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## Physicochemical and AntibioGram Study of Bacterial Species Implicated in Drinking Water Sources in Selected Communities of Ishielu, South east, Nigeria.

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### ABSTRACT

Water is a crucial necessity in public health, and the presence of safe water directly impacts the overall quality of life, as it is an essential element for survival. The objective of the present study was to investigate the physicochemical properties and antibiotic resistance patterns of bacterial species obtained from drinking water sources in certain communities within the Ishielu local government area of Ebonyi State. Water sources, specifically streams, rivers, and wells, were gathered for the purpose of conducting Physicochemical and bacteriological analysis using established analytical methods. The bacterial isolates were identified and characterised using conventional microbiological techniques. The physicochemical studies showed that the alkalinity, BOD, COD, pH, TH, TOC, DO, and turbidity values in the water samples (measured in mg/L) were all within the recommended limits for drinking water quality set by the World Health Organisation (WHO) and Nigeria standard for drinking water quality (NSDWQ), except for the alkalinity value in Azuinyaba ward. The bacteriological count results showed that water samples from diverse sources had a Total Bacterial count (TBC) of  $9.10 \times 10^{-4}$ ,  $8.10 \times 10^{-4}$ , and  $4.10 \times 10^{-4}$ , respectively. There was a statistically significant difference ( $p < 0.05$ ) in the bacterial load levels of water samples from all research locations during the study period. Prevalence investigations indicated that *Escherichia coli* had the highest occurrence, followed by *Klebsiella species*, and the least common was *Salmonella species*. The antibiotic investigations demonstrated that *Salmonella*, *Klebsiella species*, and *E. coli* exhibited significant resistance to some commonly used antibiotics, however they also displayed some vulnerability to certain drugs. Nevertheless, the significant presence of enteric bacteria and their high resistance value observed in this study strongly suggests that the samples were contaminated with faecal matter due to inadequate hygiene and sanitation practices in the respective locations. Furthermore, this finding indicates that the isolated bacteria have been exposed to multiple antibiotics, leading to the development of resistance within their bodies when infected with waterborne diseases.

**Key words:** Antibiotic susceptibility; Bacterial species; *Escherichia coli*, Ebonyi State

## INTRODUCTION

Over the past few years, countries with different economic statuses have reported numerous instances of waterborne infections caused by contaminated water (Onuoha, 2017). The growth in population and expansion of land usage result in a steady rise in sources of pathogen-contaminated waste. Consequently, there is a higher risk of water reservoirs becoming polluted with infectious agents worldwide (Gambo *et al.*, 2005). This is primarily applicable to pathogens that originate from human and animal faeces. Possible sources of faecal contamination in water include: seepage from on-site sanitation systems like septic tanks or sewers, underground storage tanks, disposal systems, animal manure and compost, accidental or intentional discharge of wastewater, and the application of sewage sludge to fields in agricultural areas (WHO, 2006).

These diseases are caused by enteric pathogenic bacteria, which are spread through both human activities and natural processes, including as grazing, spreading manure, and unregulated sewage disposal. The movement of water runoff can transport these microorganisms, which may result in the pollution of water through the infiltration of soil (Lansing *et al.*, 2002). Statistics indicate that in many developing nations, such as Nigeria, about 80% of ailments and more than 30% of deaths are attributed to water-related causes (Sundar *et al.*, 2010).

A good understanding of the ecology of antibiotic resistance, including their origins, evolution, selection and dissemination is of immense importance in confronting antibiotic resistance genes (Kummu *et al.*, 2010). In order to mitigate this issue, it is crucial to determine the specific settings in which genes that confer resistance can be favoured, and subsequently transmitted to viruses that affect humans or animals. Environments that are likely to have enough selection pressure to sustain a variety of well-known antibiotic resistance genes in significant amounts also contain a considerable number of resistance factors that have not yet been described. As a

result, these environments may act as a source for discovering new resistance traits (Onuoha, 2017).

The health of a population is directly affected by the quality of water. The provision of clean and convenient water can help fulfil one of the purposes of the millennium development goals (MDG) related to improving public health (Lansing *et al.*, 2002). Therefore, it is crucial to analyse water for its physical, biological, and chemical qualities, as well as its levels of heavy elements. This analysis is essential for conducting public health research (Fawell and Nieuwenhuijsen, 2003). Due to the significant impact of potable water on human health, it is crucial to assess the presence of bacterial pathogens in water, as they can pose serious health risks. Therefore, it is necessary to analyse and evaluate the quality of water obtained from surface and underground sources in Ishielu Communities of Ebonyi State. Regular monitoring of bacterial pathogens in is of utmost importance for public health.

## **METHODS**

### **Study area**

Ebonyi State is located in the southeastern region of Nigeria. Ebonyi State is located between latitudes 5° 40' and 6° 45'N and longitudes 7° 30' and 8° 30'E. It is predominantly inhabited by the Igbo people, with the city of Abakiliki serving as its capital and largest metropolis. Other significant cities in the state include Afikpo, Okposi, Onueke, Unwana, Ikwo, Ezzamgbo, and Nkalagu. The prevailing climatic condition in Ebonyi State is characterised by two distinct seasons: rainy and dry. The rainy season typically occurs from April to October, while the dry season spans from October to February. This region is known for its bimodal rainfall pattern, with annual rainfall ranging from 1613.8mm to 2136.27mm. Ebonyi State is primarily an agricultural region, renowned for its significant contribution to the production of rice, yam, and potatoes in Nigeria. Rice cultivation, in particular, is the primary agricultural activity in the state. The State possesses a variety of valuable mineral resources, including as lead and crude

oil.

### **Study Site**

Ishielu local government area is situated within the Ebonyi State, which is located in the South-east geopolitical zone of Nigeria. The LGA's headquarters are located in the town of Ezillo. The LGA has several towns and villages, including Umuakpu, Amalaeze, Azunyaba, Onuoji, Nkalaha, Amaokwe, Nkalagu, Iyonu, and Ntezi. The estimated population of Ishielu Local Government Area (LGA) is approximately 177,412 residents. The overall land area of Ishielu Local Government Area (LGA) is 872 square kilometres, and it experiences an average temperature of 27 degrees Celsius. The local government is responsible for the well-known Ezilo water scheme, which is intended to provide water to all residents of Ishielu local government and the state capital, Abakaliki. However, the water scheme is currently not operating at its full capacity, leading to a situation where a significant portion of the population relies on manually dug wells, boreholes, and streams for their domestic water needs, including drinking water. The likelihood of streams becoming contaminated is significantly elevated, particularly during the rainy seasons, due to the introduction of wastewater pollutants, runoff from rural areas, and agricultural practices. This contamination occurs as the streams pass through the city and its surrounding suburbs.

### **Sample collection and processing**

Each water source was sampled three times, with samples obtained at four-hour intervals, beginning at 7.00 am and concluding at 3.00 pm. The sample was undertaken during the months of December, February, and April. The samples were expeditiously delivered to the Microbiology Laboratory of Ebonyi State University on the same day of collection. Portions of the samples were subsequently employed for the specific separation of particular bacteria utilising standard microbiological techniques. The water samples were collected from several locations inside the Ishielu Local Government Area of Ebonyi State using one-liter jerry cans.

The jerry cans were first cleaned with distilled water and then filled with water samples collected from wells, rivers, and ponds, before being filled with the water for analysis.

### **Isolation, purification, and characterization of planktonic bacteria:**

The filtration process involved using a Sartorius 16824 water pump and a 0.45  $\mu\text{m}$  pore sized filter from Whatman Laboratory Division in Maidstone, England to filter three volumes of 100 mL each. Afterwards, the membrane was meticulously placed onto the plate containing nutrient-rich media, making sure that there were no air bubbles trapped underneath. The plates were thereafter placed in an incubator set at a temperature of 37 degrees Celsius for a period of 24 hours to observe the growth of bacteria. Following this time frame, the number of colonies was counted. The colonies were then transferred to specific selective media, such as Salmonella - Shigella Agar (SSA) to isolate Salmonella and Shigella species, Mannitol Salt Agar (MSA) to isolate Staphylococci, and Eosin Methylene Blue (EMB) and McConkey Agar (MA) to isolate E. coli and other Enterobacteriaceae. The media were obtained from Biolab, a subsidiary of Merck located in South Africa. The agar plates were incubated at a temperature of 37 degrees Celsius for a duration of 18 to 24 hours. The bacterial isolates were identified through a range of techniques, including assessment of colony morphology, Gram's reaction, catalase activity, motility, and biochemical tests such as oxidase activity, citrate utilisation, indole production, Methylred-Voges Proskauer (MRVP) reaction, urease activity, hydrogen sulphide production, and sugar fermentation.

### **Physicochemical Analysis**

The water samples were analysed using the approved techniques recommended by the American Public Health Association (APHA, 1998), with the exception of on-site assessments for temperature, pH, odour, and taste. These techniques are firmly established and universally acknowledged, guaranteeing the accuracy, dependability, and adherence to the defined standards of the measurements. The characteristics examined encompass alkalinity, biological

oxygen demand (BOD), chemical oxygen demand (COD), conductivity, dissolved oxygen (DO), total hardness, total organic carbon (TOC), and turbidity. The parameter was measured three times, and the average count was recorded.

### **Statistical Analysis**

The results of replicates were pooled and expressed as Mean  $\pm$  Standard error of mean. Data obtained were subjected to a one-way analysis of variance with the aid of IBM Statistical Package for Social Sciences (SPSS) version 22 and Microsoft Excel 2013 software.

Table 1. Physicochemical Composition of Water Samples Collected from **Streams** in Different Location at Ishielu Local Government Area Compared with NSDWQ and WHO Recommended Limits.

Limits	S/N	Physicochemical properties	Location of Water Sources						Standard Recommended		
			Agba	Ntezi	Ezillo	Nkalagu	Ezzagu	Azuinyaba	Amazu	NSDWQ	WHO
1		Alkalinity (mgL <sup>-1</sup> )	195.06 ± 0.04 <sup>b</sup>	168.42 ± 0.12 <sup>c</sup>	168.62 ± 0.10 <sup>c</sup>	191.63 ± 0.18 <sup>b</sup>	153.63 ± 0.10 <sup>c</sup>	287.41 ± 1.02 <sup>a</sup>	164.75 ± 0.08 <sup>c</sup>	200	200
2		BOD (mgL <sup>-1</sup> )	1.34 ± 0.12 <sup>c</sup>	1.56 ± 0.10 <sup>b</sup>	1.81 ± 0.10 <sup>a</sup>	1.67 ± 0.28 <sup>b</sup>	1.58 ± 0.50 <sup>b</sup>	1.96 ± 1.43 <sup>a</sup>	1.82 ± 0.67 <sup>a</sup>	10	1.0-2.0
3		COD (mgL <sup>-1</sup> )	8.13 ± 0.10 <sup>a</sup>	10.00 ± 0.10 <sup>a</sup>	6.44 ± 0.05 <sup>b</sup>	7.56 ± 0.2 <sup>a</sup>	8.32 ± 0.13 <sup>a</sup>	5.74 ± 0.26 <sup>b</sup>	5.48 ± 0.76 <sup>b</sup>	8-10	10
4		Conductivity (ms/L)	0.98 ± 1.00 <sup>c</sup>	0.91 ± 0.00 <sup>c</sup>	0.90 ± 0.56 <sup>c</sup>	1.74 ± 0.24 <sup>b</sup>	2.34 ± 0.67 <sup>a</sup>	1.32 ± 1.14 <sup>b</sup>	0.76 ± 0.56 <sup>c</sup>	1	0.25
5		DO (mgLp <sup>-1</sup> )	1.43 ± 1.18 <sup>b</sup>	0.72 ± 0.75 <sup>c</sup>	0.52 ± 0.45 <sup>c</sup>	3.69 ± 0.21 <sup>a</sup>	2.34 ± 0.24 <sup>a</sup>	0.74 ± 1.00 <sup>c</sup>	0.86 ± 0.00 <sup>c</sup>	7.50	1.0-2.0
6		Odour	Odour	Odour	Odour	Odour	Odour	Odour	Odour	Odour	Odour
7		PH	6.34 ± 0.08 <sup>b</sup>	6.25 ± 1.16 <sup>b</sup>	6.72 ± 0.54 <sup>b</sup>	6.74 ± 2.20 <sup>b</sup>	7.83 ± 0.56 <sup>a</sup>	6.47 ± 0.45 <sup>b</sup>	6.79 ± 0.10 <sup>b</sup>	6.0-9.0	6.5-8.5
8		Taste	Salty	Salty	Salty	Salty	Salty	Salty	Salty	T	T
9		Temp (°c)	26.52 ± 0.67 <sup>c</sup>	27.16 ± 0.12 <sup>b</sup>	27.32 ± 0.90 <sup>b</sup>	28.62 ± 1.08 <sup>a</sup>	27.04 ± 0.28 <sup>b</sup>	26.54 ± 0.78 <sup>c</sup>	26.73 ± 0.67 <sup>c</sup>	20-30	28-30
10		TH (mgL <sup>-1</sup> )	122.64 ± 0.12 <sup>c</sup>	142.54 ± 0.45 <sup>c</sup>	166.13 ± 1.00 <sup>b</sup>	109.18 ± 1.00 <sup>c</sup>	201.11 ± 1.20 <sup>a</sup>	189.41 ± 0.67 <sup>a</sup>	163.80 ± 0.00 <sup>b</sup>	150	100-200
11		TOC (mgL <sup>-1</sup> )	2.71 ± 0.65 <sup>b</sup>	3.88 ± 0.67 <sup>a</sup>	4.16 ± 0.18 <sup>a</sup>	0.89 ± 1.32 <sup>c</sup>	3.63 ± 0.90 <sup>a</sup>	3.81 ± 0.67 <sup>a</sup>	3.63 ± 0.02 <sup>a</sup>	4-5	NS
12		Turbidity (NTU)	5.75 ± 0.67 <sup>ab</sup>	6.45 ± 0.23 <sup>a</sup>	8.94 ± 1.10 <sup>a</sup>	6.89 ± 0.24 <sup>a</sup>	5.74 ± 1.24 <sup>ab</sup>	7.78 ± 0.78 <sup>a</sup>	6.13 ± 0.34 <sup>a</sup>	5	5-10

The results are mean ± SD of four stream water samples from Agba, Ntezi, Ezillo, Nkalagu, Ezzagu, Azuinyaba and Amazu. Values with different alphabets differed significantly (p<0.05) across the row. DO (Dissolved Oxygen), COD(chemical Oxygen Demand), BOD(Biochemical Oxygen Demand), BDL(Below Detectable Limits), TH(Total Hardness), TOC(Total Organic Carbon), Temp(Temperature), T(Tasteless), NSDWQ(Nigeria standard for Drinking Water Quality) and WHO(World Health Organization).

Table 2. Physicochemical Composition of Water Samples Collected from **Rivers** in Different Location at Ishielu Local Government Area Compared with NSDWQ and WHO Recommended Limits.

S/N	Physicochemical properties	Location of water sources						standard recommended limits		
		Agba	Ntezi	Ezillo	Nkalagu	Ezzagu	Azuinyaba	Amazu	NSDWQ	WHO
1	Alkalinity (mgL <sup>-1</sup> )	181.25±0.07 <sup>b</sup>	167.41 ± 0.89 <sup>c</sup>	177.15±0.54 <sup>c</sup>	191.25±0.81 <sup>b</sup>	151.51±0.78 <sup>c</sup>	243.32±0.53 <sup>a</sup>	157.17±0.98 <sup>c</sup>	200	200
2	BOD(mgL <sup>-1</sup> )	1.39 ± 0.41 <sup>c</sup>	1.68 ± 0.42 <sup>b</sup>	1.99 ± 0.12 <sup>a</sup>	1.86 ± 0.65 <sup>a</sup>	1.47 ± 0.65 <sup>c</sup>	1.91 ± 0.52 <sup>a</sup>	1.71 ± 0.36 <sup>a</sup>	10	1.0-2.0
3	COD (mgL <sup>-1</sup> )	7.41 ± 0.73 <sup>b</sup>	9.80 ± 0.45 <sup>a</sup>	7.51 ± 0.23 <sup>b</sup>	7.36 ± 0.42 <sup>b</sup>	9.02 ± 0.28 <sup>a</sup>	6.14 ± 0.02 <sup>b</sup>	5.76 ± 0.76 <sup>c</sup>	8-10	10
4	Conductivity (ms/L)	0.82 ± 0.34 <sup>d</sup>	0.99 ± 0.43 <sup>d</sup>	1.08 ± 0.18 <sup>c</sup>	1.75 ± 0.104 <sup>a</sup>	2.09 ± 0.92 <sup>a</sup>	1.56 ± 0.61 <sup>b</sup>	0.73 ± 0.42 <sup>d</sup>	1	0.25
5	DO (mgLp <sup>-1</sup> )	1.48 ± 0.54 <sup>b</sup>	0.72 ± 0.65 <sup>c</sup>	0.81 ± 0.12 <sup>c</sup>	1.99 ± 0.08 <sup>a</sup>	1.91 ± 0.82 <sup>a</sup>	2.04 ± 0.91 <sup>a</sup>	0.84 ± 0.67 <sup>c</sup>	7.50	1.0-2.0
6	Odour	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless	Odourless
7	pH	6.71 ± 0.32 <sup>a</sup>	6.61 ± 0.32 <sup>a</sup>	6.52 ± 0.61 <sup>b</sup>	6.46 ± 0.63 <sup>b</sup>	7.54 ± 0.81 <sup>a</sup>	6.36±0.52 <sup>b</sup>	6.96±0.80 <sup>a</sup>	6.0-9.0	6.5-8.5
8	Taste	Salty	Salty	Salty	Salty	Salty	Salty	Salty	T	T
9	Temp (°c)	26.51 ± 0.78 <sup>b</sup>	27.72 ± 0.57 <sup>a</sup>	27.91 ± 0.56 <sup>a</sup>	28.42 ± 0.51 <sup>a</sup>	26.06 ± 0.41 <sup>b</sup>	26.81 ± 0.73 <sup>b</sup>	27.86 ± 0.52 <sup>a</sup>	20-30	28-30
10	TH (mgL <sup>-1</sup> )	121.35±0.34 <sup>d</sup>	173.54±0.47 <sup>c</sup>	166.41±0.56 <sup>c</sup>	132.56± 0.46 <sup>a</sup>	200.51±0.42 <sup>b</sup>	193.52 ± 0.16	195.75±0.64 <sup>b</sup>	150	100-200
11	TOC(mgL <sup>-1</sup> )	2.88 ±0. 08 <sup>b</sup>	3.67 ± 0.76 <sup>a</sup>	4.18 ± 0.76 <sup>a</sup>	0.83±1.04 <sup>c</sup>	3.71 ± 0.67 <sup>a</sup>	3.84 ± 0.43 <sup>a</sup>	3.53 ± 0.61 <sup>a</sup>	4-5	NS
12	Turbidity (NTU)	5.19 ± 0.34 <sup>b</sup>	5.65 ± 0.62 <sup>b</sup>	8.75 ± 0.34 <sup>a</sup>	6.78 ± 0.53 <sup>ab</sup>	5.62 ± 0.10 <sup>b</sup>	6.56 ± 0.61 <sup>ab</sup>	6.71 ± 0.76 <sup>ab</sup>	5	5-10



Table 3. Physicochemical composition of water samples collected from **Well** in different location at Ishielu Local Government Area compared with NSDWQ and WHO recommended limits.

S/N	Physicochemical properties	Location of water sources						Standard recommended limits		
		Agba	Ntezi	Ezillo	Nkalagu	Ezzagu	Azuinyaba	Amazu	NSDWQ	WHO
1	Alkalinity (mgL <sup>-1</sup> )	148.12±1.10 <sup>c</sup>	178.42±0.65 <sup>b</sup>	147.16±0.65 <sup>c</sup>	183.28±0.65 <sup>b</sup>	171.14±0.87 <sup>b</sup>	283.12±0.14 <sup>a</sup>	163.8±0.7 <sup>ab</sup>	200	200
2	BOD (mgL <sup>-1</sup> )	1.42±0.65 <sup>b</sup>	1.74±0.76 <sup>a</sup>	1.99±0.71 <sup>a</sup>	1.78±0.71 <sup>a</sup>	1.43±0.72 <sup>b</sup>	1.74±0.26 <sup>a</sup>	1.84±0.76 <sup>a</sup>	10	1.0-2.0
3	COD (mgL <sup>-1</sup> )	8.21± 0.02 <sup>a</sup>	10.40 ±0.52 <sup>a</sup>	5.16 ± 0.56 <sup>b</sup>	7.56 ± 0.92 <sup>a</sup>	9.62 ± 0.78 <sup>a</sup>	5.71 ± 0.67 <sup>b</sup>	4.17 ± 0.65 <sup>b</sup>	8-10	10
4	Conductivity (ms/L)	0.91 ± 1.00 <sup>b</sup>	0.97 ± 0.76 <sup>b</sup>	1.0 ± 0.67 <sup>ab</sup>	1.56 ± 0.52 <sup>a</sup>	2.00 ± 0.43 <sup>a</sup>	1.41 ± 0.24 <sup>a</sup>	0.81 ± 0.81 <sup>b</sup>	1	0.25
5	DO (mgLp <sup>-1</sup> )	1.31 ± 1.08 <sup>b</sup>	0.60 ± 0.54 <sup>c</sup>	0.41 ± 0.63 <sup>c</sup>	3.44 ± 0.67 <sup>a</sup>	1.18 ± 0.39 <sup>b</sup>	0.72 ± 0.76 <sup>c</sup>	0.92 ± 0.43 <sup>ab</sup>	7.50	1.0-2.0
6	Odour	Odourless	Odourless	Odour	Odour	Odourless	Odourless	Odour	Odourless	Odourless
7	pH	6.71±0.13 <sup>a</sup>	6.15 ±0.35 <sup>a</sup>	6.55 ± 0.52 <sup>a</sup>	6.71 ± 0.24 <sup>a</sup>	7.84 ± 0.32 <sup>a</sup>	6.41 ± 0.54 <sup>a</sup>	6.71 ± 0.85 <sup>a</sup>	6.0-9.0	6.5-8.5
8	Taste	Salty	Salty	Salty	Salty	Salty	Salty	Salty	T	T
9	Temp (°c)	27.42±0.67 <sup>a</sup>	27.16 ± 0.76 <sup>a</sup>	27.41±0.47 <sup>a</sup>	28.12 ± 0.97 <sup>a</sup>	27.11 ± 0.56 <sup>a</sup>	26.34 ± 0.72 <sup>a</sup>	27.72 ± 0.45 <sup>a</sup>	20-30	28-30
10	TH (mgL <sup>-1</sup> )	121.31±0.54 <sup>c</sup>	151.51±0.62 <sup>c</sup>	167.21±0.82 <sup>b</sup>	102.79 ±0.45 <sup>c</sup>	200.39 ± 0.62 <sup>a</sup>	191.32 ± 0.67 <sup>a</sup>	179.11 ± 1.09 <sup>b</sup>	150	100-200
11	TOC (mgL <sup>-1</sup> )	2.57 ± 0.56 <sup>b</sup>	2.88 ± 0.84 <sup>b</sup>	4.18 ± 0.31 <sup>a</sup>	0.95 ± 0.61 <sup>c</sup>	3.76 ± 0.16 <sup>a</sup>	3.96 ± 1.04 <sup>a</sup>	3.61 ± 0.51 <sup>a</sup>	4-5	NS
12	Turbidity (NTU)	5.27 ± 0.61 <sup>b</sup>	8.02 ± 54 <sup>a</sup>	7.64 ± 0.76 <sup>a</sup>	5.87 ± 0.42 <sup>b</sup>	5.78 ± 0.91 <sup>b</sup>	6.61 ± 0.62 <sup>ab</sup>	5.51 ± 0.63 <sup>b</sup>	5	5-10

The results are mean ± SD of four well water samples from Agba, Ntezi, Ezillo, Nkalagu, Ezzagu, Azuinyaba and Amazu. Values with different alphabets differed significantly (p<0.05) across the row. DO (Dissolved Oxygen), COD(chemical Oxygen Demand), BOD(Biochemical Oxygen Demand), BDL(Below Detectable Limits), TH(Total Hardness), TOC(Total Organic Carbon), Temp(Temperature), T(Tasteless), NSDWQ(Nigeria standard for Drinking Water Quality) and WHO(World Health Organization)

Table 4. Total Bacterial Count (Cfu/MI) of Water Samples Collected from Different Water Sources in Ishielu Local Government Area.

<b>S/N</b>	<b>Sample Sources</b>	<b>TBC (CFU/mL)</b>
1	Streams	9.10x10 <sup>-4</sup>
2	Rivers	8.10 x10 <sup>-4</sup>
3	Wells	4.10 <sup>-</sup> x10 <sup>-4</sup>

Table 5. Percentage Frequency Distribution of Bacterial Isolated from Water Samples Collected from Different Water Sources in Ishielu Local Government Area.

<b>S/N</b>	<b>Samples Sources</b>	<b><i>Escherichia coli</i>(%)</b>	<b><i>klebsiella spp</i> (%)</b>	<b><i>Salmonella spp</i> (%)</b>
1	Streams	24 (33)	10 (32)	11 (34)
2	Rivers	27 (38)	12 (39)	14 (44)
3	Wells	21 (29)	9 (29)	7 (22)
<b>Total</b>		<b>72 (100)</b>	<b>31(100)</b>	<b>32(100)</b>

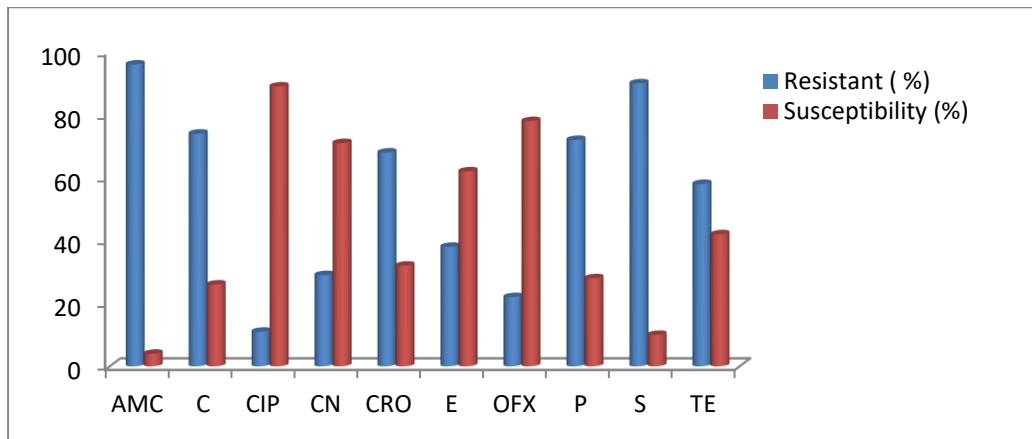


Fig 1. Antibiotic Percentage Resistance and Susceptibility of *Escherichia coli* from Different Drinking Water Sources in Ishielu Local Government Area.

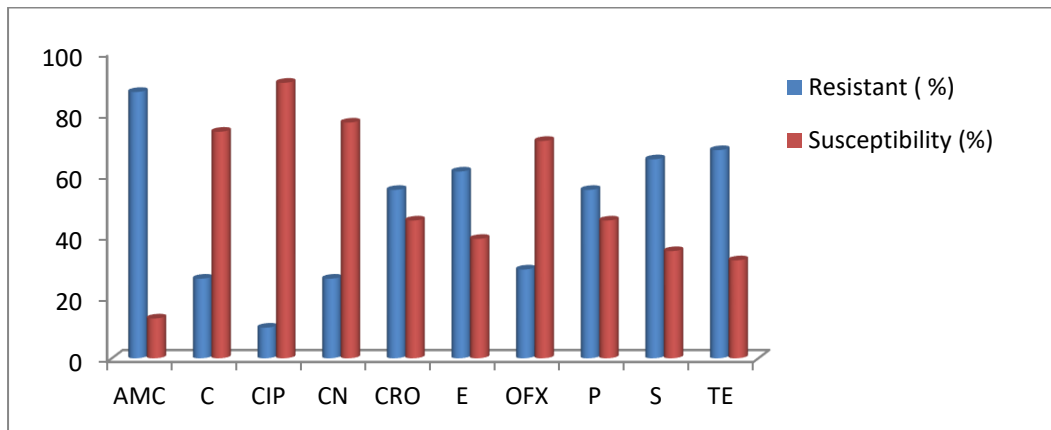


Fig 2. Antibiotic Percentage Resistance and Susceptibility of *klebsiella* species from Different Drinking Water Sources in Ishielu Local Government Area.

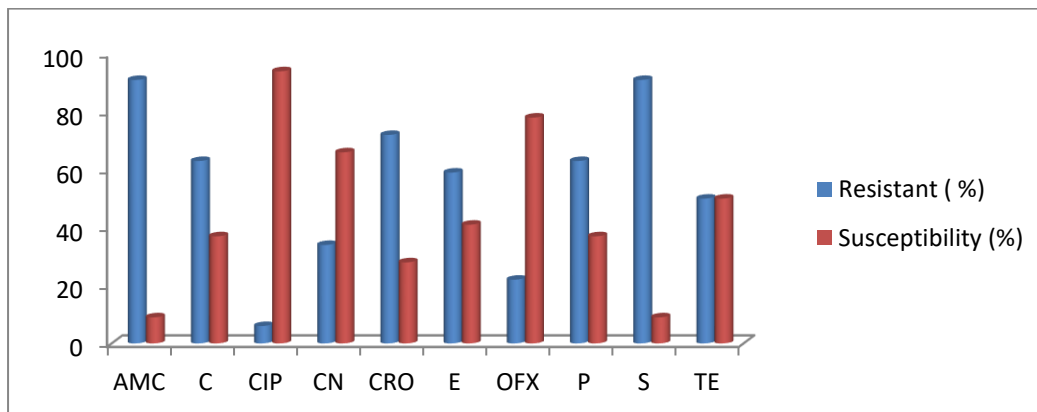


Fig 3. Antibiotic Percentage Resistance and Susceptibility of *Salmonella* species from Different Drinking Water Sources in Ishielu Local Government Area.

## RESULT AND DISCUSSION

The analysis of water samples collected from various streams in different locations within Ishielu LGA indicated the physicochemical composition, including pH, TH, TOC, DO, and turbidity. The alkalinity, BOD, and COD values in milligrammes per litre (mg/L) of stream water were within the recommended limits for drinking water set by the World Health Organisation (WHO) and the National Standard for Drinking Water Quality (NSDWQ), except for Azuinyaba. Azuinyaba had an alkalinity value that above the recommended limits for drinking water as specified in Table 1. In addition, the conductivity levels in the stream water samples were above the recommended limits set by the World Health Organisation (WHO). However, they were below the limits indicated by the National Standard for Drinking Water Quality (NSDWQ), except for Nkalagu, Ezzagu, and Azuinyaba, which exceeded the NSDWQ limit (Table 1). Overall, a statistically significant difference ( $P < 0.05$ ) was identified in alkalinity, BOD, COD, TOC, and DO across the different stream water samples, as indicated in Table 1.

The analysis of water samples collected from rivers in the study area revealed that the levels of Alkalinity, BOD, COD, pH, TH, TOC, DO, and turbidity were all within the recommended limits for drinking water set by the World Health Organisation (WHO) and the National Standard for Drinking Water Quality (NSDWQ). The only exception was Azuinyaba, which had a higher alkalinity value than the recommended limit (Table 2). The conductivity readings in river water samples exceeded the recommended limits set by the World Health Organisation (WHO), except for Ezillo, Nkalagu, Ezzagu, and Azuinyaba, which also exceeded the recommended limit set by the National Standard for Drinking Water Quality (NSDWQ) (Table 2). A statistically significant difference ( $p < 0.05$ ) was identified in the levels of alkalinity, BOD, COD, TOC, and DO among the different river water samples, as indicated in Table 2.

Table 3 displays the physicochemical composition of water samples obtained from wells located in various areas within the study site. The alkalinity, BOD, and COD values in mg/L of the well water samples were within the recommended limits for drinking water set by the WHO and NSDWQ, except for Azuinyaba. The alkalinity value for Azuinyaba exceeded the recommended limits for drinking water, as indicated in Table 3. The pH, TH, TOC, DO, and turbidity values were found to be within the acceptable levels set by the World Health Organisation (WHO) and the National Safe Drinking Water Quality (NSDWQ) guidelines (Table 3). Table 3 displays a statistically significant difference ( $p < 0.05$ ) in alkalinity, BOD, COD, TOC, and DO across the different well water samples. The presence of disinfectant in water, as indicated by alkalinity, BOD, COD, TOC, and DO levels, can lead to corrosion of metallic pipes, resulting in a decrease in the useable diameter (Siti *et al.*, 2015). Water with elevated levels of alkalinity, BOD, COD, TOC, and DO might potentially cause gastrointestinal problems, including stomach cramps, abdominal discomfort, and diarrhoea (Pandey *et al.*, 2003). The alkalinity results obtained in this study are consistent with the findings of Nweke *et al.* (2004), who reported alkalinity values of 282.50 mg/L for water samples collected from Enyigba, Ebonyi state, and Uhuo *et al.* (2014), who observed an alkalinity level of 212.25 mg/L in drinking water sources in the Abakaliki Urban Area, Ebonyi state. Consequently, the presence of calcium and magnesium ions, which are the primary cations responsible for hardness, might result in elevated levels of total hardness, BOD, COD, and TOC in water (Pallav, 2013). In their study, Peter-Ikechukwu *et al.* (2015) found that the water samples obtained from Owerri, Imo State, Nigeria had a total hardness of  $298.62 \pm 27.61$  mgL<sup>-1</sup>. Water that has a high concentration of hardness, biological oxygen demand (BOD), chemical oxygen demand (COD), and total organic carbon (TOC) can lead to significant issues in home, agricultural, and industrial environments (Okonkwo *et al.*, 2010).

Our research indicated that all of the water samples had a salty flavour. The National Safe Drinking Water Quality (NSDWQ) guidelines from 2007 and the World Health Organisation (WHO) guidelines from 2011 both state that drinking water should be devoid of any taste. According to Ugwu *et al.* (2016), the water samples collected from wells, boreholes, and streams at Michael Okpara University of Agriculture Umudike in Nigeria were found to have a salty flavour. The presence of high salinity in the research regions necessitates the implementation of suitable measures to eliminate or diminish the saltiness.

The turbidity profile of the analysed water samples remained relatively constant ( $p < 0.05$ ) across all the water bodies during the whole study period. The turbidity level achieved in this study is less than the turbidity level of  $3.2 \pm 2.01$  NTU obtained by Ajayi *et al.*, (2018) in Ibadan, Nigeria. The turbidity levels of the water sources indicate a high presence of suspended particulates, bacteria, plankton, and dissolved organic and inorganic compounds (Kadam *et al.*, 2007). Water samples exhibiting high turbidity display a visibly hazy look and are generally deemed unsuitable for consumption, unless subjected to appropriate treatment. Increased turbidity levels lead to a decrease in aquatic vegetation, resulting in a reduction of food sources for numerous aquatic creatures (Bello *et al.*, 2012). This scenario can also impede the process of disinfection and create an environment conducive to the growth of microorganisms, resulting in symptoms such as nausea, cramps, diarrhoea, and related headaches (Ezeet *et al.*, 2015). In contrast to the findings of Okoro *et al.*,(2017), low levels of alkalinity, COD, and TOC were observed in borehole and well water samples in Nsukka urban, ranging from  $4.32 \pm 0.28$  to  $3.52 \pm 0.54$  mgL<sup>-1</sup>. The discrepancies seen in this study could be attributed to disparities in hydro-geological regions, levels of exposure to sanitation, weather patterns, and potential entrance points for contaminants. Water with specific physicochemical composition, such as alkaline,

has been scientifically demonstrated to provide health benefits by promoting calcium retention and enhancing bone health. (Agwu *et al*, 2013).

The bacteriological counts of water samples from various sources revealed a statistically significant variation ( $p < 0.05$ ) in the levels of bacterial load among all the research locations (Table 4). All the values for total bacteria counts exceeded the WHO threshold of zero per 100 ml. The findings are consistent with the study conducted by Onura *et al*. (2018), which revealed that water samples obtained from several drinking water sources in Ezza South, Ebonyi State, Nigeria.

The prevalence analysis showed that *Escherichia coli* was the most commonly found bacteria, followed by *Salmonella species*, with *Klebsiella species* being the least frequently isolated (Table 5). Nevertheless, the elevated quantity of *Escherichia coli* found in drinking water sources serves as a signal of the presence of enteric pathogens. According to a report by the World Health Organisation (WHO) in 2006, the absence of proper sanitation facilities, such as pit latrines, the discharge of untreated wastewater, and the indiscriminate dumping of waste, contribute to the pollution of drinking water in areas where open defecation is practiced. This contamination can result in diseases. Allamin *et al*. (2015) found that *Escherichia coli* was the most frequently identified species in their study, with a prevalence of 98%. This was followed by *Aeromonas sobria* at 67%, *Klebsiella species* at 66%, *Salmonella species* at 44%, and *Staphylococcus species* at 25%, which had the lowest prevalence among water sources in Kaduna State, Nigeria. Bello *et al*. (2012) conducted a study where they found and identified Enterobacteriaceae in drinking water samples from North Eastern Nigeria.

The antibiogram investigations revealed varying levels of resistance exhibited by the bacterial species towards different antibiotics currently in use. *Escherichia coli* shown a high level of resistance to Amoxicillin-clavulanic acid (96%), followed by Streptomycin (90%), Chloramphenicol (74%), penicillin (72%), and Ceftiaxone (68%). The isolates exhibited a

susceptibility rate of 89% to Ciprofloxacin, 78% to Ofloxacin, 71% to Gentamicin, and 62% to Erythromycin (Fig 1).

*Klebsiella species* exhibited high levels of resistance to Amoxicillin-clavulanic acid (87%), tetracycline (68%), streptomycin (65%), penicillin (55%), and Ceftiaxone (55%). The isolate exhibited a susceptibility rate of 90% to Ciprofloxacin, 77% to Gentamicin, and 71% to Ofloxacin (Fig 2).

Furthermore, *Salmonella species* exhibited resistance to Amoxicillin-clavulanic acid at a rate of 91%, Streptomycin at 91%, Ceftiaxone at 72%, Chloramphenicol at 64%, and penicillin at 64%. The isolates exhibited a susceptibility rate of 94% to Ciprofloxacin, 78% to Ofloxacin, and 66% to Gentamicin (Fig 3).

The resistance rate to these medicines was elevated, confirming the findings, Kinge *et al.* (2010) discovered that *Escherichia coli*, *Salmonella*, *Pseudomonas*, *Shigella*, and *Klebsiella species* that were isolated also exhibited significant resistance to amoxicillin-clavulanic acid, penicillin, and ceftiaxone. However, they were found to be susceptible to ciprofloxacin, ofloxacin, and nalidixic acid. Moreover, the findings of this investigation align with the results obtained by Efuntoye and Apnapa (2010) in Nigeria, Hildebran *et al.* (2009) in South Africa, and Abd El-Salam (2012) in Egypt. These studies all found elevated levels of resistance to ampicillin, sulphamethoxazole-trimethoprim, and ofloxacin.

## CONCLUSION

In conclusion, the investigation has found that the physicochemical parameters in the water samples exceeded the acceptable limits set by NSDWQ and WHO. Our observation revealed that the bacteriological values, namely the total bacterial count, exceeded the international standard set by the World Health Organisation (WHO), which is zero per 100ml. This indicates a potential health concern for both humans and animals. The detection of enteric bacteria, including *Escherichia coli*, *Klebsiella species*, and *Salmonella species*, in samples of stream,



well, and river water, as observed in this study, indicates the presence of faecal contamination resulting from inadequate hygiene and sanitation practices at the sampling sites. This contamination could potentially contribute to the spread of various diseases among humans and animals. Therefore, the study findings indicate that there has been a widespread and unselective utilisation of the tested antibiotics, as evidenced by the high levels of antibiotic resistance observed in bacterium isolates from water and the resistance patterns of organisms in drinking water in the study areas.

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