Vol. 12, No. 2 (2026), pp.46–56

CHITKARA UNIVERSITY

Journal of Multidisciplinary Research in Healthcare

Molecular Detection of Carbapenemase Genes in Gut Enterobacteriaceae among Patients with Selected Mental Disorders

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RESEARCH ARTICLE **Open Access**

ARTICLE INFORMATION

Received: March 10, 2026
Accepted: July 01, 2026
Published Online: July 18, 2026

Keywords:
Gut-bacteria, Antibiotic resistance, Carbapenemase genes, Mental health disorders, Multidrug-resistant

DOI: 10.15415/jmrh.2026.122004

ABSTRACT

Background: Antimicrobial resistance among Enterobacterales remains a growing public health concern, particularly in vulnerable clinical populations.

Purpose: Psychiatric inpatients may be at increased risk of colonization with multidrug-resistant organisms due to prolonged hospitalization and healthcare exposure, yet data on their bacterial profiles and resistance patterns remain limited in Nigeria.

Methods: One hundred and two (102) fecal samples and biodata were collected from inpatients diagnosed with four selected mental disorders. Samples were cultured on EMB agar, and isolates were identified following standard microbiological procedures. Antibiotic sensitivity test was carried out by the Kirby-Bauer disc diffusion method. Multidrug-resistant (MDR) isolates were screened for carbapenemase production, which was distinguished by two phenotypic tests. Five selected phenotypes were further amplified for the presence of blaOXA-48, blaNDM, blaKPC, and blaIMI using the polymerase chain reaction technique.

Results: A higher percentage of the study participants (71.5%) were males, while 28.4% were females. A total of 102 Enterobacteriaceae were isolated and identified as *Escherichia coli* (43.1%), *Klebsiella* spp. (28.4%), *Enterobacter* spp. (10.7%), *Proteus* spp. (8.8%), *Salmonella* spp. (7.8%), and *Shigella* spp. (0.9%). Varying resistant patterns were observed among the isolates, with β-lactams having the highest frequency (51.0%), while the least resistance was recorded in chloramphenicol (24.4%). A total of 56 bacterial isolates (59.5%) were MDR, out of which 45 (80.3%) were carbapenem-resistant. Out of these, 41 (73.2%) were confirmed carbapenemase producers. Carbapenemase phenotypes recorded 48.2% and 7.1% as MBL and OXA-48 producers, respectively.

Conclusion: Carbapenemase genes were detected in all five selected multidrug-resistant Enterobacterales.

1. Introduction

Antimicrobial resistance (AMR) is a major global public health challenge that threatens the effective treatment of infectious diseases and contributes significantly to morbidity and mortality worldwide (Garcia-Parejo *et al.,* 2024). Among multidrug-resistant organisms, carbapenem-resistant Enterobacteriaceae (CRE) are considered critical priority pathogens because they produce carbapenemase enzymes that hydrolyze carbapenems, which are often regarded as last-resort antibiotics for severe bacterial infections (Jernigan *et al.,* 2020; Hansen, 2021). The most common carbapenemase genes, including *blaKPC, blaNDM, blaOXA-48, blaVIM*, and blaIMP, are increasingly reported among Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae*, contributing to the rapid dissemination of antimicrobial resistance worldwide (Mancuso *et al.,* 2023).

The gastrointestinal tract serves as an important reservoir for antimicrobial-resistant Enterobacteriaceae, facilitating the persistence and spread of resistance genes within both healthcare and community settings (Kang

et al., 2022; Abera *et al.,* 2023). Colonization of the gut by carbapenemase-producing Enterobacteriaceae often precedes clinical infection and has been associated with an increased risk of subsequent multidrug-resistant infections, prolonged hospitalization, and poor clinical outcomes (Heath *et al.,* 2024). Several factors, including prolonged hospitalization, previous antibiotic exposure, and underlying medical conditions, have been identified as major risk factors for CRE colonization (Quirino *et al.,* 2023). These factors are particularly relevant among patients with mental disorders, many of whom experience long-term hospital stays, recurrent healthcare exposure, and frequent antimicrobial use. Despite these potential risks, information on the molecular epidemiology of carbapenemase-producing Enterobacteriaceae among psychiatric patients remains limited, especially in Nigeria (Chen *et al.,* 2021). Thus, this study is to better understand the burden of CRE colonization, identifying circulating resistance determinants.

2. Materials and Methods

2.1. Study Design and Location

A cross-sectional study was carried out between November 2024 and February 2025 among inpatients of Neuropsychiatric Hospital Aro and Lantoro Annex, Abeokuta, Ogun State, Nigeria. The hospital is a premier federal psychiatric institution in Nigeria established in 1944 with a 735-bed capacity.

2.2. Study Population

Registered patients admitted during the time of study constituted the study population. A consecutive sampling technique was employed to include patients who met the inclusion criteria. This includes patients diagnosed with schizophrenia, mental behavioral disorders, bipolar disorder, and autism spectrum disorder, which otherwise constituted the exclusion criteria.

2.3. Sample Size

The statistics of the total registered patients of selected mental disorders for the year 2023 were received via the health information management department of Neuropsychiatric Hospital, Aro, to aid in sample calculation. The sample size was deduced to be a total of 102 psychiatry patients using the Olonite formula:

$$n = \frac{N \times Z^2 \times P \times (1-P)}{E^2 \times (N-1) + Z^2 \times P \times (1-P)} \text{(Olonite, 2021)}$$

n = Sample size required
N = Total population Size
Z = Z value corresponding to confidence level at 95% is 1.96

2.4. Ethical and Consent Approval

Ethical approval was obtained from the Ethics Review Committee of the Federal Neuropsychiatric Hospital, Aro, Abeokuta, Ogun State, Nigeria (Approval No. PRO013/24). Given that psychiatric inpatients are vulnerable participants, their capacity to consent was assessed by attending clinicians prior to recruitment. Only those considered mentally stable and capable of understanding the study were included. For individuals unable to provide informed consent, consent was obtained from legally authorized representatives or guardians in line with institutional ethical guidelines. Written informed consent was obtained from all participants after a clear explanation of the study. Participation was voluntary, confidentiality was ensured, and data were used solely for research purposes.

2.5. Data Collection

2.5.1. Sociodemographic Data

Specific biodata was collected through a retrospective review of patients' health records. Information was extracted from psychiatric case files using a structured data extraction form developed for the study. Relevant socio-demographic and clinical variables were recorded as documented by attending clinicians during routine clinical assessments. Psychosocial variables such as severe stress, social isolation, and childhood trauma were identified based on recorded clinical histories in the patients' files. Severe stress referred to documented evidence of significant psychological distress as noted in the case records. Social isolation referred to recorded descriptions of limited social interaction or lack of social support. Childhood trauma referred to a documented history of adverse childhood experiences, including abuse, neglect, or exposure to violence as recorded in the clinical notes.

2.5.2. Laboratory Data

A total of 102 fresh feces samples were collected from study participants. They had no history of gastrointestinal complaints and were not on any antibiotic therapy for more than two months as confirmed by their attending clinicians. Fecal samples were collected in a well-labeled sterile plastic universal container and transported to the laboratory for further analyses.

2.6. Laboratory Procedures

2.6.1. Bacterial Isolation and Identification

Specimens were processed in accordance with the guidelines of the Clinical and Laboratory Standard Institute (CLSI, 2023) with some modifications. One milliliter of stool suspension was transferred into 4

mL of sterile normal saline to obtain a 10^{-1} dilution. Subsequent five-fold serial dilutions were prepared up to 10^{-5}. Afterwards, an aliquot of 1 mL was inoculated on the ethylene methylene blue agar (Oxoid, Ltd., UK) and aerobically incubated at 35-37°C for 24-48 hours. Pure cultures of the isolates were obtained by a series of sub-culturing on nutrient agar and identified and confirmed by Gram stain and a series of biochemical tests. This includes the following tests: sulphur production test, indole test, motility test, urea hydrolysis test, citrate utilization test, oxidase test, gas production, hydrogen sulfide test, and triple sugar iron test.

2.6.2. Antibiotic Susceptibility Testing

The antibiotic sensitivity testing of each isolate was conducted according to the Kirby-Bauer disc diffusion method using commercially available Gram-negative antimicrobial discs (Abtek Biologicals Ltd., UK) containing ten different antibiotics with varying standard disc concentrations as specified by the manufacturer and recommended by CLSI guidelines. This included Ampiclox (Apx) 30 µg/disc, Ceftriaxone (Cef) 30 µg/disc, Penicillin (Pen) 10 µg/disc, Levofloxacin (Lev) 5 µg/disc, Ciprofloxacin (Cpx) 30 µg/disc, Ofloxacin (Ofl) 5 µg/disc, Gentamycin (Gen) 10 µg/disc, Streptomycin (Str) 10 µg/disc, Chloramphenicol 30 µg/disc, and Tetracycline (Tet) 30 µg/disc. Based on their mode of action, these antibiotics were categorized into five classes: beta-lactams, aminoglycosides, fluoroquinolones, chloramphenicols, and penicillins. A loopful of the suspension was inoculated onto Mueller-Hinton agar and allowed to set for 10 minutes before the antimicrobial sensitivity discs were placed on the surface. *Escherichia coli* strain ATCC 25922 was used as quality control to ensure the accuracy and reliability of the test procedures. Plates were incubated aerobically at 35-37°C for 24 hours. The diameter of the zones of inhibition was measured and interpreted as susceptible, intermediate, and resistant using clinical breakpoints provided by EUCAST as interpretive criteria for the respective Gram-negative bacteria. Multidrug resistant (MDR) isolates were classified based on their non-susceptibility to at least one agent in two or more antimicrobial categories. These isolates were further subjected to carbapenems (ertapenem and doripenem) and thereafter confirmed for carbapenemase production by the modified Hodges test (MHT).

*2.6.3. Phenotypic Detection of Metallo-*B*-Lactamase (MBL) Producers*

Meropenem disks were placed on a Petri dish and supplemented with a 10 µL solution of EDTA, given a drying time of 20 minutes. Non-supplemented meropenem disks, which served as control, were used in synergy with supplemented meropenem disks with EDTA on the MHA-based bacterial suspension 3 cm apart. The plates were incubated at 35°C for 18 to 24 hrs. Isolates that showed an inhibition zone difference of ≥ 5 mm only for disks supplemented with EDTA when compared with meropenem alone were considered likely producers of MBL.

2.6.4. Phenotypic Detection of OXA-48

The method of Birgy *et al.* (2012) was employed with some modifications. Cloxacillin (250 mg/ml) was homogenized and thoroughly mixed with already prepared commercial MHA, which was thereafter streaked with the bacterial suspension and impregnated with a meropenem disc. Plates inoculated with similar isolates and impregnated with a meropenem disc alone served as a control. All plates were incubated at 35°C for 18 to 24 hrs. Isolates with zone difference < 5mm for CLOXA in comparison with meropenem were considered possible producers of another β-lactamase producer (OXA-48).

2.6.5. Molecular Detection of Carbapenemase Genes

2.6.5.1. DNA Extraction

Bacteria DNA extraction was carried out using the Zymo Research (ZR) MiniPrep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's manual instructions.

2.6.5.2. Gene Amplification of Extracted DNA (PCR)

Gene amplification of the bacterial DNA was carried out using PCR. The PCR master mix consisted of 12.5 µL of Taq 2X Master Mix (New England Biolabs, M0270), 1 µL each of 10 µM forward and reverse primer (bla*NDM*, bla*KPC*, bla*IMI*, and bla*OXA*), and 2 µL of DNA template. The total reaction volume was adjusted to 25 µL with 8.5 µL of nuclease-free water.

2.6.5.3. PCR Cycling Conditions

Initial denaturation at 94°C for 5 mins, followed by 36 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec, and elongation at 72°C for 45 sec. Followed by a final elongation step at 72°C for 7 minutes, the reaction was held at 10°C indefinitely. After complete amplification, the PCR products were analyzed for gel electrophoresis by using 1% agarose gel (1 g of agarose in 100 ml of Tris buffer) with ethidium bromide as the staining agent. Fragments of the amplified DNA were then visualized under a UV transilluminator. A previously confirmed carbapenemase-producing isolate was included as the positive control, while nuclease-free water was used as the negative control in

each PCR run to monitor contamination and ensure assay validity. PCR products were separated by electrophoresis on 1.5% agarose gel stained with ethidium bromide and visualized under ultraviolet illumination.

Table 1: The Primer Sequences Used for Amplification

Target Gene	Primer Sequence (5′–3′)	Amplicon Size (bp)	Reference
blaNDM	F: GGTTTGGCGATCTGGTTTTC	621	Poirel *et al.* (2011)
	R: CGGAATGGCTCATCACGATC		
blaKPC	F: CGTCTAGTTCTGCTTAGGCG	798	Pourgholi *et al.*(2022)
	R: CTTGTCATCCTTGTTAAACG		
blaIMI	F: CTACGCTTTAGACACTGGC	818	Aubron *et al.* (2005)
	R: AGGTTTCCTTTTCACGCTCA		
blaOXA-48	F: GCGTGGTTAAGGATGAACAC	438	Poirel *et al.* (2011)

2.7. Statistical Analysis

Biodata was entered on an Excel spreadsheet and exported to the Statistical Package for the Social Sciences (SPSS) version 21 for descriptive statistics. Frequencies and percentages were calculated at the isolate level and are presented as descriptive data only, as no control group or comparative statistical analysis was used.

3. Results

Out of a total of 102 participants, the majority of them were males (67.6%) compared to females (32.3%), with the highest reported age group being 41-50 years (27.4%). About 62.7% of participants were diagnosed with schizophrenia, with the highest occurrence recorded in males (40.1%). Mental and behavioral disorders accounted for 22.5% of cases, followed by bipolar disorder (8.8%) and autism spectrum disorders (5.8%), which were commonly reported among males as well. None of the participants were on antibiotic medications (0.0%) as of the time of assessment. Family history emerged as the most prominent risk factor by 64.7%, followed by substance abuse at 14.7%, while other factors, including birth defects, social isolation, childhood trauma, loss of loved ones, and severe stress, each contribute less than 10% to the total frequency (Table 2).

In all 102 samples, a total of six Enterobacterales genera, including five genera (*Klebsiella* spp., *Enterobacter* spp., *Salmonella* spp., *Proteus* spp., and *Shigella* spp.) and one species (*Escherichia coli*), were identified. *Escherichia coli* was the most frequently isolated bacteria in all mental disorder cases, accounting for 43.1% of the total isolates. *Klebsiella spp.* follows with 28.4%, while *Enterobacter spp.* and *Proteus spp.* represent 10.7% and 8.8%, respectively. The least occurrences were observed in *Salmonella* (7.8%) and *Shigella* (0.9%) (Figure 1). All results are presented as descriptive isolate-level frequencies, with no control group or comparative statistical analysis performed.

Figure 2 shows the varying levels of antibiotic susceptibility patterns of the bacterial isolates against the tested antibiotics. High susceptibility rates were observed for streptomycin, ofloxacin, ciprofloxacin, penicillin, and levofloxacin, while high intermediate responses were recorded for cefuroxime and ampiclox. The isolates exhibited notable resistance to tetracycline, gentamycin, chloramphenicol, and ofloxacin. Out of 102 bacterial species isolated, 54.9% were multi-drug resistant (MDR). *Escherichia coli*, which is the most frequently isolated *Enterobacteriaceae* species (43.1%), showed the highest MDR occurrence (20.5%). It exhibited substantial resistance to beta-lactams (17.8%), fluoroquinolones (5.8%), aminoglycosides (11.7%), chloramphenicol (8.8%), and tetracyclines (9.8%). *Klebsiella* spp. accounted for 28.4% of the total isolates, with 15.6% being MDR while showing high resistance to beta-lactams (15.6%), fluoroquinolones (8.8%), aminoglycosides (9.8%), chloramphenicol (4.9%), and tetracyclines (10.7%). *Enterobacter* spp., making up 10.7% of the isolates, exhibited a 10.7% MDR rate, with 7.8% resistant to beta-lactams, 2.9% to fluoroquinolones, 7.8% to aminoglycosides, 3.9% to chloramphenicol, and 5.8% to tetracyclines. *Proteus* spp., comprising 8.8% of isolates, also displayed a 7.88% MDR rate, with a resistance rate of 5.8% to beta-lactams, fluoroquinolones, and aminoglycosides (Table 3).

In all 56 multidrug-resistant (MDR) *Enterobacteriaceae* isolates, 45 (80.3%) were resistant to carbapenems, with *Escherichia coli* accounting for the highest occurrence at 32.1%, followed by *Klebsiella* spp. (26.7%). *Proteus* and *Enterobacter spp.* showed the same level of resistance (10.7%). Metallo-beta-lactamase production was significantly recorded in 27 (48.2%) isolates, comprising *Escherichia coli* (26.7%) and *Proteus* spp. (10.7%). OXA-48 carbapenemase was detected in only 4 (7.1%) isolates, including *Escherichia*

coli (3.5%), *Klebsiella* spp. (1.7%), and *Enterobacter sp.* (1.7%). *Shigella* and *Salmonella* spp. showed no resistance to carbapenems or production of carbapenemases (Table 4).

Polymerase chain reaction analysis of the five selected bacterial species identified the presence of carbapenemase genes as shown in Figure 3. Gel electrophoresis results revealed that the *BlaNDM* gene, indicated by an approximately 800 bp band, was present in one out of two *Klebsiella* spp., two *Escherichia coli, and Enterobacter* spp. *The BlaKPC* gene was also identified at an approximately 500 bp band in the two *Escherichia coli* species and two *Enterobacter* species but was absent in the two *Klebsiella* spp. Additionally, *the BlaIMI* and *BlaOXA-48* genes were, however, detected at approximately 481 and 550 bp, respectively, in the five selected bacterial species.

Table 2: Distribution of Mental Disorders by Demographics and Clinical Characteristics

	Male	Female	Total (%)
Age group (years)			
0-10	6 (5.8)	0 (0.0)	6 (5.8)
11-20	1 (0.9)	0 (0.0)	1 (0.9)
21-30	10 (9.8)	4 (3.9)	14 (13.7)
31-40	9 (8.8)	7 (6.8)	16 (15.6)
41-50	18 (17.6)	10 (9.8)	28 (27.4)
51-60	13 (12.7)	7 (6.8)	20 (19.6)
61-70	8 (7.8)	4 (3.9)	12 (11.7)
71-80	2 (1.9)	1 (0.9)	3 (2.9)
81-90	2 (1.9)	0 (0.0)	1 (0.9)
Mental disorder			
Schizophrenia	41 (40.1)	23 (22.5)	64 (62.7)
Mental Behavioral disorders	19 (18.6)	4 (3.9)	23 (22.5)
Bipolar disorder	7 (6.8)	2 (1.9)	9 (8.8)
Autism spectrum	6 (5.8)	0 (0.0)	6 (5.8)
Medication with antibiotics			
Yes	0 (0.0)	0 (0.0)	0 (0.0)
No	73 (71.5)	29 (28.4)	102
Risk factors			
Birth defect	6 (5.8)	0 (0.0)	6 (5.8)
Substance abuse	14 (13.7)	0 (0.0)	14 (13.7)
Family history	38 (37.25)	28 (27.4)	66 (64.7)
Severe stress	4 (3.9)	2 (1.9)	6 (5.8)
Loss of loved ones	4 (3.9)	0 (0.0)	4 (3.9)
Social isolation	3 (2.9)	1 (0.9)	4 (3.9)

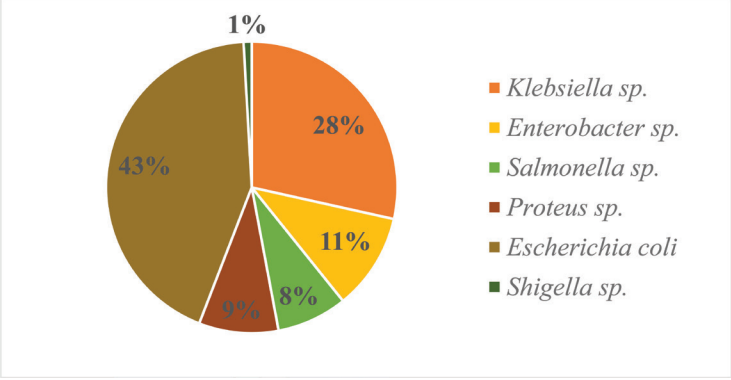

Figure 1: Prevalence of Gut Bacteria Isolated from Stool Samples of Neuropsychiatric Patients

Table 3: Antibiotics Resistance Patterns of *Enterobacteriaceae*

Bacterial isolates	Total screened (%)	Total MDR +ve (%)	Total resistat to Betalactams (%)	Total resistant to fluoroquinolones (%)	Total resistant to Aminoglycosides (%)	Total resistant to Chloramphenicol (%)	Total resistant to Tetracyclines (%)
Klebsiella sp.	29 (28.4)	16 (15.6)	16 (15.6)	9 (8.8)	10 (9.8)	5 (4.9)	11 (10.7)
Enterobacter sp.	11 (10.7)	11 (10.7)	8 (7.8)	3 (2.9)	8 (7.8)	4 (3.9)	6 (5.8)
Proteus sp.	9 (8.8)	8 (7.8)	6 (5.8)	6 (5.8)	6 (5.8)	5 (4.9)	7 (6.8)
Escherichia coli	44 (43.1)	21 (20.5)	18 (17.6)	6 (5.8)	12 (11.7)	9 (8.8)	10 (9.8)

Salmonella sp.	8 (7.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Shigella sp.	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	102	56 (54.9)	48 (47.1)	24 (23.5)	36 (35.2)	23 (22.5)	34 (33.3)

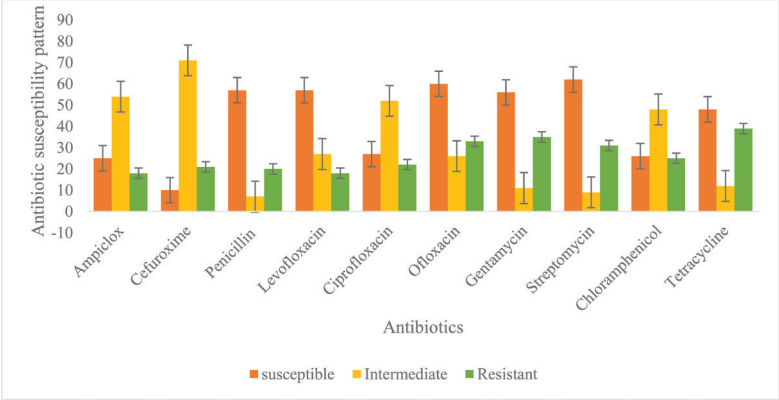

Figure 2: Frequency of Multidrug Resistance in Gram Negative Gut Bacteria from Selected Mental Disorders

Table 4: Percentage Frequency of Detection of CRE in Three Phenotypic Methods

Bacteria isolates	Total MDR+ ve screened (%)	Carbapenem resistant +ve (%)	MHT +ve (%)	MBL +ve (%)	OXA-48 +ve (%)
Klebsiella spp.	16 (28.5)	15 (26.7)	13 (23.2)	4 (7.1)	1 (1.7)
Enterobacter spp.	11 (19.6)	6 (10.7)	5 (8.9)	2 (3.5)	2 (3.5)
Proteus spp.	8 (14.2)	6 (10.7)	5 (8.9)	6 (10.7)	0 (0.0)
Escherichia coli	21 (37.5)	18 (32.1)	18 (32.1)	15 (26.7)	2 (3.5)
Total	56	45 (80.3)	41 (73.2)	27 (48.2)	4 (7.1)

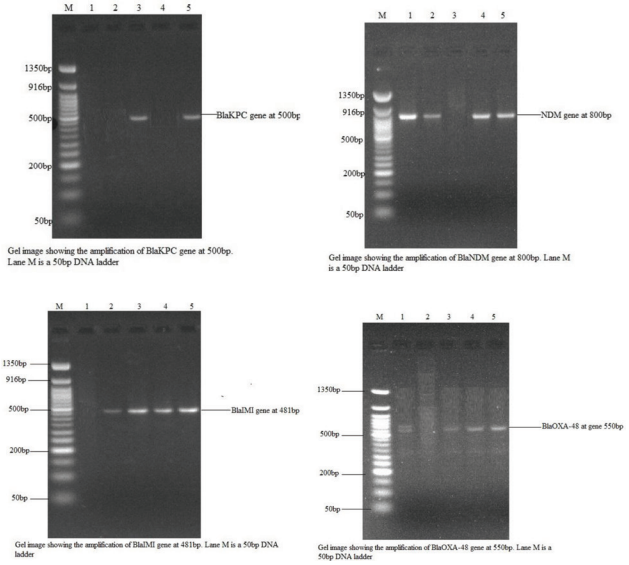

Figure 3: Agarose Gel Electrophoresis showing PCR Amplification of the blaKPC (500 bp), blaNDM (800 bp), blaIMI (481 bp), and blaOXA-48 (550 bp) Carbapenemase Genes in Representative Bacterial Isolates. Lane M, 50 bp DNA Ladder; Lanes 1–5: 1 = *Klebsiella* sp., 2 = *Escherichia coli*, 3 = *Klebsiella* sp., 4 = *Enterobacter* sp., and 5 = *Escherichia coli*.

4. Discussion

The human gastrointestinal tract serves as a significant reservoir for diverse microbial communities that play essential roles in maintaining physiological homeostasis and modulating systemic health (Hou *et al.,* 2023; Tilg *et al.,* 2020). Even though the gut-brain axis is known to play an important role in health and disease, studies on specific vulnerable patient populations are still needed to identify and understand the bacterial patterns (Cryan *et al.,* 2016; Osadchiy *et al.,* 2016). In this study, bacteriological analysis revealed a high prevalence of Gram-negative organisms belonging to the family Enterobacteriaceae, predominantly *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Salmonella* spp., and *Shigella* spp. This distribution aligns with findings by Akinjogunla *et al.* (2023), confirming these genera as common bacterial entities identified within clinical samples. While many Enterobacteriaceae are conventionally regarded as intestinal commensals, they are increasingly recognised globally as opportunistic pathogens capable of mediating severe systemic inflammation and secondary infections under favourable clinical conditions (Rizzotti *et al.,* 2024).

The notable prevalence of *Escherichia coli* and *Klebsiella* species among the recovered isolates is consistent with general epidemiological patterns reported by Zheng *et al.* (2021). In a healthy intestinal environment, the population of *E. coli* is typically maintained in functional equilibrium alongside other resident microflora. However, variations in the proportions of Enterobacteriaceae observed across different patient groups within this study indicate fluctuating colonisation patterns, which have been frequently noted in clinical demographics involving chronic illness populations (McGuinness *et al.,* 2022). A significant portion of the study population was diagnosed with schizophrenia, mirroring the high admission and treatment rates for this condition at the facility during the sampling period. Previous literature has noted statistical correlations between altered Enterobacteriaceae profiles and specific clinical diagnoses (Hsiao *et al.,* 2013; Zheng *et al.,* 2016). While external factors such as dietary habits, sugar consumption, and fibre intake are known to influence the general composition of intestinal microbiota and the production of immunomodulatory metabolites like short-chain fatty acids (Fu *et al.,* 2022; Morrison *et al.,* 2016), further longitudinal investigations are required to establish specific dietary correlation matrices for this cohort.

Characterising the antibiotic susceptibility profiles of these clinical Enterobacteriaceae isolates is important for guiding therapeutic regimens and tracking the dissemination of resistance traits. The *Klebsiella* species isolated in this study exhibited high susceptibility to fluoroquinolones, which corroborates reports by Geetha *et al.* (2020) and Zhan *et al.* (2021) highlighting the continued clinical efficacy of this drug class. Conversely, instances of resistance to ampicillin and penicillin were observed across multiple isolates. This overall pattern of fluoroquinolone sensitivity alongside beta-lactam resistance matches observations by Kawa *et al.* (2023) and Marchisio *et al.* (2015) and is traditionally associated with selective pressures driven by widespread beta-lactam utilisation or structural alterations in bacterial penicillin-binding proteins. Notably, multidrug resistance (MDR) was observed in several isolates despite no documented history of recent antibiotic use among those specific individuals. This resistance phenotype suggests potential acquisition via horizontal gene transfer mechanisms within communal environments or indirect transmission pathways (Urban-Chmiel *et al.,* 2022; Bennett, 2008). Furthermore, literature suggests that certain non-antibiotic therapeutic agents, including specific antipsychotic medications like olanzapine, carbamazepine, and fluphenazine, may exhibit secondary, intrinsic antibacterial activity that can exert selective pressure on the gut flora over extended periods (Nehme *et al.,* 2018; Laxmi *et al.,* 2025).

The detection of carbapenem resistance among these gut-derived isolates presents serious therapeutic implications, as carbapenems represent vital, last-resort interventions for severe Gram-negative infections (Codjoe *et al.,* 2017; Andrew *et al.,* 2019). Our phenotypic analysis revealed a notable pattern of ertapenem resistance. This specific pattern may be linked to the structural characteristics of ertapenem, which can limit its outer membrane permeability when facing active bacterial efflux systems or specific porin mutations (Lim *et al.,* 2013). In comparison, the isolates demonstrated moderate susceptibility to doripenem, supporting assertions by Fritsche *et al*. (2005) and Mao *et al*. (2013) that doripenem maintains superior stability against certain Gram-negative outer membrane resistance adaptations. The phenotypic resistance profiles were validated by a high percentage of positive Modified Hodge Test (MHT) results, confirming the production of carbapenem-hydrolyzing enzymes (carbapenemases) among the isolated *E. coli*, *Klebsiella* sp., *Proteus* sp., and *Enterobacter* sp. This enzymatic activity serves as a primary driver for the observed carbapenem resistance phenotypes, consistent with findings by Ravensbergen *et al.* (2018).

Carbapenemase production was prominently identified among isolates recovered from individuals within the schizophrenia group. While studies from Gashaw *et al.* (2021) and Tsamakis *et al.* (2022) have also reported carbapenemase-producing Enterobacteriaceae within similar clinical cohorts, the underlying selective drivers remain complex. Although secondary in vitro antibacterial properties reported for certain medications

could theoretically influence gene dissemination (Nehme *et al.,* 2018), a definitive causal relationship cannot be established here without comprehensive data on patient medication types, dosages, and compliance durations. Similarly, while a few carbapenemase-producing isolates were identified within the broader Mental and Behavioral Disorders (MBD) group, and relative susceptibility was maintained in isolates from bipolar and autistic spectrum disorder groups, these variations likely reflect differing environmental exposures and localized selective forces within the host environments.

Genotypic tracking highlighted *Escherichia coli* and *Klebsiella* sp. as the principal carriers of key resistance determinants, aligning with their global status as major vectors for *bla*_NDM, *bla*_KPC, and *bla*_OXA-48 genes (Zheng *et al.,* 2023; Zhu *et al.,* 2022). *Klebsiella* species emerged as prominent hosts for both *bla*_KPC (Andersson *et al.,* 2017) and *bla*_OXA-48 (Li *et al.,* 2021; Tang *et al.,* 2024). The co-occurrence of multiple carbapenemase genes within individual *E. coli* and *Enterobacter* isolates represents a critical clinical challenge, as complex resistance genotypes heavily restrict future therapeutic options (Lin and Chen, 2021). Interestingly, the *bla*_IMI gene was detected in the selected *E. coli* and *Klebsiella* isolates. Because *bla*_IMI is an Ambler Class A carbapenemase conventionally restricted to the genus *Enterobacter*, its prominent distribution among these species represents a significant departure from typical larger-scale epidemiological distribution patterns (Fang *et al.,* 2024; Antin *et al.,* 2024). Ultimately, the identification of opportunistic, carbapenemase-producing Enterobacteriaceae within the human gut underlines a strict therapeutic limitation, emphasizing the need for robust surveillance strategies to monitor the spread of critical resistance genes within vulnerable populations.

5. Limitations of the Study

Limitations of this study include the absence of a healthy control group, which precluded direct comparison between individuals with and without mental disorders. Species identification was based on phenotypic characterization because molecular identification methods were not performed, which may have affected identification accuracy. The antimicrobial susceptibility testing panel was limited to antibiotics available during the study period, excluding some clinically important agents. Additionally, carbapenemase detection was performed using the Modified Hodge Test (MHT), a method with lower specificity than newer phenotypic and molecular techniques, raising the possibility of false-positive or false-negative results.

6. Conclusion

This study demonstrated the presence of diverse Enterobacterales species among psychiatric inpatients, with *Escherichia coli* and *Klebsiella* spp. being the most prevalent isolated. A high proportion of the isolates exhibited multidrug resistance, including resistance to carbapenems, with evidence of metallo-β-lactamase production and the presence of carbapenemase genes such as blaNDM, blaKPC, blaIMI, and blaOXA-48. These findings highlight the potential role of psychiatric patients as reservoirs of antimicrobial-resistant Enterobacterales and underscore the need for continuous antimicrobial resistance surveillance and infection control measures in psychiatric healthcare settings.

Acknowledgement

The authors sincerely appreciate the Department of Microbiology, Federal University of Agriculture, Abeokuta and Staffs of Medical Microbiology unit of Federal Medical Centre Abeokuta for providing laboratory facilities. Authors also thank all colleagues who provided technical assistance during this study.

Authorship Contribution

Abidemi Esther Ojo and Regina Oghenevwede Golagha conceived and designed the study. Regina Oghenevwede Golagha performed the computational analyses, interpreted the data, and prepared the original manuscript draft. Adejare Rasaq Oloyede contributed to data analysis and interpretation. Jacob Kehinde Akintunde provided technical guidance, supervised aspects of the research, and critically reviewed the manuscript. Johnson Daniel Jemikalajah contributed to the interpretation of results and manuscript revision. Aanuoluwapo Alake assisted with the literature review, data organization, and manuscript editing. Abidemi Esther Ojo, Regina Oghenevwede Golagha, Adejare Rasaq Oloyede, Jacob Kehinde Akintunde, Johnson Daniel Jemikalajah, and Aanuoluwapo Alake reviewed, revised, and approved the final version of the manuscript.

Ethical Approval

Ethical approval was obtained from the Ethics Review Committee of Federal Neuropsychiatric hospital Aro Aeokuta, Ogun State Nigeria with approval no. PRO013/24.

Funding

The authors declare that no specific funding was received for this research work from any funding agency in the public, commercial, or not-for-profit sectors.

Declarations

The authors declare that the manuscript is original, has not been published previously, and is not under consideration for publication elsewhere. All sources of information used in this study have been appropriately acknowledged and cited.

Data Availability Statement

Authors declare that the data supporting the conclusions of this study can be obtained upon request from the corresponding author, (R.O. Golagha). The data is not publicly accessible due to information that may compromise the privacy of research participants.

Human and Animal Rights

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and relevant institutional guidelines for research involving human participants. Ethical approval was obtained from the Ethics Review Committee of the Federal Neuropsychiatric Hospital, Aro, Abeokuta, Ogun State, Nigeria (Approval No. PRO013/24). Because psychiatric inpatients are considered a vulnerable population, each participant's capacity to provide informed consent was assessed by the attending clinicians before recruitment. Only individuals deemed mentally stable and capable of understanding the study were enrolled. For participants who lacked the capacity to provide informed consent, written consent was obtained from their legally authorized representatives or guardians in accordance with institutional ethical requirements. Written informed consent was obtained from all participants or their legally authorized representatives prior to participation. Participation was entirely voluntary, participant confidentiality was maintained throughout the study, and all data were used solely for research purposes. No animals were involved in this study.

Figure Permission

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

AI Disclosure Statement

The authors declare that no artificial intelligence (AI) tools were used in the writing, analysis, or preparation of this manuscript.

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