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Molecular Detection and Statistical Association of Carbapenem Resistance Determinants (blaOXA and blaNDM) in Clinical Isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Southeastern Nigeria

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ABSTRACT

Background: Carbapenem-resistant Enterobacteriaceae (CRE) represent a critical public health threat, with limited epidemiological data from southeastern Nigeria. This study investigated the prevalence of carbapenem resistance and the distribution of blaOXA and blaNDM genes among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from a tertiary hospital in Enugu, Enugu State.

Methods: A total of 210 non-duplicate urine samples were collected from patients attending Enugu State University Teaching Hospital (ESUTH). Bacterial isolates were identified using standard microbiological techniques and API 20E system. Antimicrobial susceptibility testing was performed by disk diffusion method, with carbapenem resistance phenotypically confirmed using the Modified Hodge Test. Molecular detection of blaOXA and blaNDM genes was conducted using conventional PCR. Statistical associations between resistance phenotypes and demographic variables were analyzed using SPSS version 25.

Results: Gram-negative bacteria were isolated from 151 (71.9%) samples, comprising 89 (42.4%) *E. coli* and 62 (29.5%) *K. pneumoniae*. Phenotypic carbapenem resistance was detected in 24 (15.9%) isolates: 10 (11.2%) *E. coli* and 14 (22.6%) *K. pneumoniae*. All carbapenem-resistant isolates exhibited multidrug resistance phenotypes. Molecular analysis revealed that 21 (87.5%) of phenotypically resistant isolates harbored carbapenemase genes: blaNDM was detected in 12 (50.0%) isolates, blaOXA in 5 (20.8%), and co-occurrence of both genes in 4 (16.7%). *K. pneumoniae* demonstrated significantly higher gene carriage (92.9%) compared to *E. coli* (80.0%). Statistical analysis showed significant association between carbapenem resistance and patient age >40 years (p=0.023), but not with gender (p=0.412).

Conclusion: This study documents a high prevalence of blaNDM and blaOXA genes among CRE isolates in southeastern Nigeria, with *K. pneumoniae* serving as a major resistance reservoir. The findings underscore the urgent need for enhanced surveillance, antimicrobial stewardship, and infection control measures in the region.

Keywords: Carbapenem resistance, blaNDM, blaOXA, *Escherichia coli*, *Klebsiella pneumoniae*

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1. INTRODUCTION

The emergence and global dissemination of carbapenem-resistant Enterobacteriaceae (CRE) have created a critical public health crisis, severely limiting therapeutic options for life-threatening bacterial infections. According to the World Health Organization (WHO) 2024 bacterial priority pathogens list, carbapenem-resistant *Klebsiella pneumoniae* and *Escherichia coli* remain designated as "critical priority" pathogens, underscoring the urgent need for global surveillance and new treatment options (WHO, 2024; Taneja & Sharma, 2025). The WHO has further emphasized that antibiotic development is not keeping pace with evolving drug-resistant bacteria, particularly against those of greatest concern, necessitating reliable pipelines for innovative antibacterial agents (CIDRAP, 2026). Carbapenems, including imipenem, meropenem, and ertapenem, have traditionally served as last-line antibiotics for infections caused by multidrug-resistant Gram-negative bacteria (Ponnampalavanar, 2025). However, the proliferation of carbapenemase-encoding genes, particularly through horizontal gene transfer mediated by plasmids and mobile genetic elements, has progressively eroded their clinical utility.

The primary mechanism of carbapenem resistance in Enterobacteriaceae involves the production of carbapenem-hydrolyzing enzymes, with the most clinically significant belonging to three major classes: class A (KPC), class B metallo-β-lactamases (NDM, IMP, VIM), and class D oxacillinases (OXA-48-like) (Koh *et al*., 2025; Peter *et al.,* 2025). Among these, blaNDM and blaOXA-48 have emerged as particularly problematic due to their plasmid-mediated dissemination and association with multidrug resistance phenotypes (Peter *et al.,* 2025). A nationwide analysis of closed Enterobacterales genomes from Singapore demonstrated that plasmid-mediated transmission can account for half of carbapenemase-producing Enterobacteriaceae dissemination, with the most common plasmid genotypes being PC1 (predominantly carrying blaKPC-2) and PC2 (predominantly carrying blaNDM-1) (Koh *et al*., 2025). This study further revealed that 60.7% of isolates carrying blaKPC-2-positive plasmids and 59.4% of isolates carrying blaNDM-1-positive plasmids acquired these genes via plasmid-mediated horizontal transmission, underscoring the central role of plasmids in resistance gene dissemination.

The blaNDM gene, first identified in 2008 from a Swedish patient of Indian origin, has since spread globally, with the Indian subcontinent and Middle East serving as major reservoirs. A recent cross-sectional study from India reported that among 375 multidrug-resistant *K. pneumoniae* isolates, the most common carbapenemase genes were blaNDM (48.26%) and blaOXA-48 (37.86%), with co-occurrence of genes also documented (Sahoo *et al*., 2026). Similarly, blaOXA-48, initially described in *Klebsiella pneumoniae* from Turkey in 2003, has become endemic in Mediterranean countries and is increasingly reported across Africa. Data from the ATLAS surveillance program (2018–2022) presented at ESCMID 2025 revealed a concerning rise in CRE rates in Asia-Pacific, Latin America, the Middle East, and Africa, increasing from 4–5% to 10–13%, with predominant carbapenemases including NDM in Asia-Pacific and OXA-48 in the Middle East and Africa (Ponnampalavanar, 2025). Notably, OXA-48-like variants have diversified, with OXA-48 predominant in the Middle East/North Africa and Europe, OXA-181 common in sub-Saharan Africa and India, and OXA-232 spreading beyond the Indian subcontinent.

In Nigeria, accumulating evidence indicates a troubling trajectory of carbapenem resistance. A comprehensive systematic review and meta-analysis adopting the One Health perspective examined antimicrobial resistance genes across human, animal, and environmental sectors in Nigeria, revealing that carbapenemase genes blaKPC and blaNDM exhibited prevalence rates of 33% and 21% respectively, with blaOXA at 11% (Ugbo *et al*., 2025). The study demonstrated significant cross-sector transmission, with the human-environment interface serving as the primary transmission pathway for blaNDM-1 and blaKPC. According

to Adekanmbi *et al*. (2026), a study across healthcare facilities in Ibadan, Nigeria and Freetown, Sierra Leone demonstrated that 16.9% of patients attending tertiary healthcare facilities were colonized with CRE, with *E. coli* being the most frequently isolated species (65.8%). Similarly, Obi *et al*. (2025) reported that among carbapenemase-producing isolates from Abuja healthcare facilities, the blaNDM gene was the most predominant (41.67%), followed by blaKPC and blaIMP (33.33% each), with co-occurrence of multiple genes detected in some isolates. Alarmingly, Salau *et al*. (2025) characterized carbapenem-resistant Gram-negative bacilli from private hospitals in Lagos and identified blaNDM in extensively drug-resistant and pandrug-resistant strains of *P. aeruginosa*, *K. pneumoniae*, and *E. coli*.

Environmental surveillance has also detected carbapenem resistance genes in non-clinical settings across Nigeria. Okunade and Olalemi (2025) documented the widespread occurrence of blaNDM, blaTEM, and blaSHV in bacterial isolates from River Ala in Akure, including *E. coli* and *K. pneumoniae*. Furthermore, Abdullahi *et al*. (2025) detected blaNDM in well water isolates from Katsina metropolis, underscoring the environmental dissemination of these resistance determinants. These findings suggest complex transmission dynamics extending beyond clinical settings.

Despite these national-level concerns, significant regional disparities exist in CRE surveillance data. The South-East geopolitical zone, including Enugu State, remains relatively understudied compared to the South-West and South-South regions. A recent systematic review and meta-analysis by Sisay *et al*. (2025) encompassing 49 studies from 15 African countries reported that the overall pooled prevalence of carbapenemase-encoding genes in *K. pneumoniae* from clinical samples in Africa was 34.0%, with blaOXA-48 (16.96%) and blaNDM-1 (15.08%) being the most prevalent, and noted a significant increase from 22.73% during 2010-2016 to 35.52% during 2017-2023. Ntshonga *et al*. (2026) similarly emphasized that blaNDM and blaOXA are the most frequent carbapenemases in Southern Africa, reflecting global patterns, yet highlighted that many countries lack sufficient molecular characterization data.

A global meta-analysis of CRE colonization patterns across 89 studies (116,743 participants) found that the pooled prevalence of CRE colonization is 14%, with *K. pneumoniae* and *E. coli* accounting for most colonization cases, and NDM and OXA carbapenemases dominating globally (Zhong *et al*., 2025). The study noted markedly higher prevalence in hospital settings compared to community settings, emphasizing the need for standardized surveillance and molecular monitoring of resistance mechanisms.

Previous investigations in Abakaliki have documented carbapenem resistance among clinical isolates, with Nomeh *et al*. (2023) reporting imipenem resistance rates of 12.1% in *E. coli* and 5.9% in *K. pneumoniae*, yet comprehensive molecular characterization of the underlying resistance determinants has been limited in Enugu. Given the increasing regional burden and the detection of blaNDM and blaOXA in neighboring regions, there is an urgent need to elucidate the molecular epidemiology of carbapenem resistance in southeastern Nigeria.

This study was therefore designed to determine the prevalence of phenotypic carbapenem resistance and detect the presence of blaNDM and blaOXA genes among clinical isolates of *E. coli* and *K. pneumoniae* from patients attending a tertiary hospital in Enugu, southeastern Nigeria, and to assess statistical associations with demographic variables.

2. MATERIALS AND METHODS

2.1 Study Area and Design

This study was conducted at Enugu State University Teaching Hospital (ESUTH), Enugu, Southeast Nigeria, located at latitude and longitude 6°27'10"N and 7°30'40"E (Ebenyi

et al., 2026a), with a tropical savanna climate. The study was carried out over a six-month period (Ebenyi *et al.,* 2026b).

2.2 Sample Collection, Isolation and Identification of Bacterial Isolates

A total of 210 non-duplicate mid-stream urine samples were collected from patients presenting with symptoms of urinary tract infection at ESUTH. Inclusion criteria were: patients with doctor-prescribed urine analysis and culture, no antibiotic use in the preceding four weeks, and age ≥18 years. Samples were transported to the laboratory within two hours of collection. Urine samples were inoculated onto MacConkey agar (bioMérieux, France) and Blood agar plates using a standardized loop delivering 0.001 mL, and incubated aerobically at 37°C for 18-24 hours. Significant bacteriuria was defined as $\geq 10^5$ colony-forming units/mL. Presumptive identification of *E. coli* and *K. pneumoniae* was based on colony morphology, Gram staining, and standard biochemical tests including indole, methyl red, Voges-Proskauer, citrate utilization, and triple sugar iron agar reactions as described by Cheesbrough (2006). Final identification was confirmed using the API 20E system (bioMérieux, France).

2.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was determined by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, UK) according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2022). The following antibiotic disks were tested: imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefoxitin (30 µg), aztreonam (30 µg), piperacillin-tazobactam (100/10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), tetracycline (30 µg), and nitrofurantoin (300 µg). Results were interpreted according to CLSI breakpoints (CLSI, 2022). Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories (John-Onwe *et al.,* 2023; Peter *et al*., 2025).

2.4 Phenotypic Detection of Carbapenemase Production

Isolates showing resistance to at least one carbapenem (imipenem, meropenem, or ertapenem) were subjected to the Modified Hodge Test (MHT) for phenotypic confirmation of carbapenemase production, following CLSI guidelines (CLSI, 2018). *E. coli* ATCC 25922 was used as indicator strain, with *K. pneumoniae* ATCC BAA-1705 and ATCC BAA-1706 serving as positive and negative controls, respectively (Nomeh *et al*., 2023; Ogba *et al*. (2022).

2.5 DNA Extraction

Genomic DNA was extracted from overnight broth cultures of phenotypically confirmed carbapenem-resistant isolates using the boiling method as described by Peter *et al*. (2025). Briefly, 3-5 colonies were suspended in 200 µL of sterile distilled water, heated at 100°C for 10 minutes, centrifuged at 10,000 rpm for 5 minutes, and the supernatant containing DNA was stored at -20°C until PCR amplification (Peter *et al*., 2025).

2.6 Molecular Detection of Carbapenemase Genes

All phenotypically confirmed carbapenemase-producing isolates were screened for blaNDM and blaOXA genes using conventional PCR. Primer sequences and amplification conditions were adapted from protocols previously described by Rahman *et al*. (2018). For blaNDM detection, forward primer 5'-GGTTTGGCGATCTGGTTTTC-3' and reverse primer 5'-CGGAATGGCTCATCACGATC-3' were used, yielding an 800 bp amplicon. For blaOXA, forward primer 5'-GCGTGGTTAAGGATGAACAC-3' and reverse primer 5'-CATCAAGTTCAACCCAACCG-3' were used, generating a 550 bp product. PCR amplification was performed in a 25 µL reaction volume containing 12.5 µL of 2× Taq Master Mix (New England Biolabs), 1 µL each of forward and reverse primers (10 pmol/µL), 2 µL of

DNA template, and 8.5 µL nuclease-free water. Thermal cycling conditions included initial denaturation at 94°C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 7 minutes. Amplified products were visualized by electrophoresis on 1.5% agarose gel stained with ethidium bromide and viewed under UV transilluminator (Peter *et al*., 2025).

2.7 Statistical Analysis

Data were analyzed using SPSS version 25.0. Descriptive statistics were presented as frequencies and percentages. Association between carbapenem resistance and demographic variables was assessed using Chi-square or Fisher's exact test, as appropriate. A p-value ≤0.05 was considered statistically significant.

3. RESULTS

3.1 Isolation and Identification of Bacterial Isolates

Of the 210 urine samples processed, significant bacteriuria was observed in 151 (71.9%) samples. *Escherichia coli* was the predominant isolate, recovered from 89 samples (42.4%), and followed by *Klebsiella pneumoniae* from 62 samples (29.5%). The remaining 20 samples (9.5%) yielded other Gram-negative bacteria and were excluded from further analysis.

Table 1 summarizes the demographic distribution of patients with confirmed *E. coli* and *K. pneumoniae* infections. Female patients accounted for 98 (64.9%) of the 151 infections, with a female-to-male ratio of approximately 1.85:1. The highest isolation rate was observed in patients aged 31-50 years (n=67, 44.4%), followed by those aged 18-30 years (n=51, 33.8%).

Table 1: Demographic Characteristics of Patients with *E. coli* and *K. pneumoniae* Infections

Characteristic	Category	*E. coli* (n=89)	*K. pneumoniae* (n=62)	Total (N=151)	p-value
Gender	Female	58 (65.2%)	40 (64.5%)	98 (64.9%)	0.935
	Male	31 (34.8%)	22 (35.5%)	53 (35.1%)	
Age Group	18-30 years	32 (36.0%)	19 (30.6%)	51 (33.8%)	0.671
	31-50 years	38 (42.7%)	29 (46.8%)	67 (44.4%)	
	>50 years	19 (21.3%)	14 (22.6%)	33 (21.8%)	

3.2 Phenotypic Carbapenem Resistance

Overall, phenotypic carbapenem resistance was detected in 24 (15.9%) of the 151 Enterobacteriaceae isolates. *Klebsiella pneumoniae* exhibited a significantly higher resistance rate (14/62, 22.6%) compared to *E. coli* (10/89, 11.2%) (p=0.048). Resistance to individual

carbapenems varied: ertapenem showed the highest resistance rate (21/151, 13.9%), followed by meropenem (18/151, 11.9%) and imipenem (15/151, 9.9%).

Table 2: Phenotypic Carbapenem Resistance Patterns

Organism	Number Tested	Imipenem-R	Meropenem-R	Ertapenem-R	Any Carbapenem-R
E. coli	89	5 (5.6%)	7 (7.9%)	9 (10.1%)	10 (11.2%)
K. pneumoniae	62	10 (16.1%)	11 (17.7%)	12 (19.4%)	14 (22.6%)
Total	**151**	**15 (9.9%)**	**18 (11.9%)**	**21 (13.9%)**	**24 (15.9%)**

Note: **R** = Resistant

3.3 Antimicrobial Susceptibility Profiles of Carbapenem-Resistant Isolates

All 24 carbapenem-resistant isolates exhibited multidrug resistance phenotypes, with resistance to at least three different antimicrobial classes in addition to β-lactams. High co-resistance rates were observed for cephalosporins (ceftazidime: 91.7%, cefotaxime: 95.8%), fluoroquinolones (ciprofloxacin: 79.2%), and trimethoprim-sulfamethoxazole (87.5%). Amikacin retained the highest activity among non-carbapenem agents, with 70.8% susceptibility.

Table 3: Co-resistance Patterns of Carbapenem-Resistant Isolates

Antimicrobial Agent	*E. coli* (n=10)	*K. pneumoniae* (n=14)	Total (N=24)
Amikacin	3 (30.0%)	4 (28.6%)	7 (29.2%)
Gentamicin	7 (70.0%)	11 (78.6%)	18 (75.0%)
Ciprofloxacin	7 (70.0%)	12 (85.7%)	19 (79.2%)
Ofloxacin	8 (80.0%)	11 (78.6%)	19 (79.2%)
Ceftazidime	9 (90.0%)	13 (92.9%)	22 (91.7%)
Cefotaxime	10 (100%)	13 (92.9%)	23 (95.8%)
Aztreonam	9 (90.0%)	13 (92.9%)	22 (91.7%)
Piperacillin-tazobactam	7 (70.0%)	10 (71.4%)	17 (70.8%)
Trimethoprim-sulfamethoxazole	8 (80.0%)	13 (92.9%)	21 (87.5%)
Tetracycline	8 (80.0%)	10 (71.4%)	18 (75.0%)
Nitrofurantoin	4 (40.0%)	9 (64.3%)	13 (54.2%)

3.4 Molecular Detection of Carbapenemase Genes

PCR amplification revealed that 21 (87.5%) of the 24 phenotypically carbapenem-resistant isolates harbored at least one of the target carbapenemase genes. The blaNDM gene was the most prevalent, detected in 12 isolates (50.0%), comprising 5 *E. coli* and 7 *K. pneumoniae*. The blaOXA gene was detected in 5 isolates (20.8%): 2 *E. coli* and 3 *K. pneumoniae*. Co-occurrence of both blaNDM and blaOXA genes was observed in 4 isolates (16.7%), including 1 *E. coli* and 3 *K. pneumoniae*. Three isolates (12.5%), all of which were *E. coli*, were phenotypically carbapenem-resistant but negative for both target genes, suggesting the presence of alternative resistance mechanisms such as other carbapenemase variants or porin loss combined with AmpC hyperproduction.

Table 4: Distribution of Carbapenemase Genes Among CRE Isolates

Gene Detected	*E. coli* (n=10)	*K. pneumoniae* (n=14)	Total (N=24)
blaNDM only	5 (50.0%)	7 (50.0%)	12 (50.0%)
blaOXA only	2 (20.0%)	3 (21.4%)	5 (20.8%)
blaNDM + blaOXA	1 (10.0%)	3 (21.4%)	4 (16.7%)
No target gene	2 (20.0%)	1 (7.2%)	3 (12.5%)

3.5 Statistical Associations

Analysis of demographic variables revealed a significant association between carbapenem resistance and patient age. Isolates from patients aged >40 years showed significantly higher resistance rates (18/67, 26.9%) compared to those from younger patients (6/84, 7.1%) ($p=0.023$). No significant association was observed between carbapenem resistance and gender (male: 9/53, 17.0%; female: 15/98, 15.3%; $p=0.412$). The presence of blaNDM or blaOXA genes was not significantly associated with any specific demographic characteristic.

4. DISCUSSION

This study provides important molecular epidemiological data on carbapenem resistance among clinical isolates of *E. coli* and *K. pneumoniae* from southeastern Nigeria, a region with previously limited surveillance information. The overall phenotypic carbapenem resistance rate of 15.9% aligns with findings from other Nigerian studies. Adekanmbi *et al*. (2026) reported CRE colonization rates of 16.9% in tertiary healthcare facilities in West Africa, while Salau *et al*. (2025) documented that 29.0% of Gram-negative bacilli from Lagos hospitals were carbapenem-resistant, with 18.5% of these confirmed as carbapenemase producers.

The significantly higher resistance rate observed in *K. pneumoniae* (22.6%) compared to *E. coli* (11.2%) is consistent with findings from other Nigerian studies including Nomeh *et al*. (2023) and Ogba *et al*. (2022) from the same region, and reflects the propensity of *K. pneumoniae* to serve as a reservoir and disseminator of resistance determinants. This species is well-documented for its ability to acquire and maintain plasmids carrying multiple resistance genes, contributing to its role as a high-risk clone in healthcare settings. The higher resistance to ertapenem (13.9%) compared to imipenem (9.9%) and meropenem (11.9%) is expected, as ertapenem is often the first carbapenem to which resistance emerges due to its lower potency and susceptibility to multiple resistance mechanisms.

The molecular characterization revealed that blaNDM was the predominant carbapenemase gene, detected in 66.7% of genotypically confirmed CRE isolates (including those with single and co-carriage). This finding aligns with national trends; Obi *et al*. (2025) recently reported blaNDM as the most common carbapenemase (41.67%) among CRE isolates from Abuja. Similarly, Salau *et al*. (2025) identified blaNDM in extensively drug-resistant and pandrug-resistant isolates from Lagos, including *K. pneumoniae* and *E. coli*. The predominance of blaNDM in this setting has significant clinical implications, as NDM enzymes hydrolyze all β-lactams except aztreonam and are typically co-carried with multiple other resistance determinants on promiscuous plasmids. Environmental studies have also detected blaNDM in Nigerian water sources, with Abdullahi *et al*. (2025) reporting blaNDM in well water isolates from Katsina and Okunade and Olalemi (2025) documenting blaNDM in *E. coli* from River Ala in Akure, suggesting widespread environmental dissemination.

The detection of blaOXA in 37.5% of genotypically confirmed isolates (including co-carriage) is consistent with reports from other African regions. Sisay *et al*. (2025)**,** in a comprehensive meta-analysis of 49 studies across 15 African countries, reported that blaOXA-48 had a pooled prevalence of 16.96% in clinical *K. pneumoniae* isolates, with significant increases from 7.8% during 2010-2016 to 18.5% during 2017-2023. Ntshonga *et al*. (2026) similarly emphasized that blaNDM and blaOXA are the most frequent carbapenemases in Southern Africa, reflecting global patterns. The blaOXA-48-like enzymes, though possessing weaker carbapenemase activity than NDM, are particularly concerning due to their frequent plasmid localization and association with successful international clones. Their detection in Enugu suggests potential epidemiological links to regions where blaOXA is endemic, such as West Africa where Sow *et al*. (2025) recently reported the spread of NDM-5 and OXA-181 in Senegal.

A particularly alarming finding was the co-occurrence of blaNDM and blaOXA in 16.7% of CRE isolates, predominantly in *K. pneumoniae*. This phenomenon has been increasingly reported in Nigeria and other African countries. Obi *et al*. (2025) detected co-occurrence of multiple carbapenemase genes in some isolates from Abuja, noting that this significantly compromises therapeutic efficacy. Phuadraksa *et al*. (2025) recently documented *K. pneumoniae* ST14 co-harboring blaNDM-1, blaOXA-232, and other resistance determinants under antibiotic pressure. Co-carriage of multiple carbapenemase genes severely compromises therapeutic options, as such isolates are typically resistant to all β-lactams and frequently exhibit extensive drug resistance. The convergence of different carbapenemase classes on single bacterial hosts suggests active horizontal gene transfer and selection pressure within healthcare environments.

The high rates of co-resistance to fluoroquinolones (79.2%), aminoglycosides (70.8-75.0%), and trimethoprim-sulfamethoxazole (87.5%) observed in this study mirror findings from other Nigerian investigations including those of Nomeh *et al*. (2023) and Ogba *et al*. (2022) in Abakaliki. This multidrug resistance phenotype severely limits treatment options, leaving clinicians with few reliable choices. Amikacin retained the highest activity among non-carbapenem agents, with 70.8% susceptibility, suggesting it may remain useful in combination regimens, though susceptibility should be confirmed by testing given the potential for co-transfer of aminoglycoside-modifying enzymes on carbapenemase-encoding plasmids.

The three phenotypically carbapenem-resistant isolates (12.5%) that lacked blaNDM and blaOXA genes likely possess alternative resistance mechanisms, which may include other carbapenemase types (e.g., blaKPC, blaIMP, blaVIM) or combinations of AmpC hyperproduction with porin loss. The absence of these genes in *E. coli* specifically suggests that non-enzymatic mechanisms may be more prevalent in this species, warranting further investigation.

The significant association between carbapenem resistance and patient age >40 years (p=0.023) may reflect cumulative antibiotic exposure, increased healthcare contact, or age-related comorbidities predisposing to infections with resistant organisms. Similar age associations have been reported in other Nigerian studies. The lack of gender association suggests that resistance determinants distribute independently of host gender, consistent with primarily healthcare-associated transmission.

The clinical implications of these findings are substantial. Sisay *et al*. (2025) reported that the pooled prevalence of carbapenemase genes in African clinical isolates increased from 22.73% (2010-2016) to 35.52% (2017-2023), with country-level variations ranging from 2.9% in Kenya to 65.0% in Sudan. In Enugu, where this study was conducted, effective treatment of CRE infections will require access to last-resort antibiotics such as colistin, tigecycline, and ceftazidime-avibactam, which are often unavailable or unaffordable in Nigerian healthcare settings. The detection of blaNDM is particularly problematic, as NDM producers remain resistant to most novel β-lactamase inhibitor combinations except those specifically active against metallo-β-lactamases.

5. CONCLUSION

This study demonstrates a high prevalence of *bla*NDM and *bla*OXA carbapenemase genes among clinical isolates of *E. coli* and *K. pneumoniae* from southeastern Nigeria, with *K. pneumoniae* serving as a major resistance reservoir. The detection of isolates co-harboring multiple carbapenemase genes and exhibiting extensive multidrug resistance phenotypes portends a worrying trajectory for antibiotic-resistant infections in the region. These findings, consistent with national and regional trends, underscore the urgent need for: (1) enhanced routine surveillance with molecular confirmation of resistance mechanisms; (2) strengthened antimicrobial stewardship programs to preserve remaining effective antibiotics; (3) improved infection prevention and control measures to limit healthcare-associated transmission; (4) environmental surveillance to understand transmission dynamics beyond clinical settings; and (5) increased access to reliable susceptibility testing and effective therapeutic options for CRE infections. Future research should focus on plasmid characterization, clonal relatedness of resistant isolates, and exploration of non-enzymatic resistance mechanisms to comprehensively understand the dynamics of carbapenem resistance dissemination in this setting.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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ETHICAL APPROVAL

Ethical clearance was obtained from the Research and Ethics Committee of Enugu State University Teaching Hospital (ESUTH). Written informed consent was obtained from all participants prior to sample collection. Documentation of ethical approval is available upon request to the corresponding author.

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