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INCIDENCE OF CARBAPENEM RESISTANCE IN ENTEROBACTERIACEAE SPECIES ISOLATED FROM DIARRHOEIC PATIENTS ATTENDING A TERTIARY HOSPITAL IN NIGERIA

***Ibeneme, E. O.[1,2], Ibeneme, C. E.[3], Eyo, A. O.[1], Asuquo, E. E[4]., Orok, M. R.[4], Elizabeth, J, O., Ikwen, N. U.[1]**

[1]Department of Medical Bacteriology, Virology and Mycology, Faculty of Medical Laboratory Science, University of Calabar, Calabar-Nigeria
[2]Dr. Lawrence Henshaw Memorial Specialist Hospital Reference Laboratory, Calabar, Cross River State, Nigeria
[3] Department of Medicine and Surgery, Faculty of Clinical Sciences, College of Medical Sciences, University of Calabar, Calabar-Nigeria
[4]Department of Medical Microbiology & Parasitology, University of Calabar Teaching Hospital, Calabar-Nigeria
[5]Cross River State TB Control Programme, Department of Public Health, Ministry of Health, Calabar-Nigeria
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ABSTRACT

Introduction: Infections caused by Carbapenem-producing Enterobacteriaceae are a menace in health settings. **Objective:** A study was conducted to ascertain the incidence of carbapenem antibiotic resistance of *Enterobacteriaceae* isolated from stool samples of some patients with diarrhoea attending University of Calabar Teaching Hospital, Calabar-Nigeria. **Materials and Methods:** The study is a cross-sectional study. Stool samples were obtained from randomly-selected 100 male and female patients who attended the Out-patient clinic of the University of Calabar Teaching Hospital. Questionnaires were administered to the study participants to obtain basic demographic characteristics. Stool samples were processed by inoculating into selenite F broth and incubating for 4-6 hours at 37°C, after which they were sub-cultured onto Deoxycholate agar supplemented with meropenem and incubated for 24 hours at 37°C. Identification of isolates were carried out using Gram staining technique, standard biochemical tests and GNB Microbact 12A identification kit. Antibiotic sensitivity testing was carried out using the Kirby-Bauer disk diffusion technique and Multiple Antibiotic Resistance (MAR) Indices of Enterobacteriaceae species were calculated. **Results:** A total of 59 *Enterobacteriaceae* were isolated, comprising of eight genera, with *Citrobacter freundii* (32/59; 54.2%) as the most recurring *Enterobacteriaceae*. Gentamicin was the most potent antibiotic, while doripenem was the least potent antibiotic tested against isolates. Mean MAR index of *Enterobacteriaceae* species to carbapenems and total antibiotics tested was 0.7 and 0.4, respectively. *Providencia rettgeri* showed the highest MAR index to carbapenems and total antibiotics tested; 1.0 and 0.8 respectively. There was statistically significant difference (p=0.009) between the MAR index of carbapenems and total antibiotic tested for all groups of isolates. **Conclusion:** This study highlighted the high rate of resistance of *Enterobacteriaceae* species from stool samples of diarrhoea patients in Calabar to carbapenems. The public should be constantly educated on the judicious use of antibiotics for clinical infection as well as in animal production.

Keywords: Carbapenem resistant Enterobacteriaceae, Multiple antibiotic resistance index, Antimicrobial stewardship, Tertiary hospital, Diarrhoeic patients

Corresponding Author: **Ibeneme, E.O.**
Department of Medical Bacteriology, Virology & Mycology, Faculty of Medical Laboratory Science, University of Calabar, Calabar-Nigeria. Email: ibeneme.eo@gmail.com

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Introduction

The *Enterobacteriaceae* is a large family of Gram-negative bacilli endemic to the human gut and have long been implicated in both nosocomial and community-acquired infections worldwide. Historically, the beta-lactam antibiotics formed the cornerstone of treatment against these organisms; however, extensive and widespread indiscriminate use of penicillins, its derivatives and cephalosporins has culminated in widespread beta-lactamase-mediated resistance among *Enterobacteriaceae* species (Oduyebo *et al*., 2015; Tula *et al*., 2023). In response, carbapenems, owing to their structural resilience against many beta-lactamases, have emerged as "last-line" agents for multidrug-resistant (MDR) *Enterobacteriaceae* infections, yet the clinical utility of these lifesaving drugs is now threatened by the rise of carbapenem-resistant *Enterobacteriaceae* (CRE) (Oduyebo *et al*., 2015; Etebu *et al*., 2016).

Globally, CRE prevalence has surged, especially in resource-limited settings where antibiotic stewardship is challenging. A systematic review covering Nigeria from 2013 to 2022 reported an average carbapenem resistance rate of 21.3% among Gram-negative isolates, with regional variability from 2.2% in Lagos to 100% in certain community studies in Kogi State (Tula *et al*., 2023). Meta-analyses have further demonstrated a progressive increase in resistance over time, with pooled estimates of imipenem resistance at 11.2% and meropenem resistance at 13.5%, underscoring a worrying upward trend (Ssekataw *et al*., 2018; Irekeola *et al*., 2024). These figures not only surpass earlier reports from East Africa and Egypt, but also highlight the unique epidemiology of CRE in Nigeria's extensive and diverse geopolitical regions.

While most Nigerian studies have focused on urinary, blood, and environmental isolates, data on carbapenem resistance among Enterobacteriaceae recovered from diarrhoeic patients remain scarce. Diarrhoeal disease is a leading cause of morbidity in Nigeria, particularly among children and immunocompromised adults. Diarrhoeic patients often receive broad-spectrum empirical antibiotics—factors that promote dysbiosis and selection for resistant organisms. Gut dysbiosis is an imbalance in the gut microbiota often precipitated by frequent antibiotic use, gastrointestinal infections, or dietary disturbances. It reduces colonization resistance and is implicated in the proliferation of CRE (Korach-Rechtman *et al*., 2020). CRE-colonized individuals exhibit lower gut microbial diversity, enrichment of *Enterobacteriaceae,* and depletion of anaerobic commensals like Faecalibacterium and Barnesiella, which normally produce protective short-chain fatty acids. These changes precede or exacerbate CRE colonization and raise the risk of systemic spread (Korach-Rechtman *et al*., 2020). A 2020 study from Sokoto retrospectively analyzed stool isolates, revealing a 15.7% prevalence of carbapenem resistance, with *Escherichia coli* and *Klebsiella* spp. predominating (Iduh *et al*., 2020). However, these findings stem from a single region and lack molecular characterization of resistance mechanisms, leaving a critical gap in understanding the drivers of CRE in diarrhoeal illnesses.

Clinically, CRE infections are associated with prolonged hospital stays, increased treatment costs, and higher mortality rates compared to carbapenem-susceptible infections (Oduyebo *et al*., 2015; Iduh *et al*., 2020). In the context of diarrhoeal disease, CRE colonization or infection may exacerbate fluid losses, complicate antimicrobial therapy, and elevate the risk of extraintestinal spread. Furthermore, diarrhoeic patients often receive empirical broad-spectrum antibiotics, which may inadvertently select for and amplify resistant strains within the gut microbiota.

Given the convergence of high diarrhoeal disease burden, widespread antibiotic use, and emerging CRE in Nigeria, a focused investigation is urgently needed. To date, no study in Calabar has comprehensively assessed the prevalence and phenotypic resistance patterns of carbapenem resistance in Enterobacteriaceae isolated from adult diarrhoeic patients. This gap hampers evidence-based antibiotic stewardship and infection control protocols tailored to gastrointestinal infections.

The aim of the present study, therefore, is to determine the prevalence of carbapenem resistance among Enterobacteriaceae isolated from diarrhoeic patients attending a tertiary hospital in Nigeria, characterize their antimicrobial susceptibility profiles, and compare their Multiple Antibiotic Resistance (MAR) indices to carbapenems and antibiotics used. By illuminating the burden CRE in the population, the study seeks to inform clinical management guidelines and public health strategies aimed at curtailing the spread of these formidable pathogens.

Materials and Methods

Study area and design

This study was conducted at the University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria. It is a 400-bed facility; the biggest in Cross River State, which serves as a tertiary medical referral center for the neighbouring States and South-South region. It is a cross-sectional study which utilized convenient sampling method.

Study participants

Study participants included adult male and female out-patients with diarrhoea attending the Out-patient clinic of the University of Calabar Teaching Hospital. Only patients who consented to the study were recruited, while those who did not consent were not included in the study.

Sample collection

Stool samples were obtained from randomly-selected 100 male and female patients who attended the Out-patient clinic of the University of Calabar Teaching Hospital. Questionnaires were administered to the study participants to obtain basic demographic characteristics.

Sample size derivation

Using the sample size formula derived by Leslie (1965) [$n = Z^2P (1-P) \div d^2$], and prevalence of *bla*$_{NDM-1}$ gene in *Klebsiella pneumoniae* from a similar study by Akani *et al.* (202), in Port Harcourt, Rivers State, Nigeria (20%)

Where;

n = sample size

z = 1.96 (standard)

P = expected prevalence (0.20)

d = desired precision of 10% (0.10)

$Z^2 = (1.96)^2 = 3.8416$

$P(1-P) = 0.20 \times (1 - 0.20) = 0.20 \times 0.80 = 0.16$

$d^2 = (0.10)^2 = 0.01$

$n = Z^2P(1-P) \div d^2$

$n = (3.8416 \times 0.16) \div 0.01$

$n = 0.614656 \div 0.01$

$n = 61.4656$

$n \approx 61$ samples

Ethical considerations and approval

This study was conducted after Ethical approval had been sought for and granted by the Ethics Research Committee of the University of Calabar Teaching Hospital, Calabar, Cross Rover State (Reference Number: UCTH/HREC/33/326). Study participants gave full consent before they were recruited into the study.

Isolation and identification of Enterobacteriaceae

A total of 59 unique Enterobacteriaceae species were isolated from 100 diarrhoeaic stool samples. Samples were enriched by culturing in selenite-F broth and incubating at 37°C for 4-6 hours; the growth was then sub-cultured onto Meropenem-supplemented Deoxycholate Citrate Agar (MS-DCA). Pure isolates were sub-cultured onto nutrient agar slants and overlaid with liquid paraffin oil to preserve them for further studies. Enterobacteriaceae were identified using colony morphology, Gram staining technique, basic biochemical tests such as oxidase, indole, urease, citrate utilization, gas production, and sugar fermentation tests, and the GNB Microbact 12A (Oxoid, Basingstoke, UK).

Antibiotic sensitivity testing of Enterobacteriaceae species

The Kirby-Bauer disk diffusion technique was used to carry out antibiotic susceptibility testing,

as recommended by the Clinical Laboratory Standards Institute (CLSI, 2024). Antibiotics tested against Enterobactriaceae species included carbapenems (Ertapenem - 30µg, Imipenem-30µg and Doripenem-30µg), a cephalosporin (Cefotaxime - 30µg), a fluoroquinolone (Ofloxacin - 5µg) and an aminoglycoside (Gentamicin -15µg). Klebsiella pneumoniae ATCC® BAA-2146™ was used as a positive control for carbapenem resistance.

Determination of Multiple Antibiotic Resistance (MAR) indices of Enterobacteriaceae species

The Multiple Antibiotic Resistance (MAR) index of Enterobacteriaceae species were calculated. It is the number of antibiotics to which a particular isolate is resistant to divided by the number of antibiotics tested against the isolate and expressed as a single decimal place number (MAR index = No. of antibiotics isolate is resistant to/No. of antibiotics tested against isolate) (Osundiya *et al*., 2013).

Data and statistical analyses

Data were represented using frequencies, tables, pie and bar charts. Data analysis was carried out using Microsoft Excel Package 2016 and Statistical package for the Social Sciences (SPSS) version 25.0: $p<0.05$ was considered statistically significant.

Results

Out of the 100 diarrhoeic patients examined, Enterobacteriaceae species were isolated from 59 (59.0%). A slightly higher proportion of females (66.0%) had positive isolates compared to males (52.0%). Across age groups, isolation rates ranged from 33.3% to 63.6%, with the highest occurrence in individuals aged 38–47 years (62.9%) (Table 1).

Table 2 shows the frequency of *Enterobacteriaceae* isolated from stool samples of participants. *Citrobacter freundii* was the most isolated *Enterobacteriaceae* with frequency of 54.2 (32/59) percent followed by *Klebsiella oxytoca* with frequency of 13.6 (8/59) percent. The least *Enterobacteriaceae* isolated were *Providencia rettgeri* and *Salmonella* paratyphi A with freqeucies of 1.7 (1/59) percent each.

Table 3 shows the antibiotic resistogram of *Enterobacteriaceae* isolated from stool of participants. *Citrobacter freundii* isolates were more resistant to ertapenem and doripenem (75 percent resistance each) than any other antibiotic tested against them; they were least resistant to gentamicin and ofloxacin (6.0 percent resistance each). *Klebsiella oxytoca* isolates were more resistant to doripenem (50.0 percent) and completely susceptible to gentamicin. All isolates of *Klebsiella pneumoniae* were completely resistant to doripenem and 80% resistant to ertapenem and impenem. However, they were completely susceptible to ofloxacin and gentamicin.

Proteus vulgaris isolates were more resistant to ertapenem (83.3 percent) than any other antibiotics tested against them; they were all susceptible to ofloxacin and gentamicin. Isolates of *Proteus mirabilis* were more resistant to doripenem (66.7 percent) than any other antibiotics tested against them; they were all susceptible to cefotaxime, ofloxacin and gentamicin. Isolates of *Escherichia coli* were more resistant to doripenem (66.7 percent) than any other antibiotics tested against them; they were all susceptible to cefotaxime, ofloxacin and gentamicin. The single isolate of *Providencia rettgeri* was resistant to all antibiotics tested except ofloxacin. The single isolate of *Salmonella* paratyphi A was resistant to all antibiotics tested except imipenem, ofloxacin and gentamicin. The highest occurrence of resistance was to doripenem and the least was to gentamicin.

Table 4 shows the mean multiple antibiotic resistance index (MARI) of *Enterobacteriaceae* isolated from the stool samples of participants. In the carbapenem (last resort antibiotics) MARI, *Providencia rettgeri* had the highest MARI of 1.0 followed by *Klebsiella pneumoniae* with MARI of 0.87. The organism with the least MARI was *Klebsiella oxytoca* with MARI of 0.33. In the total antibiotic MARI, *Providencia rettgeri* also had the highest MARI of 0.83 followed by *Klebsiella pneumoniae* and *Salmonella* paratyphi A with MARI of 0.5. Escherichia coli had the least MARI of 0.22. Statistical analysis showed that there was a significant difference (p=0.009) between MARI

of carbapenems and total MARI for the isolates identified.

The multiple Antibiotic Resistance (MAR) Index of Enterobacteriaceae species is shown on Figure 1. The mean MAR index of Enterobacteriaceae species to carbapenem antibiotics and all antibiotics tested was 0.7 and 0.4 respectively. Both mean MAR indices were above the 0.2 threshold. The difference in mean MAR indices of carbapapenems and all antibiotics was statistically significant (p=0.0248).

Table 5 shows the antibiotic resistance profile and frequencies of antibiotic-resistant phenotypes of isolated *Enterobacteriaceae.* Isolates of *Citrobacter freundii* were most resistant to four (8/32; 25 per cent) out of six antibiotics tested against them. Only one isolate of *Klebsiella oxytoca* showed resistance to four out of six antibiotics tested against them. Only two out of three *Escherichia coli* isolates showed any resistance to antibiotics and each of them were resistant to two antibiotics. With *Klebsiella pneumoniae*, two isolates each showed resistance to half and four out of six antibiotics respectively. Two *Proteus vulgaris* showed resistance to half of the antibiotics and one showed resistance to four out of six antibiotics tested against them. Only one out of three *Proteus mirabilis* isolates showed resistance to half of the antibiotics tested against them. The lone isolates of *Providencia rettgeri* and *Salmonella* paratyphi A respectively showed resistance to five and three out of six antibiotics tested against them.

Table 1: Demographic characteristics of study participants in relation to isolation of Enterobacteriaceae species

Demographical characteristics	No. Examined	Frequency of isolated Enterobacteriaceae species No (%)
Gender		
Male	50	26 (52.0)
Female	50	33 (66.0)
Total	**100**	**59 (59.0)**
Age (years)		
18 – 27	14	8 (57.0)
28 – 37	21	13 (62.0)
38 – 47	35	22 (62.9)
48 – 57	10	6 (60.0)
58 – 67	9	3 (33.3)
> 67	11	7 (63.6)
Total	**100**	**59 (59.0)**

Gender: $X^2 = 3.65$; df = 3; p=0.3100 (Not sig.) **Age:** $X^2 = 23.76$; df = 11; p=0.0733 (Not sig.)

Table 2: Frequency of *Enterobacteriaceae* isolated from the stool samples of participants

Isolate	Number of isolates	Percentage (%)
Citrobacter freundii	32	54.2
Klebsiella oxytoca	8	13.6
Klebsiella pneumoniae	5	8.5
Proteus vulgaris	6	10.2
Proteus mirabilis	3	5.1
Escherichia coli	3	5.1
Providencia rettgeri	1	1.7
Salmonella Paratyphi A	1	1.7
TOTAL	**59**	**100**

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Table 3: Antibiotic resistogram of *Enterobacteriaceae* isolated from stool of participants

Enterobacteriaceae species	**No.**	**ETP No. (%)**	**IMP No. (%)**	**DOR No. (%)**	**CTX No. (%)**	**OFX No. (%)**	**CN No. (%)**
Citrobacter freundii	32	24 (75.0)	23 (72.0)	24 (75.0)	16 (50.0)	2 (6.0)	2 (6.0)
Klebsiella oxytoca	8	3 (37.5)	1 (12.5)	4 (50.0)	2 (25.0)	2 (25.0)	-
Klebsiella pneumoniae	5	4 (80.0)	4 (80.0)	5 (100)	2 (40.0)	-	-
Proteus vulgaris	6	5 (83.3)	3 (50.0)	3 (50.0)	3 (50.0)	-	-
Proteus mirabilis	3	1 (33.3)	1 (33.3)	2 (66.7)	-	-	-
Escherichia coli	3	1 (33.3)	1 (33.3)	2 (66.7)	--	-	-
Providencia rettgeri	1	1 (100)	1 (100)	1 (100)	1 (100)	-	1 (100)
Salmonella paratyphi A	1	1 (100)	-	1 (100)	1 (100)	-	-
TOTAL	**59**	**40 (67.8)**	**34 (57.6)**	**42 (71.2)**	**25 (42.4)**	**4 (6.8)**	**3 (5.1)**

Table 4: Mean MARI of *Enterobacteriaceae* isolated from the stools of participants

Isolate	**Mean Multiple Antibiotic Resistance Index (MARI)**	
	Carbapenem	**Total Antibiotics**
Citrobacter freundii	0.74	0.48
Klebsiella oxytoca	0.33	0.33
Klebsiella pneumoniae	0.87	0.50
Proteus vulgaris	0.61	0.04
Proteus mirabilis	0.44	0.34
Escherichia coli	0.45	0.22
Providencia rettgeri	1	0.83
Salmonella Paratyphi A	0.67	0.50

T-test: 3.447; p=0.009 (Significance level of p≤0.05)

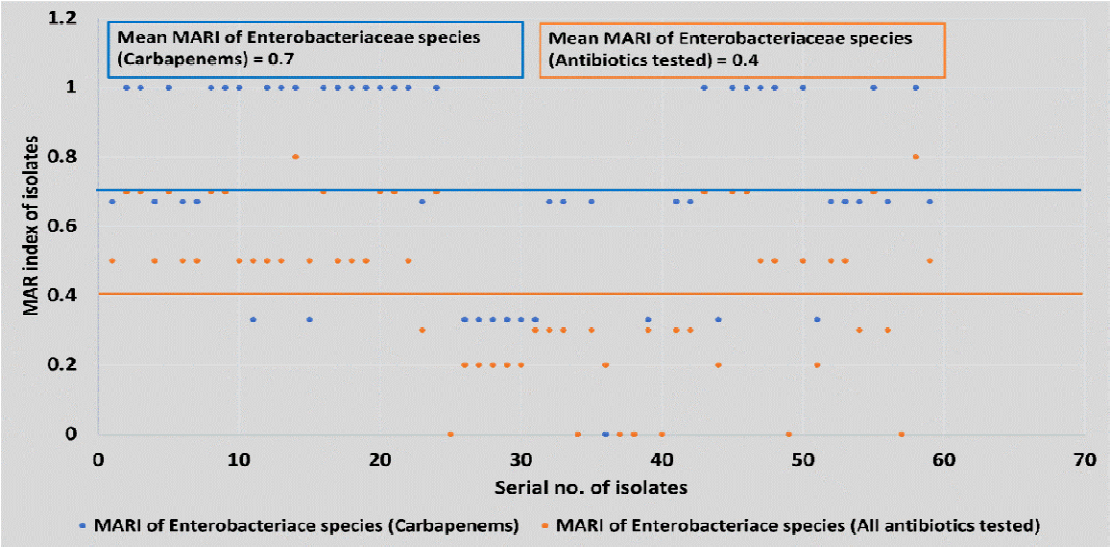

Figure 1: Multiple Antibiotics Resistance (MAR) Index of Carbapenem resistant Enterobacteriaceae phenotypes

Table 5: Antibiotic resistant profile and frequencies of antibiotic-resistant phenotypes of isolated *Enterobacteriaceae*

Isolated species	Antibiotic Resistance Profile	Frequency
***Citrobacter freundii* (n = 32)**		
Citrobacter freundii	DOR	6 (18.8)
Citrobacter freundii	DOR-CTX	1(3.1)
Citrobacter freundii	ETP-IMP	1(3.1)
Citrobacter freundii	IMP-DOR	1(3.1)
Citrobacter freundii	ETP-CTX-OFX	1(3.1)
Citrobacter freundii	ETP-IMP-OFX	1(3.1)
Citrobacter freundii	ETP-IMP-DOR	7(21.9)
Citrobacter freundii	IMP-DOR-CTX	3(9.4)
Citrobacter freundii	IMP-DOR-OFX	1(3.1)
Citrobacter freundii	ETP-IMP-DOR-CN	1(3.1)
Citrobacter freundii	ETP-IMP-DOR-CTX	8(29)
Citrobacter freundii	ETP-IMP-DOR-CTX-CN	1(3.1)
***Escherichia coli* (n = 3)**		
Escherichia coli	ETP-DOR	1(33.3)
Escherichia coli	IMP-DOR	1(33.3)
***Klebsiella oxytoca* (n = 6)**		
Klebsiella oxytoca	OFX	1(16.7)
Klebsiella oxytoca	DOR-OFX	1(16.7)
Klebsiella oxytoca	ETP-DOR	2(33.3)
Klebsiella oxytoca	ETP-IMP-DOR-CTX-OFX	1(16.7)
***Klebsiella pneumoniae* (n = 5)**		
Klebsiella pneumonia	DOR	1(20)
Klebsiella pneumonia	ETP-IMP-DOR	2(40)
Klebsiella pneumonia	ETP-IMP-DOR-CTX	2(40)
***Proteus mirabilis* (n = 3)**		
Proteus mirabilis	DOR	1(33.3)
Proteus mirabilis	ETP-IMP-DOR	1(33.3)
***Proteus vulgaris* (n = 5)**		
Proteus vulgaris	ETP-DOR	2(40)
Proteus vulgaris	IMP-DOR-CTX	2(40)
Proteus vulgaris	ETP-IMP-DOR-CTX	1(20)
***Providencia rettgeri* (n = 1)**		
Providencia rettgeri	ETP-IMP-DOR-CTX-CN	1(100)
***Salmonella* Paratyphi A (n = 1)**		
Salmonella Paratyphi A	ETA-DOR-CTX	1(100)

ETP-ertapenem; IMP-imipenem; DOR-doripenem; CTX-cefotaxime; OFX-ofloxacin; CN-gentamicin

Discussion

This study revealed a concerning incidence of carbapenem resistance among Enterobacteriaceae isolated from diarrhoeic patients in Calabar, Nigeria. *Citrobacter freundii* (54.2%) was the most frequently isolated species, followed by *Klebsiella oxytoca* (13.6%), while *Providencia rettgeri* and *Salmonella paratyphi* A accounted for 1.7% each. Although *C. freundii* is typically

not the most common diarrhoeal pathogen, it is recognized as an occasional cause of gastro-intestinal disease (Bai *et al*., 2012). This species, a common opportunistic pathogen, is frequently linked to hospital-acquired infections and outbreaks, and is adept at forming biofilms and carrying multiple resistance determinants including AmpC and ESBLs often located on transferable plasmids (Liu *et al*., 2018). The high resistance of isolates to doripenem and ertapenem, and their notable susceptibility to gentamicin and ofloxacin, reflects an emerging pattern. For instance, *C. freundii* exhibited 75% resistance to both doripenem and ertapenem, but ≥94% susceptibility to gentamicin and ofloxacin. *K. pneumoniae* was fully resistant to doripenem yet remained susceptible to gentamicin and ofloxacin.

The multiple antibiotic resistance indices (MARI) were remarkably high (0.7 for carbapenems and 0.4 overall) well above the 0.2 threshold that indicates high-risk antibiotic exposure (Adesiyan et al., 2022). *P. rettgeri* had a carbapenem MARI of 1.0 and total MARI of 0.83, while *E. coli* showed the lowest MARI of 0.22. These findings imply that many isolates likely originated from patients or environments with significant antibiotic exposure, aligning with assertions that MARI ≥0.2 indicates high-risk antibiotic contamination (Adesiyan *et al*., 2022).

These results are consistent with reports from other Nigerian and African settings. In Northwest Nigeria, nearly 90% of Gram-negative infections were multidrug-resistant, attributed to over-the-counter antibiotic sales and high selection pressure (Olowo-Okere *et al*., 2020). That study reported *E. coli*, *K. pneumoniae*, and *Pseudomonas spp*. as predominant pathogens, paralleling our recovery of opportunistic Enterobacteriaceae. Similarly, surveys across Nigeria have identified *Citrobacter, Enterobacter*, and *Proteus* spp. as emerging pathogens (Raji *et al*., 2013; Oli *et al*., 2019). In Cameroon, Bissong *et al*. (2023) observed that *Proteus* (especially *P. vulgaris*), *Providencia*, and *E. coli* were the most common carbapenem-resistant organisms. This supports our findings, where *P. vulgaris* (83.3% ertapenem-resistant), *P. mirabilis* (66.7% doripenem-resistant), and the *P. rettgeri* isolate demonstrated substantial resistance. Bissong *et al*. (2023) reported that *Proteus* and *Providencia* accounted for 63.8% of carbapenem-resistant isolates, a pattern mirrored in our data.

However, other studies present contrasting results. Akani *et al*. (2022) found *Klebsiella spp*. (mostly non-pneumoniae) and *E. coli* as dominant stool pathogens in Port Harcourt, with high sensitivity to meropenem. Similarly, Omotade *et al*. (2023) reported that diarr-hoeagenic *E. coli* from children in Ile-Ife were 100% susceptible to imipenem. This study, by contrast, found that *E. coli* showed 66.7% resistance to doripenem and 33.3% to imipenem. These differences may stem from variations in age groups, regional antibiotic practices, or sampling methods. The adult patients in this study might have had greater prior exposure to antibiotics or different resistant clones. For example, *K. pneumoniae* isolates from adults in this study were completely resistant to doripenem, unlike pediatric studies that report lower levels of carbapenem resistance.

The prevalence of carbapenem resistance here reflects broader national challenges. In Nigeria, antibiotics are frequently misused due to over-the-counter availability, lack of regulation, and empirical prescribing without susceptibility testing (Olowo-Okere *et al*., 2020). Aika and Enato (2023) note that Nigeria's AMR crisis is exacerbated by cross-sectoral antibiotic overuse and ineffective stewardship. Resistance in this study isolates to β-lactams, aminoglycosides, and fluoroquinolones aligns with their widespread use in Nigeria (Abubakar, 2020; Olowo-Okere *et al*., 2020). Although carbapenems are costly and less frequently used, resistance remains alarmingly high. Olowo-Okere *et al*. (2020) found carbapenem resistance even in hospitals where carbapenem use was limited. In this study, resistance to doripenem and ertapenem surpassed that of imipenem, suggesting either more frequent (mis)use of the former or specific resistance mechanisms targeting these drugs.

Ngbede *et al*. (2021) support this theory, reporting that 18.9% of 583 Enterobacteriaceae from humans and animals were CRE, despite lacking known carbapenemase genes. Instead, they found chromosomal mechanisms (porin

modifications and efflux pump over expression) contributing to resistance. This suggests that carbapenem resistance in Nigeria may often arise from mutational changes, independent of prior carbapenem exposure (Ngbede *et al*., 2021; Olowo-Okere *et al*., 2020). Doripenem may be due to novel carbapenemases not routinely screened for that preferentially impair doripenem's deeper structure or its ability to penetrate the bacterial cell.

The high MARI values we observed underscore the severity of antibiotic pressure in this setting. A mean carbapenem MARI of 0.7 implies isolates resisted 70% of tested carbapenems. Environmental studies use MARI greater than 0.20 to flag significant antibiotic contamination (Adesiyan et al., 2022). Nearly all the isolates had MARI greater than 0.20, indicating sustained exposure to antimicrobials either in patients or through environmental pathways. This pattern resembles that found in polluted aquatic systems in Nigeria, where 90% of isolates had high MARI scores (Adesiyan *et al*., 2022). The implications are serious. Carbapenems serve as a last line of defense against multidrug-resistant *Enterobacteriaceae*. High levels of resistance in diarrhoeal patients limit treatment options and could result in more prolonged or severe illness. Additionally, such patients may shed resistant organisms into the community, compounding the spread through poor sanitation, water systems, and human contact. This could fuel hard-to-treat diarrhoeal outbreaks. Nigeria, already burdened by high AMR rates, risks escalating mortality and morbidity. In 2019, AMR was associated with 1.27 million global deaths, with Western sub-Saharan Africa bearing the highest burden (Murray *et al*., 2022; O'Neill, 2015; Iheancho and Eze, 2022).

To combat this, Nigeria must implement robust antimicrobial stewardship. Regulatory bodies should restrict non-prescription antibiotic sales, particularly for broad-spectrum and last-line drugs. Hospitals need antimicrobial stewardship programs to promote rational antibiotic use guided by local antibiograms. Routine susceptibility testing should become standard practice to reduce reliance on empirical therapy. Education is also vital: healthcare workers must understand the risks of improper antibiotic use, and public health campaigns should discourage self-medication. Strengthening infection prevention and control including hygiene, sanitation, and CRE containment is crucial.

Additionally, antibiotic use in agriculture must be addressed. Cross-transmission between humans, animals, and the environment has been documented, and reducing antibiotic use in livestock is necessary to curb resistance spread (Emeje *et al*., 2022). This comprehensive approach is vital if Nigeria hopes to preserve the efficacy of life-saving antibiotics like carbapenems.

Conclusion

Carbapenem resistance among *Enterobacteri-aceae* in diarrhoeic patients poses a serious and escalating public health threat in Nigeria. The high prevalence of multidrug-resistant isolates, including significant resistance to last-line carbapenems such as doripenem, highlights the urgent need for coordinated national action. Key interventions must include strengthened antimicrobial stewardship, stricter regulatory enforcement, widespread public education, enhanced infection prevention practices and implementation of routine CRE surveillance in Nigerian hospitals, particularly in tertiary healthcare settings, to enable early detection, guide targeted interventions, and monitor resistance trends.

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Conflict of Interest

None to declare.

Declaration of AI generative tools

During the preparation of this work, the authors used ChatGPT Artificial Intelligence software in order to improve readability and language. After using this tool, the authors reviewed and edited the content a needed and take full responsibility for the content of the publication

References

Abubakar, U. (2020). Antibiotic use among hospitalized patients in northern Nigeria: A multicenter point-prevalence survey. *BMC Infectious Diseases, 20*(1), 86. https://doi.org/10.1186/s12879-020-4815-4

Adesiyan, I. M., Bisi-Johnson, M. A., and Okoh, A. I. (2022). Incidence of antibiotic resistance genotypes of *Vibrio* species recovered from selected freshwaters in Southwest Nigeria. *Scientific Reports, 12*, 18912. https://doi.org/10.1038/s41598-022-23479-0

Aika, I. N., and Enato, E. (2023). Antimicrobial stewardship practice among healthcare professionals: Knowledge, barriers, and facilitators in two public institutions in Nigeria. *Nigerian Journal of Clinical Pharmacy and Therapeutics, 2*(1). https://njcpt.com.n

Akani, N. P., Douglas, S. I., and Kamani, N. C. (2022). Characterization of carbapenem-resistant *Enterobacteriaceae* from patients' stool in tertiary hospitals, Port Harcourt, Rivers State, Nigeria. *Journal of Advances in Microbiology, 22*(2), 29–36. https://doi.org/10.9734/jamb/2022/v22i230435

Bai, L., Xia, S., Lan, R., Liu, L., Ye, C., Wang, Y., Jin, D., Cui, Z., Jing, H., Xiong, Y., Bai, X., Sun, H., Zhang, J., Wang, L., and Xu, J. (2012). Isolation and characterization of cytotoxic, aggregative *Citrobacter freundii*. *PLoS ONE, 7*(3), e33054. https://doi.org/10.1371/journal.pone.0033054

Bissong, M. E. A., Mobey, K. N., Muma, V., and Mkong, P. B. (2023). Reduced susceptibility to carbapenems in *Enterobacteriaceae* and antimicrobial resistance profile of *Escherichia coli* strains isolated from clinical and zoonotic sources in the Bamenda Municipality, Cameroon. *Pan African Medical Journal, 44*, 90. https://doi.org/10.11604/pamj.2023.44.90.31326

Clinical and Laboratory Standards Institute (2024). *Performance Standards for Antimicrobial Susceptibility Testing* (30th ed). Wayne, PA: Clinical and Laboratory Standards Institute. https://cdn.prod.website-files.com/66002c41caf91db80a1358cd/6712ba76cdd477bfd1aba059_dusosuvoliputunut.pdf

Emeje, M. O., Oloye, S., Bubakari, M., and Adeyeye, M. (2022). Use of antibiotics in livestock in Nigeria: Curbing antimicrobial resistance and developing a national regulatory guideline towards monitoring antibiotic use in animals and animal foods. *Review*. https://orcid.org/0000-0002-0202-5426

Etebu, E., and Arikekpar, I. (2016). Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. *International Journal of Applied Microbiology and Biotechnology Research*, *4*, 90–101. https://www.researchgate.net/publication/319881509

Iduh, M. U., Mohammed, K., Garba, M. K., Nataala, S. U., Ashcroft, O. F., and Abdulkadir, A. (2020). Prevalence of Carbapenem-Resistance among Enterobacteriaceae Isolated from Patients Attending Uduth Sokoto, North West Nigeria. *International Journal of TROPICAL DISEASE & Health*, *41*(6), 55–64. https://doi.org/10.9734/ijtdh/2020/v41i630288

Iheanacho, C. O., and Eze, U. I. (2022). Antimicrobial resistance in Nigeria: Challenges and charting the way forward. *European Journal of Hospital Pharmacy, 29*(2), 119. https://doi.org/10.1136/ejhpharm-2021-002762

Irekeola, A. A., Shueb, R. H., Engku Abd Rahman, E. N. S., Afolabi, H. A., Wada, Y., Elmi, A. H., Hakami, M. A.,

Alghzwani, S. M., Elnoubi, O. A., and Alshehri, A. A. (2024). High prevalence of carbapenem-resistant Enterobacterales (CRE) in human samples from Nigeria: A systematic review and meta-analysis. *Heliyon*, *10*(15), e34926. https://doi.org/10.1016/j.heliyon.2024.e34926

Korach-Rechtman, H., Hreish, M., Fried, C., Gerassy-Vainberg, S., Azzam, Z. S., Kashi, Y., and Berger, G. (2020). *Intestinal dysbiosis in carriers of carbapenem-resistant Enterobacteriaceae*. *mSphere, 5*(2), e00173-20. https://doi.org/10.1128/mSphere.00173-20

Murray, C. J., Ikuta, K. S., Sharara, F., and the Antimicrobial Resistance Collaborators (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet, 399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Ngbede, E. O., Adekanmbi, F., Poudel, A., Kalalah, A., Kelly, P., Yang, Y., Adamu, A. M., Salem, D. T., Adikwu, A. A., Akwuobu, C. A., Abba, P. O., Mamfe, L. M., Maurice, N. A., Adah, M. I., Lockyear, O., Butaye, P., and Wang, C. (2021). Concurrent resistance to carbapenem and colistin among *Enterobacteriaceae* recovered from human and animal sources in Nigeria is associated with multiple genetic mechanisms. *Frontiers in Microbiology, 12*, 740348. https://doi.org/10.3389/fmicb.2021.740348

Oduyebo, O. O., Falayi, O. M., Oshun, P., and Ettu, A. O. (2015). Phenotypic Determination of Carbapenemase Producing Enterobacteriaceae Isolates from Clinical Specimens at a Tertiary Hospital in Lagos, Nigeria. *Nigerian Postgraduate Medical Journal,* 22(4), 223-227. https://doi.org/10.4103/1117-1936.173973

Oli, A. N., Ogbuagu, V. I., Ejikeugwu, C. P., Iroha, I. R., Ugwu, M. C., Ofomata, C. M., Okeke, K. N., Emechebe, G. O., Okoro, J. C., Okani, C. O., and Onah, S. K. (2019). Multi-Antibiotic Resistance and Factors Affecting Carriage of Extended Spectrum β-Lactamase-Producing Enterobacteriaceae in Pediatric Population of Enugu Metropolis, Nigeria. *Medical sciences (Basel, Switzerland)*, *7*(11), 104. https://doi.org/10.3390/medsci7110104

Olowo-Okere, A., Ibrahim, Y. K. E., Nabti, L. Z., and Olayinka, B. O. (2020). High prevalence of multidrug-resistant Gram-negative bacterial infections in Northwest Nigeria. *Germs, 10*(4), 310–321. https://doi.org/10.18683/germs.2020.1223

Omotade, T. I., Babalola, T. E., Anyabolu, C. H., and Japhet, M. O. (2023). Rotavirus and bacterial diarrhoea among children in Ile-Ife, Nigeria: Burden, risk factors and seasonality. *PLoS ONE, 18*(9), e0291123. https://doi.org/10.1371/journal.pone.0291123

O'Neill, J. (2015). *Antimicrobials in agriculture and the environment: Reducing unnecessary use and waste – The review on antimicrobial resistance*. London, UK. https://edepot.wur.nl/365663

Osundiya, O. O., Oladele, R. O., and Oduyebo, O. O. (2013). Multiple antibiotic resistance (MAR) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital. *African Journal of Clinical and Experimental Microbiology*, 14:164-8. https://doi.org/10.4314/ajcem.v14i3.8

Raji, M. A., Jamal, W., Ojemhen, O., and Rotimi, V. O. (2013). Point-surveillance of antibiotic resistance in *Enterobacteriaceae* isolates from patients in a Lagos teaching hospital, Nigeria. *Journal of Infection and Public Health, 6*, 431–437. https://doi.org/10.1016/j.jiph.2013.05.002

Ssekatawa, K., Byarugaba, D. K., Wampande, E., and Ejobi, F. A. (2018). Systematic review: The current status of carbapenem

resistance in East Africa. *BMC Research Notes*, *11*, 629. https://doi.org/10.1186/s13104-018-3738-2

Tula, M. Y., Enabulele, O. I., Ophori, E. A., Aziegbemhin, A. S., Iyoha, O., and Filgona, J. (2023). A systematic review of the current status of carbapenem resistance in Nigeria: Its public health implication for national intervention. *Nigerian Postgraduate Medical Journal*, *30*(1), 1–11. https://doi.org/10.4103/npmj.npmj_240_22