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Presence, distribution and antimicrobial resistance profiles of Microorganisms in surface and underground water in Owo Metropolis

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ABSTRACT: Microbial contamination in both surface and groundwater sources presents a significant public health concern (World Health Organization, 2020). This study aimed to evaluate bacterial diversity and antimicrobial resistance patterns in water samples from various locations within Owo Metropolis. Standard microbiological methods were used to isolate, characterize, and identify bacterial strains. Hemolytic activity was assessed to determine potential pathogenicity. Antibiotic susceptibility was tested using the disk diffusion method. The multiple antibiotic resistance index (MARI) was calculated to evaluate the extent of resistance. Molecular techniques, including polymerase chain reaction (PCR) and plasmid curing, were employed to investigate the genetic basis of antibiotic resistance. A total of thirty-four (34) bacterial isolates were identified, with Bacillus spp. being the most prevalent. High bacterial counts were observed across all water samples. The isolates displayed varying levels of resistance to multiple antibiotics, with several strains harboring high-molecular-weight plasmids. Plasmid curing experiments indicated that antibiotic resistance in these isolates was plasmid mediated. These findings underscore the urgent need for effective water treatment and sanitation practices to reduce the risks associated with waterborne infections and antimicrobial resistance in Owo Metropolis.

Keywords: *Surface and ground water, Microbial diversity, Antimicrobial resistance, Plasmid profile, Plasmid curing, Polymerase chain reaction (PCR)*

Introduction

Access to safe and clean water is a fundamental human right, as water is essential for life. The quality of water sources, including both surface and groundwater, significantly influences public health. Waterborne diseases remain a major global health threat, contributing to considerable illness and death (World Health Organization, 2020). In Nigeria, many communities depend on unprotected water sources such as rivers, boreholes, and dams for various domestic purposes, including drinking. Unfortunately, these water bodies are frequently contaminated with pathogenic microorganisms. The increasing prevalence of antimicrobial resistance (AMR) among these pathogens further intensifies public health risks (Manetu and Karanja, 2021). To safeguard public health, it is crucial to assess the microbiological quality and antibiotic resistance profiles of water sources. By understanding the types of microorganisms present and their resistance patterns, effective strategies can be implemented to prevent the spread of waterborne diseases and mitigate the impact of AMR (Kussi et al., 2022).

Literature Review

Human activities significantly impact the quality of water sources (Umar et al., 2003). Assessing the physicochemical parameters of water is essential before its use for consumption, domestic, agricultural, or industrial purposes. These parameters vary based on the intended use and the required purity level (Umar et al., 2003). Recent research has indicated alarming trends in the microbial quality of surface waters, revealing the presence of disease-causing bacteria and the increasing occurrence of antibiotic-resistant strains. For example, Ahmed et al. (2019) found high levels of antibiotic-resistant bacteria in urban lakes, highlighting the influence of urbanization on microbial profiles and linking antibiotic resistance to pollution from sewage and industrial wastewater.

Antimicrobial resistance is a growing global health crisis. The overuse and misuse of antibiotics in both human and animal medicine have created selective pressure,

leading to the development of resistant bacteria (Levy et al., 2020). Groundwater sources are also susceptible to contamination by multidrug-resistant bacteria. Hassan et al. (2023) found high levels of resistance in bacteria such as *Klebsiella pneumoniae* and *Acinetobacter baumannii* in groundwater samples. These findings suggest that agricultural practices, particularly the use of antibiotics in livestock, contribute to groundwater contamination and pose risks to public health.

The mechanisms driving antimicrobial resistance in aquatic environments are complex. Sharma et al. (2023) reviewed the role of environmental factors, including heavy metals, antibiotics, and biocides, in selecting for resistant microorganisms. Horizontal gene transfer among microbial populations facilitates the rapid spread of resistance genes. Lajqi et al. (2024) emphasized the role of environmental reservoirs in maintaining AMR, noting that water bodies can act as both carriers and amplifiers of resistance genes. The presence and distribution of microorganisms in surface and groundwater are influenced by various environmental and human-related factors. The increasing prevalence of antimicrobial resistance underscores the urgent need for effective monitoring and management strategies to protect public health and water quality.

Materials and Methods

2.1 Sample Collection

Water samples, including both surface and groundwater, were collected from four different locations in Owo. At each location, three samples were collected using sterile bottles. The samples were immediately transported to the Microbiology Laboratory at Achievers University, Owo, and stored at appropriate temperatures.

2.2 Heterotrophic Plate Count

The heterotrophic plate count was determined using the pour plate technique. Serial dilutions of each water sample were prepared. A 1 ml aliquot from the 10^{-5} and 10^{-6} dilutions was transferred to sterile Petri dishes. Molten nutrient agar was poured into each plate, gently mixed, and allowed to solidify. The inoculated plates were incubated at 37°C for 24 hours. Colony-forming units (CFUs) were counted using a colony counter. The CFU/ml was calculated using the formula:

CFU/ml=Volume Plated (Number of Colonies × Total Dilution Factor)

2.3 Enumeration of Total Coliforms

The Most Probable Number (MPN) procedure was used to enumerate total coliforms, following APHA (2017) guidelines. This method involves three stages: presumptive, confirmed, and completed tests.

2.3.1. Presumptive Test: Ten-ml portions of single-strength and double-strength lactose broth were inoculated with appropriate dilutions of the water sample. Durham tubes were inverted into the broth tubes to collect any gas produced. The tubes were incubated at 37°C for 24 hours. Gas production and acid formation indicated the potential presence of coliforms. The MPN index was determined using the MPN (McCrady) table.

2.3.2. Confirmed Test: A loopful of bacterial culture from a positive presumptive test tube (2.3.1) was transferred to a brilliant green bile broth (BGLB) tube. The tube was incubated at 37°C for 24-48 hours. Gas production in the Durham tube confirmed the presence of coliforms (Adetunde and Glover, 2010).

2.3.3. Completed Test: A loopful of culture from a positive confirmed test tube was streaked onto an Eosin Methylene Blue (EMB) agar plate. The plate was incubated at 37°C for 24-48 hours. The presence of colonies with a green metallic sheen on the EMB agar confirmed the presence of *Escherichia coli* (Adetunde and Glover, 2010).

2.4. Isolation, Characterization, and Identification of Bacterial Isolates

The procedures described by Negera et al. (2017) and Cheesebrough (2010) were followed for preparing water samples and standardizing inocula, using sterile distilled water as the diluent. The second dilution factor was chosen as the standard for microbial isolation to ensure accurate numerical estimation of colony-forming units on agar plates. Nutrient agar was used for the isolation of heterotrophic bacteria. MacConkey agar and Eosin Methylene Blue agar were used to isolate coliforms and fecal coliforms, respectively, as described by Vulindlu et al. (2004).

2.5. Biochemical Identification of Isolates

Distinct subcultured colonies were subjected to a series of biochemical tests, following the methods outlined by Negera et al. (2017), Cheesebrough (2010), and Olutiola et al. (2001), to characterize and identify the isolates.

2.6. Pathogenicity Tests

To assess pathogenicity, the isolates were cultured on MacConkey agar plates (MAP), Blood agar plates (BAP), and Chocolate agar plates (CAP).

2.6.1 Pathogenicity Test on BAP: The hemolytic properties of hydrolytic bacterial isolates were determined by streaking a loopful of each single colony onto the surface of sheep blood agar plates, followed by incubation at 37 °C for 18-24 hours and further incubation for another 24 hours for confirmation. BAP media was prepared according to the manufacturer's instructions (Thermo Scientific, UK). Following a technique first reported by Buxton (2005), sterile defibrinated blood, warmed to room temperature, was added to sterile blood agar base (melted and cooled to 45-50°C) at a concentration of 5% (v/v). The mixture was gently swirled to ensure homogeneity while avoiding bubble formation. The mixture was then carefully dispensed into sterile plates to prevent bubbles and froth on the agar surface. Hemolysis was observed after 24-36 hours of incubation based on color changes in the zone surrounding the bacterial colonies.

2.6.2. Pathogenicity Test on CAP: CAP media was prepared following the manufacturer's instructions (Thermo Scientific, UK). The plates were observed after 24 hours of incubation for specific colony morphology and hemolysis (Darmawati et al., 2021).

2.6.3. Pathogenicity Scoring System: A plate-based pathogenicity scoring system was established in this study based on observation techniques previously reported for MAP, BAP, and CAP (Buxton, 2016).

2.7. Standardization of Inocula

Bacterial cultures were incubated on nutrient agar plates at 37°C for 24 hours. A 0.1 ml volume of bacterial cells was transferred into sterile normal saline, prepared by mixing 0.6 ml of 1% barium chloride with 99.4 ml of sulfuric acid, to achieve a turbidity equivalent to the 0.5 McFarland standard (CLSI, 2016). The standardized bacterial suspension was evenly spread over Mueller–Hinton agar (Oxoid) to ensure confluent growth. Antibiotic discs were promptly placed on the agar surface using sterile forceps, followed by aerobic incubation at 37°C for 16 hours. Zones of inhibition were measured, and susceptibility was categorized as sensitive,

intermediate, or resistant based on Clinical Laboratory Standards Institute guidelines (CLSI, 2012).

2.8. Evaluation of Antimicrobial Susceptibility to Antibiotics

Antibiotic susceptibility testing was performed using a modified Kirby-Bauer disk diffusion method (Bauer et al., 1966) and standardized according to the Clinical Laboratory Standards Institute protocols (CLSI, 2006).

2.9. Calculation of Multiple Antibiotic Resistance (MAR) Indices

The Multiple Antibiotic Resistance (MAR) Index was calculated using the formula:

MAR Index for isolates=ba

Where “a” represents the number of antibiotics to which the isolate shows resistance, and “b” is the total number of antibiotics tested (Osundiya et al., 2013).

2.10 Plasmid Analysis of Resistant Isolates

Plasmid isolation was performed using the QIAGEN Plasmid Purification Mini Kit, following the provided protocol.

2.10.1. Gel Integrity Assessment: The method for plasmid isolation described by Zang and Calahan (2007) was used. The integrity of the extracted plasmids was assessed using a 1% agarose gel to confirm amplification. A 1X TAE buffer was prepared and used for gel preparation. To create the gel, an agarose suspension was heated in a microwave for 5 minutes until molten. After cooling to 60°C, the solution was stained with 3 µl of 0.5 g/ml ethidium bromide. A comb was placed in the casting tray to form wells, and the molten agarose was poured into the tray. After 20 minutes of solidification, the gel was submerged in 1X TAE buffer within the gel tank. For sample preparation, 2 µl of 10X blue gel loading dye was added to each sample. To facilitate loading and track electrophoresis progress, 2 µl of 10X blue gel loading dye was added to 10 µl of each PCR product. The samples were loaded into the wells, with a 100–3000 bp DNA ladder loaded into the first well as a molecular weight marker. Electrophoresis was performed at 120 V for 45 minutes. The gel was visualized under ultraviolet (UV) trans-illumination and photographed. The sizes of the PCR products were estimated by comparing their mobility with that of the DNA ladder.

2.11. Plasmid Curing of Resistant Isolates

To determine if the resistance markers were plasmid-borne or chromosomal, plasmid curing experiments were conducted using 10% sodium dodecyl sulfate (SDS). An overnight culture (0.2 ml) was added to 5 ml of nutrient broth containing 10% SDS and incubated at 37°C. After 24 hours, the broth cultures were thoroughly mixed and sub-cultured onto Mueller-Hinton agar (MHA) plates. These plates were then incubated at 37°C for another 24 hours. Following incubation, colonies were tested for antibiotic resistance using the disk diffusion method. Differences in the antibiograms of isolates before and after curing were used to identify cured resistance markers (Akortha et al., 2011).

3. Results

3.1 Heterotrophic Plate Count (HPC) of Bacteria in Water Samples

The heterotrophic plate count (HPC) of bacteria isolated from water samples collected across the selected areas is presented in Table 1. The HPC values ranged from 5.3×10^4 to 9.2×10^4 CFU/ml. Samples from Isaipen exhibited the highest bacterial colony counts, ranging from 9.0×10^4 to 9.2×10^4 CFU/ml. This was followed by samples from AUO, with counts ranging from 7.5×10^4 to 8.4×10^4 CFU/ml, and samples from AUFH, which had counts ranging from 7.0×10^4 to 7.9×10^4 CFU/ml. Samples from Okeogun recorded the lowest colony counts, with values ranging from 5.3×10^4 to 6.4×10^4 CFU/ml.

3.2 Coliform Bacterial Enumeration from Water Samples

3.2.1 Presumptive Test: The results of the presumptive test using the Most Probable Number (MPN) method are presented in Table 2. Water samples collected from Isaipen exhibited the highest coliform counts, ranging from 900 to over 1600 MPN/100ml. This was followed by samples from AUFPO, with counts between 4 and 14 MPN/100ml, and AUFH, with counts ranging from 4 to 8 MPN/100ml. The lowest coliform counts were recorded in samples from Okeogun, which ranged from values less than 2 to 2 MPN/100ml.

3.2.2 Confirmatory and Completed Test Results Using the MPN Method: The confirmatory and completed test results indicated the presence of both coliforms and

fecal coliforms (*E. coli*) in all water samples from Isaipen. However, water samples from the other locations investigated were free from fecal coliforms, as shown in Table 3.

3.3 Morphological and Biochemical Characterization of Isolates

The organisms isolated from the water samples were identified based on their biochemical, colonial, and morphological characteristics, with confirmation using Bergey's Manual of Determinative Bacteriology (9th edition). The identified isolates included *Bacillus* sp., *Streptococcus* sp., *E. coli*, *Edwardsiella* spp., *Salmonella* spp., *Staphylococcus* spp., *Enterobacter* spp., *Citrobacter* sp., and *Klebsiella* sp. (Table 4).

3.4 Percentage Occurrence of Bacterial Isolates

A total of 34 bacterial isolates were identified during the study. Their percentage occurrences were as follows: *Bacillus* sp. (38.2%), *Streptococcus* sp. (17.65%), *E. coli* (11.76%), *Edwardsiella* sp. (8.82%), *Salmonella* sp. (5.88%), *Staphylococcus* sp. (5.88%), *Enterobacter* sp. (5.88%), *Citrobacter* sp. (2.94%), and *Klebsiella* sp. (2.94%) (Figure 1). Additionally, the overall distribution of Gram-positive and Gram-negative bacteria across the nine species is illustrated in Figure 5, with Gram-negative organisms showing a higher prevalence (66.7%).

3.5 Pathogenicity Test

The pathogenicity test results for resistant isolates are presented in Table 5. Fermenting bacteria, including *E. coli*, *Enterobacter* sp., *Klebsiella* sp., and *Edwardsiella* sp., exhibited violet coloration on MacConkey agar. On blood agar plates, *E. coli* and *Klebsiella* sp. displayed beta (β) hemolysis, while *Enterobacter* sp. showed alpha (α) hemolysis, and *Edwardsiella* sp. exhibited gamma (ϒ) hemolysis.

3.6 Antibiotic Susceptibility

Antibiotic susceptibility and resistance profiles for Gram-positive bacteria are summarized in Table 6. All isolates demonstrated 100% susceptibility to Pefloxacin (PEF). Gram-positive organisms, such as *Staphylococcus* sp. and *Bacillus* sp., showed complete susceptibility to Ciprofloxacin (CPX), although some *Streptococcus* sp. exhibited resistance, with a susceptibility rate of 83.3%. *Micrococcus* sp. displayed 100% susceptibility to Pefloxacin (PEF), Gentamycin (CN), Ampiclox (APX), Rocephin (R), Streptomycin (S), Septrin, and Erythromycin

(E), but only 50% susceptibility to Zinnaclef (Z) and Amoxicillin (AM). High resistance rates were observed in *Streptococcus* sp. (66.7%), *Bacillus* sp. (53.8%), and *Staphylococcus* sp. (50%) against Amoxicillin (AM).

Klebsiella sp. was resistant to all tested antibiotics except Gentamycin (CN) and Ampiclox (APX). Similarly, *Citrobacter* sp. was resistant to Amoxicillin (AM), Rocephin (R), Septrin (SXT), and Erythromycin (E). *Salmonella* sp. exhibited 100% resistance to PEF, AM, R, SXT, and E, while *E. coli*, *Enterobacter*, and *Edwardsiella* sp. demonstrated mixed resistance and susceptibility profiles (Table 7).

The MAR indices for both Gram-positive and Gram-negative isolates, as detailed in Table 8, ranged from 0.9 to 1. A MAR value exceeding 0.2 is indicative of multiple antibiotic resistance (Osundiya et al., 2013).

3.8 Plasmid Profile

Figure 2 displays the electrophoretic gel plate resulting from plasmid characterization of the isolates. Isolates B4, C3, and WA exhibited band sizes greater than 3000 bp when compared to the 100–3000 bp ladder. In contrast, isolate A3 showed no detectable plasmid bands. These results suggest that antibiotic resistance in isolates B4, C3, and WA is likely mediated by high molecular weight plasmids, while resistance in isolate A3 may be chromosomally encoded.

3.9 Plasmid Curing

Following the plasmid curing process, bacteria that initially showed resistance to specific antibiotics became susceptible, as presented in Table 9. This indicates the potential role of plasmids in mediating this resistance.

4.0 Discussion

Water intended for drinking or domestic purposes must be free of disease-causing microorganisms. However, surface and groundwater sources can harbor microorganisms that pose significant health risks (World Health Organization, 2020). In this study, the heterotrophic plate count (HPC) ranged from 5.3×10^4 to 9.2×10^4 CFU/ml, surpassing the WHO permissible limit of ≤500 CFU/ml. Similarly, coliform counts from the presumptive test exceeded the WHO standard of zero MPN/ml in all samples except those from Okeogun. This suggests that the majority of the examined water sources are unsafe for consumption without prior treatment.

Elevated coliform counts are indicative of fecal contamination, a finding consistent with EPA (2002) guidelines. The comparatively lower coliform count in Okeogun might be attributed to the depth of its underground water sources; however, inadequate well construction and proximity to sanitation facilities can still compromise water quality. The confirmed and completed tests detected *E. coli* in all samples from Isaipen, which is a strong indicator of fecal contamination and the potential presence of other pathogens such as *Salmonella* spp., *Shigella* spp., and *Vibrio* spp., all known to be associated with waterborne diseases (Isa et al., 2015).

The study successfully identified a diverse range of bacteria, including *Bacillus* spp., *Streptococcus* spp., *E. coli*, *Salmonella* spp., *Staphylococcus* spp., *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp., and *Edwardsiella* spp., suggesting contamination originating from domestic sources (Okiki & Ivbijaro, 2013). Gram-negative bacteria, particularly *E. coli*, *Klebsiella* spp., *Enterobacter* spp., and *Edwardsiella* spp., exhibited high levels of antibiotic resistance. These observations are consistent with the findings of Atobatele and Owoseni (2023), who also reported similar resistance patterns. Notably, *E. coli* strains displayed resistance to all tested antibiotics, exhibiting a multidrug resistance (MDR) index of 1, which raises significant public health concerns (Oteo et al., 2005; Bartoloni et al., 2006).

The increasing prevalence of multidrug-resistant bacteria, potentially driven by antibiotic overuse in agriculture, poses serious health risks to humans, animals, and the environment (Allen et al., 2013). Pathogenicity tests confirmed that all highly resistant Gram-negative bacteria exhibited pathogenic characteristics. These antibiotic-resistant pathogens contribute to the global challenge of antimicrobial resistance, as emphasized by Kaboore et al. (2018) and CDC (2017). The observed resistance patterns in these strains align with findings from previous research (Wang et al., 2016; Zhang et al., 2017). Plasmid profiling, a valuable tool in epidemiological investigations of bacterial resistance outbreaks, revealed that *Enterobacter* spp., *Klebsiella* spp., and *Edwardsiella* spp. harbored large plasmids (>3000 bp). These plasmids may be responsible for the multidrug resistance observed in these organisms and could facilitate the horizontal transfer of antibiotic resistance genes to other bacterial strains (Bengtsson-Palme et al., 2018). Plasmid curing experiments demonstrated that all resistant isolates, with the exception of *E. coli*, became

susceptible to various antibiotics after the curing process, indicating that plasmid-mediated mechanisms were primarily responsible for the observed resistance in these isolates.

Conclusion

The analysis of water samples in this study revealed the presence of pathogenic bacteria, posing significant risks to public health. Furthermore, the identification of isolates exhibiting multidrug antibiotic resistance (MDR) underscores a critical concern. Based on these findings, it is evident that most of the examined water samples are not suitable for direct consumption or domestic use and necessitate appropriate treatment before utilization.

Appendix (Tables and Figures)

Table 1; Heterotrophic Plate Count of Bacterial isolates

Samples/Sites	Colony Forming Unit (Cfu/ml) = $\frac{\text{Number Of Colonies} \times \text{Total Dilution Factor}}{\text{Volume Of Culture Plated}}$			
	Aufh	Aufpo	Isaipen	Okeogun
Sample 1	7.9×10^4	8.4×10^4	9.2×10^4	6.4×10^4
Sample 2	7.5×10^4	8.0×10^4	9.1×10^4	5.5×10^4
Sample 3	7.0×10^4	7.5×10^4	9.0×10^4	5.3×10^4
Control (Nestle water)	0	0	0	0

Key : Aufh – Achievers University Female Hostel, Aufpo- Achievers University Flexible Programme Office.

Table 2: Presumptive Coliform Count by MPN (Most Probable Number) Method

Sites /Samples	5 of 10ml each	5 of 1ml each	5 of 0.1ml each	MPN/100ml
Aufh Fh1	3	0	0	8
Fh2	2	0	0	4
Fh3	2	0	0	4
Aufpo Fp1	3	2	0	14
Fp2	3	1	0	11
Fp3	2	0	0	4
Isaipen Is1	5	5	5	>1600
Is2	5	5	3	900
Is3	5	5	4	1600
Okeogun Ok1	0	0	0	<2
Ok2	2	0	0	4
Ok3	1	0	0	2

Key : Aufh – Achievers University Female Hostel, Aufap- Achievers University flexible academic program.

Table 3: Confirmed and Completed test

Samples	Growth on LB	Growth on EMB	Green Metallic Sheen
Aufh Fh1	+ve	- ve	-ve
Fh2	+ve	-ve	-ve
Fh3	+ve	-ve	-ve
Aufap	+ve	+ve	-ve
Fo1	+ve	-ve	-ve
Fo2	+ve	-ve	-ve
Fo3	+ve	-ve	-ve
Isaipen Is1	+ve	+ve	+ve
Is2	+ve	+ve	+ve
Is3	+ve	+ve	+ve
Okeogun Ok1	-ve	-ve	-ve
Ok2	+ve	-ve	-ve
Ok3	+ve	-ve	-ve

Key : Aufh – Achievers University Female Hostel, Aufap- Achievers University flexible academic program

Table 4: Characterization and identification of isolates.

isolates	Gram reaction	Cellular Morphology	Triple Sugar Fermentation		Indole	Methyl red	Vogues Proskeur	Citrate	Urease	Motility	Oxidase	Catalase	Sugar fermentation						H2S	Probable organisms
			Acid	GAS									Lactos	Maltos	Glucos	Galactose	Sucros	Fructose		
1	+ve	R	+	+	-	-	+	+	-	+	+/-	+	+/-	+	+	+/-	+	+	-	*Bacillus* sp
2	+ve	C	+	+	+	+	-	+	-	-	-	-	+	+	+	+	+	+	+	*Streptococus* sp
3	-ve	R	+	+	+	+	-	-	-	+	-	+	+	+	+	-		+	-	*Echerichia coli*
4	-ve	R	-	+	-	+	-	-	-	+	-	+	-	+	+	+	-	+	+	*Salmonella* sp
5	+ve	C	-	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	-	*Staphylococcus* sp
6	-ve	R	+	+	-	-	+	+	-	+	-	+	-	+	+	+	+	+	-	*Enterobacter* sp
7	-ve	R	+	+	-	+	-	+	+/-	+	-	+	+	+	+	+	+	+	+	*Citrobacter* sp
8	-ve	R	+	+	-	-	+	+	+	-	-	+	+	+	+	-	+	-	-	*Klebsiella* sp
9	-ve	R	_	+	+	+	-	-	-	+	-	+	-	-	+	-	+/-	-	+	*Edwardsiella* sp

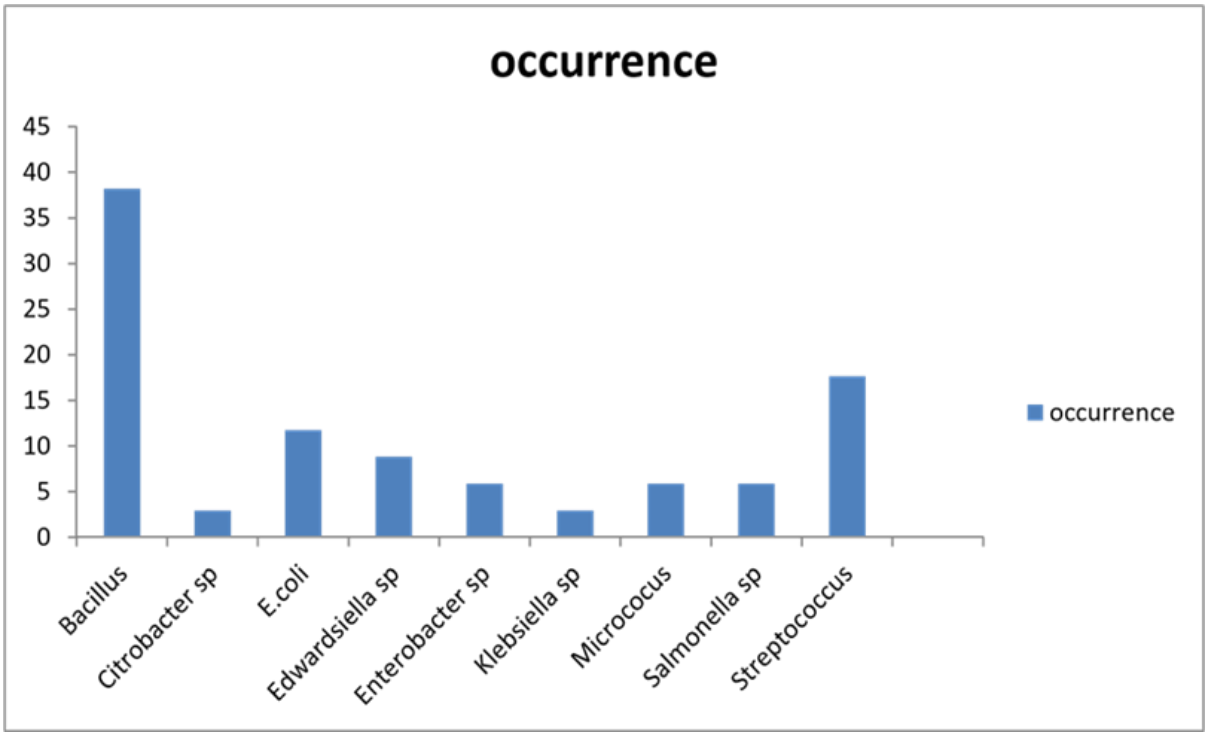

Fig 1. Percentage occurrence of bacterial isolates.

Organisms	MacConkey Agar	Blood Agar	Chocolate Agar	Pathogenicity scores
E. coli	+	β	-	High
Enterobacter sp	+	α	-	Medium
Klebsiella sp	+	β	-	High
Edwardsiella sp	+	γ	-	Medium

Table 5. Pathogenicity of resistant Bacterial Isolates.

Table 6: Percentage Antibiotics Susceptibility and Resistant Profiles of Gram-positive Isolates

Bacteria	PEF			CN			APX			Z			AM			R			CPX			S			SXT			E		
Bacillus sp (n=13)	30	≥18	S	30	≥18	S	25	≥18	S	22	≥18	S	20	≥18	S	20	≥18	S	20	≥18	S	11	≤13	R	15	14-17	I	11	≤13	R
Streptococus sp (n=6)	13	≤13	R	13	≤13	R	20	≥18	S	30	≥18	S	-	≤13	R	-	≤13	R	15	14-17	I	15	14-17	I	14	14-17	I	-	≤13	R
Staphylococus spp (n=2)	23	≥18	S	18	≥18	S	20	≥18	S	22	≥18	S	-	≤13	R	15	14-17	I	30	≥18	S	30	≥18	S	19	≥18	S	20	≥18	S

Key: Pefloxacin – PEF, Gentamycin- CN, Ampiclox- APX, Zinnaclef – Z, Amoxacillin – AM, Rocephin – (R), CiprofloxacinCPX, Streptomycin – (S), Septrin – SXT, Erytromycin – E S-Susceptible, R-Resistant, I-Moderate Inhibition.

Table 7: Percentage Antibiotics Susceptibility and Resistant Profiles of Gram negative Isolates

Bacteria		SXT		CH			SP				CPX			AM			CN			PEF			OFX			S			AU		
		Value obtained	CLSI		Valve obtained	CLSI		Valve obtained	CLSI		Valve obtained	CLSI		Valve obtained	CLSI		Valve obtained	CLSI		Valve gobtained	CLSI		Valve obtained	CLSI		Valve btained	CLSI		Valve obtained	CLSI	
Edwardsiella spp (n=2)		-	≤13	R	-	≤13	R	14	14-17	S	19	≥18	S	-	≤13	R	-	≤13	R	11	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R
Enterobacter sp (n=2)		-	≤13	R	-	≤13	R	15	14-17	S	22	≥18	S	-	≤13	R	-	≤13	R	10	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R

https://zenodo.org/records/15448075

E. coli sp N=1		-	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R
Samonella spp (n=3)		-	≤13	R	13	≤13	R	16	14-17	S	19	≥18	S	-	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R	-	≤13	R

Key; R- Resistant, S- Susceptib

Table 8: Multiple Antibiotic Resistant Indices of Bacteria

Isolates code	SXT	CH	SP	CPX	AM	CN	PEF	OFX	S	AU	MAR Index	Organisms
A3	-R	-R	-R	-R	-R	-R	-R	-R	-R	-R	1	*E. coli*
B4	-R	08R	21S	30S	-R	-R	-R	10R	10R	-R	0.8	*Enterobacter sp*
C3	-R	07R	20S	-R	-R	-R	-R	-R	-R	-R	0.9	*Klebsiella sp*
WA	-R	-R	10R	19S	-R	-R	09R	-R	-R	-R	0.9	*Edwardsiella sp*

Key; R- Resistant, S- Susceptible

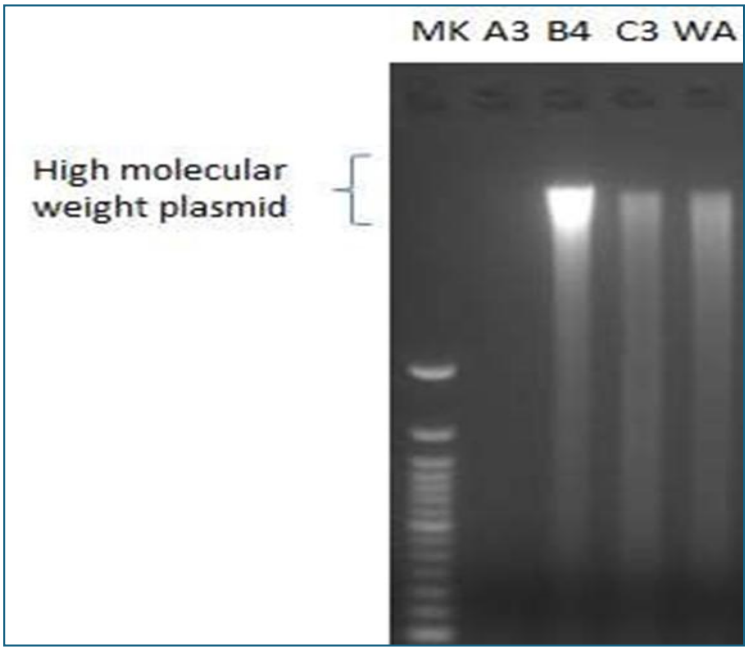

Figure 2. Agarose gel electrophoresis showing the profiling of the plasmid extracted from selected bacteria .

Key; A3- *E. coli*, B4- *Enterobacter sp*, C3- *Klebsiella sp*, WA- *Edwardsiella sp*

Table 9: Isolates with cured plasmid and their post curing status

	Escherichia sp		*Enterobacter sp*		*Klebsiella sp*		*Edwardsiella sp*	
Antibiotics	Precuring	Post curing	Pre-curing	Post curing	Precuring	Post curing	Precuring	Post curing
SXT	R	R	R	S	R	S	R	S
CH	R	R	R	S	R	S	R	S
SP	R	R	S	S	S	S	R	S
CPX	R	R	S	S	R	S	S	S
AM	R	R	R	S	R	S	R	S
CN	R	R	R	S	R	S	R	S
PEF	R	R	R	S	R	S	R	S
OFX	R	R	R	S	R	S	R	S
S	R	R	R	S	R	S	R	S
AU	R	R	R	S	R	S	R	S

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