

Occurrence of Multidrug-Resistant *Escherichia coli* in Abuja Wastewater Treatment Plant and Wupa River

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Abstract: The discharge of multidrug-resistant bacteria into the environment can promote the transfer of resistance genes to clinically important bacteria, thereby posing a substantial public health risk. This study evaluated *Escherichia coli* isolated from the Abuja wastewater treatment plant and the Wupa River in Abuja, Nigeria, for multidrug resistance (MDR) and antibiotic resistance genes (ARGs). Isolates were characterised as presumptive using morphological and biochemical methods. Susceptibility to 10 different antibiotics was determined using the Kirby-Bauer disc diffusion technique. Confirmation of *E. coli* (*uidA* gene presence) and detection of ARGs were achieved by conventional polymerase chain reaction (PCR). Of 39 presumptive *E. coli* isolates, 100% were resistant to cefpodoxime, with high rates of resistance to ampicillin (79.5%) and cefuroxime (71.8%). Susceptibility to ciprofloxacin (89.7%), fosfomycin (84.6%), nitrofurantoin (84.6%), and chloramphenicol (74.4%) remained high, but up to 7.7% of the isolates showed carbapenem resistance. Multidrug resistance was observed in 87.2% (34/39) of *E. coli* isolates, with MAR index above the critical threshold of 0.2. Seventeen isolates, comprising 15 multidrug-resistant (MDR) and 2 extensively drug-resistant (XDR), were selected for confirmation, and the *uidA* gene was detected in 16 (94.1%). Of the ARGs screened for, *aadA* (75%), *acrA* (88%), *bla_{CTX-M}* (69%), *bla_{NDM-1}* (75%), and *tetA* (25%) were detected. In all, 81.3% of the confirmed isolates harboured at least three resistance genes. These findings highlight the environmental dissemination of MDR *E. coli* and ARGs and underscore the need for enhanced wastewater management.

Key word: Antibiotic resistance genes, *E. coli*, multidrug-resistant, public health risk, surface water pollution, wastewater treatment plants.

INTRODUCTION

Municipal wastewater is a complex mixture of domestic, commercial, and industrial liquid waste, making wastewater treatment plants (WWTPs) major reservoirs of a diverse array of pathogens originating from human faeces and urine and hotspots for the development of antimicrobial resistance (AMR) (Kumar *et al.*, 2024). The global health threat of AMR spread is heightened by the extensive use of antibiotics in medicine that led to the emergence of multidrug-resistant bacteria in both human and animal gut microbiomes (Bürgmann *et al.*, 2018) and the fact that bacteria from the gut confluence with the WWTP microbiome (Yu *et al.*, 2022; Abdelgalel *et al.*, 2025), fostering horizontal gene transfer.

Horizontal gene transfer facilitates the acquisition of resistance genes, particularly through integrons in *Escherichia coli*. This results in the release of both multidrug-resistant microorganisms and antibiotic-resistant genes into the environment (Papajová *et al.*, 2022). Effective wastewater treatment, therefore, is crucial for safeguarding public health and controlling infectious diseases. *Escherichia coli* is a highly diverse bacterium that occurs naturally in the intestinal tracts of humans and some animals. Although a part of the normal flora, commensal organisms, some pathogenic *E. coli* strains have emerged (Sora *et al.*, 2021). Pathogenic *E. coli* consists of two main groups: intestinal *E. coli*, which includes enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC),

enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC), and extra-intestinal *E. coli* (ExPEC), which consists of neonatal meningitis *E. coli* (NMEC), uropathogenic *E. coli* (UPEC), avian pathogenic *E. coli* (APEC), and sepsis-associated *E. coli* (SEPEC) (Sora *et al.*, 2021). The intestinal *E. coli* pathotypes are responsible for most food- and waterborne diseases, causing diarrhoea and haemolytic uremic syndrome (Biswas *et al.*, 2024). In contrast, extraintestinal pathogenic *E. coli* is linked to urinary tract infections (UTIs), sepsis, and meningitis (Biswas *et al.*, 2024). *Escherichia coli* significantly contributes to the spread of antibiotic resistance among bacteria, leading to treatment failure in human and veterinary medicine. It can readily obtain and transfer resistance genes among bacterial populations through horizontal gene transfer, primarily because these resistance genes are plasmid-borne (Poirel *et al.*, 2018). The occurrence of MDR *E. coli* pathotypes carrying several antibiotic-resistance genes in wastewater treatment plants (WWTPs) and associated rivers has been well documented (Adefisoye and Okoh, 2016; Adegoke *et al.*, 2020; Calhau *et al.*, 2015; Chigor *et al.*, 2020; Martínez-Organiz *et al.*, 2020; Mbangha *et al.*, 2021; Savin *et al.*, 2021). Additional studies have demonstrated that these resilient *E. coli* strains have acquired unique characteristics that enable the strains to evade treatment processes and persist in effluent and surrounding aquatic environments (Anastasi *et al.*, 2010, 2012; Zhi *et al.*, 2016, 2020). These studies have demonstrated the potential role of wastewater treatment plant effluent in the distribution of multidrug-resistant and virulent *E. coli* and the possible transmission to humans and animals through the water and food chains.

The World Health Organization (WHO) reported that waterborne infections are the leading cause of human disease in developing countries, accounting for

approximately 80% of all diseases in the region (Osman *et al.*, 2024). Also, studies have shown that activated sludge technology can promote the transmission of resistance genes and the multiplication of resistant bacteria (Zhang, 2016). Abuja WWTP uses activated sludge technology to treat domestic wastewater and discharges effluent into the Wupa River. Hence, releasing contaminated effluent into the environment will not only increase the disease burden, but it can also contribute to the distribution of multidrug resistance. Although previous researchers have established the presence of *E. coli* in the effluent of the Abuja WWTP (Audu, 2016; Ibangha *et al.*, 2023), there are no data on antibiotic resistance profiles and the presence of resistance genes in *E. coli* strains from the plant and the Wupa River. This study examined the phenotypic and genotypic antibiotic resistance in *E. coli* from the Abuja WWTP and the Wupa River.

MATERIALS AND METHODS

Study Site Description: The study was conducted at the Abuja Wastewater Treatment Plant (AWWTP), Abuja, Nigeria, from February to November 2020. It is situated between latitudes 90°1'18" and 90°3'12" north and longitudes 70°22'43" east and 70°23'3" east. The treatment process consists of two stages: mechanical and biological, followed by sterilization with continuous UV light. It operates a conventional activated sludge process and discharges its effluent into the Wupa River. The Wupa River is the principal river in Abuja, Nigeria's federal capital, and runs through many towns (Balogun and Ogwueleka, 2021; Ibangha *et al.*, 2023). The geographical map of the study area, highlighted by the sampling sites, is shown (Plate 1).

Wastewater and River Water Sample Collection and Processing: A hundred millilitres (100 ml) of grab samples were collected monthly for 10 months (February to November 2020) from five sampling points, S1 to S5 (Plate 1): the sewage treatment plant (STP) influent point, the STP

effluent point, upstream of the discharge point (DP), the discharge point, and downstream of DP, respectively. The sampling points included the influent to the Abuja WWTP (S1), the effluent of the Abuja WWTP (S2), the Wupa River upstream of the Abuja WWTP discharge point (S3), the Abuja WWTP discharge point into the Wupa River (S4), and the Wupa River downstream of the Abuja WWTP discharge point. The samples were collected using sterile sample bottles, transported in a chest box to the Abuja WWTP laboratory, and subsequently processed within 6 hours (Baird *et al.*, 2017). All samples were subjected to a ten-fold serial dilution before inoculation onto selective media.

Isolation and Presumptive Identification of *Escherichia coli*: The water samples were analysed for *E. coli* in duplicates on Chromocult Coliform Agar (CCA; Merck Millipore) using the spread plate method. Wastewater and water samples (1 ml) were inoculated onto CCA and incubated at 37°C for 24 hours. The *E. coli* isolates were randomly selected from each plate after incubation, purified, and further confirmed on Methylene Blue (EMB) agar. Morphological and biochemical characteristics were used for preliminary identification. Subsequently, Gram-negative rod and indole-positive isolates were selected for additional analysis (Hara *et al.*, 2018; Crettels *et al.*, 2023).

Phenotypic Antibiotic Susceptibility Testing of *Escherichia coli* from Abuja WWTP and Wupa River: The modified Kirby-Bauer disc diffusion method was used to determine the antibiotic pattern of the *E. coli* isolates. A total of ten (10) antibiotic discs (Oxoid, UK) from nine (9) different classes were employed, which include ampicillin 10 µg (penicillins), cefpodoxime 10 µg (cephalosporins), cefuroxime 30 µg (cephalosporins), chloramphenicol 30 µg (phenicols), ciprofloxacin 5 µg (fluoroquinolones), doripenem 10 µg (carbapenems), fosfomycin 50 µg (phosphonic acid), tetracycline 30 µg (tetracyclines), nitrofurantoin 30 µg

(nitrofuran), and trimethoprim-sulfamethoxazole 25 µg (folate pathway inhibitor). The *E. coli* isolates and the control (*E. coli* ATCC 25922) were inoculated onto Mueller-Hinton agar plates impregnated with antibiotic discs and incubated at 35°C for 18 hours. The CLSI breakpoint was used to interpret inhibition zone sizes (CLSI, 2022; Osman *et al.*, 2024). Phenotypic multidrug resistance (MDR) was defined as resistance to at least one agent in three antibiotic classes. Extensively drug-resistant (XDR) colonies were defined as resistance to at least one agent in nearly all antibiotic classes tested. Finally, pan-drug-resistant (PDR) colonies were defined as resistance to all agents in all antibiotic classes tested (Adefisoye and Okoh, 2016; Almakrami *et al.*, 2024). *E. coli* strains that demonstrated multiple resistance to the antibiotics tested were chosen for resistant gene profiling. The multiple antibiotic resistance (MAR) index for each isolate was calculated by comparing the sum of antibiotics to which the isolate demonstrates resistance (X) with the total number of antibiotics tested against the isolate (Y) (Adegoke *et al.*, 2020).

$$\text{MAR Index} = X/Y$$

Equation 1: Multiple antibiotic resistance (MAR) index formula.

Bacterial DNA Extraction: Bacterial DNA was isolated following the modified protocol detailed by Nishiguchi *et al.* (2002). Bacterial cells were harvested by centrifuging the nutrient broth at 4,600 g for 5 minutes. The cell pellet was suspended in 520 µl of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). Subsequently, 20% SDS (15 µl) and 3 µl of Proteinase K (20 mg/ml) were added, and the mixture was incubated for 1 hour at 37°C. Then, 100 µl of 5 M NaCl and 80 µl of a CTAB/NaCl solution (0.7 M NaCl, 10% CTAB) were added after incubation, mixed, and incubated again at 65°C for 10 minutes. The mixture was cooled for 15 minutes on ice. Subsequently, chloroform: isoamyl alcohol (24:1) was added, incubated on ice for 5 minutes, and centrifuged for 20 minutes at

7,200 g. The aqueous solution was then transferred to a fresh tube, and isopropanol was added at a 1:0.6 (v/v) ratio. Bacterial DNA was precipitated for 16 hours at -20°C, separated by centrifugation for 10 minutes at 7,200 g, washed with 70% ethanol (500 µl), and dried at room temperature. The DNA integrity was assessed on a 2% agarose gel and photographed with a UV transilluminator (BioRad, USA) at 302 nm. The DNA pellet was stored in TE buffer (50 µl) and kept at 4 °C for PCR analysis.

Polymerase Chain Reaction (PCR)

Confirmation of *E. coli*: The *E. coli* strains exhibiting multiple antibiotic resistance phenotypic traits were confirmed by PCR detection of the *uidA* target gene (Table 1). The mixtures (12.5 µl) comprise 0.25 µl each of forward and reverse primers (Inqaba Biotech, South Africa), 5 U/µl Taq polymerase (0.06 µl), template DNA (2 µl), 25 mM MgCl₂ (0.75 µl), 10 mM dNTPs (0.25 µl), 10X green buffer (2.5 µl), and nuclease-free water (6.44 µl). The PCR cycling conditions, as described by Cebula *et al.* (1995), were modified slightly (Table 1). The amplification products were resolved by electrophoresis at 100 V for 1 hour and visualised under UV light for the 252-bp band.

Detection of Antibiotic-Resistant Genes:

The *E. coli* strains exhibiting multiple drug resistance phenotypes were further analysed for antibiotic resistance genes using specific oligonucleotide primers (Table 1). The PCR reaction mixture (12.5 µl) for multiplex PCR (*tetA* and *aadA*) comprises DNA template (2 µl), 5 U/µl Taq Polymerase (0.06 µl), 25 mM MgCl₂ (0.75 µl), 10X green buffer (2.5 µl), nuclease-free H₂O (5.94 µl), and 5.94 µl of 10 mM dNTPs, 10 pM forward primer *tetA*, 10 pM reverse primer *tetA*, 10 pM forward primer *aadA*, and 10 pM reverse primer *aadA* (Inqaba Biotechnical Industries, South Africa) (Nontongana *et al.*, 2014). The singleplex PCR for *acrA*, *blaCTX*, and *blaNDM-1* was performed using gene-specific primers (Table 1). The PCR reaction mixture (12.5 µl) comprised 2 µl DNA template, 6.44 µl of nuclease-free

H₂O, 0.06 µl of 5 U/µl Taq polymerase, 0.75 µl of 25 mM MgCl₂, 2.5 µl of 10X green buffer, and 0.25 µl each of 10 mM dNTPs, 10 pM forward primers, and 10 pM reverse primers. The cycling conditions for the *acrA* gene (Jaktaji and Jazayeri, 2013), *blaCTX* (Poirel *et al.*, 2018), and *blaNDM-1* (Nordmann *et al.*, 2011) were detailed in Table 1. The product was evaluated on agarose gel (1.5%) and visualised on a UV transilluminator (BioRad, USA) at a wavelength of 302 nm.

RESULTS

All the *E. coli* isolates (39) were selected based on morphological and biochemical traits. *Escherichia coli* was identified as a Gram-negative, indole-positive rod with blue and shiny green metallic colonies on CCA and EMB agar, respectively. The presumptive *E. coli* from different sampling points is shown in Table 2.

Escherichia coli strains from wastewater and river samples demonstrated alarming resistance to β-lactam antibiotics (penicillins and cephalosporins). All 39 isolates (100%) were resistant to third-generation cephalosporin (cefepodoxime). Similarly, high resistance rates were observed for ampicillin (79.5%, 31/39) and cefuroxime (71.8%, 28/39), with no isolates showing full susceptibility to these agents (Figure 1). Doripenem, a carbapenem, showed low resistance rates (7.7%, 3/39), although a significant proportion (53.8%, 21/39) showed intermediate susceptibility. In contrast, susceptibility to fluoroquinolones, fosfomycin, and nitrofurantoin remained relatively preserved, with 89.7% (35/39) of isolates susceptible to ciprofloxacin and 84.6% (33/39) each for fosfomycin and nitrofurantoin. Chloramphenicol also showed high effectiveness, with a susceptibility rate of 74.4% (29/39) among isolates.

Also, trimethoprim/sulfamethoxazole and tetracycline showed moderate susceptibility of 53.8% (21/39) each, with substantial resistance rates of 46.2% (18/39) and 41.0% (16/39), respectively, as illustrated in Figure

1. The multiple antibiotic resistance (MAR) index and resistance profile of the *E. coli* are shown in Table 3. This study identified 17 distinct profiles of multiple resistance among the *E. coli* isolates, each encompassing 2 to 7 antibiotic classes. In this study, phenotypic multidrug resistance was observed in 87.2% (34/39) of *E. coli* strains. The MAR index ranged from 0.2 to 0.8, with 87.2% (34) of the *E. coli* displaying an index above the critical threshold of 0.2.

A total of 16 out of 17 MDR *E. coli* strains screened were found to possess the *uidA* gene, confirming their *E. coli* identity. The PCR product of amplified antibiotic resistance genes: *aadA*, *tetA*, *acrA*, *bla*_{CTX-M},

and *bla*_{NDM-1} (Plates 2–5 and Table 4) showed that 4 (25%) *E. coli* strains harboured *tetA*, 12 (75%) showed the presence of the *aadA* gene, 14 (88%) harboured the *acrA* gene, 11 (69%) harboured *bla*_{CTX-M} genes, and 12 (75%) possessed the *bla*_{NDM-1} gene. In summary, 13 (81%) of 16 MDR *E. coli* profiled for resistance genes harboured at least three antibiotic resistance genes, confirming the potential for multidrug resistance in *E. coli*. Table 5 presents the frequencies of multidrug-resistant phenotypes and the presence of resistance genes among MAR *E. coli*. Of the 16 *E. coli* isolates with MDR phenotype, 13 (81.3%) harboured at least three (3) antibiotic resistance genes tested.



Plate 1: Aerial View of Abuja Wastewater Treatment Plant and Wupa River

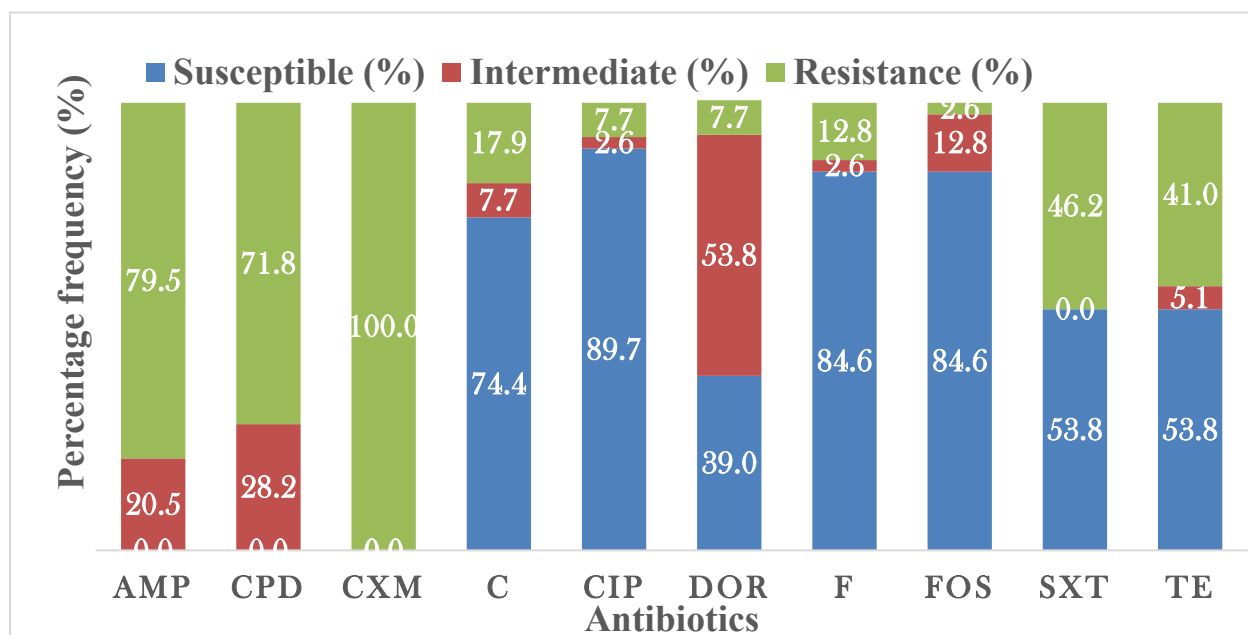
Table 1: Primers Used for *E. coli* Identification and Antibiotic Resistance Genes (ARGs)

Strain/Antibiotic agent	Target gene	Oligonucleotide sequence (5'→3')	Size (bp)	PCR Conditions	Reference
<i>E. coli</i>	<i>uidA</i>	F: GCGAAACTGTGGAATTGGG R: TGATGCTCCATAACTTCCTG	252	94°C for 2 min, 30 cycles of 94°C for 30 s, 64°C for 30 s and 72°C for 45 s, 72°C for 5 min	Cebula et al., 1995
Ciprofloxacin	<i>acrA</i>	F: TTGAAATTACGCTTCAGGAT R: CTTAGCCCTAACAGGATGTG	189	94°C for 2 min, 30 cycles of 94°C for 45 s, 51°C for 45 s, and 72°C for 45 s 72°C for 5 min.	Jaktaji and Jazayeri, 2013
Streptomycin	<i>aadA</i>	F: TATCCAGCTAAGCGCGAAC R: ATTTGCCGACTACCTTGTC	147	94°C for 2 min, 30 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 45 s 72°C for 5 min	Nontongana et al., 2014
Tetracycline	<i>tetA</i>	F: CCTCAGCTTCTCAACGCGTG R: GCACCTTGCTGATGACTCTT	402	94°C for 2 min, 30 cycles of 94°C for 30 s, 57°C for 30 s, and 72°C for 45 s 72°C for 5 min	Nontongana et al., 2014
β- lactam	<i>bla</i> _{CTX-M}	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	550	94°C for 5 min, 35 cycles of 95°C for 30 s, 60°C for 30 s 72°C for 30 s 72°C for 5 min	Poirel et al., 2001
β- lactam	<i>bla</i> _{NDM-1}	F: GGGTTGGCGATCTGGTTTTC R: CGGAATGGCTCATCACGATC	621	94°C for 5 min, 36 cycles of 94°C for 30 s, 52°C for 40 s and 72°C for 50 s, 72°C for 5 min	Nordmann et al., 2011

Keys: *uidA* gene encodes the β-D-glucuronidase gene (*uidA*) in the *E. coli* species. *AcrA*, *aadA*, *tetA*, *bla*_{CTX-M}, and *bla*_{NDM-1} are genes that code for resistance to ciprofloxacin, streptomycin, tetracycline, and β-lactamase, respectively.

Table 2: *Escherichia coli* Contamination Rate in Water Samples

Sampling point	Location	Samples analysed	Presumptive <i>E. coli</i> -positive samples
S1	STP Influent point	10	6 (60%)
S2	STP Effluent point	10	9 (90%)
S3	Upstream of the Discharge point	10	5 (50%)
S4	Discharge point (DP)	10	10 (100%)
S5	Downstream of DP	10	9 (90%)
	Total	50	39 (78%)

**Figure 1: Resistance Pattern of Presumptive *E. coli***

Keys: AMP: Ampicillin; CPD: Cefuroxime; CXM: Cefpodoxime; C: Chloramphenicol; CIP: Ciprofloxacin; DOR: Doripenem; F: Nitrofurantoin; FOS: Fosfomycin; SXT: Trimethoprim/Sulfamethoxazole; TE: Tetracycline

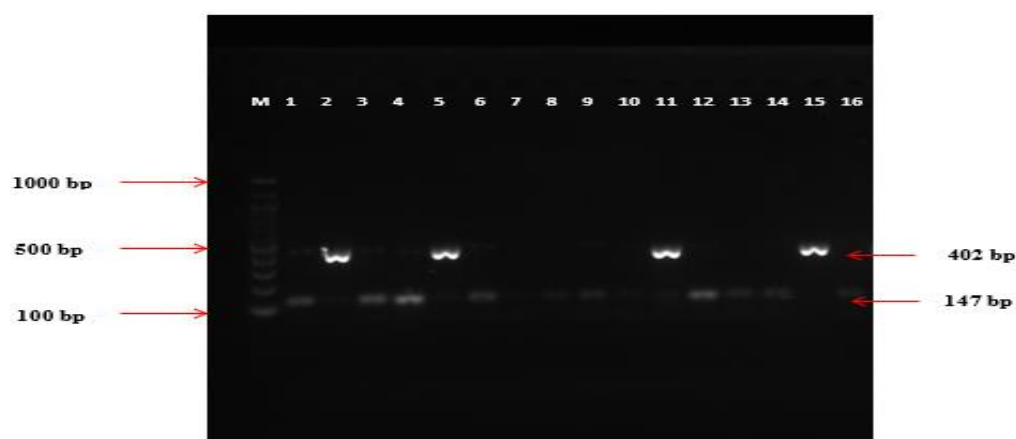
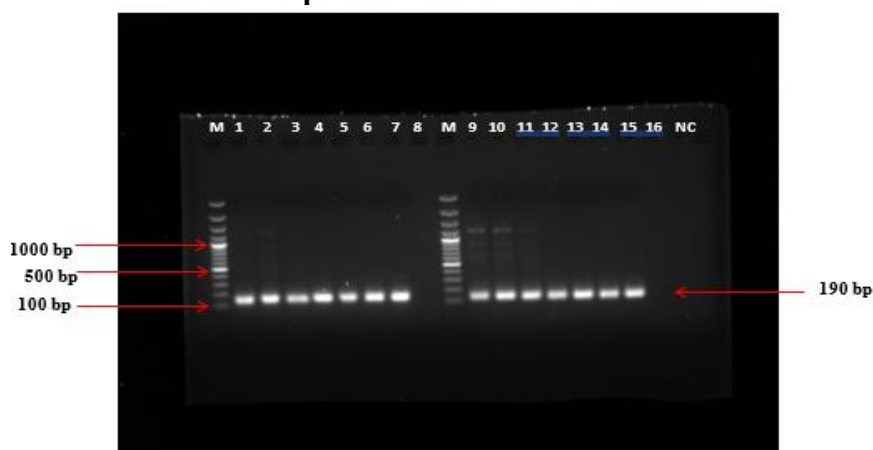
Table 3: Antibiotic resistance profile of *E. coli* from the Abuja WWTP and Wupa River

Antibiotic classes	Resistance Pattern	Category of resistance pattern	Number of isolates	MAR Index
2	AMP, CPD, CXM	non-MDR	5	0.2
3	AMP, CPD, CXM, C	MDR	1	0.3
3	AMP, CPD, CXM, SXT	MDR	1	0.3
3	AMP, CPD, CXM, DOR	MDR	12	0.3
4	AMP, CPD, CXM, DOR, F	MDR	1	0.4
4	AMP, CPD, CXM, SXT, TE	MDR	2	0.4
4	AMP, CPD, CXM, DOR, TE	MDR	1	0.4
4	AMP, CPD, CXM, DOR, FOS	MDR	1	0.4
5	AMP, CPD, CXM, C, SXT, TE	MDR	1	0.6
5	AMP, CPD, CXM, DOR, SXT, TE	MDR	2	0.6
5	AMP, CPD, CXM, CIP, DOR, SXT	MDR	1	0.6
6	AMP, CPD, CXM, DOR, F, SXT, TE	MDR	1	0.7
6	AMP, CPD, CXM, C, DOR, SXT, TE	MDR	3	0.7
6	AMP, CPD, CXM, CIP, FOS, SXT, TE	MDR	1	0.7
6	AMP, CPD, CXM, CIP, DOR, SXT, TE	MDR	1	0.7
7	AMP, CPD, CXM, C, F, FOS, SXT, TE	XDR	4	0.8
7	AMP, CPD, CXM, C, CIP, DOR, SXT, TE	XDR	1	0.8

Keys: AMP: Ampicillin; CPD: Cefuroxime; CXM: Cefpodoxime; C: Chloramphenicol; CIP: Ciprofloxacin; DOR: Doripenem; F: Nitrofurantoin; FOS: Fosfomycin; SXT: Trimethoprim/Sulfamethoxazole; TE: Tetracycline; MDR: multidrug resistance; XDR: Extensively drug-resistant

Table 4: Antibiotic Resistance Genes (ARGs) Incidence in *E. coli*

Antibiotic resistance genes	Resistance encoded	Number of isolates tested	Positives (%)
<i>tetA</i>	Tetracycline	16	4 (25%)
<i>aadA</i>	Streptomycin	16	12 (75%)
<i>acrA</i>	Ciprofloxacin	16	14 (88%)
<i>bla</i> _{CTX-M}	β -Lactamase	16	11 (69%)
<i>bla</i> _{NDM-I}	Carbapenems	16	12 (75%)

**Plate 2: Gel Electrogram with the Amplification of the *tetA* at 402 bp and *aadA* at 147 bp Genes within *E. coli* from Samples****Plate 3: Gel Electrogram with the Amplification of the *E. coli* *acrA* Resistant Gene at 190bp. M= Molecular Weight Marker; Lane 1 -16 = Bacterial Isolates; NC: Negative Control****Plate 4: Gel Electrogram with the Amplification of the *E. coli* *bla*_{CTX-M} Gene at 550 bp. M = Molecular Weight Marker; Lanes 1-16 = Bacterial Isolates**

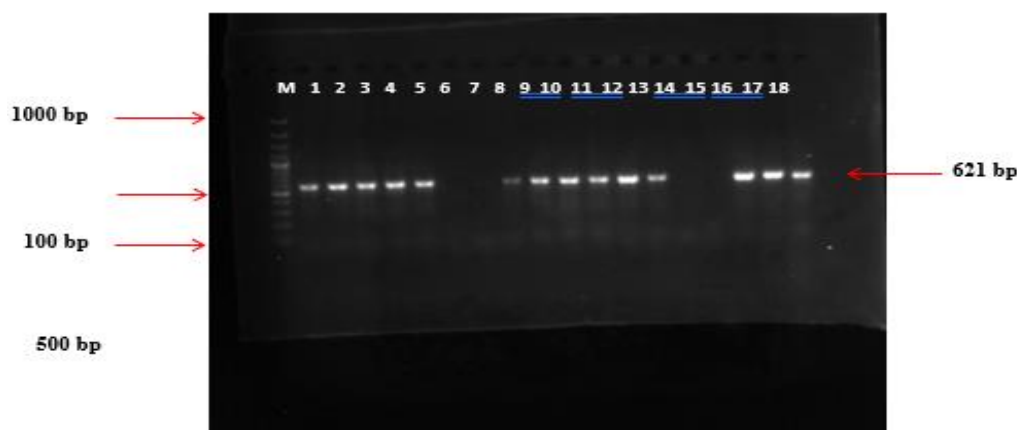


Plate 5: Gel Electrogram with the Amplification of the *E. coli* *blNDM-1* Gene at 621 bp. M = Molecular Weight Marker; Lanes 1–16 = Bacterial Isolates

DISCUSSION

The continuous discharge of WWTP effluent into surface waters has been connected to the distribution of multidrug-resistant microorganisms, posing a notable public health risk. Wastewater treatment plants promote the spread of plasmid-borne resistance genes within bacterial communities and also play a vital role in the emergence of multidrug-resistant bacteria (Zhang, 2016).

The present study revealed high MAR *E. coli* with unusually high resistance to cefuroxime, ampicillin, and cefpodoxime. The findings agree with reports of notable researchers on surface waters and wastewater treatment facilities (Blaak *et al.*, 2015; Adefisoye and Okoh, 2016; Adegoke *et al.*, 2020; Chigor *et al.*, 2020; Ibangha *et al.*, 2023). Also, the isolates' resistance to ampicillin is similar to findings reported by Chigor *et al.* (2020) and Gumede *et al.* (2021). However, it disagrees with the finding of Adefisoye and Okoh (2016).

The high prevalence of cefuroxime resistance (71.8%) agrees with the findings of Gumede *et al.* (2021) but differs from the discoveries of Chigor *et al.* (2020), who reported a lower resistance rate of 7%. This pattern indicates widespread beta-lactamase production among these *E. coli* strains from the study area, raising public health concerns because beta-lactam antibiotics are first-line treatment for bacterial infections.

Furthermore, *E. coli* strains showed low resistance (7.7%) to carbapenem (Doripenem), but a significant proportion (53.8%) showed intermediate susceptibility. The findings support those of Gumede *et al.* (2021), who also reported a low carbapenem resistance rate (15%). This pattern suggests the emergence of carbapenem resistance, as intermediate-resistant isolates quickly attain full resistance and can also be referred to as resistant to the antibiotic (Ozochi *et al.*, 2025). The situation is further worrisome since carbapenems are often reserved as last-resort antibiotics for multidrug-resistant infections. Ciprofloxacin (89.7%), fosfomycin (84.6%), and nitrofurantoin (84.6%) exhibit excellent inhibition against most of the strains from the Abuja WWTP and Wupa River, contrasting sharply with Adegoke *et al.* (2020), who reported 47.1% resistance, and much lower than the 5% resistance observed by Chigor *et al.* (2020) in *E. coli* isolated from a university WWTP in Nigeria.

In this study, approximately 87.2% of isolates had a MAR index exceeding 0.2, which is somewhat consistent with previous findings by Chigor *et al.* (2020), Gumede *et al.* (2021), and Ozochi *et al.* (2025), which reported MAR indices in over 80% of test isolates. Although the study by Adefisoye and Okoh (2016) in the Eastern Cape, South Africa, reported a significantly lower MAR index of 32.7%, Adegoke *et al.* (2020)

reported a higher value of more than 94% in *E. coli* isolated from municipal wastewater in Durban, South Africa. An MAR index above 0.2 indicates high-risk contamination sources, suggesting that these wastewater isolates originate from environments with frequent antibiotic use. The level of antibiotic resistance observed in the study may be linked to self-medication, the sale and use of antibiotics without prescriptions, underdosing, unnecessary consumption, and the availability of antibiotics over the counter, which is common in developing countries, including Nigeria (Torres *et al.*, 2019; Abubakar and Tangiisuran, 2020). The *E. coli* strains isolated in this study carry β -lactamase genes (*bla*_{CTX-M}), a tetracycline resistance gene (*tetA*), and an aminoglycoside resistance gene (*aadA*). These findings supported previous research that illustrated the presence of antibiotic resistance genes in *E. coli* strains from wastewater treatment facilities and nearby aquatic ecosystems (Adefisoye and Okoh, 2016; Adegoke *et al.*, 2020; Gumede *et al.*, 2021). In addition, the study revealed a notable prevalence of the *bla*_{CTX-M} (69%), *bla*_{NDM-1} (75%), and *aadA* (75%) genes in the *E. coli* strain. The finding is contrary to the observations of Adefisoye and Okoh (2016), who reported a 30.4% prevalence of the *tetA* gene, a higher rate than the 25% observed in the study. The observation, however, was linked to treatment options and antibiotic usage in the region. The occurrence of MAR *E. coli* in treated wastewater and the Wupa River poses a significant public health risk to the community. Furthermore, the elevated multiple antibiotic resistance (MAR) indices and the presence of clinically relevant resistance genes (*bla*_{CTX-M}, *bla*_{NDM-1}, and *aadA*) suggest possible transmission to human and animal populations in the study area.

CONCLUSION

The discovery of multidrug-resistant *E. coli* possessing clinically significant resistance genes (*bla*_{CTX-M}, *bla*_{NDM-1}, *aadA*, and *tetA*)

highlighted the role of WWTPs in the accumulation and dissemination of these genes. Also, the prevalence of MDR *E. coli* with MAR indices above the high-risk threshold, along with the presence of carbapenemase genes in the environment, indicates that using water from the Wupa River for domestic, agricultural, and recreational purposes poses a significant public health risk. This study concurs with previous reports, indicating that the discharge of treated wastewater into the environment is a significant factor in the spread of antibiotic-resistant bacteria and resistance genes.

Many researchers have reported the efficiency of the Abuja WWTP in producing final effluent with physicochemical parameters that meet the discharge standard for the Wupa River (Akpen *et al.*, 2016; Chukwu and Oranu, 2018; Ibangha *et al.*, 2023; Abubakar *et al.*, 2025). Although Balogun and Ogwueleka (2021) reported higher total coliform and *E. coli* removal efficiency using secondary data from 2013 to 2017, recent studies reported bacteria such as total coliforms, *E. coli*, and *Vibrio* species in the final treated effluent, indicating inefficient removal (Ibangha *et al.*, 2023, 2025; Abubakar *et al.*, 2025). The Abuja WWTP tertiary treatment regime employs continuous-flow UV disinfection, a physical process that prevents the formation of harmful disinfection by-products that could pose ecological or human health risks. However, UV disinfection requires continuous electrical power, and high turbidity attenuates shortwave radiation, reducing its efficacy (Obayomi *et al.*, 2024). Maintenance and upgrade of Abuja WWTP and inclusion of other treatment processes, such as ozonation and membrane filtration in conjunction with UV radiation or application of advanced treatment options like advanced oxidation processes (AOPs), coagulation-flocculation, electrocoagulation, and nanotechnology can assist in high bacterial removal efficiency and prevention of public health risks associated with the discharge of multiple drug-resistant bacteria into the

aquatic system. In addition, water from the Wupa River should be treated to ensure it is

safe for domestic use and to protect the Zhidu community along the river.

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