

## Widespread occurrence of antibiotic resistance genes in bacterial isolates from River Ala in Akure, Nigeria

### ABSTRACT

Antibiotic resistance genes can be naturally occurring in bacteria or can be acquired through horizontal gene transfer. This study investigated the antibiotic resistance genes in bacterial isolates from River Ala. Water samples were collected bi-weekly over a period of 24 weeks from three representative points in River Ala. The load and identities of bacteria in the water samples were determined using standard microbiological methods. Antibiotic susceptibility pattern of the isolates were determined using disc diffusion method. Antibiotic resistance genes (ARGs) were detected using molecular methods. Results showed that the total plate count ranged from  $7.0 \times 10^1$  to  $1.3 \times 10^4$  cfu/100 ml and total enterobacteriaceae count ranged from  $9.2 \times 10^2$  to  $4.2 \times 10^4$  cfu/ 100 ml. The bacterial isolates detected in the samples were *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Enterobacter cloacae*. Antibiotic susceptibility pattern of the isolates revealed that all the bacterial isolates had 100% resistance to penicillin, while 92.86% of the isolates were resistant to nalidixic acid and ceporex. ARGs such as blaNDM, blaTEM, blaSHV and tetA were detected in *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae*. There were positive correlations between *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae*, ARGs such as blaNDM, blaTEM, blaSHV and tetA. The findings of this study demonstrated the presence of ARGs such as blaNDM, blaTEM, blaSHV and tetA in *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae* in water samples from River Ala. Understanding the presence and dynamics of these genes in aquatic environments is essential for managing public health risks.

**Keywords:** Antibiotic resistance genes, antibiotic susceptibility pattern, enterobacteriaceae.

### HIGHLIGHTS

1. Widespread of antibiotic resistance genes (ARGs) were investigated.
2. Load and identities of bacteria in the water samples were determined.
3. Antibiotic susceptibility pattern of the isolates were determined using disc diffusion method.
4. The relationships between pollution levels in the river and ARGs in the bacterial isolates were determined.
5. River Ala is a potential environmental reservoir for antibiotic resistant bacteria.

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## INTRODUCTION

Antibiotics are one of the most transformative discoveries in the history of medicine, revolutionizing the strategies for combating bacterial infections (Jamal *et al.*, 2023). It has saved many lives and significantly extended human life expectancy. Resistance occurs when bacteria evolve mechanisms to withstand antibiotics designed to inhibit or eliminate their cells (Amente *et al.*, 2023). This natural evolutionary process has been exacerbated by the widespread and often indiscriminate use of antibiotics in healthcare, agriculture, and veterinary medicine (Tiedje *et al.*, 2023). Antibiotic resistance genes can be naturally occurring in bacteria or can be acquired through horizontal gene transfer (Kavya *et al.*, 2023; Mancuso *et al.*, 2023).

As a result of the direct selection pressure that antibiotics exert on organisms carrying antibiotic resistance genes (ARGs), the transport pathways of antibiotic-resistant microorganisms and the ARGs that they carry are expected to be similar to the pathways of antibiotics used as pharmaceuticals. It is likely that ARGs persist further in the pathway, considering that in many cases they are maintained in the microbial populations even after the antibiotic selection pressure has been removed (Li *et al.*, 2023). Aquatic environments are known to be a reservoir for antibiotic-resistant bacteria (ARB), which are a major global public health concern due to their prevalence and dissemination (Muteeb *et al.*, 2023).

Antibiotics found in sewage and agricultural runoff, which result from the widespread and increased use of antibiotics, select for and enrich naturally occurring antibiotic-resistant genes (ARGs) and ARBs in the aquatic environment (Manaia *et al.*, 2024). Many of these bacteria are hosts of antibiotic resistance genes, water ecosystems are seriously contaminated by these antibiotic-resistant microorganisms (Mounzer *et al.*, 2024). Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, and multi-resistant pseudomonads are threat to ecological public health (Bai *et al.*, 2024). The use of antibiotics in medical settings, by veterinarians, and in animal husbandry may contribute to the promotion of antibiotic resistance and its genetic material transmission in bacteria (Duwor *et al.*, 2024).

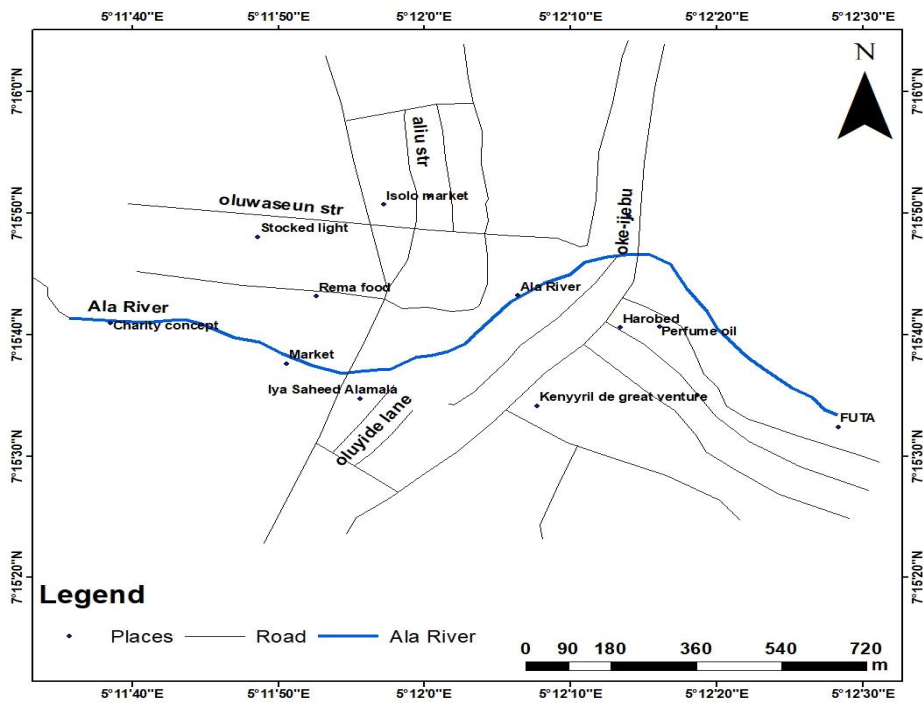
The degree of simplicity of the DNA that makes an organism resistant to antibiotics and the ease with which it can obtain DNA from other microbes are often linked to the development of antibiotic resistance (Muteeb *et al.*, 2023). Two essential components must come together for antibiotic resistance to form: an antibiotic that can inhibit most of the bacteria in a colony and a heterogeneous colony of bacteria, where at least one bacterium has a genetic determinant that can express antibiotic resistance (Tran *et al.*, 2024). Susceptible bacteria in the colony are inhibited, while resistant strains survive. The genetic determinants of the surviving bacteria specify the kind and level of resistance that the bacterial cell will express (Pepi and Focardi, 2021).

Antibiotic resistance can occur naturally (intrinsic) or be acquired, and it can spread both vertically and horizontally (Liang *et al.*, 2022). Antimicrobial resistance genes can be transferred from other bacteria to susceptible bacteria, or they can be acquired genetically (Djordjevic *et al.*, 2024). The study was aimed at determining the antibiotic resistance genes in bacterial isolates from River Ala.

## **METHODS**

### **Study area**

The study area is the upper region of River Ala catchment in Akure, Ondo State, Nigeria. The catchment lies between Latitudes 7° 14' N, and 7° 17' N, and Longitudes 5° 8' E and 5° 16' E covering a total area of 55 km<sup>2</sup> (Figure 1). The River Ala and its tributaries is one of the main tributaries of River Ogbese in Southwestern, Nigeria. River Ala has a total length of about 57 km of which 14.8 km traverses the thickly populated built up area of Akure Township. The river takes its source from northwestern part of Akure town and flow southeastern direction of the town. The study area experiences an intermittent rain fall between February and July with a short break in August and continues between September and November, with the heaviest rainfall in July. The river was selected due to its close proximity to sources of faecal contamination, its use for recreational activities as well as the use of the water for irrigation.



**Figure 1:** The location of River Ala in Akure, Ondo State, Nigeria

**Sample collection**

Water samples were collected during wet and dry season from the three sampling points. Sample collection was done at the three different sampling points bi-weekly for 24 weeks, 36 samples were collected in total for the study. Sterile bottles of 500 ml were used for sample collection. Samples for microbial analysis were collected aseptically, labelled and stored in ice packed plastic coolers and transported to the laboratory in the Department of Microbiology at the Federal University of Technology, Akure, Nigeria where analysis was done within one hour of collection.

**Determination of total plate count and total enterobacteriaceae count in the water samples**

Serial dilutions of the water samples were carried out aseptically up to  $10^{-4}$  dilution in order to obtain countable bacteria colonies on the agar plate. Dilution  $10^{-3}$  and  $10^{-4}$  were plated on different media (Mac agar, SSA, MFCA and MLSA). Colonies with different morphologies were observed on the plates and

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streaked out on Nutrient Agar plate for purification. Colonies were later stored at 4°C on Nutrient Agar (NA) slant.

#### **Biochemical identification of bacterial isolates from water samples from River Ala**

Biochemical tests such as Gram staining, catalase, oxidase, sugar fermentation, citrate, indole etc. were carried out on the bacterial isolates from the water samples (Dimri *et al.*, 2020).

#### **Enumeration of faecal coliforms in water samples from River Ala**

The concentrations of *Escherichia coli*, faecal coliforms, *Salmonella* and *Shigella* in the water samples were determined using standard microbiological methods. Using membrane filters (0.45 µm), the concentrations of the bacteria were determined by placing the filters on freshly prepared selective media: M-lauryl sulphate agar (MLSA), eosin methylene blue (EMB), membrane faecal coliform agar (m-FC agar) and *Salmonella-Shigella* agar (SSA). Agar plates were incubated at 37°C for 24 h (MLSA, EMB, SSA) and 44°C for 24 h (m-FC). Colonies were counted recorded and expressed as colony forming unit (CFU) per 100 ml of water utilizing a colony counter.

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#### **Determination of antibiotic susceptibility pattern of the isolates**

The antibiotics susceptibility of the isolates was determined by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The isolates were tested against ten antibiotic discs (Mast Diagnostics, UK) which comprised of ampicillin (PN), septrin (SXT) streptomycin (S), erythromycin (E), ciprofloxacin (CPX), ceporex (CEP), nalidixic acid (NA), azithromycin (AZM), augmentin (AU), and Gentamycin (CN). The inoculums were standardized by adjusting their densities to the turbidity of a Barium sulphate (BaSO<sub>4</sub>) (0.5 McFarland turbidity standard). 100 µl of bacterial suspension were spread-plated on Mueller-Hinton agar plates, the antibiotic discs were placed on the plates and plates were incubated for 24 hours at 37°C. After incubation, zones of inhibition (mm) were measured and results obtained were used to classify isolates as resistant, intermediate resistant, or susceptible using standard reference values according to Clinical and Laboratory Standards Institute (CLSI) (Ardila *et al.*, 2023).

#### **Detection of antibiotic resistant genes (ARGs) in bacterial isolates**

Primer sequences described by Ismael and Nasser (2017) were adopted. Reaction cocktail used for all PCR per primer set included (Reagent Volume µl) - 5X PCR SYBR green buffer (2.5), MgCl<sub>2</sub> (0.75), 10pM DNTP (0.25), 10pM of each forward and backwards primer (0.25), 8000U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to which 2 µl template was added. Buffer control was also added to eliminate any probability of false amplification Table below shows the primer sequence and PCR

profile used in amplifying each fragment. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair (Table 1).

**Table 1: Primers used for detection of antibiotic resistance genes**

Multiplex	Gene	Primer	Primer sequence 5'-3'	Profile
<b>Multiplex1</b>	blaVIM	VIM F	TCGTTTGAAGAAGTTAACG	An initial denaturing 5min at 94°C, then 35 cycles of 94°C for 30s, 50°C for 40s 72°C for 40s and terminate at 72°C for 10min
	blaNDM	VIM R	ATGTAAGTTTCAAGAGTGATGC	
		NDM F	GGTGTGGTTCGCATATCGCAA	
<b>Multiplex2</b>	blaIMP	NDM R	ATTCAGCCAGATCGGCATCGGC	
		IMP F	GGTTTGGCGATCTGGTTTTTC	
	blaKPC	IMP R	CGGAATGGCTCATCACGATC	
		KPCF	CATTCAAGGGCTTTCTTGCTGC	
	blaOXA	KPCR	ACGACGGCATAGTCATTTGC	
<b>Multiplex3</b>	blaTem	OXA R	TTCTGTGTGGTTGGGTTTCGC	
		OXA R	ACGCAGGAATTGAATTTGTT	
	blaSHV	Tem F	GTCGCCGCATACACTATTCTCA	
		Tem R	CGCTCGTCGTTTGGTATGG	
<b>Multiplex4</b>	tet(A)	SHV F	GCCTTGACCGCTGGGAAAC	
		SHV R	GGCGTATCCCGCAGATAAAT	
	tet(B)	tet(A)F	GGTTCACTCGAACGACGTCA	
		tet(A)R	CTGTCCGACAAGTTGCATGA	
VanA	tet(B)F	CCTCAGCTTCTCAACGCGTG		
	tet(B)R	GCACCTTGCTCATGACTCT		
<b>Multiplex5</b>	VanB	VanAF	TCTGCAATAGAGATAGCCGC	
		VanAR	GGAGTAGCTATCCCAGCATT	
	VanB	VanBF	ATGGGAAGCCGATAGTC	
		VanBR	GATTTTCGTTCTCGACC	

#### Statistical analysis

All data obtained from the study were subjected to descriptive statistics. Two-way Analysis of Variance (ANOVA) were carried out using SPSS version 22 (IBM, NY) and means were separated using Duncan's

New Multiple Range Test at 95% confidence interval. The relationship between the pollution levels and ARGs were determined using Pearson correlation coefficients.

## RESULTS

### Total plate count and total enterobacteriaceae count in water samples from River Ala

The total plate count (TPC) ranged from  $7.0 \times 10^1$  cfu/100 ml to  $1.67 \times 10^4$  cfu/100 ml. Sample 9 had the highest TPC of  $1.67 \times 10^4$  cfu/100 ml, while sample 3 had the lowest TPC of  $7.0 \times 10^1$  cfu/100 ml and the total enterobacteriaceae count (TEC) ranged from  $9.2 \times 10^2$  cfu/100 ml to  $4.17 \times 10^4$  cfu/100 ml. Sample 12 had the highest TEC of  $4.17 \times 10^4$  cfu/100 ml, while Sample 1 exhibited the lowest TEC of  $9.2 \times 10^2$  cfu/100 ml (Table 2).

### Morphological and biochemical characteristics of bacterial isolates in water samples from River Ala

The morphological characteristics of the bacterial isolates from the water samples such as size, shape, texture, opacity, margin, colour and elevation were determined while the biochemical tests carried out on the bacterial isolates revealed the presence of *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumonia*, *Proteus mirabilis* and *Enterobacter cloacae* in the water samples (Table 3 and Table 4).

**Table 2: Total plate count and total enterobacteriaceae count in water samples from River Ala**

Samples	Total plate count (cfu/100 ml)	Total enterobacteriaceae count (cfu/100 ml)
1	$5.3 \times 10^2$	$9.2 \times 10^2$
2	$2.0 \times 10^3$	$4.9 \times 10^3$
3	$7.0 \times 10^1$	$2.1 \times 10^3$
4	$3.9 \times 10^3$	$9.5 \times 10^3$
5	$3.9 \times 10^3$	$9.7 \times 10^3$
6	$3.7 \times 10^3$	$1.9 \times 10^4$
7	$1.0 \times 10^2$	$9.9 \times 10^2$
8	$2.5 \times 10^3$	$8.8 \times 10^3$
9	$1.7 \times 10^4$	$2.7 \times 10^4$
10	$5.0 \times 10^2$	$3.3 \times 10^3$

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11	$9.8 \times 10^3$	$3.2 \times 10^4$
12	$1.3 \times 10^4$	$4.2 \times 10^4$

**Table 3: Morphological characteristics of bacterial isolates in water from River Ala**

Morphological Tests							
Sample	Size	Shape	Texture	Opacity	Margin	Colour	Elevation
1	medium	circular	shiny	translucent	entire	pink	flat
2	medium	rhizoid	dry	transparent	irregular	cream	flat
5	small	circular	dry	translucent	entire	black	flat
6	small	circular	shiny	opaque	entire	cream	flat
9	small	circular	dry	opaque	irregular	cream	flat
11	large	irregular	dry	opaque	irregular	cream	flat
23	small	irregular	dry	opaque	irregular	cream	flat
24	small	circular	dry	opaque	entire	cream	flat
26	medium	rhizoid	dry	transparent	irregular	cream	flat
29	large	irregular	dry	opaque	irregular	cream	flat
31	medium	circular	shiny	translucent	entire	cream	flat
32	medium	circular	shiny	transparent	entire	cream	raised
35	small	circular	shiny	translucent	entire	pink	flat
36	small	irregular	shiny	translucent	irregular	pink	flat
37	small	irregular	shiny	translucent	irregular	pink	flat
38	small	irregular	shiny	opaque	entire	cream	raised
39	small	circular	shiny	opaque	entire	cream	raised
40	small	circular	dry	opaque	entire	cream	flat
41	small	circular	dry	opaque	entire	cream	flat

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**Table 4: Biochemical characteristics of bacterial isolates in water from River Ala**

Biochemical Tests													
Sample	Shape											Probable Organisms	
		G-RXT	Motility	Catalase	Oxidase	Citrate	Indole	Glucose	Lactose	Sucrose	MR		VP
1	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	<i>Escherichia coli</i>
2	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	<i>Escherichia coli</i>
5	rod	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	<i>Samonella enterica</i>
6	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	<i>Escherichia coli</i>
9	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	<i>Escherichia coli</i>
11	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	<i>Escherichia coli</i>
23	rod	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	<i>Enterobacter cloacae</i>
24	rod	-ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve	<i>Proteus mirabilis</i>
26	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	<i>Klebsiella pneumonia</i>
29	rod	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	<i>Samonella enterica</i>
31	rod	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve	<i>Klebsiella pneumonia</i>
32	rod	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	<i>Proteus mirabilis</i>
35	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	<i>Escherichia coli</i>

36	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	<i>Escherichia coli</i>
37	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	<i>Escherichia coli</i>
38	rod	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	<i>Enterobacter cloacae</i>
39	rod	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	<i>Enterobacter cloacae</i>
40	rod	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	<i>Enterobacter cloacae</i>
41	rod	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	<i>Enterobacter cloacae</i>

Keys: G-RXT- Gram reaction, MR- Methyl red, VP- Vogues-Proskauer

### Load of bacteria in water samples from River Ala

The mean load of faecal coliforms ranged from  $1.1 \times 10^2$  to  $1.2 \times 10^4$  cfu/100 ml. The highest was recorded in July ( $1.2 \times 10^4$  cfu/100 ml. while the least was recorded in February ( $1.1 \times 10^2$  cfu/100 ml) (Figure 2). The mean load of *E. coli* ranged from  $5.3 \times 10^2$  to  $1.1 \times 10^4$  cfu/100 ml. The highest was recorded in July ( $1.1 \times 10^4$  cfu/100 ml), while the least was recorded in February ( $5.3 \times 10^2$  cfu/100 ml) (Figure 3). The mean load of *Salmonella* spp. ranged from  $2 \times 10^2$  to  $1.3 \times 10^4$  cfu/100 ml. The highest was recorded in July ( $1.3 \times 10^4$  cfu/100 ml), while the least was recorded in February ( $2 \times 10^2$  cfu/100 ml) (Figure 4). The mean load of *Shigella* spp. ranged from  $0.8 \times 10^2$  to  $1.3 \times 10^3$  cfu/100 ml. The highest was recorded in July ( $1.3 \times 10^3$  cfu/100 ml), while the least was recorded in February ( $0.8 \times 10^2$  cfu/100 ml) (Figure 5).

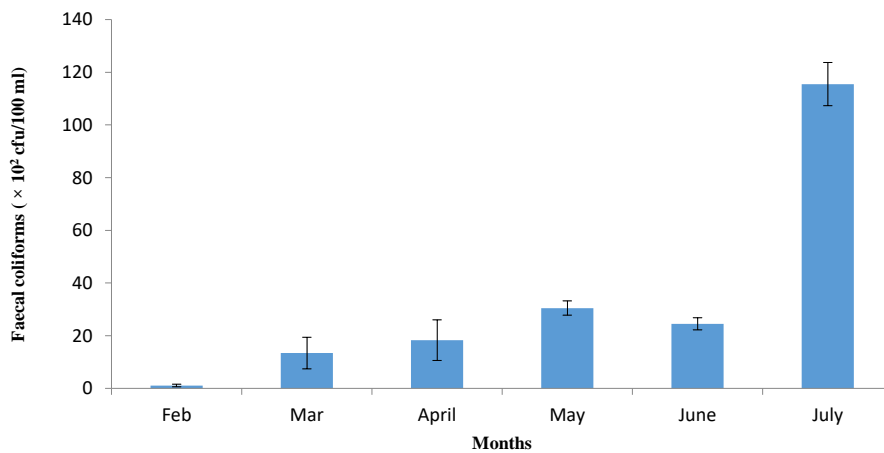
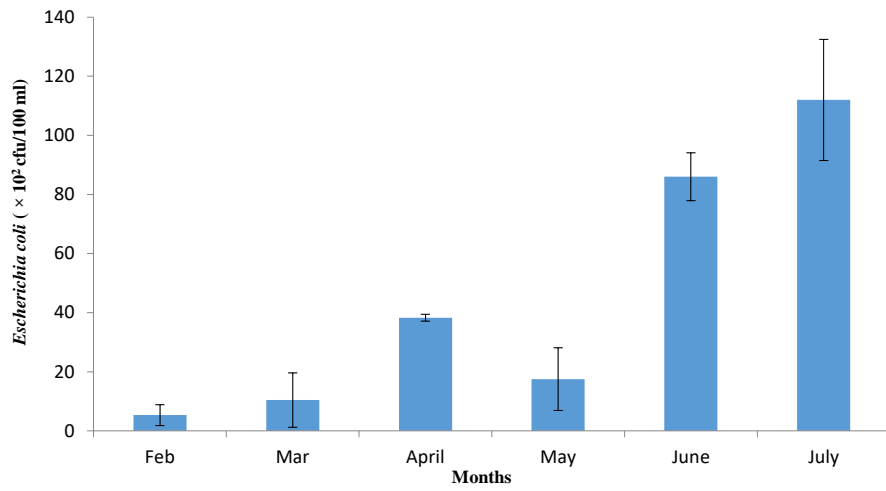
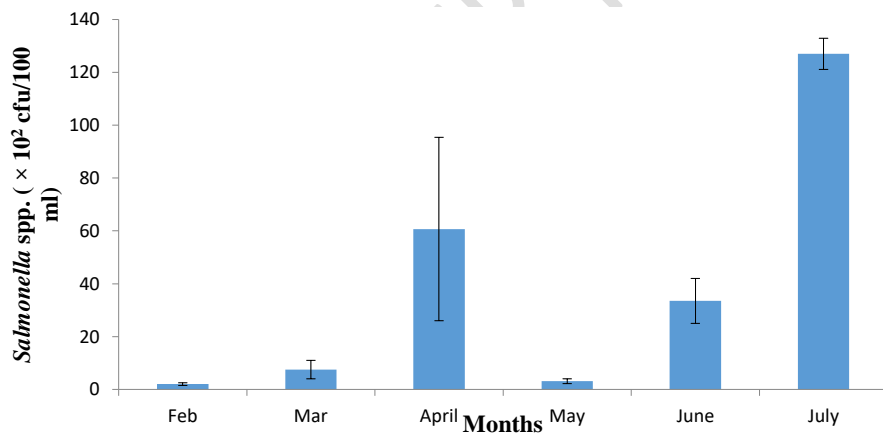


Figure 2: Mean load of faecal coliforms in water samples from River Ala (n=36)

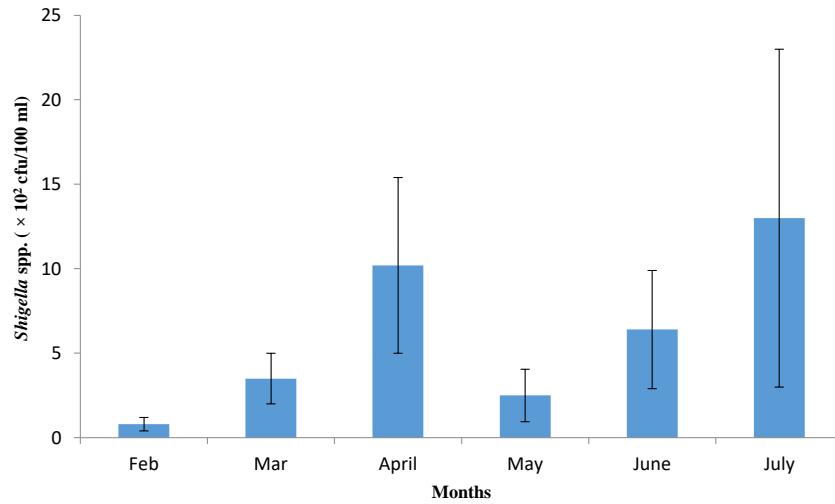
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**Figure 3:** Mean load of *Escherichia coli* in water samples from River Ala (n=36)



**Figure 4:** Mean load of *Salmonella* spp. in water samples from River Ala (n=36)

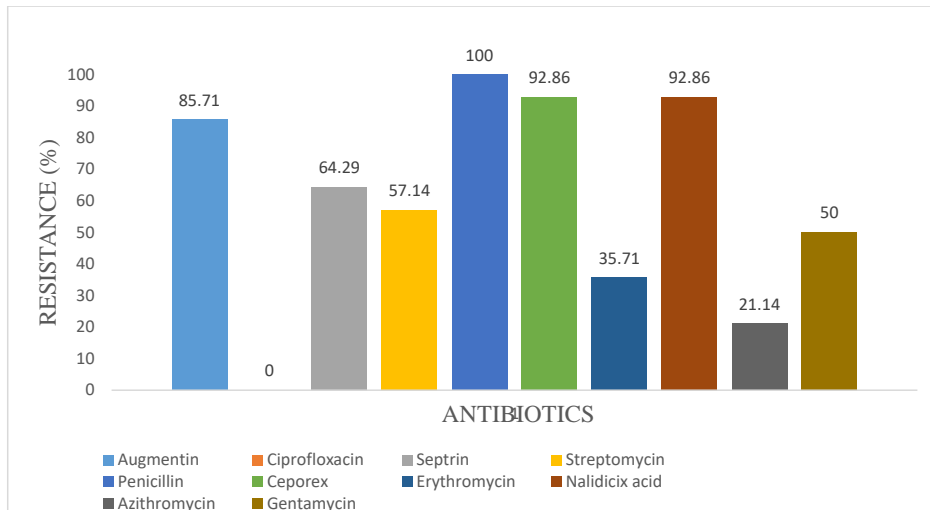


**Figure 5:** Mean load of *Shigella* spp. in water samples from River Ala (n=36)

**Antibiotic resistance pattern of bacterial isolates from River Ala**

The antibiotic resistance patterns observed in bacterial isolates from River Ala against ten (10) different antibiotics with varying resistance rates to commonly used antibiotics such as penicillin (100%), ceporex (92.86%), nalidixic acid (92.86%), augmentin (85.71%) (Figure 6).

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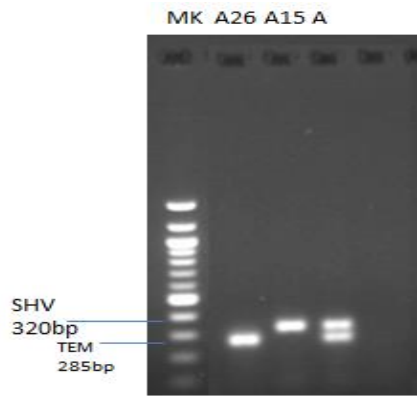
**Figure 6:** Antibiotic resistance pattern of the bacterial isolates against 10 different antibiotics

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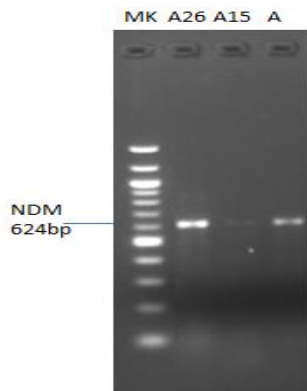
#### ARGs in bacterial isolates in water samples from River Ala

The antibiotic resistant genes tetA, tetB, blaVIM, blaNDM, blaTEM, blaSHV, vanA and vanB were detected in bacteria isolated from River Ala (Table 5). The gel electrophoresis of the PCR products showed positive amplification of the ESBL TEM gene in samples A26 and A and the SHV gene in samples A15 and A by the presence of specific DNA bands at the expected sizes (Plate 1). This confirms the presence of these resistance genes in the respective bacterial isolates. The agarose gel electrophoresis shows positive amplification of the ESBL NDM gene (624 bp) in samples A26 and A, indicating the presence of this resistance gene in these isolates. The absence of a 502 bp band in all samples indicates that the VIM gene is not present in any of the tested bacterial isolates (Plate 2).

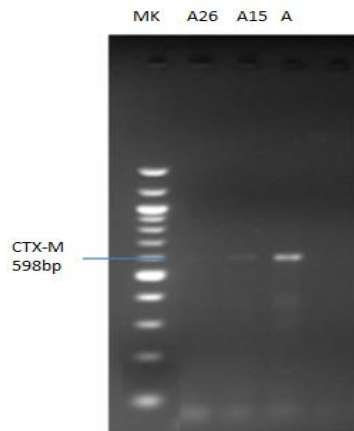
The agarose gel electrophoresis shows positive amplification of the ESBL CTX-M gene (598 bp) in samples A and A15, indicating the presence of this resistance gene in these isolates. No band is present in sample A26, indicating it is negative for the CTX-M gene (Plate 3). The agarose gel electrophoresis shows positive amplification of the tetracycline resistance gene tetA (577 bp) in samples A15 and A, indicating the presence of this resistance gene in these isolates (Plate 4). No band at 634 bp indicates that the tetB gene is absent in all tested samples. The multiplex electrophoresis of the PCR products shows no bands at 400 bp or 740 bp, indicating that neither the vanA gene (400 bp) nor the vanB gene (740 bp) was amplified in any of the bacterial isolates (Plate 5). This shows the absence of both vanA and vanB resistance genes in the tested samples.



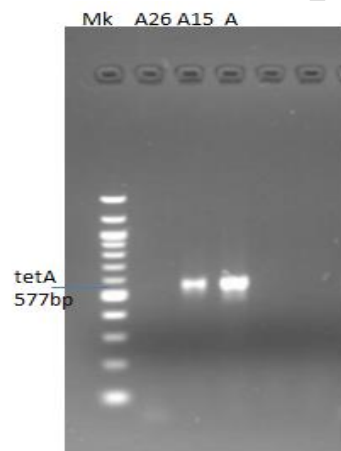
**Plate 1:** Gel image indicates a positive of TEM in sample A26 and A and SHV in A15 and A. (A- *Escherichia coli*, A26- *Salmonella enterica* and A15- *Enterobacter cloacae*)



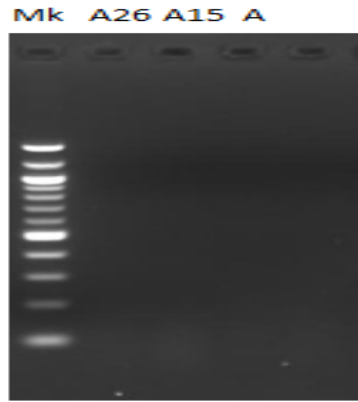
**Plate 2:** Gel image indicates a positive of NDM in samples A26 and A while VIM absent in all samples. (A- *Escherichia coli*, A26- *Salmonella enterica* and A15- *Enterobacter cloacae*)



**Plate 3:** Gel image indicates a positive of CTX-M in only sample A and A15 but negative in A26. (A- *Escherichia coli*, A26- *Salmonella enterica* and A15- *Enterobacter cloacae*)



**Plate 4:** Gel image indicates a positive of tetA in only sample A15 and A and tetB gene in none of the sample. (A- *Escherichia coli*, A26- *Salmonella enterica* and A15- *Enterobacter cloacae*)



**Plate 5:** Gel image indicates no amplification for vanA and vanB gene. (A- *Escherichia coli*, A26- *Salmonella enterica* and A15- *Enterobacter cloacae*)

**Table 5: ARGs in bacterial isolates in water samples from River Ala**

Bacterial/strain ID	blaVIM	blaNDM	blaTEM	blaSHV	blaCTX-M	tetA	tetB	vanA	vanB
<i>Escherichia coli</i> (A)	-	+	+	+	+	+	-	-	-
<i>Salmonella enterica</i> (A26)	-	+	+	-	-	-	-	-	-
<i>Enterobacter cloacae</i> (A15)	-	-	-	+	+	+	-	-	-

**Keys:** Present (+), Absent (-) Note: Only isolates that gave a positive signal in at least one PCR experiment were included in this table.

#### ARGs and enteric bacterial isolated from River Ala

The bacterial strains isolated from River Ala exhibited a high prevalence of antibiotic resistance genes. The antibiotic resistant genes tetA, blaVIM, blaNDM, blaTEM and blaSHV were detected in 66.67% of the bacterial isolates (Figure 7). blaNDM showed negative correlation with *Escherichia coli* and *Salmonella* spp. blaTEM showed a significant positive correlation with *Escherichia coli*, *Salmonella* spp. and *Enterobacter cloacae*. blaCTX and tetA showed a significant positive correlation with *Escherichia coli* and *Enterobacter cloacae* (Table 6).

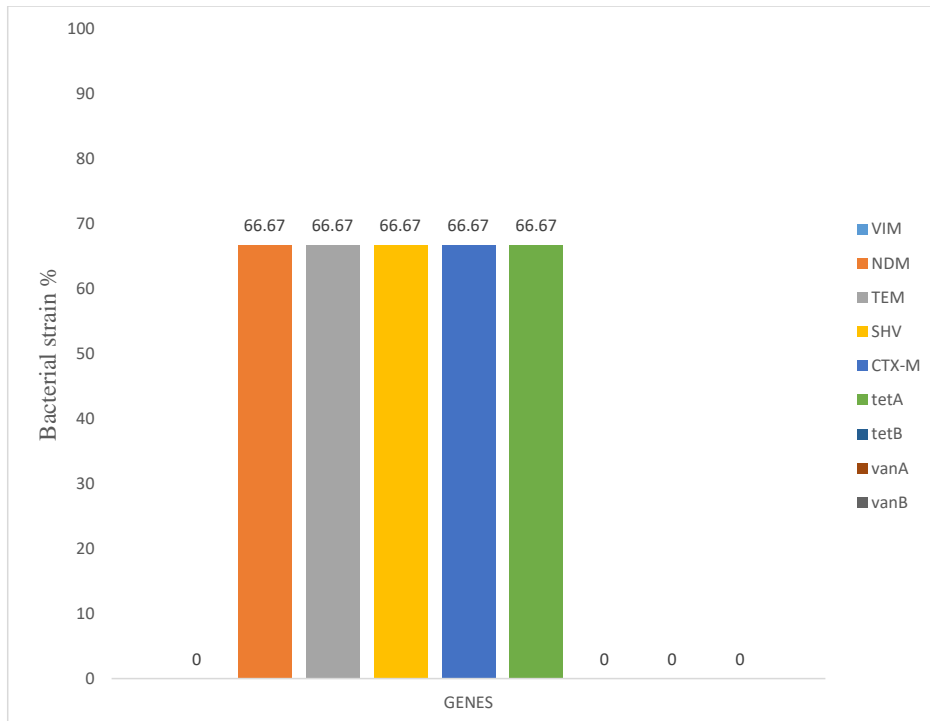


Figure 7: Percentage of bacterial strain in ARGs.

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Table 6: Correlation between the loads of enteric bacteria and ARGs in water samples from River Ala

	blaNDM	blaTEM	blaSHV	blaCTX	tetA
<i>Escherichia coli</i>	<b>-0.56**</b>	<b>0.71**</b>	0.39	<b>0.58**</b>	<b>0.93**</b>
<i>Salmonella</i> spp.	<b>-0.55**</b>	<b>0.63**</b>	0.19	0.38	-0.11
<i>Shigella</i> spp.	-0.21	0.48*	0.34	-0.28	0.31
Faecal coliform	0.25	0.32	0.23	-0.44	-0.36
<i>Enterobacter cloacae</i>	0.32	<b>0.58**</b>	0.37	<b>0.62**</b>	<b>0.81**</b>

Keys: \*\*Correlation is significant at the 0.01 level (2-tailed)

\*Correlation is significant at the 0.05 level (2-tailed)

## DISCUSSION

High total plate counts and total enterobacteriaceae counts suggests bacterial contamination. This is in agreement with Kilonzo-Nthenge *et al.* (2018) where the authors reported high bacterial loads to poor sanitary conditions and faecal pollution in river. Morphological characteristics and biochemical tests, confirmed the presence of *Escherichia coli*, *Salmonella enterica*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Enterobacter cloacae*. The result of this study is in line with Hanna *et al.* (2020), who reported a significant presence of *E. coli*, *Salmonella*, *Klebsiella* and *Enterobacter* in river samples due to higher occurrence of thermotolerant (faecal) coliform in temperate environments, contrasting with the infrequent occurrence of *Escherichia coli*. The presence of *E. coli* and faecal coliforms in the water samples is in line with Hanna *et al.* (2020), who reported a significant presence of *E. coli* (faecal coliform) in river water samples.

High faecal coliforms counts in water samples was as a result of the weather change in Nigeria characterized by intermittent rain. This observation is in agreement with Adenola *et al.* (2021) and Egberongbe *et al.* (2021) where the authors reported high faecal coliform counts in surface waters during periods of heavy rainfall. High mean load of *Escherichia coli* in water samples indicates indiscriminate discharge of sewage. Borja-Serrano *et al.* (2020) reported that the presence of higher load of *Escherichia coli* was attributed to the polluted water conditions. The high mean loads of *Salmonella* spp. observed in the water samples suggests direct discharge of waste water. Akrong *et al.* (2019) reported that high loads of *Salmonella* was as a result of heavy rainfall, land run-off and direct discharge of untreated or partially treated wastewater into the water sources.

Antibiotic susceptibility pattern of the isolates revealed that all bacterial isolates were 100% resistance to penicillin. This is in agreement with Tesfaye *et al.* (2019) where authors reported high *E.coli*, *Salmonella* and *Enterobacter* were resistance penicillin, while 92.86% of the isolates were resistant to nalidixic acid and cefepex. 85.71% of the isolates were resistant to augmentin, 64.29% of the isolates were resistant to septrin, and 57.14% of the isolates were resistant to streptomycin, while 50% of the isolates were resistant to gentamycin. Also, 35.71% of the bacterial isolates were resistant to erythromycin and the 21.14% of the isolates were resistant to azithromycin which is in line with Bamidele *et al.* (2022) who reported a low rate of resistance.

Resistance to tetracyclines is often associated with the presence of the tetA and tetB genes, which encode efflux pumps that actively expel tetracycline from bacterial cells, the moderate resistance rates to other antibiotics like streptomycin (57.14%) and septrin (64.29%) suggest the potential presence of these genes. Li *et al.* (2024), reported a high prevalence of tetA and tetB in environmental bacterial isolates from water bodies contaminated by agricultural runoff and sewage. The authors noted that the wide usage of tetracyclines in animal farming had led to the enrichment of tet genes in aquatic environments. Sung *et al.* (2024) further corroborated these findings, showing over 70% of bacterial isolates from rivers in agricultural areas harboring tetA or tetB, with corresponding high levels of tetracycline resistance (80-90%).

Beta-lactam resistance genes, including blaVIM, blaNDM, blaTEM, and blaSHV, play critical roles in the high resistance observed to penicillin (100%), ceporex (92.86%), and augmentin (85.71%). These genes encode beta-lactamases, enzymes that hydrolyze beta-lactam antibiotics and render them ineffective. blaTEM and blaSHV genes are commonly associated with resistance to penicillins and first-generation cephalosporins, explaining the near-complete resistance observed in this study. Taha *et al.* (2023) reported a 90% prevalence of blaTEM in bacterial isolates from hospital wastewater, which correlated with over 85% resistance to beta-lactam antibiotics, including penicillin and amoxicillin. Similarly, Rana *et al.* (2024) found that blaTEM and blaSHV were prevalent in over 80% of environmental isolates from wastewater treatment plants, contributing to the widespread beta-lactam resistance observed in aquatic environments.

blaNDM (New Delhi metallo-beta-lactamase) and blaVIM (Verona integron-encoded metallo-beta-lactamase) genes are primarily linked to carbapenem resistance but are often co-located with other resistance genes. Javid and Ahmed (2024) found a rising occurrence of blaNDM in bacteria isolated from rivers near healthcare facilities, with co-resistance to multiple beta-lactams, including cephalosporins like ceporex. This supports the high resistance rates observed in this study, where ceporex resistance was at 92.86%. Zhang *et al.* (2024) also observed the co-occurrence of blaVIM and blaNDM in bacterial isolates from rivers in urban areas of China, contributing to multidrug resistance and posing a significant threat to public health.

ARGs such as blaNDM, blaTEM, blaSHV, blaCTX and tetA were detected in *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae*. Li *et al.* (2024) highlighted the role of these elements in horizontal gene transfer, facilitating the spread of antibiotic resistance. *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae* that showed positive correlation with blaNDM, blaTEM, blaCTX and tetA may be as a result of waste disposed in river water (An *et al.*, 2024). In contrast, *Shigella* spp. and faecal coliforms

exhibit weaker correlations between ARGs and MGEs, particularly for blaNDM and blaCTX. Zhao *et al.* (2021) noted that the environment plays a significant role in the maintenance and dissemination of ARGs, with factors such as microbial community composition and the presence of antibiotics in the environment influencing the dynamics of ARG transfer. Zhao *et al.* (2020) suggested that the presence of ARGs in environmental bacteria is often linked to the local selective pressures, such as the concentration of antibiotics in the environment, which can vary significantly.

Recent studies consistently highlight the interplay between human activities e.g., agriculture, healthcare, and wastewater discharge and the dissemination of ARGs in aquatic environments. The results of this study align with these findings, particularly the widespread resistance to beta-lactams, aminoglycosides, and other antibiotics, suggesting that River Ala, like many other water bodies, serves as a reservoir for ARGs. Beshiru *et al.* (2024) demonstrated that the presence of ARGs, particularly blaNDM and tetA, in bacterial isolates from rivers in Nigeria correlates with high levels of multidrug resistance. The authors linked this trend to the poor regulation of antibiotic usage in healthcare and agriculture, which contaminates water bodies with antibiotic residues and resistant bacteria. Blanco-Peña *et al.* (2024) reported similar findings in their assessment of ARG prevalence in river water used for irrigation in Latin America, where blaTEM, blaSHV, and tetB were among the most common ARGs detected. Their study found over 90% resistance to first-generation cephalosporins, in line with the high resistance levels to ceporex observed in River Ala. Barathan *et al.* (2024) also noted the spread of ARGs like blaNDM and blaVIM in water bodies near industrial areas in Southeast Asia, underscoring the global nature of the ARG problem. The authors emphasized the importance of monitoring water quality and implementing stricter antibiotic regulations to curb the environmental spread of ARGs.

The negative correlation observed between blaNDM and *E. coli* and *Salmonella spp.* suggests that these bacterial species are less likely to harbor this particular carbapenem resistance gene in this environment. This finding is consistent with Chen *et al.* (2023), who reported that blaNDM is prevalent in environmental and clinical settings. Similarly, Khaledi *et al.* (2024) found that in Nigerian rivers, *Salmonella spp.* were less likely to carry blaNDM, suggesting environmental factors or species-specific genetic barriers limiting the spread of this gene in these bacterial populations.

The negative correlation may also indicate selective pressures where other beta-lactamase genes like blaTEM and blaCTX are more dominant in *E. coli* and *Salmonella spp.*, reducing the likelihood of co-occurrence with blaNDM in these species. This supports the theory that different bacterial species may prefer certain resistance genes based on ecological and evolutionary factors, as observed by Zhang *et al.*

(2023), who noted that *E. coli* strains often prioritize extended-spectrum beta-lactamase (ESBL) genes over metallo-beta-lactamases like blaNDM.

The significant positive correlation of blaTEM with *E. coli*, *Salmonella spp.*, and *Enterobacter cloacae* is consistent with a growing body of evidence. Aslan *et al.* (2024) reported that blaTEM was highly prevalent in *E. coli* and *Enterobacter cloacae* from wastewater and river systems, the widespread distribution of blaTEM is linked to the ability of *E. coli* and *Salmonella* to efficiently acquire and disseminate plasmids carrying this gene.

The positive association with *Enterobacter cloacae* underscores the role of this gene in promoting resistance across various Enterobacteriaceae species. According to Molina *et al.* (2024), blaTEM is often found in plasmids or integrons that are highly mobile among *E. coli*, *Salmonella spp.*, and *Enterobacter spp.*, contributing to the rapid spread of resistance in both clinical and environmental settings.

The blaCTX gene showed a significant positive correlation with *E. coli* and *Enterobacter cloacae*, indicating its widespread presence in these bacteria. This finding aligns with Bhattacharya *et al.* (2024), who found that blaCTX was highly prevalent in *E. coli* and *Enterobacter spp.* isolated from rivers contaminated with hospital and industrial effluents. The positive correlation suggests that both bacterial species serve as major reservoirs for blaCTX, facilitating the dissemination of ESBLs in aquatic environments.

Similarly, the positive correlation of tetA with *E. coli* and *Enterobacter cloacae* indicates a strong association with tetracycline resistance. The tetA gene encodes an efflux pump that confers resistance to tetracyclines, and its presence in both bacterial species is consistent with findings from Ramírez-Castillo *et al.* (2023), who reported high levels of tetA in Enterobacteriaceae isolated from agricultural runoff and polluted rivers. The combination of blaCTX and tetA in *E. coli* and *Enterobacter* points to their co-selection in environments where both beta-lactam and tetracycline antibiotics are present, driving multidrug resistance in these species.

## CONCLUSION

The findings of this study demonstrated high total plate count and total enterobacteriaceae count in the water samples from River Ala. *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae*, *Proteus mirabilis* and *Klebsiella pneumonia* were the predominant bacterial genera. All bacterial isolates exhibited

**Commented [NA12]:** State the recommendations that should be followed when using this river water after the results obtained.

marked resistance to penicillin. blaNDM, blaTEM, blaSHV, tetA were the antibiotic resistance genes detected in the isolates. *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae* had positive correlation with antibiotic resistance genes.

## DECLARATIONS

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable

### Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## REFERENCES

1. Adenola, O. J., Olalemi, A. O. and Ogundare, A. O. (2021). Antibacterial effect of *Nymphaea lotus* (Linn) extracts on enteric bacteria isolated from River Ogbese, Nigeria. *Journal of Advances in Microbiology*, 21(11): 65-87.
2. Akinwale, I. O., Elabor, I. and Alaiya, G. I. (2022). Assessment and Spatio-seasonal Variation of Physicochemical Parameters and Heavy Metals in the Warri River, Delta State, Nigeria. *Advanced in Analytical Chemistry*, 12(1): 1-6.
3. Akpe, A. R., Femi, I. J., Okwu, G. I. and Obiazi, H. (2018). Seasonal variations in the physicochemical parameters of Ikpoba river water samples. *International Journal of Life Science Resources*, 2455: 1716-1725.
4. Akrong, M. O., Amu-Mensah, F. K., Amu-Mensah, M. A., Darko, H., Addico, G. N. D. and Ampofo, J. A. (2019). Seasonal analysis of bacteriological quality of drinking water sources in communities surrounding Lake Bosomtwe in the Ashanti Region of Ghana. *Applied Water Science*, 9:1-6.
5. Amente, D. T., Mulatu, H., and Shafi, W. (2023). Review on Ethical Approaches to Antimicrobial Resistance. *J Trop Dis*, 11, 377.
6. An, L., Wu, Y., Zhang, B., Xu, Q., Liao, L., Wu, S. and Chen, J. (2024). Transmission chains and molecular characterizations of extended-spectrum  $\beta$ -lactamase producing Enterobacteriaceae at a veterinary hospital in Chengdu, China. *Infection, Genetics and Evolution*, 124, 105658.

7. Ardila, C. M., Zuluaga-Gómez, M., and Vivares-Builes, A. M. (2023). Applications of Lab on a Chip in Antimicrobial Susceptibility of *Staphylococcus aureus*: A Systematic Review. *Medicina*, 59(10), 1719.
8. Aslan, S., Demir, C., Kurtoğlu, E. L., Altındış, M., Kurtoğlu, E., and ALTINDIŞ, M. (2024). Antibiotic Resistance Profiles of *Escherichia coli* and *Salmonella* spp. Isolated From Dairy Farms and Surroundings in a Rural Area of Western Anatolia, Turkey. *Cureus*, 16(8).
9. Bai, H. J., Geng, Q. F., Jin, F., and Yang, Y. L. (2024). Epidemiologic analysis of antimicrobial resistance in hospital departments in China from 2022 to 2023. *Journal of Health, Population and Nutrition*, 43(1), 1-11.
10. Bamidele, O., Yakubu, A., Joseph, E. B., and Amole, T. A. (2022). Antibiotic resistance of bacterial isolates from smallholder poultry droppings in the Guinea savanna zone of Nigeria. *Antibiotics*, 11(7), 973.
11. Barathan, M., Ng, S. L., Lokanathan, Y., Ng, M. H. and Law, J. X. (2024). Unseen Weapons: Bacterial Extracellular Vesicles and the Spread of Antibiotic Resistance in Aquatic Environments. *International Journal of Molecular Sciences*, 25(6), 3080.
12. Beshiru, A., Isokpehi, N. A., Igbinsola, I. H., Akinnibosun, O., Ogofure, A. G. and Igbinsola, E. O. (2024). Extended-spectrum beta-lactamase (ESBL)-and non-ESBL producing *Escherichia coli* surveillance in surface water sources in Edo State, Nigeria: a public health concern. *Scientific Reports*, 14(1), 21658.
13. Bhattacharya, C., Dinc, M. T., Basu, S., Chaudhuri, R., Fu, C., Cervantes, G. and Mason, C. E. (2024). Role and Relation of Microbiome to Address Antimicrobial Resistance. In *Antimicrobial Resistance: Factors to Findings: Omics and Systems Biology Approaches* (pp. 179-237). Cham: Springer International Publishing.
14. Blanco-Peña, K., Quesada-Alvarado, F., Salas-González, D., Estrada-König, S., Salom-Pérez, R., Arroyo-Arce, S. and Chaverri-Fonseca, F. (2024). A multidisciplinary approach to analyze the antimicrobial resistance in natural ecosystems. *Environmental Research*, 251, 118549.
15. Borja-Serrano, P., Ochoa-Herrera, V., Maurice, L., Morales, G. and Cristian, M. (2020). Determination of the microbial and chemical loads in rivers from the Quito capital province of Ecuador (Pichincha)-A preliminary analysis of microbial and chemical quality of the main river. *International Journal of Environmental Research and Public Health*, 17(14): 5048.
16. Chen, K., Xie, M., Dong, N., Chan, E. W. C., Zhang, R. and Chen, S. (2023). Deciphering mechanisms of bla<sub>NDM</sub> gene transmission between human and animals: a genomics study of bacterial isolates from various sources in China, 2015 to 2017. *Eurosurveillance*, 28(37), 2200925.

17. Dimri, A. G., Chaudhary, S., Singh, D., Chauhan, A., and Aggarwal, M. L. (2020). Morphological and biochemical characterization of food borne Gram-positive and Gram-negative bacteria. *Science Archives*, 1(1), 16-23.
18. Djordjevic, S. P., Jarocki, V. M., Seemann, T., Cummins, M. L., Watt, A. E., Drigo, B., and Howden, B. P. (2024). Genomic surveillance for antimicrobial resistance—a One Health perspective. *Nature Reviews Genetics*, 25(2), 142-157.
19. Duwor, S., Brites, D., and Mäser, P. (2024). Phylogenetic Analysis of Pyruvate-Ferredoxin Oxidoreductase, a Redox Enzyme Involved in the Pharmacological Activation of Nitro-Based Prodrugs in Bacteria and Protozoa. *Biology*, 13(3), 178.
20. Egberongbe, H. O., Bankole, M. O., Popoola, T. O. S. and Olowofeso, O. (2021). Seasonal variation of enteric bacteria population in surface water sources among rural communities of Ijebu North, Ogun State, Nigeria. *Agro-Science*, 20(2): 81-85.
21. Eliku, T. and Leta, S. (2018). Spatial and seasonal variation in physicochemical parameters and heavy metals in Awash River, Ethiopia. *Applied Water Science*, 8, 1-13.
22. Hanna, N., Purohit, M., Diwan, V., Chandran, S. P., Riggi, E., Parashar, V. and Lundborg, C. S. (2020). Monitoring of water quality, antibiotic residues, and antibiotic-resistant escherichia coli in the kshipra river in india over a 3-year period. *International Journal of Environmental Research and Public Health*, 17(21), 7706.
23. Ismaeel, G. K., and Nasser, H. H. (2017). Molecular determination of extended spectrum  $\beta$ -lactamases antibiotics resistance genes in *E. coli* isolated from diarrhea in cattle. *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, 16(1), 67-73.
24. Jamal, A. (2023). Antibiotics in Contemporary Medicine: Advances, Obstacles, and the Future. *BULLET: Journal Multidiscipline Ilmu*, 2(2), 548-557.
25. Javid, A. and Ahmed, M. (2024). A computational odyssey: uncovering classical  $\beta$ -lactamase inhibitors in dry fruits. *Journal of Biomolecular Structure and Dynamics*, 42(9), 4578-4604.
26. Kavya, I. K., Kochhar, N., Ghosh, A., Shrivastava, S., Rawat, V. S., Ghorai, S. M., and Kumar, M. (2023). Perspectives on systematic generation of antibiotic resistance with special emphasis on modern antibiotics. *Total Environment Research Themes*, 8, 100068.
27. Kilonzo-Nthenge, A., Liu, S., Hashem, F., Millner, P., and Githua, S. (2018). Prevalence of Enterobacteriaceae on fresh produce and food safety practices in small-acreage farms in Tennessee, USA. *Journal of Consumer Protection and Food Safety*, 13, 279-287.

28. Khaledi, M., Khatami, M., Hemmati, J., Bakhti, S., Hoseini, S. A. and Ghahramanpour, H. (2024). Role of Small Non-Coding RNA in Gram-Negative Bacteria: New Insights and Comprehensive Review of Mechanisms, Functions, and Potential Applications. *Molecular Biotechnology*, 1-19.
29. Kondum, F. A., Iwar, R. T. and Kon, E. T. (2021). A comparison of water quality indexes for an inland river. *Journal of Engineering Research and Reports*, 20(4): 1-15
30. Li, J., Li, C., Han, Y., Yang, J., Hu, Y., Xu, H. and Wang, H. (2024). Bacterial membrane vesicles from swine farm microbial communities harboring and safeguarding diverse functional genes promoting horizontal gene transfer. *Science of The Total Environment*, 951, 175639.
31. Li, Y., Wang, T., Li, Y., Xu, C., Wang, T., Huang, L. and Dong, N. (2024). Fitness cost of tet (A) type I variant-mediated tigecycline resistance in *Klebsiella pneumoniae*. *Journal of Global Antimicrobial Resistance*, 38, 158-162.
32. Li, S., Ondon, B. S., Ho, S. H., Zhou, Q., and Li, F. (2023). Drinking water sources as hotspots of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs): Occurrence, spread, and mitigation strategies. *Journal of Water Process Engineering*, 53, 103907.
33. Li, Q., Chang, W., Zhang, H., Hu, D., and Wang, X. (2019). The role of plasmids in the multiple antibiotic resistance transfer in ESBLs-producing *Escherichia coli* isolated from wastewater treatment plants. *Frontiers in microbiology*, 10, 633.
34. Liang, X., Dai, N., Sheng, K., Lu, H., Wang, J., Chen, L., and Wang, Y. (2022). Gut bacterial extracellular vesicles: important players in regulating intestinal microenvironment. *Gut Microbes*, 14(1), 2134689.
35. Manaia, C. M., Aga, D. S., Cytryn, E., Gaze, W. H., Graham, D. W., Guo, J., and Zhang, T. (2024). The complex interplay between antibiotic resistance and pharmaceutical and personal care products in the environment. *Environmental Toxicology and Chemistry*, 43(3), 637-652.
36. Mancuso, G., De Gaetano, S., Midiri, A., Zummo, S., and Biondo, C. (2023). The Challenge of overcoming antibiotic resistance in carbapenem-resistant Gram-negative bacteria: "Attack on Titan". *Microorganisms*, 11(8), 1912.
37. Molina, D., Carrión-Olmedo, J. C., Jarrín-V, P., and Tenea, G. N. (2024). Genome characterization of a multi-drug resistant *Escherichia coli* strain, L1PEag1, isolated from commercial cape gooseberry fruits (*Physalis peruviana* L.). *Frontiers in Microbiology*, 15, 1392333.
38. Mounzer, C. K., Baydoun, S. A., Amer, R. A., and Borjac, J. M. (2024). Occurrence of antibiotics and antibiotic-resistant bacteria in the Lebanese polluted Litani River. *Environmental Monitoring and Assessment*, 196(1), 90.

39. Muteeb, G., Rehman, M. T., Shahwan, M., and Aatif, M. (2023). Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. *Pharmaceuticals*, 16(11), 1615.
40. Pepi, M., and Focardi, S. (2021). Antibiotic-resistant bacteria in aquaculture and climate change: A challenge for health in the Mediterranean area. *International journal of environmental research and public health*, 18(11), 5723.
41. Rahman, A., Jahanara, I. and Jolly, Y. N. (2021). Assessment of physicochemical properties of water and their seasonal variation in an urban river in Bangladesh. *Water Science and Engineering*, 14(2): 139-148.
42. Ramírez-Castillo, F. Y., Guerrero-Barrera, A. L., and Avelar-González, F. J. (2023). An overview of carbapenem-resistant organisms from food-producing animals, seafood, aquaculture, companion animals, and wildlife. *Frontiers in Veterinary Science*, 10, 1158588.
43. Rana, S., Chowdhury, K., and Muchemi, J. (2024). Molecular characterization of biofilm producing *Escherichia coli* isolated from beef value chain in Bangladesh, *Food Sci. Anim. Prod.*, 2, 9240059.
44. Samuel, O., Dominic, N. and Patrick, O. (2020). Effects of Seasonal Variation on the Physicochemical Characteristics of Iyifeyi Stream in Ugwobi Abbi, Enugu State Nigeria. *Environment and Ecology Research*, 8(3): 76-84.
45. Sung, K., Nawaz, M., Park, M., Chon, J., Khan, S. A., Alotaibi, K., and Khan, A. A. (2024). Whole-Genome Sequence Analysis of Antibiotic Resistance, Virulence, and Plasmid Dynamics in Multidrug-Resistant *E. coli* Isolates from Imported Shrimp. *Foods*, 13(11), 1766.
46. Taha, M. S., Shouib, S. M., ELmonir, W., Zahra, S. W., and Hegazy, E. E. (2023). Microbiological profile of blood stream infection: Genetic relatedness of carbapenemase producing *Klebsiella pneumoniae* isolates using ERIC PCR. *Microbes and Infectious Diseases*, 4(1), 168-181.
47. Tesfaye, H., Alemayehu, H., Desta, A. F., and Eguale, T. (2019). Antimicrobial susceptibility profile of selected Enterobacteriaceae in wastewater samples from health facilities, abattoir, downstream rivers and a WWTP in Addis Ababa, Ethiopia. *Antimicrobial Resistance & Infection Control*, 8, 1-11.
48. Thai-Hoang, L., Thong, T., Loc, H. T., Van, P. T. T., Thuy, P. T. P. and Thuoc, T. L. (2022). Influences of anthropogenic activities on water quality in the Saigon River, Ho Chi Minh City. *Journal of Water and Health*, 20(3): 491-504.
49. Tiedje, J. M., Fu, Y., Mei, Z., Schäffer, A., Dou, Q., Amelung, W., and Wang, F. (2023). Antibiotic resistance genes in food production systems support One Health opinions. *Current Opinion in Environmental Science and Health*, 100492.

50. Tran, H. M., Prathan, R., Hein, S. T., and Chuanchuen, R. (2024). Microbiological Quality and Antimicrobial Resistance of Commercial Probiotic Products for Food-Producing Animals. *Antibiotics*, 13(2), 148.
51. Tula M, Enabulele O, Ophori E and Aziegbemhin A. (2022). A comprehensive study on the physicochemical characteristics of two water sources from the Adamawa North senatorial zone, Nigeria. *Public Health Toxicol.* 2(4), 1–13.
52. Wali, K. B., Essiet, U. U., Ajayi, A., Akintunde, G., Olukoya, D. K., Adeleye, A. I., and Smith, S. I. (2024). Molecular diversity and antimicrobial susceptibility profile of *Vibrio* species and distribution of other bacteria isolated from water Hyacinth (*Eichornia crassipes*) and Lagos lagoon. *Biologia*, 1-14.
53. Zhang, Y., Hu, Y., Li, X., Gao, L., Wang, S., Jia, S. and Li, A. (2024). Prevalence of antibiotics, antibiotic resistance genes, and their associations in municipal wastewater treatment plants along the Yangtze River basin, China. *Environmental Pollution*, 348, 123800.
54. Zhao, Q., Guo, W., Luo, H., Xing, C., Wang, H., Liu, B., and Ren, N. (2021). Deciphering the transfers of antibiotic resistance genes under antibiotic exposure conditions: driven by functional modules and bacterial community. *Water Research*, 205, 117672.
55. Zhao, R., Yu, K., Zhang, J., Zhang, G., Huang, J., Ma, L., and Li, B. (2020). Deciphering the mobility and bacterial hosts of antibiotic resistance genes under antibiotic selection pressure by metagenomic assembly and binning approaches. *Water research*, 186, 116318.