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Antibiogram Profiles of Heterotrophic Bacteria and Coliforms Isolated from Orogodo River in Agbor - Southern Nigeria

Jaboro A. G.[1,5*], Oyem M. I.[1,5], Obayagbona O. N.[2], Igborgbor J. C.[1], Konyeme T. E.[3], Oghene U. J.[4] & Ikechukwu C.[5]

[1]Microbiology Unit, Department of Biological Sciences, Faculty of Science, University of Delta, Agbor, Delta State
[2]Department of Environmental Management Toxicology, Faculty of Life Science, University of Benin, Benin City Edo State
[3]Plant Science & Biotechnology Unit, Department of Biological Sciences, Faculty of Science, University of Delta, Agbor, Delta State.
[4]Department of Environmental Management Toxicology, Faculty of Science, Dennis Osadebey University, Asaba, Delta State.
[5]Alpha Research Group, Microbiology Unit, Faculty of Science, University of Delta, Agbor, Delta State.

[*]Corresponding Author Email: grandy.jaboro@unidel.edu.ng

ABSTRACT
This study examined selected bacteriological attributes as well as the antibiogram and plasmid profiling of identified surface water borne bacterial cultures. Several water samples were collected from 3 locations along the Orogodo River in Delta State, Nigeria. Using serial dilution and pour plate procedures, the mean total heterotrophic bacterial (THBC) and coliform counts (TCC) of the water samples were determined. Tentative identification of the bacterial isolates was achieved by subjecting the respective isolates to several biochemical tests which included Gram staining and catalase production tests. The overall mean THBC and TCC ranged from 3.59 ± 241 × 10^5cfu/ml to 7.06 ± 177 × 10^5 cfu/ml and 3.32 ± 79 × 10^4 cfu/ml to 4.85 ± 186 × 10^4cfu/ml respectively. The observed difference between the overall mean THBC and TCC were not insignificant ($P> 0.05$). The tentatively identified bacterial isolates were; *Bacillus* sp., *Weissela*sp., *Pseudomonas* sp., *Corynebacterium* sp., *Micrococcus*sp., *Pseudomonas*sp., *Shigella*sp., *Serratia* sp., *Klebsiella* sp., *Eschericia coli* and *Citrobacter* sp. respectively. Antibiotic susceptibility/resistance testing of the bacterial isolates revealed that both *Corynebacterium* sp. and *Serratia* sp. Exhibited maximal multiple drug resistance index (MDRI) value of 0.6 respectively, while *Citrobacter* sp. showed the lowest MDRI value (0.1). Plasmid profiling indicated that *Bacilllus* sp, *Weissela* sp., *Corynebacterium* sp., *Micrococcus* sp. and *Shigella* spharbored plasmids while no plasmid was detected in *Klebsiella* sp. And *E. coli* respectively.Findings from this study suggested an urgent need for continuous monitoring of the river as well as implementation of pollution control strategies to protect both public health and the Orogodo river aquatic ecosystem respectively.

Keywords:
Antibiogram,
Antibiotic Resistance,
Orogodo, River,
Plasmid profiling

INTRODUCTION

Surface water bodies such as rivers are major sources of water for many communities but are vulnerable to contamination from human activities. In Nigeria, rivers like the Orogodo River in Delta State serve as important water sources for domestic and agricultural uses but face increasing pollution pressures (Iweriolor and Anyiam, 2023).

Anthropogenic activities which include; industrial discharge, agricultural runoff and improper waste disposal are known to contribute notably to water contamination (Okeke and Nwosu, 2021). Heterotrophic bacteria, known to rely on organic compounds for growth, are commonly used as indicators of microbial water quality and overall contamination levels (Onyango *et al.*, 2018).

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Elevated heterotrophic bacterial count can be utilized as an indicator of fecal pollution and the potential presence of pathogenic microorganisms (Adebami *et al*., 2020; Lipp *et al*., 2001).

Antibiotic resistance among bacteria in freshwater environments is a growing global health concern due to the potential for resistant strains to spread through water sources and impact human and animal health (Adesoji *et al.*, 2015). In Nigerian rivers, several studies have reported a high prevalence of multi-drug-resistant bacteria isolated from water samples, highlighting the role of environmental contamination in antibiotic resistance dissemination (Akpan *et al.*, 2020). For example, Gram-negative bacteria isolated from the Ogun River and abattoir effluents showed resistance to commonly used antibiotics including tetracycline, ampicillin, and ciprofloxacin (Akpan *et al.*, 2020). Resistance to tetracycline, often mediated by *Tet* genes, is particularly widespread in Nigerian aquatic bacteria, as noted in southwestern water distribution systems (Adesoji *et al.*, 2015). The presence of drug resistant bacteria in water sources has often linked to anthropogenic activities such as improper disposal of pharmaceutical waste, agricultural runoff, and inadequate wastewater treatment (Obayiuwana *et al.*, 2021). Pharmaceutical wastewaters have been identified as hotspots for the accumulation and spread of antibiotic resistance genes (ARGs), which can be horizontally transferred between bacteria, exacerbating resistance issues in the environment (Obayiuwana *et al.*, 2021). Moreover, the proliferation of antibiotic-resistant bacteria in aquatic environments has become a critical global health issue (Ogbolu*et al.,* 2013). Resistance genes are often carried on plasmids, which are mobile genetic elements capable of transferring antibiotic resistance traits between bacteria.

The presence of plasmid-bearing bacteria in surface waters facilitates the spread of resistance genes and complicates treatment of infections. Recent investigations have demonstrated that plasmids in environmental bacteria from Nigerian waters contribute significantly to antimicrobial resistance dissemination (Radhouani*et al*., 2014). Generally surface water bodies in the Niger Delta region, including parts of Delta State, have shown elevated bacterial loads due to domestic sewage discharge, oil exploration activities as well as agricultural runoffs (Eze and Okafor, 2021). These bacteria not only degrade water quality but can also contribute to the spread of antibiotic resistance genes through horizontal gene transfer (Adebayo *et al.*, 2023).

The suspected contamination of the Orogodo River with plasmid-bearing, antibiotic-resistant bacteria can pose potential health risks to local populations who depend on the water body as a source of drinking water (Iweriolor and Anyiam, 2023). The reported outcome of this study will contribute to understanding the microbial contamination status of Orogodo river, the spread of antibiotic resistance, and inform water quality management strategies to safeguard public health. The aim of this study was to determine the heterotrophic bacterial and coliform counts, antibiogram as well as the plasmid profiles of bacterial isolates cultured from water samples collected Orogodo River in Delta State, Nigeria.

MATERIALS AND METHODS

Description of Study Site

The Orogodo river originates from the dense rainforest region of Mbiri and flows through several towns in Delta State, Nigeria, including Owa, Agbor, Abavo, and Oyoko, before eventually discharging into the Ethiope River (Efe, 2013). Agbor, one of the major towns through which River Orogodo flows, is located at approximately latitude 6°15′13.5″ N and longitude 6°11′39.1″ E (Manpower Nigeria, 2020). The town lies at an elevation of around 160 meters above sea level and experiences a humid tropical climate, with an average annual rainfall of over 2,000 mm and mean annual temperature of about 24°C (Okolie *et al.*, 2022). The geographical attributes of Agbor is characterized by undulating terrain with both residential and industrial zones. The river plays a significant role as a water source for various local industries, and domestic activities (Ehiagbonare*et al.*, 2011). Notably, a slaughterhouse is located along the riverbank, contributing to the anthropogenic pollution of the river. Figure 1 shows the map of River Orogodo with sampling coordinates indicating upstream, midstream, and downstream collection points.

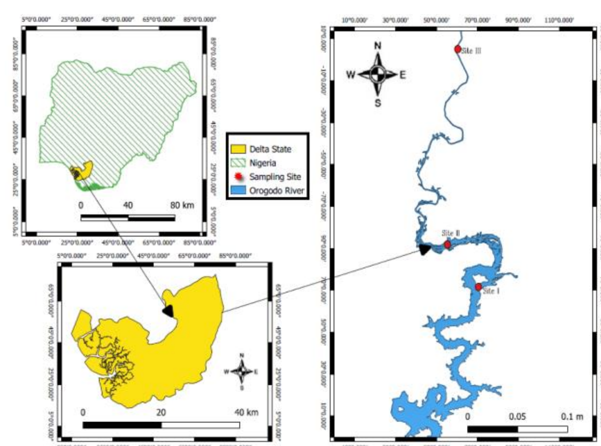


Fig 1: Map of Orogodo river showing the respective sampling points

Sample collection

The water samples were collected from the respective sampling points on the water body in February 2025. The water samples were collected in duplicates with the aid of clean and sterile 1 liter plastic containers and the samples were immediately placed in a cooler containing ice blocks to preserve its microbial integrity. Immediately after collection, all samples were transported to the microbiology laboratory for the determination of **heterotrophic bacterial count (HBC), antibiogram profiling** and subsequent plasmid analysis. Samples were taken from three strategic points along the Orogodo River namely station I (Umuaja village), station II (Umutu village) and station III (Agbor town). These stations represented upstream, midstream, and downstream of Orogodo river, respectively.

Bacteriological analysis of the water samples

The total heterotrophic bacterial and coliform counts of the various water samples were enumerated using both serial dilution and pour plate methods as described by Harley and Prescott (2002) as well as Cappuccino and Welsh (2020) with Peptone water (PW), Nutrient agar (NA) and MaConkey agar (MCA) utilized as serial diluent, general purpose as well as differential culture media respectively. The procedures were done in aseptic settings and the inoculated NA and MCA plates were labeled and incubated at 35^{o}C for 48h.

Sub-culturing and identification of bacterial isolates

Unique pure representatives of the bacterial isolates were sub-cultured, characterized and tentatively identified using relevant physiological and biochemical tests such as; Gram staining, Spore staining, oxidase test, catalase production, starch hydrolysis and urease production. The results were collated and compared with identification charts stated by Cullimore (2000), Collins *et al*. (2004) and Cheesbrough, (2006).

Bacterial inoculum standardization and antibiotic susceptibility/resistance testing

Prior to antibiogram determination, standardized liquid inocula for each purified and identified bacterial isolate directly equivalent to 0.5 McFarland standard was prepared using barium sulphate solution {0.5ml of 1% barium in chloride to 99.5ml of 1% H2SO4} (0.36 Normal) as described by Asowataet al. (2013). The standardized test bacterial inoculawere subjected to antibiotic susceptibility/ resistance test utilizing the modified Kirby-Bauer disc diffusion technique as described by Vandepitte*et al.* (2003). The culture media utilized was Muellar Hinton agar prepared according to manufacturer's instructions. The elicted growth inhibitory zones were interpreted as either resistant (R), intermediate (I) and sensitive (S) using the Clinical and Laboratory Standards Institute (CLSI) breakpoint guidelines (CLSI 2020). Commercially available antibiotic discs utilized were; Pefloxacin (10 µg, 30 µg PEF), Gentamicin (10 µg, 30 µg CN), Ampliclox (30 µg APX), Zinnacef (30 µg Z), Amoxicillin (30 µg AM), Rocephin (25 µg R), Ciprofloxacin (10 µg,30 µg CPX), Azithromycin, (12 µg AZ), Levofloxacin (20 µg LEV), Erythromycin (10 µg E), Septrin (30 µg SXT), Chloramphenicol (30 µg CH), Sparifloxacin (10 µg SP), Augmentin (10 µg AU), Tarivid (10 µg OFX) and Streptomycin (30 µg S). The plates were then inverted and incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition around each disc was measured in millimeters (mm) using a meter rule.

Determination of the multiple drug resistance index (MDRI) value

The multiple drug resistance index (MDRI) of the exposed bacterial isolates was evaluated using a formula described by Mir *et al.* (2021). The formula is given below:

MAR= A/B

wherein;

A: represent the number of antibiotics to which the isolate was resistant

B: total number of antibiotics to which the isolate was exposed

MARI was categorized as resistance to more than two classes of antibiotics among all tested antibiotics (Mir *et al*., 2021).

Plasmid DNA extraction, profiling and gel electrophoresis

Plasmid DNA was extracted from bacterial isolates using the **Zyppy™ Plasmid Miniprep Kit** (Zymo Research, Catalog No. D4036) according to the manufacturer's instructions. Six hundred (600 µl) of test bacterial culture grown in LB medium was added to a 1.5 ml microcentrifuge tube and it was centrifuged at 14,000 rpm. The resultant supernatant was discarded. About 100 µl of 7X Lysis Buffer (Blue) 1 was added and mixture was homogenized by inverting the tube 4-6 times. To this mixture, 350 µl of cold Neutralization Buffer (Yellow) was also and mix thoroughly. The mixture was centrifuged at 11,000 – 16,000 x g for 2-4 minutes and the resultant supernatant (~900 µl) was transferred into the provided Zymo-Spin™ IIN column. The column was then placed into a collection tube and centrifuged for 15 seconds. The flow-through was discarded and the column was placed back into the same collection tube. To the collection tube, 200 µl of Endo-Wash Buffer was added and the mixture was centrifuged for 30 seconds. To this column, about 400 µl of Zyppy™ Wash Buffer was added and the mixture was centrifuged for 1 minute. The column was then transferred into a clean 1.5 ml microcentrifuge tube and 30 µl of Zyppy™ was added to the tube. Elution Buffer was directly added to the column matrix and left to stand for one minute at room temperature. This mixture was centrifuged for 30 seconds to elute the plasmid DNA and the final eluted bacterial DNA was stored at 4°C for further analysis. Agarose gel electrophoresis was performed to determine the plasmid profiles of the bacterial isolates, following standard molecular protocols (Sambrook and Russell, 2001). To prepare the agarose gel, 2g of agarose was weighed and mixed with 100 mL 1xTAE in a microwavable flask. The mixture was microwaved for 3 min until the agarose was completely dissolved. The agarose solution was then allowed to cool down to about 50 °C for about 5 minutes upon which 10µL EZ vision DNA stain was added. The agarose was then poured into a gel tray with the well comb in place. The newly poured gel was then left to cool at 4 °C for 10-15 min until it had completely solidified. The solidified agarose gel was placed into the gel box (electrophoresis unit). Fill gel box with 1xTAE until the gel is covered and carefully load a molecular weight ladder into the first lane of the gel. The DNA samples were carefully loaded into the additional wells of the gel and the gel was at 100V for about 1hour. Upon attaining the 1 hour, the agarose gel was switched off and the electrodes were disconnected from the power source. The purified plasmid fragments were visualized under ultraviolet (UV) light, and plasmid sizes were estimated by comparison with the molecular weight DNA ladder.

Statistical analysis

The overall mean values for the respective counts were derived using Microsoft Excel 2016 software. The respective overall mean THBC and TCC values were subjected to Mann Whitney unpaired T test using SPSS version 21. The test was conducted at 95% probability level.

RESULTS AND DISCUSSION

The mean total heterotrophic bacterial counts (THBC) and total coliform counts (TCC) obtained from samples collected at the three different stations, namely, Umuaja, Umutu, and Agbor is shown in Table 1. The overall mean THBC ranged from $3.59 \pm 241 \times 10^5$ cfu/ml for Umutu station to $7.06 \pm 177 \times 10^5$ cfu/ml for Agbor station respectively. The overall mean TCC ranged from $3.32 \pm 79 \times 10^4$ cfu/ml for Agbor station to $4.85 \pm 186 \times 10^4$ cfu/ml for Umutu station respectively. The observed difference between the overall mean THBC and TCC was not significant ($P > 0.05$). The tentatively identified bacterial isolates were; *Bacillus* sp., *Weissela* sp., *Pseudomonas* sp., *Corynebacterium* sp., *Micrococcus* sp., *Pseudomonas* sp., *Shigella* sp., *Serratia* sp., *Klebsiella* sp., *Escherichia coli* and *Citrobacter* sp. respectively. The distribution pattern of the heterotrophic bacterial isolates from the different sampling points are shown in Table 2. Amongst the identified isolates, *E coli* and *Citrobacter* sp. Had maximal prevalence values as both isolates were cultured from 2 stations respectively.The heterotrophic bacterial and coliform counts were elevated and varied across the three sampling stations along the Orogodo River. This trend suggests that microbial loads were relatively elevated across all sites, possibly indicating on-going organic pollution of the water body. This trend is not surprising, given the fact that flowing water bodies usually constitute the primary inland water resources for domestic, industrial and irrigation purposes; and as such, are among the most vulnerable water bodies to pollution activities (Adedokun *et al*., 2008). The high microbial counts generally observed for all the samples collected from the respective sampling locations could be indicative of the high saprophytic microbial bio load of the water samples. The observed elevated bacterial counts could also be reflective of the fact that the examined water source is both natural and untreated, and is continually exposed to various forms of organic and inorganic pollution which can invariably boost the growth of various prokaryotic species. The elevated bacterial counts could also be reflective of the effects or impact of a plethora of both anthropogenic activities such as bathing, disposal of raw domestic wastes and environmental factors such as surface run offs arising from precipitation across the catchment area of the river. The range of total heterotrophic bacterial counts observed in this study was higher and at variance with mean values reported by Ogbonna (2010) for water samples obtained from the Imo, River, Eastern Nigeria and as well as Agbabiaka and Oyeyiola (2012) with respect to water samples sourced from several sampled points on the Foma river, Ilorin, Kwara State, North Central Nigeria. The isolation and identification of *Micrococcus* sp. and *Pseudomonas* sp. from the analysed water samples was in agreement with a report by Aluyi *et al*. (2006) which indicated the isolation of these bacterial isolates from surface water samples collected from Udu River, Warri, Delta State, Southern Nigeria.

Table 1: Mean heterotrophic bacterial and coliform count

Sample	**Site**	**Heterotrophic bacterial counts (cfu/ml)**	**Mean coliform count (cfu/ml)**
RP1(b)	Umuaja	6.33×10^5	4.67×10^4
RP1(c)	Umuaja	7.17×10^5	3.53×10^4
RP1(d)	Umuaja	6.87×10^5	4.20×10^4
Site 1 Mean	Umuaja	$6.79 \pm 43 \times 10^5$	$4.13 \pm 57 \times 10^4$
RP2(b)	Umutu	1.25×10^6	4.07×10^4
RP2(c)	Umutu	3.47×10^5	6.97×10^4
RP2(d)	Umutu	6.07×10^5	3.50×10^4
Site 2 Mean	Umutu	$3.59 \pm 241 \times 10^5$	$4.85 \pm 186 \times 10^4$
RP3(b)	Agbor	5.17×10^5	4.23×10^4
RP3(c)	Agbor	7.30×10^5	2.90×10^4
RP3(d)	Agbor	8.70×10^5	2.83×10^4
Site 3 Mean	Agbor	$7.06 \pm 177 \times 10^5$	$3.32 \pm 79 \times 10^4$

Table 2: Distribution pattern of the bacterial isolates form Orogodo River

	Bacterial isolates											
Stations	***Bacillus* sp.**	***Weissela* sp.**	***Pseudomonas* sp.**	***Corynebacterium* sp.**	***Micrococcus* sp.**	***Pseudomonas* sp.**	***Shigella* sp.**	***Shigella* sp.**	***Serratia* sp.**	***Klebsiella* sp.**	***Eschericia coli***	***Citrobacter* sp.**
RP1(b)								+			+	
RP1(c)		+					+					
RP1(d)	+											
RP2(b)			+									
RP2(c)				+								+
RP2(d)					+				+			
RP3(b)												
RP3(c)						+				+		
RP3(d)											+	+

The antibiotic sensitivity/resistance (antibiogram) profiles of the bacterial isolates recovered from the respective samples is shown in Table 3. The antibiogram results revealed varying degrees of antibiotic susceptibility /resistance among the bacterial isolates (Table 3). Among the Gram-positive organisms, *Bacillus sp.* strains recorded a MDRI value of 0.3 and were resistant to older beta-lactam antibiotics such as ampicillin, ampiclox, and zinnacef but remained sensitive to fluoroquinolones like ciprofloxacin and azithromycin, as well as rifampicin. *Weissella sp.* Strains exhibited a slightly higher MDRI value of 0.4, with resistance to erythromycin and beta-lactams, though it displayed sensitivity to ciprofloxacin and levofloxacin. Exposed *Corynebacterium sp.* strains displayed the highest resistance among the Gram-positive isolates, with a MDRI value of 0.6. The bacterial strains was resistant to a variety of antibiotics, including chloramphenicol and ampicillin, but remained sensitive to ciprofloxacin and levofloxacin. *Micrococcus sp.* strains had an MDRI value of 0.4, with resistance to beta-lactams but were susceptible to quinolones and macrolides.

The Gram-negative isolates demonstrated a wider range of resistance profiles. Strains of Pseudomonas *sp.*[1] and *Pseudomonas sp.*[2] both had MDRI value of 0.3 and were resistant to sulfonamides and augmentin but sensitive to ciprofloxacin, ofloxacin as well as pefloxacin. *Shigella sp.*[1] strains had a MDRI value of 0.4, showing resistance to four antibiotics including streptomycin and sulfonamides, while *Shigella sp.*[2] had a slightly lower MDRI of 0.3. Strains of *Serratia sp.* had a MDRI value of 0.6 with resistance to multiple antibiotic classes and limited sensitivity to fluoroquinolones. *Klebsiella sp.* strains had a MDRI value of 0.4 with susceptibility to pefloxacin and ofloxacin but resistance to several beta-lactam and sulfonamides respectively. Strains of E. *coli* had a MDRI value of 0.2 and were resistant to sparifloxacin and augmentin respectively. Strains of *Citrobacter sp.* had the lowest MDRI value of 0.1, showing susceptibility to nearly all antibiotics tested.

Overall, the results indicate that while some of the exposed bacterial isolates were susceptible, especially to fluoroquinolones as well as pefloxacin, there was expressed resistance to older and commonly used antibiotics, particularly amongst the Gram-negative isolates. However, the MDRI values for majority (5 out of 8) of the Gram-negative isolates were lower than 0.4. Generally, the percentage of the all the isolates which had an MDRI value higher than 0.2 was 83% (10 out of 12). This observation was in agreement with a report by Popoola *et al.* (2024) which revealed that about 88% of exposed water borne bacterial isolates had MDR index values greater than 0.2. The antibiotic sensitivity profile of the bacterial isolates revealed varying levels of resistance, with some isolates such as *Cornyebacterium* sp. and *Serratia* sp. displaying appreciable multidrug resistance as these cultures exhibited MDRI index value of 0.6 respectively. This trend suggested the likely involvement of acquired resistance mechanisms.

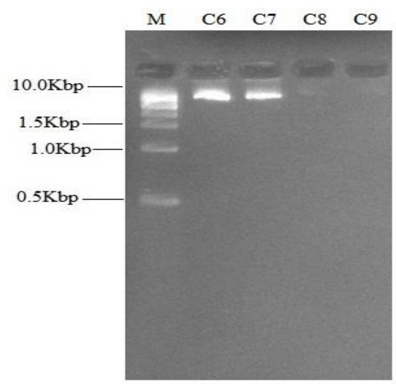
The detection of multiple drug resistant (MDR) strains of *Cornyebacterium* sp. in this study was in agreement with an earlier report by Popoola *et al.* (2024) which detailed the isolation of MDR *Corynebacterium* spp. from five rivers in Oyo town, Oyo State, SouthWestern Nigeria. The detection of multidrug-resistant water borne bacterial strains could be reflective of indiscriminate usage of these drugs amongst residents living within the catchment area of the water body. This trend has also been documented by Popoola *et al*. (2024) and the authors also opined that these waterborne isolates might have been exposed to antibiotic residues likely present in human feaces which could be present in the water body. A conseqence of this exposure would be the isolates developing resistance to these drug residues as a means of survival. The presence of antibiotic residues in human feaces has been attributed to the widespread practice of self-prescription and consumption of antibiotics in the treatment of gastrointestinal infections, which can invariably lead to development of fewer effective and alternative therapies (Delgado-Gardea *et al*., 2016).

Table 3: Antibiogram profile of the bacterial isolates from Orogodo River

Gram positive disc											
ISOLATES	**PEF (10µg)**	**CN (10µg)**	**APX (30µg)**	**Z (20µg)**	**AM (30µg)**	**R (25µg)**	**CPX (10µg)**	**AZ (12µg)**	**LEV (20µg)**	**E (10µg)**	**MDRI**
Bacillus sp.	*100 (I)	100 (I)	100 (R)	100 (R)	100 (R)	100 (I)	100 (S)	100 (S)	100 (S)	100 (S)	0.3
Weissella sp.	100 (S)	100 (I)	100 (R)	100 (R)	100 (R)	100 (I)	100 (S)	100 (S)	100 (S)	100 (R)	0.4
Corynebacterium sp.	100 (I)	100 (R)	100(R)	100 (R)	100 (R)	100 (R)	100 (S)	100 (R)	100 (S)	100 (I)	0.6
Micrococcus sp.	100 (S)	100 (R)	100 (R)	100(R)	100(R)	100 (I)	100 (I)	100 (I)	100 (S)	100 (S)	0.4
Gram negative disc											

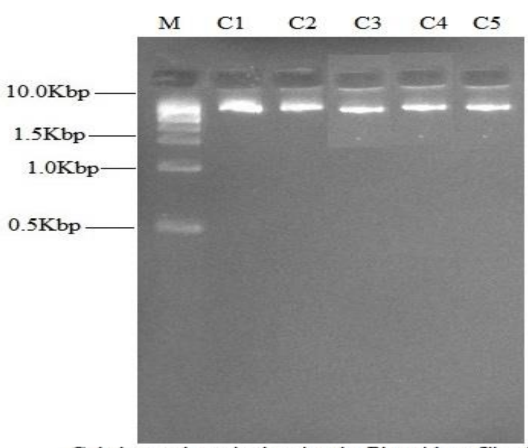
	SXT (30µg)	CH (30µg)	SP (10µg)	CPX (30µg)	AM (30µg)	AU (10µg)	CN (30µg)	PEF (30µg)	OFX (10µg)	S (30µg)	MDRI
Pseudomonas sp.[1]	100 (R)	100 (I)	100 (S)	100 (S)	100 (S)	100 (R)	100 (I)	100 (I)	100 (S)	100 (R)	0.3
Pseudomonas sp.[2]	100 (R)	100 (I)	100 (I)	100 (I)	100 (R)	100 (R)	100 (I)	100 (S)	100 (S)	100 (I)	0.3
Shigella sp.[1]	100 (R)	100 (I)	100 (I)	100 (I)	100 (R)	100 (R)	100 (I)	100 (I)	100 (S)	100 (R)	0.4
Shigella sp.[2]	100 (R)	100 (R)	100 (I)	100 (I)	100 (I)	100 (R)	100 (I)	100 (S)	100 (S)	100 (S)	0.3
Serratia sp.	100 (R)	100 (R)	100 (R)	100 (R)	100 (R)	100 (R)	100 (I)	100 (S)	100 (S)	100 (I)	0.6
Klebsiella sp.	100 (I)	100 (R)	100 (R)	100 (R)	100 (I)	100 (R)	100 (S)	100 (S)	100 (S)	100 (I)	0.4
Escherichia coli	100 (R)	100 (I)	100 (S)	100 (I)	100 (I)	100(R)	100 (I)	100 (I)	100 (I)	100 (I)	0.2
Citrobacter sp.	100 (S)	100 (I)	100 (S)	100 (I)	100 (I)	100 (R)	100 (I)	100 (I)	100 (I)	100 (S)	0.1

Key: * Percentage values of exposed bacterial strains, PEF – Pefloxacin, CN – Gentamicin, APX – Ampliclox, Z – Zinnacef, AM – Amoxicillin, R – Rocephin, CPX – Ciprofloxacin, AZ – Azithromycin, LEV – Levofloxacin, E – Erythromycin, SXT – Septrin, CH – Chloramphenicol, SP – Sparifloxacin, AU – Augmentin, OFX – Tarivid, S – Streptomycin, MDRI - Multiple Drug Resistance Index Resistance R- Resistant, I-Intermediate, S-Sensitive



Gel electrophoresis showing the Plasmid profiling for the isolates. Isolates C6 and C7 has high concentration of Plasmids of about 10kbp while isolate C8 and C9 has no Plasmids. Lane M is 1kbp DNA ladder used to estimate the size of the Plasmids.

The results of the plasmid profiling analysis are shown in Fig 2 and Fig 3. Gel electrophoresis results showed the presence of plasmids in *Bacilllus* sp, *Weissela* sp., *Corynebacterium* sp., *Micrococcus* sp. and *Shigella* sp. [1], all of which showed plasmid bands of approximately 10.0 kilobase pairs (kbp) with high band intensity, indicating a high concentration of plasmid DNA. No plasmid was detected in Klebsiella sp. *a*nd Escherichia *coli* respectively.



Gel electrophoresis showing the Plasmid profiling for the isolates. Isolates C1-C5 has high concentration of Plasmids of about 10kbp. Lane M is 1kbp DNA ladder used to estimate the size of the Plasmids.

Fig 2: Plasmid profile of the Gram-Positive bacterial isolates

Fig 3: Plasmid profile of the Gram-Negative bacterial isolates

The plasmid profiling results revealed that some of the water borne bacterial isolates; Bacilllus sp., *Weissela* sp., *Corynebacterium* sp., *Micrococcus* sp., and *Shigella* sp[1] from the Orogodo River harbored plasmids of approximately 10kbp in size, while two isolates *Klebisiella* sp. and *E. coli*, did not harbor any detectable plasmid. The presence of plasmids in these isolates indicates a high prevalence of plasmid-bearing bacteria within the aquatic environment (Thomas and Nielsen, 2005). Similar results as reported by Igbinosa *et al.* (2022), found a high proportion of plasmid-bearing *Enterobacteriaceae* in surface water samples from the Ikpoba River in Southern Nigeria. Their study also noted plasmid sizes ranging from 8 to 12 kbp, suggesting a shared pattern in environmental plasmid profiles across different river systems within the region. The consistent size and intensity of the plasmid bands would infer that these plasmids may be conserved among different bacterial species or strains, potentially due to selective environmental pressures (Davies and Davies, 2010). Likewise, Radhouani *et al.* (2014) reported plasmid-mediated antibiotic resistance in bacteria isolated from aquatic environments in Portugal. The authors also noted that plasmids frequently harbored genes conferring resistance to β-lactams and tetracyclines, often as part of mobile genetic elements that facilitate horizontal gene transfer. The absence of plasmids in both Klebisiella sp., *E. coli*, could be attributed to natural variation in plasmid carriage, plasmid loss, or detection limitations. The results are also consistent with studies that link plasmid carriage with polluted environments. Akinde *et al.*, (2016), observed that plasmid-bearing bacteria were more abundant in regions with anthropogenic influence, especially near areas of sewage discharge. The Orogodo River, which flows through urban and agricultural zones, is likely subjected to similar pressures ranging from domestic wastewater to runoffs containing agrochemicals thus promoting plasmid retention. However, some differences exist when comparing with Soge *et al.* (2009), who reported more variable plasmid sizes (ranging from 2 to >50 kbp) among environmental isolates from U.S. rivers. This discrepancy may arise from differing pollution types, local antibiotic usage patterns, or bacterial species composition in the environment. Additionally, the plasmid detection technique used (agarose gel electrophoresis) may limit the resolution of smaller or low-copy plasmids, potentially underestimating the actual diversity present.

The absence of plasmids in the two isolates *Klebisiella* sp. and *E. coli*, may also be reflective of the prevailing natural microbial diversity within the river ecosystem. According to Ogbolu *et al.* (2013), not all environmental bacteria carry plasmids, and plasmid presence can fluctuate depending on environmental conditions, species involved, and even laboratory handling. Plasmid loss can occur due to the absence of selective pressure or during culturing when plasmid maintenance is metabolically costly (Wein *et al.*, 2019). This may explain the lack of detectable plasmids in these two isolates. Nonetheless, this heterogeneity could be reflective of the prokaryotic diversity of the river ecosystem. These findings suggest that the bacterial populations in the Orogodo River have adapted to environmental stress, possibly *via* the expression of plasmid-mediated traits (Madigan *et al.*, 2021).

CONCLUSION

Surface water samples collected from Orogodo River at three sampling points harbored a variety of both heterotrophic bacteria and coliforms. Several isolates were identified from the samples and they include; *Bacillus* sp., *Weissela* sp., *Pseudomonas* sp., *Corynebacterium* sp., *Micrococcus* sp., *Pseudomonas* sp., *Shigella* sp., *Serratia* sp., *Klebsiella* sp., *E. coli* as well as Citrobacter sp. Two of these isolates (*Corynebacterium* sp. *and Serratia* sp.) had an MDRI value of 0.6 respectively. It was revealed in this study that five of the tentatively identified isolates harbored extrachromosonal plasmids. The plasmids, estimated at around 10kbp, might play notable roles in enhancing bacterial adaptability and survival in the river's potentially contaminated environment.Further molecular analysis, including sequencing of the plasmids, is recommended to understand the genetic content and ecological implications of these mobile elements. The documented findings would suggest an urgent need for continuous monitoring of the river as well as implementation of pollution control strategies to protect both public health and the Orogodo river ecosystem respectively.

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