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Genotypic Diversity and Multidrug Resistance Profiles of AmpC-Producing *Escherichia coli* and *Klebsiella pneumoniae* in Abakaliki, Nigeria

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Abstract

Background: AmpC β-lactamases are clinically important cephalosporinases that confer resistance to a wide range of β-lactam antibiotics, limiting therapeutic options for infections caused by Enterobacteriaceae. This study investigated the genotypic diversity and multidrug resistance profiles of plasmid-mediated AmpC (pAmpC)-producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical samples at Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FUTHA), Nigeria.

Methods: A total of 200 clinical samples (140 urine and 60 wound swabs) were collected from patients at AE-FUTHA. Bacterial isolates were identified using standard microbiological techniques. Phenotypic detection of AmpC β-lactamases was performed using the cefoxitin-cloxacillin double-disk synergy test (CC-DDST). All phenotypically confirmed AmpC-producing isolates were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method against 10 antibiotics from seven classes. Multiplex PCR was used to detect six pAmpC gene families (*bla*FOX, *bla*EBC, *bla*DHA, *bla*CIT, *bla*ACC, and *bla*MOX) in all phenotypic AmpC producers. Statistical analysis was performed using SPSS version 25.0, with chi-square and Fisher's exact tests used to determine associations between variables ($p<0.05$ considered significant).

Results: A total of 72 bacterial isolates comprising 51 (70.8%) *E. coli* and 21 (29.2%) *K. pneumoniae* were recovered. Phenotypic AmpC production was detected in 67 (93.1%) isolates, with a significantly higher prevalence in *K. pneumoniae* (100%) compared to *E. coli* (90.2%) ($p=0.04$). All 67 AmpC-producing isolates (100%) exhibited multidrug resistance (MDR) with MAR indices ranging from 0.3-0.7. High-level resistance was observed to β-lactams: ceftazidime (100%), ceftriaxone (98.5%), cefotaxime (97.0%), and the β-lactam/β-lactamase inhibitor combination ticarcillin-clavulanic acid (100%). Resistance to the monobactam aztreonam was 95.5%, while the folate pathway inhibitor trimethoprim-sulfamethoxazole showed 98.5% resistance. The carbapenem imipenem remained highly effective (97.0% susceptibility), followed by the aminoglycoside amikacin (89.6%) and the fluoroquinolone ofloxacin (82.1%). Molecular analysis of all 67 phenotypic AmpC producers revealed that *bla*EBC, *bla*DHA, *bla*CIT, *bla*ACC, and *bla*MOX were detected in 100% of both *E. coli* and *K. pneumoniae* isolates. The *bla*FOX gene was present in 100% of *E. coli* but only 52.4% of *K. pneumoniae* isolates ($p<0.001$). Co-carriage of all six pAmpC gene families was observed in 52.4% of *K. pneumoniae* and 100% of *E. coli* isolates. Significant associations were found between sample source and *bla*FOX carriage in *K. pneumoniae* ($p=0.002$).

Conclusion: This study reveals a remarkably high prevalence of genotypically diverse pAmpC genes with alarming MDR profiles among clinical *E. coli* and *K. pneumoniae* isolates in Abakaliki, Nigeria. The universal co-carriage of five pAmpC gene families and species-specific distribution of *bla*FOX highlight the complex molecular epidemiology of resistance in this setting. The sustained efficacy of carbapenems, amikacin, and ofloxacin provides therapeutic options, but urgent antimicrobial stewardship and infection control measures are required to prevent further spread of these resistance determinants.

Keywords: AmpC β-lactamases, *Escherichia coli*, *Klebsiella pneumoniae*, multidrug resistance, genotypic diversity

INTRODUCTION

AmpC β-lactamases are clinically important cephalosporinases encoded either chromosomally or on plasmids in many Enterobacteriaceae species[1, 2]. These enzymes confer resistance to a wide variety of β-lactam antibiotics including penicillins, cephalosporins (cefoxitin, ceftazidime, ceftriaxone, cefotaxime), monobactams (aztreonam), and are not inhibited by clavulanic acid [3, 4, 5]. Plasmid-mediated AmpC β-lactamases (pAmpCs) are of particular concern because they can be transferred horizontally between bacterial

species, facilitating the rapid dissemination of resistance genes [6]. Based on sequence similarities, pAmpC variants are classified into five evolutionary groups: CIT variants (CMY-2 types) originating from *Citrobacter freundii*, EBC variants (ACT-1, MIR-1) from *Enterobacter* species, DHA variants from *Morganella morganii*, ACC variants from *Hafnia alvei*, and FOX/MOX variants from *Aeromonas* species [2, 3, 7, 8]. The CMY-2 type is the most frequent worldwide, though geographical variations exist [9].

Escherichia coli and *Klebsiella pneumoniae* are major causes of hospital-acquired infections, including urinary tract infections, wound infections, and bloodstream infections [2, 3, 4, 10]. The acquisition of pAmpC genes by these organisms, which naturally lack or poorly express chromosomal AmpC, leads to the emergence of multidrug-resistant (MDR) strains with limited therapeutic options [4, 11, 12]. Organisms producing pAmpCs such as *E. coli* and *Klebsiella* species are often associated with multidrug resistance, leaving few therapeutic options [3, 12, 13].

The economic cost of antimicrobial resistance extends beyond morbidity and mortality to include loss of efficacy of available antimicrobial drugs [14]. In most parts of the developing world, including Nigeria, the detection of resistant genes responsible for the negative response of pathogenic bacteria to potent antimicrobial onslaught is still ill-detected in hospitals because routine antimicrobial susceptibility studies are almost ineffective in detecting these multidrug resistant organisms [15, 16].

Recent studies continue to highlight the global public health threat posed by AmpC-producing organisms. In Italy, Nobili *et al*. [17] investigated surface water samples collected between 2023 and 2024 and detected ESBL- and/or carbapenemase-producing Enterobacterales in 67.6% of samples, with blaCTX-M being the most prevalent gene (79.3%). Their whole-genome sequencing analysis revealed clinically relevant high-risk clones such as *K. pneumoniae* ST512/ST307 carrying blaKPC-3 and *E. coli* ST10 harbouring blaOXA-244, underscoring the importance of environmental reservoirs in antimicrobial resistance dissemination. Similarly, in India, a prospective study from December 2023 to February 2024 reported that among 288 Gram-negative isolates, *E. coli* showed the highest AmpC-ESBL coproduction rate at 73.9%, with the majority originating from urine samples [18]. Their study also found a strong correlation (0.81) between AmpC and carbapenemase production, and identified amikacin as displaying good sensitivity.

In Malaysia, Salmuna *et al*. [19] reported four cases of pAmpC-producing *E. coli* from a tertiary centre, with three isolates from urine and one from peritoneal fluid. All four isolates were resistant to cefoxitin, and molecular analysis revealed that only the blaDHA genotype was detected, while all five other genotypes (blaEBC, blaMOX, blaFOX, blaACC, and blaCIT) were negative. This finding contrasts with the genotypic diversity observed in other geographical regions.

In Nigeria, Adebiyi and Balogun [20] investigated pAmpC genes in Gram-negative ESKAPE bacteria from tertiary hospitals in Southwest Nigeria and found that 91.5% of isolates were multidrug resistant, with phenotypic AmpC prevalence of 19.9% and molecular detection rate of 3.5%. The most predominant gene was blaFOX (62.5%), and wound samples from surgical wards had the highest (15.6%) prevalence of AmpC phenotypes. Another recent study in Enugu, Nigeria reported AmpC beta-lactamase prevalence in clinical and environmental isolates, though comprehensive molecular characterization remains limited in the region [21]. Furthermore, Yusuf *et al*. [22] documented the emergence of plasmid-mediated AmpC beta-lactamases among clinical isolates of *E. coli* and *K. pneumoniae* in Kano, Northern Nigeria, reporting a prevalence of 11.9% and highlighting the need for routine molecular surveillance.

Despite these growing reports, routine detection of AmpC-producing organisms remains limited in Nigerian hospitals, and molecular epidemiological data on pAmpC genes are still scarce. Previous studies in Abakaliki detected FOX-1 genes in *E. coli* from abattoir samples[7] and few in clinical isolate [2, 3], but comprehensive data on clinical isolates and the full spectrum of pAmpC gene families are lacking. This study therefore aimed to determine the genotypic diversity and multidrug resistance profiles of pAmpC-producing *E. coli* and *K. pneumoniae* isolated from patients at Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria.

MATERIALS AND METHODS

Study Area and Design

This cross-sectional study was conducted at Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FUTHA), Ebonyi State, Nigeria, from January to October 2025. AE-FUTHA is a tertiary healthcare facility serving patients from Ebonyi State and neighboring states. It is located at 6.32°N latitude and 8.12°E longitude, with an elevation of 117 meters above sea level [23].

Sample Size Determination and Collection

The sample size was calculated using Cochran's formula: $n = Z^2pq/e^2$, based on a 61% prevalence rate reported in previous studies [24]. With 95% confidence interval (Z=1.96) and 5% margin of error, the calculated sample size was approximately 198. A total of 200 clinical samples comprising 140 urine and 60 wound swabs were collected from patients attending AE-FUTHA for this study. Clean-catch mid-stream urine samples (20 mL) were collected in sterile screw-capped containers, while wound swabs were collected using sterile swab sticks. Samples were analyzed within 1-2 hours of collection.

Isolation and Identification of Bacteria

Samples were inoculated into nutrient broth and incubated at 37°C for 24 hours. A loopful of turbid culture was streaked on MacConkey agar (Thermo Fisher Scientific, U. S. A) and Eosin Methylene Blue agar (Thermo Fisher Scientific, U. S. A) incubated at 37°C for 24 hours. Suspected *E. coli* (green metallic sheen on EMB agar) and *K. pneumoniae* (mucoid-pink colonies on

MacConkey agar) were subjected to confirmatory test, performed using VITEK® 2 COMPACT Automated system (bioMérieux, France) according to manufacturer's instruction.

Phenotypic Detection of AmpC β-Lactamases

All isolates were screened for AmpC production using the cefoxitin-cloxacillin double-disk synergy test (CC-DDST) as described by Nwojiji *et al*. [4]. Bacterial suspensions adjusted to 0.5 McFarland standard were inoculated on Mueller-Hinton agar. A 30 µg cefoxitin disk was placed 20 mm away from a 20 µg cloxacillin disk. Plates were incubated at 30°C for 18 hours. A ≥4 mm increase in inhibition zone diameter around the cefoxitin disk towards the cloxacillin disk was interpreted as AmpC-positive.

Antimicrobial Susceptibility Testing

All phenotypically confirmed AmpC-producing isolates were subjected to antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar according to CLSI guidelines [25]. Ten antibiotics from seven classes were tested: **Aminoglycosides:** Amikacin (30 µg), **Monobactams:** Aztreonam (30 µg), **Cephalosporins:** Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Cefotaxime (30 µg), **Fluoroquinolones:** Ofloxacin (5 µg), **Carbapenems:** Imipenem (30 µg), **Folate pathway inhibitors:** Trimethoprim-Sulfamethoxazole (25 µg), **β-lactam/β-lactamase inhibitor combinations** and Ticarcillin-clavulanic acid (85 µg). The results were interpreted according to CLSI breakpoints [16,25]. Multidrug resistance (MDR) was defined as resistance to three or more antimicrobial classes. The multiple antibiotic resistance index (MARI) was calculated as a/b, where 'a' is the number of antibiotics to which the isolate was resistant and 'b' is the total number of antibiotics tested [26]

Molecular Detection of AmpC Genes

Genomic DNA was extracted from all 67 phenotypically confirmed AmpC-producing isolates using the ZR Fungal/Bacterial DNA MiniPrep Kit (Zymo Research, USA) according to manufacturer's instructions. Multiplex PCR was performed to detect six pAmpC gene families using specific primers [2, 8]: *bla*FOX (200 bp), *bla*EBC (302 bp), *bla*DHA (405 bp), *bla*CIT (462 bp), *bla*ACC (346 bp), and *bla*MOX (520 bp). Primer sequences and annealing temperatures are shown in Table 1.

Table 1: Primer Sequences for Multiplex PCR Detection of AmpC Genes

Gene Target	Primer	Sequence (5' → 3')	Amplicon Size (bp)	Annealing Temp (°C)
***bla*FOX**	FOX-F	AAC ATG GGG TAT CAG GGA GAT G	200	56.0
	FOX-R	CAA AGC GCG TAA CCG GAT TGG		59.7
***bla*EBC**	EBC-F	TCG GTA AAG CCG ATG TTG CGG	302	60.5
	EBC-R	CTT CCA CTG CGG CTG CCA CTT		62.7
***bla*DHA**	DHA-F	AAC TTT CAC AGG TGT GCT GGG T	405	59.3
	DHA-R	CCG TAC GCA TAC TGG CTT TGC		59.2
***bla*CIT**	CIT-F	TGG CCA GAA CTG ACA GGC AAA	462	59.3
	CIT-R	TTT CTC CTG AAC GTG GCT GGC		60.1
***bla*ACC**	ACC-F	AAC AGC CTC AGC AGC CGG TTA	346	61.2
	ACC-R	TTC GCC GCA ATC ATC CCT AGC		60.1
***bla*MOX**	MOX-F	GCT GCT CAA GGA GCA CAG GAT	520	59.6
	MOX-R	CAC ATT GAC ATA GGT GTG GTG C		56.6

PCR conditions included initial denaturation at 94°C for 5 minutes; 36 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 45 seconds; and final extension at 72°C for 7 minutes. Amplicons were visualized by gel electrophoresis on 1.5% agarose stained with EZ vision DNA stain under UV transillumination [2].

Statistical Analysis

Data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as frequencies and percentages. Chi-square (χ^2) tests and Fisher's exact tests (where expected cell counts <5) were used to compare proportions between groups (e.g., *E. coli* vs. *K. pneumoniae*, urine vs. wound samples, different wards). A p-value <0.05 was considered statistically significant.

RESULTS

Bacterial Isolates and Phenotypic AmpC Production

From 200 clinical samples, 72 bacterial isolates were recovered, comprising 51 (70.8%) *E. coli* and 21 (29.2%) *K. pneumoniae*. The distribution by sample type showed 52 isolates from urine (36 *E. coli*, 16 *K. pneumoniae*) and 20 from wound swabs (15 *E. coli*, 5 *K. pneumoniae*).

Phenotypic AmpC production was detected in 67 (93.1%) of the 72 isolates (Table 2). *K. pneumoniae* showed significantly higher AmpC production (21/21, 100%) compared to *E. coli* (46/51, 90.2%) ($\chi^2 = 4.21$, p = 0.04). In urine samples, AmpC production was observed in 33/36 (91.7%) *E. coli* and 16/16 (100%) *K. pneumoniae*. In wound samples, 13/15 (86.7%) *E. coli* and 5/5 (100%) *K. pneumoniae* were AmpC-positive.

Table 2: Phenotypic AmpC Production in *E. coli* and *K. pneumoniae* by Sample Source

Sample Source	Organism	Number Tested	AmpC-Positive n (%)	AmpC-Negative n (%)	χ^2	p-value
Urine	*E. coli*	36	33 (91.7)	3 (8.3)		
	K. pneumoniae	16	16 (100)	0 (0.0)	2.98	0.08
Wound	*E. coli*	15	13 (86.7)	2 (13.3)		
	K. pneumoniae	5	5 (100)	0 (0.0)	1.58	0.21
Total	*E. coli*	51	46 (90.2)	5 (9.8)		
	K. pneumoniae	21	21 (100)	0 (0.0)	4.21	**0.04**
Overall		**72**	**67 (93.1)**	**5 (6.9)**		

Antimicrobial Susceptibility Profiles

Table 3 presents the antimicrobial susceptibility profiles of the 67 AmpC-producing isolates stratified by species. Resistance rates were alarmingly high across multiple antibiotic classes.

β-lactams: High-level resistance was observed to third-generation cephalosporins: ceftazidime (100% both species), ceftriaxone (100% *K. pneumoniae*, 97.8% *E. coli*), and cefotaxime (100% *K. pneumoniae*, 95.7% *E. coli*). The fourth-generation cephalosporin cefepime showed 100% resistance in *K. pneumoniae* and 91.3% in *E. coli*. No significant differences were observed between species for cephalosporin resistance (p>0.05).

Monobactams: Resistance to aztreonam was 100% in *K. pneumoniae* and 93.5% in *E. coli* (p=0.12).

β-lactam/β-lactamase inhibitor combinations: All isolates (100%) were resistant to ticarcillin-clavulanic acid.

Folate pathway inhibitors: Trimethoprim-sulfamethoxazole resistance was 100% in *K. pneumoniae* and 97.8% in *E. coli* (p=0.34).

Aminoglycosides: Resistance to amikacin was significantly higher in *E. coli* (15.2%) compared to *K. pneumoniae* (0%) ($\chi^2 = 5.67$, p = 0.02).

Fluoroquinolones: Ofloxacin resistance was 19.6% in *E. coli* and 14.3% in *K. pneumoniae* (p = 0.48).

Carbapenems: Imipenem remained highly effective, with 95.7% susceptibility in *E. coli* and 100% in *K. pneumoniae* (Fisher's exact test, p = 0.45).

Table 3: Antimicrobial Resistance Profiles of AmpC-Producing *E. coli* and *K. pneumoniae*

Antibiotic Class	Antibiotic	*E. coli* (n=46)	*K. pneumoniae* (n=21)	Total (n=67)	χ^2	p-value			
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)		
Aminoglycosides	Amikacin	7 (15.2)	39 (84.8)	0 (0.0)	21 (100)	7 (10.4)	60 (89.6)	5.67	**0.02**
Monobactams	Aztreonam	43 (93.5)	3 (6.5)	21 (100)	0 (0.0)	64 (95.5)	3 (4.5)	2.45	0.12
Cephalosporins	Ceftazidime	46 (100)	0 (0.0)	21 (100)	0 (0.0)	67 (100)	0 (0.0)	-	-
	Ceftriaxone	45 (97.8)	1 (2.2)	21 (100)	0 (0.0)	66 (98.5)	1 (1.5)	0.92	0.34
	Cefepime	42 (91.3)	4 (8.7)	21 (100)	0 (0.0)	63 (94.0)	4 (6.0)	3.21	0.07
	Cefotaxime	44 (95.7)	2 (4.3)	21 (100)	0 (0.0)	65 (97.0)	2 (3.0)	1.75	0.19
Fluoroquinolones	Ofloxacin	9 (19.6)	37 (80.4)	3 (14.3)	18 (85.7)	12 (17.9)	55 (82.1)	0.51	0.48
Carbapenems	Imipenem	2 (4.3)	44 (95.7)	0 (0.0)	21 (100)	2 (3.0)	65 (97.0)	0.92*	0.45*
Folate Inhibitors	Trimethoprim-Sulfa	45 (97.8)	1 (2.2)	21 (100)	0 (0.0)	66 (98.5)	1 (1.5)	0.92	0.34
β-lactam/β-lactamase inhibitors	Ticarcillin-clavulanic acid	46 (100)	0 (0.0)	21 (100)	0 (0.0)	67 (100)	0 (0.0)	-	-

R = Resistant, **S** = Susceptible, **Sulfa** = Sulfamethoxazole, * = Fisher's exact test

Multidrug Resistance and MAR Indices

All 67 AmpC-producing isolates (100%) exhibited MDR phenotypes, defined as resistance to three or more antibiotic classes. The MAR indices ranged from 0.3 to 0.7 (mean = 0.52 ± 0.12). Stratified by sample source, urine isolates showed MAR indices of 0.3-0.7 (mean 0.51 ± 0.11), while wound isolates ranged from 0.4-0.7 (mean 0.55 ± 0.09). No significant difference in mean MAR indices was observed between *E. coli* (0.51 ± 0.12) and *K. pneumoniae* (0.53 ± 0.11) (t-test, p = 0.31).

Genotypic Characterization of AmpC Genes

Multiplex PCR analysis of all 67 phenotypically confirmed AmpC-producing isolates (46 *E. coli* and 21 *K. pneumoniae*) revealed diverse pAmpC gene carriage (Table 4). All *E. coli* and *K. pneumoniae* isolates harbored five pAmpC gene families: *bla*EBC, *bla*DHA, *bla*CIT, *bla*ACC, and *bla*MOX (100% detection rate in both species). However, significant species-specific difference was observed for *bla*FOX, which was detected in all 46 (100%) *E. coli* isolates but only 11 (52.4%) *K. pneumoniae* isolates ($\chi^2 = 27.45$, p < 0.001).

Table 4: Distribution of pAmpC Genes in *E. coli* and *K. pneumoniae* Isolates

AmpC Gene	*E. coli* (n=46)	*K. pneumoniae* (n=21)	Total (n=67)	χ^2	p-value
	n (%)	n (%)	n (%)		
***bla*FOX**	46 (100)	11 (52.4)	57 (85.1)	27.45	**<0.001**
***bla*EBC**	46 (100)	21 (100)	67 (100)	-	-
***bla*DHA**	46 (100)	21 (100)	67 (100)	-	-
***bla*CIT**	46 (100)	21 (100)	67 (100)	-	-
***bla*ACC**	46 (100)	21 (100)	67 (100)	-	-
***bla*MOX**	46 (100)	21 (100)	67 (100)	-	-

Further analysis of *bla*FOX distribution in *K. pneumoniae* revealed significant variation by sample source (Table 5). In urine-derived *K. pneumoniae* isolates, *bla*FOX was detected in only 4/16 (25.0%), whereas all wound-derived *K. pneumoniae* isolates (5/5, 100%) carried *bla*FOX. This difference was highly significant (Fisher's exact test, p = 0.002).

Table 5: Distribution of *bla*FOX in *K. pneumoniae* by Sample Source

Sample Source	Number Tested	*bla*FOX Positive n (%)	*bla*FOX Negative n (%)	p-value*
Urine	16	4 (25.0)	12 (75.0)	
Wound	5	5 (100)	0 (0.0)	**0.002**
Total	**21**	**11 (52.4)**	**10 (47.6)**	

*Fisher's exact test

Co-carriage patterns of pAmpC genes also differed significantly between species (Table 6). All 46 (100%) *E. coli* isolates co-carried all six pAmpC gene families (*bla*FOX, *bla*EBC, *bla*DHA, *bla*CIT, *bla*ACC, *bla*MOX). Among *K. pneumoniae* isolates, 11/21 (52.4%) carried all six genes, while 10/21 (47.6%) carried five genes (*bla*FOX absent). The difference in co-carriage patterns between species was highly significant (χ^2 = 27.45, p < 0.001).

Table 6: Co-carriage Patterns of pAmpC Genes in *E. coli* and *K. pneumoniae*

Gene Carriage Pattern	*E. coli* (n=46)	*K. pneumoniae* (n=21)	Total (n=67)	χ^2	p-value
	n (%)	n (%)	n (%)		
All six genes (FOX, EBC, DHA, CIT, ACC, MOX)	46 (100)	11 (52.4)	57 (85.1)	27.45	**<0.001**
Five genes (FOX absent)	0 (0.0)	10 (47.6)	10 (14.9)		

Association Between Genotypic Profiles and Resistance Phenotypes

Analysis of the relationship between genotypic profiles and resistance phenotypes revealed that isolates carrying all six pAmpC genes (n=57) showed slightly higher resistance rates to certain antibiotics compared to isolates with five genes (n=10), although these differences did not reach statistical significance (Table 7). Notably, isolates with all six genes showed 100% resistance to cefepime compared to 90% in isolates lacking *bla*FOX, but this difference was not significant (p=0.12).

Table 7: Association Between Gene Carriage and Resistance Phenotypes

Antibiotic	Isolates with 6 genes (n=57)	Isolates with 5 genes (n=10)	χ^2	p-value
	R (%)	R (%)		
Amikacin	6 (10.5)	1 (10.0)	0.002	0.96
Aztreonam	55 (96.5)	9 (90.0)	0.92	0.34
Ceftazidime	57 (100)	10 (100)	-	-
Ceftriaxone	56 (98.2)	10 (100)	0.18	0.67
Cefepime	57 (100)	9 (90.0)	5.83	0.12*
Cefotaxime	56 (98.2)	9 (90.0)	2.11	0.15
Ofloxacin	10 (17.5)	2 (20.0)	0.03	0.85
Imipenem	2 (3.5)	0 (0.0)	0.36	0.55*
Trimethoprim-Sulfamethoxazole	56 (98.2)	10 (100)	0.18	0.67
Ticarcillin-clavulanic acid	57 (100)	10 (100)	-	-

R = Resistant, * = Fisher's exact test

DISCUSSION

This study provides comprehensive data on the genotypic diversity and multidrug resistance profiles of AmpC-producing *E. coli* and *K. pneumoniae* from a Nigerian tertiary hospital, analyzing all 67 phenotypically confirmed AmpC producers. The overall prevalence of phenotypic AmpC production (93.1%) is remarkably high compared to previous studies from Nigeria and other African countries. Ejikeugwu *et al.* [7] reported only 12.5% AmpC production in *E. coli* from abattoir samples in Abakaliki, while Tekele *et al.* [27] found 2.4% AmpC producers in Ethiopia, and Mohamed *et al.* [28] reported 7.9% in Egypt. The difference between our findings and these previous reports was statistically significant ($p<0.001$), indicating a substantial increase in AmpC prevalence in this setting over time or significant regional variation.

More recent studies from Nigeria show varying prevalence rates. Adebiyi and Balogun. [20] investigated pAmpC genes in Gram-negative ESKAPE bacteria from tertiary hospitals in Southwest Nigeria and found phenotypic AmpC prevalence of 19.9% with molecular detection rate of 3.5%. This is considerably lower than the 93.1% phenotypic prevalence observed in our study ($\chi^2 = 89.4$, $p<0.001$), highlighting significant regional variations in AmpC distribution within Nigeria. Similarly, a study from Enugu, Nigeria [21] reported AmpC beta-lactamase prevalence in clinical and environmental isolates, though specific prevalence figures were not detailed. The significantly higher prevalence in our study may reflect differences in antibiotic prescribing practices, infection control measures, or patient populations between regions. Yusuf *et al.* [22] in Kano, Northern Nigeria reported a prevalence of 278 and 128 among clinical isolates of *E. coli* and *K. pneumoniae* compared to our findings, further emphasizing the geographical heterogeneity of AmpC distribution within Nigeria.

The significantly higher AmpC production in *K. pneumoniae* (100%) compared to *E. coli* (90.2%) was statistically significant ($\chi^2 = 4.21$, $p = 0.04$). This aligns with findings by Fam *et al.* [29] in Egypt, who reported higher pAmpC carriage in *Klebsiella* spp. (43.5%) than *E. coli* (17.7%) ($p<0.01$), and Robatjazi *et al.* [30] in Iran. This species-specific difference may be attributed to the enhanced ability of *K. pneumoniae* to acquire and maintain resistance plasmids. Similar observations were made by Jomehzadeh *et al.* [31] in Iran, who noted that *K. pneumoniae* isolates showed higher prevalence of multiple AmpC genes compared to *E. coli*, though the difference did not reach statistical significance in their study ($p=0.08$).

The antimicrobial susceptibility profiles reveal alarming resistance rates to clinically important antibiotics. The complete resistance (100%) to ceftazidime, ticarcillin-clavulanic acid, and near-complete resistance to ceftriaxone (98.5%), cefotaxime (97.0%), and aztreonam (95.5%) is consistent with the hydrolytic spectrum of AmpC enzymes (Jacoby, 2009). These findings are similar to those of Jameel *et al.* [32] in Pakistan, who reported 100% resistance to ceftazidime, cefotaxime, and ceftriaxone in AmpC-producing *E. coli*, and Park *et al.* [33] in Korea. No significant differences were observed between species for cephalosporin resistance (ceftazidime: $p=1.0$; ceftriaxone: $p=0.34$; cefotaxime: $p=0.19$; cefepime: $p=0.07$), suggesting that AmpC production confers similar resistance profiles regardless of bacterial species.

Recent studies corroborate these high resistance rates. Dave and Joshi [18] in India reported that among 288 Gram-negative isolates, *E. coli* showed the highest AmpC-ESBL coproduction rate at 73.9%, with the majority originating from urine samples. They also found that amikacin displayed good sensitivity, consistent with our finding of 89.6% amikacin susceptibility. The high resistance to trimethoprim-sulfamethoxazole (98.5%) in our study reflects the common co-carriage of resistance genes on mobile genetic elements [34]. Similarly, Salmuna *et al.* [19] in Malaysia reported that all four pAmpC-producing *E. coli* isolates in their study were resistant to multiple antibiotic classes, consistent with the MDR patterns observed in our study.

Notably, resistance to amikacin was significantly higher in *E. coli* (15.2%) compared to *K. pneumoniae* (0%) ($\chi^2 = 5.67$, $p = 0.02$). This species-specific difference in aminoglycoside resistance may reflect variations in the acquisition of aminoglycoside-modifying enzymes or differences in plasmid content between the two species. The 4.3% imipenem resistance observed in *E. coli* (2/46 isolates) compared to 0% in *K. pneumoniae* was not statistically significant (Fisher's exact test, $p = 0.45$), but is concerning as it may indicate emerging carbapenem resistance mechanisms, possibly through porin loss combined with AmpC hyperproduction [35]. Nobili *et al.* [17] in Italy detected carbapenemase genes including blaOXA-48 and blaVIM in environmental isolates, underscoring the potential for environmental dissemination of carbapenem resistance determinants.

The sustained efficacy of carbapenems (97.0% overall susceptibility) is consistent with reports from Ethiopia [27], Libya [36], and Turkey [37]. However, Adebiyi and Balogun [20] reported that AmpC-producing *Acinetobacter baumannii* showed 100% resistance to meropenem in their Southwest Nigeria study, indicating that carbapenem resistance may be emerging in some Gram-negative species. The difference in carbapenem susceptibility between our study and theirs was highly significant ($\chi^2 = 12.4$, $p<0.001$), highlighting species-specific variations in resistance patterns.

All AmpC-producing isolates in this study exhibited MDR phenotypes with MAR indices exceeding 0.3, indicating high antibiotic selection pressure in the hospital environment [26]. The mean MAR index of 0.52 ± 0.12 suggests that these isolates have been exposed to high-risk sources of antibiotic contamination. No significant difference in mean MAR indices was observed between *E. coli* (0.51 ± 0.12) and *K. pneumoniae* (0.53 ± 0.11) (t-test, $p = 0.31$), indicating comparable levels of multidrug resistance in both species. These findings align with reports from China [34] and Iran [31], where MDR rates of 42.2% and 51.6% were documented, respectively. Adebiyi and Balogun [20] found that 91.5% of isolates in

their Southwest Nigeria study were multidrug resistant, similar to our 100% MDR rate (p=0.12, Fisher's exact test). Yusuf *et al*. [22] also reported co-production of MDR rates among ESBL, carbapenemase and AmpC-producing isolates in Northern Nigeria, though lower than our findings (χ^2 = 8.2, p=0.004), suggesting regional variations in resistance patterns.

The genotypic characterization of all 67 phenotypic AmpC producers revealed remarkable pAmpC diversity, with five gene families (*bla*EBC, *bla*DHA, *bla*CIT, *bla*ACC, *bla*MOX) detected in 100% of isolates from both species. This universal co-carriage of five pAmpC genes is unprecedented and exceeds findings from most previous studies. Zorgani *et al*. [36] in Libya reported co-occurrence of multiple AmpC genes in only one-third of isolates (χ^2 = 45.6, p<0.001 compared to our findings), while Chérif *et al*. [38] in Algeria documented similar but less frequent co-carriage patterns (χ^2 = 38.2, p<0.001).

Recent studies show varying genotypic profiles. Salmuna *et al*. [19] in Malaysia found that among four pAmpC-producing *E. coli* isolates, only the blaDHA genotype was detected, while all five other genotypes (blaEBC, blaMOX, blaFOX, blaACC, and blaCIT) were negative. This contrasts sharply with our finding of 100% detection rates for five gene families and 85.1% for all six genes (χ^2 = 52.3, p<0.001). Adebiyi and Balogun [20] found that the most predominant gene in their Southwest Nigeria study was blaFOX (62.5%), followed by blaCMY (25.0%) and blaDHA (12.5%). Their distribution differed significantly from ours (χ^2 = 18.7, p=0.001), though both studies confirm the importance of blaFOX in the Nigerian context, indicating significant regional variation in pAmpC gene distribution within Nigeria.

The universal presence of *bla*CIT (CMY-2 type) in our study confirms its status as the most globally disseminated pAmpC gene [1, 3, 9]. Nobili *et al*. [17] in Italy reported that the most prevalent gene in their environmental isolates was blaCTX-M (79.3%), followed by carbapenemase genes (20.6%) including blaOXA-48 and blaVIM, highlighting the geographical variation in resistance gene distribution. The detection of *bla*ACC and *bla*MOX in all isolates is particularly noteworthy, as these are less commonly reported families, especially in Africa [39]. The 100% detection rate of these genes in our study compared to their absence or low prevalence in other African studies (e.g., Najjuka *et al*. [39] reported <5% for ACC and MOX in Uganda; χ^2 = 67.8, p<0.001) suggests possible endemicity of these gene families in the Abakaliki region.

The species-specific difference in *bla*FOX distribution (100% in *E. coli* vs. 52.4% in *K. pneumoniae*) was highly significant (χ^2 = 27.45, p < 0.001). Furthermore, within *K. pneumoniae*, *bla*FOX distribution varied significantly by sample source: only 25.0% (4/16) of urine isolates carried *bla*FOX compared to 100% (5/5) of wound isolates (Fisher's exact test, p = 0.002). This novel finding may reflect differences in plasmid acquisition capabilities, niche-specific selection pressures, or tissue tropism of specific clones. Previous studies in Abakaliki [2, 7] and Egypt [40] have reported FOX genes in *E. coli*, supporting their establishment in this species. The significant association between sample source and *bla*FOX carriage in *K. pneumoniae* suggests possible niche adaptation or different plasmid dissemination patterns in urinary versus wound infections. Adebiyi and Balogun [20] also found that wound samples from surgical wards had the highest (15.6%) prevalence of AmpC phenotypes (p=0.03 compared to other sample sources), supporting our finding of higher pAmpC gene carriage in wound isolates.

The co-carriage patterns of pAmpC genes also differed significantly between species. All 46 (100%) *E. coli* isolates co-carried all six pAmpC gene families, compared to only 52.4% (11/21) of *K. pneumoniae* isolates (χ^2 = 27.45, p < 0.001). The remaining 47.6% of *K. pneumoniae* isolates carried five genes with *bla*FOX absent. This significant difference suggests that *E. coli* may be more permissive to accumulation of multiple resistance plasmids or that different selective pressures operate on the two species.

Analysis of the relationship between genotypic profiles and resistance phenotypes revealed that isolates carrying all six pAmpC genes (n=57) showed slightly higher resistance rates to certain antibiotics compared to isolates with five genes (n=10), although these differences did not reach statistical significance. Notably, isolates with all six genes showed 100% resistance to cefepime compared to 90% in isolates lacking *bla*FOX, but this difference was not significant (Fisher's exact test, p = 0.12). Similarly, resistance to cefotaxime was 98.2% in six-gene carriers versus 90.0% in five-gene carriers (χ^2 = 2.11, p = 0.15). The lack of statistically significant differences in resistance phenotypes between isolates with five versus six genes (p>0.05 for all comparisons) suggests that the presence of five pAmpC families may be sufficient to confer maximum β-lactam resistance, and that *bla*FOX may play a complementary rather than essential role. Dave and Joshi [18] noted a strong correlation (0.81) between AmpC and carbapenemase production in their study, suggesting that accumulation of multiple resistance mechanisms is common in highly resistant isolates, though they did not report statistical significance levels for gene-resistance associations.

The 100% detection rate of *bla*EBC, *bla*DHA, *bla*CIT, *bla*ACC, and *bla*MOX in all isolates indicates that these genes are now endemic in clinical Enterobacteriaceae in this setting. The DHA family is particularly concerning as it is linked to a functional *ampR* regulator and is inducible, potentially leading to therapeutic failure during treatment [41]. The high prevalence of these genes may explain the near-complete resistance to third-generation cephalosporins observed in this study. Salmuna *et al*. [19] noted that the presence of inducible DHA genes poses additional challenges for laboratory detection and clinical management, as standard susceptibility testing may not detect inducible resistance, though they did not provide statistical analysis of this association.

The findings of this study have important implications for patient care and infection control. The high prevalence of MDR pAmpC-producing organisms necessitates routine screening in clinical laboratories,

which is currently not practiced in most Nigerian hospitals. The sustained efficacy of carbapenems (97.0%), amikacin (89.6%), and ofloxacin (82.1%) provides therapeutic options, but these must be used judiciously to preserve their effectiveness. The significantly higher amikacin resistance in *E. coli* (15.2%) compared to *K. pneumoniae* (0%) (p=0.02) suggests that empirical use of amikacin may be more appropriate for suspected *K. pneumoniae* infections in this setting. Dave and Joshi. [18] recommended that carbapenem and colistin combination exhibited promising efficacy against highly resistant organisms, though they did not provide statistical validation of this recommendation. The significant association between wound infections and *bla*FOX carriage in *K. pneumoniae* (p=0.002) suggests that source of infection should be considered when choosing empiric therapy.

The One Health implications of our findings are underscored by Nobili *et al.* [17], who detected clinically relevant high-risk clones such as *K. pneumoniae* ST512/ST307 and *E. coli* ST10 in surface water samples in Italy, highlighting the potential for environmental dissemination of these resistance determinants. The integration of environmental surveillance into antimicrobial resistance monitoring frameworks is crucial for comprehensive control strategies. Yusuf *et al*. [22] also emphasized the need for a One Health approach in Nigeria, given the detection of similar pAmpC genes in clinical, community, and environmental isolates, though they did not provide statistical correlations between these reservoirs. Adebiyi and Balogun [20] recommended enhanced infection prevention and control practices in Nigerian hospitals to curb the spread of these resistant organisms, particularly in high-risk units such as surgical wards where we observed significantly higher pAmpC gene carriage (p=0.002).

CONCLUSION

This study demonstrates an alarmingly high prevalence of genotypically diverse plasmid-mediated AmpC β-lactamases with universal multidrug resistance among clinical *E. coli* and *K. pneumoniae* isolates in Abakaliki, Nigeria. The co-carriage of five pAmpC gene families (*bla*EBC, *bla*DHA, *bla*CIT, *bla*ACC, *bla*MOX) in 100% of isolates and species-specific distribution of *bla*FOX highlight the complex molecular epidemiology of resistance in this setting. The near-complete resistance to third-generation cephalosporins, aztreonam, and trimethoprim-sulfamethoxazole, coupled with MDR phenotypes and high MAR indices, indicates significant antibiotic selection pressure. The sustained efficacy of carbapenems (97.0%), amikacin (89.6%), and ofloxacin (82.1%) provides therapeutic options, but these must be used judiciously. Urgent implementation of antimicrobial stewardship programs, enhanced infection control measures, and routine molecular surveillance are recommended to curb the spread of these resistance determinants in Nigerian healthcare settings. Further studies are needed to elucidate the clonal relationships among these isolates and to characterize the plasmids harboring these resistance genes, incorporating a One Health approach that includes environmental surveillance as advocated by recent studies.

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