

Research Article

Detection of Mobile Genetic Elements in Enteric Bacteria and Physicochemical Characteristics of Water from River Ala in Akure, Nigeria

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Abstract

Antibiotic-resistant bacteria can release resistance genes into the rivers, potentially spreading them through mobile genetic elements such as transposable elements, bacteriophages, plasmids, and gene cassettes play a crucial role in the dissemination of antibiotic resistance and horizontal gene transfer. This study investigated the mobile genetic elements in enteric bacteria and physicochemical characteristics of water from River Ala. Water samples were collected bi-weekly over a period of 24 weeks from three representative points in River Ala. Standard microbiological methods were employed to isolate and identify enteric bacteria. The physicochemical characteristics of the water samples including temperature, pH, turbidity, total dissolved solids (TDS), total dissolved solids (TSS), conductivity and biological oxygen demand (BOD) were determined using established protocols. Mobile genetic elements (MGEs) were detected using polymerase chain reaction (PCR) techniques. Results showed that the turbidity ranged from 2.80 ± 0.06 to 13.19 ± 1.05 NTU, temperature ranged from 27.52 ± 0.48 to 31.50 ± 0.83 °C, total dissolved solids (TDS) ranged from 108.09 ± 0.27 to 207.33 ± 7.06 mg/L, pH ranged from 7.13 ± 0.34 to 8.40 ± 0.05 , biological oxygen demand ranged from 3.22 ± 0.42 to 4.63 ± 0.32 mg/L and dissolved oxygen ranged from 4.05 ± 0.58 to 6.94 ± 0.14 mg/L. MGEs such as plasmid and integron were detected in *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae*. Findings revealed that there is a need for improved water quality monitoring and public health interventions to mitigate the risks associated with antibiotic resistant bacteria in water from River Ala.

Keywords

Mobile Genetic Elements, Enteric Bacteria, Physicochemical Characteristics

1. Highlights

- 1) Mobile genetic elements (MGEs) in enteric bacteria were detected.
- 2) Physicochemical characteristics of the water samples were determined.
- 3) The relationships between physicochemical characteristics of the water samples and MGEs in the bacterial isolates were determined.
- 4) The spread of MGEs and antibiotic resistant bacteria occurred due to the poor water quality and contamination in River Ala
- 5) Indiscriminate faecal discharge and disposal of untreated wastewater in River Ala should be discouraged.

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2. Introduction

In low- and middle-income countries, waterborne illnesses continue to be a leading source of mortality and morbidity [38]. Based on global spatial distribution, a substantial portion of diseases such as gastroenteritis, cholera, typhoid fever, paratyphoid, bacillary dysentery, amoebic dysentery, and infectious hepatitis are prevalent in Africa and Asia. Children under the age of five are especially negatively impacted. In this age group, diarrhea is thought to be the cause of 15–18% of deaths. However, the same diseases still plague adults (United State Agency for International Development, 2005). Waterborne pathogens are transmitted via contaminated food or water. A number of these gastrointestinal tract-related illnesses manifest as epidemics. This is due to the fact that they typically impact a large geographic area where residents share a common source of water supply [34]. Major contributing factors to the occurrence of waterborne diseases include lack of access to clean and adequate water supply, unsanitary conditions and poor hygiene.

Mobile genetic elements (MGEs) are DNA sequences found within the genetic material of organisms, including bacteria that have the remarkable ability to move from one location in the genome to another or between different organisms [8]. These elements (e.g. transposable elements, bacteriophages, plasmids, gene cassettes) play a pivotal role in the spread of antibiotic resistance and horizontal gene transfer [31, 33].

Antibiotic-resistant bacteria (ARB) can release antibiotic resistance genes into the rivers and are carried by mobile genetic elements like plasmids and integrons, which can transfer resistance to other bacteria, potentially creating a reservoir of antibiotic resistance in the environment. This poses a risk to human and animal health as these resistant genes may find their way back into clinical settings. Antibiotic-resistant bacteria may be transported into rivers through various means, including wastewater discharge. These resistant bacteria may potentially infect humans through contact with contaminated water, fish, or other exposure routes. Understanding the presence and dynamics of these genes is crucial for managing public health risks [19].

Resistance gene dissemination is known to be facilitated by mobile elements such as integrons, transposons and plasmids. Although they are all made of double-stranded DNA, they vary greatly in terms of their sizes, shapes, biological characteristics, and modes of propagation [40]. Extra chromosomal genetic elements known as plasmids are capable of autonomous replication and have replication mechanisms. Large plasmids known as conjugative plasmids can carry the transfer gene (*tra* gene), allowing them to autonomously migrate from one host cell to another [37].

Some smaller, non-conjugative plasmids can be mobilized by the conjugative plasmids. High frequency plasmid conjugation is capable of co-transferring many resistance genes, both within and between bacterial species, as well as between

distinct species [24]. More recently, plasmid-mediated resistance to expanded-spectrum cephalosporins, which are encoded by the CMY-2 AmpC beta-lactamase in human and animal strains of *E. coli* and *Salmonella* spp. The importance of newly discovered plasmid-mediated processes is further demonstrated by the global spread of Enterobacteriaceae quinolone resistance caused by plasmids [27].

According to [12], integrons have the ability to mobilize or integrate gene cassettes expressing antibiotic resistance determinants, such as resistance to trimethoprim, aminoglycosides, chloramphenicol, or tetracyclines. Class 1 integrons are the most common among clinical isolates among the three classes of integrons that have been found. Two conserved segments, the 5' conserved segment (5'-CS) and the 3' conserved segment (3'-CS), as well as an internal variable region containing gene cassettes encoding antibiotic resistance determinants, were the original definitions of class 1 integrons [10, 23]. This study was aimed at determining the mobile genetic elements in enteric bacteria and physico-chemical characteristics of water from River Ala.

3. Methods

3.1. 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 km² (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.

3.2. 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.

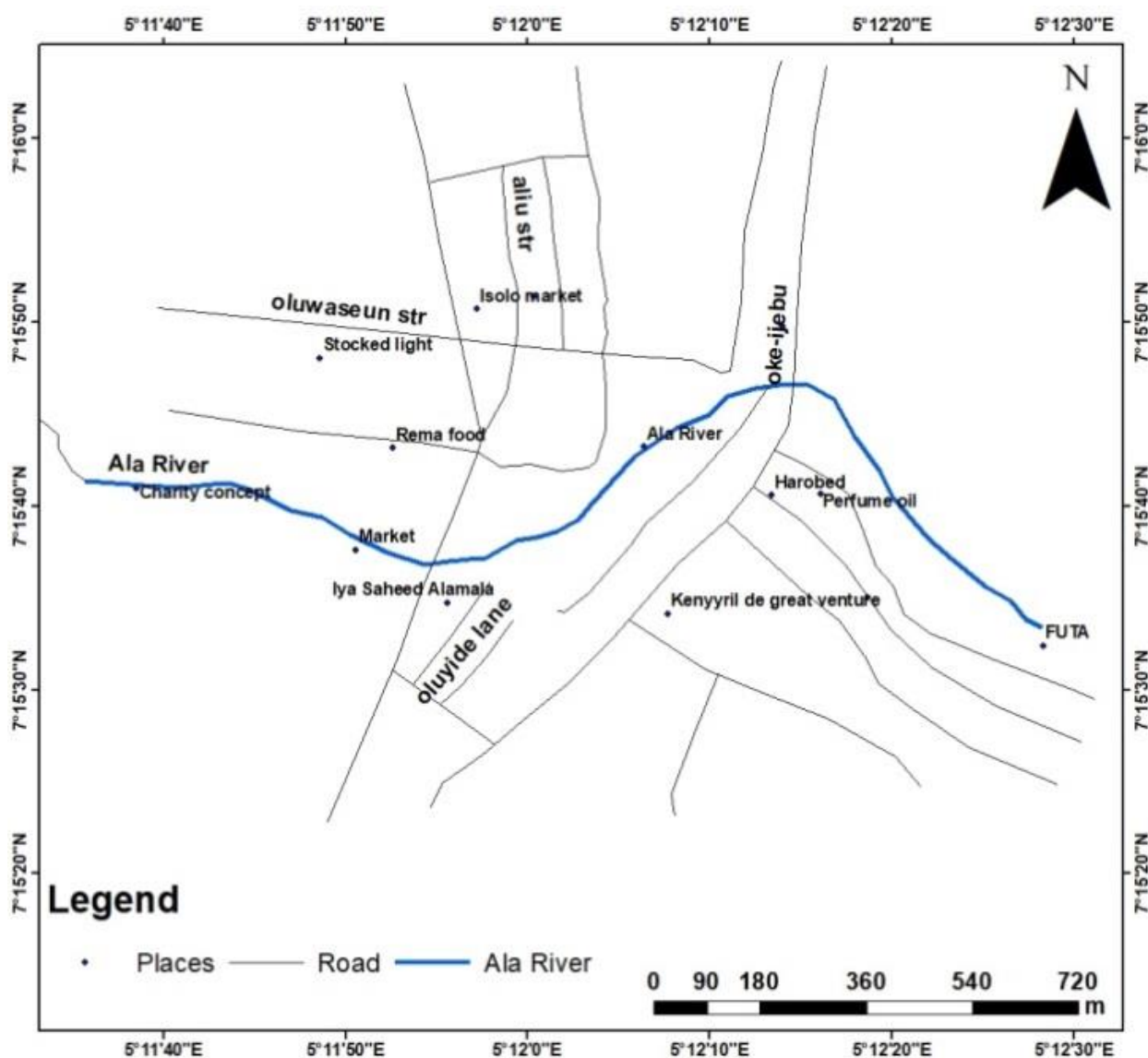


Figure 1. The location of River Ala in Akure, Ondo State, Nigeria.

3.3. Determination of the Physicochemical Characteristics of the Water Samples from River Ala

The physiochemical characteristics of the water samples determined were dissolved oxygen, pH, turbidity, salinity, biochemical oxygen demand, total dissolved solids, temperature, electrical conductivity, nitrate and nitrite. All the parameters were measured in duplicate.

The pH of water samples were enumerated (*in-situ*) at the site of sample collection. About 400 ml of the water sample was measured in a clean beaker. The water content was sufficient enough for the tip of the probe to be submerged. The tip of the calibrated pH meter (Model Hi9828) probe was rinsed with distill water and then the probe was lowered and dipped to a depth of about 0.3 m of the water sample, allowing it to stabilize and the pH value was read and recorded [13].

The pH value obtained was expressed as $\text{pH} = -\log \text{H}^+$. Biological oxygen demand (BOD) was determined using dissolved oxygen values taken initially (DO_0) at sample collection and the dissolved oxygen value after five (5) days of incubation [15]. Appropriate dilutions of samples were prepared and transferred in two BOD bottles corked with a stopper. The difference between the two values (in mg/L) represent the quantity of oxygen consumed during the stabilization of organic matter present in the water sample by microorganism to degrade and mineralize organic matter present during the five day incubation period, at 20 °C under anaerobic condition [22].

The BOD was calculated as follows:

$\text{BOD (mgL}^{-1}\text{)} = (\text{DO}_0 - \text{DO}_5) \times \text{BOD bottle volume of sample used}$. Where, DO_0 = dissolved oxygen of diluted water sample taken immediately after preparation and, DO_5 = dissolved oxygen of diluted sample taken after five days of incubation at 20 °C [16].

A multi range conductivity meter (Model 913) was used to measure and record the conductivity of the water samples. The meter was standardized with a known concentration of conductance of potassium chloride [13, 35]. Winkler's method was used to determine the oxygen concentration in the river water sample. Standard thiosulphate solution is then titrated with the liberated iodine. The results were taken at unit value of mg L⁻¹. A blank determination was also carried out [5].

The turbidity of the water sample was determined *in-situ* using turbidity meter. The turbidity meter was calibrated with buffer of 4 and 9. About 10ml of the water sample was poured into the sampling tube and it was gently placed in the turbidimeter and the reading was recorded in Nephelometric Turbidity Units (NTU) [13]. The temperature of the water sample was carried out on-site by dipping the calibrated thermometer (Model 913) probe into the sample and the readings were recorded.

3.4. 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.

3.5. Detection of Mobile Genetic Elements (MGEs) in Bacterial Isolates

The tool MobileElementFinder was developed to enable rapid detection of MGEs and their genetic context in assem-

bled sequence data. MGEs in the bacterial isolates were determined using sequence similarity to a database of 4452 known elements augmented with annotation of plasmids [32].

Plasmids were isolated using Zyppy™ Plasmid Miniprep Kit Catalog Nos. D4019. Briefly, Plasmid analysis protocol (Zyppy™ Plasmid Miniprep Kit Catalog Nos. D4019) was performed on representative bacterial isolates, 600 µl of bacterial culture grown in LB medium was added to 1.5 ml microcentrifuge tube, tube containing the culture was centrifuged for 30 seconds at 14,000 rpm, then the supernatant was discarded. 100 µl of 7X Lysis Buffer (Blue) 1 was added and mixed by inverting the tube 4-6 times. (After addition of 7X Lysis Buffer the solution changed from opaque to clear blue indicating complete lysis. 350 µl of cold Neutralization Buffer (Yellow) was added and mixed thoroughly. The sample turned yellow when the neutralization was completed and a yellowish precipitate was formed. The sample was inverted for 2-3 times to ensure complete neutralization. Then it was centrifuged at 11,000 – 16,000 x g for 2-4 minutes and the supernatant (~900 µl) was transferred into the Zymo-Spin™ IIN column. The column was placed inside the collection tube and was centrifuged for 15 seconds. The flow-through was discarded and the column was placed back into the same collection tube. Then, 200 µl of Endo-Wash Buffer was added to the column and centrifuged for 30 seconds. 400 µl of Zyppy™ Wash Buffer was added to the column and centrifuged for 1 minute. This was transferred into a clean 1.5 ml microcentrifuge tube column then 30 µl of Zyppy™ Elution Buffer 2 was added directly to the column matrix and allowed to stand for one minute at room temperature. Then centrifuged for 30 seconds to elute the plasmid DNA. Plasmid were profiled in a 1.5% Agarose gel and integron were detected using primer sets as shown in Table 1. The amplification of all the genes was carried out using 5 µl of the chelex extracted DNA as temp late for the PCR reaction mixture with 5 µl of PCR buffer (1x), 2 µl of MgCl₂ (2mM), 1 µl of dNTPs 0.8mM and 1 µl (0.2 µl) each of the forward and reverse primers in a thermal cycler (Model: Bio Rad Laboratories, Richmond, CA, USA).

Table 1. Primers used for detection of mobile genetic elements.

Gene	Primer	Primer sequence 5'-3'	Profile
Class 1 integron variable region	5'_CS	GGCATCCAAGCAGCAG	An initial denaturing 1min at 95 °C, then 30 cycles of 96 °C for 30s, 60 °C for 30s and 72 °C for 30s. and terminate at 72 °C for 10mins
	3'_CS	AAGCAGACTTGACCTGA	
Class 1 integrase gene	IntI1_F	CCTCCCGCACGATGATC	An initial denaturing 1min at 95 °C, then 30 cycles of 96 °C for 30s, 60 °C for 30s and 72 °C for 30s. and terminate at 72 °C for 10mins
	IntI1_R	TCCACGCATCGTCAGGC	
Inc	rep 1	CAAGTTCTTCTGTTGGGATTCCG	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
	rep 2	CAAGTTCTTCTGTTGGGATTCCG	

3.6. 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 among mobile genetic elements (MGEs) and physicochemical characteristics was assessed using Pearson correlation coefficients.

4. Results

4.1. Physicochemical Characteristics of Water Samples from River Ala

The mean pH of the water samples from River Ala ranged

from 3.2 to 8.34. The highest mean value of 8.34 was observed in July, while the lowest value of 3.2 was observed in February (Figure 2). The mean biological oxygen demand (BOD) ranged from 3.2 to 4.58 mg/L. The highest mean value of 4.58 mg/L was observed in July, while the lowest mean value of 3.2 mg/L was observed in February (Figure 3). The mean electrical conductivity (EC) ranged from 217 to 383.5 $\mu\text{S}/\text{cm}$. The highest mean value of 383.5 $\mu\text{S}/\text{cm}$ was observed in May, while the lowest mean value of 217 $\mu\text{S}/\text{cm}$ was observed in February (Figure 4). The mean dissolved oxygen concentration of the water samples from River Ala ranged from 4.72 to 6.69 mg/L. The highest mean value of 6.69 mg/L was observed in July, while the lowest value of 4.72 mg/L was observed in May (Figure 5).

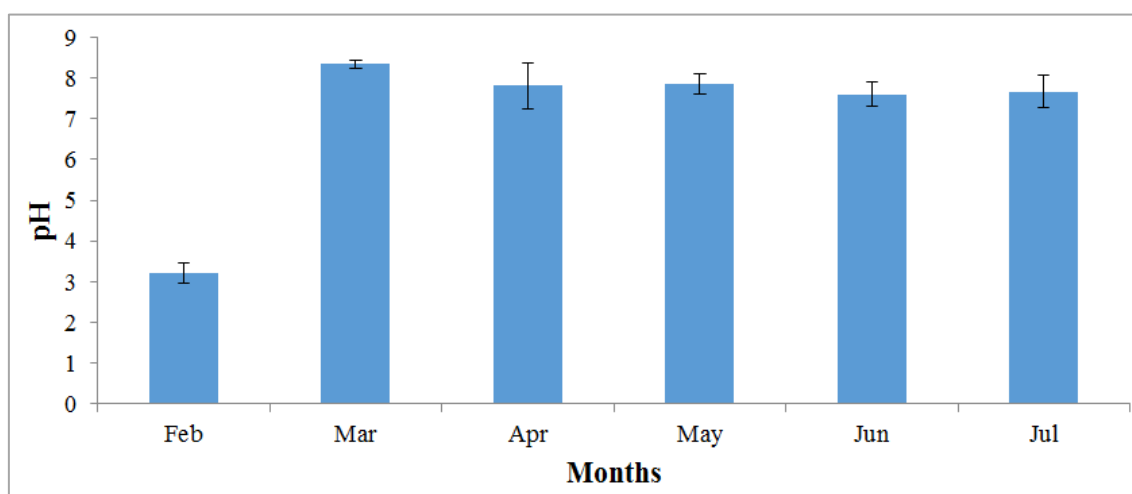


Figure 2. Mean value of pH in water samples from River Ala (n=36).

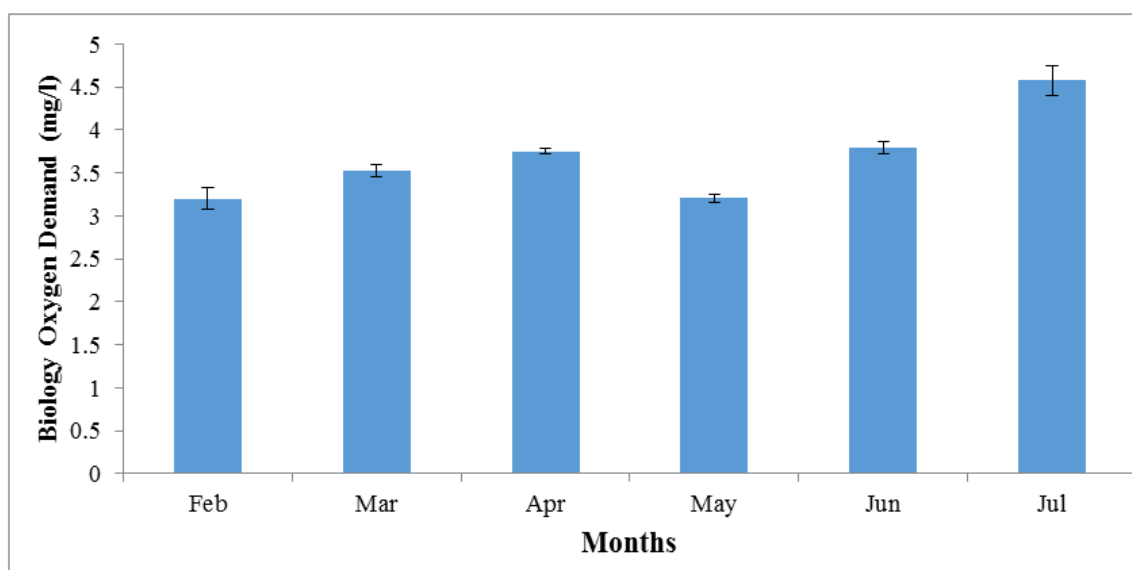


Figure 3. Mean concentration of biological oxygen demand in water samples from River Ala (n=36).

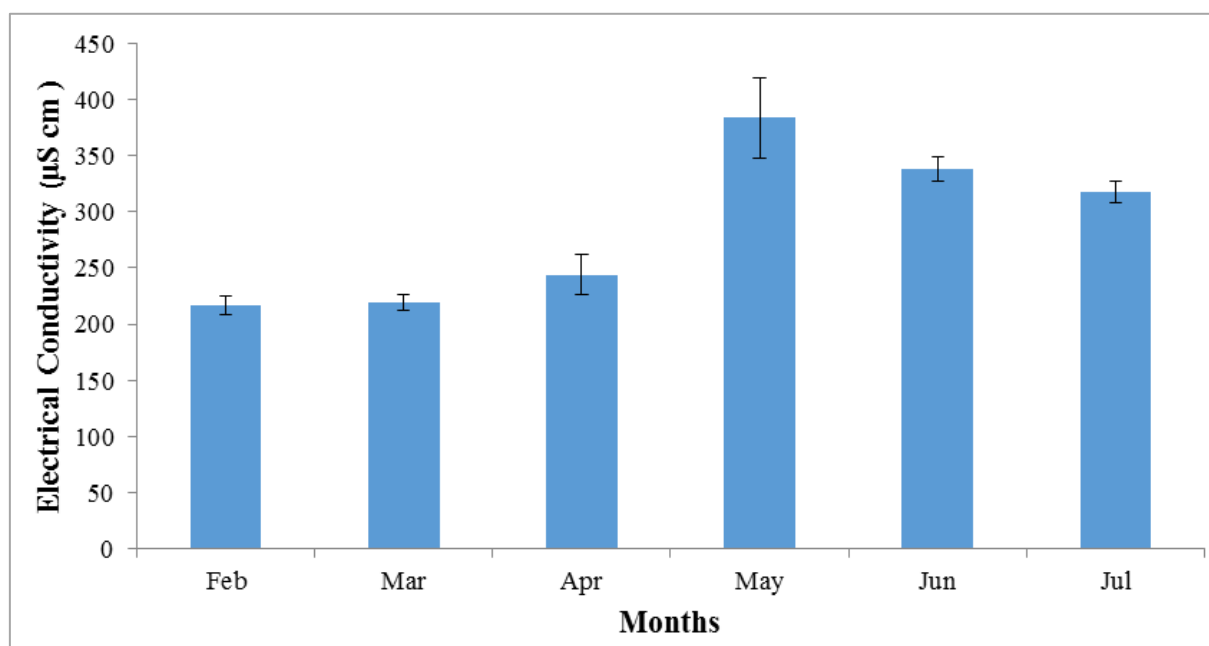


Figure 4. Mean value of electrical conductivity in water samples from River Ala (n=36).

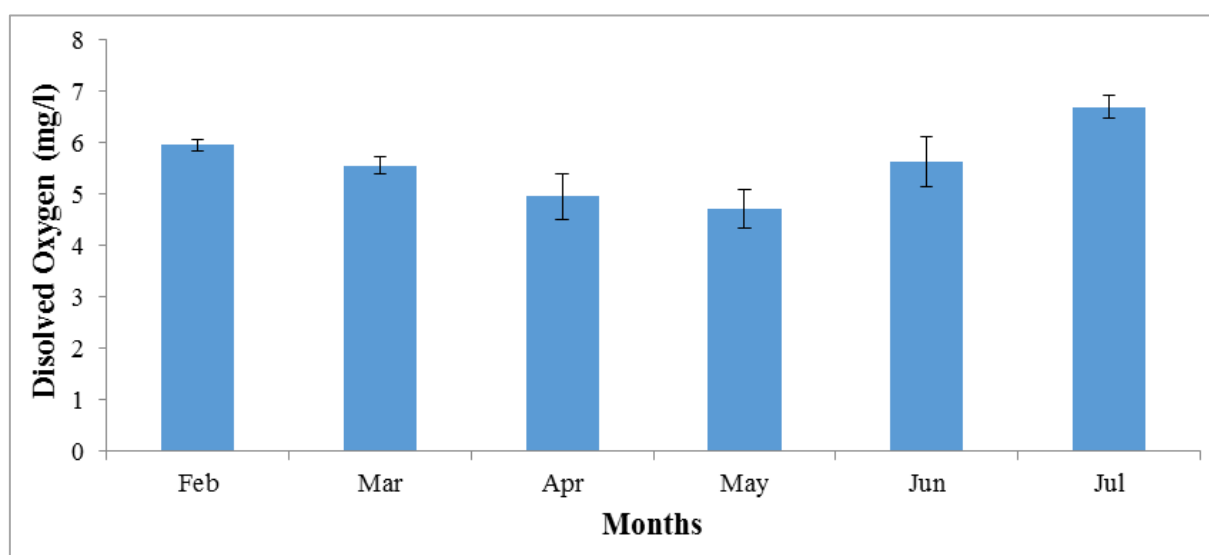


Figure 5. Mean concentration value of dissolved oxygen in water samples from River Ala (n=36).

The mean turbidity concentration of the water samples from River Ala ranged from 2.8 to 12.2 NTU. The highest mean value of 12.2 NTU was observed in June, while the lowest mean value of 2.8 NTU was observed in February (Figure 6). The mean temperature of the water sample from River Ala ranged from 29.2 to 31.28 °C. The highest mean value of 31.28 °C was observed in June, while the lowest mean value of 29.3 °C was observed in February (Figure 7). The mean total dissolved solids of the water sample from River Ala ranged from 108.5 to 192 mg/L. The highest mean value of 192 mg/L was observed in May, while the lowest mean value of 108.5 mg/L was observed in February (Figure 8). The

mean nitrate concentration of the water sample from River Ala ranged from 3.2 to 6.62 mg/L. The highest mean value of 6.62 mg/L was observed in July, while the lowest mean value of 3.2 mg/L was observed in February (Figure 9).

The mean hardness concentration of the water sample from River Ala ranged from 185 to 281.75 mg/L. The highest mean value of 281.75 mg/L was observed in March, while the lowest mean value of 185 mg/L was observed in May (Figure 10). The mean salinity concentration of the water sample from River Ala ranged from 0.15 to 5.9 mg/L. The highest value of 5.9 mg/L was observed in February, while the lowest mean value of 0.15 mg/L was observed in July (Figure 11).

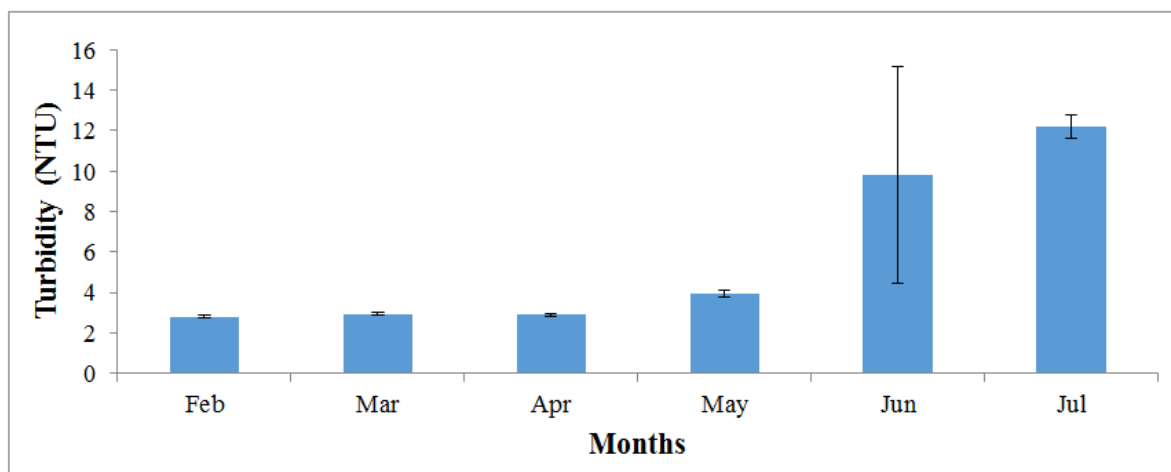


Figure 6. Mean concentration value of turbidity in water samples from River Ala (n=36).

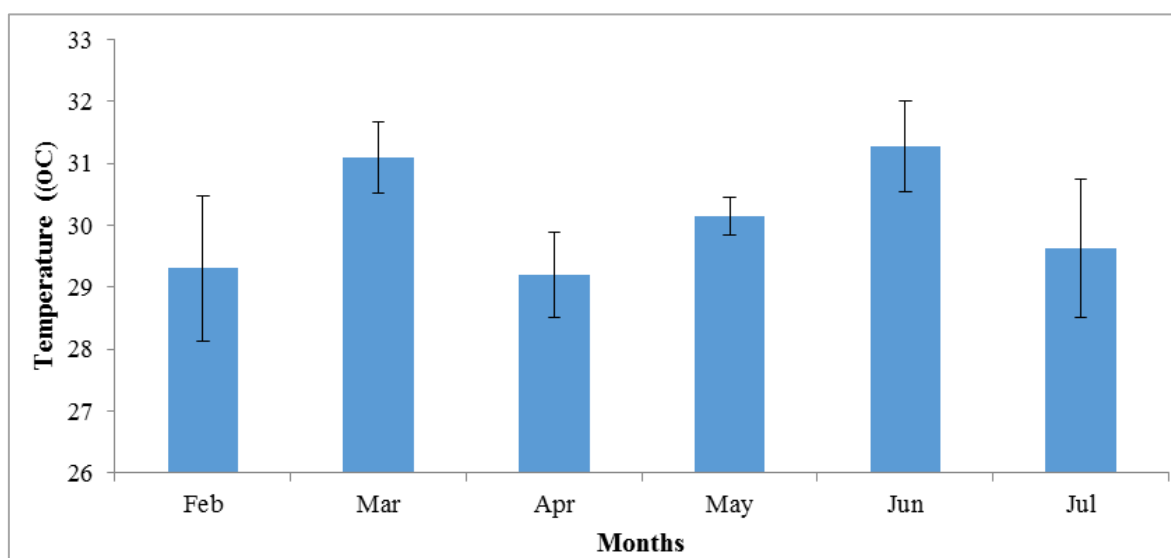


Figure 7. Mean value of temperature in water samples from River Ala (n=36).

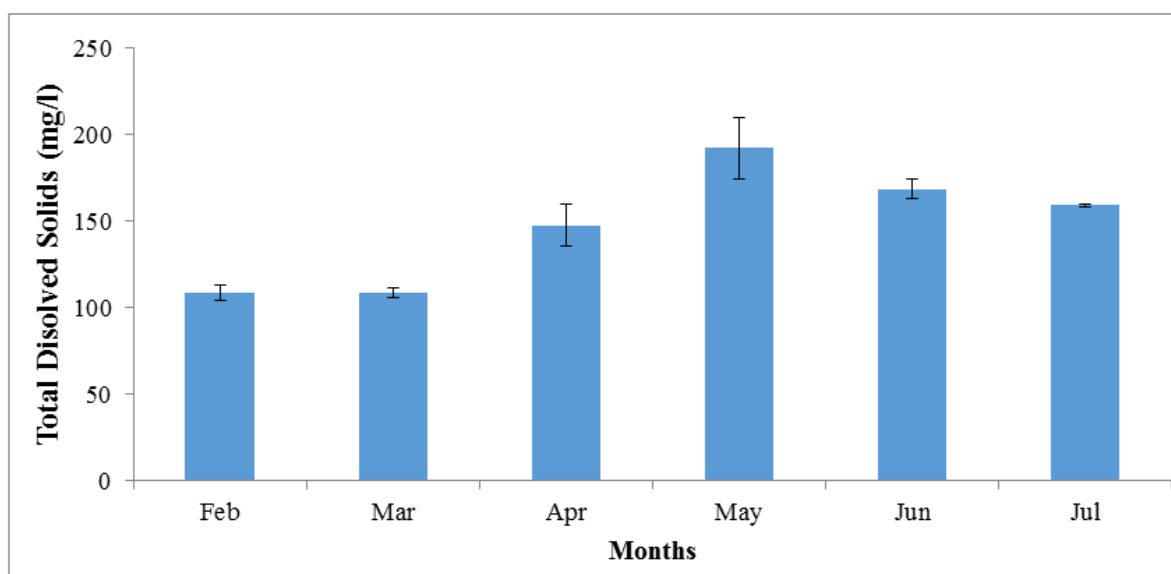


Figure 8. Mean concentration value of total dissolved in water samples from River Ala (n=36).

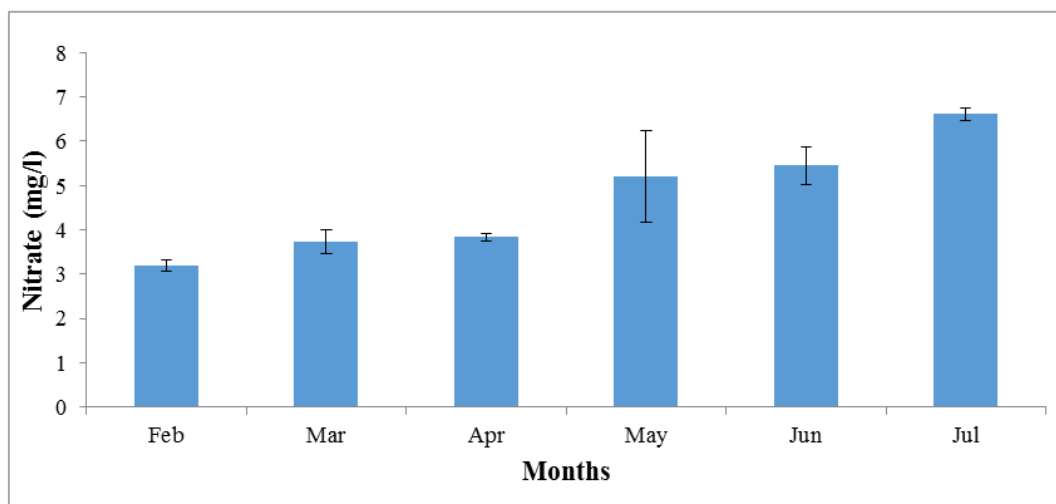


Figure 9. Mean nitrate concentration value in water samples from River Ala (n=36).

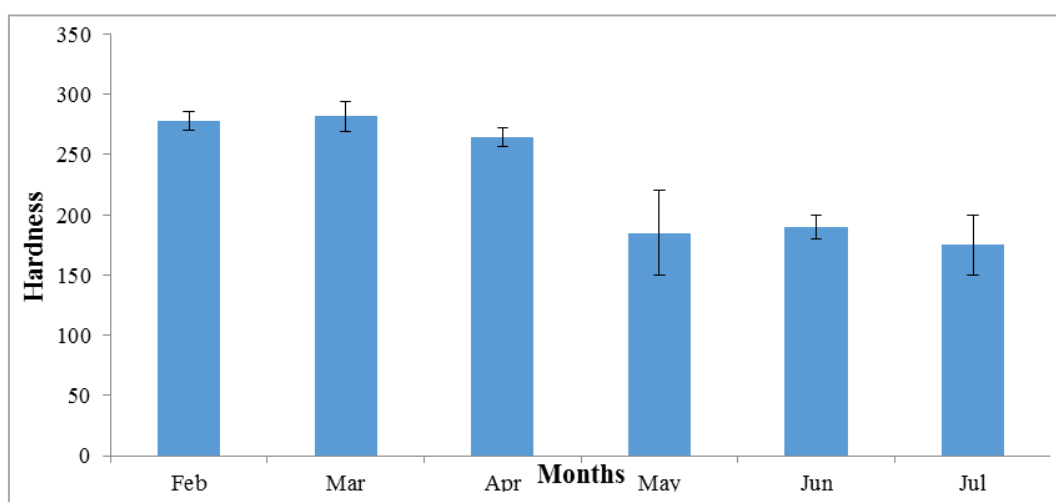


Figure 10. Mean concentration value of hardness in water samples from River Ala (n=36).

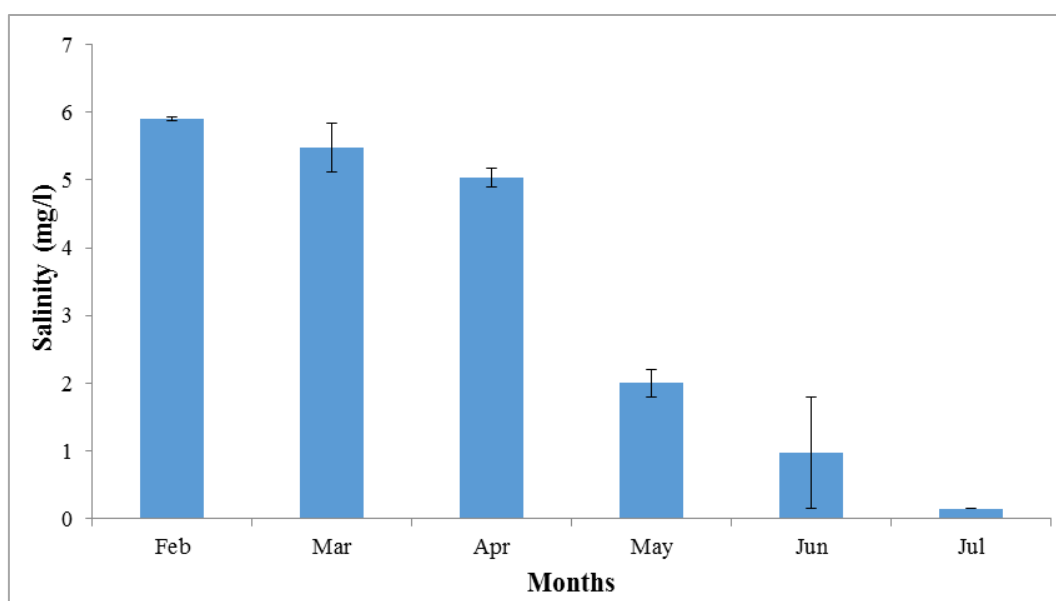


Figure 11. Mean concentration value of salinity in water samples from River Ala (n=36).

4.2. MGEs in Bacterial Isolates from River Ala

The mobile genetic elements present in bacteria isolated from river Ala possess plasmids and integron of 10 kb in size respectively (Table 2). The gel electrophoresis revealed that all bacterial isolates have a high molecular weight of approximately 10 kbp, as indicated by the bands' migration patterns. The presence of these bands at the same position across all isolates suggests uniform plasmid sizes. Lane M contains a 1 kbp DNA ladder, which allows you to confirm

that the observed plasmid bands are indeed around 10 kbp (Figure 12 and Figure 13).

Plasmids has a strong positive correlation with turbidity 0.85, temperature 0.95, total dissolved solid 0.73, nitrate 0.81, salinity 0.77 while hardness 0.57, pH 0.61 showed a moderate positive correlation and negative correlation with dissolved oxygen -0.67. Integrons revealed negative correlation with electrical conductivity -0.66 and a strong negative correlation with dissolved oxygen -0.99 (Table 3).

Table 2. MGEs in bacteria isolated from Ala River.

Bacterial/strain ID	Number of plasmid and size	Number of integron and size
<i>Escherichia coli</i> (A)	1 (10kb)	1(10kb)
<i>Salmonella enterica</i> (A26)	1(10kb)	1(10kb)
<i>Enterobacter cloacae</i> (A15)	1(10kb)	1(10kb)

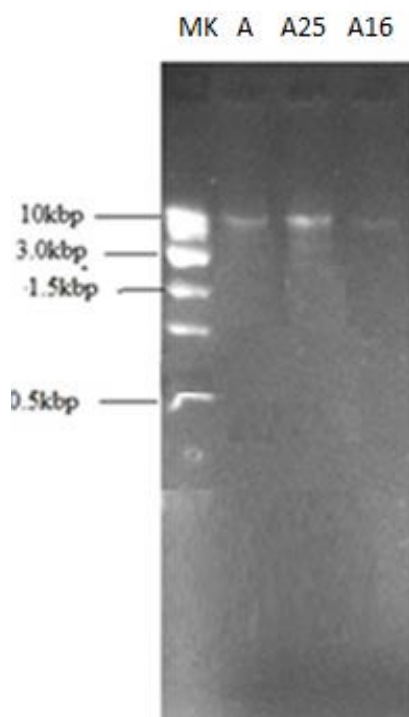


Figure 12. Gel electrophoresis image showing the profiling of the bacterial isolates. The Plasmids are of high molecular weight of approximately 10kbp. All the isolates had the 10.0kbp Plasmids. Lane M is 1kbp DNA ladder. (A- *Escherichia coli*, A25- *Salmonella enterica*, A16- *Enterobacter cloacae*).

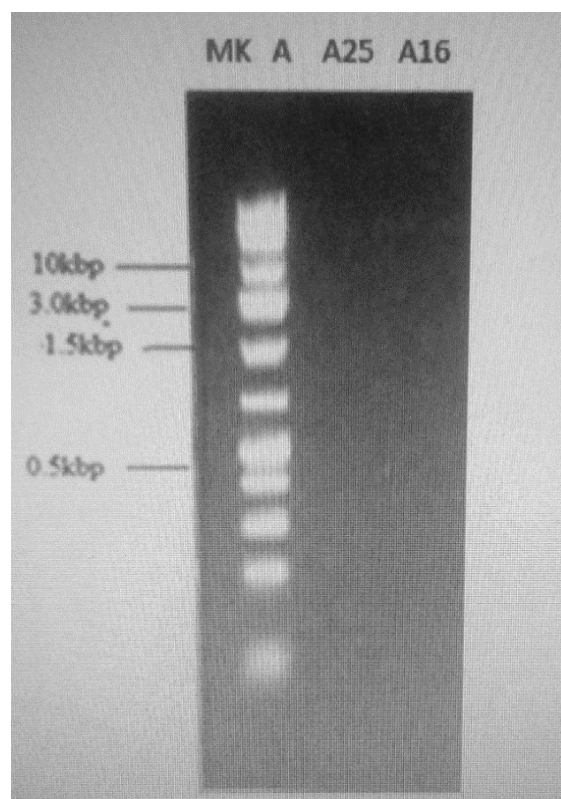


Figure 13. Gel image showing the integron of the bacterial isolates. (A- *Escherichia coli*, A25- *Salmonella enterica*, A16- *Enterobacter cloacae*).

Table 3. Relationship between physicochemical characteristics of the water from River Ala and mobile genetic elements (MGEs).

	Plasmids	Integrans
Turbidity (NTU)	0.85**	0.02
Temperature (°C)	0.95**	-0.09
Total dissolved solid (mg/L)	0.73**	0.14
Nitrate (mg/L)	0.81**	0.25
Hardness (mg/L)	0.57**	0.16
Salinity (mg/L)	0.77**	0.27
pH	0.61**	-0.46
Biological oxygen demand (mg/L)	0.12	-0.47
Electrical conductivity (Mscm)	0.48	-0.66**
Dissolved oxygen (mg/L)	-0.67**	-0.99**

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

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

5. Discussion

The physicochemical characteristics of the water samples showed that the mean value of turbidity was high. [26, 29] reported that high turbidity may have devastating effects on the water quality. The pH value of water from the river Ala was slightly alkaline. The occurrence of alkaline pH is in line with [34] who asserted that the presence of higher pH value may be due to storm-water runoff from persistence or daily rain that move organic pollutant to surface water. Dissolved oxygen in the water samples from the river was in line with [9] who reported agricultural runoff, fauna manure waste, high level of organic matter and nutrient, mineralizing of organic matter and the metabolism activities by microorganism.

The mean concentration of temperatures is in line with [36], who suggested that increased temperatures have negative impacts, including preventing oxygen dissolution, and generating thermal pollution. Similarly, [4, 5] reported a significant reduction in availability of gases when there is an increase in chemical and biological processes of aquatic microorganisms. The concentration of nitrate in the water samples had an increasing peak value. This result is in agreement with [4, 18] where the authors reported several related factors such as sewage, synthetic fertilizer, organic materials and manure runoff from agricultural land.

High mean values of electrical conductivity in the water samples is in line with [2, 5] where the authors reported high concentration of dissolved salt and inorganic materials such as chlorides, sulfides and carbonate compound that originate from wastewater discharge and agricultural runoff. The average concentration of total dissolved solid in the water sam-

ples was less than the maximum permissible limit of (less than 500 mg/l) as recommended by WHO (2011). This is in agreement with [26, 28] where the authors reported high concentration of dissolved substances, presence of toxicological additives, chemical used for water treatment, industrial effluents that pollute the water at high rate and high evaporation rate leading to reduction in the volume of water. [6] reported that dilution effects in water may alter values of salinity. The mean concentration of hardness in the water samples are in line with [1] who reported an average total hardness of 132.5 ± 47.41 mg/L in water samples from locations in Nigeria. The level of biological oxygen demand in the water samples. This is in agreement with [11, 23] where the authors recorded continuous disposal of biodegradable organic waste in the river.

MGEs such as plasmid and integron were detected in *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae*. [20] highlighted the role of these elements in horizontal gene transfer, facilitating the spread of antibiotic resistance. The plasmid and integron detected were 10kb which was similar to the observation of [30] who reported that plasmid and integron are the most important facilitating agents in the fast spreading of antibiotics resistance among bacteria. The microbial resistance genes frequently carried on plasmid and integron have the ability to replicate and possibly the potential for self-transmission. The incidence of plasmid and integron among bacteria with resistance to antibiotics in this study is alarming because plasmid and integron have been identified as movable elements through which resistance and foreign genes are being transmitted in niches [25, 39] reported that genes that influence resistance bacteria are also frequently found on plasmid and integron. Consequently, non-pathogenic and antibiotic susceptible bacteria can be-

come pathogenic and resistant to antibiotics over time as a result of transmission of plasmid and integrin which can pose a health threat to the public.

pH plays a key role in bacterial growth and plasmid stability, affecting the mobility of genetic elements. In River Ala, pH values ranged from 3.2 to 8.34. Alkaline conditions, favor bacterial survival and promote the stability of plasmids and integrons, leading to more efficient horizontal gene transfer (HGT). [41] suggested that higher pH enhances bacterial conjugation, facilitating the spread of ARGs and acidic conditions may reduce bacterial activity and MGE transfer. BOD, an indicator of organic matter in water, correlates with microbial activity and biofilm formation. In River Ala, BOD values ranged from 3.2 mg/L to 4.58 mg/L, with higher BOD reflecting increased bacterial growth. Biofilms are important for MGE exchange as they provide a favorable environment for HGT. [14] demonstrated that lower BOD limits bacterial activity and the spread of MGEs.

Electrical conductivity (EC), which indicates ion concentration in water, influences bacterial cell interactions and biofilm development. In River Ala, EC ranged from 217 $\mu\text{S}/\text{cm}$ to 383.5 $\mu\text{S}/\text{cm}$. Higher EC promotes bacterial aggregation and biofilm formation, which facilitates close contact between bacteria, enhancing MGE exchange. [39] observed that increased EC levels encourage biofilm formation, promoting the transfer of ARGs in bacterial populations and lower EC values reduced MGE transfer. Dissolved oxygen (DO) impacts bacterial metabolism and survival, influencing MGE transfer. In River Ala, DO levels ranged from 4.72 mg/L to 6.69 mg/L. Higher DO supports the growth of aerobic bacteria, which are significant carriers of MGEs like plasmids and integrons. [17] found that higher DO concentrations promote bacterial metabolism, increasing the likelihood of MGE transfer and lower DO levels may limit bacterial activity and the dissemination of MGEs.

Turbidity, reflecting suspended particles in water, facilitates biofilm formation and HGT. In River Ala, turbidity ranged from 2.8 NTU to 12.2 NTU. Higher turbidity increases bacterial attachment to particles, leading to biofilm development where plasmids and integrons are more easily exchanged. [3] highlighted that lower turbidity in February likely reduces the opportunities for MGE exchange. Temperature affects bacterial growth rates and MGE transfer efficiency. In River Ala, temperatures ranged from 29.2 °C to 31.28 °C. Warmer temperatures accelerate bacterial metabolism and HGT, enhancing MGE transfer, particularly plasmids. [7] reported that lower temperatures significantly slow bacterial activity, reducing MGE exchange. Nitrate concentrations, indicative of nutrient availability, support bacterial growth and biofilm formation. In River Ala, nitrate levels ranged from 3.2 mg/L to 6.62 mg/L. Higher nitrate levels provide a nutrient-rich environment that boosts bacterial growth and MGE transfer. [21] demonstrated that nutrient-rich conditions enhance bacterial density, increasing opportunities for MGE exchange.

6. Conclusion

The findings of this study demonstrated that the level of turbidity in the water samples were high especially for samples collected during wet periods and low during the dry period. The results implied that the River Ala is contaminated with the faecal material of man or other animals and the presence of resistant bacteria genes such as *Escherichia coli*, *Salmonella enterica* and *Enterobacter cloacae*, which pose a potential health threat to individuals using it for agricultural and recreational purposes. In addition, detection of plasmid and integron were good marker for the identification of mobile genetic elements in the isolates, then the need for continuous monitoring and implementation of management strategies to mitigate pollution and curb the spread of antibiotic resistance in River Ala.

Abbreviations

ARB	Antibiotic Resistant Bacteria
BOD	Biological Oxygen Demand
DNA	Dissolved Oxygen
DO	Deoxyribonucleic Acid
MGEs	Mobile Genetic Elements
TDS	Total Dissolved Solid
WHO	World Health Organization

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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.

Funding

None was received.

Conflicts of Interest

The authors declare no conflicts of interest.

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