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 7.0×10^1 to 1.3×10^4 cfu/100 ml and total enterobacteriaceae count ranged from 9.2×10^2 to 4.2×10^4 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).

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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^2 (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.

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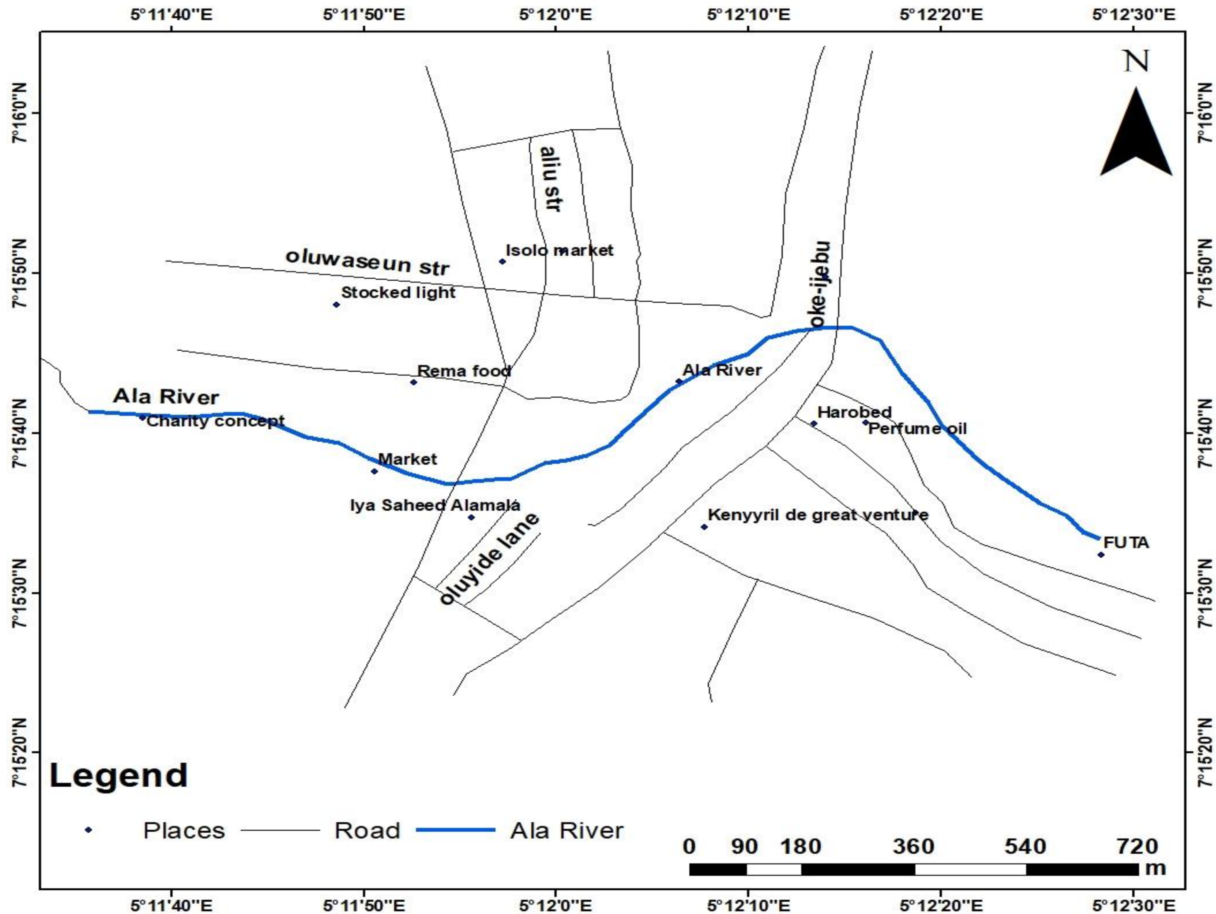

Figure 1: The location of River Ala in Akure, Ondo State, Nigeria

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

streaked out on Nutrient Agar plate for purification. Colonies were later stored at 4ºC 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.

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 (BaSO4) (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 Ismaeel 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), MgCl2 (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

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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
Multiplex1	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
		VIM R	ATGTAAGTTTCAAGAGTGATGC	
	blaNDM	NDM F	GGTGTTTGGTCGCATATCGCAA	
		NDM R	ATTCAGCCAGATCGGCATCGGC	
Multiplex2	blaIMP	IMP F	GGTTTGGCGATCTGGTTTTC	An initial denaturing 5min at 94°C, then 35 cycles of 94°C for 30s, 47°C for 40s and 72°C for 30s. and terminate at 72°C for 10mins
		IMP R	CGGAATGGCTCATCACGATC	
	blaKPC	KPCF	CATTCAAGGGCTTTCTTGCTGC	
		KPCR	ACGACGGCATAGTCATTTGC	
	blaOXA	OXA R	TTCTGTTGTTTGGGTTTCGC	
		OXA R	ACGCAGGAATTGAATTTGTT	
Multiplex3	blaTem	Tem F	GTCGCCGCATACACTATTCTCA	An initial denaturing 5min at 94°C, then 35 cycles of 94°C for 30s, 49°C for 40s 72°C for 35s and terminate at 72°C for 10min
		Tem R	CGCTCGTCGTTTGGTATGG	
	blaSHV	SHV F	GCCTTGACCGCTGGGAAAC	
		SHV R	GGCGTATCCCGCAGATAAAT	
Multiplex4	tet(A)	tet(A)F	GGTTCACTCGAACGACGTCA	An initial denaturing 5min at 94°C, then 35 cycles of 94°C for 30s, 48°C for 40s 72°C for 40s and terminate at 72°C for 10min
		tet(A)R	CTGTCCGACAAGTTGCATGA	
	tet(B)	tet(B)F	CCTCAGCTTCTCAACGCGTG	
		tet(B)R	GCACCTTGCTCATGACTCT	
Multiplex5	VanA	VanAF	TCTGCAATAGAGATAGCCGC	An initial denaturing 5min at 94°C, then 35 cycles of 94°C for 30s, 50°C for 30s 72°C for 60s and terminate at 72°C for 10min
		VanAR	GGAGTAGCTATCCCAGCATT	
	VanB	VanBF	ATGGGAAGCCGATAGTC	
		VanBR	GATTTCGTTCCTCGACC	

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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 7.0×10^1 cfu/100 ml to 1.67×10^4 cfu/100 ml. Sample 9 had the highest TPC of 1.67×10^4 cfu/100 ml, while sample 3 had the lowest TPC of 7.0×10^1 cfu/100 ml and the total enterobacteriaceae count (TEC) ranged from 9.2×10^2 cfu/100 ml to 4.17×10^4 cfu/100 ml. Sample 12 had the highest TEC of 4.17×10^4 cfu/100 ml, while Sample 1 exhibited the lowest TEC of 9.2×10^2 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	5.3×10^2	9.2×10^2
2	2.0×10^3	4.9×10^3
3	7.0×10^1	2.1×10^3
4	3.9×10^3	9.5×10^3
5	3.9×10^3	9.7×10^3
6	3.7×10^3	1.9×10^4
7	1.0×10^2	9.9×10^2
8	2.5×10^3	8.8×10^3
9	1.7×10^4	2.7×10^4
10	5.0×10^2	3.3×10^3

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11	9.8×10^3	3.2×10^4
12	1.3×10^4	4.2×10^4

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	transluscent	entire	pink	flat
36	small	irregular	shiny	transluscent	irregular	pink	flat
37	small	irregular	shiny	transluscent	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

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

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

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36	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	*Escherichia coli*
37	rod	-ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	*Escherichia coli*
38	rod	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	*Enterobacter cloacae*
39	rod	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	*Enterobacter cloacae*
40	rod	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	*Enterobacter cloacae*
41	rod	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	*Enterobacter cloacae*

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

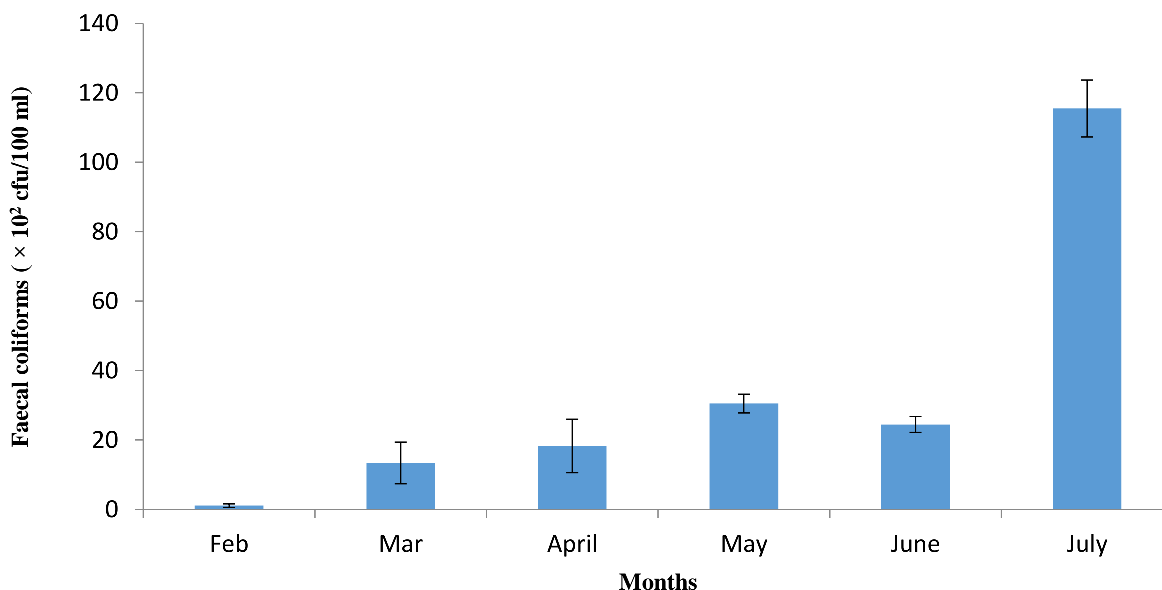

Figure 2: Mean load of faecal coliforms in water samples from River Ala (n=36)

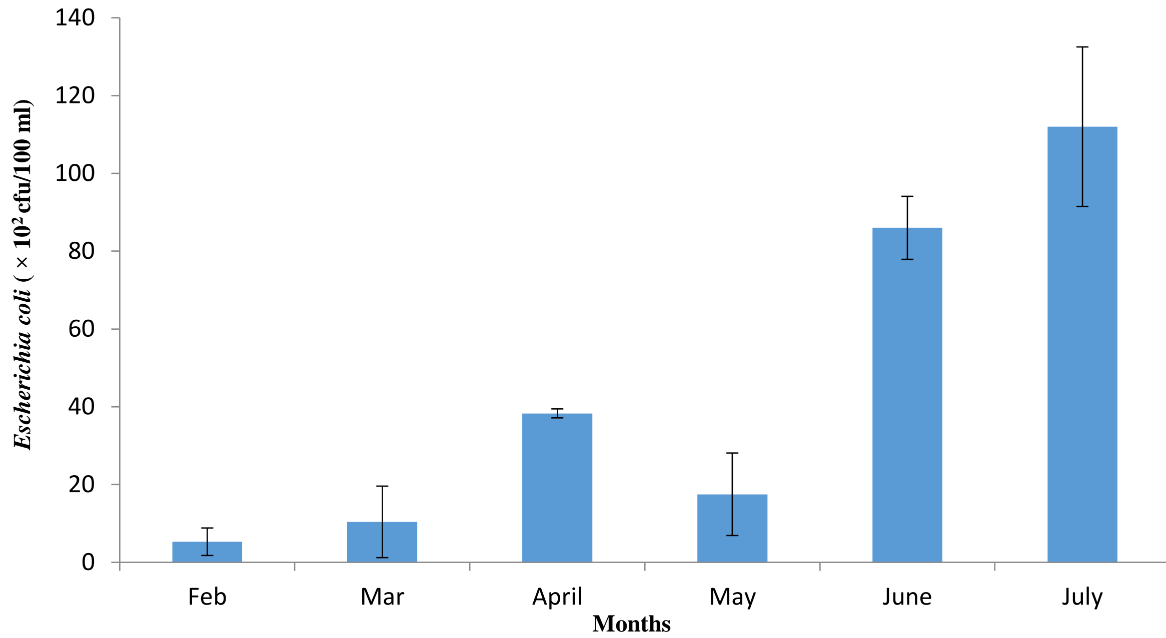

Figure 3: Mean load of *Escherichia coli* in water samples from River Ala (n=36)

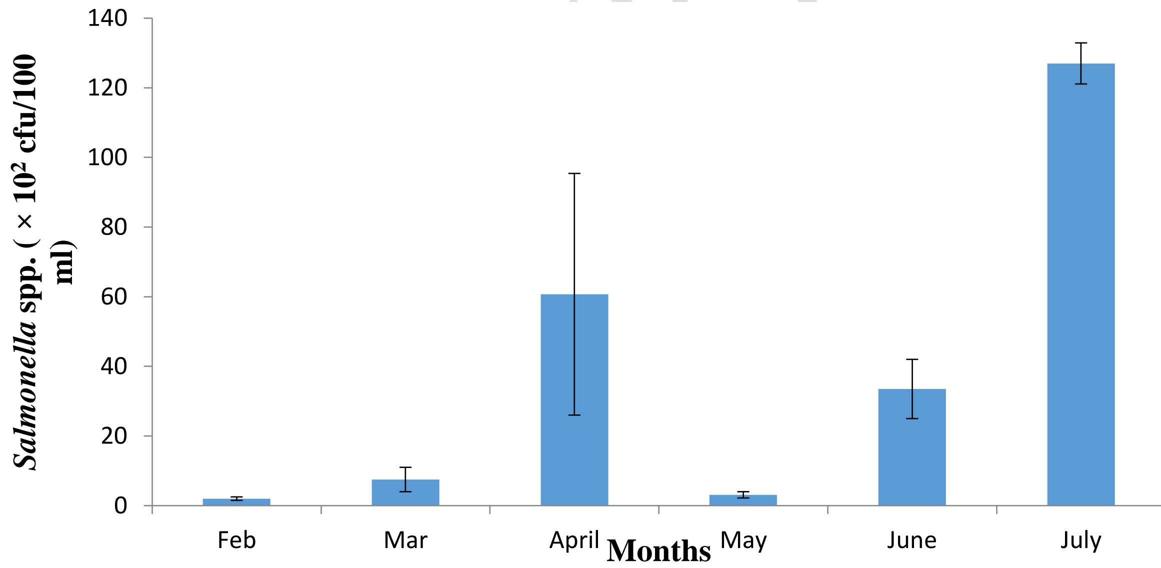

Figure 4: Mean load of *Salmonella* spp. in water samples from River Ala (n=36)

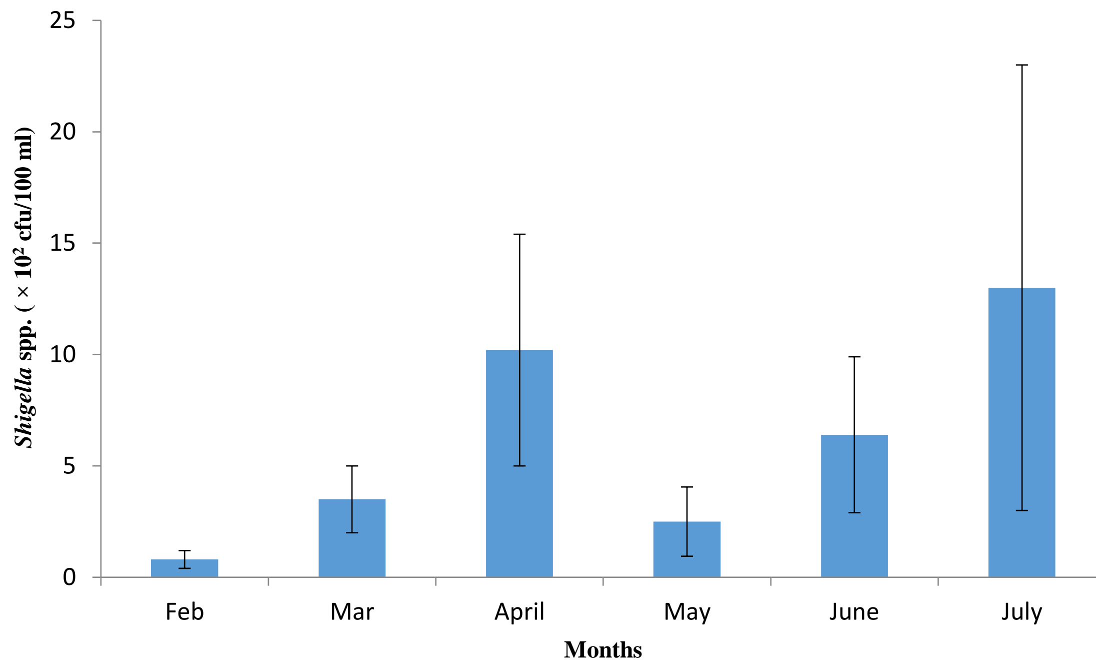

Figure 5: Mean load of *Shigella* spp. in water samples from River Ala (n=36)

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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).

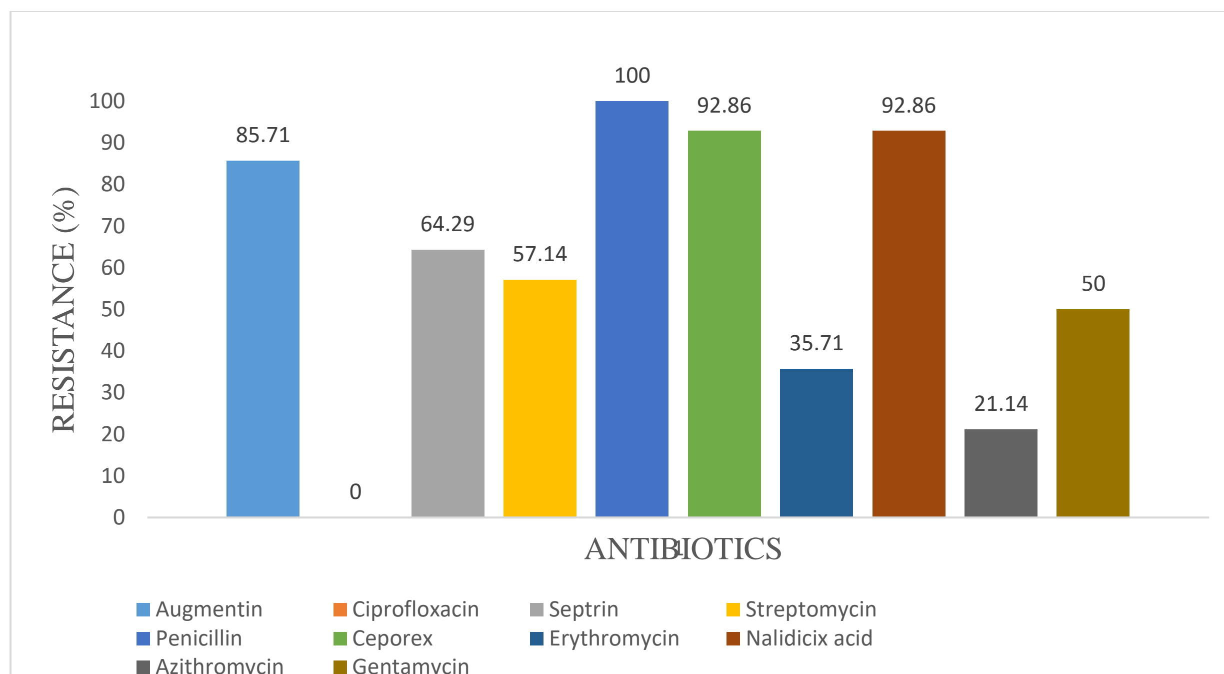

Figure 6: Antibiotic resistance pattern of the bacterial isolates against 10 different antibiotics

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.

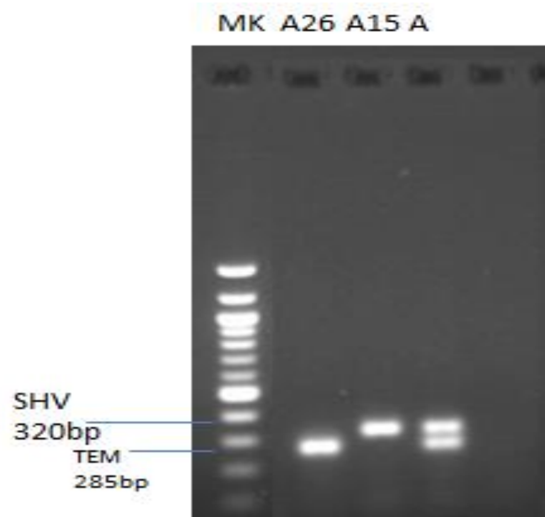

Plate 1: Gel image indicates a positive of TEM in sample A26 and A and SHV in A15 and A. (**A**- *Escherichia coli*, **A26**- *Salmonella enterica* and **A15**- *Enterobacter cloacae*)

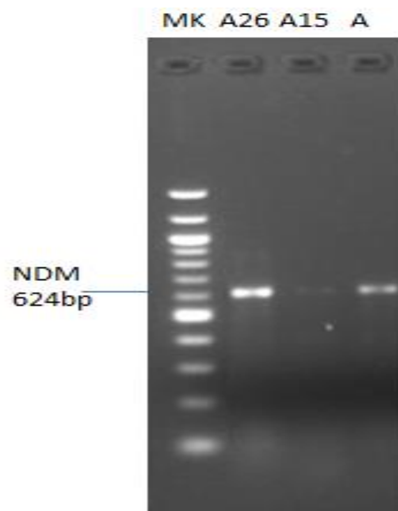

Plate 2: Gel image indicates a positive of NDM in samples A26 and A while VIM absent in all samples. (**A**- *Escherichia coli*, **A26**- *Salmonella enterica* and **A15**- *Enterobacter cloacae*)

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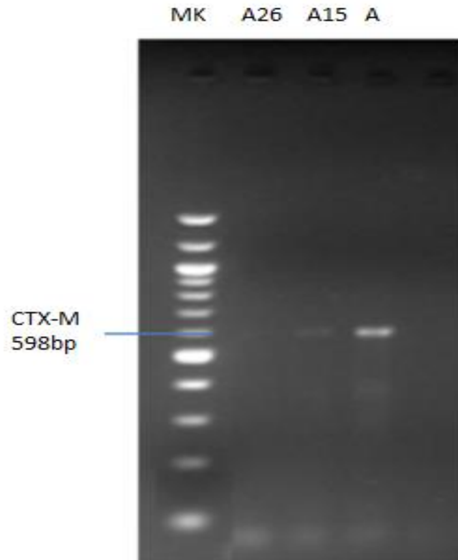

Plate 3: Gel image indicates a positive of CTX-M in only sample A and A15 but negative in A26. (**A**- *Escherichia coli*, **A26**- *Salmonella enterica* and **A15**- *Enterobacter cloacae*)

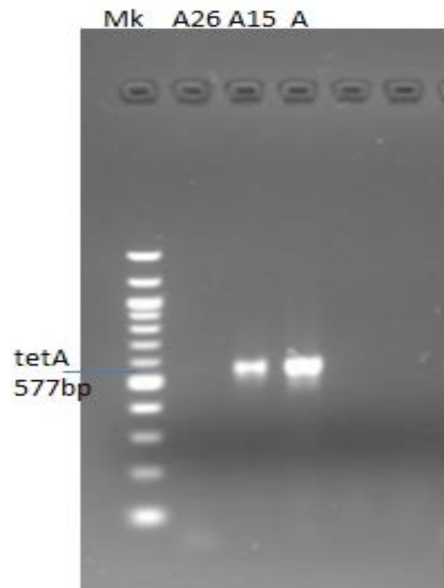

Plate 4: Gel image indicates a positive of tetA in only sample A15 and A and tetB gene in none of the sample. (**A**- *Escherichia coli*, **A26**- *Salmonella enterica* and **A15**- *Enterobacter cloacae*)

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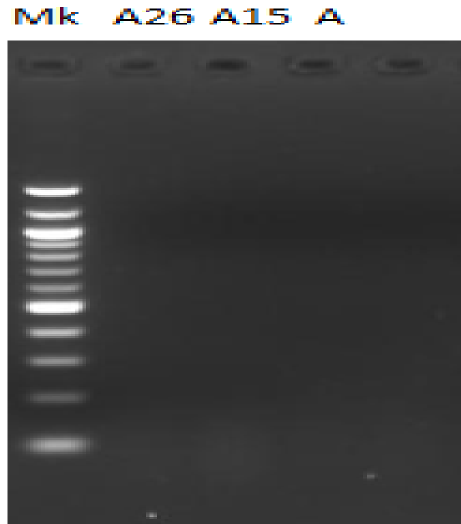

Plate 5: Gel image indicates no amplification for vanA and vanB gene. (**A**- *Escherichia coli*, **A26**- *Salmonella enterica* and **A15**- *Enterobacter cloacae*)

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

Bacterial/strain ID	bla_{VIM}	bla_{NDM}	bla_{TEM}	bla_{SHV}	bla_{CTX-M}	tetA	tetB	vanA	vanB
Escherichia coli (A)	-	+	+	+	+	+	-	-	-
Salmonella enterica (A26)	-	+	+	-	-	-	-	-	-
Enterobacter cloacae (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).

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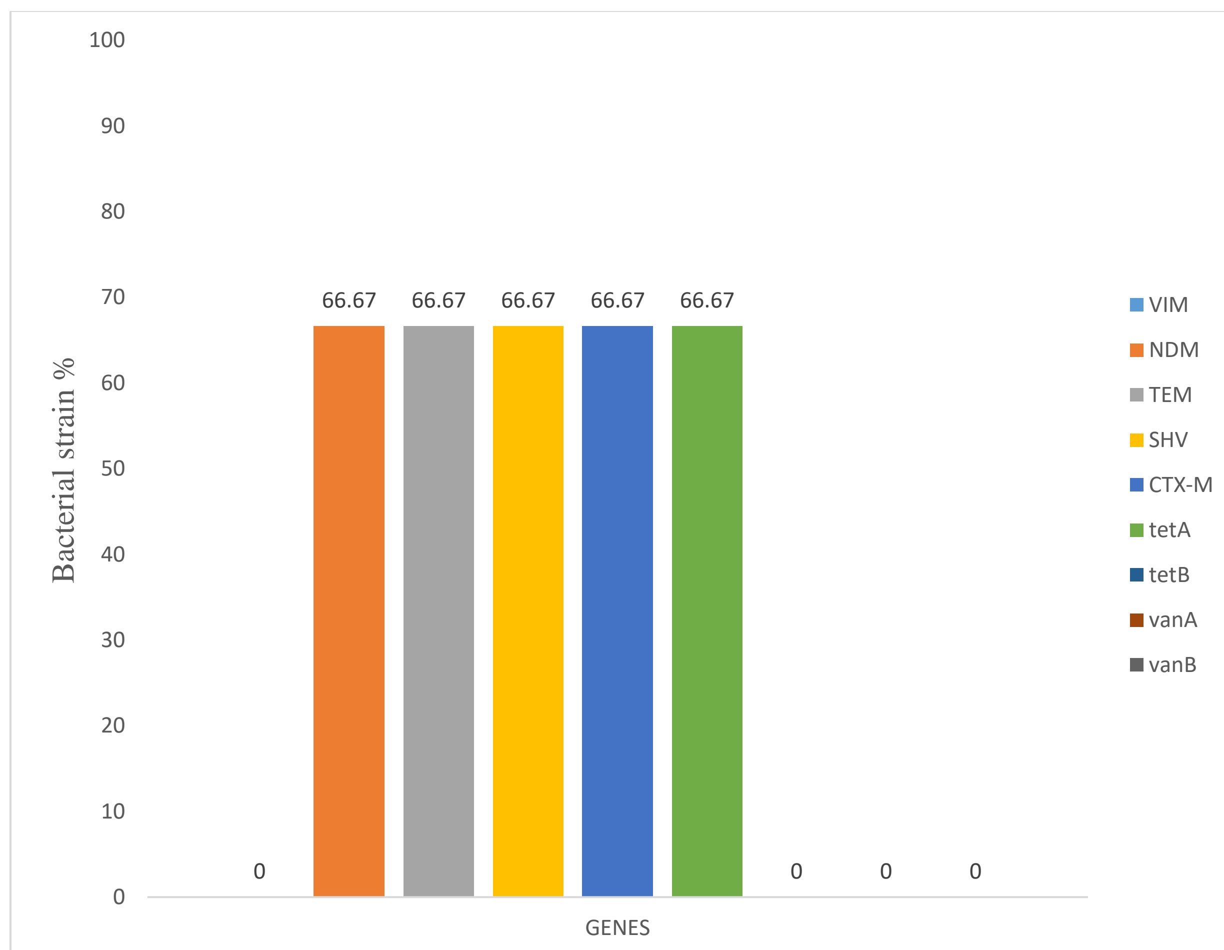

Figure 7: Percentage of bacterial strain in ARGs.

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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
Escherichia coli	**-0.56****	**0.71****	0.39	**0.58****	**0.93****
Salmonella spp.	**-0.55****	**0.63****	0.19	0.38	-0.11
Shigella spp.	-0.21	0.48*	0.34	-0.28	0.31
Faecal coliform	0.25	0.32	0.23	-0.44	-0.36
Enterobacter cloacae	0.32	**0.58****	0.37	**0.62****	**0.81****

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 ceporex. 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.

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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

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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*.

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(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.

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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

marked resistance to pencillin. 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.

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