using sterile swab sticks moistened with normal saline within the labour rooms of the four hospitals. The samples were transported in ice packs as recommended by WHO (2020) to the Microbiology laboratory, Department of Microbiology, Federal University Dutsin-Ma.

Media Preparation

All media used in the study were prepared according to the manufacturer's instructions, which include: eosin Methylene Blue Agar (EMB), Nutrient Agar (NA), Simon Citrate, Methyl Red-Voges Proskauer (MR-VP), and Mueller Hinton Agar (MHA) (Mousum, 2021).

Bacterial Isolation

Sterile swab sticks moistened with sterile normal saline were used to swab the selected surfaces in the maternity ward by rubbing the swab firmly over an approximately 10 cm × 10 cm area using a rotating motion to ensure adequate sample collection. Immediately after collection, each swab was streaked onto pre-prepared sterile Eosin Methylene Blue (EMB) agar plates. The inoculated plates were incubated at 37°C for 24 hours. After incubation, colonies exhibiting a green metallic sheen on EMB were presumed to be *Escherichia coli*. After incubation, the colonies were further sub-cultured on NA to obtain a pure culture. The resulting pure isolates were then transferred onto NA slants and stored at 4 °C for subsequent analyses (Idris, 2021).

Gram's Staining

A bacterial smear was prepared on a clean, grease-free microscope slide using colonies from 24 hour-old cultures grown on Eosin Methylene Blue (EMB) agar. The smears were air-dried and heat-fixed by passing them over a Bunsen burner flame. Crystal violet, serving as the primary stain, was applied to the smear and left for 60 seconds, followed by gentle rinsing with tap water for approximately 3 seconds to remove excess stain. Lugol's iodine (mordant) was then added and left for 60 seconds to fix the crystal violet complex, after which the slide was rinsed gently with water. Decolorization was carried out using alcohol for a few seconds, followed by immediate rinsing with water. Carbol fuchsin, used as a counterstain, was applied for 30 seconds and then washed off with water for 5 seconds. The slide was allowed to air-dry and subsequently examined under a light microscope using the oil immersion objective lens (100× magnification). Gram-negative rod-shaped cells observed were presumptively identified as *Escherichia coli* (Idris, 2021).

Biochemical Tests for *E. coli*

The presumptive *E. coli* isolates were subjected to a series of standard biochemical tests (Indole test, Citrate utilization, Methyl red test, and Voges-Proskauer) for further identification as described by (Mousum, 2021; Idris, 2021).

Antibiotic Susceptibility Testing

The antibiotic susceptibility of the bacterial isolates was assessed using the Kirby-Bauer disc diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2022). The isolates were tested against various antibiotics from different classes, including β -lactams (amoxicillin-clavulanic acid, 30 μ g), carbapenems (imipenem, 10 μ g), aminoglycosides (gentamicin, 10 μ g), macrolides (erythromycin, 15 μ g), fluoroquinolones (ciprofloxacin, 5 μ g), and cephalosporins (cefoxitin, 10 μ g; ceftazidime, 30 μ g; cefuroxime, 30 μ g) using commercially available Mast discs (Mast Diagnostics). A 0.5 McFarland

standard suspension of each isolate was prepared and evenly swabbed onto Mueller-Hinton agar plates with sterile cotton swabs (Dimri *et al.*, 2020). The plates were left for about 5 minutes to allow absorption, then antibiotic discs were aseptically placed onto the agar surface with sterile forceps. The plates were incubated at 37°C for 24 hours. After incubation, the diameters of the zones of inhibition around each antibiotic disc were measured in millimeters with a 30 cm ruler. Susceptibility results were interpreted based on CLSI (2022) breakpoints, classifying isolates as susceptible, intermediate, or resistant. Isolates resistant to at least three different antibiotic classes were classified as multidrug-resistant (MDR) (Dimri *et al.*, 2020).

DNA Extraction for PCR Amplification

Genomic DNA extraction was performed using the AccuPrep® Genomic DNA Extraction Kit (Bioneer, South Korea) in accordance with the manufacturer's protocol. DNA templates were prepared from pure colonies of *Escherichia coli* isolates. Colonies from overnight cultures were suspended in 200 μ L of phosphate-buffered saline (PBS) in a sterile 1.5 mL microcentrifuge tube. Subsequently, 200 μ L of cell lysis buffer was added, and the mixture was vortexed for 10 seconds to ensure homogenization. The suspension was then incubated at room temperature for 10 minutes. Following incubation, 200 μ L of absolute ethanol was added and the mixture was vortexed again for 10 seconds to precipitate the nucleic acids. The entire mixture was transferred to an adsorption column placed in a collection tube and centrifuged at 8,000 rpm for 1 minute. The flow-through was discarded. The column was then washed with 600 μ L of washing buffer 1 and centrifuged at 8,000 rpm for 1 minute. The filtrate was discarded and the washing step was repeated using 600 μ L of Washing Buffer 2, followed by centrifugation under the same conditions. To ensure complete removal of residual wash buffer, the column was reinserted into the collection tube and centrifuged at 8,000 rpm for an additional 2 minutes. The collection tube and flow-through were discarded, and the adsorption column was transferred to a new sterile RNase/DNase-free 1.5 mL microcentrifuge tube. A total of 50 μ L of elution buffer was applied to the center of the membrane within the column and incubated at room temperature for 1 minute. Elution was carried out by centrifugation at 8,000 rpm for 1 minute. The resulting purified genomic DNA was collected in a microcentrifuge tube and stored at -20 °C for subsequent molecular analysis (Iseghohi *et al.*, 2020).

Confirmation of *E. Coli*

Escherichia coli uidA gene (encoding β -glucuronidase) was targeted for its molecular identification. The expected amplicon size was 168 bp. Amplification was carried out using a specific pair of uidA primers (Table 1). Each 20 μ L reaction contained 10 μ L of 2× EasyTaq® PCR SuperMix, 1 μ L of each primer (forward and reverse), 2 μ L of template DNA (i.e. amplified DNA above), and 6 μ L of nuclease-free water. The thermal cycling conditions for this reaction are presented in Table 1. To determine the size of the amplified fragments, DNA bands were visualized and documented using a gel documentation system (Fisher Biotech Fb 105, USA) (Idris, 2021).

Table 1: Primers used for the Molecular Confirmation in *Escherichia Coli* from Labour Rooms from selected Hospitals within Katsina State, Nigeria

Bacteria	Target Genes	Primer Sequences (5'→3')	Product Sizes (bp)	Annealing Temperatures (°C)	Final Extension	Bacteria
E. coli	UidA	F: TGGTAATTACCGACGAAAACGGC				E. coli
						UidA

Detection of Antibiotic Resistance Genes (*bla*_{TEM}, *bla*_{CTX}, *parC*, *qnrB* and *qnrD*)

Multiplex PCR was employed to simultaneously detect two antibiotic resistance genes (*bla*_{TEM} and *bla*_{CTX}) within the bacterial isolates. Extracted genomic DNA served as the template for PCR amplification. The specific primers used for each target gene are detailed in (Table 2). The PCR reaction was prepared in a final volume of 20 µL, comprising 10 µL of premix containing dNTPs, Taq DNA polymerase, and 1.5 mM MgCl₂; 3 µL of a primer mix (forward and reverse primers, 3 µL each); 2 µL of template DNA; and 5 µL of nuclease-free water. Amplification was performed in a thermal cycler (PTC-100™, USA) under the following

cycling conditions: an initial denaturation at 95°C for 5 minutes; then 35 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute; followed by a final extension at 72°C for 5 minutes. The resulting PCR products were resolved on a 1.5% agarose gel stained with ethidium bromide (0.5µg/mL). Electrophoresis was run at 100 V for approximately 45 minutes alongside a 100 bp DNA ladder (Intron Biotechnology) to determine the size of the amplified fragments. DNA bands were visualized and documented using a gel documentation system (Fisher Biotech Fb 105, USA) (Musa *et al.*, 2026).

Table 2: Primers used for detection of antibiotic resistance genes in *Escherichia coli* from labour room within hospitals from Katsina State, Nigeria

Bacteria	Target Genes	Primer Sequences (5'→3')	Product Sizes (bp)	Annealing Temperatures (°C)	Final Extension Temperatures	References
<i>E. coli</i>	<i>bla</i> _{TEM}	F: GTATCCGCTCATGAGACAATAACCCAR: CCAATGCTTAATCAGTGAGGCACC	918	58°C	72°C for 5 minutes	Egwatu <i>et al.</i> , 2023
<i>E. coli</i>	<i>bla</i> _{CTX}	F: CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	R: 550	60°C	72°C for 5 minutes	Egwatu <i>et al.</i> , 2023

RESULTS AND DISCUSSION

In this study, twenty-four distinct sampling sites across four secondary and tertiary healthcare centers were assessed. *E. coli* contamination was found on three specific environmental surfaces: the sink, toilet seat, and floor. Sinks had the highest cumulative recovery rate, contributing 3 isolates (50.00%) of the total recovered bacteria (n = 6), followed by toilet seats with 2 isolates (33.33%), and floors with 1 isolate (16.67%). All other sampled surfaces, including medical equipment, furniture, door handles, and clinical instruments, showed no signs of bacterial growth (0.00%). Facility-wise, it became

clear that the contamination was limited to two institutions: Comprehensive Hospital and Turai Children Hospital, each reporting 3 isolates (50.00%) of the total *E. coli* found. At Comprehensive Hospital, the isolates were mainly from sinks (2 isolates, 50.00%) and floors (1 isolate, 20.00%). On the other hand, Turai Children Hospital had contamination from toilet seats (2 isolates, 100%) and sinks (1 isolate, 25.00%). Interestingly, General Hospital and the Federal Teaching Hospital showed no bacterial recoveries (0.00%) at any of the tested sites.

Table 3: Distribution of *E. coli* isolates from different sites at various Hospital within Katsina State, Nigeria

Sites	Comprehensive Hospital <i>E. coli</i> (%)	General Hospital <i>E. coli</i> (%)	Turai Children Hospital <i>E. coli</i> (%)	Federal Teaching Hospital <i>E. coli</i> (%)	Total Occurrence of isolate (%) <i>E. coli</i> (%)
Bed	-	-	-	-	0 (0.00)
Curtains	-	-	-	-	0 (0.00)
Chair	-	-	-	-	0 (0.00)
Door Handles	-	-	-	-	0 (0.00)
Dripping Hanger	-	-	-	-	0 (0.00)
Drawer	-	-	-	-	0 (0.00)
Floor	1 (20.0)	-	-	-	1 (16.67)
Forcep	-	-	-	-	0 (0.00)
Fridge	-	-	-	-	0 (0.00)
Fan Regulator	-	-	-	-	0 (0.00)
Infant Radiant Warmer	-	-	-	-	0 (0.00)
Light Switch	-	-	-	-	0 (0.00)
Medication Drawer	-	-	-	-	0 (0.00)
Mopping Stick	-	-	-	-	0 (0.00)
Nasal Tube	-	-	-	-	0 (0.00)
Oxygen Cylinder	-	-	-	-	0 (0.00)
Sink	2 (50.00)	-	1 (25.00)	-	3 (50.00)
Scissors	-	-	-	-	0 (0.00)
Toilet seat	-	-	2 (100)	-	2 (33.33)

Sites	Comprehensive Hospital <i>E. coli</i> (%)	General Hospital <i>E. coli</i> (%)	Turai Children Hospital <i>E. coli</i> (%)	Federal Teaching Hospital <i>E. coli</i> (%)	Total Occurrence of isolate (%) <i>E. coli</i> (%)
Tap	-	-	-	-	0 (0.00)
Table	-	-	-	-	0 (0.00)
Trays	-	-	0 (0.00)	-	0 (0.00)
Wheel chair	-	-	-	-	0 (0.00)
Weighing machine	-	-	-	-	0 (0.00)
Wall	-	-	-	-	0 (0.00)
Total	3 (100)	0 (0.00)	3 (100)	0 (0.00)	6 (100)

Key: - = Not isolated

In Table 4, *E. coli* isolates demonstrated 100% resistance to each of ciprofloxacin, erythromycin, amoxicillin-clavulanate, and cefoxitin in Comprehensive Healthcare Centre. In contrast, imipenem was 100% effective against *E. coli* in both

hospitals. Gentamicin showed some variability: only 33.33% of isolates were susceptible at the Comprehensive Healthcare Centre compared to 100% susceptibility at Turai Hospital.

Table 4: Antibiotic Susceptibility Profiles for *E. Coli* Isolated from Hospitals within Katsina State, Nigeria

Antibiotics	Comprehensive Healthcare Centre			Turai Children and Maternity Hospital		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Ciprofloxacin	0 (0.00)	0 (0.00)	3 (100)	2 (66.67)	0 (0.00)	1 (33.33)
Erythromycin	0 (0.00)	0 (0.00)	3 (100)	1 (33.33)	0 (0.00)	2 (66.67)
Imipenem	3 (100)	0 (0.00)	0 (0.00)	3 (100)	0 (0.00)	0 (0.00)
Amoxicillin clavulanate	0 (0.00)	0 (0.00)	3 (100)	2 (66.67)	0 (0.00)	1 (33.33)
Gentamicin	1 (33.33)	0 (0.00)	2 (66.67)	3 (100)	0 (0.00)	0 (0.00)
Cefoxitin	0 (0.00)	0 (0.00)	3 (100)	2 (66.67)	0 (0.00)	1 (33.33)

Keys: S= Susceptible, I= Intermediate, R= Resistant

Among the four identified multidrug-resistant isolates, it was found that the presence of MDR *E. coli* was limited to two healthcare facilities: the Comprehensive Health Centre and Turai Umaru Yar'Adua Hospital, with each site reporting two isolates, which is about 6.67%. On the flip side, multidrug resistance phenotypes in the *E. coli* strains from the General

Hospital or the Federal Teaching Hospital Katsina (both at 0.00%) was not detected. Overall, these results point to a low yet localized occurrence of multidrug-resistant *E. coli* in the clinical settings we examined throughout the state.

Table 5: Prevalence of Multidrug Resistant *E. Coli* from Various Hospitals in Katsina State, Nigeria

Hospitals	No. of MDR <i>E. coli</i>
General hospital Katsina	0 (0.00%)
CHC	2 (6.67%)
FTHK	0 (0.0%)
TCMH	2 (6.67%)
Total	4

Keys: MDR= multidrug resistant, FTHK =Federal Teaching Hospital Katsina, TCMH= Turai children and maturity Hospital, CHC = Comprehensive health centre

Table 6 presents the resistant phenotypes of MDR *Escherichia coli* isolates obtained from selected hospitals within Katsina State, Nigeria. A total of four MDR *E. coli* isolates were identified, with resistance patterns involving combinations of four or five antibiotics. Comprehensive Hospital Dutsin-Ma reported two MDR isolates: one resistant

to four antibiotics, ciprofloxacin, erythromycin, amoxicillin-clavulanate, and cefoxitin and one also resistant to a broader five-antibiotic profile that additionally included gentamicin. Similarly, Turai Hospital Katsina and Comprehensive Health Centre Dutsin-Ma recorded all isolates showing resistance to the same four-drug combination.

Table 6: Resistant Phenotypes of MDR *Escherichia Coli* Isolates Isolated from selected Hospitals within Katsina State, Nigeria

Hospitals	No. of Antibiotics	MDR Patterns	No. of Isolates
Comprehensive Hospital Dutsin-Ma	4	CIP, ERY, AUG, FOX	1
	5	CIP, ERY, AUG, GEN, FOX	1
		Total	2
Turai Hospital Katsina	4	CIP, ERY, AUG, FOX	1
	5	CIP, ERY, AUG, FOX, GEN,	1
		Total	2

Hospitals	No. of Antibiotics	MDR Patterns Grand Total	No. of Isolates 4
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Keys: CIP = Ciprofloxacin, ERY = Erythromycin, AUG = Amoxicillin-clavulanate (Augmentin) FOX = Cefoxitin, GEN = Gentamicin

Table 7 showed the distribution of ESBL-producing *E. coli* isolates from two hospital environments. Out of six isolates, four (66.67%) were ESBL producers while two (33.33%) were non-producers. Both Turai Children and Maternity

Hospital (TCMH) and Comprehensive Healthcare Centre (CHC) recorded an equal proportion of ESBL-producing (66.67%) and non-producing (33.33%) isolates.

Table 7: Extended Beta Lactamases (ESBL) producing *E. coli* isolated from two hospital environments

Hospitals	ESBL-producing (%)	Non-ESBL-producing (%)
TCMH	2 (66.67)	1 (33.33)
CHC	2 (66.67)	1 (33.33)
Total	4 (100)	2 (100)

Keys: TCMH= Turai children and maturity Hospital, CHC = Comprehensive health centre

Table 8 revealed consistent presence of the *uidA* gene in all (100%) tested strains, confirming the identity of the isolates as *E. coli*. The *uidA* gene, which encodes the enzyme beta-glucuronidase, is a well-established molecular marker used for *E. coli* confirmation. Regarding antibiotic resistance

genes, 50% of the isolates carried the *bla*_{TEM} gene, and an equal proportion harbored the *bla*_{CTX} gene. Both *bla*_{TEM} and *bla*_{CTX} genes are responsible for encoding beta-lactamase enzymes that confer resistance to beta-lactam antibiotics, such as penicillins and cephalosporins.

Table 8: Molecular Confirmation of MDR *E. Coli* Isolates and Detection of Antibiotics Resistance Genes Among *E. Coli* from Selected Hospitals Environment within Katsina State, Nigeria

Strain IDs	<i>uidA</i>	<i>bla</i> _{TEM}	<i>bla</i> _{CTX}
1	+	+	+
2	+	-	-
3	+	-	-
4	+	+	+
Total (%)	4 (100)	2 (50)	2 (50)

Keys: *uidA*= *E. coli* beta –glucuronidase, *bla*_{TEM} and *bla*_{CTX} = beta lactam resistance

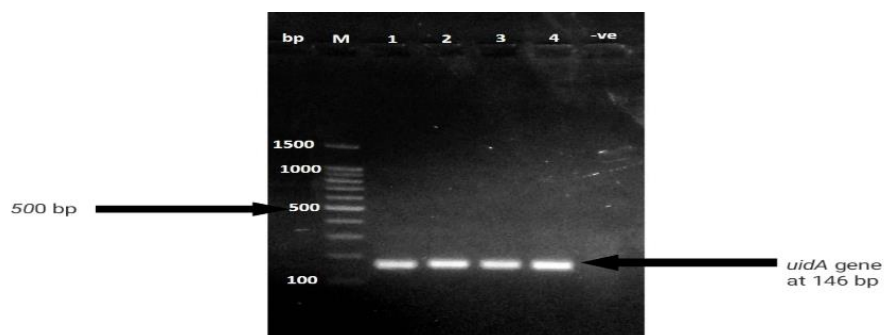


Plate 1: Gel electrophoregram of *uidA* genes in *E. coli*

Lane M represents the DNA ladder (100 bp to 1500 bp) used to estimate the size of the PCR products. Lanes 1 through 4 shows the confirmation PCR amplicons of *E. coli* isolates from hospital environments within Katsina State, Nigeria. All displayed clear and distinct bands of approximately 146 bp,

corresponding to the expected size of the *uidA* gene fragment. The negative control in lane -ve shows no amplification, validating the specificity of the PCR reaction and the absence of contamination.

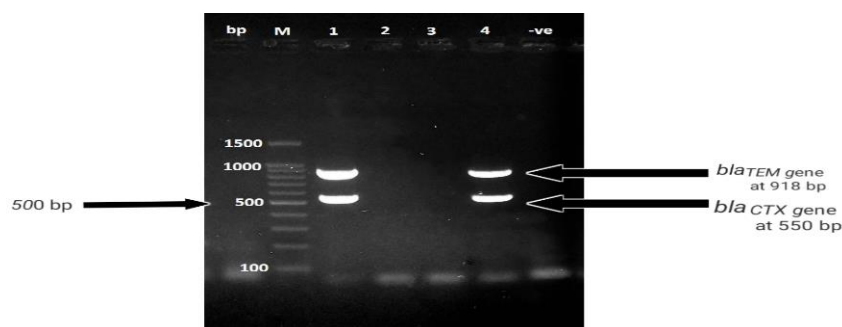


Plate 2: Gel electrophoregram of *bla*_{TEM} and *bla*_{CTX} genes amplified and detected in *E. coli*

It illustrates the amplification of extended-spectrum beta-lactamase (ESBL) encoding genes *bla*_{TEM} and *bla*_{CTX} from *Escherichia coli* isolates using conventional PCR. The gel electrophoresis shows two distinct bands at approximately 918 bp and 550 bp in lanes 1 and 4, corresponding to *bla*_{TEM} and *bla*_{CTX}, respectively. No amplification is observed in the negative control (-ve), confirming the assay's specificity.

Discussion

The spread of extended-spectrum β -lactamase (ESBL)-producing and multidrug-resistant (MDR) *Escherichia coli* in hospital labour wards pose a significant challenge for infection prevention and control (IPC), especially in low- and middle-income countries (LMICs). This study sheds light on the local distribution, resistance mechanisms, and genetic characteristics of *E. coli* contaminants found in four healthcare facilities in Katsina State, Nigeria. *E. coli*'s preference for sinks and toilet seats is consistent with menace cause by Gram-negative enteric bacilli. Sinks create damp environments filled with organic matter and stagnant water, which are perfect for biofilm formation, allowing these bacteria to evade standard cleaning products. Likewise, toilet seats are often re-contaminated due to fecal aerosolization during flushing and direct contact (Lam et al., 2025). These findings highlight that hospital sanitation facilities can serve as ongoing reservoirs for fecal pathogens in healthcare settings. Interestingly, it was observed that no *E. coli* on critical clinical equipment or high-touch surfaces like delivery beds and door handles. This stands in stark contrast to broader studies in African maternity wards, where delivery beds, baby cots, and incubators often show heavy microbial contamination (Odoyo et al., 2023). This difference might indicate varying hygiene practices in the hospitals surveyed, where routine cleaning focuses on visible clinical equipment right after delivery, while non-clinical areas like sinks, drainage points, and communal toilets may not receive the same level of attention or effective disinfectant use.

Interestingly, contamination was found only at the Comprehensive Healthcare Centre (CHC) and Turai Children and Maternity Hospital (TCMH), while General Hospital and Federal Teaching Hospital Katsina (FTHK) had no isolates. This variation between institutions really underscores how much infrastructure quality and adherence to standard infection prevention and control (IPC) protocols can make a difference. Tertiary facilities like FTHK usually have the advantage of organized cleaning schedules, dedicated sanitation teams, and rigorous environmental monitoring. In contrast, secondary and comprehensive centers often struggle with overcrowding, insufficient water, sanitation, and hygiene (WASH) facilities, and limited resources (Valzano et al., 2024).

The fact that there's still a high susceptibility to imipenem shows that carbapenems are still the go-to treatment for severe infections caused by ESBL-producing Gram-negative bacteria in this area. This aligns with surveillance studies from Nigeria, which indicate that carbapenem resistance in environmental *E. coli* is relatively low when compared to β -lactams and fluoroquinolones (Beshiru et al., 2024). It's quite concerning to see that amoxicillin-clavulanate (Augmentin) and ciprofloxacin have completely failed at CHC. These medications are often the first choice for treating maternal urinary tract infections, puerperal sepsis, and intra-partum infections in Nigerian healthcare settings (Oladokun et al., 2023). The high resistance rates to fluoroquinolones and co-amoxiclav can be attributed to the easy, unregulated access to antibiotics and the tendency to over-prescribe them in the

community (Adegboyega et al., 2025). Additionally, the high resistance to cefoxitin, a second-generation cephamycin, hints at a possible increase in chromosomal AmpC β -lactamases or plasmid-mediated AmpC production alongside ESBLs.

The 66.67% prevalence of ESBL among environmental isolates is consistent with the high rates seen in both clinical and environmental contexts in Nigeria (Adenipekun et al., 2016; Beshiru et al., 2024). The presence of multidrug-resistant (MDR) ESBL-producing *E. coli* in labour rooms is a serious concern for maternal and neonatal health. Environmental transmission during labor or in the early days of neonatal care can result in puerperal sepsis and early-onset neonatal sepsis, conditions that carry a high risk of mortality due to delays in effective treatment (Joseph et al., 2023). Although the total number of isolates recovered in this study is relatively small, which limits broad epidemiological conclusions, the high proportion of MDR/ESBL phenotypes among these isolates highlights the strong selective pressure in hospital microenvironments. Even a small number of environmental pathogens can pose a considerable risk, especially when these organisms have developed resistance to crucial first-line treatments.

The fact that both *bla*_{TEM} and *bla*_{CTX} were observed in 50% of the MDR strains really points to a genetic reason behind the observed traits. *bla*_{CTX-M} type β -lactamases are well-known as the main players in extended-spectrum cephalosporin resistance globally (Adelowo et al., 2017). The *bla*_{TEM} resistant gene, which usually codes for either narrow-spectrum TEM-1 or its extended-spectrum variants, shows up alongside *bla*_{CTX} on mobile genetic elements like conjugative plasmids or insertion sequences such as *ISE*_{cp1}, makes it easier for these genes to spread between Gram-negative bacteria, especially in crowded hospital settings (Ajekiigbe et al., 2025). On the flip side, two MDR isolates that tested negative for both *bla*_{TEM} and *bla*_{CTX} were found, even though they still showed ESBL and MDR characteristics. This difference really underscores how complex β -lactam resistance can be. For these strains that lack the usual genes, resistance might be due to other ESBL variants (like *bla*_{SHV}, *bla*_{OXA}, or *bla*_{PER}), plasmid-mediated AmpC β -lactamases (such as *bla*_{CMY}), carbapenemases, or even non-enzymatic methods like porin loss (think *OmpF*/*OmpC* changes) paired with the overactivity of multidrug efflux pumps (like *AcrAB-TolC*) (Edupuganti, 2021).

CONCLUSION

In conclusion, this study highlights a concerning issue: sanitary utilities like sinks and toilet seats in hospital labour rooms are acting as breeding grounds for *bla*_{TEM} and *bla*_{CTX}-carrying multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL) producing *E. coli*.

To reduce the risk of hospital-associated infections for mothers and newborns in Katsina State, there is need to focus on some key infection prevention and control (IPC) strategies:

- i. Enhanced Environmental Decontamination: specific disinfection protocols that target wet utilities, including sinks, drain traps, and sanitary facilities, in addition to clinical surfaces should be embraced.
- ii. Antimicrobial Stewardship: it is crucial to limit the unnecessary use of fluoroquinolones and co-amoxiclav in obstetric wards, ensuring that carbapenems and aminoglycosides (when available) are reserved for severe infections that are microbiologically confirmed.

- iii. Routine Molecular Surveillance: it is needful to broaden surveillance efforts on plasmid-mediated resistance factors and the dynamics of horizontal gene transfer across secondary and tertiary maternal care units in Nigeria

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