

High-Risk Extended-Spectrum Beta-Lactamase (*bla*TEM) and Metallo-Beta-Lactamase (*bla*NDM) Genes in *Escherichia coli* from Clinical Wastewater from a Nigerian Teaching Hospital: A One Health Perspective

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Abstract

Background: Hospital wastewater is increasingly recognized as a critical reservoir for multidrug-resistant Gram-negative bacteria, contributing to the global antimicrobial resistance crisis. *Escherichia coli* harboring extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) genes pose significant public health threats, particularly in resource-limited settings where wastewater treatment is inadequate. However, comprehensive data on the molecular characterization of these resistance determinants from clinical wastewater in southeastern Nigeria remain scarce.

Objective: This study aimed to molecularly characterize ESBL (*bla*TEM) and MBL (*bla*NDM) genes in *Escherichia coli* isolated from clinical wastewater at Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria.

Methods: A total of 30 effluent samples (500 mL each) were collected from clinical wastewater lines (CWL) of Alex Ekwueme Federal University Teaching Hospital, Abakaliki. Bacterial isolation and identification were performed using standard microbiological techniques. Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method, and Multiple Antibiotic Resistance (MAR) indices were calculated. Molecular characterization of *bla*TEM and *bla*NDM genes was performed by Polymerase Chain Reaction (PCR) using specific primers on all 40 isolated *E. coli* isolates.

Results: *E. coli* was isolated from all sampling points, accounting for 40 (18.5%) of total bacterial isolates. All *E. coli* isolates exhibited high resistance to aztreonam (100%), ceftriaxone (97.5%), colistin (95.0%), and nalidixic acid (97.5%), while gentamicin (100%) and ciprofloxacin (87.5%) remained the most effective antibiotics. MAR indices ranged from 0.65 to 0.84, exceeding the 0.2 high-risk threshold. Molecular analysis revealed *bla*TEM and *bla*NDM genes in all 40 (100%) *E. coli* isolates, confirming the genetic basis for observed resistance patterns.

Conclusion: This study provides the first molecular evidence of alarmingly high prevalence of *E. coli* co-harboring *bla*TEM and *bla*NDM genes in hospital wastewater from Abakaliki, Nigeria. The high MAR indices confirm hospital effluent as a high-risk source for antimicrobial resistance dissemination. These findings underscore the urgent

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need for on-site wastewater treatment interventions, enhanced antimicrobial stewardship, and integrated One Health surveillance to prevent environmental and community transmission of these multidrug-resistant pathogens.

Keywords: *Escherichia coli*; *bla*TEM; *bla*NDM; Extended-spectrum beta-lactamase; Metallo-beta-lactamase; Hospital wastewater; One Health; Antimicrobial resistance

1. Introduction

Hospital effluents are increasingly recognized as critical reservoirs for multidrug-resistant (MDR) Gram-negative bacteria, contributing significantly to the global antimicrobial resistance (AMR) crisis. The proliferation of antibiotic-resistant bacteria and the occurrence of antibiotic resistance genes (ARGs) in wastewaters are notable factors that contribute to the decline in the potency of antibiotic interventions globally [1,2]. The aquatic ecosystem, serving as a major recipient of inadequately treated hospital wastewater, has become a crucial pathway for the environmental dissemination of clinically significant resistance determinants [3].

Escherichia coli remains the most studied indicator organism for antimicrobial resistance surveillance in wastewater systems globally. Its ubiquitous presence in human and animal gastrointestinal tracts, coupled with its capacity to acquire and disseminate resistance genes through horizontal gene transfer, positions it as a sentinel organism for monitoring AMR in the environment [4]. The emergence and global spread of extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) producing *E. coli* from hospital wastewater represent a critical public health concern, as these organisms are often resistant to last-resort antibiotics, including carbapenems [5,6].

The World Health Organization has classified carbapenem-resistant Enterobacteriaceae, including *E. coli*, as a critical priority pathogen requiring urgent research and development of new antibiotics [7]. Similarly, ESBL-producing Enterobacteriaceae are classified as a high priority threat. The convergence of ESBL and MBL resistance mechanisms in the same organism creates a particularly dangerous phenotype that is difficult to treat and challenging to eliminate from environmental reservoirs [8].

The One Health approach recognizes the interconnectedness of human, animal, and environmental health in the emergence and spread of antimicrobial resistance [9]. Hospital wastewater serves as a critical nexus within this framework, receiving antibiotics, antimicrobial-resistant bacteria, and resistance genes from human patients, which are then discharged into the environment, potentially affecting wildlife, agricultural systems, and surrounding communities [10,11]. Understanding the molecular epidemiology of resistance genes in hospital wastewater is therefore essential for developing effective interventions under the One Health paradigm.

Numerous healthcare-associated outbreaks have been linked to contaminated water used for patient care, particularly sinks, faucets, or shower heads associated with hand washing and cleaning of medical devices [12]. Hospital wastewater is named as a major source of clinically important drug-resistant bacteria [13]. Recent AMR studies have reported the prevalence of antibiotic-resistant bacteria by detection of ARGs via polymerase chain reaction (PCR) due to its high sensitivity and specificity [14,15].

Alex Ekwueme Federal University Teaching Hospital, Abakaliki, is a major tertiary healthcare facility in southeastern Nigeria with numerous departments and high patronage. Despite the potential risk of poor hospital wastewater management, studies linking antibiotic resistance to hospital effluent management in Ebonyi State remain scarce. Specifically, comprehensive molecular data on ESBL and MBL genes in *E. coli* from clinical wastewater in this setting are lacking.

Water is considered the most important medium for the dissemination of resistant bacteria in the environment due to its interaction with microbes associated with humans. The most significant source of pollution of aquatic ecosystems by antimicrobial residues and resistant bacteria comes from hospitals [16,17]. This study is therefore justified by the need to fill the knowledge gap regarding the molecular epidemiology of ESBL and MBL genes in *E. coli* from hospital wastewater in southeastern Nigeria.

2. Materials and methods

2.1. Study Area

The study was conducted in Abakaliki, the capital city of Ebonyi State, Nigeria (6.3231°N, 8.1121°E). Wastewater effluent samples were collected from the clinical wastewater line (CWL) of the Alex Ekwueme Federal Teaching Hospital, Abakaliki. Ebonyi State is characterized by a sub-humid tropical climate. The hospital was selected due to its high patient volume, numerous active departments, and status as a major referral center in the region.

2.2. Ethical Approval

The research protocols for this study (NHREC/17/06/23/393) were reviewed and approved by the Research and Ethics Committee of the Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State, Nigeria. All experimental procedures were conducted in accordance with ethical guidelines for research involving human subjects.

2.3. Sample Collection

A total of 30 effluent samples (500 mL each) were collected from the clinical wastewater line (CWL) of Alex Ekwueme Federal University Teaching Hospital, Abakaliki. The CWL comprises wastewater from Intensive Care Unit (ICU), Accident and Emergency (AE), Ultrasound Ward (UW), Female Ward (FW), and Male Ward (MW). Samples were collected in sterile bottles and transported to the microbiology laboratory at approximately 8°C and analyzed within two hours of collection.

2.4. Bacterial Isolation and Identification

Ten-fold serial dilutions were prepared following standard microbiological procedures. After dilution, 1 mL from dilution factor 10^2 was transferred to sterile Petri dishes before pouring sterilized Plate Count Agar. Plates were incubated at room temperature for 24 hours. Colony enumeration was performed using a conventional colony counting machine. Each colony was aseptically picked from the agar plate and sub-cultured onto selective and differential media (Eosin Methylene Blue agar, MacConkey agar) for proper identification. A single colony from predominant morphologically similar colonies was picked and sub-cultured on Nutrient Agar to obtain pure culture. Identification was performed using Gram staining and various biochemical tests (Indole, Methyl Red, Voges Proskauer, Citrate utilization, Triple Sugar Iron, and carbohydrate fermentation tests) according to standard protocols [18]. Further confirmation of *E. coli* was performed using the VITEK® 2 COMPACT Automated system according to manufacturer instruction.

2.5. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [19]. A suspension was prepared from a 24-hour growth of test organisms in sterile water to match the 0.5 McFarland turbidity standard. This suspension was seeded onto Mueller-Hinton agar plates. The following antibiotic discs were used: amoxicillin/clavulanic acid (30 µg), aztreonam (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), colistin sulphate (10 µg), ofloxacin (10 µg), ciprofloxacin (10 µg), imipenem (10 µg), ertapenem (10 µg), meropenem (10 µg), streptomycin (10 µg), tetracycline (30 µg), nalidixic acid (30 µg), and trimethoprim-sulfamethoxazole (25 µg). Plates were incubated at 35–37°C for 18–24 hours, and inhibition zone diameters were interpreted according to CLSI breakpoints [19].

2.6. Multiple Antibiotic Resistance (MAR) Index

The Multiple Antibiotic Resistance (MAR) index was calculated for each isolate as the ratio of the number of antibiotics to which the isolate was resistant to the total number of antibiotics tested [20,21].

2.7. Molecular Analysis

2.7.1. DNA Extraction

All 40 isolated *E. coli* isolates were grown in nutrient broth for 24 hours at 37°C. Bacterial genomic DNA was extracted using a ZR Fungal/Bacterial DNA MiniPrep™ (Zymo Research, USA) according to the manufacturer's instructions. Extracted DNA was stored at -20°C until use.

2.7.2. PCR Amplification

PCR amplification was performed using specific primers for *bla*TEM and *bla*NDM genes. The primer sequences and expected amplicon sizes are shown in Table 1.

Table 1 PCR Primers and Conditions for *bla*TEM and *bla*NDM Genes

Gene	Primer Sequence (5' → 3')	Amplicon Size (bp)	Annealing Temperature
<i>bla</i> TEM F	5'-ATGAGTATTCAACATTTCC-3'	445	61°C
<i>bla</i> TEM R	5'-ACAAACCGTTAGCGACAG-3'		
<i>bla</i> NDM F	5'-GGGTTTGGCGATCTGG-3'	893	60°C
<i>bla</i> NDM R	5'-CGGTGCGTGGGTAGTGC-3'		

Source: Adapted from Khalefa et al., 2025 [22]

The PCR reaction mixture (25 µL) contained 12.5 µL of Taq 2x Master Mix (New England Biolabs), 1 µL each of forward and reverse primers (10 µM), 2 µL of DNA template, and 8.5 µL of nuclease-free water. PCR conditions were: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 61°C (for *bla*TEM) or 60°C (for *bla*NDM) for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes.

2.7.3. Gel Electrophoresis

PCR products were separated by electrophoresis on 2% agarose gel in 1× TAE buffer at 80–100 V for 1 hour. Gels were stained with EZ-Vision DNA stain and visualized under UV transillumination. A 50 bp DNA ladder was used as a molecular weight marker.

3. Results

3.1. Bacterial Isolation and Identification

Escherichia coli was consistently isolated from all clinical wastewater sampling points. The distribution of *E. coli* isolates is presented in Table 2.

Table 2 Distribution of *E. coli* from Clinical Wastewater Samples

Sampling Point	Total Isolates (n)	<i>E. coli</i> (n)	<i>E. coli</i> (%)
ICU	45	8	17.8
Accident/Emergency	52	10	19.2
Ultrasound Ward	38	7	18.4
Female Ward	41	8	19.5
Male Ward	40	7	17.5
Total	216	40	18.5

E. coli accounted for 40 (18.5%) of total bacterial isolates, with prevalence rates ranging from 17.5% to 19.5% across different sampling points.

3.2. Antibiotic Susceptibility Profile

The antibiotic susceptibility profiles of *E. coli* isolates are presented in Table 3.

High Resistance: *E. coli* isolates exhibited complete resistance (100%) to aztreonam. High resistance (90–100%) was observed to amoxicillin-clavulanic acid, ceftazidime, ceftriaxone, imipenem, ertapenem, streptomycin, colistin, and

nalidixic acid. **Low Resistance:** Gentamicin demonstrated 100% susceptibility across all *E. coli* isolates. Ciprofloxacin showed 87.5% susceptibility. **Carbapenem Resistance:** Imipenem resistance was 90.0%, meropenem resistance was 75.0%, and ertapenem resistance was 95.0%.

Table 3 Antibiotic Susceptibility Profile of *E. coli* Isolates (n=40)

Antibiotic	Resistance n (%)	Susceptible n (%)
Aztreonam (30)	40 (100.0)	0 (0.0)
Amoxicillin-Clavulanic Acid (30)	38 (95.0)	2 (5.0)
Ceftazidime (30)	37 (92.5)	3 (7.5)
Ceftriaxone (30)	39 (97.5)	1 (2.5)
Imipenem (10)	36 (90.0)	4 (10.0)
Meropenem (10)	30 (75.0)	10 (25.0)
Ertapenem (10)	38 (95.0)	2 (5.0)
Ciprofloxacin (5)	5 (12.5)	35 (87.5)
Gentamicin (15)	0 (0.0)	40 (100.0)
Streptomycin (25)	37 (92.5)	3 (7.5)
Colistin (30)	38 (95.0)	2 (5.0)
Ofloxacin (5)	15 (37.5)	25 (62.5)
Trimethoprim-Sulfamethoxazole (25)	26 (65.0)	14 (35.0)
Tetracycline (5)	29 (72.5)	11 (27.5)
Nalidixic Acid (15)	39 (97.5)	1 (2.5)

3.3. Multiple Antibiotic Resistance (MAR) Index

The MAR indices for *E. coli* isolates are presented in Table 4.

Table 4 Multiple Antibiotic Resistance Index of *E. coli* Isolates

Sampling Point	MAR Index
ICU	0.84
Accident/Emergency	0.82
Ultrasound Ward	0.79
Female Ward	0.74
Male Ward	0.65
Overall	0.77

All MAR indices exceeded the 0.2 threshold, indicating that these bacterial isolates originated from high-risk environments where antibiotics are frequently used. The highest MAR index (0.84) was observed in ICU isolates, while the lowest (0.65) was observed in Male Ward isolates. The overall MAR index was 0.77.

3.4. Molecular Detection of *bla*TEM and *bla*NDM Genes

Molecular analysis of all 40 *E. coli* isolates revealed the presence of *bla*TEM and *bla*NDM genes in all isolates tested (100%, n=40). The detection rates are shown in Table 5.

Table 5 Molecular Detection of *bla*TEM and *bla*NDM Genes in *E. coli* Isolates

Gene	Total Isolates Tested (n)	Positive n (%)
<i>bla</i> TEM	40	40 (100)
<i>bla</i> NDM	40	40 (100)

Table 6 Distribution of Resistance Genes by Sampling Point

Sampling Point	Total <i>E. coli</i> (n)	<i>bla</i> TEM Positive n (%)	<i>bla</i> NDM Positive n (%)
ICU	8	8 (100)	8 (100)
Accident/Emergency	10	10 (100)	10 (100)
Ultrasound Ward	7	7 (100)	7 (100)
Female Ward	8	8 (100)	8 (100)
Male Ward	7	7 (100)	7 (100)
Total	40	40 (100)	40 (100)

The universal detection of both *bla*TEM and *bla*NDM genes in all 40 isolates confirms the genetic basis for the observed resistance patterns. The co-occurrence of these resistance genes in the same isolates indicates the presence of mobile genetic elements capable of simultaneous transmission of multiple resistance determinants.

4. Discussion

This study revealed that *E. coli* was consistently present in clinical wastewater from Alex Ekwueme Federal University Teaching Hospital, Abakaliki, with a prevalence rate of 18.5% of total bacterial isolates. This finding is consistent with the global literature, which identifies *E. coli* as the predominant Gram-negative bacterial contaminant in hospital wastewater [23–25]. The prevalence rates observed in this study (17.5–19.5%) are comparable to those reported by Mustapha and Imir [23] in North-East Nigeria, who identified *E. coli* at 24.0% prevalence in hospital sewage. Onuoha et al. [25] reported *E. coli* at 26.2% prevalence in hospital effluents from South-Eastern Nigeria, which is slightly higher than the rates observed in the present study.

E. coli is recognized as a primary indicator of fecal contamination and its ubiquity in hospital wastewater globally makes it a sentinel organism for monitoring antimicrobial resistance [4]. The recovery of *E. coli* from clinical wastewater lines, including ICU and Accident/Emergency units, indicates widespread fecal contamination and highlights the need for improved infection prevention and control measures. The presence of *E. coli* in high-risk clinical areas such as the ICU (17.8%) and Accident/Emergency (19.2%) is particularly concerning, as these units handle critically ill patients who are more susceptible to infections.

The antibiotic susceptibility profiles revealed alarmingly high levels of resistance to multiple classes of antibiotics. The complete resistance to aztreonam (100%) and high resistance to third-generation cephalosporins (ceftazidime 92.5%, ceftriaxone 97.5%) are consistent with the ESBL phenotype, which confers resistance to oxyimino-cephalosporins and monobactams [26]. The 100% resistance to aztreonam is particularly concerning, as aztreonam is often used as an alternative agent in patients with beta-lactam allergies. This finding is consistent with El garraoui et al. [27] who reported high resistance to aztreonam among ESBL-producing *E. coli* from municipal wastewater in Marrakech.

The high resistance rates to imipenem (90.0%), meropenem (75.0%), and ertapenem (95.0%) indicate significant carbapenemase activity. Carbapenem-resistant *E. coli* are classified as a critical priority pathogen by the WHO [7], and the presence of these organisms in hospital wastewater represents a direct threat to public health. These findings are consistent with reports from other settings. Lamba et al. [28] reported that hospital wastewaters in New Delhi had up to nine orders of magnitude greater concentrations of carbapenem-resistant bacteria than local sewers. Zurfluh et al. [29] detected NDM-5-producing *E. coli* ST617 in the effluent of a WWTP discharged into the river Rhine. The higher resistance to ertapenem (95.0%) compared to meropenem (75.0%) may reflect differential activity of the specific carbapenemases present, as different MBLs have varying substrate profiles [30].

Gentamicin demonstrated 100% susceptibility across all *E. coli* isolates, which is consistent with some reports from other settings [24,31] but contrasts with studies that have reported high gentamicin resistance in Enterobacteriaceae from wastewater [32]. This selective susceptibility to gentamicin but not streptomycin (92.5% resistance) reflects the presence of specific aminoglycoside modifying enzymes (AMEs) capable of inactivating streptomycin but not gentamicin [33]. Ramirez and Tolmasky [33] documented that streptomycin resistance is frequently conferred by the *strA* and *strB* genes encoding APH(6)-Ia and APH(6)-Id phosphotransferases, while gentamicin resistance typically requires AAC(3) or AAC(6') acetyltransferases.

Ciprofloxacin demonstrated 87.5% susceptibility, which is higher than many reports in the literature. Gumede et al. [34] reported variable ciprofloxacin resistance rates among ESBL-producing *E. coli* from wastewater treatment plants in South Africa. Dolejska et al. [35] reported 100% ciprofloxacin resistance in ESBL-positive *E. coli* from a WWTP in the Czech Republic, which is higher than the rates observed in the present study. Nalidixic acid resistance (97.5%) suggests the presence of first-step mutations in the quinolone resistance determining region (QRDR), while preserved ciprofloxacin susceptibility indicates that higher-level mutations or plasmid-mediated quinolone resistance genes may not yet be fully established [36]. The literature review explains that mutations in *gyrA* and *parC* are the primary mechanisms of fluoroquinolone resistance, while plasmid-mediated quinolone resistance (PMQR) genes such as *qnrA*, *qnrB*, and *qnrS* confer low-level resistance [37].

Colistin resistance (95.0%) is a critical finding, as colistin is a last-resort antibiotic for the treatment of carbapenem-resistant Gram-negative infections. The emergence of colistin resistance in wastewater bacteria, potentially mediated by *mcr* genes, represents a terminal threat to antibiotic therapy [38]. Hayashi et al. [38] detected colistin- and tigecycline-resistant *Klebsiella pneumoniae* ST29 in WWTP influents from Japan. The high colistin resistance observed in this study may reflect the increasing use of colistin in clinical settings and the potential dissemination of *mcr*-mediated resistance.

The MAR indices (0.65–0.84) substantially exceed the 0.2 threshold, indicating that these bacteria originated from high-risk environments with frequent antibiotic use. MAR indices >0.2 indicate high-risk sources where antibiotics are frequently used [20]. The highest MAR index (0.84) observed in ICU isolates reflects the cumulative antibiotic pressure in critical care settings and the selection of MDR organisms. This is consistent with the literature, which identifies ICUs as hotspots for the selection of MDR organisms due to the high intensity of antibiotic use.

These findings are consistent with studies from other Nigerian settings. Uzoije et al. [39] reported MAR indices ranging from 0.5–0.8 for bacterial isolates from different hospital environments in Umuahia, Nigeria. Mustapha and Imir [23] found MAR indices ranging from 0.2–1.0 for Gram-negative bacteria from hospital sewage in North-East Nigeria. Fadare and Okoh [32] reported MAR indices ranging from 0.5 to 1.0 for Enterobacteriaceae isolated from hospital wastewater in South Africa. The overall MAR index of 0.77 in this study indicates a high-risk environment and suggests that the hospital effluent is a significant source of MDR organisms.

The universal detection of *bla*TEM (100%) and *bla*NDM (100%) genes in all 40 *E. coli* isolates is one of the most important findings of this study. The 100% detection of *bla*TEM is consistent with the global literature, which identifies TEM-type ESBLs as the most prevalent ESBL genes in wastewater bacteria [40,27]. El garraoui et al. [27] reported *bla*TEM at 81.25% among ESBL-producing *E. coli* from municipal wastewater in Marrakech. Adekanmbi et al. [41] detected *bla*TEM in combination with *bla*CTX-M genes in ESBL-producing *E. coli* from a hospital treatment plant in Nigeria. The higher prevalence observed in the present study (100%) may reflect the targeted selection of *E. coli* isolates from clinical wastewater with high antibiotic pressure.

The universal detection of *bla*NDM represents one of the most alarming findings of this study. NDM-type carbapenemases have emerged as a major global public health threat since their first description in 2009 [42]. The presence of *bla*NDM in 100% of tested isolates indicates widespread contamination of the hospital effluent with NDM-producing organisms. Lamba et al. [28] reported that 55% of CRE isolates from large hospitals in New Delhi carried at least one *bla*NDM-1 gene. Wang et al. [43] found that *bla*NDM-5 was the most prevalent carbapenemase gene in carbapenem-resistant *E. coli* isolates from a WWTP in China, detected in 75 of 77 isolates. Mannan et al. [44] reported that *bla*NDM-1 was present in 100% of beta-lactamase-producing bacterial isolates from biomedical wastewater. The universal detection of *bla*NDM in the present study surpasses most reports in the literature and indicates an extraordinarily high burden of NDM-producing organisms in the study setting.

The co-occurrence of *bla*TEM and *bla*NDM in the same isolates (100%) indicates the presence of mobile genetic elements capable of capturing and maintaining multiple resistance cassettes. This is consistent with the literature, which extensively documents the role of integrons, transposons, and conjugative plasmids in the dissemination of

multiple ARGs [45,46]. Alouache et al. [47] and Hassen et al. [48] demonstrated that ESBL and quinolone resistance genes are frequently located on class 1 integrons with various gene cassette arrays, as well as on IncP-1 and IncFIB plasmids. The universal co-carriage observed in this study suggests the presence of similar complex integron or plasmid structures capable of accommodating multiple resistance cassettes.

The distribution of resistance genes across sampling points showed that all isolates from all clinical areas (ICU, Accident/Emergency, Ultrasound Ward, Female Ward, and Male Ward) harbored both *bla*TEM and *bla*NDM genes. This uniform distribution indicates widespread contamination of the entire clinical wastewater system and suggests that the resistance genes are not confined to specific clinical areas but are pervasive throughout the hospital wastewater network.

The findings of this study have significant implications under the One Health framework. Hospital wastewater serves as a critical nexus connecting human healthcare activities with the environment [9]. The discharge of *E. coli* harboring *bla*TEM and *bla*NDM genes into the environment creates a pathway for the dissemination of clinically significant resistance determinants into surrounding communities, water bodies, and agricultural systems [10,11].

The persistence of ESBL and MBL-producing *E. coli* in hospital wastewater, even after the organisms have left the clinical setting, represents a "slow-moving pandemic" [49]. These organisms can colonize wildlife, domestic animals, and plants through contaminated water and soil, creating a reservoir for future human infections [50]. The high MAR indices (>0.2) confirm the hospital effluent as a high-risk source of AMR dissemination, reinforcing the need for integrated surveillance systems that monitor clinical, environmental, and community reservoirs simultaneously [9,10].

The detection of *bla*NDM in 100% of tested isolates is particularly concerning from a One Health perspective. Carbapenems are last-resort antibiotics, and the environmental dissemination of NDM-producing organisms threatens the continued efficacy of these critically important agents. The potential for horizontal gene transfer from environmental *E. coli* to clinically relevant pathogens, as documented by Szczepanowski et al. [45], further amplifies the public health risk.

The presence of these resistance genes in wastewater from all clinical areas, including the Ultrasound Ward and Female/Male Wards, suggests that antimicrobial resistance is not confined to high-acuity areas but is pervasive throughout the hospital environment. This underscores the need for comprehensive infection prevention and control measures across all hospital departments, as well as effective wastewater treatment strategies.

5. Conclusion

This study provides the first molecular evidence of alarmingly high prevalence of *E. coli* co-harboring *bla*TEM and *bla*NDM genes in clinical wastewater from Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Nigeria. The high antibiotic resistance rates (90–100% to multiple classes), high MAR indices (0.65–0.84), and universal detection of *bla*TEM and *bla*NDM genes in all 40 isolates confirm hospital effluent as a high-risk source of antimicrobial resistance dissemination. The uniform distribution of these resistance genes across all clinical areas indicates widespread contamination of the entire hospital wastewater system.

These findings underscore the urgent need for:

- On-site wastewater treatment interventions to reduce the microbial load and ARG burden of hospital effluent before discharge into the environment
- Enhanced antimicrobial stewardship programs to reduce selective pressure in the hospital setting and preserve the efficacy of last-resort antibiotics
- Integrated One Health surveillance to monitor the transmission of resistance genes across human, animal, and environmental compartments
- Regulatory enforcement of wastewater treatment standards for healthcare facilities, with particular attention to the removal of antibiotic-resistant bacteria and resistance genes
- Further research using Whole Genome Sequencing to determine gene variants, plasmid types, and genetic context of resistance genes, as well as metagenomic analysis to characterize the full resistome of the hospital effluent

Infection prevention and control measures across all hospital departments to reduce the dissemination of MDR organisms from patients into the wastewater system

Under the One Health framework, addressing the challenge of AMR in hospital wastewater requires a collaborative, multi-sectoral approach that recognizes the interconnectedness of human, animal, and environmental health. The findings of this study contribute critical baseline data for the molecular epidemiology of antibiotic resistance in hospital wastewater in Nigeria and provide a foundation for future surveillance, intervention, and policy development efforts aimed at mitigating the spread of antimicrobial resistance in the context of the One Health framework.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

Statement of ethical approval

This study was approved by the Research and Ethics Committee of Alex Ekwueme Federal University Teaching Hospital, Abakaliki (NHREC/17/06/23/393). All procedures were conducted in accordance with ethical guidelines for research involving human subjects.

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