Seasonal variation and antibiotic susceptibility patterns of bacteriological parameters in groundwater sources in Oyi LGA, Anambra State, Nigeria

ABSTRACT

Potable water is essential for health and survival of all life forms. Contamination of groundwater sources by microbes, organic and inorganic matter has continued to negatively affect the well-being of most people in developing countries such as Nigeria. Thus, this study aimed to determine the seasonal variation and antibiotic susceptibility patterns of bacteriological parameters in groundwater sources in Oyi LGA, Anambra State, Nigeria. Hand-dug well and borehole water sites were randomly selected in five communities of Oyi LGA, and water samples were aseptically collected and analyzed using standard microbiological procedures. The total heterotrophic counts of bacteria isolated from groundwater samples during both seasons were between 1.3×10^2 and 2.87×10^4 CFU/mL, total coliform counts ranged from 2 to 165 MPN/100mL and faecal coliform ranged from 0 to 32 MPN/100mL. Probable bacterial isolates in groundwater samples were *Escherichia coli*, *Klebsiella aerogenes*, *Shigellaflexneri*, *Enterococcusfaecalis* and *Enterobacter* sp. Seasonal variations showed there were more bacterial loads in sampled water during wet season than dry season. Antibiotic susceptibility test revealed that all the bacterial isolates were sensitive to Levofloxacin and Ofloxacin, and are recommended for treatment of diseases caused by some of these pathogens. *Shigellaflexneri* exhibited highest susceptibility (60%) to the antibiotics, while *Enterobacterfaecalis* showed highest resistance (70%). *Escherichia coli* exhibited 50% susceptibility and 50% resistance. The sampled water sources did not meet the WHO and NSDWQ guidelines for drinking water, and therefore not fit for human consumption without adequate treatment.

Keywords: Antibiotic sensitivity; Borehole; Coliform counts; Hand-dug well; Oyi LGA.

1. INTRODUCTION

Water is an important natural resource used for domestic, agricultural and industrial purposes [1]. Despite its importance in the sustenance of life and livelihood, the inability to access safe and potable water has been identified as a major cause of morbidity and mortality [2]. It is estimated that 28% of the world's population lack access to sufficient safe and potable water [3].Although significant progress has been made in increasing access to clean drinking water and sanitation, billions of people, especially in rural areas of low and middle-income countries, still lack drinking water. Globally, one in every three persons lack access to safe drinking water, two out of every five persons lack access to a basic hand-washing facility with soap and water, and more than 673 million people continue to practice open defaecation [4]. About 2 billion people drink water that has been polluted by faeces [5]. Lack of safe water and poor sanitation practices are leading causes of preventable water-borne diseases, which are the world's second cause of child mortality [6]. The WHO estimated that 4.1% of the total daily global burden is linked to diarrhoeal as a result of contaminated water [7]. This disease is responsible for the deaths over 1.8 million people globally each year. Unsafe water, poor sanitation and hygiene have been attributed to the cause of many water-related illnesses globally [8].

Studies have shown that over 5 million people, majorly from developing countries, die due to consumption of contaminated water and food [6]. In the year 2012, sub-Sahara Africa recorded about 200,000 deaths that were as a result of unsafe drinking water [9].Sufficient supply of potable water is critical in the prevention of gastrointestinal diseases; in other words, drinking water quality has a significant impact on public health [10].

Groundwater is usually obtained from underground aquifers through deep wells, boreholes or springs, and it is always assumed to be unpolluted due to its depth and clarity [11]. This isa fallacy, as many sources of groundwater are increasingly being polluted, mainly from anthropogenic activities. Although well and borehole waters are sourced from deep groundwater aquifers, they can be contaminated by seepages from pesticides and fertilizer-applied soil, human and animal excreta, pit latrines, septic tanks, domestic, industrial and municipal wastes, and refuse dumps[12,2].

Bacterial contamination in drinking water is a major public health threat, as it may encourage the transmission of waterborne diseases [7]. Coliforms are generally used as indicators for presence of bacterial contamination in drinking water quality. They are the bacteria frequently recovered from water, linked to several diseases such as diarrhoea, typhoid, dysentery, and have also been associated with mortality across the globe, especially in sub-Sahara Africa [9]. Presence of bacteria especially coliform bacteria and *Escherichia coli* in water is an indication of pathogenic/faecal contamination[13]. The prevalence of water-borne disease outbreaks in Nigeria is a clear indication that transmission of infectious agents through consumption of contaminated drinking water is a significant cause of illness. Seasonal variations may have direct and indirect effects on the quality and quantity of groundwater. Several studies have reported higher levels of microbial loads in groundwater samples during wet season than dry season [11]. This could be attributed to intensity in water runoff, increase in organic matter, agricultural wastes and agro-allied products during planting period in wet season, etc.

Antibiotics such as Augmentin, Ciprofloxacin, Gentamycin, Tetracycline, Streptomycin, etc have been administered for treatment of various forms of water-related illnesses such as typhoid fever, diarrhoea, cholera, dysentery, etc[14,15].In the last two decades, several studies have reportedantimicrobial resistance (AMR) from members of *Enterobacteriaceae*family, isolated from water[16-20]The dissemination of AMR among pathogenic bacteria is a serious threat in the natural environment. AMR may occur either by mutation or acquisition of antibiotic resistance genes (ARGs) through horizontal gene transfer (HGT). HGT is one of the major mechanisms used to spread ARGs from environmental and commensal species to pathogenic ones [21,22]. Thus, water bodies can be efficient vehicles for the dissemination of AMR.The emergence and spread of antimicrobial resistance (AMR) among bacteria are two of the most important public health challenges worldwide [23]. *ASM Microbe* 2023 opined that AMR would be the leading cause of death in the next decade. Globally, it is estimated that by 2050, 10 million premature deaths could occur as a result of AMR annually [24]. The WHO recommends that practicing WASH (water, sanitation and hygiene), the scourge of antimicrobial resistance would drastically reduce [5].

The main sources of domestic water supply in Oyi LGA are boreholes, hand-dug wells, rainwater and streams, but the present study focuses on boreholes and hand-dug wells. This is due to the fact that these two sources of groundwaterare frequently used by Oyi residents,for both drinking and domestic purposes. Public boreholes are situated in village squares, market centers, church and school fields, etc where villagers line up to fetch, whereas hang-dug wells are situated in people's compounds. The bacteriological quality of underground well water in Oyi LGA, Anambra State, Nigeria, was evaluated by [25], but excluded the

seasonal variation and antibiotic susceptibility patterns. This study, therefore, examines the seasonal variation and antibiotic susceptibility patterns of the bacteriological parameters in borehole and hand-dug well water samples in Oyi LGA, Anambra State, Nigeria.

2. MATERIALS AND METHODS

2.1Study area

The study area was Oyi Local Government Area (LGA) in Anambra State, Nigeria. Oyi LGA is one of the 21 LGAs in Anambra State, geographically located at Latitude $6^{o}10'36.1^{o}$N (6.1767000^{o}) and Longitude $6^{o}51'42.7^{o}$E (68618700^{o}). Oyi is inthe tropical rainforest belt of West Africa with rainforest climate, and the relative humidity is about 75%, reaching 80% during wet season with an annual rainfall of 2000mm. The mean temperature ranges from 22 ^{o}C to 30 ^{o}C in wet season and 28 ^{o}C to 32 ^{o}C in dry season. The major occupations of the inhabitants are agriculture, trading, artisans and administrative work. Oyi LGA is blessed with significant groundwater resources which the residents rely on for their domestic, drinking and agricultural purposes. Awkuzu, Nkwelle-Ezunaka, Nteje, Ogbunike and Umunya communities in Oyi LGA were the study areas.

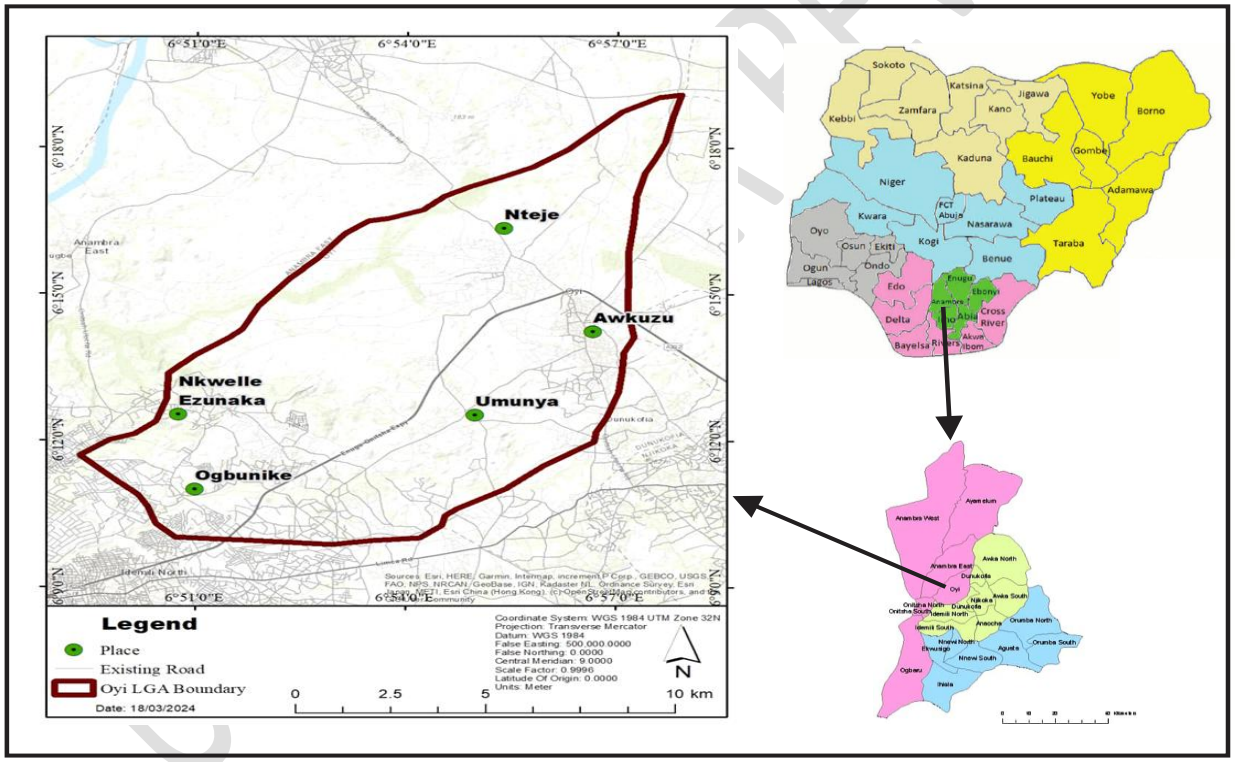

Fig. 1: Map of Oyi LGA in Anambra State, Nigeria showing the study area

2.2 Study design

A total of fifteenstudy sites, comprising eight hand-dug wellsand seven boreholes, were selectedfor the study based on the populations of residents that used them. The water samples were sampled from December, 2022 to August, 2023, covering both dry and wet seasons. The study sites wereAwkuzu (designated as **A1, A2, A3**), Nkwelle-Ezunaka (designated as **Nk1**, **Nk2**, **Nk3**), Nteje (designated as**Nt1**, **Nt2**, **Nt3**), Ogbunike (designated as**Og1**, **Og2**, **Og3**) and Umunya (designated as**U1**, **U2**, **U3**).

2.3 Sample collection

Duplicate water samples from each of the study sites were aseptically collected in 1Lsterile containers during dry and wet seasons. The collected water samples were transported to the Microbiology laboratory of Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Nigeria under aseptic conditions for bacteriological analysis. The following were hand-dug well locations: **A3**, **Nk3**, **Nt1**, **Nt2**, **Og1**, **Og2**, **U1** and **U3**, while **A1**, **A2**, **Nk1**, **Nk2**, **Nt3**, **Og3** and **U2** were borehole locations. Overall, 60 water samples were collected in the study area during both seasons.

2.4 Bacteriological analyses

2.4.1 Total heterotrophic bacterial count (THB)

The total heterotrophic bacterial count was carried out using the method described by [26]. Nutrient agar was prepared, autoclaved, dispensed into sterile Petri dishes and cooled. After a 10^{-5} serial dilution of water samples from various sources and sites, already solidified NA plates were inoculated with 1.5 mL of the samples from 10^{-2} tubes and plated on agar surface using spread plate technique. They were incubated at 37 ^{o}C for 24hrs, and after incubation, total heterotrophic bacterial counts were obtained. Plates with 30-300 colonies were selected for calculating the colony forming unit (CFU) in 100 mL using the equation below:

$$\text{CFU/100 mL} = \frac{\text{number of colonies} \times \text{dilution factor}}{1}$$

Upon macroscopic identification of discrete bacterial colonies, isolates were picked and further sub-cultured on Blood agar (BA), Salmonella-Shigella agar (SSA), MacConkey agar (MCA) to obtain pure cultures of enteric organisms. Isolates from these pure cultures were then identified using standard microbial methods according to [26], and tests such as Gram's reaction and biochemical tests (catalase, motility, indole, methyl red, Voges-Proskauer, citrate utilization, sugar fermentation, oxidase and urease tests) were employed to identify the bacteria species. Cultural and morphological appearances of isolates from the pure culture were categorized using Bergey's manual of determinative bacteriology[27].

2.4.2 Membrane filter technique

The membrane filtration technique was employed. From the 1L sampled containers, a 100 mL aliquot of each water sample was taken and filtered through a membrane filter (0.45 µm membrane filter manufactured by Merck Millipore, Darmstadt Germany). Sterile forceps were used to remove the membrane filter, and thereafter aseptically transferred onto the surface of already prepared plates of Eosin Methylene blue (EMB) agars as described by [28].Before performing the agar plate Coliform count method as described by [29], the plates were incubated at 37 ^{o}C for 24-48hrs for *E. coli* detection through the Most Probable Number (MPN/100mL),which involvedthree stages of test being the presumptive, confirmatory and completed tests.

2.5 Antibiotic susceptibility patterns of isolates

This was determined using the method described by [30]. A loopful of bacterial colony from the pure culture plate was inoculated using a sterile inoculating loop into two millilitres of Mueller Hinton broth. The bacterial suspension was matched with 0.5 McFarland Standard using UV spectrophotometer. The Mueller-Hinton agar was prepared based on the manufacturer's instruction and sterilized using an autoclave. It was introduced into culture and allowed to gel and labelled. A sterile cotton swab stick containing the broth was

streaked on to the Mueller Hinton agar plate in zig-zag style. Antibiotic discs: Amoxicillin-Clavulanate (AUG) (30µg), Cefuroxime (CFX) (30µg), Nalidixic acid (NA) (30µg), Gentamycin (CN) (10µg),Ofloxacin (OFX) (5µg), Levofloxacin (LBC) (5µg), Streptomycin (ST) (10µg), Imipenem (IMI) (10µg), Ceftazidime (CFT) (10µg)and Ciprofloxacin (CPX) (5µg) (CelTech Diagnostic, Belgium Inc.) were placed on the agar culture using sterile forceps. The culture was incubated at 37 ^{o}C for 24 hours. The zones of inhibition of the isolates were measured with a meter rule and clear zones of inhibition were recorded and interpreted using the Clinical Laboratory Standard Institute (CLSI) guidelines [31].

2.6 Data analysis

The collected data was analyzed using descriptive statistics and Analysis of Variance (ANOVA). Turkey *post-hoc* was used for multiple comparisons of all sites, while Pair-wise was used for seasonal comparison at statistical significance of $P < 0.01$.

3. RESULTS

3.1 Total heterotrophic bacterial (THB), total coliform (*E. coli*) and faecal coliform counts

The results of bacteriological analysis of the water samples obtained in this study during both dry and wet seasons are shown in Tables 1 and 2 respectively. Table 1 revealed that*E. coli*were the predominant bacterial isolates during the dry season, followed by *Klebsiella aerogenes* and *Enterobacter* sp. Others were *Enterococcus faecalis* and *Shigella flexneri*. These bacteria isolated from these two sources of groundwater presentedtotal heterotrophic bacterial (THB) and total coliform (TC) counts with well water from **A3** having the most contamination (i.e. 5.5×10^4 CFU/mL) and (115MPN/100mL) respectively, and 17MPN/100mL forfaecal coliforms (FC). **A3** was followed by well water from **U1** which recorded 94MPN/100mL as TC count, and then 82MPN/100mL from **Nt1**. **A1** had the least bacterial contamination with total heterotrophic bacterial counts as 1.3×10^2 CFU/mL; total coliform as 2 MPN/100mL and 0 MPN/100mL as faecal coliform. During dry season, borehole water samples had the least faecal contamination (0 MPN/100mL to 3MPN/100mL), unlike well water samples which recorded 6 MPN/100mL to 17 MPN/100mL.Borehole water had the least faecal contamination and presence of other enteric organisms, compared with hand-dug well water samples.

Table 1:Bacteriological parameters in hand-dug well and borehole water samples inOyi LGA during the dry season

S/N	Water source	Sites	Bacteriological parameters						
			THB (CFU/mL)	**TC(*E. coli*) (MPN/100mL)**	**FC (MPN/100mL)**	***Enterobacter* sp. (CFU/mL)**	***Klebsiella* sp.(CFU/mL)**	***Enterococcus* sp.(CFU/mL)**	***Shigella* sp. (CFU/mL)**
1	**Hand-dug well**	**A3**	5.5×10^4	115	17	7.2×10^3	1.18×10^3	1.32×10^3	2.4×10^3
2		**Nk3**	1.25×10^3	64	12	4.2×10^3	4.8×10^3	8.0×10^2	2.1×10^2
3		**Nt1**	1.5×10^3	82	15	3.6×10^3	3.4×10^3	6.8×10^2	1.8×10^2
4		**Nt2**	1.16×10^3	42	8	3.9×10^3	5.3×10^3	ND	1.3×10^2
5		**Og1**	1.86×10^4	74	15	6.2×10^3	7.8×10^2	4.4×10^2	2.4×10^2
6		**Og2**	1.38×10^3	58	10	5.8×10^3	1.03×10^3	ND	1.4×10^2
7		**U1**	2.14×10^4	94	8	4.0×10^3	4.5×10^3	6.3×10^2	1.2×10^2
8		**U3**	1.68×10^4	34	6	ND	ND	ND	2.2×10^2
9	**Borehole**	**A1**	1.3×10^2	2	0	3.4×10^2	1.8×10^2	ND	ND
10		**A2**	2.6×10^2	4	0	4.6×10^2	3.5×10^3	4.5×10^3	ND
11		**Nk1**	1.5×10^3	18	2	ND	4.6×10^3	3.5×10^3	1.3×10^2
12		Nk2	3.8×10^3	34	1	ND	ND	4.5×10^2	1.4×10^2
13		**Nt3**	2.2×10^3	20	3	4.2×10^3	3.3×10^3	5.5×10^2	ND
14		**Og3**	3.4×10^3	25	2	3.5×10^3	4.6×10^2	4.2×10^2	ND
15		**U2**	2.3×10^3	3	0	3.3×10^2	3.8×10^2	4.6×10^3	ND

KEY**:** THB= Total heterotrophic bacterial count, TC = Total coliform, FC = Faecal coliform, *E. coli* = *Escherichia coli*, ND = Not Detected.

Similarly, Table 2 reveals the bacteriological parameters in hand-dug well and borehole water samples in Oyi LGA during the wet season. The results reveal that well water samples from **A3** recorded the most bacterial contamination with 8.7×10^4CFU/mL as total heterotrophic bacterial counts, while the total coliforms and faecal coliforms were 165 MPN/100mL and 32 MPN/100mL respectively. Borehole water from **A2** recorded the least bacterial contamination, with 2.3×10^3 CFU/mL as total heterotrophic bacterial counts, 6 MPN/100mL as total coliforms, and 0 MPN/100mL as faecal coliform.*E. coli* were the predominant bacterial isolates during the wet season, followed by *Shigella flexneri*, and then *Klebsiella aerogenes*.*Enterococcus faecalis* were the least bacterial isolates during the wet season, as they were not detected in two well sites and three borehole sites (50%), followed by *Enterobacter* sp., which were not detected in three borehole sites (30%).

Table 2: Bacteriological parameters in hand-dug well and borehole water samplesinOyi LGA during the wet season

S/N	Water source	Sites	Bacteriological parameters					
			THB (CFU/mL)	TC (*E. coli*) (MPN /100mL)	FC (MPN/ 100mL)	*Shigella* sp. (CFU/mL)	*Enterococcus* sp. (CFU/100mL)	*Enterobacter* sp. (CFU/mL)	*Klebsiella*sp (CFU/mL)
1	Hand-dug well	A3	8.7×10^4	165	32	2.8×10^3	3.7×10^3	6.8×10^3	2.5×10^3
2		Nk3	3.8×10^4	38	5	2.2×10^3	5.3×10^2	3.7×10^2	2.2×10^3
3		Nt1	4.9×10^4	127	27	2.6×10^3	ND	2.9×10^3	1.8×10^3
4		Nt2	2.07×10^3	45	12	1.4×10^2	ND	8.6×10^2	2.1×10^3
5		Og1	3.6×10^4	95	21	1.8×10^3	4.3×10^2	4.6×10^3	2.3×10^2
6		Og2	5.3×10^4	64	17	4.2×10^2	3.5×10^2	2.1×10^3	1.5×10^3
7		U1	2.5×10^4	144	22	3.2×10^2	4.8×10^2	1.7×10^3	1.5×10^2
8		U3	2.8×10^4	58	14	6.4×10^2	5.2×10^2	2.3×10^2	ND
9	Borehole	A1	3.9×10^3	15	8	1.8×10^3	2.2×10^3	3.3×10^3	1.2×10^2
10		A2	2.3×10^3	6	0	ND	ND	ND	ND
11		Nk1	1.8×10^3	25	4	1.4×10^3	1.2×10^2	3.2×10^3	2.1×10^3
12		Nk2	6.4×10^4	79	12	4.5×10^2	ND	4.2×10^3	2.8×10^2
13		Nt3	4.2×10^3	18	2	2.2×10^2	ND	ND	3.0×10^2
14		Og3	3.4×10^3	30	6	1.2×10^3	1.2×10^3	ND	2.5×10^2
15		U2	2.2×10^3	15	10	1.3×10^3	1.5×10^3	3.0×10^2	2.3×10^2

3.2 Characterization and identification of bacterial isolates: The colonial morphologies and biochemical features ofthe bacterial isolates in hand-dug well and borehole water samples in Oyi LGA are presented in Table 3. Probable bacterial isolates were *Klebsiellaaerogenes*, *Shigellaflexneri*, *Enterobacter* sp., *Enterococcusfaecalis* and *E. coli*.

Table 3:Colonial morphology and biochemical features of bacterial isolates in groundwater sources in Oyi LGA

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Ox	Ur	Identity
A	Irregular	Glistening	Cream	Entire	Raised	Opaque	- Rod	+	-	-	+	-	+	+	+	+	-	+	-	+	*Klebsiella aerogenes*
B	Circular	Smooth	Greyish	Entire	Convex	Translucent	- Rod	+	-	Var	+	-	-	-	+	-	+	Var	-	-	*Shigella flexneri*
C	Circular	Shiny	White	Entire	Convex	Moist	- Rod	+	+	-	-	+	+	+	+	+	+	+	-	-	*Enterobacter* sp.
D	Circular	Smooth	Cream	Entire	Convex	Opaque	+Coccus	-	-	-	-	+	-	+	+	+	-	+	-	-	*Enterococcus faecalis*
E	Circular	Smooth	Whitish	Entire	Convex	Translucent	- Rod	+	+	+	+	-	-	+	+	var	-	-	-	-	*E. coli*

Gram = Gram reaction, Cat = Catalase, Mot = Motility, Indole = Indole, MR = Methyl red, VP = Voges-Proskauer, Cit = Citrate, Lac = Lactose, Glu = Glucose, Suc = Sucrose, Fru = Fructose, Mal = Maltose, Ox = Oxidase, Ur = Urease, Var = Variable, + = positive result, - = negative result.

3.3 Antibiotic susceptibility patterns

Results of the antibiotic susceptibility testing of bacterial isolates in hand-dug well and borehole water samples in Oyi LGA are shown in Table 4.

All the isolates showed 100% sensitivity to Ofloxacin and Levofloxacin, while being 100% resistant to Augmentin and Cefuroxime.

Table 4:Antibiotic susceptibility patterns of isolated bacteria (measured in mm as zones of inhibition)

Antibiotics (µg)	**Standard values**			***E. coli***	***Enterococcus faecalis***	***Klebsiella aerogenes***	***Enterobacter*sp.**	***Shigella flexneri***
	R ≤	I	S≥					
AUG (30)	13	14-17	18	R (5.8)	R (6.0)	R (6.2)	R (-)	R (5.0)
CFX (30)	14	15-17	18	R (6.5)	R (5.7)	R (8.1)	R (7.2)	R (6.2)
NA (30)	13	14-18	19	R (6.0)	S (25)	R (10.0)	S (20.0)	S (26.0)
OFX (5)	12	13-15	16	S (18.0)	S (20.0)	S (22.0)	S (20.0)	S (17.0)
CPR (5)	21	22-25	26	S (30.0)	R (10.0)	S (28.0)	R (8.1)	S (30.0)
ST (10µg)	11	12-14	15	S (20.0)	R (8.0)	R (6.0)	S (18.0)	S (16.0)
LBC (5)	16	17-20	21	S (36.0)	S (24.5)	S (30)	S (24.0)	S (28.0)
CFT (30µg)	17	18-20	21	R (10.4)	R (8.9)	R (8.5)	I (18.0)	R (9.80)
IMI (10µg)	19	20-22	23	S (26.0)	R (6.0)	I (20.0)	R (6.0)	I (20.0)
CN (10µg)	12	13-14	15	R (5.0)	R (-)	S (18.0)	R (-)	S (20.0)

Key: R = Resistance, S = Sensitive, I = intermediate, AUG = Amoxicillin Clavulanate, CFX = Cefuroxime, NA = Nalidixic acid, OFX = Ofloxacin, CPR = Ciprofloxacin,ST = Streptomycin, LBC = Levofloxacin, CFT = Ceftaxidime, IMI = Imipenem and CN = Gentamycin; R (-) = No recorded zone of inhibition

3.4 Frequency of occurrence of bacteriological parameters during both seasons

Comparison between the frequency of occurrence of the bacteriological parameters in wet and dry seasons are shown in Fig. 2. It clearly shows that the parameters were higher in the wet season than the dry season (all the bars of wet season for each parameter are higher than that of the dry season).

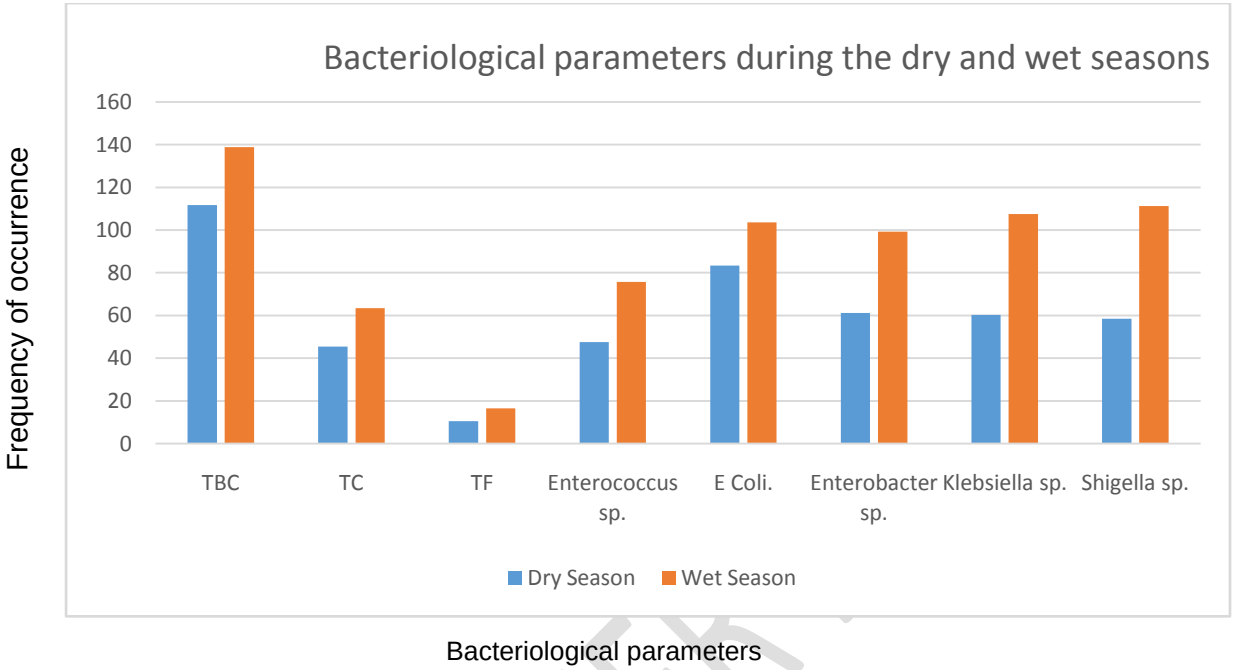

Fig. 2: Comparison of frequency of occurrence of bacteriological parameters in both seasons

4. DISCUSSION

Natural and human activities induce changes in water quality that are mostly observed in its physical, chemical and microbiological parameters [32]. The transmission of waterborne diseases, caused by bacteriological entities in drinking water is still a global public health challenge, despite several attempts made by the United Nations (UN), World Health Organization (WHO), International Water and Health Alliance (IWHA) and other agencies to ensure access to potable drinking water for the world's population.The THB counts obtained in this study (Tables 1 and 2) revealed presence of various heterotrophic bacteria in all the water sources. The heterotrophic bacterial limit in drinking water should not exceed 100 CFU/mL, as recommended by [8]. But in this study, the THB counts exceeded the WHO limits, signifying that the water sources had high bacterial contamination, and therefore, are unfit for direct consumption. These THB count results ranged from 1.3×10^2 CFU/mL in **A1** borehole to 8.7×10^4 CFU/mL in **A3** well. This result was similar with several research findings on bacteriological qualities of well and borehole water samples [16, 18,33,34], and most recent result from Oyi LGA in particular [25]. This rampant water contamination can be attributed to poor dumping and unsanitary conditions that persist in most rural parts of Nigeria. Bacterial contamination can arise due to seepage and runoffs from waste and faecal materials, which happens mostly during wet season.

Seasonal variations revealed there were more THB counts during wet season (1.8×10^3 – 8.7×10^4 CFU/mL) (Table 2) than dry season (1.3×10^2 – 5.5×10^4 CFU/mL) (Table 1), and this was attributed to the above mentioned reasons for higher bacterial contamination. A comparison of frequency of occurrence of the bacteriological parameters during both seasons showed elevated levels of all examined parameters during wet season (Fig. 2). Contaminated water sources are mostly implicated in causing diseases such as typhoid fever, cholera, dysentery, gastroenteritis, shigellosis, etc [14, 35, 4]. Borehole water recorded minimal bacterial contamination, compared to hand-dug well water, and this could be due to the fact that boreholes are drilled in deep depth (200ft to 500ft) , and they are mostly well covered and protected. The presence of *Escherichia coli* being the most common faecal indicator of water contamination as reported in the research findings, is a sign of the occurrence of other enteric agents [35-39].

Well water samples had the most bacterial contamination, possibly due to the fact hand-dug wells serve as main water sources in rural areas, and are more susceptible to pollution from dirty surroundings, as opined by [18], or lack of proper well sanitary seals, use of unclean drawer containers and ropes, or non-hygienic practices done by people while fetching water from wells, as explained by [40]. As was observed, the probable causes of groundwater contamination in Oyi LGA were unprotected hand-dug wells, poor handling of water by fetchers, poor sanitary conditions, indiscriminate dumping of solid wastes (refuse) as well as the practice of open defecation. It was also observed that hand-dug wells and boreholes were situated in compounds with limited land spaces, and therefore were at variance with WHO recommendations of situating deep wells or boreholes 20-30 m away from any source of contamination such as septic tanks, pit latrines and refuse dumpsites [41].

The most prominent bacteria isolated from the water sources in this study (*E. coli*) implied a faecal contamination of all the water sources, probably given the poor sanitary and hygienic conditions seen in the study area.The WHO recommends that there should be zero coliforms (*E. coli*) and faecal coliforms in 100 mL of drinking water. [2], also reported elevated levels of coliforms in well water sources, and attributed it to close proximity between water source and septic tanks. He further stated that the seepage can be prevented by increasing the distance of well water sources from the source of contamination.Similarly, [35], reported that coliforms in wellsprings of Baturiti district, Indonesia were above the WHO and Indonesian Regulatory standards. He further opined that the high coliforms were as a result of contamination by human and animal excreta, through water runoff and seepage leaks from soak-away pits. This also implies a potential for various pathogenic organisms to live in the gastrointestinal tracts and transmigrate in the water. Several other researchers such as [40,15,41,31], also reported that coliform bacteria were above the WHO and NSDWQ standards in drinking water samples.

Seasonal variation of the coliform showed there were higher levels of coliform during the wet season (6MPN/100 mL – 165MPN/100 mL) (Table 2) than that of dry season (2MPN/100 mL – 115MPN/100 mL) (Table 1). This result is similar to that of [43], in a studyof the impact of seasonal variation on the physicochemical and bacteriological properties of spring water in Oji LGA, Enugu State,

Nigeria.Seasonal variations also revealed that all the water sources had total coliforms during both seasons, whilethere were 12 out of 15 sites (80%) and 14 out of 15 sites (93.3%) during dry and wet seasons respectively, hadfaecal coliforms beyond WHO acceptable limits for drinking water. This result conformed to other research findings [44-46], which reported increased levels of coliforms during wet season than dry season.**A3** was shown to be the most contaminated site while **A1** was the least contaminated with coliform. This could be attributed to the water source, as **A1**is a borehole water source located in a village square, where every clan participates in its sanitation.

Klebsiella aerogenes, *Enterobacter*sp and *Enterococcus faecalis* isolated in these water sources are most likely to be inhabitants of soil and vegetation, and are therefore not of faecal origin. They are referred to as atypical coliforms. Probable bacterial isolates characterized and identified in this study include*Escherichiacoli*, *Shigellaflexneri*, *Klebsiellaaerogenes*, *Enterococcusfaecalis* and *Enterobacter* sp (Table 3). This finding is similar to the reports of [47], in their study of bacterial analysis of selected drinking water sources in Mbarara Municipality, Uganda.

Bacterial isolates were screened for their antimicrobial susceptibility patternson multi-drug disks,and results revealed that all the isolated bacteriawere susceptible to Ofloxacin (OFX) and Levofloxacin(LBC) (Table 4), while being resistant to most of the other antibiotics at varying degrees. This result shows that OFX and LBC are first choice of antibiotics to be administered to patients suffering from diseases caused by the isolated pathogens. In the study of [20], it was reported that all Gram-negative bacteria isolated in feed and water samples of poultry farms in Awka metropolis were susceptible to OFX. Similarly, [19] alsoreported that all isolates were susceptible to fluoroquinolones in their study of bacteriological quality and antibiogram of isolates from potable water sources in Ekosodin Community, Edo State, Nigeria. All the isolates exhibited 100% resistance to Augmentin (AUG) and Cefuroxime (CFX), and 80% resistance to Ceftazidime (CFT).*E. coli*, *Klebsiellaaerogenes* and *Shigellaflexneri*were also susceptible to Ciprofloxacin (CPR), while beingresistant to CFT. *Enterococcusfaecalis* exhibited highest resistance to seven out of the ten antibiotics, while *S.flexneri* showed the highest susceptibility to six out of the ten antibiotics they were exposed to. *K.flexneri* and *S.flexneri* showed intermediate values to Imipenem (IMI), while *E. coli* exhibited 50% susceptibility and 50% resistance to the ten antibiotics.

The findings of this study, suggests that the isolates may have acquired resistant genes to the tested antibiotics, probably due to exposure to sub-lethal doses in the environment or possession of intrinsic genes by the isolates. Several researchers have also elucidated on the occurrence of antibiotics in trace amounts in the environments including domestic wastewaters and sewage systems, e.g. septic tanks which are viable sources where these isolates can possibly acquire resistant genes [48, 47]. Antimicrobial resistance of isolates from the *Enterobacteriaceae* family where the isolates from this study belongs, is well documented [50, 51]. For instance, *Enterobacter cloacae* and *E. aerogenes*,which are well known opportunistic pathogens causing nosocomial infection, exhibit intrinsic resistance towards Augmentin and third-generation Cephalosporins like Ceftazidime and Cefotaxime [52-54, 17]**.**In this study, *E. aerogenes* were also resistant to Ceftazidime and Augmentin.

[53],reported that 75% - 100% of Enterococcus species including *E. faecalis* from groundwater sources were resistant to Gentamycin and Imipenem amongst other antibiotics. Similarly, as observed in this study, the sensitivity of all isolates to Ofloxacin and Levofloxacin was also reported by [19], whose results revealed that all isolates which included *E. coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Shigella* sp. and *Enterobacter*sp were completely sensitive to CPR, OFX and LBC. In another study by [18], *E. coli* from well water sources exhibited sensitivity to CPR and ST, and this is similar to the result of this study.

5. CONCLUSION

This study revealed that the bacteriological parameters in hand-dug well and borehole water samples in Oyi LGA were above the WHO and NSDWQ recommended guidelines for drinking water. The isolation of coliform bacteria is a confirmation of the presence of pathogens in the water samples. Hence, water samples from hand-dug wells and boreholes should be treated by filtration, flocculation or boiling before consumption. This would reduce prevalence of water-related diseases in Oyi LGA. Seasonal variations revealed there were higher levels of bacteriological parameters during the wet season than dry season. The high levels of coliform in hand-dug well water samples is an indicative of faecal contamination, poor sanitary conditions around the wells and poor hygiene of water handlers.Antibiotic susceptibility testing revealed that all the isolated bacteria were sensitive to Ofloxacin and Levofloxacin, and therefore, would be effective in treating water-related diseases caused by the isolated bacteria. *Enterobacter faecalis* exhibited highest resistance to the assayed antibiotics, while *S. flexneri* exhibited highest susceptibility.

The study recommends more enlightenment programmes by the Environment and Health Department of Oyi Local Government Area totheir villagers, especially landlords and clan heads on criteria for citing wells and boreholes, and the need for improved sanitation and hygiene. Additionally, water sources used for drinking and other domestic purposes in Oyi LGA should be undergoing quality checks periodically, to ascertain their statuses on physicochemical and microbiological qualities, and to be on track towards achieving SDG 6.

Overall, this study will serve as an invaluable reference for future studies on drinking water quality assessment on bacteriological parameters and antibiotic susceptibility patterns. It will also have application in efficacious drinking water quality monitoring through the provision of data that can be employed for the water quality index (WQI) of different drinking water sources in Oyi LGA. This may assist policy makers and stakeholders in the water sector to provide the LGA with guidelines and advisories on potable water consumption to prevent water-related disease outbreaks.

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APPENDIX

Some of the selected sites for collection of borehole water samples

Fig 3. Central borehole in a community primary school

fig 4. Children fetching water from a borehole

Fig.5 Village borehole in a market square