IJMAAS International Journal of Microbiology and Applied Sciences
ISSN 1115-4004 Volume 5 issue 1 Jan, 2026 Research Article

Extended Spectrum-Beta-Lactamase Genes in Multidrug-Resistant Gram-Negative Bacteria from Clinical and Environmental Sources in Hospitals in Makurdi, Nigeria

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ABSTRACT

A major contributing factor to Gram Negative Bacteria (GNB) resistance to third and fourth generation cephalosporin is the formation of Extended Spectrum Beta-Lactamases (ESBLs). Antibiotic resistance, particularly mediated by ESBL enzymes, poses a major global public health challenge. This study investigated the distribution of GNB, ESBL resistance genes, and their occurrence in clinical and environmental samples from two healthcare facilities. A total of 566 bacterial were isolated from clinical and environmental samples at the hospitals using MacConkey agar. The GNB were identified using standard microbiological methods and subjected to antibiotic susceptibility testing using the Kirby Bauer disk diffusion method. Isolates (137) that showed resistance to at least eight antibiotics were selected for ESBLs resistance genes (*bla-$_{TEM}$*, *bla-$_{CTX-M}$*, *bla-$_{SHV}$*) detection using primer specific PCR. Isolates that haboured resistance genes were identified using 16S rRNA sequencing. Data were analysed using descriptive statistics. Out of a total of 566 bacteria isolated, 409(72.3%) were GNB and were tested for Multidrug Resistance (MDR). Molecular characterization revealed that 66 (16.1%) of the isolates harbored ESBL genes. The distribution of ESBL genes showed that *bla-$_{TEM}$* (53.0%) was the most prevalent, followed by *bla-$_{CTX-M}$* (37.9%) and *bla-$_{SHV}$* (9.1%). Clinical isolates accounted for the majority of ESBL genes (77.3%), compared to environmental isolates (22.7%). *Escherichia coli* was the predominant ESBL-producing organism, accounting for 74.5% of clinical isolates and 40.0% of environmental isolates. Molecular identities of the isolates were *Enterobacter hormaechei*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella quasipneumoniae*, *Proteus mirabilis* and *Pseudomonas* sp. The presence of ESBL genes in environmental samples underscores the role of hospital environments as reservoirs for antibiotic resistance and supports the potential for horizontal gene transfer among bacterial populations.

Keywords: Multidrug resistance, Gram-negative bacteria, Extended Spectrum-Beta-Lactamase genes, *bla-$_{TEM}$*, *bla-$_{CTX-M}$*, *bla-$_{SHV}$*.

Introduction

Gram-negative bacteria have evolved highly specialized strategies to pass both cellular and biochemical barriers, as well as to elicit unusual reactions from the host organism that aid in the organism's survival and growth (Holmes *et al*., 2021). Antibiotic-resistant strains, particularly those of Gram-negative bacteria, are emerging at an alarming rate, posing significant challenges to effective treatment (Tanni *et al*., 2025). Infections caused by multidrug-resistant (MDR) bacteria significantly increase morbidity and mortality, leading to extended hospital stays, consumption of more expensive antibiotics, and the risk of developing antimicrobial resistance.

Although, MDR infections not only threaten patient outcomes but also result in substantial financial losses for healthcare systems (Tanni *et al*., 2025). Consequently, hospitalisation duration, healthcare infections, mortality, and morbidity rates are all significantly increased by Enterobacteria producing Extended-spectrum Beta-Lactamases (ESBLs) (Ghenea *et al*., 2022).

Hospitals and communities around the world are home to Enterobacteriaceae that produce Extended-Spectrum Beta-Lactamases (ESBLs), with differing levels of prevalence (Ouattara *et al*., 2023).

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Each year, ESBL-producing Enterobacteriaceae cause 1,700 fatalities and 26,000 hospital acquired infections (HAIs) due to their broad-spectrum beta-lactamase enzyme, which makes them resistant to a wide range of penicillin and cephalosporin antibiotics (Jha *et al*., 2023). Research on antibiotic resistance was spurred by the 1940 European description of beta-lactamase enzymes, which were isolated from *E. coli* (Bastidas-Caldes *et al*., 2022). Enterobacteriaceae develop plasmid-encoded enzymes called ESBLs, which hydrolyse β-lactam antibiotics to cause bacterial resistance (Wei *et al*., 2023). Common β-lactamase inhibitors inhibit the hydrolysis of oxyimino-β-lactams, including cefotaxime, ceftriaxone, ceftaxidime, and the monobactam aztreonam. This enzyme is crucial because it breaks down the β-lactam ring of many antibiotics, including Aztreonam, Penicillin, and all generations of Cephalosporins, while carbapenems, cephamycin, and doxalactam are left unaltered (Ikram *et al*., 2023; Inam *et al*., 2023).

Extended-spectrum β-lactamases are widely distributed amongst Enterobacteriaceae due to the overuse or abuse of antibiotics (Zhu *et al.*, 2021). The widespread use of these agents quickly created selection pressure. The generation of β-lactamases, which can render even the strongest β-lactams, such as carbapenems inactive, is the most common way that β-lactam antibiotic resistance occurs. These drugs' efficacy is weakened by carbapenemases, cephalosporinases and extended spectrum β-lactamases. The ESBLs genes are frequently carried by Plasmids, which has facilitated their proliferation and increased the persistence and worldwide transmission of high-risk clones (Dalazen *et al*., 2023). A ESBLs with broader substrate profiles are produced when mutations occur in the genes encoding these enzymes (Shirisha *et al*., 2023). There are hundreds of varieties of ESBLs, among which Temoneira (TEM), Sulfhydryl variable (SHV) and the Munich cefotaximase (CTX-M) are the most common genotypes (Zhu *et al.*, 2021). Of them, TEM, SHV, OXA, and CTX-M have been the most studied in a number of studies and commonly linked to antibiotic resistance (Ghenea *et al*., 2022). According to Ghenea *et al*. (2022), there are approximately 130 distinct types of CTX-M enzymes, classified into five groups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25. The CTX-M-15 variant is the most widespread type found in both hospitals and the community (Ghenea *et al*., 2022).

The ascendency of CTX-M is not due to greater β-lactamase activity compared to TEM or SHV types, but rather to their genes' capacity to be mobilised and spread via mobile genetic elements, allowing efficient transfer between replicons and cells, especially within highly successful bacterial lineages. Amongst ESBLs, CTX-M-type β-lactamases have demonstrated the greatest ability to disseminate in clinical environments and are now the most globally prevalent ESBLs (Ghiglione *et al*., 2021).

Also, there are over 140 distinct TEM beta-lactamase varieties identified. Additionally, SHV comes in over 100 varieties (Ghenea *et al*., 2022). Shirisha *et al*. (2023) assertion that the penicillinase that produces the TEM and SHV forms of the antibiotic is capable of hydrolysing extended range cephalosporins due to multiple single amino acid changes. Some, such as TEM-1 and TEM-2, do not display ESBL activity and solely hydrolyse beta-lactams, including penicillins and narrow-spectrum cephalosporins (Jacoby and Munoz-Price, 2005). The first SHV-ESBL isolate was discovered in clinical samples from Chile in 1989 and Argentina in 1988 (Bastidas-Caldes *et al*., 2022). Various enzyme types have now been identified (Khalid, 2023). Among the most prevalent are SHV-2, SHV-5, SHV-7, and SHV-12 (Castanheira *et al*., 2013). According to Jacoby and Munoz-Price (2005), not all SHVs are ESBL, and some, like SHV-1, exclusively hydrolyse beta-lactams like penicillins and narrow-spectrum cephalosporins. This study aimed to detect the prevalence, distribution, and antibiotic resistance patterns of MDR GNB isolated from clinical and environmental sources in two tertiary healthcare hospitals in Makurdi, Benue State, Nigeria.

Materials and Methods

Study Area

This study was carried out in two hospitals namely, The Federal Medical Centre (FMC) and Benue State University Teaching Hospital (BSUTH), both in Benue State of Nigeria. Benue State is situated in Nigeria's north-central geopolitical zone. The Federal Medical Centre (FMC) and Benue State University Teaching Hospital (BSUTH) were selected for this study because, they are the two major tertiary healthcare institutions that serve as referral Centres for Benue State and neighboring regions.

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Ethical clearance

The Benue State Ministry of Health granted ethical approval with Ref: No. MOH/STA/204/VOL.1/31 for this study and the management of the two hospitals gave their consent.

Sample collection

Wastewater and Swab Samples

Samples of wastewater were collected from the drainages of the two healthcare facilities. Drains from different wards (as stated below) were chosen for the investigation, including pooled data from two channels (Channels 1 and 2) from FMC and three channels (Channels A, B, and C) from BSUTH.

The FMC sampling sites include two channels, source of generation designated as (1) and the exit channel designated as (2). These two channels also include pooled of wastewater coming from the entire hospitals.

From BSUTH, channel A comprises of the theatre, female surgical ward, pediatric ward, male and female medical ward, Amenity ward (ward block), resident doctor's hostel and cafeteria, isolation centre, Gynaecology ward, and Postnatal ward; channel B comprises the laboratories; channel C includes Consultant block and flood water from Cameron dam (Lagdo dam).

Swab samples were collected from the entire surface of toilet bowls, tap faucets and hand washing sinks in various wards using sterile swab sticks (CJ SMART®, China).

The wards from which swabs were taken in BSUTH included; General operating unit (GOPD), Gynaecology, Post natal, Pediatric surgical children ward and ORL ward.

While the wards from which swabs samples were taken in FMC, included; Dialysis ward, Postnatal, Emergency pediatric ward, Antenatal, APIN (Aids Prevention and Initiative in Nigeria) ward, and Gynaecology ward.

The collected wastewater samples and swab specimens were transported immediately to BSUTH laboratory for analyses.

Bacteriological Analysis

Wastewater samples

The wastewater samples underwent a 10-fold to 10^{-6} serial dilution. Nine milliliters of sterile distilled water were put into six test tubes. The initial series of dilutions was followed by addition with 1 mL of wastewater sample, mix and labeled as 10^{-1}.

In the second series, 1 mL of the sample from the 10^{-1} dilution was added to a tube that contained 9 mL of distilled water, and the mixture was then mixed. Several dilutions were made up until a 10^{-6} dilution was achieved. Lastly, MacConkey's agar plates (Hi-media, India) on cooling at 45°C, were poured into the sterile Petri dishes inoculated with 1 mL of the 10^{-1}, 10^{-2} , 10^{-6} dilution and was rocked properly.

After the plates have solidified, they were incubated in inverted positions for 18 to 24 hours at 37°C (Asghari *et al*., 2021). To isolate pure colonies from the primary plates (MacConkey's agar plates), nutrient agar plates were prepared. After selecting and streaking a colony on nutrient agar plates, it was cultured for 18 to 24 hours at 37°C and then stored for further analysis.

Swab samples

On arrival at the BSUTH laboratory, the swab specimens were cultured by inoculating/streaking of the swab on MacConkey agar plates (Hi-media, India), and then incubated for 18-24 hours at 37^0C. Overall, 1-5 different distinct colonies were selected from each plate, each colony was subculture on sterile Nutrient agar plates to isolate pure colonies from the primary plates (MacConkey's agar plates). They were incubated for 18 to 24 hours at 37°C and then stored in nutrient agar slant for further analysis.

Collection of Clinical and Environmental Bacterial Isolates

A total of three hundred and forty (340) clinical isolates were isolated comprising 210 from Benue State University Teaching Hospital (BSUTH). While one hundred and thirty (130) were collected from The Federal Medical Centre (FMC).

The samples were collected from September 15, 2022, to December 22, 2022.

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Morphological characterization and Gram staining

Distinct bacterial colonies isolated with non-duplicating morphological types observed on the MacConkey agar plates after 18 to 24 hours incubation were selected and identified using Gram staining reaction and morphological characteristics as size, form, colour, pigmentation, texture, elevation, and edge of the isolates (Alhassan *et al*., 2022; Amare *et al*., 2023).

Antibiotic Susceptibility testing of the Gram-negative Bacteria

Using Kirby-Bauer disk diffusion method, the antibiotic susceptibility testing was carried out on the Gram-negative bacteria using Mueller-Hinton agar (Ali *et al.*, 2020). Ten antibiotics (BIOANALYSE®, Turkey) belonging to six different classes were tested against the isolates including β-lactam/cephalosporin (cefepime (30 µg), cefotaxime (30 µg) and ceftazime (30 µg)); β-lactam combination (piperacillin tazobactam (100 µg) and Amoxicillin clavulanic acid (20 µg); Quinolone (ciprofloxacin (5 µg); Aminoglycoside (Amikacin (30 µg); Monobactam ((azteronam) and Carbapenem (ertapenem (10 µg) and impenem (10 µg)). The bacterial isolates were subcultured on Mueller Hinton Agar (MHA) for 18 to 24 hours at 37ºC. The 18-24 hours culture of the isolates were inoculated into 5 mL sterile NaCl (0.9 percent) suspension using a sterile wire loop and adjusted to match 0.5 McFarland standard (Amare *et al*., 2023). The standardised bacterial suspension was inoculated onto MHA plates using a sterile swab stick (CJ SMART®, China). The swab was streaked in three different ways across the MHA surface. They were left for three to four minutes in order to allow the surfaces to dry. Discs of the antibiotics were placed with a space of (20mm) from one another on the surface of MHA plates. After being inverted, the plates were incubated at 35ºC for 18 to 24 hours. The zone of inhibition's diameter (mm) was measured, recorded and interpreted in accordance with the Clinical and Laboratory Standard Institute (Alhassan *et al*., 2022; CLSI, 2022).

Detection and sequencing of ESBLs Genes Using Polymerase Chain Reaction (PCR)

The extraction of deoxyribonucleic acid (DNA) was done by boiling method (Takawira and Mbanga, 2023). This was used for gene detection and and 16S rRNA sequencing. Three pairs of primers (Inqaba Biotech), were used in this study. The 137 Gram-negative bacteria isolates with multidrug resistance profile were subject to PCR for the investigation of the ESBL encoding genes *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$. The 1.5% agarose gel electrophoresis was used to identify the amplified product. A gel imaging and documentation system called E-BOX (Vilber Lourmat, Italy) was utilised for this in accordance with the manufacturer's instructions. Isolates with positive ESBL genes were identified using PCR amplification and 16S rRNA sequencing. Sequences obtained were blasted against reference sequences in the GenBank (http://blast.ncbi.nlm.nih.gov/ blast.cgi) to identify the bacteria strains. By manually matching the 16S rRNA sequences with recognised species sequences on the NCBI website, the isolates were identified to species level. The following ESBL genes, the amplicon sizes and their primers (5'-3') sequences and base pairs (bp) used for this study were;

1. *TEM* with primers (5'-3');
F: TCGCCGCATACACTATTCTCAGAATGAC
R: CAGCAATAAACCAGCCAGCCGCAAG (bp422).
2. *SHV* with primers (5'-3');
F: TGTATTATC(C/T) CTGTTAGCC (A/G) CCCTC
R: GCTCTGCTTTGTTATTCGGGCCAAGC (bp739).
3. *CTX-M* with primers (5'-3');
F: ATGTCGAGYACCAGTAARGTKATGGC
R: GGTRAARTARGTSACCAGAAYCAGCGG (bp 590).

The PCR conditions for the three genes (*bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$) is same and is as followed; Denaturation for 30 sec at 95ºC; 36 cycles of 95ºC for 30 sec, Annealing 58ºC for 60 sec, Extension 72ºC for 60 sec; and final extension of 72ºC for 5 min. Hold 4ºC infinite.

Results

A total of 566 bacterial isolates were isolated from clinical and environmental samples, of which 409 (72.3%) were identified as Gram-Negative Bacteria (GNB) and subjected to Multidrug Resistance (MDR) testing. Among the MDR GNB isolates, 137 (33.5%) were resistant to at least eight antibiotics and were used for ESBLs gene determination. Furthermore, 66 (16.1%) of the 137 MDR GNB isolates harbored Extended-Spectrum Beta-Lactamase (ESBL) genes.

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Table 1: Distribution of Multidrug Resistance and ESBL Genes Detection among Bacterial Isolates

General Information on Gram Negative Isolates	Number (n)	Percentage (%)
Total isolates (clinical and environmental samples)	566	100.0
Gram-negative bacteria tested for MDR	409	72.30
Isolates resistant to ≥ 8 antibiotics	137	33.50
ESBL genes detected	66	16.10

Table 2 shows the distribution of ESBL resistance genes (*bla-TEM, bla-**CTX-M**,* and *bla-SHV*) across clinical and environmental samples from two healthcare facilities Benue State University Teaching Hospital (BSUTH) and Federal Medical Centre (FMC), with a total of 66 ESBL-positive isolates. The results showed that *bla-TEM* genes were the most prevalent, accounting for 35 (53.0%) of all ESBL genes detected, followed by *bla-CTX-M* genes with 25 (37.9%) prevalence while SHV genes were the least common, with 6 (9.1%) prevalent. From BSUTH, clinical samples contributed the highest number of ESBL genes 36 (54.6%), *bla-**TEM*** 21 (31.8%), *bla-CTX-M* 11 (16.7%) and SHV 4 (6.1%) while the environmental samples had fewer ESBL genes detected with 8 (12.1%) prevalence. Equal distribution of *bla-**TEM*** and *bla-CTX-M* (4 each) and no SHV gene detected. This suggests that clinical settings in BSUTH are major reservoirs of ESBL-producing organisms, with a broader diversity of genes compared to the environment. From FMC, clinical samples had a prevalence of 15 (22.7%) with equal presence of *bla-TEM* and *bla-CTX-M* (7 each) with Minimal prevalence in *bla-SHV* (1 isolate).

The Environmental samples had 7 (10.6%) prevalence with equal distribution of *bla-TEM* and *bla-CTX-M* (3 each) and *bla-SHV* also present (1 isolate). Compared to BSUTH, FMC shows a more balanced distribution of *bla-TEM* and *bla-CTX-M* genes, but overall lower ESBL burden. Clinical isolates (51/66; 77.3%) carried the majority of ESBL genes compared to environmental isolates (15/66; 22.7%) which contributed a smaller but notable proportion.

Table 2: Distribution of ESBL Genes in the Healthcare Facilities

Health centers	ESBL Resistance Genes			
	TEM	CTX-M	SHV	Total
BSUTH Clinical	21(31.80)	11(16.70)	4(6.10)	36(54.60)
BSUTH Environmental	4(6.10)	4(6.10)	0(0.00)	8(12.10)
FMC Clinical	7(10.60)	7(10.60)	1(1.50)	15(22.70)
FMC Environmental	3(4.60)	3(4.60)	1(1.50)	7(10.60)
Total	35(53.00)	25(37.90)	6(9.10)	66(100.0)

Key: CTX-M: Cefotaximase; SHV: Sulfhydryl Variable; TEM: Temoneira.

Figure 1 shows the distribution of ESBL resistance genes (*bla-TEM, bla-CTX-M and bla-SHV*) among Gram-negative bacteria isolated from clinical samples in the two healthcare facilities (BSUTH and FMC), with a total of 51 ESBL-positive isolates. *E. coli* has a total of 38 (74.5%) as the predominant organism with a gene distribution of 19 (37.3%) *bla-TEM* [BSUTH: 14; FMC: 5], 15 (29.5%) *bla-CTX-M* [BSUTH: 9; FMC: 6] and 4 (7.8%) SHV.

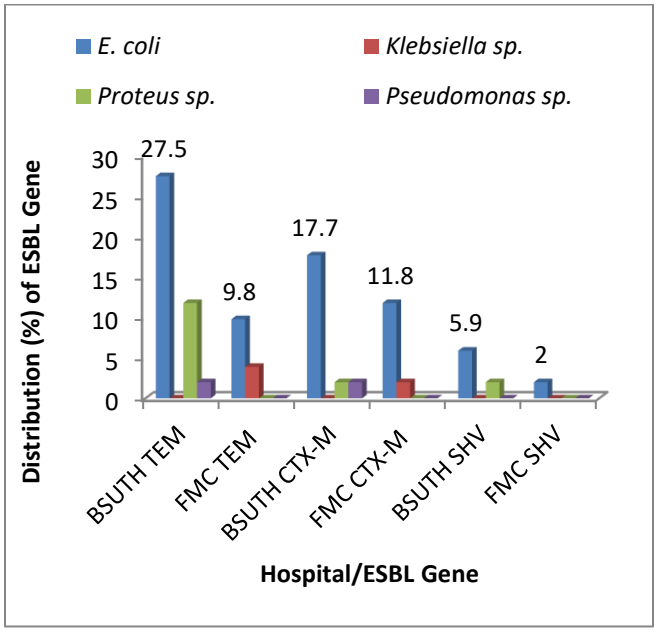

Fig. 1: Distribution of the ESBL resistance genes in the Gram-negative bacteria from clinical isolates (n = 51).

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E. coli is the major reservoir of ESBL genes, carrying all three gene types and accounting for nearly three-quarters of all isolates. Proteus spp. has a total of 8 (15.7%) with gene distribution of predominantly *bla-TEM* (6 isolates, all from BSUTH) with least *bla-CTX-M* and *bla-SHV* (1 each). *Klebsiella* spp. has a total of 3 (5.9%) with gene distribution of two *bla-TEM* (FMC only), *bla-CTX-M* one (FMC only) while *Pseudomonas* spp. has a total of 2 (3.9%) with gene distribution of one *bla-TEM* and one *bla-CTX-M*. The gene *bla-SHV* is rare, found only in *E. coli* and *Proteus* sp. BSUTH contributed the majority of ESBL genes with higher counts across all organisms, especially *E. coli* and *Proteus* sp. while FMC showed lower counts.

Figure 2 presents the distribution of ESBL resistance genes (*bla-TEM, bla-CTX-M and bla-SHV*) among Gram-negative bacteria isolated from environmental samples in two healthcare facilities (BSUTH and FMC), with a total of 15 ESBL-positive isolates.

Unlike the clinical isolates (Table 2), *bla-TEM* and *bla-CTX-M* are equally prevalent in environmental samples, suggesting a more balanced distribution of ESBL gene types in the environment.

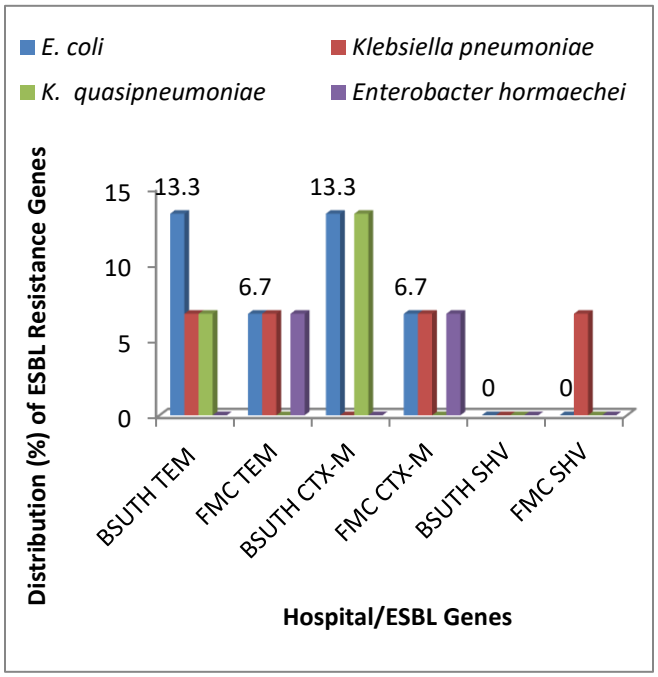

Fig. 2: Distribution of the ESBL resistance genes in the Gram-negative bacteria from environmental isolates (n = 15)

*E. coli has a t*otal of 6 (40.0%) making it the most prevalent environmental isolate with gene distribution of three *bla-TEM* (BSUTH: 2; FMC: 1) and three *bla-CTX-M* (BSUTH: 2; FMC: 1), *E. coli* remains the dominant reservoir of ESBL genes even in environmental samples. *Klebsiella pneumoniae has a t*otal of 4 (26.7%) with gene distribution of two *bla-TEM* (1 each from BSUTH and FMC), one *bla-CTX-M* (FMC) and one *bla-SHV*) (FMC). This organism shows greater gene diversity, being the only species harboring all three ESBL gene types in environmental samples. *Klebsiella quasipneumoniae has a t*otal of 3(20.0%) with gene distribution of one *bla-TEM* (BSUTH) and two *bla-CTX-M* (BSUTH). *Enterobacter hormaechei has a t*otal of 2(13.3%) with gene distribution of one *bla-TEM* (FMC) and one *bla-CTX-M* (FMC). BSUTH has slightly higher contribution to *bla-TEM* and *bla-CTX-M* genes with more species diversity (*E. coli*, *K. quasipneumoniae*) while FMC has the presence of all three ESBL genes (including the only *bla-SHV*) gene detected) with broader gene spread across species. Both facilities contribute to environmental ESBL dissemination, though with slightly different patterns. Compared to clinical isolates (Table 3), Environmental samples show lower overall ESBL burden (n = 15 versus 51), Equal distribution of *bla-TEM* and *bla-CTX-M* (versus *bla-TEM* dominance in clinical isolates) with very low *bla-SHV*) prevalence.

Discussion

The prevalence of Multidrug Resistance (MDR) (33.5%) in this study, although lower than the 70 to 90% reported in similar settings (Abayneh and Worku, 2020; Manandhar *et al*., 2020), remains clinically significant. The presence of isolates resistant to multiple antibiotic classes (≥8) suggests sustained selective pressure, likely driven by inappropriate antibiotic use and suboptimal antimicrobial stewardship. Variability in MDR rates across studies may reflect differences in antibiotic panels, study design, and regional prescribing practices.

Extended-spectrum beta-lactamase (ESBL) genes were detected in 16.1% of isolates, a relatively lower prevalence compared to reports ranging from 22% to over 70% (Legese *et al*., 2022; Sharma *et al*., 2023; Rahaman *et al*., 2025).

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This disparity indicates that ESBL production alone does not account for the resistance burden observed, suggesting the involvement of additional mechanisms such as carbapenemase production, efflux pumps, and porin loss (Ali and Masoom, 2025). The concurrent detection of resistant organisms in both clinical and environmental samples reinforces the One Health concept, highlighting the interconnected roles of human, environmental, and institutional reservoirs in antimicrobial resistance transmission (Avatsingh *et al*., 2023; Ariyanti *et al*., 2025). Investigation of ESBL gene distribution revealed a predominance of TEM-type enzymes (53.0%), followed by *bla-CTX-M* (37.9%) and SHV (9.1%). This pattern contrasts with global trends where *bla-CTX-M* enzymes are typically dominant but aligns with regional reports demonstrating persistence of *bla-TEM,* variants (Azab *et al*., 2021; Dirar *et al*., 2020). The observed pattern likely reflects localised antibiotic selection pressures and gene circulation dynamics. Notably, *bla-SHV* genes were least prevalent, consistent with studies suggesting a gradual decline in their contribution relative to other ESBL types (Djifahamaï *et al*., 2026).

Clinical isolates accounted for the majority of ESBL gene carriage (77.3%), significantly exceeding environmental isolates (22.7%). This finding underscores the role of healthcare settings as primary reservoirs of resistant organisms, where intense antibiotic exposure promotes selection and persistence (Nuhu *et al*., 2024; Cocker *et al*., 2024). The higher ESBL burden observed in BSUTH compared to FMC further indicates possible inter-facility differences in antibiotic usage, infection control practices, and patient demographics, emphasising the importance of localised antimicrobial surveillance. Despite the lower prevalence in environmental samples, the detection of ESBL-producing organisms in hospital surroundings remains concerning. Environmental matrices including surfaces, wastewater, and medical equipment serve as reservoirs and transmission pathways for resistance genes, facilitating their persistence and spread beyond clinical settings (Avatsingh *et al*., 2023; Ariyanti *et al*., 2025). This underscores the need to integrate environmental monitoring into antimicrobial resistance control strategies.

Species-level analysis identified *Escherichia coli* as the predominant ESBL-producing organism in both clinical (74.5%) and environmental (40.0%) samples. This finding is consistent with its recognised role as a major reservoir and disseminator of resistance genes globally (Ihorimbere, 2020; Nuhu *et al*., 2024). Its ecological versatility and genetic plasticity enhance its capacity to acquire and spread resistance determinants. Other organisms, including *Proteus* spp. (15.7%) and *Klebsiella* spp. (5.9%), contributed to the ESBL burden, although to a lesser extent. The relatively low prevalence of *Klebsiella* spp. contrasts with some studies where it is a leading ESBL producer (Ramatla *et al*., 2023), suggesting potential epidemiological variation.

In environmental isolates, ESBL gene distribution was more balanced, with *bla-TEM,* and *bla-CTX-M* genes equally represented (46.7% each) and minimal *bla-SHV* contribution (6.7%). This diversity likely reflects multiple contamination sources rather than direct antibiotic selection pressure. Notably, *Klebsiella pneumoniae* was identified as a carrier of all three ESBL gene types, highlighting its role as a potential reservoir for diverse resistance determinants and its significance in nosocomial transmission (Azab *et al*., 2021). Although the number of environmental isolates was relatively small, the presence of ESBL genes across multiple bacterial species emphasizes the potential for horizontal gene transfer within environmental niches. This supports existing evidence that hospital environments and associated ecosystems act as critical reservoirs for antimicrobial resistance, facilitating gene exchange and transmission across human and environmental interfaces (Avatsingh *et al*., 2023; Ariyanti *et al*., 2025).

Conclusion

This study demonstrates a substantial burden of multidrug-resistant Gram-negative bacteria, with ESBL gene prevalence lower than the proportion of highly resistant isolates, indicating that additional resistance mechanisms may contribute significantly to the observed MDR patterns. Extended-spectrum beta-lactamase mediated resistance was mainly driven by *bla-TEM* and *bla-CTX-M* genes, with *E. coli* acknowledged as the principal reservoir, while other Gram-negative organisms like *Klebsiella* and *Proteus* spp., played subsidiary roles. The significantly higher distribution of ESBL genes in clinical isolates particularly in BSUTH highlights healthcare settings as major drivers of antimicrobial resistance, likely due to sustained antibiotic pressure and infection dynamics.

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Nevertheless, the detection of ESBL genes in environmental isolates, including *E. coli* and *Klebsiella* species, underscores the role of hospital environments as important reservoirs that facilitate persistence and transmission.

Acknowledgement: The authors acknowledge the funding of this study by the Tertiary Education Trust Fund (TETFund) under the TETFund award of scholarship programme.

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