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Research article

Physicochemical and Bacteriological Properties of Water from Owo Local Government Area, Nigeria

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Abstract

Water is indispensable for healthy living, but it can be a vehicle for disease transmission. This study evaluated physicochemical properties, bacteria and their antibiotic resistance patterns in water from Owo Local Government Area. Twenty one water samples from boreholes (BW), stream (SW), and wells (WW) were collected using aseptic techniques. Bacteria were isolated and subjected to plate counts and identified using biochemical and molecular characterization methods, while antimicrobial susceptibility test (AST) was carried out using the Kirby-Bauer method. The physicochemical parameters including temperature, pH, electrical conductivity, total dissolved solids, dissolved oxygen, turbidity, chloride, and fluoride content were determined using standard methods. Analysis of Variance (ANOVA) was employed for data analysis (p-values < 0.05). The findings revealed that BW had the lowest plate count value of 3.3×10^3-6.0×10^3 cfu/mL, followed by WW (3.9×10^3 cfu/mL-8.7×10^3 cfu/mL), and SW (9.2×10^3 cfu/mL) while coliform counts ranged from 0 to 1600 MPN/100 mL. Borehole water samples were less contaminated (0-48 MPN/100 mL) when compared to SW and WW (150-1600 MPN/100 mL). AST results showed that some isolates (*Salmonella* spp., *Citrobacter* spp., *Vibrio* spp., *Klebsiella* spp., and *Pseudomonas* spp.) were resistant to more than three antibiotics and were thus considered to be multidrug resistant (MDR) bacteria. The physicochemical parameters were within the permisible limit (as recommended by WHO), except for chloride (122-255 mg/L) and flouride (0.39-1.99 mg/L) concentrations, which were above the standards (200 mg/L and 1.5 mg/L, respectively). Most of the water samples from wells and stream did not meet the standard criteria for drinking and domestic purposes. The presence of resistant bacteria poses serious health risks to individuals and the communities. Hence, this study recommends proper water treatment, monitoring, and good personal hygiene to avert the dangers associated with possible disease outbreaks in the study area.

Keywords: antibacterial activity; antibiotics resistance; bacteria; physicochemical parameters; water quality

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1. Introduction

Water is a vital component of life and is essential for growth and development of humans, animals, and plants. It is a colorless, odorless, and tasteless liquid that plays a crucial role in maintaining various bodily functions. Drinking enough water helps to forestall dehydration, modulates body temperature, facilitates joint lubrication and cushioning, protects sensitive tissues like spinal cord, and facilitates waste removal through urination, perspiration, and bowel movements (EFSA, 2010). Despite its importance, access to clean water remains a significant challenge worldwide. Approximately 1.1 billion people lack access to safe water, 1.9 billion lack fundamental sanitation and 1.2 billion are unable to access clean drinking water (Jamal et al., 2020; WHO/UNICEF, 2021). Limited access to clean water has severe consequences, particularly in terms of public health. Insufficient access to clean water contributes to the spread of waterborne diseases, such as diarrhea, which claims approximately 1.6 million lives annually (Jamal et al., 2020). The presence of disease-causing microorganisms in drinking water poses a significant threat to global health, making waterborne illness a critical concern that requires immediate attention and action.

In developing countries, water contamination due to improper waste disposal is one of the pollution courses. Once water is contaminated, it is costly to remove the pollutants. About 80% of untreated global wastewater released into the water bodies contains materials from human waste to toxic industrial discharges. Pollution of freshwater ecosystems can impact the habitat and quality of life of fish and other wildlife (Jannat et al., 2019). Water physicochemical parameters are among the basic indices used in determining water quality. Parameters such as pH, temperature, electrical conductivity, total dissolved solids, turbidity, fluorides, chlorides, and dissolved oxygen are most used. Jannat et al. (2019) reported some physicochemical parameter values that were within the standard limit of Bangladesh Environmental Quality Standard (BEQS). In another study by Bilewu et al. (2022), the physicochemical parameters of water from the Oyo and Lagos sates in Nigeria were compared with the WHO standards for water. The pHs, conductivity, TDS, and salinity values of the water samples were high when compared to the threshold level.

Good quality water requires the absence of pathogenic and antibiotic-resistance organisms and physicochemical characteristics that fall within permissible limits. The World Health Organization (WHO) and National Standard for Drinking Water Quality (NSDWQ) standards for drinking water quality specify acceptable limits that include; pH (6.5 - 8.5), turbidity (<5NTU), conductivity (<1000 µS/cm), Total Dissolved Solids (TDS) (<500 mg/L), chloride (< 250 mg/L), fluoride (<1.5 mg/L), and temperature (ambient) (NSDWQ, 2017; WHO, 2017). Pollution of water with heavy metals such as lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), and chromium (Cr) can exacerbate various health and environmental issues (Balali-Mood et al., 2021). Moreover, bacteria such as *Salmonella* spp., *Shigella* spp., *Vibrio cholerae*, and *Escherichia coli* are common with water contamination, leading to outbreaks of typhoid fever, cholera, and gastroenteritis (Al-Abdan et al., 2021; Ali et al., 2021). Some of these Gram-negative bacteria are resistant to antibiotics and are of public health concern (Rossolini et al., 2017). Also, contamination of water resources with heavy metals can pose a significant threat to human health because of their potent toxicity at low concentrations (Marcovecchio et al., 2007). In addition, pollution of water can exacerbate various health issues such as cardiovascular diseases, cancers, respiratory illness, and pulmonary disease which can substantially shorten life expectancy (Balali-Mood et al., 2021).

The emergence and spread of antibiotic resistance is of global concern as it retards the treatment of common infections. Antibiotics refer to the substances that have the ability to inhibit the growth of microorganisms while antibiotic resistance involves the antagonistic effect of microorganisms on antibiotics, which can be attributed to overuse and misuse of antimicrobial drugs (Blair et al*.,* 2015). Antimicrobial resistance (AMR) poses a significant threat to modern medicine, undermining decades of progress. It also jeopardizes the safety and efficacy of various medical procedures, including surgery, cesarean sections, and cancer chemotherapy, making them more perilous. Globally, bacterial antimicrobial resistance claimed approximately 1.27 million lives in 2019 and contributed to 4.95 million deaths (Antimicrobial Resistance Collaborators, 2022).

Frequent use of antibiotics, poor sanitation and waste disposal system can promote the discharge of pathogenic or resistant organisms into the environment such as water bodies, air and soils resulting in the increased risk of transmission of resistance from the environment to human beings. Ashbolt et al*.* (2013) reported that antibiotics find their way into water through many pathways which include the discharge of municipal sewage, landfill leachates, and animal husbandry. The spread of resistance among bacteria is attributed to horizontal gene transfer (HGT). Horizontal gene transfer is thought to occur through various mobile genetic elements, including plasmids, integrons, transposons, and prophages. These elements enable the exchange of genetic material, specifically antibiotic resistance genes, among bacterial cells, thereby promoting the dissemination of resistance (Gupta et al*.,* 2021). Resistance of microorganisms to antibiotics continues to escalate globally with few available solutions (Carlet et al*.,* 2012).

Access to clean water is crucial for human and animal survival. Interestingly, humans can endure prolonged periods without food, with some studies suggesting up to 28 days. However, the same cannot be said for water deprivation, as the human body can only survive for approximately 72 h without hydration (Akin-Osanaiye et al*.,* 2018). Protecting the health and well-being of residents of any community should be concerned with rigorous testing and monitoring of the quality of their drinking water. Water should be tested for trace metals, heavy metals, organic materials as well as biological contaminants. By doing so, authorities can mitigate the risk of water-borne diseases and ensure a safer environment for the community. In a study by Adegoke et al*.* (2023) on sanitation and occurrence of waterborne diseases in Local Government Areas of Ondo State, Nigeria, the Owo Local Government Area (LGA) had one of the highest percentages of occurrence, which highlighted the need for further investigation. Hence, this study examined the bacteriological and physicochemical properties of water from three sources: boreholes, wells, and stream in five selected areas within Owo LGA.

2. Materials and Methods

2.1 Study area

Owo LGA is situated in Ondo State, Nigeria, and is one of the State's 18 LGAs. Geographically, it is situated at latitude of 7.183°N and a longitude of 5.583°E, with a sizable population projected to be around 341,400 residents at 2.7% annual population change [2006 → 2022] in 2022, according to City Population (2025). The five rural settlements examined in this study included Ijebu, Emure, Idasen, Iyere, and Upemen (Figure 1).

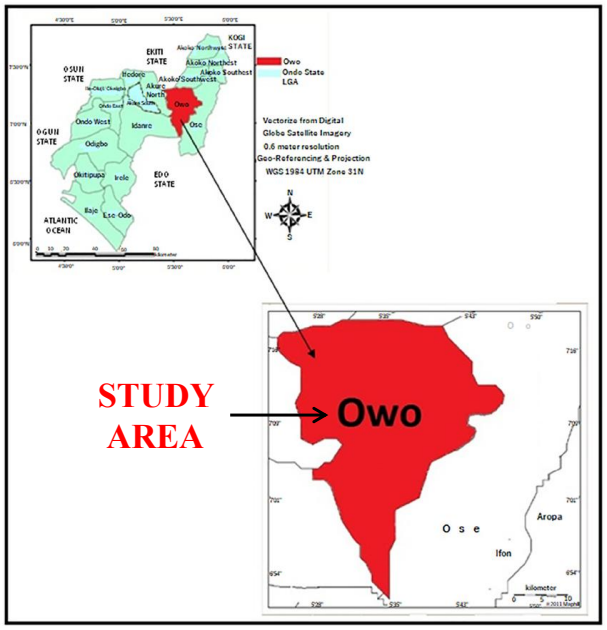

Figure 1. Map of Owo Local Government, Ondo State, Nigeria (Source: Oladeji et al., 2022)

2.2 Collection of water samples

A total of 21 water samples were purposefully collected from the five study areas (Ipele, Emure, Uso, Upeme, and Idasen). A single (1) stream sample (S1) (consumed by some people because of their belief that the water is a miracle water), and two well (W1 and W2) and two borehole (B1 and B2) water samples were collected from each site. Each water sample was aseptically collected into a sterile bottle, stored on ice, and immediately transported using laboratory cooler box to the Department of Microbiology laboratory, Adekunle Ajasin University, Akungba-Akoko and then stored at 4°C throughout the study period.

2.3 Physicochemical analysis of water samples

Physicochemical parameter such as pH, temperature, electrical conductivities (EC), dissolve oxygen (DO), total dissolved solids (TDS), turbidity, biochemical oxygen demand (BOD), floride and chloride levels of the water saamples were determined using standard procedures (Barghouthi & Amereih, 2017; Karki & Thapa, 2022).

2.4 Determination of pH

The pH was measured with an automated digital pH meter. The pH meter was calibrated using a standard buffer solution that had pH values of 7.0 and 4.0. The sample water was introduced after the gas electrode had been cleaned with distilled water. The pH value was then recorded after the glass electrode had been dipped in the beaker containing each water sample until the reading had stabilized.

2.5 Determination of temperature

Each water sample was placed in a beaker to determine the temperature. A mercury-filled Celsius thermometer was used to record the temperature.

2.6 Determination of electrical conductivity (EC)

Electrical conductivity was measured using a conductivity meter. After washing the gas electrode and a 250 mL beaker with distilled water, 100 mL of the sample water was added into the 250 mL beaker. Then, the glass electrode was dipped into the sample and left there until the reading stabilized, at which point the EC was recorded (Karki & Thapa, 2022).

2.7 Determination of dissolved oxygen (DO)

N/80 of $Na_2S_2O_3.5H_2O$ solution was first prepared in a 250 mL volumetric flask by weighing 0.775 g of $Na_2S_2O_3.5H_2O$ and dissolved in distilled water with a few drops of 0.1N Na_2CO_3, the volume was then made up to the mark by adding distilled water. Preparation of 50% $MnSO_4$ solution: 50 g of $MnSO_4.5H_2O$ was weighed and dissolved in distilled water; the volume was made 100 mL in a 100 mL volumetric flask. Preparation of 20% alkaline KI solution: 49 g of NaOH, 20 g of KI, and 0.5 g of sodium azide were dissolved in distilled water; the volume was made up to 100 mL by adding distilled water. DO of each water sample was determined by withdrawing 5 mL of water from each sample bottle, 2 mL of $MnSO_4$ (manganese sulphate) was poured from the bottle wall, and 2 mL of alkaline KI solution was added deep below the surface. The stopper was tightened after a precipitate appeared and the bottle was shaken frequently by inverting it to ensure thorough mixing of the contents. The precipitate was allowed to settle, each bottle was filled with 85% concentrated H_3PO_4 and shaken vigorously to dissolve all of the precipitates. Then, 50 mL of each water sample was placed in a conical flask and titrated against a 0.0125N $Na_2S_2O_3$ (sodium thiosulphate) using starch as an indicator (Karki & Thapa, 2022). The original blue colour faded to a colorless state towards the end of the experiment. DO was calculated using the following formula:

$$\text{DO (mg/L)} = \frac{\text{(mL x N) of Titrant x 8 x 1000}}{\frac{V_2 \text{ x } (V_1 - V)}{V_2}}$$

Where, V_1 = volume of sample bottle after placing the stopper
V_2 = volume of part of content titrated
V = volume of $MnSO_4$ and KI added

2.8 Determination of total dissolved solids (TDS)

A 150 mL volume clear dry beaker was weighed. After that, a 100 mL sample of water was put into the beaker and heated to the appropriate temperature until it had completely evaporated. After full evaporation, the beaker was cooled and weighed. The heating, chilling and weighing procedure was repeated until constant weight was attained. Lastly, the weight of the empty, clean beaker was subtracted from the weight of the solids in the

beaker. Total dissolved solids were calculated using the following formula (Karki & Thapa, 2022).

$$\text{Dissolved Solids (mg/L)} = \frac{(A-B) \times 1000}{\text{mL of Sample}}$$

Where, A = weight of dried residue + beaker
B = weight of empty beaker

2.9 Determination of turbidity

Turbidity was measured in nephelometric turbidity units (NTU). A turbidimeter was used to measure the intensity of light scattered at 90 degrees as a beam of light passed through each water sample.

2.10 Determination of chloride

Potassium chromate indicator solution was first prepared. Five gram of potassium chromate was dissolved in a small amount of distilled water and silver nitrate solution was added till the formation of the red precipitate. After allowing the solution to stand for 12 hours, it was filtered and distilled water was used to get the volume up to 100 mL. Standardization of N/50 $AgNO_3$ with N/50 NaCl solution was conducted by pipetting 10 mL of N/50 NaCl solution and placed in a 250 mL conical flask and 1 mL of 2% K_2CrO_4 solution was added to this solution. $AgNO_3$ was then titrated against this solution till a faint red color precipitate appeared. For chloride determination in each water sample, a burette was filled with standard $AgNO_3$ and titrated against 50 mL of water sample (containing 2 mL of 2% K_2CrO_4) until a faint red color precipitate emerged in the volumetric flask. It was noted how that $AgNO_3$ was required for this endpoint. The chloride was precipitated as silver chloride, and the titration was completed when the color of the potassium chromate indicator changed from yellow to pinkish yellow (Karki & Thapa, 2022). Chloride present was calculated by using the following formula:

$$\text{Chloride (mg/L)} = \frac{(a-b) \times N \times 35.5 \times 1000}{V}$$

Where, a = Volume of titrant (silver nitrate) for sample
b = Volume of titrant (silver nitrate) for blank
V = volume of the sample in mL
N = normality of silver nitrate

2.11 Determination of fluoride

Fluoride was colorimetrically analyzed using Hack-DR/2010 as a spectrophotometer and SPADNS as a fluoride reagent. Fluoride was measured after spiking each water samples with 0.5 $mg \cdot \ell^{-1}$ fluoride. The results obtained were then compared with those reported by the Central Public Health Laboratory (Barghouthi & Amereih, 2017).

2.12 Media preparation

Media used included nutrient agar (NA), nutrient broth, MacConkey agar (MAC), eosin methylene blue (EMB) agar, lactose broth and Mueller-Hinton agar. Media preparation was conducted in strict accordance with the manufacturer's protocols (Atmanto et al., 2022), with subsequent sterilization achieved through 15-min autoclaving at 121°C.

2.13 Determination of heterotrophic bacteria in water samples

The heterotrophic plate count was determined using serial dilution and the pour plate techniques. Each water sample was serially diluted and 1 mL from a x1000 dilution test tube was aseptically dispensed into each sterile empty Petri dish. Molten nutrient agar was then poured into the Petri dish containing the water sample. The plate was then swirled and left to gel (solidified). The plates were then subjected to a 24-h incubation period at 37°C, after which the colonies were meticulously enumerated using a specialized counting device (colony counter). The results were expressed as:

$$\text{Colony forming unit (CFU/mL)} = \frac{\text{Number of colonies x Total dilution factor}}{\text{Volume of culture plated}}$$

2.14 Enumeration of total and fecal coliform bacteria in water

Multiple tube fermentation tests were employed to enumerate total and fecal coliforms (APHA, 2017). Total coliform counts and fecal coliform were determined using the most probable number (MPN) method, which included the presumptive test, the confirmed test and the completed test. These were carried out according to the procedure highlighted by Adetunde and Glover (2010). Water samples that exhibited minimal coliform bacteria presence and no traceable fecal coliform contaminants, including *E. coli* were deemed suitable for consumption and categorized as potable while samples that failed to meet these standards were considered unsafe and categorized as not potable (APHA, 2017).

2.15 Isolation of bacteria in water samples

The methods of Cheesbrough (2010) and Negera et al. (2017) were adopted for water sample preparation and inoculum standardization. Sterile distilled water served as a diluent in these procedures. After serial dilution, each water sample was inoculated on each medium (NA, MAC or EMB) and incubated at 37°C for 24 h. Each culture plate was observed for colony forming units (CFU). Each colony was sub-cultured on fresh agar medium to obtained pure culture and used as a stock culture for further identification (APHA, 2017).

2.16 Macroscopic and microscopic examination of bacteria in water

A macroscopic examination was conducted to assess the physical morphology of the samples, considering characteristics such as dimensions, chromatic appearance, surface texture, pigmentary features, and overall consistency. The microscopic examination was carried out through Gram staining. The Gram staining technique was employed to differentiate between Gram-positive and Gram-negative isolates, adhering to the methodology described by Cheesbrough (2010).

2.17 Biochemical characteristics of bacteria in water samples

Methods described by Cheesbrough (2010) and Negera et al. (2017) were adopted. Pure distinct colonies were subjected to a comprehensive characterization process involving biochemical tests that included catalase, citrate tests, coagulase, indole, methyl red test, motility, oxidase, sugar fermentation, triple sugar iron (TSI), urease, and Voges Proskauer.

2.18 Molecular identification of bacterial isolates

The DNA of each bacterium was extracted using the protocol described by Trindade et al. (2007). To ensure the accuracy of the results, the entire extracted genomic DNA underwent integrity verification by gel electrophoresis method. The universal bacterial 16S-rDNA gene was targeted for DNA amplification using the following oligonucleotide primers 27F (5′- AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5′-AAGGAGGTGWTCC ARCC-3′) with genomic DNA as template. This was followed by PCR and Sanger sequencing methods. DNA sequences were analyzed with DNAstar Lasergene software in combination with the BLAST program (available on NCBI http://www.ncbi.nlm.nih.gov/) (Chan et al., 2007; 2009).

2.19 Antimicrobial susceptibility testing

The isolates underwent antibiotic susceptibility testing using the standardized Kirby-Bauer method, as evaluated and recommended by the Clinical Laboratory Standards Institute (CLSI, 2020). The conventional antibiotics used for investigating Gram positive and Gram negative bacteria were: amoxicillin (30 µg), cefotaxime (25 µg), imipenem/ cilastatin (10/10 µg), ofloxacin (5 µg), gentamicin (10 µg), nalidixic acid (30 µg), nitrofurantoin (300 µg), cefuroxime (30 µg), ceftriaxone (30 µg), ampiclox (10 µg), cefixime (5 µg), levofloxacin (5 µg), erythromycin (15 µg), and azithromycin (15 µg).

2.20 Standardization of inoculum

Bacterial cultures were grown on nutrient agar plates at 37°C for 24 h in an incubator. The concentration of each bacterial culture was adjusted to 0.5 McFarland standard before use (CLSI, 2016).

2.21 Multiple antibiotics resistance (MAR) index

The MAR index was calculated as follows: MAR index for isolate = a/b. Where “a” is the number of antibiotics to which the isolates is resistant while “b” represents the number of antibiotics tested (Afunwa et al., 2020).

2.22 Statistical analysis

Microsoft Excel was used to calculate various statistical parameters, including mean, standard deviation, correlation, and analysis of variance (ANOVA), to enable the identification of any significant differences in the average concentrations of compounds/bacteria across different sample sites. The results were deemed statistically significant for $p \leq 0.05$

3. Results and Discussion

3.1 Results

3.1.1 Physicochemical analysis of water samples

In the findings of this study, the temperatures of the water samples investigated ranged from 22.09 to 24.95°C. The pH ranged from 7.18 to 8.48, the electrical conductivity values ranged from 83 to 193 μS/cm, the TDS values ranged from 40 to 363 mg/L, the DO values ranged from 5.11 mg/L to 5.34 mg/L, the turbidity values ranged from -2.8 to 2.8 NTU, chloride values ranged from 122 to 255 mg/L, and the fluoride values ranged from 0.39 to 1.99 mg/L (Table 1).

3.1.2 Enumeration of total coliform bacteria

The presumptive coliform count results by the MPN method revealed that well samples had total coliform counts that ranged from 150 MPN/100 mL to >1600 MPN/100 mL, the borehole samples had total coliform counts between 0 MPN/100 and 48 MPN/100 mL and the stream sample had total coliform counts of >1600 MPN/100 mL (Table 2). The results for the confirmed and completed test of MPN method revealed the presence of coliforms and fecal coliforms (*E. coli*) in the stream sample tested and in all the well samples, while no *E. coli* was recorded in borehole samples with the exception of a sample from Idasen community (Table 3).

3.1.3 Heterotrophic bacterial counts in water samples

The heterotrophic plate count (HPC) in all the samples ranged from 3.3×10^3 cfu/mL to 9.2×10^3 cfu/mL. The boreholes had the lowest bacterial counts that ranged from 3.3×10^3 cfu/mL to 6.0×10^3 cfu/mL while the stream sample had the highest total plate count of 9.2×10^5 cfu/mL, followed by well samples that ranged from 3.9×10^3 cfu/mL to 8.7×10^3 cfu/mL (Table 4).

3.1.4 Isolation and identification of bacteria in water samples

A total of 71 bacterial species were isolated. After identification, the bacteria were noted to belong to thirteen genera: *Escherichia* (14), *Pseudomonas* (11), *Proteus* (10), *Klebsiella* (9), *Citrobacter* (7), *Vibrio* (2), *Salmonella* (3), *Enterobacter* (4), *Shigella* (1), *Serratia* (2), *Providencia* (1), *Staphylococcus* (4), and *Bacillus* (3) (Table 5). Gram-negative bacteria had 90.14% of occurrence, while 9.86% were Gram-positive bacteria (Figure 2). Figure 3 shows the overall percentages of organisms isolated including: *E. coli* (19.71%), *Pseudomonas* sp. (15.49%), *Proteus* sp. (14.08%), *Klebsiella* sp. (12.68%), (19.71%), *Pseudomonas* sp. (15.49%), *Proteus* sp. (14.08%), *Klebsiella* sp. (12.68%), *Citrobacter* sp. (9.86%), *Vibrio* sp. (2.82%), *Salmonella* sp. (4.23%), *Enterobacter* sp. (5.63%), *Shigella* sp. (1.41%), *Serratia* sp. (2.82%), *Providencia* sp. (1.41%), *Staphylococcus* sp. (5.63%), and *Bacillus* sp. (4.23%). The percentage of occurrence of bacteria in each sampled area was depicted in Figure 4. *Escherichia coli, Pseudomonas* sp., and *Proteus* sp. were present in all the sampled areas while *Klebsiella* sp. was not observed at Uso community. *Citrobacter* sp. was also absent at Emure. Other organisms were least predominant in these areas.

Table 1. Physicochemical properties of water samples from selected areas in Owo

Site	Sample	Temperature (°C)	pH	EC (μS/cm)	TDS (mg/L)	DO (%)	DO (mg/L)	Turbidity	Fluoride (mg/L)	Chloride (mg/L)
IPELE	W1	24.19	8.45	148.00	74.50	63.50	5.15	-02.80	0.89	248.00
	W2	24.85	8.47	149.00	74.59	57.50	5.17	-01.90	0.92	239.00
	B1	23.35	7.18	191.00	97.00	59.90	5.18	2.10	1.64	141.00
	B2	22.69	7.20	194.00	98.00	52.60	5.21	1.90	1.43	130.00
EMURE	**W1**	23.61	8.27	180.00	90.00	45.15	5.16	-1.80	0.79	251.00
	W2	24.45	8.48	183.00	93.00	43.49	5.17	0.10	1.20	238.00
	B1	23.86	8.39	109.00	55.00	51.22	5.18	2.40	1.91	155.00
	B2	24.91	8.42	120.00	59.00	50.81	5.19	2.10	1.86	126.00
USO	**W1**	24.89	8.50	142.00	76.50	60.95	5.16	-1.50	1.15	242.00
	W2	24.95	8.47	144.00	77.59	52.72	5.19	-0.18	0.96	190.00
	B1	23.05	7.23	193.00	95.00	55.90	5.20	2.80	1.99	122.00
	B2	22.75	8.08	127.00	363.00	62.83	5.19	1.90	0.39	165.00
UPEMEN	W1	23.22	8.47	148.00	74.30	59.02	5.16	-1.60	0.91	219.00
	W2	23.19	8.40	147.00	74.00	61.80	5.15	-1.10	0.86	231.00
	B1	22.85	7.68	195.00	98.00	62.83	5.19	1.90	1.20	123.00
	B2	22.92	7.70	197.00	99.00	60.32	5.20	2.20		165.00
IDASEN	W1	23.45	8.09	181.00	93.00	57.60	5.12	-1.20	0.82	231.00
	W2	22.61	8.17	179.00	90.00	60.39	5.11	-1.80	0.76	223.00
	B1	22.86	8.19	103.00	51.00	58.05	5.16	2.10	1.70	131.00
	B2	22.09	8.22	105.00	54.00	55.96	5.17	1.90	1.52	142.00
	S1	22.65	8.24	83.00	40.00	64.39	5.34	1.20	0.96	240.00

Key: W1 = Well sample 1, W2 = Well sample 2, B1 = Borehole sample 1, B2 = Borehole sample 2, and S1 = Stream sample 1, DO = Disolved oxygen

Table 2. Presumptive coliform counts by MPN method

Site	Sample	5 of 10 mL each	5 of 1 mL each	5 of 0.1 mL each	MPN/100 mL
IPELE	W1	5	4	5	430
	W2	5	5	5	>1,600
	B1	4	0	0	13
	B2	5	0	3	25
EMURE	W1	5	5	5	>1,600
	W2	5	5	5	>1,600
	B1	4	5	0	41
	B2	0	0	0	0
USO	W1	5	4	4	350
	W2	5	2	4	150
	B1	4	3	0	25
	B2	4	3	1	35
UPEMEN	W1	5	5	5	>1,600
	W2	5	5	5	>1,600
	B1	4	5	0	40
	B2	4	5	1	38
IDASEN	W1	5	5	5	>1,600
	W2	5	5	5	>1,600
	B1	4	5	1	48
	B2	4	5	0	40
	S1	5	5	5	>1,600

Key: W1 = Well sample 1, W2 = Well sample 2, B1 = Borehole sample 1, B2 = Borehole sample 2, and S1 = Stream sample

Table 3. Confirmed and completed tests by MPN method

Site	Sample	EMB	LB broth	Green Metallic Sheen	Microscopic morphology	Result
IPELE	W1	+	+	+	Short, G(-VE) Straight rods	Non Potable
	W2	+	+	+	Short, G(-VE) Straight rods	Non Potable
	B1	-	-	-	-	Potable
	B2	+	+	-	-	Potable
EMURE	W1	+	+	+	Short, G(-VE) Straight rods	Non Potable
	W2	+	+	+	Short, G(-VE) Straight rods	Non Potable
	B1	+	+	-	-	Potable
	B2	-	-	-	-	Potable
USO	W1	+	+	+	Short, G(-VE) Straight rods	Non Potable
	W2	+	+	+	Short, G(-VE) Straight rods	Non Potable
	B1	-	+	-	-	Potable
	B2	+	-	-	-	Potable
UPEMEN	W1	+	+	+	Short, G(-VE) Straight rods	Non Potable
	W2	+	+	+	Short, G(-VE) Straight rods	Non Potable
	B1	+	+	-	-	Potable
	B2	+	-	-	-	Potable
IDASEN	W1	+	+	+	Short, G(-VE) Straight rods	Non Potable
	W2	+	+	+	Short, G(-VE) Straight rods	Non Potable
	B1	+	+	-	-	Potable
	B2	+	-	+	Short, G(-VE) Straight rods	Non Potable
	S1	+	+	+	Short, G(-VE) Straight rods	Non Potable

Key: EMB = EOSIN METHELYNE BLUE, LB- Lactose broth, G(-VE) = Gram negative, W1 = Well sample 1, W2 = Well sample 2, B1 = Borehole sample 1, B2 = Borehole sample 2, and S1 = Stream sample1

Table 4. Heterotrophic bacterial counts of water samples

Site	Ipele (CFU/mL)	Emure (CFU/mL)	Uso (CFU/mL)	Upemen (CFU/mL)	Idasen (CFU/mL)
W1	5.0×10^3	6.8×10^3	7.1×10^3	8.7×10^3	4.7×10^3
W2	3.9×10^3	8.1×10^3	6.4×10^3	5.7×10^3	5.8×10^3
B1	3.5×10^3	4.8×10^3	6.1×10^3	3.8×10^3	5.9×10^3
B2	3.3×10^3	3.4×10^3	6.0×10^3	5.2×10^3	5.0×10^3
S1	-	-	-	-	9.2×10^3
Control	0	0	0	0	0

Key: W1 = Well sample 1, W2 = Well sample 2, B1 = Borehole sample 1, B2 = Borehole sample 2, S1 = Stream sample 1 and - = not detected

Table 5. Morphological and biochemical characteristics of bacteria isolated from water in Owo local government

Gram reaction	Morphology	Tripple Sugar Iron		Indole	Methy Red	Voges Proskauer	Citrate	Urease	Motility	Oxidase	Catalase	Lactose	Maltose	H_2S	Gas	Probable organism
		Slant	**Butt**													
-ve	R	Acid	Acid	+	+	-	-	-	+	-	+	+	+	-	+	*Escherichia coli*
-ve	R	Alk	Alk	-	-	-	+	-	+	+	+	-	-	-	+	*Pseudomonas* sp.
-ve	R	Alk	Acid	-	+	-	+	+	+	-	+	-	-	+	+	*Proteus* sp.
-ve	R	Acid	Acid	-	-	+	+	+	-	-	+	+	+	-	+	*Klebsiella* sp.
-ve	R	Acid	Acid	-	+	-	+	+/-	+	-	+	+	+	+	+	*Citrobacter* sp.
-ve	R	Acid	Acid	+	-	-	+/-	-	+	+	+	+/-	+	-	-	*Vibrio* sp.
-ve	R	Alk	Acid	-	+	-	-	-	+	-	+	-	+	+	-	*Salmonella* sp.
-ve	R	Acid	Acid	-	-	+	+	-	+	-	+	-	-	-	+	*Enterobacter* sp.
-ve	R	Alk	Acid	+/-	+	+	-	-	-	-	+	-	+/-	-	+	*Shigella* sp.
-ve	R	Alk	Acid	-	-	+	+	+	+	-	+	-	+	-	+/-	*Serratia* sp.
-ve	R	Alk	Acid	+	+	-	+	-	+	-	+	-	-	-	-	*Providencia* sp.
+ve	C	Acid	Acid	-	+	+	+	+	-	-	+	+	+	-	-	*Staphylococcus* sp.
+ve	R	Acid	Acid	-	-	+	+	-	+	+/-	+	+/-	+	-	-	*Bacillus* sp.

Key: -ve = Gram negative, +ve = Gram positive, R = Rod, C = Coccus, - = negative result, + = positive result, Alk=Alkaline

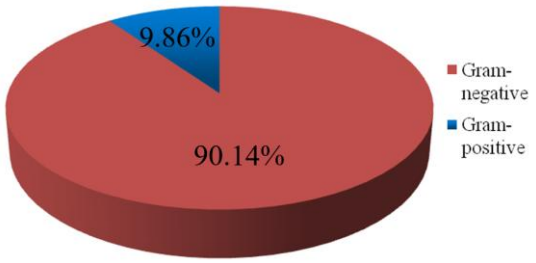

Figure 2. Percentage occurrence of Gram positive and Gram negative bacteria in water from selected sources in Owo

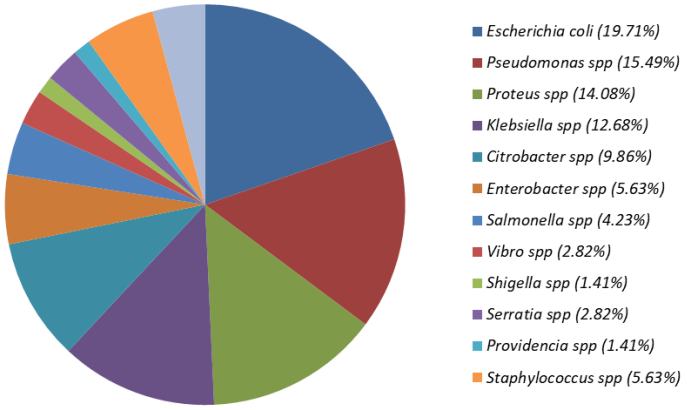

Figure 3. Overall percentage occurence of bacterial species across all areas investigated

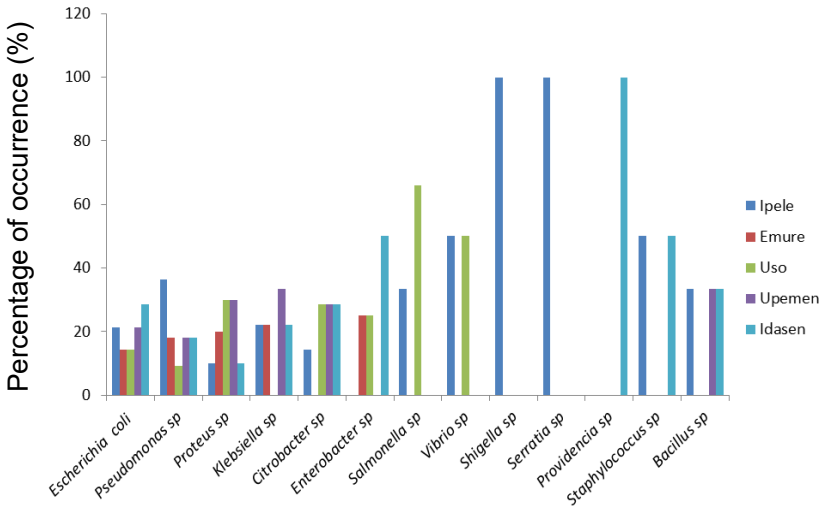

Figure 4. Prevalence of different bacterial species in each community

3.1.5 Molecular identification of bacterial isolates

Figure 5 reveals the amplification of the 16S region amplified from selected bacterial isolates using Agarose gel. The DNA ladder (Mk) with 1500 bp served as a reference point for determining the size of the amplified fragments. Lanes sm1, sm2, sm3, sm4 and sm5 represented each bacterial isolate with fragment size of approximately 1500 bp. Figure 6 shows the phylogenetic tree of bacterial isolates indicating the phylogenetic relationships among the isolates. The phylogenetic tree shows that *Klebsiella pneumoniae* (CP 021695) shared a common accestor but was distantly related to other bacteria as reperesented by the longer branches. *Escherichia coli* (MW 368769) was more closely related to *Proteus vulgaris* (NR 115878) and *Citrobacter freundii* (NR 028894) compared to *Klebsiella pneumoniae* CP 021695. *Pseudomonas aeruginosa* (KT 285549) was more closely related to *Pseudomonas stutzeri* (JN 378750) compare to *Acinetobacter baummani* (LN 611374) and was distantly related to other organisms present on the phylogentic tree.

Similarly, Table 6 displays the NCBI BLAST results, revealing significant matches between the query sequence and edited sequences from selected isolates. The sequences of the amplified fragments of bacterial isolates were compared to those of the gen bank and were found to be *Escherichia coli* (MW 368769), *Pseudomonas aeruginosa* (KT 285549), *Proteus vulgaris* (NR 115878), *Citrobacter freundii* (NR 028894), and *Klebsiella pneumoniae* (CP 021695). The matches were characterized by high max identity, query value and lower E value of zero (0.0) that indicate a more significant match with corresponding accession.

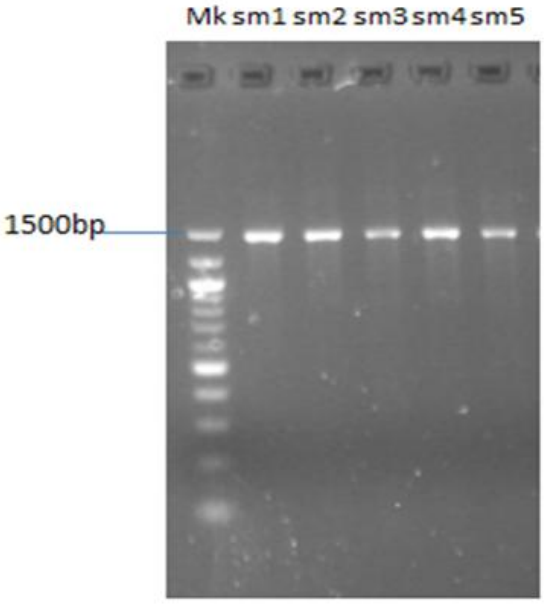

Figure 5. Positive amplification of the 16S gene region amplified from selected bacterial isolates (sm1- *Pseudomonas aeruginosa*, sm2 - *Escherichia coli*, sm3 - *Proteus vulgaris*, sm4 - *Citobacter freundii*, and sm5 - *Klebsiella pneumoniae*)

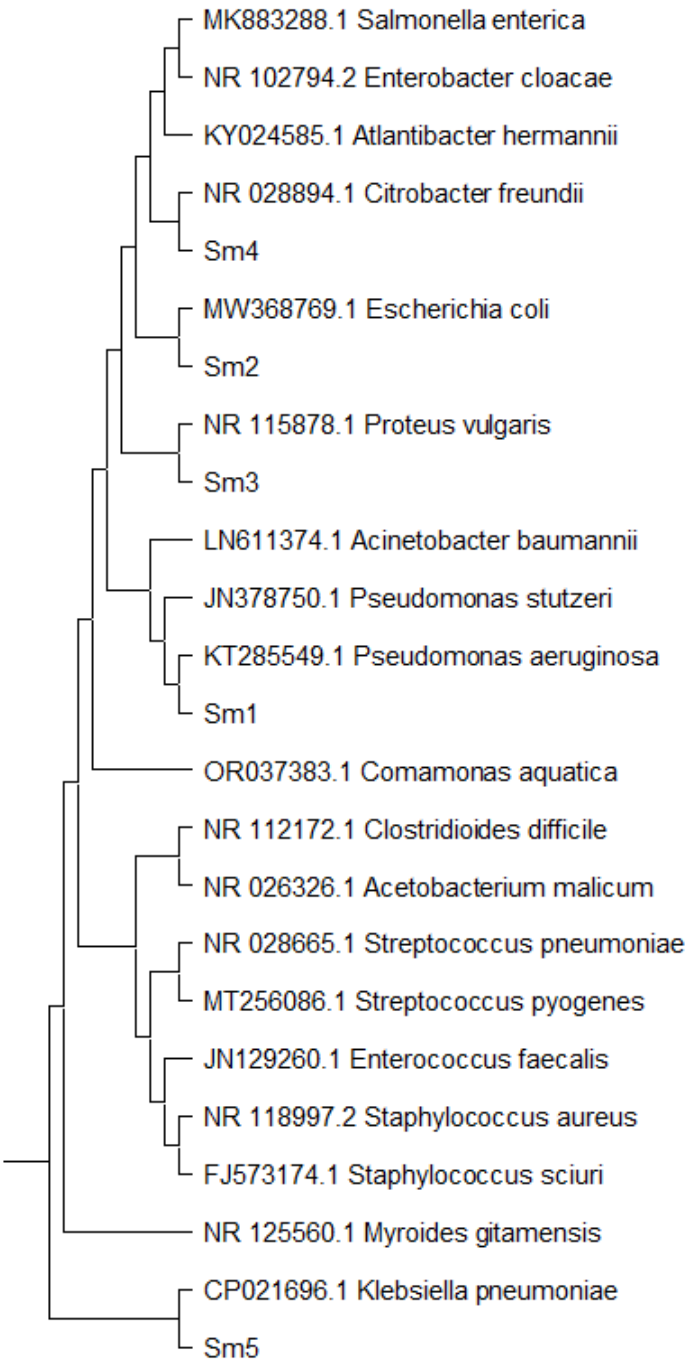

Figure 6. Phylogenetic tree showing the relationships of the identified organisms (sm1 *Pseudomonas aeruginosa*, sm2 - *Escherichia coli*, sm3 - *Proteus vulgaris*, sm4 - *Citobacter freundii*, and sm5 - *Klebsiella pneumoniae*)

Table 6. Molecular identity of selected bacteria

S/N	Scientific Name	Max Score	Total Score	Query Cover (%)	E value	% Identity	Accession Number
1	*Pseudomonas aeruginosa*	2375	2375	99	0.0	99.85	KT285549.1
2	*Escherichia coli*	2344	2344	99	0.0	99.53	MW368769.1
3	*Proteus vulgaris*	2385	16620	99	0.0	99.92	NR115878.1
4	*Citrobacter freundii*	2379	18998	100	0.0	99.86	NR028894.1
5	*Klebsiella pneumoniae*	2399	2399	100	0.0	99.77	CP021696.1

3.1.6 Antibiotic susceptibility profile of Gram-negative bacteria

Figure 7 shows the percentage susceptibility and resistant Gram negative bacteria. A high percentage (71.97%) of Gram negative organisms were resistant to the conventional antibiotics under study, while 27.17% were susceptible and 0.86% showed intermediate sensitivity. Table 7 displays the antibiotic susceptibility of Gram-negative bacteria isolates, indicating that the majority of bacteria exhibited resistance to the multiple antibiotics tested. *Escherichia coli* showed 100% resistance to ampiclox and all tested classes of cephalosporins, specifically the third generation cephalosporins (cefotaxime, cefuroxime, ceftriaxone, and cefixime) with 92.86% resistance to amoxillin and imipenem, while 92.86%, 92.86%, 78.57%, 64.29%, and 50% susceptibility were shown to ofloxacin, levofloxacin, gentamycin, nitrofurantoin, and nalidixic acid, respectively.

Pseudomonas species also showed 100% resistance to ampiclox and all tested classes of cephalosporins. In addition, 100% resistance was also shown to amoxicillin, imipenem and nitrofurantoin while 90.91%, 81.82%, 72.73%, and 36.36% susceptibility were shown to levofloxacin, ofloxacin, gentamycin and nalidixic acid, respectively. The antibiotic resistance profile of the Gram negative isolates revealed 100% resistance to cefixime, high resistance rates to amoxicillin (99.35%), ampiclox (98.99%), imipenem (94.81%), cefotaxime (90.15%), nitrofurantoin (84.87%), and ceftriaxone (71.17%). In contrast, the isolates showed significant susceptibility to ofloxacin (80.09%), levofloxacin (79.62%), and gentamicin (65.81%). Both ofloxacin and levofloxacin demonstrated high potency of activities against majority of bacteria that were resistant to other antibiotics, as revealed in Figure 10.

3.1.7 Antibiotic susceptibility profile of Gram-positive bacteria isolates

The percentage susceptibility and resistance of Gram positive bacteria is revealed in Figure 8. Gram positive bacteria showed 55.9% susceptibility, 43.06% resistance and 1.04% intermediate sensitivity. *Staphylococcus* and *Bacillus* species demonstrated varying degrees of susceptibility to the tested antibiotics, with 87.5% of strains susceptible to ofloxacin, levofloxacin, and imipenem. Significant susceptibility was also observed, with 75% of bacterial species susceptible to gentamicin and cefuroxime. Moderate susceptibility was noted with 58.33% of bacteria susceptible to cefixime and ciprofloxacin while susceptibility of 54.16% was shown to azithromycin (Table 8).

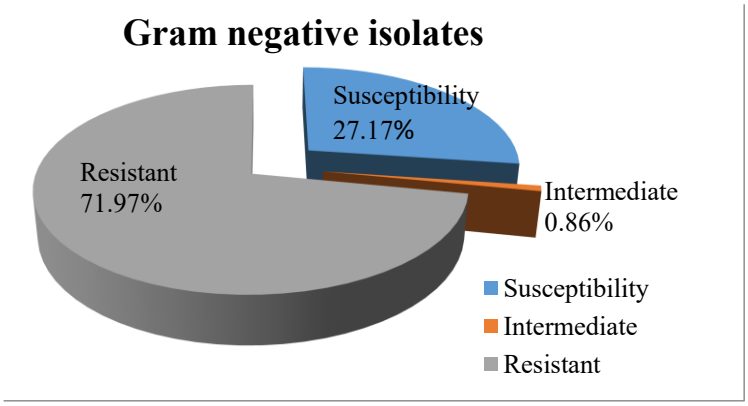

Figure 7. Percentage susceptibility and resistant of Gram negative bacteria isolated from water sources in Owo Local Government Area

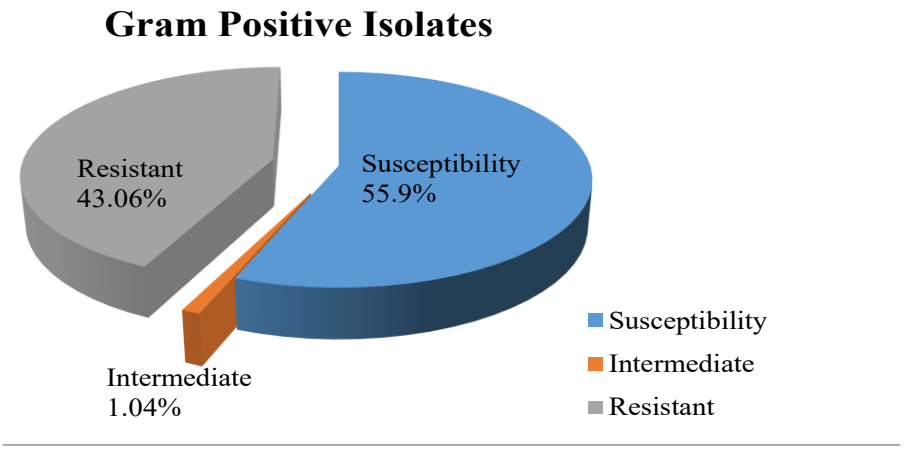

Figure 8. Percentage susceptibility and resistant of Gram positive bacteria isolated from water sources in Owo Local Government Area

Table 7. Antibiotic susceptibility and resistance profiles of Gram-negative bacteria isolated from water in Owo

Bacteria		AMX	CTX	IMP	OFX	GEN	NAL	NIT	CXM	CRO	ACX	CFM	LVX
Escherichia coli (n=14)	S	(1) 7.14%	0	(1) 7.14%	(1) 92.86%	(11) 78.57%	(7) 50%	(9) 64.29%	0	0	0	0	(13) 92.86%
	R	(13) 92.86%	(14) 100%	(13) 92.86%	(1) 7.14%	(3) 21.43%	(7) 50%	(5) 35.71%%	(14) 100%	(14) 100%	(14) 100%	(14) 100%	(1) 7.14%
Pseudomonas spp. (n =11)	S	0	0	0	(9) 81.82%	(9) 81.82%	(4) 45.45%	0	0	(1) 9.09%	0	0	(10) 90.91%
	R	(11) 100%	(11) 100%	(11) 100%	(2) 18.18	(2) 18.18%	(6) 54.55	(11) 100%	(11) 100%	(10) 90.91%	(11) 100%	(11) 100%	(1) 9.09%
Proteus spp. (n =10)	S	0	0	0	(9) 90%	(10) 100%	(8) 80%	(2) 20%	(1) 10%	(6) 60%	0	0	(9) 90%
	R	(10) 100%	(10) 100%	100%	(1) 10%	0	(2) 20%	(8) 80%	(9) 90%	(4) 40%	(10) 100%	(10) 100%	(1) 10%
Klebsiella spp. (n= 9)	S	0	(3) 33.33%	0	(8) 88.89%	(1) 11.11%	(7) 77.78%	0	(1) 11.11%	(1) 11.11%	(1) 11.11%	0	(8) 88.89%
	R	(9) 100%	(6) 66.67%	(9) 100%	(1) 11.11%	(8) 88.89%	(2) 22.22%	(9) 100%	(8) 88.89%	(8) 88.89%	(8) 88.89%	(9) 100%	(1) 11.11%
Citrobacter spp. (n = 7)	S	0	0	0	(6) 85.71%	(6) 85.71%	(6) 85.71%	(4) 57.14%	0	(2) 28.57%	0	0	(5) 71.43%
	R	(7) 100%	(7) 100%	(7) 100%	(1) 14.29%	(1) 14.29%	(1) 14.29%	(3) 42.86%	(7) 100%	(5) 71.43%	(7) 100%	(7) 100%	(2) 28.57%
Enterobacter spp. (n = 4)	S	0	(1) 25%	0	(3) 75%	(2) 50%	(1) 25%	(1) 25%	0	25%	0	0	75%
	R	(4) 100%	(3) 75%	(4) 100%	(1) 25%	(2) 50%	(3) 75%	(3) 75%	(4) 100%	75%	100%	100%	25%
Salmonella spp. (n= 3)	S	0	0	0	(2) 66.67%	(2) 66.67%	(2) 66.67%	0	0	(1) 33.33%	0	0	(2) 66.67%
	R	(3) 100%	(3) 100%	(3) 100%	(1) 33.33%	(1) 33.33%	(1) 33.33%	(3) 100%	(3) 100%	(2) 66.67%	(3) 100%	(3) 100%	(1) 33.33%
Vibrio spp. (n = 2)	S	0	0	(1)50%	(2) 100%	(2) 100%	0	0	0	(2) 100%	0	0	(2) 100%
	R	(2) 100%	(2) 100%	(1) 50%	0	0	(2) 100%	(2) 100%	(2) 100%	0	(2) 100%	(2) 100%	0
Shigella spp. (n =1)	S	0	0	0	(1) 100%	(1) 100%	(1) 100%	0	0	0	0	0	(1) 100%
	R	(1) 100%	(1) 100%	(1) 100%	0	0	0	(1) 100%	(1) 100%	(1) 100%	(1) 100%	100%	0

Table 7. Antibiotic susceptibility and resistance profiles of Gram-negative bacteria isolated from water in Owo (continued)

Bacteria		AMX	CTX	IMP	OFX	GEN	NAL	NIT	CXM	CRO	ACX	CFM	LVX
Serratia spp. (n = 2)	S	0	(1) 50%	0	(2) 100%	(1) 50%	0	0	0	(1) 50%	0	0	(2) 100%
	R	(2) 100%	(1) 50%	(2) 100%	0	(1) 50%	(2) 100%	(2) 100%	(2) 100%	(1) 50%	(2) 100%	(2) 100%	0
Providencia spp. (n= 1)	S	0	0	0	0	0	0	0	0	0	0	0	0
	R	(1) 100%	(1) 100%	(1) 100%	(1) 100%	(1) 100%	(1) 100%	(1) 100%	(1) 100%	(1) 100%	(1) 100%	(1) 100%	(1) 100%
Total N = 64	S	0.65%	9.85%	5.19%	80.09%	65.81%	48.24%	15.13%	1.92%	28.83%	1.01%	0	79.62%
	R	99.35	90.15%	94.81%	19.91%	34.19%	51.76%	84.87%	98.08%	71.17%	98.99%	100%	20.38%

Key: AMX: Amoxicillin, CTX: Cefotaxime, IMP: Imipenem/Cilastatin, OFX: Ofloxacin, GEN: Gentamicin, NAL: Nalidixic Acid, NIT: Nitrofurantoin, CXM: Cefuroxime, CRO: Ceftriaxone, ACX: Ampiclox, CFM: Cefixime, LVX: Levofloxacin.

Table 8. Antibiotic susceptibility and resistance profiles of Gram-positive bacteria isolated from water in Owo

Bacteria		AMX	CTX	CRO	CFM	LVX	CIP	IMP	CXM	OFX	ERY	GEN	AZM
Staphylococcus spp. n = 4	S	0	(1) 25%	(2) 50%	(2) 50%	(3) 75%	(2) 50%	(3) 75%	(2) 50%	(3) 75%	(1) 25%	(2) 50%	(3) 75%
	R	(4) 100	(3) 75%	(2) 50%	(2) 50%	(1) 25%	(2) 50%	(1) 25%	(2) 50%	(1) 25%	(3) 75%	(2) 50%	(1) 25%
Bacillus spp. n= 3	S	0	(1) 33.33%	(1) 33.33%	(2) 66.67%	(3) 100%	(2) 66.67%	(3) 100%	(3) 100%	(3) 100%	(1) 33.33%	(3) 100%	(1) 33.33%
	R	(3) 100%	(2) 66.67%	(2) 66.67%	(1) 33.33%	0	(1) 33.33%	0	0	0	(2) 66.66%	0	66.66%
Total N = 7	S	0	29.16%	41.67%	58.33%	87.5%	58.33%	87.5%	75%	87.5%	29.16%	75%	54.16%
	R	100%	70.84%	58.33%	41.67%	12.5%	41.67%	12.5%	25%	12.5%	70.84%	25%	45.84%

Key: AMX: Amoxicillin, CTX: Cefotaxime, CRO: Ceftriaxone, CFM: Cefixime, LVX: Levofloxacin, CIP: Ciprofloxacin, IMP: Imipenem/Cilastatin, CXM: Cefuroxime, OFX: Ofloxacin, ERY: Erythromycin, GEN: Gentamicin, AZM: Azithromycin.

3.1.8 Multiple antibiotic resistance (MAR) indices of bacteria

Figure 9 reveals the multiple antibiotic indices of Gram positive and Gram negative bacteria with mean MAR indices that ranged from 0.36 to 1.0. The Gram-positive bacteria (*Bacillus* spp. and *Staphylococcus* spp.) showed the lowest MAR indices, which ranged from 0.36 to 0.5. In contrast, the MAR indices of the Gram-negative bacteria were significantly higher, ranging from 0.63 to 1.00. Figure 10 reveals the susceptibility of bacterial isolates to multiple antibiotics while still being resistant to some antibiotics (more than three) (MAR index greater than 0.2) indicating a MDR bacterium.

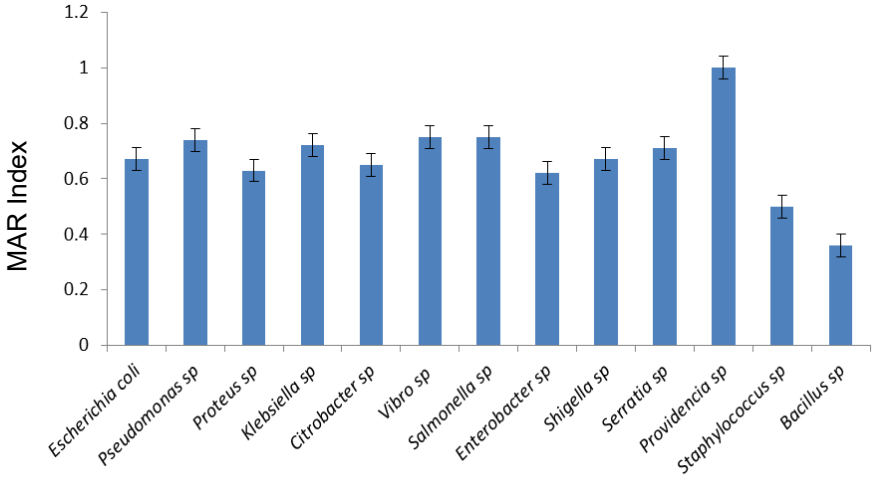

Figure 9. Mean distribution of MAR Index for all bacteria

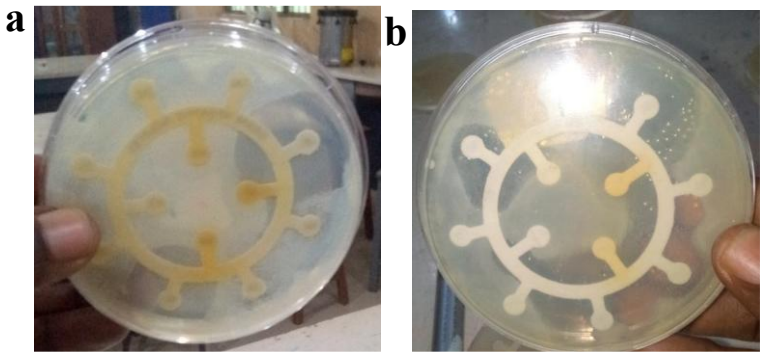

Figure 10. (a) Resistance of *Escherichia coli* to multiple antibiotics while being susceptible to fluoroquinolones (levofloxacins and oxfloxacin), (b) Susceptibility of *Proteus vulgaris* to multiple antibiotics while resistance was shown to ofloxacin, levofloxacin, gentamicin, nalidixic acid, nitrofurantoin, and ceftriaxone.

3.2 Discussion

The quality of any water source can be assessed by analysis of the water's physical, chemical, and biological parameters (Spellman, 2017). This study revealed that 75% (6/8) of the physicochemical parameters (pH, temperature, turbidity, TDS, DO, and EC) for drinking water, sampled across all the 5 settlements were within the World Health Organization permissible level of pH (6.5-8.5), turbidity (0-5 NTU), TDS (≤1000 mg/L), DO (5 mg/L) and EC (≤400 μS/cm) (WHO, 2017; WHO, 2024). The study of Fasoranti et al. (2017) showed a 70% overall safety index of physicochemical parameters of well water samples in Owo LGA. Some fluoride (0.39-1.99 mg/L) and chloride (122-255 mg/L) values in this study slightly exceeded the permissible limits set by WHO which were ≤1.5 mg/L and ≤ 250 mg/L, respectively. High concentrations of chloride and related chemicals may render water unfit for consumption and can impair the aesthetic properties of the water and cause problem such as stomach discomfort and body irritation (Ngala et al., 2022). The statistical analysis of water samples across the five selected areas showed a $p>0.05$ for temperature (0.11), pH (0.34), electrical conductivity (0.33), TDS (0.37), DO (0.15), turbidity (0.96), fluoride (0.68), and chloride (0.99). The ANOVA revealed that there was no significant difference in the physicochemical parameters of water samples collected from the five locations.

Heterotrophic plate counts were considerably high and above the permissible limit set by WHO (≤ 500 cfu/mL), indicating the need for urgent intervention although the borehole samples recorded the lowest counts followed by well, and stream. This correlates with two previous studies that reported the lowest bacterial counts in boreholes, stream, and well samples in Akungba-Akoko, Ondo State, Nigeria (Ajayi & Adejumo, 2011; Adeoyo & Omaku, 2022). The coliform test showed a high occurrence of coliforms in the water samples investigated, indicating a high level of contamination. The presence of *E. coli* is an indication of fecal contamination that may indicate a higher risk of pathogens being present, which can lead to waterborne diseases (Bukar et al., 2015). Borehole samples had the lowest total coliform counts, which might be due to the bore depths or differences in population and human activities around the boreholes (Seth et al., 2013). All the well samples and stream sample had higher coliform counts. The safety level of water for drinking in these areas can then be said to be very low. However, a borehole sample from Emure showed no coliform or fecal presence. This agrees with the results of a previous study that reported the absence of fecal coliforms in water samples of some boreholes and wells in Ijebu-Ode, Southwestern Nigeria (Bello et al., 2013). The borehole water's fecal coliform-free status suggested a well-executed construction process and diligent maintenance routine. Possible factors contributing to this outcome include strategic siting, effective waste management, and robust water treatment measures, such as regular tank sanitization and purification systems (Okoro et al., 2017).

A total of 71 bacteria belonging to 13 genera were identified. This agrees with several studies that identified these bacteria (*E. coli, Salmonella* sp., *Shigella* sp., and *Vibrio cholerae*) to be of public health importance (Osiemo et al., 2019; Ferdous et al., 2021). Similarly, the results agree with the findings of Gwimbi et al. (2019), in which several bacteria of public health importance such as *Escherichia, Enterobacter, Bacillus, Pseudomonas,* and *Klebsiella* species were identified. The most commonly isolated bacterium was *E. coli*, and this correlated with the findings of Gautam (2021) who stated that *E. coli* was the predominant strain among the coliforms isolated. The presence of *E. coli* in water indicates a strong fecal contamination. Although *E. coli* is widespread in the environment, an elevated level can be indicative of fecal pollution, unhygienic practices,

prevalence of gastroenteritis, and location of the water source close to source of contaminants (Osunla & Okoh, 2017).

A noteworthy finding of this investigation was the cleared disparity in the antibiotic resistance profiles of Gram-negative and Gram-positive bacteria, with the former demonstrating a significantly greater propensity for resistance. This finding is consistent with the observations of Breijyeh et al. (2020) who noted that Gram-negative bacteria, including *E. coli* and *Klebsiella pneumoniae* displayed elevated resistance rates to certain antibiotics, such as ciprofloxacin and ceftazidime relative to Gram-positive bacteria, like *Staphylococcus aureus.* Furthermore, a study by Odonkor et al. (2022) revealed high rates of multidrug resistance, with *Klebsiella* and *E. coli* exhibiting resistance to multiple antibiotics at frequencies of 30% and 27.8%, respectively. Moreover, a hospital-based study revealed that Gram-negative bacteria, particularly *A. baumannii* and *P. aeruginosa* exhibited enhanced antibiotic resistance compared to Gram-positive bacteria, resulting in elevated mortality rates (Nasr et al*.,* 2024). Similarly, a study focusing on waterborne pathogens, conducted by Berendonk et al*.* (2015), uncovered a notable difference in antibiotic resistance profiles between Gram-negative and Gram-positive bacteria. Specifically, *Salmonella typhi* and *Vibrio cholerae* (Gram-negative bacteria) exhibited more resistance to antibiotics compared to the Gram-positive species *Bacillus subtilis* (Berendonk et al*.,* 2015).

This present study revealed high level of resistance of Gram negative bacteria to cefixime, amoxicillin, ampiclox, imipenem, cefotaxime, nitrofurantoin, and ceftriaxone. The resistance showed to imipenem is a pressing concern, as carbapenems are typically reserved as a final therapeutic resort for combating infections caused by the highly resilient Enterobacteriaceae. In contrast, high susceptibility was recorded in ofloxacin and levofloxacin. These are fluoroquinolones that target two critical enzymes essential for bacterial DNA maintenance (DNA gyrase and topoisomerase IV). By inhibiting these enzymes, fluoroquinolones disrupt DNA replication and repair processes, ultimately leading to bacterial cell death. This study corroborates the observations of Onduru et al*.* (2021) who demonstrated that ciprofloxacin exhibited 34% susceptibility against all tested isolates while Armin et al*.* (2023) reported that levofloxacin had 26% susceptibility against carbapenem-resistant Enterobacteriaceae. Notably, this study also demonstrated a high degree of susceptibility to gentamicin, a finding that holds significant promise, given the antibiotic's established role in combating Enterobacteriaceae infections.

This present study revealed a trend of elevated resistance most especially in Gram negative isolates, underscoring the pressing need for antibiotic stewardship and novel therapeutic strategies. The MAR indices obtained in this study exceeded the critical threshold of 0.2, indicating that the bacterial isolates exhibited a multidrug-resistant phenotype. This finding concurred with the observations of Atobatele & Owoseni (2023), who reported that Gram-negative bacteria exhibited a broad spectrum of resistance, with a significant proportion demonstrating resistance to three or more antibiotics. The observed antibiotic resistance in the bacterial isolates may be attributed to the presence of specific resistance genes that confer the ability to withstand various antibiotics. Studies have identified various resistance genes in bacteria isolated from water sources, including bla genes (e.g., blaCTX-M, blaSHV): encoding beta-lactamases that confer resistance to beta-lactam antibiotics (e.g., ampicillin, cefotaxime), tet genes (e.g., tetA, tetB): encoding efflux pumps that confer resistance to tetracycline antibiotics, qnr genes: encoding proteins that protect DNA gyrase and topoisomerase IV from quinolone antibiotics (e.g., ciprofloxacin) and mph genes: encoding macrolide phosphotransferases that confer resistance to macrolide antibiotics (e.g., erythromycin) (Ranjbar et al*.,* 2019; Belotindos et al*.,* 2021; Salvador-Membreve & Rivera, 2021).

The antibiotic resistance rates observed may also be attributed to the facilitation of horizontal gene transfer, a process that enables the rapid dissemination of antibiotic resistance genes among bacterial populations. Several studies have investigated the horizontal transfer of antibiotic resistance genes among bacteria in aquatic environments, revealing high frequencies of gene transfer (Mutuku et al*.,* 2022; Michaelis & Grohmann, 2023). The presence of antibiotic-resistant pathogens in water samples poses significant public health risks, particularly in communities relying on substandard water sources, as highlighted by the World Health Organization. Waterborne pathogens can lead to increased morbidity and mortality due to treatment failures (Berisha et al*.,* 2024). The risk of waterborne diseases is a major concern (Prüss-Ustün et al*.,* 2019), and the reduced effectiveness of antibiotics in treating waterborne infections can have severe consequences.

4. Conclusions

This study revealed that 12 out of 21 water samples fell short of the required standards for potable water and domestic use, with wells and stream samples being particularly non-compliant. The presence of fecal coliforms, potential pathogens and antibiotic resistant bacteria in the samples poses serious health risk to the communities. Hence, adequate preventive, monitoring, and control measures should be put in place to avert the dangers associated with the use of contaminated water in the study area. Also, a well-structured public education initiative is necessary to inform residents about the need to purify water, promote household water storage, improve personal hygiene practices, and prevent antibiotic resistance. Proper location and construction of wells and boreholes, and proper waste disposal in the environment should also be encouraged. Therefore, this research confirms the state of water consumed in some selected areas in Owo local government. It provides information on the risk of human exposure to waterborne diseases and contributes to the achievement of sustainable development goal 6 (SDG 6) “Clean water and sanitation for all”. It also proffered necessary information to avert diseases outbreak. Finally, future studies should focus on the use of omics technology to analyze water samples from the environment and there should be further investigation into the machanism(s) of antibiotic resistance patterns.

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6. Authors’ Contributions

All authors designed the research. A.S.A. performed all the experiments and wrote the main manuscript. All authors were involved in data interpretation. T.O.A. and O.R.A. reviewed the manuscript. All authors approved the final manuscript.

7. Conflicts of Interest

Authors declare that they have no competing interests.

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