

Carbapenemase Resistance Genes among Gram-Negative Bacteria Isolated from Clinical and Environmental Samples in Selected Tertiary Hospitals in Makurdi, Nigeria

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Research Article

Keywords: Antibiotic resistance, Gene detection, Carbapenem, blaOXA-48, blaOXA-23, blaVIM, blaNDM, blaKPC, Healthcare facilities

Posted Date: October 9th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10710359/v1>

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Additional Declarations: No competing interests reported.

Abstract

The increasing occurrence of Multidrug-Resistant (MDR) Gram negative bacteria harbouring carbapenem resistance genes pose a serious threat to public health. The focus of most studies has either on clinical or environmental isolates. This study was designed to determine the antibiotic susceptibility patterns and detect carbapenemase resistance genes in Gram-negative bacteria isolated from both clinical and environmental sources in selected hospitals in Makurdi, Nigeria.

Clinical samples including urine (150), blood (2), endocervical (3), ear (3)-high vaginal (7)-wound (61) swabs, sputum (1), stools (10), semen (1) and cerebrospinal fluid (1) were collected from the two tertiary hospitals. Environmental samples: comprises of wastewater and swabs (toilet, taps and sink) from selected hospitals were collected. Gram negative bacteria were isolated and identified using standard microbiological methods and subjected to antibiotic susceptibility testing using Kirby Bauer disk diffusion method against cefepime, cefotaxime, ceftazime, ciprofloxacin, amoxicillin clavulanic acid, piperacillin tazobactam, amikacin, aztreonam, ertapenem and imipenem. Bacteria isolates that showed resistance to at least eight antibiotics were selected for carbapenem resistance genes (*bla*_{NDM}, *bla*_{KPC}, *bla*_{VIM}, *bla*_{OXA-23} and *bla*_{OXA-48}) detection using primer specific PCR. Isolates that harboured resistance genes were identified using 16S rRNA sequencing. Data were analysed using descriptive statistics.

Out of the 566 bacteria isolated, 409 were Gram-negative bacteria and were resistant to cefotaxime, amoxicillin clavulanic acid, ceftazime, cefepime, ciprofloxacin, imipenem and aztreonam were 83.4, 79.5, 78.0, 71.1, 57.2, 54.3 and 51.6%, respectively. The carbapenem resistant genes detected from clinical isolates were: *bla*_{OXA-48} (68.8%), *bla*_{VIM} (18.5%), *bla*_{NDM} (6.3%), *bla*_{KPC} (6.3%), while from the environmental isolates, *bla*_{OXA-48} (41.7%), *bla*_{VIM} (33.3%), *bla*_{OXA-23} (16.7%) and *bla*_{NDM} (8.3%) were detected. The *bla*_{OXA-23} was detected only in environmental isolates, while *bla*_{KPC} was detected only in the clinical isolates. Molecular identity of the isolates were *Alcaligenes faecalis*, *Alcaligenes ammonioxydans*, *Enterobacter hormaechei*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella quasipneumoniae*, *Proteus mirabilis*, *Providencia manganooxydans*, *Providencia rettgeri*, *Pseudomonas fragi* and *Shigella sonnei*.

Multidrug-resistant isolates that harboured carbapenem resistance genes were recovered from both the clinical and environmental source in Makurdi; this could pose transmission risk, hence effective infection control is recommended.

Introduction

The fight against infectious diseases, especially the emergence of Multidrug-Resistant Gram-Negative Bacteria (MDR-GNB) poses a significant threat to public health due to the lack of effective therapies (Kostyanov et al., 2021; Chukamnerd et al., 2022; Gonzalez et al., 2022). These bacteria are highly resistant to the existing antibiotic classes, particularly carbapenems which are regarded as one of the antibiotics in the last-line of treatments (Pormohammad et al., 2020; Theriault et al., 2021; Wareth et al., 2021). Increased selective pressure has been the primary cause of the current acceleration of the evolution of resistant microorganisms. Infections caused by Gram negative bacteria are becoming more prevalent globally and are sometimes resistant to all medications. These bacteria known as Multidrug Resistant (MDR) resist the effect of at least antibiotics from three or more classes (Gonzalez et al., 2022).

However, the healthcare system is increasingly threatened by the rise of Gram negative bacteria that are Extensively Drug-Resistant (XDR) or Pan Drug-Resistant (PDR). The XDR is resistance to all but one or two antimicrobial categories, while PDR is resistance to all available antimicrobial categories, and both are becoming increasing public health issue (Stewart et al., 2022). The main causes of XDR Gram negative bacteria include changes in outer membrane permeability and the synthesis of a variety of antibiotic resistance enzymes (Stewart et al., 2022). Beta-Lactams are among the most frequently prescribed antibiotics used to treat bacterial infections, but β -lactamase-mediated resistance does not spare even the most powerful β -lactams. Despite being one of the most prescribed antibiotics against bacterial infections, even the strongest β -lactams like carbapenems, are susceptible to β -lactamase-mediated resistance. These antibiotics' activity is hindered by carbapenemases like *Klebsiella pneumoniae* carbapenemases (KPC) and Oxacillinate-48 (OXA-48). Similarly, drug resistance may arise as a result of the use of substandard antibiotics in various regions of the world, especially in developing nations like Nigeria (Ullah and Ali, 2021).

Despite that carbapenem antibiotics are the preferred last-line β -lactam medications in case of deadly diseases caused by Gram-negative bacteria, their efficacy is severely compromised by the wide proliferation of Enterobacteriaceae that showed resistance to carbapenem and many other drugs (Bao et al., 2022; Stewart et al., 2022). Three of the most common carbapenemases that have been extensively documented in Carbapenem Resistant Enterobacteria (CRE) are class A carbapenemases (KPC), class B metallo- β -lactamases (NDM), and class D carbapenemases (OXA-48).

Globally, carbapenem resistance poses a serious threat; most serious infections caused by MDR Gram negative bacteria producing ESBL are often treated with carbapenems, a powerful and broad-spectrum β -lactam antibiotics which remain effective even against some universal

antibiotic resistance mechanisms, making them invaluable as last-line treatments (Behzadi et al., 2021; Amsalu et al., 2021; Elshamy et al., 2020; Darnal et al., 2021). Carbapenems share structural similarities with penicillin but rather than freely diffusing through the bacterial cell wall, carbapenems enter the cell via specialised proteins called porins, which are outer membrane proteins, this special structure greatly increases their stability against β -lactamases.

However, the primary source of MDR in bacteria is carbapenem resistance, triggered by the development of the enzyme carbapenemases. This is typically the final stage before PDR develops (Pramodhini, 2023). The global emergence and rapid dissemination of MDR-GNB, driven by carbapenemases resistance genes, pose a significant threat to public health. These resistance mechanisms severely limit treatment options, leading to increased morbidity, mortality, and healthcare costs. In Nigeria, the situation is further compounded by inadequate laboratory capacity and limited access to molecular diagnostic tools necessary for the accurate detection of resistance genes. This study was therefore aimed at determining the pattern of MDR and detecting Carbapenemase resistance genes in Gram negative bacteria isolated from clinical and environmental samples of two tertiary hospitals in Makurdi, North-central Nigeria.

Materials and methods

Study location

This study was carried out in Benue State situated in north-central geopolitical zone of Nigeria. With a total land area of about 34,059 km², the state is located between latitudes 6°25' and 8°8' N and longitudes 7°47' and 10°0' E. It is sharing boundaries with Kogi State to the (west), Nasarawa State to the (north), Taraba State to the (east), Cross River State (southeast). It also shares a 22-kilometer international border to the southeast with the Republic of Cameroon.

The two hospitals selected for the study were Federal Medical Centre (FMC) and Benue State University Teaching Hospital (BSUTH). These hospitals are the major tertiary healthcare institutions that serve as referral Centre's for the state and neighboring regions. The BSUTH is a state-owned teaching hospital established in 2012 with a 450-bed space, while FMC is a Federal Government-owned tertiary healthcare institution, originally established in 1927 as a railway clinic but was later converted into a general hospital and became a Federal Medical Centre in 1995. It serves as a major referral hospital.

Sample collection and bacteria isolation

A total of 340 clinical isolates comprising 210 and 130 were isolated from BSUTH and FMC, respectively between September and December 2022. Also, within same period, wastewater samples were collected from the drainages of the two healthcare facilities including pooled samples from two channels in FMC and three channels in BSUTH. The BSUTH channels include: theatre, female surgical ward, pediatric ward, male and female medical ward, amenity ward and resident doctor's hostel. Others include: isolation center, gynaecology ward, postnatal ward, laboratories drains and consultant block. Moreover, in FMC, the samples were the pool of wastewater coming out of the entire hospital. The samples were aseptically collected in sterile sample bottles using disposable Pasteur pipette and were transported in ice packs to the Microbiology Laboratory Department in BSUTH for immediate processing and bacteria isolation using MacConkey agar.

Swab Samples collection

Swab samples were collected from various wards of the hospitals using sterile swab sticks (CJ SMART®, China). Swabs of toilet bowls, tap faucets and washing hand sinks were taken. Also, swabs general operating unit, gynaecology, post-natal, pediatric surgical children ward and ORL in BUSTH were taken, while from FMC, it was the swabs of dialysis, postnatal, emergency pediatric antenatal, APIN, and gynaecology wards. The sampling sites were scrubbed with sterile swabs stick for 15–30 s, and placed in transport medium. The samples were transported in ice packs and processed upon arrival at the Microbiology laboratory Department in BSUTH and bacteria were isolated using MacConkey agar. The samples were also collected within same period with the isolates and wastewater.

Morphological characterisation of the isolates

Distinct bacterial colonies with non-duplicating morphological types observed on the plates after 18 to 24 hours incubation were selected and identified using Gram staining reaction and morphological characteristics (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 GNB using Mueller-Hinton agar (Ali et al., 2020). Ten antibiotics (BIOANALYSE®) that belongs to six different classes were tested against the isolates including β -lactam/cephalosporin (cefepime (30 μ g), cefotaxime (30 μ g) and ceftazime (30 μ g)); β -lactam combination (piperacillin tazobactam (100 μ g) and Amoxicillin clavulanic acid (20 μ g)); Quinolone (ciprofloxacin (5 μ g)); Aminoglycoside (Amikacin (30 μ g)); Monobactam ((azteronam) and Carbapenem (ertapenem (10 μ g) and impenem (10 μ g)).

Preparation of the inoculum for antibiotic susceptibility testing

The bacterial isolates were grown on MacConkey agar for 18 to 24 hours at 37°C and then on Mueller Hinton Agar (MHA). An 18–24 hours old culture of the isolates on MHA were inoculated into five mL sterile NaCl (0.9 percent) suspension using a sterile wire loop and adjusted to 0.5 McFarland standard (Amare et al., 2023). The standardised bacterial suspension was inoculated into MHA plates using a sterile swab stick (CJ SMART®, China). The swab was streaked in three different ways across the MHA surface and 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 and inverted for incubation at 35°C for 18 to 24 hours. The diameter of the zones of inhibition (mm) was measured, recorded and interpreted in accordance with the Clinical and Laboratory Standard Institute (Alhassan et al., 2022; CLSI, 2022).

Determination of Multiple Antibiotics Resistance Index (MARI)

The Multiple Antibiotic Resistance Index (MARI) was determined using previously described formula, MAR index = a/b; where "a" is the number of antibiotics to which an individual isolate is resistant and "b" is the total number of antibiotics to which an individual isolate was tested (Mthembu et al., 2008).

Detection of Carbapenemase Resistance Genes

DNA Extraction

Deoxyribonucleic acid (DNA) of the 137 selected bacteria was done by boiling method after it was freshly cultured on Nutrient agar at 37°C for 24hrs (Takawira and Mbanga, 2023). Briefly, in 200 μ L sterile Eppendorf tubes, a loopful of each of the bacterial isolates was suspended in sterile distilled water, left to remain at room temperature for five minutes and boiled for 20 minutes at 100°C. The extracted DNA were stored at -20°C in a deep freezer until needed for further analysis

Polymerase Chain Reaction

The primers used were reconstituted by dissolving 100 pmol/mL of each primer in sterile distilled water as instructed by the manufacturer. The primers were further dissolved to 10 pmol/mL by adding 90 μ L of sterile distilled water to 10 μ L from the initial constituent of 100 pmol/mL. The total reaction volume (12.5 μ L) in 0.2 mL PCR tube consisted of; 4.75 μ L nuclease free water (BioConcept), 6.25 μ L Master mix (NEW ENGLAND, BioLabs_{inc.}), 0.25 μ L forward primer (Inqaba Biotech), 0.25 μ L reverse primer (Inqaba Biotech), 1.0 μ L DNA (template DNA).

Five pairs of specific primers (Inqaba Biotech) for the detection of carbapenemase encoding genes (*bla*_{KPC}, *bla*_{OXA-23}, *bla*_{OXA-48}, *bla*_{NDM}, and *bla*_{VIM}) were used. The 137 Gram-negative bacteria with multidrug resistance profile were subject to PCR for the detection of the genes. The total reaction mixture was calculated and multiplied by the number of isolates. The amplicon sizes and primer sequences as well as the PCR conditions are shown in Tables 1 and 2, respectively. The 1.5% agarose gel electrophoresis was used to identify the amplified product.

Agarose Gel Electrophoresis

In a 50 μ L of 1X TBE (Tris Borate EDTA) buffer was mixed with 0.5 grams of Agarose powder to prepare the Agarose gel. To prevent buffer loss, the solution was microwaved until completely dissolved, avoiding boiling, and then allowed to cool at 45–50°C. Then stained with a safe-view solution (Applied Biological Materials Inc.) to enhance the visibility of the bands. The comb was inserted and gel poured in the electrophoresis tray to make a cast. After carefully pouring the gel to avoid creating air bubbles, it was left to gelatinize for 20 to 30 minutes at room temperature. After which the comb was gently removed from the solid agarose and submerged in 1X TBE buffer inside the electrophoresis tank.

A 5 μ L molecular weight ladder was loaded into the first lane, and 5 μ L each of the amplicon (PCR product) were added to other wells. The size of each target gene was confirmed using a 100 bp DNA ladder ((NEW ENGLAND, BioLabs_{inc.}) as DNA molecular weight standard to estimate the DNA band. The gel was allowed to run in an Electric current at 100 volts for 40 minutes till the tincture has reached to the other

side of the gel. The gel was carefully taken out, the electrodes unplugged, and the electricity turned off. The PCR products was viewed using the UV trans-illuminator. A gel imaging and documentation system (Vilber Lourmat, Italy).

Molecular Characterisation of the Selected Multidrug Resistant Carbapenemase Genes Carrier

The universal primers used for PCR targeted the conserved regions of the 16S rRNA gene were 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT T-3') forward and reverse primers respectively. A total PCR Master Mix (NEW ENGLAND, BioLabs_{inc.}) of 12.5 µL contained 0.5 µL of each primer, 9.5 µL nuclease-free water (BioConcept), and 2 µL of DNA template. The PCR conditions were five minutes of initial denaturation at 94°C, 30 seconds amplification at 94°C for 35 cycles, 30 seconds annealing at 55°C, one minute thirty seconds extension at 68°C, 10 minutes final extension at 68°C, and holding temperature at 4°C. Two microliters of each amplicon were loaded onto a 1% agarose gel stained with five microliters of Safe-view Red. After PCR amplification, photograph was taken using the gel documentation system E-BOX (Vilber Lourmat, Italy) and was kept at -20°C for 16S rRNA gene sequencing.

Sequencing of 16S rRNA gene amplicons

For 16S rRNA gene sequencing, the isolates with predicted band widths on the gel were selected and the amplified PCR products was cleaned up using 50 µL to 20 U/µL Exonuclease 1 (catalogue No. NEB M0293L) and 200 µL of 1 U/µL Shrimp Alkaline Phosphatase (catalogue No. NEB M0371) added to 0.6 mL micro-centrifuge tube to create the ExoSAP master mix. The reaction mixture contained 10 µL of the generated PCR products and 2.5 µL of ExoSAP mix and was incubated at 37°C for 15 minutes and at 80°C for 15 minutes. The fragments were sequenced using the Nimagen Brilliant Dye Terminator cycle sequencing kit V3. 1, BRD3-100/1000. The tagged products were subsequently cleaned using the ZR-96 DNA Sequencing Cleanup Kit (catalogue No. D4053). The cleaned products were injected using POP7 into the 50 cm array of the Applied Biosystems ABI 3500XL Genetic Analyser. Sequence data was then gathered and examined using bioinformatics tools. By manually matching the 16S rRNA sequences with recognised species sequences on the NCBI website, the isolates were identified to species level.

Table 1
Primers Used in this Study

S/No.	TARGET GENE	PRIMER (5'-3')	BASE PAIR (bp)	PCR CONDITIONS
1	VIM	F: GATGGTGT TTTGGTCGCATA R: CCAATGCGCAGCACCAG	390	Denaturation for 30 sec at 95°C; 36 cycles of 95°C for 30 sec, Annealing 52°C for 60 sec, Extension 72°C for 60 sec; and final extension of 72°C for 5 min. Hold 4°C infinite
2	NDM	F: GGTTTGCGCATCTGGTTTTC R: CGGATTGGCTACCGAOGAT	621	Same as VIM
3	KPC	F: CGTCTAGTTCGTCTGTCTTG R: CTTGTCATCCTTAGGCG	798	Same as VIM
4	OXA-23	F: CCTGATCGGATTGGAGAACCAG R: GATGCCGGAATTTCTGACCG	516	Denaturation for 30 sec at 95°C; 36 cycles of 95°C for 30 sec, Annealing 60°C for 60 sec, Extension 72°C for 60 sec; and final extension of 72°C for 5 min. Hold 4°C infinite
5	OXA-48	F: CCAAGCATTTTACCCGCATCKACC R: GYTTGACCATACGCTGRCTGCG	389	Same as OXA-23

Results

A total of 566 bacteria were isolated comprising 340 and 226 from clinical and environmental samples, respectively. Out of the clinical isolates, 239 Gram negative bacteria were obtained comprising 166 from BSUTH and 73 from FMC. Moreover, out of the 170 from the environmental samples 105 and 65 were from BSUTH and FMC, respectively (Table 2).

The distribution of the Gram negative bacteria based on the clinical and environmental samples types showed that 36.7% were from urine, 0.5% (blood), 0.7% (endocervical swabs), 0.7% (ear swabs), 1.7% (high vaginal swabs), 14.9% (wound swabs), 0.2% (sputum), 2.4% (stool), 0.2% (semen) and 0.2% (cerebrospinal Fluid). In addition, the isolates from the environmental samples were from wastewater (30.6%), swabs of toilet (5.1%), taps (1.5%) and sink (4.4%) (Table 3).

Table 2
The Gram-negative bacteria isolated from the two hospitals

Source	Gram-negative Bacteria Isolated	Confirmed Gram-negative Bacteria	Percentage confirmed Gram-negative bacteria
Clinical			
BSUTH	210	166	79.10
FMC	130	73	56.20
Environmental			
BSUTH	142	105	73.90
FMC	84	65	77.40
Total	566	409	72.30
Key:			
BSUTH: Benue State Teaching Hospital			
FMC: Federal Medical Centre			

Table 3
The Confirmed Gram negative bacteria Isolated from the Clinical and Environmental Samples (n = 409)

CLINICAL		ENVIRONMENTAL	
Name of Specimen	Number of GNB (%)	Name of Specimen	Number of GNB (%)
Blood	2(0.50)	Sink swab	18(4.40)
Cerebrospinal fluid	1(0.20)	Toilet swab	21(5.10)
Ear swab	3(0.70)	Tap swab	6(1.50)
Endocervical swab	3(0.70)	Wastewater	125(30.60)
High vaginal swab	7(1.70)		
Semen	1(0.20)		
Sputum	1(0.20)		
Stool	10(2.40)		
Urine	150(36.70)		
Wound swab	61(14.90)		

The susceptibility profile of the isolates

The pattern of the susceptibility of the Gram negative bacteria showed that 71.1% were resistant to Cefipime, while it was 83.4%, 78.0%, 57.2%, 79.5%, and 30.8% to cefotaxime, ceftazidime, ciprofloxacin, amoxicillin clavulanic acid and piperacillin tazobactam respectively. In addition, 5.4%, 51.6%, 22.7% and 54.3% showed resistance to amikacin, aztreonam, ertapenem and imipenem, respectively (Table 4).

Table 4
The susceptibility pattern of the GNB to different antibiotics (N = 409)

Antibiotic Susceptibility (%)				
S/No.	Antibiotics used	S	I	R
1	ATM	161(39.40)	35(8.60)	211(51.60)
2	CAZ	72(17.60)	18(4.40)	319(78.00)
3	AK	340(83.10)	47(11.50)	22(5.40)
4	PTZ	211(51.60)	72(17.60)	126(30.80)
5	CPM	81(19.80)	37(9.00)	291(71.10)
6	IPM	169(41.30)	18(4.40)	222(54.30)
7	CTX	46(11.20)	22(5.40)	341(83.40)
8	AUG/AMC	52(12.70)	32(7.80)	325(79.50)
9	ETP	265(64.80)	51(12.50)	93(22.70)
10	CIP	109(26.70)	66(16.10)	234(57.20)

KEY:

ATM- Aztreonam, CAZ- Ceftazime, AK- Amikacin, PTZ- Piperacillin Tazobactam, CPM- Cefepime, IPM- Imipenem, AUG- Amoxicillin Clavulanic Acid, ETP- Ertapenem, CIP- Ciprofloxacin, CTX- Cefotaxime, S- Sensitive, I- Intermediate and R- Resistance.

Table 5
Antibiotics resistance Pattern of the GNB Isolated from Samples Obtained from BSUTH

Antibiotics resistance pattern of the GNB (%)						
S/No.	Antibiotics	<i>E. coli</i> n = 79	<i>Klebsiella</i> sp. n = 7	<i>Proteus</i> sp. n = 57	<i>Pseudomonas</i> sp. n = 22	<i>Salmonella</i> sp. n = 1
1	ATM	55(69.60)	5(71.00)	33(57.90)	12(54.50)	0(0.00)
2	CAZ	75(94.90)	6(85.70)	53(93.00)	18(81.80)	1(100.00)
3	AK	13(16.50)	0(0.00)	6(10.50)	3(13.60)	1(100.00)
4	PTZ	52(65.80)	2(28.60)	23(40.40)	8(36.40)	0(0.00)
5	CPM	66(83.50)	5(71.40)	49(86.00)	15(68.20)	1(100.00)
6	IMP	57(72.20)	3(42.90)	44(77.20)	14(63.60)	1(100.00)
7	CTX	74(93.70)	4(57.10)	52(91.20)	20(90.90)	1(100.00)
8	AUG	76(96.20)	3(42.90)	53(93.00)	20(90.90)	1(100.00)
9	ETP	31(39.20)	3(42.90)	17(29.80)	14(63.60)	0(0.00)
10	CIP	63(79.70)	5(71.40)	40(70.20)	10(45.50)	0(0.00)

Table 6
Antibiotics resistance Pattern of the Gram negative bacteria Isolated from Samples Obtained from FMC

Antibiotics resistance pattern of the GNB (%)				
S/No.	Antibiotics	E. coli n = 48	Klebsiella sp. n = 12	Pseudomonas sp. n = 13
1	ATM	35(72.90)	8(66.70)	9(69.20)
2	CAZ	43(89.60)	11(91.70)	9(69.20)
3	AK	11(22.90)	1(8.30)	7(53.80)
4	PTZ	24(50.00)	6(50.00)	7(53.80)
5	CPM/PEP	41(85.40)	10(83.30)	9(69.20)
6	IMP	15(31.30)	5(41.70)	6(46.20)
7	CTX	43(89.60)	11(91.70)	13(100.00)
8	AUG/AMC	32(66.70)	9(75.00)	7(53.80)
9	ETP	16(33.30)	3(25.00)	6(46.20)
10	CIP	42(87.50)	12(100.00)	10(76.90)

The multiple antibiotic resistance phenotypes in the isolates

The multiple resistance phenotypes exhibited by the isolates obtained from BSUTH showed that one of the *Proteus* sp. and two other *Pseudomonas* spp. showed resistance to a combination of three antibiotics (CTX-AUG-ETP), while another set of two *E. coli* and one *Proteus* sp. showed resistance to a combination of three antibiotics (CAZ-AUG-CIP). In addition, it was observed that 15 *E. coli*, four *Proteus* spp. and three *Pseudomonas* spp. showed resistance to a combination of nine antibiotics that included ATM-CAZ-PTZ-CPM-IMP-CTX-AUG-ETP-CIP, while another set of nine *E. coli* and three *Proteus* species were resistant to a combination of the following eight antibiotics: ATM-CAZ-PTZ-CPM-IMP-CTX-AUG-CIP (Table 7). Similarly, among the GNB isolated from FMC, six *E. coli* and one *Klebsiella* spp. were resistant to a combination of six antibiotics that included ATM-CAZ-CPM-CTX-AUG-CIP, while a different set of four *E. coli* and two *Klebsiella* spp. Also exhibited resistance to a combination of the following eight antibiotics: ATM-CAZ-CPM-CTX-AUG-CIP. In addition, one *E. coli* and three *Proteus* spp also showed resistance to a combination of 10 antibiotics including ATM-CAZ-AK-PTZ-CPM-IMP-CTX-AUG-ETP-CIP (Table 8).

The distribution of carbapenemase genes in the healthcare facilities

A total of 28 carbapenemase-producing isolates were detected across the studied healthcare facilities. The distribution of carbapenemase genes showed marked variability between hospitals and sample sources (clinical and environmental). The *bla*_{OXA-48} was the most prevalent gene, detected in 16 isolates (57.1%) and followed by *bla*_{VIM} (25.0%), while *bla*_{NDM} (7.1%) and *bla*_{OXA-23} (7.1%) were less frequent. The *bla*_{KPC} gene was rare, occurring in only 1 isolate (3.6%). At the facility level, isolates from BSUTH clinical samples accounted for the highest proportion (42.9%) of all carbapenemase genes identified, with a strong predominance of *bla*_{OXA-48} (32.1%) within this group. In contrast, BSUTH environmental samples contributed 25.0%, with *bla*_{VIM} (10.7%) and *bla*_{OXA-48} (10.7%) being the dominant genes. At FMC, carbapenemase genes were less prevalent. Clinical isolates contributed 14.3%, while environmental isolates accounted for 17.9%. In both sample types, *bla*_{OXA-48} and *bla*_{VIM} were the most frequently detected genes, although at lower proportions compared to BSUTH. Notably, *bla*_{NDM} genes were exclusively detected in FMC (clinical and environmental samples) and were absent in BSUTH. Similarly, *bla*_{KPC} was only detected in BSUTH clinical isolates, suggesting possible localized gene dissemination patterns (Table 9).

Distribution of the carbapenemase resistance genes in the Gram-negative bacteria from clinical and environmental isolates

A total of 16 carbapenemase-producing Gram-negative bacterial isolates were identified from clinical samples across the two healthcare facilities. The distribution of carbapenemase genes varied across bacterial species and between hospitals. *Escherichia coli* (*E. coli*) was the predominant organism, accounting for 9 isolates (52.3%), followed by *Proteus* species (18.8%), while *Klebsiella* species and *Pseudomonas* species each contributed 12.5% of the total isolates. Across all isolates, *bla*_{OXA-48} was the most frequently detected carbapenemase gene, predominantly observed in *E. coli* (25.0% in BSUTH; 6.3% in FMC), and also present in *Proteus*, *Klebsiella*, and *Pseudomonas* species. In contrast, *bla*_{VIM} was the second most prevalent gene and was mainly associated with *E. coli* and *Proteus* species.

Table 7. The multiple antibiotic phenotypes of the GNB isolated from clinical samples from BSUTH

No. of antibiotic	Resistance pattern	<i>E. coli</i>	<i>Klesiella</i> sp.	<i>Proteus</i> sp.	<i>Pseudomonas</i> sp.	<i>Salmonella</i> sp.	Total (%)
3	CTX/AUG/ETP	0	0	1	2	0	3(1.90)
	CAZ/AUG/CIP	2	0	1	0	0	3(1.90)
4	CAZ/AK/AUG/CIP	1	0	0	0	0	1(0.60)
	CTX/AUG/ETP/CIP	1	0	0	0	0	1(0.60)
	CAZ/CTX/AUG/CIP	1	0	0	0	0	1(0.60)
	CAZ/CTX/AUG/ETP	0	0	0	1	0	1(0.60)
5	ATM/CAZ/CTX/ETP/CIP	1	0	0	0	0	1(0.60)
	ATM/CAZ/CTX/AUG/CIP	1	0	0	0	0	1(0.60)
	ATM/CAZ/CPM/CTX/AUG	2	0	1	0	0	3(1.90)
	CAZ/IMP/CTX/AUG/CIP	0	0	2	0	0	2(1.30)
	ATM/CAZ/CPM/CTX/CIP	0	1	0	0	0	1(0.60)
	CAZ/CPM/IMP/CTX/AUG	2	1	4	0	0	7(4.50)
	CAZ/PTZ/IMP/CTX/AUG	1	0	1	0	0	2(1.30)
6	ATM/CAZ/CPM/CTX/AUG/CIP	1	0	2	1	0	4(2.60)
	ATM/CPM/IMP/CTX/AUG/ETP	0	0	1	0	0	1(0.60)
	CAZ/CPM/IMP/CTX/AUG/CIP	3	0	8	0	0	11(7.00)
	CAZ/PTZ/CPM/IMP/CTX/CIP	1	0	0	0	0	1(0.60)
	CAZ/CPM/IMP/CTX/AUG/ETP	1	0	0	1	0	2(1.30)
	CAZ/AK/CPM/IMP/CTX/AUG	1	0	0	0	0	1(0.60)
	CAZ/PTZ/CPM/IMP/CTX/AUG	3	0	1	0	1	5(3.20)
	ATM/CAZ/CPM/IMP/CTX/AUG	1	0	0	0	0	1(0.60)
	CAZ/PTZ/IMP/CTX/AUG/CIP	2	0	0	0	0	2(1.30)
7	ATM/CAZ/CPM/IMP/CTX/AUG/ETP	2	0	3	2	0	7(4.50)
	ATM/CAZ/PTZ/CTX/AUG/ETP/CIP	0	0	0	1	0	1(0.60)
	ATM/CAZ/PTZ/CPM/CTX/AUG/CIP	3	0	1	0	0	4(2.60)
	ATM/CAZ/CPM/IMP/CTX/AUG/CIP	1	0	5	2	0	6(3.80)
	ATM/CAZ/AK/CPM/CTX/AUG/CIP	1	0	0	0	0	1(0.60)
	ATM/CAZ/CPM/CTX/AUG/ETP/CIP	1	1	1	0	0	3(3.20)
	CAZ/CPM/IMP/CTX/AUG/ETP/CIP	1	0	0	0	0	1(0.60)
	CAZ/AK/CPM/IMP/CTX/AUG/ETP	0	0	0	1	0	1(0.60)
	CAZ/PTZ/CPM/IMP/CTX/AUG/CIP	1	0	3	1	0	5(3.20)
	CAZ/PTZ/CPM/IMP/CTX/AUG/ETP	0	0	0	1	0	1(0.60)
8	ATM/CAZ/PTZ/CPM/CTX/AUG/ETP/CIP	0	1	1	0	0	2(1.30)
	CAZ/PTZ/CPM/IMP/CTX/AUG/ETP/CIP	1	0	1	0	0	2(1.30)
	ATM/CAZ/PTZ/CPM/CTX/AUG/ETP/CIP	2	0	1	0	0	3(1.90)
	ATM/CAZ/PTZ/CPM/IMP/CTX/AUG/CIP	9	0	5	0	0	14(8.90)
	CAZ/AK/PTZ/CPM/IMP/CTX/AUG/CIP	1	0	0	0	0	1(0.60)

No. of antibiotic	Resistance pattern	<i>E. coli</i>	<i>Klesiella</i> sp.	<i>Proteus</i> sp.	<i>Pseudomonas</i> sp.	<i>Salmonella</i> sp.	Total (%)
	ATM/CAZ/PTZ/CPM/IMP/CTX/AUG/ETP	1	0	0	0	0	1(0.60)
9	ATM/CAZ/PTZ/CPM/IMP/CTX/AUG/ETP/CIP	15	1	4	3	0	23(14.70)
	ATM/CAZ/AK/PTZ/CPM/IMP/CTX/AUG/CIP	4	0	3	0	0	7(4.50)
10	ATM/CAZ/AK/PTZ/CPM/IMP/CTX/AUG/ETP/CIP	6	0	3	3	0	12(7.60)
	TOTAL						157

Table 8
The multiple antibiotic resistance Phenotypes of the GNB isolated from clinical samples from FMC

No. of antibiotic	Resistance pattern	<i>E. coli</i>	<i>Klesiella sp.</i>	<i>Proteus sp.</i>	<i>Pseudomonas sp.</i>	Total (%)
3	CTX/AUG/ETP	0	0	0	1	1(1.40)
	CTX/AUG/CIP	1	0	0	0	1(1.40)
	ATM/CAZ/CPM	1	0	0	0	1(1.40)
4	ATM/CTX/AUG/CIP	0	0	0	1	1(1.40)
	CAZ/CTX/AUG/CIP	1	1	0	0	2(1.30)
	ATM/CAZ/CTX/CIP	0	0	0	1	1(1.30)
5	ATM/CAZ/CTX/AUG/CIP	0	0	0	1	1(1.30)
	ATM/CAZ/CPM/CTX/CIP	1	0	0	0	1(1.30)
	CAZ/CPM/CTX/AUG/CIP	1	0	0	0	1(1.30)
6	ATM/CAZ/CPM/CTX/AUG/CIP	6	1	0	0	7(9.70)
	CPM/IMP/CTX/AUG/ETP/CIP	0	0	0	1	1(1.30)
	ATM/CAZ/CPM/CTX/AUG/ETP	2	0	0	0	2(2.80)
	CAZ/PTZ/CPM/CTX/AUG/CIP	0	1	0	0	1(1.30)
	CAZ/AK/CPM/CTX/ETP/CIP	1	0	0	0	1(1.30)
	ATM/CAZ/CPM/CTX/ETP/CIP	1	0	0	0	1(1.30)
	ATM/CAZ/PTZ/CPM/AUG/CIP	1	0	0	0	1(1.30)
	CAZ/PTZ/IMP/CTX/AUG/CIP	1	0	0	0	1(1.30)
	CAZ/CPM/IMP/CTX/AUG/CIP	0	1	0	0	1(1.30)
7	ATM/CAZ/PTZ/CTX/AUG/ETP/CIP	0	0	0	1	1(1.30)
	ATM/CAZ/PTZ/CPM/CTX/AUG/CIP	4	0	0	1	5(6.90)
	ATM/CAZ/CPM/CTX/AUG/ETP/CIP	0	3	0	0	3(4.20)
	ATM/CAZ/AKCPM//CTX/AUG/CIP	1	0	0	0	1(1.30)
	CAZ/PTZ/CPM/IMP/CTX/AUG/CIP	0	1	0	1	2(2.80)
	ATM/CAZ/CPM/IMP/CTX/AUG/CIP	1	1	0	0	2(2.80)
	CAZ/CPM/IMP/CTX/AUG/ETP/CIP	1	0	0	0	1(1.30)
	ATM/CAZ/PTZ/CPM/IMP/CTX/AUG	0	0	0	1	1(1.30)
8	ATM/CAZ/PTZ/CPM/CTX/AUG/ETP/CIP	4	2	0	0	6(8.30)
	ATM/CAZ/CPM/IMP/CTX/AUG/ETP/CIP	3	0	0	0	3(4.20)
	ATM/CAZ/AK/PTZ/CPM/CTX/AUG/CIP	3	0	0	0	3(4.20)
	ATM/CAZ/PTZ/CPM/IMP/CTX/AUG/CIP	2	1	0	0	2(2.80)
	CAZ/AK/PTZ/CPM/IMP/CTX/AUG/CIP	3	0	0	0	3(4.20)
9	ATM/CAZ/AK/PTZ/CPM/IMP/CTX/AUG/CIP	0	1	0	0	1(1.30)
	ATM/CAZ/PTZ/CPM/IMP/CTX/AUG/ETP/CIP	3	0	0	0	3(4.20)
10	ATM/CAZ/AK/PTZ/CPM/IMP/CTX/AUG/ETP/CIP	1	0	0	3	4(5.60)
TOTAL						72

KEY: ATM- Aztreonam, CAZ- Ceftazime, AK- Amikacin, PTZ- Piperacillin Tazobactam, CPM- Cefepime, IPM- Imipenem, AUG- Amoxicillin Clavulanic Acid, ETP- Ertapenem, CIP- Ciprofloxacin, CTX- Cefotaxime.

Table 9
Distribution of the Carbapenemase Genes in the healthcare facilities (n = 28)

Health centers	Carbapenemase resistance genes					
	NDM	KPC	VIM	OXA-23	OXA-48	Total
BSUTH Clinical	0(0.00)	1(3.60)	2(7.10)	0(0.00)	9(32.10)	12(42.90)
BSUTH Environmental	0(0.00)	0(0.00)	3(10.70)	1(3.60)	3(10.70)	7(25.00)
FMC Clinical	1(3.60)	0(0.00)	1(3.60)	0(0.00)	2(7.10)	4(14.30)
FMC Environmental	1(3.60)	0(0.00)	1(3.60)	1(3.60)	2(7.10)	5(17.90)
Total	2(7.10)	1(3.60)	7(25.00)	2(7.10)	16(57.10)	28(100.00)

The *bla*_{KPC} gene was exclusively detected in *E. coli* isolates from BSUTH (6.3%), while *bla*_{NDM} was only identified in *E. coli* isolates from FMC (6.3%). Notably, *bla*_{OXA-23} was not detected in any of the clinical isolates (Table 10) whereas in the environmental isolates a total of 12 carbapenemase-producing Gram-negative bacterial isolates were detected. The isolates comprised a diverse group of organisms, with *Enterobacter hormaechei* being the most frequent three isolates (25.0%), followed by *Escherichia coli* (16.7%), while other species including *Alcaligenes faecalis*, *Alcaligenes ammonioxydans*, *Klebsiella pneumoniae*, *Providencia rettgeri*, *Providencia manganooxydans*, *Proteus mirabilis*, and *Shigella sonnei* each accounted for 8.3% of the isolates. In terms of carbapenemase gene distribution, *bla*_{OXA-48} was the most prevalent gene, detected in 5 isolates (41.7%) and followed by *bla*_{VIM} (33.3%). The *bla*_{KPC} (16.7%) and *bla*_{NDM} (8.3%) were less frequently detected, while *bla*_{OXA-23} was not identified in any environmental isolate. Species-specific analysis showed that *bla*_{OXA-48} was distributed across multiple organisms, including *E. coli*, *Klebsiella pneumoniae*, and *Alcaligenes ammonioxydans*, indicating broad dissemination in the hospital environment. *bla*_{VIM} genes were identified in *Alcaligenes faecalis*, *Enterobacter hormaechei*, and *Providencia manganooxydans*, while *bla*_{KPC} was uniquely detected in *Providencia rettgeri*. The only NDM-positive isolate was observed in *Enterobacter hormaechei* from FMC (Table 11).

Table 10
Distribution of the carbapenemase resistance genes in the Gram-negative bacteria from clinical isolates (n = 16)

Bacterial Isolates	Carbapenemase resistance genes										Total
	NDM		KPC		VIM		OXA-23		OXA-48		
	BSUTH	FMC	BSUTH	FMC	BSUTH	FMC	BSUTH	FMC	BSUTH	FMC	
<i>E. coli</i>	0(0.00)	1(6.30)	1(6.30)	0(0.00)	1(6.30)	1(6.30)	0(0.00)	0(0.00)	4(25.00)	1(6.30)	9(52.30)
<i>Proteus</i> sp.	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(6.30)	0(0.00)	0(0.00)	0(0.00)	2(12.50)	0(0.00)	3(18.80)
<i>Klebsiella</i> sp.	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(6.30)	1(6.30)	2(12.50)
<i>Pseudomonas</i> sp.	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(12.50)	0(0.00)	2(12.50)
Total	1(6.30)		1(6.30)		3(18.80)		0(0.00)		11(68.80)		16(100.00)

Table 11
Distribution of the carbapenemase resistance genes in the Gram-negative bacteria from environmental isolates (n = 12)

Bacterial Isolates	Carbapenemase resistance genes										
	NDM		KPC		VIM		OXA-23		OXA-48		Total
	BSUTH	FMC	BSUTH	FMC	BSUTH	FMC	BSUTH	FMC	BSUTH	FMC	
<i>Alcaligenes faecalis</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(8.30)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(8.30)
<i>Alcaligenes ammonioxydans</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(8.30)	0(0.00)	1(8.30)
<i>E. coli</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(8.30)	1(8.30)	2(16.70)
<i>Enterobacter hormaechei</i>	0(0.00)	1(8.30)	0(0.00)	0(0.00)	1(8.30)	1(8.30)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	3(25.00)
<i>Klebsiella pneumoniae</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(8.30)	0(0.00)	1(8.30)
<i>Providencia manganooxydans</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(8.30)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(8.30)
<i>Providencia rettgeri</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(8.30)	0(0.00)	0(0.00)	0(0.00)	1(8.30)
<i>Proteus mirabilis</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(8.30)	0(0.00)	0(0.00)	1(8.30)
<i>Shigella sonnei</i>	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(8.30)	1(8.30)
Total	1(8.30)		0(0.00)		4(33.30)		2(16.70)		5(41.70)		12(100.00)

Discussion

Urine samples accounted for the highest number of Gram-negative bacterial isolates 150 (36.70%). This observation align with the findings of Debabza et al. (2023), who reported high frequency of bacterial isolation from urine samples, it also agrees with a multicenter study in Nigeria where urine was the leading source of Gram negative bacteria (56.9%) (Omeregbe and Fagade, 2020). Similarly, in a study from Gombe, Nigeria, urine accounted for 53.4% of all Enterobacteriaceae isolates (Saleh et al., 2024). A study in Pakistan also confirmed that most Gram-negative bacteria were from urine samples (71.1%) (Abbas et al., 2020) though, contrasts with those of Flamerz et al. (2023), who reported a lower frequency. Urine is frequently the most common source of Gram Negative Bacteria (GNB) isolates, primarily due to the high prevalence of infections of the urinary tract (UTIs), especially in both community and hospital settings. The gastrointestinal tract serves as repository for many GNB like *Escherichia coli*, which can ascend the urethra and colonise the urinary tract, particularly in females due to anatomical proximity (Behzadi et al., 2010). High rate of GNB were also isolated from wound samples 61 (4.9%). This may be due to hospital-acquired infections, surgical site infections, or trauma-related contamination.

Wastewater accounted for highest number of Gram negative bacteria isolated from the environmental samples 125 (30.6%) and the lowest from tap 6(1.5%). This distribution highlights significant variation in bacterial contamination across different environmental sources. Numerous studies have confirmed that wastewater systems, particularly hospital and urban effluents, serve as significant reservoirs for multidrug-resistant GNB. High bacterial loads are typical due to the accumulation of organic waste, antibiotics, and human pathogens, which create favorable conditions for microbial growth and resistance gene exchange (Zagui et al., 2020; Tarannum et al. 2024). Tap water typically contains the lowest levels of GNB, may be due to rigorous water treatment and disinfection processes. However, some studies have still detected opportunistic and antibiotic-resistant strains in drinking water systems, such as *Pseudomonas*, *Acinetobacter*, and *Ralstonia*, (Vincenti et al., 2014; Vaz-Moreira et al., 2016).

The most prevalent Gram-negative bacteria is *E. coli*. These results are consistent with patterns observed in other clinical settings. Studies from several regions, including Nigeria and India, reported that *the most prevalent Gram-negative bacteria is E. coli* (Ali, 2020; Omeregbe and Fagade, 2020; Olaoye and Olajide, 2024). *Escherichia coli* is a frequent pathogen in the gastrointestinal and urinary systems, as evidenced by the high incidence of the bacteria in urine stool, sputum and HVS. Frequent isolation of *Pseudomonas* spp. from CSF, urine, and wounds samples shows that *it is an* opportunistic bacteria found in moist environments and is known for being highly prevalent in such clinical specimens and frequently causes hospital-acquired infections (Ruhil et al., 2009; Awanye et al., 2021).

The antibiotics resistance profile of the 409 GNB shows a concerning trend of multidrug resistance (MDR). High Resistance to Cephalosporins (CPM, CTX, CAZ). Cefepime (CPM) 71.1% resistance, cefotaxime (CTX) 83.4% resistance and ceftazidime (CAZ) 78.0% resistance were observed. This is in agreement with the report that hospital-acquired infections had comparable high rates of ceftazidime and cefotaxime resistance (Yaman et al., 2004). Distinct resistance patterns were observed among *E. coli*, *Klebsiella* spp., *Proteus* spp., and *Pseudomonas* spp., with *Salmonella* spp. showing notable resistance. *Escherichia coli* shows high multidrug resistance, particularly to AUG, CAZ, and CTX. *Escherichia coli* displayed the highest resistance to Augmentin (AUG) at 96.2%. This trend aligns with several studies reporting high resistance of *E. coli* to β -lactams, especially due to extended-spectrum β -lactamase production (Starling et al., 2021; Dodani and Nasim, 2023; Olaoye and Olajide, 2024).

Klebsiella spp. showed resistance to all antibiotics tested except amikacin. This reflects growing carbapenem and β -lactam resistance in hospital settings due to carbapenemase production. Similar findings were reported in hospital isolates from Nigeria and Bangladesh (Uddin et al., 2017). *Pseudomonas* spp. had the highest resistance to CTX and AUG (90.9%), this is consistent with its intrinsic resistance mechanisms and ability to acquire further resistance under antibiotic pressure (Karungamye et al., 2023; Morales et al., 2024). *Salmonella* spp. show notable resistance. Though less frequently isolated, *Salmonella* spp. displayed resistance to several antibiotics (100.0%), reflecting sporadic but significant resistance emergence, particularly in settings with poor infection control and antibiotic misuse (Moremi et al., 2016). Resistance to carbapenems like IPM (31.3.0%) and ETP (33.3%) was lower, which aligns with international trends where carbapenems still retain efficacy, although increasing resistance is being observed globally (Starling et al., 2021).

The predominance of *bla*_{OXA-48} (57.1%) observed in this study agrees with global and African reports identifying OXA-48-like enzymes as major drivers of carbapenem resistance among Gram-negative bacteria (GNB). Global surveillance data have consistently highlighted the increasing dissemination of *bla*_{OXA-48}, particularly in low- and middle-income settings (Pitout et al., 2019; Manenzhe et al., 2015). The high burden observed in BSUTH clinical isolates suggests ongoing nosocomial transmission and possible clonal expansion within the facility. The second most prevalent carbapenemase gene, *bla*_{VIM} (25.0%) underscore the growing role of metallo- β -lactamases (MBLs) in antimicrobial resistance. Although less dominant than *bla*_{OXA-48} and *bla*_{NDM} globally, *bla*_{VIM} has been increasingly reported in both clinical and environmental settings, contributing to multidrug resistance and limiting therapeutic options (Logan and Weinstein, 2017; Gajdács et al., 2020). Its detection across hospitals and sample types in this study supports environmental persistence and bidirectional transmission between patients and hospital surfaces.

The occurrence of *bla*_{NDM} (7.1%), restricted to FMC, is epidemiologically significant. The *bla*_{NDM} is widely recognized for its rapid global dissemination via mobile genetic elements, with increasing prevalence reported across Africa (~ 15%) (Manenzhe et al., 2015; Wu et al., 2019). Its localized detection in FMC suggests recent introduction or facility-specific transmission dynamics, warranting targeted surveillance and containment measures. In contrast, the low prevalence of *bla*_{KPC} (3.6%) aligns with regional trends in Africa and parts of Asia, where *bla*_{OXA-48} and *bla*_{NDM} predominate, unlike Europe and North America (Nordmann and Poirel, 2019). Its confinement to BSUTH clinical isolates suggests limited dissemination or sporadic introduction. The detection of *bla*_{OXA-23} (7.1%), typically associated with *Acinetobacter* spp., highlights the contribution of non-fermenting GNB to hospital-acquired infections. Its presence in both clinical and environmental samples further supports the role of hospital environments as reservoirs of persistent resistant organisms (Hamidian and Nigro, 2019). Notably, the substantial proportion of carbapenemase genes in environmental samples (42.9%) underscores the critical role of hospital environments in the epidemiology of antimicrobial resistance.

Environmental contamination has been identified as a key driver of resistance transmission, particularly in resource-limited settings with suboptimal infection prevention practices (Tacconelli et al., 2018). The predominance of *Escherichia coli* (52.3%) among carbapenemase-producing isolates highlights its role as a major reservoir and vector for resistance genes, driven by its genetic plasticity and capacity for horizontal gene transfer (Alvisi et al., 2025). The widespread distribution of *bla*_{OXA-48} across multiple species including *E. coli*, *Klebsiella*, *Proteus*, and *Pseudomonas* further supports extensive interspecies dissemination mediated by mobile genetic elements (Pitout et al., 2019; Alvisi et al., 2025). Environmental analysis revealed diverse carbapenemase-producing organisms, with *Enterobacter hormaechei* predominating, consistent with its emerging role in hospital contamination and outbreaks (Davin-Regli et al., 2019).

The high prevalence of *bla*_{OXA-48} (41.7%) and *bla*_{VIM} (33.3%) in environmental isolates aligns with recent surveillance studies, while detection in less commonly implicated species such as *Alcaligenes faecalis* and *Providencia manganoxydans* highlights the role of opportunistic environmental bacteria as reservoirs of resistance genes (Davin-Regli et al., 2019; Gajdács et al., 2020). The detection of *bla*_{KPC} and *bla*_{NDM} in environmental isolates further suggests surfeit from clinical sources and reinforces the interconnectedness of patient infections and environmental contamination (Tacconelli et al., 2018). Although, *bla*_{OXA-23} was not detected in environmental isolates, its known association with *Acinetobacter* spp. suggests species-specific distribution patterns (Hamidian and Nigro, 2019).

Conclusion

This study shows a high burden of Gram-negative bacteria in both clinical and environmental samples from the two healthcare facilities in Benue State, Nigeria. The findings reveal alarming levels of antimicrobial resistance, particularly to commonly used antibiotics. Although, carbapenems retained relatively better activity, the detection of carbapenem-resistant isolates and carbapenemase genes is of serious concern.

RECOMMENDATIONS

Special attention should be given to environmental reservoirs identified in this study to minimise nosocomial transmission through the strengthening of comprehensive Infection Prevention and Control (IPC) strategies. In addition, there is a critical need to establish or reinforce antimicrobial stewardship programs aimed at optimizing antibiotic use, alongside routine screening for multidrug-resistant and carbapenemase-producing organisms to enable early detection and containment.

Abbreviations

Not applicable

Declarations

AVAILABILITY OF DATA AND MATERIALS

Following verification of our NCBI submission, we have confirmed the correct and publicly accessible accession number as PV535558-PV535579.

The deposited sequence data are accessible through NCBI database at:

<https://www.ncbi.nlm.nih.gov/nuccore/PV535558.1/>

The Diversity of Environmental Bacterial Isolates as Identified By 16S rRNA

S/No.	Isolates code	Gram-negative Bacterial	Percentage similarity (%)	Accession Number
1	7TA ³	<i>Alcaligenes faecalis</i>	98.96	PV5335568
2	8F1 ³	<i>Alcaligenes faecalis</i>	99.89	PV5335571
3	9TA ⁷	<i>Alcaligenes faecalis</i>	98.85	PV535572
4	6TA ²	<i>Alcaligenes ammonioxydans</i>	98.95	PV535575
5	FTb3 ²	<i>Enterobacter hormaechei</i>	99.54	PV535559
6	4F2 ^a	<i>Enterobacter hormaechei</i>	98.39	PV535564
7	TS1 ²	<i>Enterobacter hormaechei</i>	96.55	PV535567
8	2F2 ^b	<i>Escherichia coli</i>	99.08	PV535560
9	TTb4 ²	<i>Escherichia coli</i>	99.20	PV535561
10	TTb3 ¹	<i>Escherichia coli</i>	98.73	PV535563
11	5F1 ³	<i>Escherichia coli</i>	98.97	PV535577
12	TA ³	<i>Escherichia coli</i>	97.13	PV535579
13	6TC ⁴	<i>Klebsiella pneumoniae</i>	99.54	PV535558
14	6F1 ¹	<i>Klebsiella pneumoniae</i>	98.60	PV535562
15	TTb5 ¹	<i>Klebsiella pneumoniae</i>	99.50	PV535565
16	3F2 ^d	<i>Klebsiella pneumoniae</i>	99.54	PV535566
17	4TA ⁴	<i>Klebsiella quasipneumoniae</i>	98.61	PV535578
18	6F1 ²	<i>Proteus mirabilis</i>	98.60	PV535576

ETHICS APPROVAL AND CONSENT TO PARTICIPATE:

We confirm that the study was submitted to and approved by the **Research Ethical Committee, Ministry of Health and Human Services, Benue State, Nigeria**. Ethical approval was granted under Reference No. **MOH/STA/204/VOL.1/31**.

The clinical isolates used in the study were obtained from routine diagnostic laboratory samples, and no identifiable personal or patient information was collected or used. Environmental samples were obtained from selected hospital environments.

CONSENT FOR PUBLICATION

All authors gave their consent for publication of this work

COMPETING INTERESTS

The authors declare that they have no competing interests.

FUNDING

Tertiary Education Trust Fund (TETFund) Nigeria

AUTHORS' CONTRIBUTIONS

A.A Ogunjobi and O.E. Fagade supervised the research work while O.I Falodun work with me extensively in the manuscript writing.

ACKNOWLEDGEMENT

I sincerely acknowledge the funding body for supporting this work, the Tertiary Education Trust Fund (TETFund) Nigeria under the TETFund award of scholarship programme.

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