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Molecular Detection of Ampicillinase blaEBCM, blaFOX and blaDHAM resistant genes in multi-drug resistant Gram-negative bacteria

Esther Chigbaziru Nwojiji [1, 2], Ijeoma Onyinye Okolo [3], Chigoziri Akudo Osuji [4], Ugonna Cassandra Aniokete [5, 6], Sikiru Oladimeji Akomolafe [7], Uchechukwu Okekeaji [8], Christiana Inuaesiet Edemekong [7, 9], Ikemesit Udeme Peter [10, *] and Ifeanyichukwu Romanus Iroha [1]

[1] *Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, P.M.B. 53, Nigeria.*
[2] *Department of Microbiology and Parasitology, David Umahi Federal University of Health Science, Uburu, Ebonyi State, P. M. B 211, Nigeria*
[3] *Department of Biochemistry, Faculty of Basic Medical Sciences, Federal University of Allied Health Science, Trans-Ekulu, P. M. B 01473, Enugu, Nigeria.*
[4] *Department of Biochemistry, College of Natural and Applied Sciences, Gregory University Uturu, Abia State, Nigeria.*
[5] *Department of Medical Laboratory Science, David Umahi Federal University of Health Science, Uburu, Ebonyi State, P. M. B 211, Nigeria.*
[6] *International Institute of for Infectious Disease, Biosafety and Biosecurity Research, Uburu, Ebonyi State, Nigeria.*
[7] *Department of Public Health, Faculty of Healthcare Services, Federal University of Allied Health Sciences, Trans-Ekulu, P. M. B 01473, Enugu, Nigeria.*
[8] *Department of Pharmaceutical Microbiology and Biotechnology, College of Pharmacy, Gregory University Uturu, P. M. B 1012, Abia State, Nigeria.*
[9] *Department of Biotechnology, Faculty of Science, Ebonyi State University, Abakaliki, P.M.B. 53, Nigeria.*
[10] *Department of Microbiology, Faculty of Basic Medical Science, Federal University of Allied Health Science, Trans-Ekulu, P. M. B 01473, Enugu, Nigeria.*

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Abstract

Background: Multidrug-resistant (MDR) Gram-negative bacteria (GNB) harboring AmpC β-lactamase determinants represent a significant global public health threat, contributing to life-threatening infections in humans. Despite their growing prevalence, the detection of AmpC β-lactamase remains underreported in low- and middle-income countries (LMICs), where clinical microbiology laboratories often lack the resources for accurate identification. This study aimed to investigate the presence of AmpC resistance genes (*blaEBC*, *blaFOX*, and *blaDHA*) in MDR Gram-negative bacteria to address this critical gap.

Materials and methods: Over a six-month period, 290 non-replicated urine samples were collected from hospitalized patients, yielding clinical isolates of *E. coli* (n=95), *K. pneumoniae* (n=34), and *Pseudomonas* species (n=17). Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method, and multidrug resistance (MDR) was determined. Isolates resistant to cefoxitin were preliminarily identified as potential AmpC β-lactamase producers and further confirmed using the Cefoxitin-Cloxacillin Double Disk Synergy Test (CC-DDST). AmpC β-lactamase phenotypes were subsequently analyzed using polymerase chain reaction (PCR) to detect the presence of *blaFOX-M*, *blaEBC-M*, and *blaDHA-M* genes.

Results: The study identified 146 MDR Gram-negative bacteria with high resistance rates to multiple antibiotic classes, including β-lactams, cephalosporins, carbapenems, anti pseudomonals, folate inhibitors, and protein synthesis inhibitors. Resistance was notably lower to macrolides (e.g., azithromycin), aminoglycosides (e.g., gentamicin), and

* Corresponding author: Ikemesit Udeme Peter.

fluoroquinolones (e.g., ciprofloxacin). Among the MDR isolates, 23 (7.9%) were confirmed as AmpC producers, with prevalence rates of 1.0% in *P. aeruginosa*, 1.7% in *K. pneumoniae*, and 5.2% in *E. coli*. Genetic analysis revealed the presence of *FOXM* (100%), *EBC* (95.7%), and *DHAM* (13.0%) genes among the MDR isolates.

Conclusion: This study highlights the alarming prevalence of MDR Gram-negative bacteria with high resistance to critical antibiotic classes, particularly β-lactams, carbapenems, and cephalosporins. The widespread detection of AmpC β-lactamase genes (*FOXM*, *EBC*, and *DHAM*) underscores the complexity of managing MDR infections. Our findings suggest that combination therapy involving azithromycin, gentamicin, and ciprofloxacin may offer a viable treatment strategy for MDR infections. Regional variations in resistance patterns emphasize the urgent need for enhanced surveillance, routine detection of resistance mechanisms, and stricter antibiotic stewardship to combat the global threat of antimicrobial resistance.

Keywords: Gram-negative bacteria; Ampc β-lactamase; Multidrug-resistant bacteria; MDR infections

1. Introduction

Gram-negative bacteria (GNB) are ubiquitous in nature and are significant causative agents of life-threatening infections in humans [1, 2, 3]. Key pathogens within this group, such as *Enterobacteriaceae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, are particularly notorious for their resistance to multiple antibiotics [4, **5,** 6]. The emergence and spread of multidrug-resistant (MDR) GNB pose a severe global public health threat. MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories [6, 7]. According to Antimicrobial Resistance Collaborators [7], antimicrobial resistance (AMR) was directly responsible for 1.27 million of the predicted 4.95 million fatalities that occurred worldwide in 2019. Although surveillance and resistance data are scarce in Western sub-Saharan Africa, the region has the largest burden, with 27.3 AMR-attributable and 114.8 AMR-associated deaths per 100,000 people [8]. Healthcare expenditures are expected to rise by USD 0.22 trillion annually in low-AMR settings and over USD 1.2 trillion in high-AMR situations by 2030, demonstrating the profound economic impact of AMR [8].

In Nigeria and globally, the overuse of cephalosporin beta-lactam antibiotics, favored for their low toxicity and high efficacy, has exacerbated resistance [9-13]. The production of beta-lactamase (β-lactamase) enzymes, particularly class C cephalosporinases (AmpCs), in GNB is a major driver of antibiotic resistance, leading to therapeutic failures, prolonged hospital stays, increased healthcare costs, and higher mortality rates [9,11, 14]. These enzymes hydrolyze the beta-lactam ring, rendering antibiotics ineffective, and can be encoded chromosomally or acquired via mobile genetic elements (MGEs) such as plasmids, integrons, and transposons [14, 15].

AmpC resistance among GNB has surged globally over the past decade, with reports from both hospital and community settings [11, 16, 17, 18, 19]. Resistance mechanisms include reduced membrane permeability due to porin loss, often linked to AmpC β-lactamase production (Akpu *et al*., 2023a; John-Onwe *et al*., 2023a). Plasmid-mediated AmpC β-lactamases (e.g., *blaFOX-M*, *blaDHA-M*, *blaCMY-M*, and *blaACC-M*) confer resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and β-lactamase inhibitor combinations [14, 15, 20]. Hyperproduction of AmpC also leads to resistance against extended-spectrum cephalosporins like cefotaxime, ceftazidime, and ceftriaxone [10, 11, 19, 21].

The rapid emergence of plasmid-mediated AmpC variants, particularly in *K. pneumoniae* and *E. coli*, has been documented globally. These variants, classified based on nucleic acid sequence homology, have spread across bacterial genera, with notable families including CMY, FOX, MOX, ACC, LAT, MIR, ACT, and DHA [14, 22, 23]. Chromosome-mediated AmpC β-lactamases in organisms like *Enterobacter cloacae*, *Citrobacter freundii*, *Serratia marcescens*, and *Morganella morganii* further complicate resistance patterns, particularly in nosocomial infections [24]. The co-expression of AmpC β-lactamases with other resistance mechanisms in MDR pathogens poses a significant diagnostic and therapeutic challenge [15].

AmpC β-lactamase genes, such as *MOXM*, *CITM*, *DHAM*, *EBCM*, *FOXM*, and *ACCM*, confer broad-spectrum resistance to β-lactams, except cefepime and carbapenems. Unlike class A enzymes, AmpC β-lactamases are not inhibited by clavulanic acid, sulbactam, or tazobactam, though some variants may be partially inhibited by tazobactam or sulbactam [14, 25]. The hyperproduction of AmpC β-lactamases, coupled with upregulated efflux pumps, can synergistically enhance resistance to carbapenems, further limiting treatment options [26]. AmpC-producing bacteria are a leading cause of healthcare-associated infections, contributing to severe outcomes and outbreaks in both hospital and community [11, 14, 16].

Despite the growing threat, AmpC β-lactamase detection remains underreported in low- and middle-income countries (LMICs), where routine clinical microbiology laboratories often lack the resources for accurate identification. Recent studies across Africa, however, have highlighted the prevalence of AmpC β-lactamases, with high rates reported in Ghana [3], Nigeria (94.1% in *E. coli*) [14], Burkina Faso [18], Nepal [22], Saudi Arabia [27], and Sudan (49.3% in *Acinetobacter baumannii*) [28]

In Abakaliki, Nigeria, efforts to combat drug-resistant pathogens are ongoing, but studies on AmpC β-lactamases remain limited. The lack of precise diagnostic methods and resources often results in undetected cases, underscoring the need for reliable and accessible detection protocols. Molecular techniques such as polymerase chain reaction (PCR) has emerged as a valuable tool for identifying plasmid-encoded AmpC genes, offering a more accurate and efficient approach to diagnosing AmpC β-lactamase-producing infections.

While the prevalence of β-lactamase-producing GNB is well-documented, most studies focus on single isolates, extended-spectrum β-lactamases (ESBLs), or carbapenemases, primarily in *E. coli* and *K. pneumoniae*. There is a critical gap in research examining the concurrent presence of AmpC β-lactamases in diverse MDR GNB from clinical sources. Comprehensive data on AmpC β-lactamase-producing organisms are essential for guiding antibiotic therapy, enhancing surveillance, and improving infection prevention and control strategies. This study therefore investigated the presence of AmpC resistance genes (*blaEBCM*, *blaFOX*, and *blaDHAM*) in multidrug-resistant Gram-negative bacteria, contributing to a deeper understanding of this pressing public health challenge.

2. Materials and Methods

2.1. Sampling and Identification of bacterial strains

This study was conducted at Alex Ekwueme Federal University Teaching Hospital in Abakaliki, Ebonyi State, Nigeria. Abakaliki is geographically situated at latitude 6.3231° N and longitude 8.1121° E [2, 19]. Over a six-month period, a total of 290 non-replicated urine samples were collected from hospitalized patients, yielding clinical isolates of *E. coli* (n=95), *K. pneumoniae* (n=34), and *Pseudomonas* species (n=17). The isolates were cultured on selective media, including Cetrimide agar, MacConkey agar, and *Klebsiella* ChromoSelect agar (Thermo Scientific™, U.S.A.), and incubated at 35°C for 18–24 hours. Following incubation, isolates exhibiting characteristic colony morphologies of green for *Pseudomonas*, non-mucoid pink for *E. coli*, and purple-magenta for *Klebsiella pneumoniae* were further identified using the API 20E system (Biomérieux, Marcy-l'Étoile, France) in accordance with the manufacturer's instructions.

2.2. Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby–Bauer disk diffusion method. A bacterial suspension adjusted to the 0.5 McFarland standard was inoculated onto Mueller–Hinton agar plates. The following antibiotics were tested: azithromycin (10 µg), amoxicillin/clavulanic acid (30 µg), aztreonam (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), meropenem (30 µg), imipenem (30 µg), gentamicin (15 µg), trimethoprim-sulfamethoxazole (25 µg), tetracycline (30 µg), and ertapenem (30 µg) (Oxoid). Results were interpreted in accordance with the 2022 guidelines of the Clinical and Laboratory Standards Institute (CLSI) [29]. Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories, consistent with previous studies [6, 7]. Bacterial isolates exhibiting a cefoxitin inhibition zone diameter of less than 18 mm (<18 mm) were preliminarily identified as potential AmpC β-lactamase producers. These isolates were subsequently confirmed using the Cefoxitin-Cloxacillin Double Disk Synergy Test (CC-DDS) to further validate AmpC β-lactamase production.

2.3. Phenotypic Identification of Ampicillin-Resistant β-Lactamase (AmpC) Producers

2.3.1. Cefoxitin Cloxacillin-Double disk synergy test (CC-DDST)

A total of 146 presumptive AmpC β-lactamase-producing bacterial isolates, including *E. coli* (*n*=95), *K. pneumoniae* (*n*=34), and *Pseudomonas aeruginosa*. (*n*=17), were tested for AmpC β-lactamase production using the Cefoxitin-Cloxacillin Double Disk Synergy Test (CC-DDST). This method relies on the inhibitory effect of cloxacillin on AmpC β-lactamase activity. The isolates were inoculated onto Mueller-Hinton agar, and a cefoxitin/cloxacillin disk (200 µg/30 µg) alongside a cefoxitin disk (30 µg) were applied. A difference of ≥4 mm in the inhibition zones between the two disks was considered indicative of AmpC β-lactamase production, as per the Clinical and Laboratory Standards Institute (CLSI, 2022) guidelines [11, 29].

2.4. Molecular Characterization of AmpC β-Lactamase Resistance Genes

Isolates that tested positive for AmpC β-lactamase phenotypes were further analyzed using polymerase chain reaction (PCR) to detect genes encoding AmpC, including *blaFOX-M*, *blaEBC-M*, and *blaDHA-M* [14, 15]. Crude DNA was extracted from pure overnight cultures by suspending 10 µL of the culture in 200 µL of molecular-grade nuclease-free water, heating at 98°C for 10 minutes, and centrifuging at 20,000 × g for 5 minutes at 4°C, as described by Quansah *et al*. [30]. The supernatant was transferred to sterile 1.5 mL Eppendorf® tubes and used as the DNA template for PCR.

Each 25 µL PCR reaction mixture consisted of 12.5 µL of Green PCR Master Mix (2x) (DreamTaq, Thermo Scientific, Waltham, MA, USA), 4.5 µL of primer mix, 6 µL of molecular-grade nuclease-free water, and 2 µL of crude DNA template, following the protocol outlined by Khurana *et al*. (2017). The primers are detailed in Table 1 and the cycling conditions used for PCR amplification were as follows: Amplification was carried out at 94 ^{o}C for 15 min as the initial step for denaturation, 25 cycles of denaturation at 94 ^{o}C for 30 s, annealing at 64 ^{o}C for 90 s and extension at 72 ^{o}C for 60 s [31]. Final elongation was at 72 ^{o}C for 10 min. PCR amplicons were analyzed by horizontal gel electrophoresis on a 2% (weight/volume) agarose gel (SeaKem® GTG® Agarose, Lonza, Basel, Switzerland) using Tris/Acetate/EDTA (TAE) 50x buffer.

Table 1 PCR amplification parameters, primer sequences, and cycling conditions for the detection of the AmpC gene

AmpC Gene	Primers (5'-3')	Size (Bp)	References
DHAM	F: AACTTTCACAGGTGTGCTGGGT R: CCGTACGCATACTGGCTTTGC	405	[14]
FOXM	F:AACATGGGGTATCAGGGAGATG R: CAAAGCGCGTAACCGGATTGG	190	[15]
EBCM	F:TCGGTAAAGCCGATGTTGCGG R: CTTCCACTGCGGCTGCCAGTT	302	[20]

3. Results

Gram-negative bacteria accounted for 146 isolates (50.3%), with *E. coli* being the most prevalent at 95 isolates (32.8%), followed by *K. pneumoniae* at 34 isolates (11.7%) and *P. aeruginosa* at 17 isolates (5.8%), as detailed in Table 2.

These Gram-negative bacteria exhibited high levels of resistance to several antibiotic classes, including cephalosporins, β-lactam inhibitors, antipseudomonal agents, folate inhibitors, and protein synthesis inhibitors. Carbapenem resistance ranged from 25.3% to 100%, while resistance to aminoglycosides ranged from 41.1% to 54.5%. In contrast, macrolides and fluoroquinolones showed low resistance rates of 0.0%, as presented in Table 3.

Among the Gram-negative bacteria, 146 isolates were multidrug-resistant (MDR), demonstrating resistance to at least one agent in three or more antibiotic classes. *E. coli* (n=95) was the most common MDR pathogen, followed by *K. pneumoniae* (n=34) and *P. aeruginosa* (n=17), as illustrated in Figure 1.

The study further revealed that 23 isolates (7.9%) of MDR Gram-negative bacteria exhibited phenotypic AmpC production. The prevalence of AmpC production was 1.0% in *P. aeruginosa*, 1.7% in *K. pneumoniae*, and 5.2% in *E. coli*. Non-AmpC producers accounted for 123 isolates (42.4%), including *P. aeruginosa* (n=14, 4.8%), *K. pneumoniae* (n=29, 10.0%), and *E. coli* (n=80, 27.6%), as shown in Table 4.

Genetic analysis of MDR Gram-negative bacteria identified the presence of resistance genes, including *FOXM* (100%), *EBC* (95.7%), and *DHAM* (13.0%). Among *P. aeruginosa*, *EBC* and *FOXM* were detected in 3 isolates each (13.0%), while *DHAM* was found in 1 isolate (4.3%). In *E. coli*, *EBC* was present in 14 isolates (60.9%), *FOXM* in 15 isolates (65.2%), and *DHAM* in 1 isolate (4.3%). For *K. pneumoniae*, *EBC* and *FOXM* were detected in 5 isolates each (21.7%), and *DHAM* was found in 1 isolate (4.3%), as detailed in Table 5.

Table 2 Distribution of Gram-negative bacteria isolate in clinical samples

Sample Source	No. Sampled	Bacterial strain	Frequency (%)
		P. aeruginosa	17(5.8)
Urine	290	*E. coli*	95(32.8)
		K. pneumoniae	34(11.7)
			146(50.3)

Table 3 Antibiotic Resistance profile of Gram-negative Bacteria isolated from urine samples

	Antibiotics (µg)	*P. aeruginosa* (n=17)		*E. coli* (n=95)		*K. pneumoniae* (n=34)	
Drug classes		**R (%)**	**S (%)**	**R (%)**	**S (%)**	**R (%)**	**S (%)**
Macrolide	AZM (10)	0(0.0)	17(100)	0(0.0)	95(100)	0(0.0)	34(100)
β-lactam inhibitor	AMC (30)	17(100)	0(0.0)	95(100)	0(0.0)	34(100)	0(0.0)
Antipseudomonas	ATM (30)	17(100)	0(0.0)	95 (100)	0(0.0)	34(100)	0(0.0)
Cephalosporins	CTX (30)	17(100)	0(0.0)	95 (100)	0(0.0)	34(100)	0(0.0)
	CRO (30)	17(100)	0(0.0)	95(100)	0(0.0)	34(100)	0(0.0)
	FOX (30)	17(100)	0(0.0)	95(100)	0(0.0)	34(100)	0(0.0)
Fluoroquinolones	CIP (5)	0(0.0)	17(100)	0(0.0)	95(100)	0(0.0)	34(100)
Carbapenem	ETP (30)	17(100)	0(0.0)	95(100)	0(0.0)	34(100)	0(0.0)
	MEM (30)	9(0.0)	1(100)	45(0.0)	50(100)	24(70.6)	10(29.4)
	IPM (30)	7(41.2)	10(58.8)	24(25.3)	71(74.7)	0(0.0)	34(100)
Aminoglycosides	GN (15)	6(54.5)	5(45.5)	0(0.0)	95(100)	14(41.1)	20(58.8)
Folate inhibitor	SXT (25)	17(100)	0(0.0)	95(100)	0(0.0)	34(100)	0(0.0)
Protein synthesis inhibitor	TE (30)	17(100)	0(0.0)	95(100)	0(0.0)	34(100)	0(0.0)

Key: AZM-Azithromycin, AMC- Amoxicillin-Clavulanic acid, ATM-Azetronam, CTX-Cefotaxime, CRO- Ceftriaxone, FOX-Cefoxitin, CIP- Ciprofloxacin, ETP-Ertapenem, MEM-meropenem, IPM-Imipenem, GN- Gentamicin, SXT- Trimethoprim-Sulfamethoxazole, TE-Tetracycline

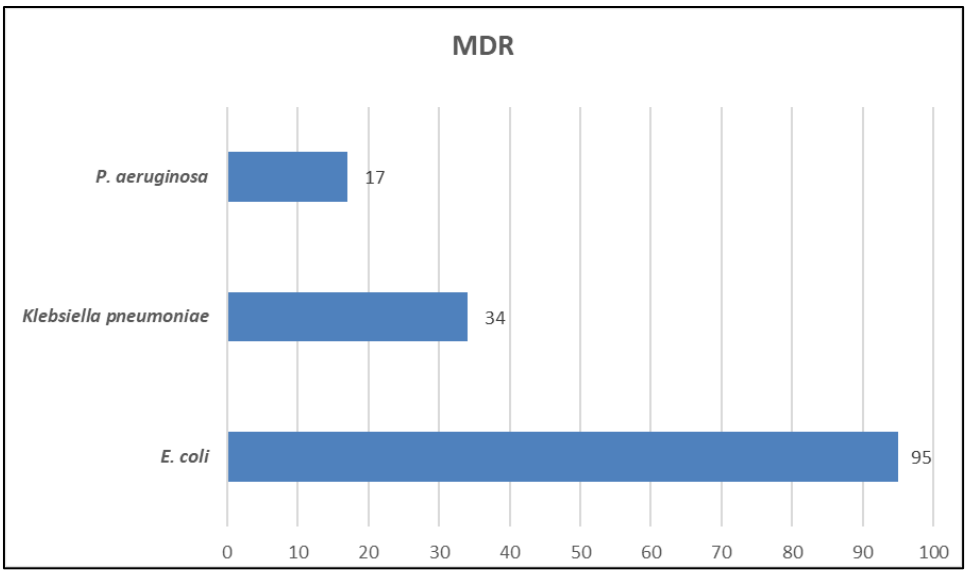

Figure 1 Bar chart showing the proportion of Multidrug resistance phenotypes in Gram-negative bacteria

Table 4 Distribution of phenotypically identified AmpC β-lactamase in MDR Gram-negative bacteria isolates in urine samples

Sample Source	No. Sampled	Bacterial strain	No of Isolate	AmpC (%)	Non- AmpC (%)
		P. aeruginosa	17	3(1.0)	14(4.8)
Urine	290	*E. coli*	95	15(5.2)	80(27.6)
		K. pneumoniae	34	5(1.7)	29(10.0)
			146	23(7.9)	123(42.4)

Table 5 Distribution of AmpC β-lactamase gene in MDR Gram-negative bacteria isolates

Bacterial strain	AmpC phenotype (%)	AmpC Genotype		
		EBCM (%)	FOXM (%)	DHAM (%)
P. aeruginosa	3	3(13.0)	3(13.0)	1(4.3)
E. coli	15	14(60.9)	15(65.2)	1(4.3)
K. pneumoniae	5	5(21.7)	5(21.7)	1(4.3)
	23	22(95.7)	23(100)	3 (13.0)

4. Discussion

Our study identified 146 MDR Gram-negative bacteria (GNB) with high resistance rates across multiple antibiotic classes, including protein synthesis inhibitors, β-lactams, anti-pseudomonals, carbapenems, folate inhibitors, and cephalosporins. Notably, resistance was lower for macrolides (azithromycin), aminoglycosides (gentamicin), and fluoroquinolones (ciprofloxacin). These findings align with similar studies conducted in Southwest and Eastern Nigeria [6, 7, 11, 32], Ouagadougou, Burkina Faso [18], Annapurna, Nepal [22], and Bisha, Saudi Arabia [27]. The treatment of MDR GNB can be quite expensive and challenging, exacerbated by the high prevalence of nosocomial infections. This may be attributed to the overuse of broad-spectrum antibiotics, prolonged treatment durations, and prior antimicrobial exposure in referred patients. Misuse of antibiotics during hospitalization further contributes to the selection of MDR strains [7].

Additionally, multidrug resistance can arise from the aggregation and expression of resistance genes on (R) plasmids or through multidrug efflux pumps [2, 33, 34]. Additionally, β-lactamase genes are often linked to resistance against non-β-lactam antibiotics, such as aminoglycosides and fluoroquinolones [15, 35].

Our study revealed that 23 (7.9%) of MDR GNB were AmpC producers, with prevalence rates of 1.0%, 1.7%, and 5.2% among *P. aeruginosa*, *K. pneumoniae*, and *E. coli*, respectively. AmpC β-lactamase production has been documented in Enterobacterales across Africa at varying rates, such as 49.3% in Sudan [28], 15.2–94.1% in Nigeria [11, 32, 36], 2.5% in Ethiopia [37], and 36.5% in Uganda [38]. These findings underscore the need for routine AmpC β-lactamase detection in African hospitals, as its presence complicates treatment, increasing morbidity and mortality [11, 14, 15].

Not all cefoxitin-resistant isolates (146 GNB) were positive for CC-DDST. This indicates that Cefoxitin resistance may result from other mechanisms, such as extended-spectrum β-lactamases (ESBLs), metallo-β-lactamases (MBLs), or porin mutations [39]. Overexpression of chromosomal AmpC genes due to promoter/attenuator mutations or active efflux pumps may also contribute [40, 41].

In this study, MDR GNB harbored *FOXM* (100%), *EBC* (95.7%), and *DHAM* (13.0%) genes. The *FOX* AmpC gene, a plasmid-encoded β-lactamase, confers resistance to broad-spectrum cephalosporins and cephamycins, likely driving the observed resistance. Similar findings were reported by Owusu *et al*. [3], who detected $bla_{\text{FOX-M}}$ (64%) and $bla_{\text{DHA-M}}$/$bla_{\text{EBC-M}}$ (27%) in GNB. Ejikeugwu *et al*. [16] also identified bla_{FOX} in *E. coli* (11.8%), *K. pneumoniae* (5.6%), and *P. aeruginosa* (12.5%). In contrast, Akpu *et al*. [14] reported a high prevalence of *DHAM* in *E. coli* and *K. pneumoniae*, while Thonda *et al*. [32] found *FOX*-type gene to be the second most prevalent gene (38%). In Iran, bla_{EBC} was most common,

followed by *bla*$_{FOX}$ and *bla*$_{DHA}$ [42]. Song *et al*. [43] highlighted the clinical significance of *bla*$_{DHA}$, an inducible AmpC gene prevalent in Korea and Japan, associated with higher mortality rates [44].

Twenty GNB isolates lacked *DHAM* genes, possibly due to gene mutations, the presence of other β-lactamases (e.g., ESBLs or carbapenemases) [45, 46], or primer limitations. The prevalence of *bla*$_{FOX}$ varied significantly across studies, reflecting strain diversity and regional differences in infection control and treatment practices and study design. For instance, Fam *et al*. [47] found *bla*$_{CMY}^{-1}$ more common in *Klebsiella* species [47], while our study focused on different strains.

The findings of these studies exhibit notable differences in the prevalence of AmpC genes compared to the present study, suggesting a rising trend in resistance gene prevalence over time, warranting further investigation. Comparisons indicate that the frequency of beta-lactamase enzymes varies significantly among strains isolated from different countries and even across hospitals within the same country. These variations may be attributed to differences in infection control measures and patient treatment practices. A key limitation of this study was the inability to conduct large-scale screening of bacterial genomes, which would have enabled the identification and comparison of diverse AmpC-like genes in various genetic environments, both mobile and non-mobile, as well as the exploration of their evolutionary relationships, including cross-species transfer events. The findings from our study highlight the urgent need for sustained surveillance to track emerging resistance mechanisms, particularly the rising prevalence of AmpC β-lactamase genes and other resistance determinants in Gram-negative bacteria. Continuous monitoring is essential to track evolving resistance patterns, guide evidence-based treatment protocols, and inform targeted mitigation strategies. Strengthening antimicrobial stewardship programs, improving infection control practices, and promoting regional collaboration are vital to curb the spread of multidrug-resistant infections and safeguard public health.

5. Conclusion

This study highlights the alarming prevalence of multidrug-resistant Gram-negative bacteria (MDR GNB) with high resistance to multiple antibiotic classes, particularly β-lactams, carbapenems, and cephalosporins. The widespread presence of AmpC β-lactamase genes, such as FOXM, EBCM, and DHAM, underscores the complexity of treating MDR infections. Our study suggests that combination therapy using azithromycin, gentamicin, and ciprofloxacin may offer a promising approach for treating infections caused by multidrug-resistant bacteria. Regional variations in resistance patterns emphasize the need for enhanced surveillance, routine detection of resistance mechanisms, and stricter antibiotic stewardship to mitigate the global threat of antimicrobial resistance.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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