



## Research Article

# Molecular Detection of Multiple Resistance Genes from Extended Spectrum $\beta$ -Lactamase (ESBL) and Metallo- $\beta$ -lactamases (MBLs) Producing *E. coli* Isolated from Patients Attending Selected Hospitals in Maiduguri, Nigeria

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### ABSTRACT

The rising prevalence of multidrug-resistant *Escherichia coli* (*E. coli*) represents a growing threat to public health, particularly in hospital settings where the incidence of infections is high. This study aimed to investigate the prevalence of *E. coli* in stool and urine samples collected from three hospitals in Maiduguri, Nigeria, and to assess their resistance profiles, including the production of extended-spectrum beta-lactamases (ESBL). Three hundred sixty-four (364) clinical samples (150 stool and 214 urine) were analyzed, revealing an 80.0% prevalence of *E. coli* in stool and 59.6% in urine. Multidrug resistance was significantly higher in urine isolates (72.0%) compared to stool isolates (44.2%). Among the MDR isolates, 32.17% produced ESBL. Molecular identification using PCR and gel electrophoresis detected key resistance genes, including *bla*TEM, *bla*SHV, *CTX-M*, and *NDM*, highlighting the genetic mechanisms driving resistance. These findings stress the pressing need for improved antimicrobial stewardship, targeted infection control measures, and the development of novel therapeutic strategies to combat the rising threat of resistant *E. coli* infections.

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### INTRODUCTION

*Escherichia coli* (*E. coli*), a facultative anaerobe of the Enterobacteriaceae family, is an essential component of the gut microbiota in humans and warm-blooded animals. While it primarily exists as a harmless commensal, certain strains have evolved to become significant pathogens, causing a range of infections such as urinary

tract infections (UTIs), septicemia, and diarrheal diseases (Kaper *et al.*, 2004). This dual role as a commensal and a pathogen stresses its clinical and scientific importance. Furthermore, *E. coli* is a cornerstone organism in industrial microbiology and molecular biotechnology, including recombinant DNA technology (Rosano & Ceccarelli, 2014). Transmission of

pathogenic *E. coli* commonly occurs via the fecal-oral route, often through consumption of contaminated or undercooked food and water. This is a critical public health concern, especially in resource-limited settings where sanitation is inadequate. In recent years, *E. coli* has emerged as a multidrug-resistant (MDR) pathogen, producing extended-spectrum  $\beta$ -lactamases (ESBLs) and metallo- $\beta$ -lactamases (MBLs) that confer resistance to  $\beta$ -lactam antibiotics, including cephalosporins and carbapenems (Wakil *et al.*, 2021). These enzymes degrade the antibiotics, rendering them ineffective and complicating treatment options. ESBL-producing *E. coli* strains typically harbor resistance genes such as *blaTEM*, *blaSHV*, and *blaCTX-M*, while MBL-producing strains exhibit genes like *blaVIM*, *blaIMP*, and *blaNDM*. The coexistence of these resistance determinants often results in strains with broad-spectrum antibiotic resistance, posing a formidable challenge to clinicians and public health authorities (Bush and Bradford, 2019; Banin *et al.*, 2017). The prevalence of these genes in hospital environments, where antibiotic pressure is high, accelerates their dissemination and threatens effective antimicrobial therapy. In Maiduguri, Nigeria, antimicrobial resistance among clinical isolates of *E. coli* remains underexplored despite the region's high burden of infectious diseases. With reports of increasing resistance to multiple antibiotic classes, the molecular detection and characterization of resistance genes are critical for understanding the scope of the problem and guiding clinical management (Pitout, 2012; Tzouveleki *et al.*, 2014).

This study seeks to address the rising challenge of antimicrobial resistance by focusing on the molecular detection and characterization of resistance genes in *E. coli* isolates from patients in Maiduguri, Nigeria. By examining the prevalence and diversity of extended-spectrum  $\beta$ -lactamases (ESBL), it aims to elucidate the mechanisms driving multidrug resistance. The findings will provide critical insights into resistance patterns, inform clinical treatment strategies, and support public health interventions to combat the spread of these challenging pathogens.

## METHODOLOGY

### Study Design and Sampling

This cross-sectional study was conducted in Maiduguri, Nigeria, involving one tertiary hospital (State Specialist Hospital), two secondary hospitals (Umaru Shehu Ultra-Modern Hospital, Maiduguri and Mamman Shuwa Memorial Hospital). A purposive sampling technique was employed, with 364 clinical samples divided into two groups: 214 urine samples from patients with urinary tract infections (UTIs) and 150 stool samples from diarrheic patients.

### Ethical Clearance

Ethical approval was sought from the Research and Ethics Committees of the participating hospitals and healthcare centres prior to the study's commencement, ensuring compliance with research ethics and participant rights.

### Sample Collection and Bacterial Identification

Urine and stool samples were collected aseptically using sterile 200 mL universal containers. Each container was labelled with a unique participant identifier. The samples were cultured under aseptic conditions: urine on CLED agar and stool on MacConkey agar. The cultures were incubated at 37°C for 24 hours. Colonies obtained were subcultured onto Eosin Methylene Blue (EMB) agar for confirmatory identification, followed by further incubation at the same temperature and duration (Cheesbrough, 2006).

### Preliminary Bacterial Identification

Preliminary identification involved gram staining and a series of biochemical tests, including the citrate test, methyl red test, triple sugar iron (TSI) test, and indole test. These tests helped characterize the isolates based on morphology, metabolic activity, and gram reaction (Cheesbrough, 2006).

### Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disk diffusion method in adherence to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2016). The selected antibiotics include ampicillin (30  $\mu$ g), ceftazidime (30  $\mu$ g), cefixime (5  $\mu$ g), imipenem (10  $\mu$ g), ciprofloxacin (5  $\mu$ g), amikacin (30  $\mu$ g), ceftriaxone (30  $\mu$ g), nalidixic acid (30  $\mu$ g), amoxicillin (10  $\mu$ g), cefotaxime (30  $\mu$ g),

gentamicin (30 µg), doripenem (30 µg), levofloxacin (5 µg), tigecycline (15 µg), and nitrofurantoin (10 µg). Isolates resistant to three or more antibiotics, including β-lactams, were analyzed further.

#### Detection of Extended Spectrum β-Lactamase (ESBL)

The detection of ESBL production involved testing the isolates with third-generation cephalosporins: ceftazidime (30 µg), cefotaxime (30 µg), and ceftriaxone (30 µg). Bacterial colonies tested positive for multidrug resistance and were cultured on Mueller-Hinton agar. Antibiotic disks were placed at a distance of 15 mm, and plates were incubated at 37°C for 24 hours. Zones of inhibition <27 mm for ceftazidime, <22 mm for cefotaxime, and <25 mm for ceftriaxone indicate ESBL production (Sambrook & Russell, 2006; CLSI, 2016).

#### Confirmatory Testing for ESBL

Confirmatory tests used an Augmentin (30 µg) disk placed at the centre of Mueller-Hinton agar plates, surrounded by ceftazidime, cefotaxime, and ceftriaxone disks at a 15 mm distance. Plates were incubated at 37°C for 24 hours. A clear zone of inhibition extending toward the Augmentin disk confirmed ESBL production (CLSI, 2016; Livermore, 1995). Molecular Detection of Resistance Genes

#### DNA Extraction and Quantification

Following the manufacturer's instruction, DNA was extracted from the bacterial culture using the Accupower genomic DNA extraction kit (Bioneer Corporation). Briefly, the bacterial cells were transferred into a clean labelled 1.5ml tube containing 200 µl PBS, then 200 µl of genomic lysis buffer (GB buffer), 20 µl of proteinase k and 10 µl of RNase were added to the tube. The tubes were vortexed and incubated at 60 for 30 minutes; after the incubation, 400 µl of absolute ethanol was added and mixed by pipetting. The lysates were transferred to a binding column attached to a collecting tube and centrifuge at 8000rpm for 1m. The lysates in the collection tubes were discarded, and the tubes were re-attached to the binding column. Two stages of washing were followed by using wash buffers 1 and 2. After the washing, the empty binding columns were centrifuged at 13000rpm for 1 minute to remove excess ethanol. 50 µl of elution buffer was added to the binding column to elute the DNA. The extracted DNA was quantified using a Nanodrop spectrophotometer to obtain the quantity and purity of the DNA.

#### PCR Amplification of Resistance Genes

PCR was performed using Accupower hot start PCR premix (Bioneer corporation) to amplify the beta-lactamase *blaSHV* gene, *blaTEM* gene, beta-lactamase *CTXM* gene and NDM genes using the following primers as presented in Table 1

**Table 1:** Sequences of the primer pairs utilized in the multiplex polymerase chain reaction for amplification of extended-spectrum β-lactamase encoding resistance genes

Resistance Genes	Product size (bp)	Sequences (5'-3' direction)	Amplicons size in bp	References
<i>bla<sub>TEM</sub></i>	<i>bla<sub>TEM</sub>-F</i>	CAGCGGTAAGATCCTTGAGA	680	Ensor <i>et al.</i> , 2009
	<i>bla<sub>TEM</sub>-R</i>	ACTCCCCGTCGTGTAGATAA-		
<i>bla<sub>SHV</sub></i>	<i>bla<sub>SHV</sub>-F</i>	CGTTCATCCATAGTTGCCTGAC	450	Ghorbani-Dalini <i>et al.</i> , 2015
	<i>bla<sub>SHV</sub>-R</i>	AGCCGCTTGAGCAAATTAAC		
<i>bla<sub>CTX-M</sub></i>	<i>bla<sub>CTX-M</sub>-F</i>	TCTTCCAGAATAAGGAATCCC	909	Ghorbani-Dalini <i>et al.</i> , 2015
	<i>bla<sub>CTX-M</sub>-R</i>	CCGTTTCCGCTATTACAAAC		
<i>bla<sub>NDM</sub></i>	<i>bla<sub>NDM</sub>-F</i>	GGGCAGTCGCTTCCAACGGT	475	Ejaz <i>et al.</i> , 2020
	<i>bla<sub>NDM</sub>-R</i>	GTAGTGCTCAGTGTCGGCAT		

## RESULTS

The findings of the study revealed significant variations in the prevalence of *E. coli* isolated from stool and urine samples collected at three hospitals in Maiduguri: Borno State Specialist Hospital (B.S.S.H.), Umaru Shehu Ultramodern Hospital (U.S.U.H.), and Mamman Shuwa Memorial Hospital (M.S.M.H.). For stool samples, 150 were analyzed, with 120 *E. coli* isolated, resulting in an overall prevalence of 80.0%. Among the hospitals, B.S.S.H. contributed the highest number of stool samples (60), with 41.7% testing positive for *E. coli*. Similarly, M.S.M.H. reported a prevalence of 33.3% from its 50 stool samples, while U.S.U.H. recorded the lowest prevalence of 25.0% from 40 stool samples (Table 2).

A total of 214 were collected for urine samples, with *E. coli* isolated in 125 cases, reflecting an overall prevalence of 59.6%. M.S.M.H. exhibited the highest prevalence (66.1%), isolating *E. coli* in 39 of 59 urine samples. U.S.U.H. followed with a prevalence of 59.3%, isolating *E. coli* from 32 of 54 urine samples. B.S.S.H., despite contributing the most significant number of urine samples (101), reported the lowest prevalence at 53.5% (Table 2). These results demonstrate a higher prevalence of *E. coli* in stool samples compared to urine samples and highlight disparities in isolation rates across the hospitals, suggesting differences in the bacterial burden or sample types at these healthcare facilities.

**Table 2:** The occurrence of *E. coli* in clinical samples collected from patients at hospitals in Maiduguri

Hospital	Stool		Urine	
	No. of Samples Collected (%)	No. of <i>E. coli</i> Isolated (5%)	No. of Samples Collected (%)	No. of <i>E. coli</i> Isolated (5%)
B.S.S.H	60 (40.0)	50 (41.7)	101 (47.2)	54 (53.5)
U.S.U.H	40 (26.7)	30 (25.0)	54 (25.2)	32 (59.3)
M.S.M.H	50 (33.3)	40 (33.3)	59 (27.6)	39 (66.1)
<b>Total</b>	<b>150 (100)</b>	<b>120 (80.0)</b>	<b>214 (100)</b>	<b>125 (59.6)</b>

**Key:** B.S.S.H= Borno State Specialist Hospital, Maiduguri, U.S.U.H= Umaru Shehu Ultramodern Hospital, Maiduguri. M.S.M.H= Mamman Shuwa Memorial Hospital, Maiduguri,

The study's results indicate a concerning prevalence of multidrug resistance (MDR) among *E. coli* isolates obtained from clinical stool and urine samples. Out of a total of 245 *E. coli* isolates analyzed, 143 (58.4%) were found to be multidrug-resistant (Table 3). When analyzed by sample type, significant differences in MDR prevalence were observed. From stool samples, 120 isolates were tested, of which 53 (44.2%) exhibited resistance to multiple antibiotics. In contrast, urine samples showed a much higher prevalence of MDR, with 90 (72.0%) out of 125 isolates demonstrating resistance (Table 3). These

findings suggest that *E. coli* isolates from urine samples have a significantly higher propensity for multidrug resistance compared to those from stool samples. This disparity may reflect differences in the selection pressure exerted by antibiotic use in urinary tract infections compared to gastrointestinal infections, or variations in the clinical management of these conditions. Overall, the study stresses the widespread issue of multidrug resistance among clinical *E. coli* isolates, particularly in urine samples, posing a serious challenge to effective treatment.

**Table 3:** Prevalence of Multidrug Resistance Isolated from Clinical Samples

Source of Isolates	Total No. of Isolates	Multidrug Resistant Isolates	Multidrug Resistant Isolates (%)
Stool	120	53	44.2
Urine	125	90	72.0
<b>Total</b>	<b>245</b>	<b>143</b>	<b>58.4</b>

The study reveals the prevalence of ESBL-producing *E. coli* among multidrug-resistant (MDR) isolates obtained from stool and urine samples. Of the 143 MDR isolates identified in the study, 46 (32.17%) produced ESBL, indicating that a substantial proportion of the MDR *E. coli* isolates possess this resistance mechanism. When analyzed by sample source, the results show comparable ESBL prevalence in stool and urine isolates. Among the 53 MDR *E. coli* isolates from stool samples, 18 (33.96%) were ESBL producers. Similarly, from the 90 MDR isolates obtained from urine samples, 28 (31.11%) were confirmed to produce ESBL. These findings suggest that the prevalence of ESBL production among MDR *E. coli* isolates is relatively consistent across the two sample types, with stool isolates showing a slightly higher percentage (Table 4).

Regarding the previously discussed results, the high prevalence of MDR in urine samples (72.0%) compared to stool samples (44.2%) is notable. However, the proportion of ESBL producers among MDR isolates is similar in both sample types. This suggests that while urine-derived *E. coli* isolates may exhibit higher overall multidrug resistance, the likelihood of ESBL production is independent of the source and remains a common resistance mechanism. The findings stress the dual challenge of MDR and ESBL production in *E. coli*, which complicates antibiotic therapy and increases the risk of treatment failure. These results highlight the need for continuous surveillance and targeted antimicrobial stewardship strategies to combat the spread of these resistant strains.

**Table 4:** Prevalence of ESBL producing *E. coli* Isolated from Clinical Samples

Source of Isolates	Multidrug Resistant Isolates	ESBL producing <i>E. coli</i>	ESBL producing <i>E. coli</i> (%)
Stool	53	18	33.96
Urine	90	28	31.11
<b>Total</b>	<b>143</b>	<b>46</b>	<b>32.17</b>

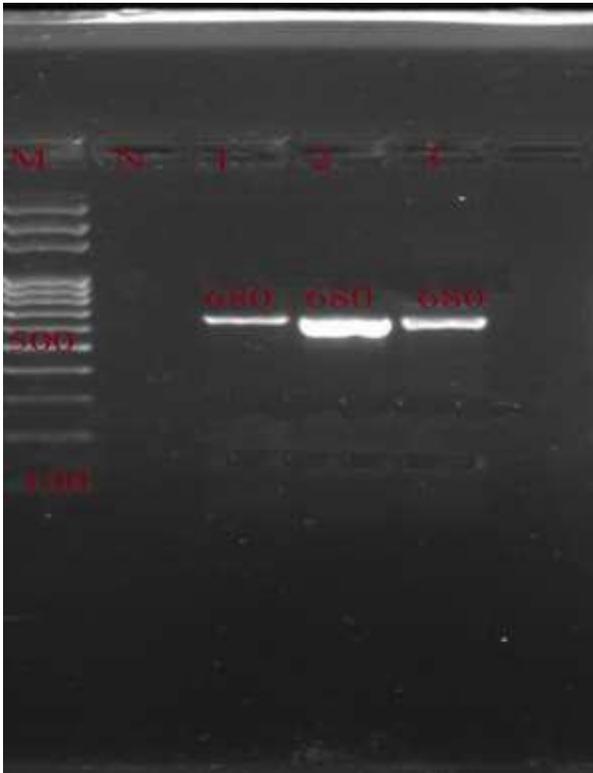
The molecular analysis of *E. coli* isolates using PCR and gel electrophoresis revealed the presence of key resistance genes (*blaTEM*, *blaSHV*, *CTX-M*, and *NDM*), which confer ESBL and metallo- $\beta$ -lactamase MBL activity. Out of the 46 ESBL-producing isolates initially identified, 10 were selected for further molecular testing. Among these, 4 isolates

harboured all 4 resistance genes, stressing their significant multidrug-resistant potential.

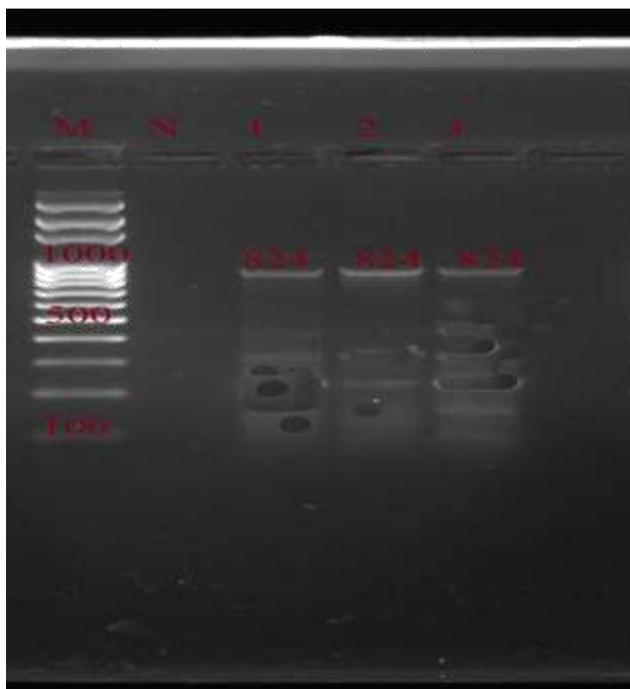
For the *blaTEM* gene, positive bands were observed at approximately 680 bp in all three tested samples, as shown in Figure 1. This finding aligns with the widespread role of *blaTEM* in mediating resistance to penicillins

and first-generation cephalosporins, suggesting that this gene is a common determinant of resistance among the tested isolates. Similarly, Figure 2 demonstrates positive bands at 824 bp for the *blaSHV* gene in all three samples, indicating its role in resistance to third-generation cephalosporins. The universal detection of these genes in tested samples suggests a baseline resistance mechanism among the isolates. The CTX-M gene, a hallmark of ESBL production, was detected in Samples 2 and 3, as evidenced by bands at 550 bp in Figure 3. However, its absence in Sample 1 suggests heterogeneity in the genetic makeup of ESBL-producing *E. coli*. This finding complements the earlier results, where only a subset of multidrug-resistant isolates demonstrated ESBL production. Similarly, the NDM gene, which encodes a metallo- $\beta$ -lactamase capable of

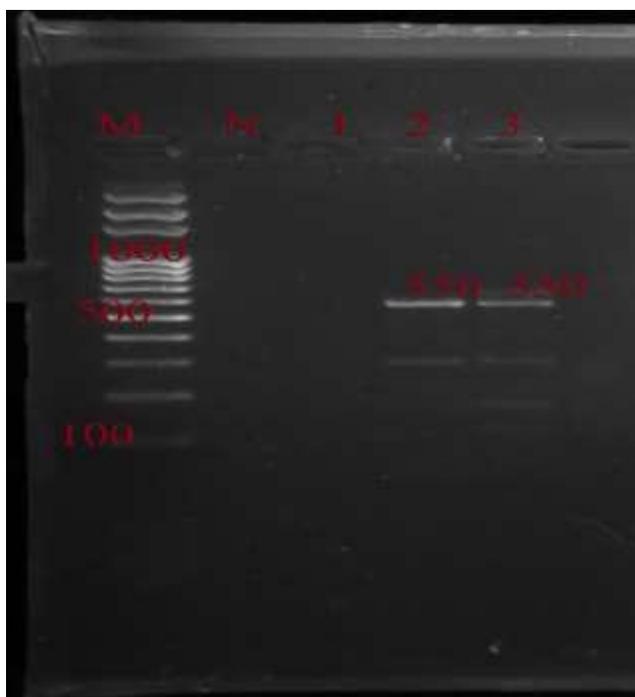
hydrolyzing carbapenems, was detected as faint bands at 475 bp in Samples 2 and 3, as shown in Figure 4. The faint bands may reflect lower gene expression or amplification efficiency, but their presence signifies a critical resistance mechanism against last-resort antibiotics. These molecular findings correlate with earlier results indicating a 32.17% prevalence of ESBL production among multidrug-resistant isolates. Identifying specific resistance genes further clarifies the molecular basis for this phenomenon, highlighting the coexistence of multiple  $\beta$ -lactamase genes within the identical isolates. Notably, detecting the NDM gene in ESBL-producing isolates introduces an additional layer of concern, suggesting a broader resistance spectrum that includes carbapenems.



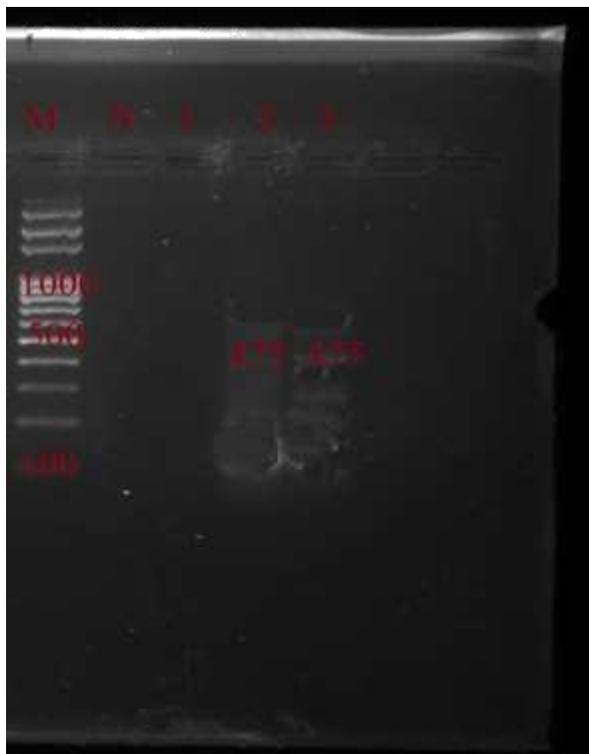
**Figure 1:** *bla*TEM Gel Picture Showing Positive in all the Three Samples at around 680 bp  
**KEY:** M = Molecular Marker, N = Negative control, 1 = Sample 1, 2 = Sample 2, 3 = Sample 3



**Figure 2:** Gel picture showing positive band of around 824 bp for blaSHV in all the three samples  
**KEY:** M = Molecular Marker, N = Negative control, 1 = Sample 1, 2 = Sample 2, 3 = Sample 3



**Figure 3:** Gel picture showing a band size of around 550 bp for the presence of CTX-M gene in sample 2 and 3  
**KEY:** M = Molecular Marker, N = Negative control, 1 = Sample 1, 2 = Sample 2, 3 = Sample 3



**Figure 4:** Gel picture showing faint bands of around 475 in samples 2 and 3 for presence of NDM genes  
**KEY:** M = Molecular Marker, N = Negative control, 1 = Sample 1, 2 = Sample 2, 3 = Sample 3

## DISCUSSION

The study's results provide an understanding of the prevalence and resistance mechanisms of *E. coli* from stool and urine samples in Maiduguri hospitals, which revealed significant multidrug resistance (MDR) rates. The higher prevalence of *E. coli* in stool (80.0%) compared to urine (59.6%) aligns with the bacteria's role as a gut commensal organism and its lesser colonization in the urinary tract. However, the greater MDR prevalence in urine samples (72.0% versus 44.2% in stool) suggests more substantial antibiotic selection pressure in urinary tract infections (UTIs), likely due to more frequent or inappropriate antibiotic treatments. Identifying key resistance genes, such as *blaTEM*, *blaSHV*, *CTX-M*, and *NDM*, stresses the bacteria's ability to counteract critical antibiotic classes (Muhammad *et al.*, 2013; Isa *et al.*, 2013). The *blaTEM* and *blaSHV* genes, detected universally in tested isolates, are pivotal in conferring resistance to penicillins and cephalosporins. In contrast, *CTX-M* and *NDM* genes expand resistance to third-generation cephalosporins and carbapenems. The latter finding, particularly the

presence of *NDM*, highlights an alarming trend towards resistance against last-resort antibiotics, complicating infection management and raising the risk of treatment failure (Muhammad *et al.*, 2013).

This study aligns with global and regional research on *E. coli* resistance mechanisms, particularly the prevalence of MDR and ESBL-producing strains. A study by Olowe *et al.* (2018) in Nigeria reported MDR prevalence rates of up to 62%, comparable to the 58.4% observed here. Similarly, Shaikh *et al.* (2015) reported ESBL production in approximately 30–40% of clinical *E. coli* isolates globally, consistent with the 32.17% prevalence in this study.

The widespread presence of *blaTEM* and *blaSHV* genes in *E. coli* aligns with findings by Jacoby and Munoz-Price (2005), who described these genes as primary mediators of ESBL activity in clinical isolates. The *CTX-M* gene's detection in a subset of isolates mirrors findings by Canton *et al.* (2012), who highlighted its emerging dominance among ESBL producers. Moreover, although less common, identifying

the NDM gene reflects the increasing incidence of carbapenem resistance documented in studies such as Kumarasamy *et al.* (2010).

The disparity in MDR prevalence between stool and urine samples, despite similar ESBL rates, is noteworthy. Research by Pitout and Laupland (2008) indicated that urinary isolates often face higher antibiotic exposure, which explains their elevated MDR prevalence. However, the comparable ESBL production rates in stool and urine align with findings by Ruppé *et al.* (2015), suggesting that the ESBL phenotype is driven by genetic factors independent of the sample source.

### CONCLUSION

This study explains the prevalence, multidrug resistance (MDR), and extended-spectrum beta-lactamase (ESBL) production in *Escherichia coli* isolates from stool and urine samples collected from three hospitals in Maiduguri, Nigeria. The findings stress the high prevalence of *E. coli* in both stool and urine samples, with a notable variation across different healthcare facilities. Notably, the study stresses a concerning level of MDR in urine samples, where 72.0% of isolates exhibited resistance to multiple antibiotics. This reflects significant antibiotic pressure in treating urinary tract infections (UTIs). Identifying ESBL-producing strains in a substantial proportion of MDR isolates (32.17%) further complicates treatment procedures, given the resistance to key beta-lactam antibiotics. Molecular analysis revealed the presence of several key resistance genes, including blaTEM, blaSHV, CTX-M, and NDM, indicating the genetic basis of resistance. These findings reinforce the urgency for improved antimicrobial stewardship, targeted surveillance, and developing alternative treatment strategies to manage *E. coli* infections effectively.

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