Antibiotic Susceptibility Pattern of Bacterial Species Isolated from Selected Underground Water Bodies in Ohaukwu Metropolis, Ebonyi State, Nigeria

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Abstract: Infections caused by using contaminated water are common in developing nations. Indiscriminate use of antibiotics has led to increased spread of antibiotic-resistance bacteria, even in underground water. This study investigated the susceptibility pattern of bacterial isolates from borehole water and hand-dug wells in Ohaukwu, Ebonyi State, Nigeria. Twenty (20) water samples were collected at random from hand-dug wells and underground boreholes located throughout Ohaukwu Local Government Area, and were examined for their physicochemical parameter, presence of bacteria as well as the antibiogram of the bacteria isolates using standard techniques. Our test results showed most of the physicochemical parameters were within permissible limits. The bacteriological analysis however exceeded the WHO limit of 0Cfu/100ml for drinking water with the presence of *Escherichia coli* 22 (42.5%), *Pseudomonas* spp. 3 (8.9%), *Klebsiella* spp.15 (36.0%), Salmonella spp. 15 (36.0%), and *Staphylococcus aureus* 6 (12.6%) make up the percentage distribution of the bacteria isolates. The antibiogram analysis revealed that most isolates were resistant to Mupirocin (97%), Bacitracin (91%), Gentamycin (84%) and Clindamycin (76.3%), but were sensitive to Ofloxacin (99.4%), Ceftazidime (86%) and Amikacin (72.3%). The study revealed that while *Salmonella* species was susceptible to Amikacin (72%), Ceftazidime (82.1%), and Ofloxacin (86.9%), it was resistant to the other three tested drugs. The resistance profile and low bacteriological quality of the drinking water sources evident in this study, is of great importance for the public health to the people resident in Ohaukwu Local government Area of Ebonyi State. It emphasizes the need for public education campaigns against the risks of building substandard wells and boreholes, and maintaining stringent standards for sanitation and personal hygiene.

Key word: Groundwater, antibiotics, resistance, Ebonyi

INTRODUCTION

A new era in the practice of contemporary medicine began with the discovery of antibiotics (Zainab *et al.*, 2020; Ejaz *et al.*, 2021). The development of antibiotic resistance by pathogenic organisms has presented a counterattack to this achievement, though. This therapeutic achievement is currently under jeopardy due to the notable rise in antibiotic resistance among common bacterial infections, which puts critically sick patients' chances of recovery at risk and creates a serious public health emergency (Pazda *et al.,* 2020). The global community recognizes the emergence of resistance among the most significant bacterial diseases as a serious danger to public health. Recent researches have shown that the environment has a significant role in the establishment of resistant infections and the spread of resistant bacteria, contributing to the rising healthcare issue caused by antibiotic resistance (Larsson and Flach, 2022).

It is estimated that seventy percent (70%) of the Earth’s surface is made up of water (Atobatele and Owoseni, 2023). This underground water may be found almost anywhere on Earth if one digs deep enough, but most accessible groundwater is generally found within 1 km of Earth’s surface (Atobatele and Owoseni, 2023). In many parts of the world, it is regarded as the most important source of public water supply (Zainab *et al.,* 2020). Underground water is made up of water bodies that are not directly exposed to the atmosphere, but are submerged within the earth's crust. Examples include wells, springs, and so on. It is generally believed that groundwater is the purest form of naturally occurring water since the passage of water through the soil sediments tends to retain contaminating agents, (Orogu *et al.*, 2017). The fact that ground water is not always free of microorganisms however, defeats this assumption as recent studies have shown that the quality of groundwater in most urban areas in Nigeria is deteriorating fast due to contamination with pathogenic microorganisms (Ekhosuehi *et al.,* 2018). It has been demonstrated that pathogenic organisms, including bacteria and fungi, are prevalent in the soil and eventually the ground water. However, because several contaminants have the ability to enter the aquifers, anthropogenic activities, particularly those related to agriculture and industry, pose a threat to groundwater quality (Zainab *et al.,* 2020). Some recently identified types of environmental pollutants, such as pharmaceuticals and personal care products (PPCPs), have drawn attention from the scientific community and the general public because of their possible bioactive qualities and unclear consequences on the aquatic environment, (Pompei *et al.*, 2019). Groundwater quality pollution has been linked to well construction and placement, well proximity to residential waste disposal sites, abattoirs, and sanitary systems such septic tanks and pit latrines (Ekhosuehi *et al.,* 2018; Obayiuwana and Ibekwe, 2020).

Access to clean water is a global concern as water is responsible for a variety of human illnesses, including urinary tract infections, wound infections, gastroenteritis, meningitis, septicemia, and pneumonia. A lot of enterobacterial infections are distributed by faecal-oral transmission and are commonly associated with poor hygienic conditions (Olufeyikemi and Abimbola, 2020; Uzairue *et al.*, 2023). Countries with poor water decontamination have more illness and death from enterobacterial infection. Infections gotten through the consumption and use of contaminated water are common in poor and developing nations (Atobatele and Owoseni, 2023). A significant issue for humanity is the availability of sufficient and high-quality water, particularly in developing and impoverished countries. It is now extremely difficult to meet all of the water requirements in terms of quantity, quality, and consistency due to the population growth in most towns and cities and the ensuing rise in demand for social amenities (Atobatele and Owoseni, 2023). In the past, only bacteria linked to illnesses like cholera and typhoid fever were a major issue when it came to water contamination. However, more recently, it has been reported that newly identified microbial pollutants, including microbial strains carrying virulence genes and antibiotic resistance determinants designated as severe public health risk, represent more serious problems to water safety (Ateba *et al.,* 2020), especially when the organisms develop resistance to antibiotics.

In Nigeria, particularly in the state of Ebonyi, the government is unable to offer purified water via pipes, people resort to groundwater for their water needs. The most readily available sources of potable water for residents of Ohaukwu Local Government Area in Ebonyi State, similar to many other African and Nigerian communities, are boreholes, wells, and streams. These water sources may be home to pathogens that cause diseases like cholera, diarrhea, typhoid fever, river blindness, and schistosomiasis, among others (Onifade *et al.,* 2019). Most people agree that one of the purest sources of water is groundwater. Pipe borne and groundwaters have been shown to contain pathogenic bacteria, including *Escherichia coli*, *Aerobacter aerogenes*, *Klebsiella* sp., *Pseudomonas* sp., *Proteus* sp., *Staphylococcus* sp., and *Acaligenes* spp. (Ogu *et al.*, 2017; Ekhosuehi *et al.,* 2018). Groundwater and pipe-borne *Acaligenes* sp.

have been isolated (Ogu *et al.*, 2017; Babatunde *et al.*, 2022). Particularly in individuals with impaired immune systems, these isolates, *Pseudomonas aeruginosa* and *Klebsiella* sp., have the potential to cause infections. Public health is at risk due to the spread of new pollutants like bacteria and genes that resist antibiotics. One unattractive side effect of this is the rise in the prevalence of bacterial illnesses in the general population. The fact that viruses that persist longer and are distributed throughout the environment pose a higher risk to public health than those that are contagious is the reason for their significance (Tangwa *et al.*, 2019). Since antibiotic-resistant genes and bacteria settle in aquatic environments, the spread of these resistant bacteria in the environment poses a serious risk to public health. Any direct or indirect contact with contaminated water for drinking or recreational purposes increases the risk of antibiotic-resistant pathogens harming and infecting the human population (Tangwa *et al.,* 2019).

The existence of antibiotic-resistant genes and bacteria in treated and untreated drinking water has drawn more attention in recent years (Sanganyado and Gwenzi, 2019). This study is necessary because there are no known comprehensive studies on the antibiogram pattern of bacterial species isolated from specific underground water bodies in residential environments in Ohaukwu Local Government Area of Ebonyi State, Nigeria. While many studies of antimicrobial resistance around the state have focused more on environments and samples considered to be antibiotic resistance hotspots, such as sewage, Abattoir effluents, municipal wastewater, medical environments, and effluents, this one will address these issues. This study was designed to ascertain the bacteriological quality and antibiotic susceptibility pattern of bacterial species isolated from selected underground water bodies in Ohaukwu metropolis, Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study area: This study was carried out in Ohaukwu Local Government Area of Ebonyi State, Nigeria. Ohaukwu Area Council is one of the 13 Area Councils in Ebonyi State, South-East, Nigeria and it has three main communities namely Izhia, Ngbo and Effium with an estimated human population of over 196,000 (NPC, 2006). The area lies within latitudes 6^0 3' N to 6^0 50' N and longitudes 7^0 80' E to 8^0 00' E with climatic conditions such as rainy season (March-October) and dry season (October-February). Two distinct vegetative regions exist in the study area: The Savannah in the Northern part of the study area, and tropical rainforests in the southern parts. More than 70% of the inhabitants of Ohaukwu metropolis engage in economic activities such as petty trading, subsistent agriculture, hunting and fishing.

Underground water sample collection: A total of twenty (20) underground water samples were randomly collected from different locations at Ohaukwu metropolis (Ngbo, Izhia) aseptically. Ten (10) water samples from boreholes and ten (10) samples from hand dug wells used by households and public outlets/markets as a source of drinking, cooking and bathing purposes. Samples from the wells were aseptically collected by lowering a clean plastic container tied to a synthetic rope down the well. Samples from borehole water were also aseptically collected by first pushing the handle of the borehole so that water will flow for about 2 minutes, before putting the sterile beaker to collect the water samples. All the water samples (100 ml each) collected in beaker were labeled and transported to Microbiology laboratory unit of Ebonyi State University Abakaliki, Nigeria for bacteriological analysis. Samples were analyzed within 4 h of collection.

Physicochemical analysis of the underground water samples: A portion of the water samples collected for the physicochemical analysis such as total dissolved solids, turbidity, total alkalinity and temperature were determined according to American Public Health Association (APHA) methods.

Culture, isolation and identification of bacteria: The water samples were shaken thoroughly and one milliliter from each sample location was added to 9 ml sterile peptone water, and serially diluted up to 10^{-4}. Thereafter, 0.1 ml was aseptically collected from all the dilutions and inoculated on sterile nutrient agar (Oxoid, UK). This was done by using a sterilized, flamed wire loop to get a colony of the organism, then streak on the new petri dish containing nutrient agar (Oxoid, UK), medium, it was incubated at 37^0C for 24 hours. Pure culture was prepared by sub-culturing the organisms in new petri dishes containing new sterile MacConkey agar, Eosin methylene blue agar (Flumedia, UK) and Mannitol salt agar. At the end of incubation, the number of distinct colonies were counted and used to calculate the bacterial load of each organism. The total viable counts of the colonies were counted and recorded with all the colony counts expressed as Cfu/m of water sample. Plates that showed significant growth were separated for biochemical and Gram staining. Identification of isolated bacteria to species level was carried out using Gram stain, microscopic examination and biochemical tests such as catalase test, coagulase test, citrate test, indole test, VP test, motility test, methyl red, oxidase test, and nitrate reduction test as described by (Cheesebrough, 2010).

Antibiogram: Bacterial species isolated were tested for their sensitivity against a total of seven antibiotics by means of M2-A6 disc diffusion method recommended by the National Committee for Clinical Laboratory Standards, NCCLS (NCCLS, 2004) using Mueller-Hinton agar. Aliquots of the test isolates were exposed to gentamicin (10 μg), mupirocin (5 μg), amikacin (30 μg), bacitracin (10 μg), ofloxacin (5 μg), ceftazidime (30 μg), and clindamycin (10 μg) (Oxoid UK) and incubated at 37°C for 24 h. The diameter zone of inhibition (DZI) around each disc was measured in millimeter using a transparent plastic rule, and interpreted accordingly. Test isolates were classified as resistant, intermediate or susceptible based on the zone of inhibition following the standard interpretive chart (NCCLS, 2004).

Statistical analysis: The mean and standard error of the mean were calculated. Relationships between variables of physicochemical parameters were determined by Pearson correlation. Analysis was carried out with the aid of Statistical Package for Social Science (Version 21) and antimicrobial susceptibility profile of isolated bacteria against commonly used antibiotics were analyzed using one-way analysis of variance (ANOVA) with the level of significance set at $p<0.05$.

RESULTS

The findings in Table 1 below shows the bacterial loads of borehole water samples from different locations in Ohaukwu L.G.A of Ebonyi State, Nigeria. From the samples code, BWS2 had the highest counts of 7.2×10^4 cfu/ml followed by BWS10, BWS8, BWS1 and BWS4, while BWS5 and BWS3 had the least count of 8.5×10 cfu/ml and 8.8×10^2 CFU/ml respectively. The bacterial loads of well water samples from different locations in Ohaukwu L.G.A of Ebonyi State. From the samples code, WWS6 and WWS1 had the highest count of 4.0×10^5 cfu/ml and 2.3×10^5cfu/ml respectively followed by WWS3, WWS5, WWS10 and WWS2 while WWS8, WWS9 and WWS7 had the least count of 5.2×10 cfu/ml, 3.0×10^2 cfu/ml and 3.1×10^2cfu/ml respectively. Table 2 below shows the morphological, microscopic and biochemical characteristics of bacteria isolated from 10 borehole water samples examined. The probable isolates were *Escherichia coli, Staphylococcus aureus, Staphylococcus* sp, *Pseudomonas* sp, and *Klebsiella* sp. The findings of morphological, microscopic and biochemical characteristics of bacteria isolated from 10 borehole water samples examined revealed that the probable isolates were *Escherichia coli, Staphylococcus aureus, Staphylococcus* sp, *Pseudomonas* sp, *Salmonella* sp *and Klebsiella* sp as shown in Table 3.

Table 3 illustrates the distribution of bacterial isolates from borehole water samples from different locations in Ohaukwu metropolis of Ebonyi State. The findings shows that the borehole water habours various degrees of microbes such as *Escherichia coli, Pseudomonas* spp, *Staphylococcus aureus* and *Klebsiella* spp. Samples tagged BWS8 and BWS1 has greater percentage bacteria distribution, while BWS9 had the least percentage distribution. It also revealed the distribution of bacterial isolates from hand dug well water samples from different locations in Ohaukwu metropolis of Ebonyi State. The findings showed the presence of *Escherichia coli, Salmonella spp, Pseudomonas* sp, *Staphylococcus aureus* and *Klebsiella spp.* In the hand dug well water at varying degrees. From the table below, samples WWS1 and WWS4 has a greater percentage of bacteria distribution while WWS5 has the least percentage distribution. Table 4 below depicts the findings of the physicochemical parameters of underground borehole and well water samples in Ohaukwu. The minimum, maximum, standard deviation and mean value of the temperature, pH, total dissolved solid (TDS), turbidity and alkalinity were measured and the mean temperature of the borehole water samples was 28.45^{o}C and the mean pH value of the borehole was at 7.42, while the turbidity value of the borehole was 5.85 NTU. The mean value of total dissolved solids for the borehole source was 250.14 mg/l and the mean value of total alkalinity of the borehole of 304.30 mg/l was recorded for borehole water samples. Similar to the borehole water samples, the minimum, maximum, standard deviation and mean value of the Temperature, pH, Total dissolved solid (TDS), Turbidity and Alkalinity were measured and the mean temperature of the well water samples was 28.90^{o}C and the mean pH value of the well was at 6.85 while the turbidity value of the borehole was 5.20 NTU. The mean value of total dissolved solids for the well water sample was 230.20 mg/l and the mean value of total alkalinity of the well sample of 256.90Mg/l was recorded for well water samples. The findings were compared with WHO standard value and the findings obtained shows that the physicochemical parameter of these underground waters meets the WHO standard value for safe drinking water as shown in Table 4 below.

Table 1: Bacterial loads of the borehole water sample (BWS) and well water samples (WWS)

	Samples Code	Bacterial load (cfu/mL)
Borehole water samples	BWS1	9.4×10^3
	BWS2	7.2×10^4
	BWS3	8.8×10^2
	BWS4	6.3×10^4
	BWS5	8.5×10^1
	BWS6	1.12×10^2
	BWS7	1.09×10^2
	BWS8	9.6×10^3
	BWS9	1.02×10^2
	BWS10	7.1×10^4
Well water samples	WWS1	2.3×10^5
	WWS2	2.7×10^3
	WWS3	2.8×10^4
	WWS4	4.2×10^4
	WWS5	3.4×10^3
	WWS6	4.0×10^5
	WWS7	3.1×10^2
	WWS8	5.2×10^1
	WWS9	3.0×10^2
	WWS10	3.8×10^3

Key: BWS= Borehole water sample; WWS= well water samples

Table 2: Morphological and biochemical characteristics of bacterial species isolated from the borehole water sample and well water samples

Morphological Characteristics				Microscopic Characteristics	Biochemical Test									Probable Organism
Source	Cell Shape	Cell arrange-ment	Colour	Gram reaction	Motility Test	Catalase Test	Citrate Test	Coagulase Test	Indole Test	Oxidase Test	Methyl red	Nitrate reduction test	VP Test	
Bore hole water samples	Rod	Single	Pink	-	+	+	-	-	+	-	+	+	-	*Escherichia coli*
	Cocci	Cluster	Golden-yellow	+	-	+	+	+	-	-	+	+	+	*Staphylococcus aureus*
	Cocci	Cluster	Yellow	+	-	+	+	-	-	-	+	+	+	*Staphylococcus* sp
	Rod	Pairs	Greenish	-	+	+	+	-	-	+	-	+	-	*Pseudomonas* sp
	Rod	Single	Shiny and dark pink	-	-	-	+	-	+	-	-	+	+	*Klebsiella* sp
Well water samples	Rod	Pair	Pink	-	+	+	-	-	+	-	+	+	-	*Escherichia coli*
	Cocci	Cluster	Golde-yellow	+	-	+	+	+	-	-	+	+	+	*Staphylococcus aureus*
	Cocci	Cluster	Golden-yellow	+	-	+	+	-	-	-	+	+	+	*Staphylococcus* sp
	Cocci	pairs	Greenish	-	+	+	+	-	-	+	-	+	-	*Pseudomonas* sp
	Rod	chains	Grayish white	-	+	+	-	-	-	-	+	+	-	*Salmonella* sp
	Rod	Single	Shiny pink	-	-	-	+	-	+	-	-	+	+	*Klebsiella* sp

Key: + = Positive Reaction; - = Negative Reaction

Table 3: Percentage distribution of bacterial species from the borehole water samples (Bws) and well water samples (Wws)

Source	Isolates	BWS1 (%)	BWS2 (%)	BWS3 (%)	BWS4 (%)	BWS5 (%)	BWS6 (%)	BWS7 (%)	BWS8 (%)	BWS9 (%)	BWS10 (%)
Borehole water samples	*E. coli*	2 (10)	1 (5)	0 (0.0)	3 (15)	2 (10)	3 (15)	0 (0.0)	5 (25)	0 (0.0)	7 (35)
	S. aureus	3 (15)	2 (10)	5 (25)	0 (0.0)	3(15)	0 (0.0)	4 (20)	2 (10)	2 (10)	0 (0.0)
	P. sp	7 (35)	3 (15)	1 (5)	2 (10)	4 (20)	0 (0.0)	2 (10)	6 (30)	3 (15)	5 (25)
	K. sp	3 (15)	2 (10)	6 (30)	4 (20)	1 (5)	6 (30)	0 (0.0)	4(20)	0 (0.0)	1 (5)
	Total	15(75)	8 (40)	12(60)	9 (45)	10(50)	9 (45)	6 (30)	17(85)	5 (25)	13 (65)
Well water samples	Isolates	WWS1 (%)	WWS2 (%)	WWS 3 (%)	WWS 4 (%)	WWS 5 (%)	WWS 6 (%)	WWS 7 (%)	WWS 8 (%)	WWS 9 (%)	WWS10 (%)
	E. coli	5 (25)	3 (15)	2(10)	3 (15)	0 (0.0)	1 (5)	0 (0.0)	3 (15)	0 (0.0)	4 (20)
	S. aureus	7 (35)	2 (10)	8 (40)	2 (10)	3(15)	2 (10)	4 (20)	0 (0.0)	2 (10)	5(25)
	S. sp.	3 (15)	4 (20)	0 (0.0)	3(15)	3(15)	4 (20)	0 (0.0)	1 (5)	2 (10)	6 (30)
	P. sp.	4 (20)	3 (15)	4 (20)	5 (25)	0 (0.0)	1 (5)	6 (30)	2 (10)	5(25)	2(10)
	K. sp.	1 (5)	3 (15)	4 (20)	6 (30)	1 (5)	5 (25)	0 (0.0)	5(25)	3 (15)	0 (0.0)
	Total	20(100)	15 (75)	18 (90)	19 (95)	7 (35)	13 (65)	10 (50)	11 (55)	12(60)	17 (85)

Table 4: Physicochemical parameters of the underground borehole water and well water samples analyzed

	Borehole water samples					Well water samples					
Sample Code	Temp	pH	TDS	Turbidity	Total alkalinity	Sample code	Temp	pH	TDS	Turbidity	Total alkalinity
BWS1	28.40	6.80	350.0	2.30	330	WWS1	32.30	6.60	320.0	3.20	295
BWS2	28.10	6.60	250.0	5.50	343	WWS2	29.20	6.40	210.0	4.50	324
BWS3	29.00	6.50	310.0	6.50	345	WWS3	29.00	6.30	280.0	5.50	320
BWS4	28.40	7.80	320.0	7.40	334	WWS4	30.40	7.10	310.0	6.40	240
BWS5	28.50	6.40	215.0	5.50	270	WWS5	32.50	5.40	210.0	5.30	250
BWS6	28.20	7.80	240.0	5.70	370	WWS6	29.20	6.80	220.0	5.60	360
BWS7	29.00	7.40	230.0	6.50	170	WWS7	29.70	7.40	260.0	6.30	210
BWS8	28.70	6.80	240.0	5.40	336	WWS8	31.60	6.70	240.0	5.40	305
BWS9	28.30	8.40	250.00	5.40	265	WWS9	29.30	7.20	230.00	5.10	235
BWS10	28.60	8.50	220.0	5.30	280	WWS10	30.00	8.00	220.0	5.40	270
Mean	28.45	7.42	250.14	5.85	304.30	Mean	28.90	6.85	230.20	5.20	256.90
STD	0.45	0.82	46.33	1.18	22.16	STD	0.72	0.64	41.24	1.12	16.10
MIN	28.10	6.40	215.0	2.30	170	Min	29.00	6.30	210.00	3.20	210
MAX	29.00	8.50	350.0	7.40	370	Max	32.50	8.00	320.0	6.40	360
WHO (2006) (≤)	26.00-32.00	6.50 – 9.20	500	2.20 – 7.50	500	WHO (2006) (≤)	26.00-32.00	6.40 - 8.90	500	2.20 -8.30	500

Key: Temp = Temperature; pH = Acidity/Alkalinity; TDS= Total Dissolved Solid; BWS= Borehole water samples; WWS = well water samples; STD = Standard deviation; MIN= minimum; Max=maximum

Table 5: Antibiogram pattern of different bacteria isolated from the underground water samples

Antibiotics	Conc. (μg)	*Escherichia coli*				*Pseudomonas spp*				*Staphylococcus aureus*				*Klebsiella spp*				*Salmonella spp*			
		Borehole water sample n = 8		Well water sample n = 12		Borehole sample n = 7		Well water sample n = 9		Borehole sample n = 6		Well water sample n = 8		Borehole sample n = 3		Well water sample n = 5		Borehole sample n = 5		Well water sample n = 14	
		R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)
DA	2	8(100)	0(0.0)	9(75)	3(25)	5(71.4)	2(28.6)	8(88.9)	1(11.1)	4(66.7)	2(33.3)	7(87.5)	1(12.5)	1(33.3)	2(66.7)	5(100)	0(0.0)	3(60)	2 (40)	8(57.1)	6(42.9)
B	10	6(75)	2(25)	8(66.7)	4(33.3)	7(100)	0(0.0)	9(100)	0(0.0)	5(83.3)	1(16.7)	8(100)	0(0.0)	3(100)	0(0.0)	4(80)	1(20)	4(80)	1(20)	10(71.4)	4(28.6)
OFX	5	4(50)	4(50)	1(8.3)	11(91.7)	3(42.9)	4(57.1)	0(0.0)	9(100)	2(33.3)	4(66.7)	3(37.5)	5(62.5)	1(33.3)	2(66.7)	2(40)	3(60)	1(20)	4(80)	3(21.4)	11(78.6)
CAZ	30	0(0.0)	8(100)	5(41.7)	7(58.3)	1(14.3)	6(85.7)	7(77.8)	2(22.2)	1(16.7)	5(83.3)	2(25)	6(75)	0(0.0)	3(100)	0(0.0)	5(100)	0(0.0)	5(100)	6(42.9)	8(57.1)
MUP	5	5(62.5)	3(37.5)	7(58.3)	5(41.7)	4(57.1)	3(42.9)	6(66.7)	3(33.3)	2(33.3)	4(66.7)	5(62.5)	3(37.5)	2(66.7)	1(33.3)	4(80)	1(20)	5(100)	0(0.0)	10(71.4)	4(28.6)
CN	10	7(87.5)	1(12.5)	9(75)	3(25)	6(85.7)	1(14.3)	5(55.6)	4(44.4)	4(66.7)	2(33.3)	8(100)	0(0.0)	3(100)	0(0.0)	4(80)	1(20)	3(60)	2(40)	7(50)	7(50)
AK	30	2(25)	6(75)	2(16.7)	10(83.3)	2(28.6)	5(71.4)	2(22.2)	7(77.8)	0(0.0)	6(100)	2(25)	6(75)	1(33.3)	2(66.7)	2(40)	3(60)	1(20)	4(80)	5(35.7)	9(64.3)

Where**:** DA= Clindamycin, B = Bacitracin, OFX = Ofloxacin, CAZ = Ceftazidime, MUP = Mupirocin, CN = Gentamicin, AK = Amikacin

Table 6: Percentage antibiotics susceptibility/resistance profile of the bacteria species isolated from the underground water samples

Antibiotics	Conc. (µg)	Borehole water samples		Well water samples	
		Total No of species Susceptible (%) n = 29	Total No of species Resistant (%) n = 29	Total No of species Susceptible (%) n = 48	Total No of species Resistant (%) n = 48
Amikacin	2	23 (79.31)	6 (20.69)	35 (72.92)	13 (27.08)
Bacitracin	10	4 (13.79)	25 (86.21)	9 (18.75)	39 (81.25)
Ceftazidime	5	27 (93.10)	2 (6.90)	28 (58.33)	20 (41.67)
Clindamycin	30	8 (25.59)	21 (72.41)	11 (22.92)	37 (77.08)
Gentamicin	5	6 (20.69)	23 (79.31)	15 (31.25)	33 (68.75)
Mupirocin	10	11 (37.93)	18 (62.07)	16 (33.33)	32 (66.67)
Ofloxacin	30	18 (62.07)	11 (37.93)	39 (81.25)	9 (18.75)

DISCUSSION

The underground water samples from hand-dug wells and borehole water in Ohaukwu Local Government Area were assessed using physicochemical and microbiological methods. The underground water was found to contain some bacteria of public health importance. The total aerobic plate count of bacteria from boreholes and well water samples with total aerobic plate count ranged from 4.3×10^2 cfu/ml to 7.5×10^5 cfu/ml in the borehole samples while well samples ranged from 9.4×10^2 cfu/ml to 1.12×10^5 cfu/ml). This finding is in line with the findings of Ekhosuehi *et al.* (2018) on the microbial quality of borehole and well water in Ijebu-Ode and Ago-Iwoye communities in South Western Nigeria, but disagrees with the result reported by Onuoha *et al*. (2017) from bacterial species in Surface Waters in Afikpo, Ebonyi South Eastern Nigeria, which exceeded the WHO limit of cfu/ml for drinking water (WHO, 2011).

The findings support earlier research on the microbiological quality of potable water sources in the Ekosodin Community, Benin City, Nigeria, by demonstrating that the underground water samples were polluted with many mesophilic bacteria of public health significance (Ekhosuehi *et al.,* 2018). Similar to the reports of Orogu *et al.* (2017), Atobatele, and Owoseni (2023); Babatunde *et al.* (2022); Ogu *et al.* (2017), and Gao and Sui (2021), our findings showed that most bacteria present in these underground water samples examined where from the Enterobacteriaceae group.

In this study, the findings revealed a percentage distribution of *Escherichia coli* 22 (42.5%), *Pseudomonas* spp. 3 (8.9%), *Salmonella* spp. 15 (36.0%), and *Staphylococcus aureus*. 6 (12.6%) Which suggests a potential faecal contamination of the underground waters in Ohaukwu Local Government Area of Ebonyi State. Similar investigations also demonstrated that surface water bacteria are involved, as previously reported from water samples by Atobatele, and Owoseni (2023); as well as Ajoke, and Adetokunboh (2018). Since these are intestinal bacteria, their existence suggests that residents of Ohaukwu Local Government Area of Ebonyi State do not practice good hygiene which may be the cause of their underground water contamination. The high total aerobic bacterial counts in the water samples under investigation may result from the study area's poor hygiene standards, as some boreholes and wells were drilled within 15 meters of sanitary facilities like pit latrines, soak-away pits, and septic tanks, where the underground water sources were located. In other cases, the study location's basic observation revealed that the majority of the indigenous people lack proper sewage systems and toilet facilities, hence resort to the use of any nearby bush or open space to dispose of their waste and defecate.

The presence of *Pseudomonas*, *Salmonella*, and *Staphylococcus* species have been linked to a variety of human illnesses, their presence is especially noteworthy. The *E. coli* isolation may have resulted from faecal contamination of underground water and storage environments. There have been reports linking *Escherichia coli* to several water-borne illnesses, including diarrheal disorders. In this investigation, *Pseudomonas* species and *Staphylococcus aureus* were also identified. *Pseudomonas* species have been linked to nosocomial infections, such as urinary tract infections after catheterization, eye and ear infections that can be dangerous in hospitalized patients, diarrhea patients who drink untreated water, and localized and/or generalized infections after surgery or burns (Sanganyado and Gwenzi, 2019).

Since *Staphylococcus aureus* may cause a variety of diseases, including foodborne poisoning, its identification is also crucial for public health. Other investigations have documented the isolation of *Staphylococcus aureus* from water samples. *Staphylococcus aureus* produces poisonous chemicals called enterotoxins, which is commonly associated with gastroenteritis. The presence of this bacterium in underground water is indicative of inadequate sanitation practices by the residents.

For drinking water, a pH between 6.5 and 7.5 is suitable. The pH levels of the well and borehole water were found to be, 7.42 and 6.85 respectively, the lowest and highest values. According to our findings, the average pH values of the well and borehole are 6.85 ± 0.64 and 7.42 ± 0.82, respectively, and there is a significant difference between them at $p<0.05$. The world Health Organization's (WHO) range (6.5 to 7.5) is not met by the well water quality. The pH levels of the well and borehole water samples under investigation varied from 6.10 to 6.90. During the study period, no notable differences were observed. Every drinking water source examined fell between 5.0 and 9.5 on the stated criterion, with an average value of 6.62±0.25. The weakly acidic nature of drinking water may be traceable to some dissolved matter in the water. This finding shows that the pH of these waters has an acidic tendency (pH below 7). The water sources (well) with pH below 6.5 may be attributed to the discharge of acidic products into this source by the agricultural and domestic activities. Studies have shown that 85% of all underground water worldwide is related to the geological nature of the aquifer formations and the lands traversed (Gothwal and Shashidhar, 2014; Karimi *et al.,* 2023).

Water temperature is a physical and ecological characteristic that significantly impacts both living and non-living elements of the environment, hence influencing organisms and the efficiency of an ecosystem. The well's average temperature of 28.90 ± 0.72 degrees Celsius is much higher than the borehole water's (28.45 ± 2.55). However, our finding shows that there is no significant difference (28.90 ± 0.72), even at $p<0.05$, between the well and borehole water. The highest and minimum values (28.10 and 32.50) were found in the well and borehole water, respectively. They all, however, exceed the WHO's recommended drinking water temperature of 25°C.

This study revealed that the turbidity values of the water Samples measured were somewhat greater than the WHO advised standard. The measurement of total dissolved solids (TDS), indicates whether or not the sources of drinking water included all suspended materials. The TDS was between 150.00 and 260.00. From the foregoing, it is evidently clear that there were no appreciable variations when compared to World Health Organization (WHO) standard value for drinking water. average TDS levels were 211.47±26.02 mg/l, within the 1000 mg/L drinking water standard. With the exception of the well sample designated W8, which has a high degree of turbidity and TDS, all drinking water sources from all boreholes and wells studied had TDS levels within the advised ranges and might not have any detrimental effects. For appropriate monitoring and treatment, however, regions with significant fluctuation in physicochemical parameters and microbial burden can be identified in the research.

An antibiotic susceptibility test of the isolated species of bacteria against commonly used antibiotics showed different levels of bacterial susceptibility pattern. The widely used antibiotics that were evaluated showed a general order of antibacterial ineffectiveness: Mupirocin (97%) > bacitracin (91%) > gentamycin (84%) > clindamycin (76.3%). The majority of the isolates tested positive for more than three different antibiotics, according to the findings of the antibiotic susceptibility testing. This result is consistent with the findings of recent studies conducted in Ife East local government area (LGA), Ile-Ife, Osun State, Nigeria, by Babatunde *et al.* (2022) and Atobatele, and Owoseni (2023) on the distribution of various antibiotic-resistant Gram-negative bacteria in potable water from hand-dug wells in Iwo, Nigeria.

Majority of the bacterial species isolated were sensitive to Amikacin (72.3%), Ceftazidime (86%), and Ofloxacin (99.4%). It also showed variations in the susceptibility and patterns of resistance to popular antibiotics amongst the bacterial isolates. Ninety-five percent of the *Escherichia coli* isolation from the Ogiri Playground borehole was sensitive to amikacin and ceftazidime. The findings of this study are in line with the reports of Babatunde *et al.* (2022) and Odonkor *et al.* (2022) who had previously reported a 91.75% susceptibility profile *Escherichia coli* isolates from various water sources. Furthermore, it was discovered that the vast majority of the isolated bacteria were resistant to Bacitracin, Clindamycin, Gentamycin, and Mupirocin, and very susceptible to Ofloxacin, Ceftazidime, and Amikacin. It implies that these medications may not be useful in treating illnesses brought on by these bacteria.

The finding that *Bacillus* and *Staphylococcus* isolated from well waters in Iworoko-Ekiti, Nigeria, were completely resistant to ampicillin and Bacitracin is consistent with the result of Ogunleye *et al.* (2017). This result, however, conflicts with the reports of Soge (2009); as well as Samie *et al.* (2011) who reported that Ampicillin resistance rates in South Africa and Uganda were 92% and 50%, respectively. The *Pseudomonas* species isolated from the Okwo borehole in this study, exhibited varying degrees of sensitivity to antibiotics, Ceftazidime, and Amikacin, ranging from 95% to 86%. The majority of the antibiotics examined in this study were shown to be resistant in *Escherichia coli* isolates, although were found to be 99% sensitive to Ofloxacin, 87.4% susceptible to Ceftazidime, and 78.6% susceptible to Amikacin.

Salmonella species found in the water sources examined, with significant prevalence rates of 25% and 45% found in the Ekwashi playground well and the Ogiri playground borehole in Ohaukwu city, respectively. The *Salmonella* species isolated in this study, were found to be 86.9% Sensitive to Ofloxacin, 82.1% susceptible to Ceftazidime, and 72% susceptible to Amikacin. Despite this, three of the tested antibiotics were ineffective against the *Salmonella* isolates. Since *Salmonella* species are not very stable in aquatic settings, their presence in drinking water suggests recent contamination from human.

Therefore, the majority of the bacteria isolates from water sources are becoming resistant to routinely used antibiotics, as previously reported by Morshed *et al.* (2018); Sanganyado and Gwenzi (2019); Ateba *et al.*, 2020; Gao and Sui (2021); and Babatunde *et al.*, 2022) which this study validates. Any previous research on the bacterial diversity of some underground water in Ohaukwu, the capital of Ebonyi State, is improved by the findings of this study. The findings from this study, outside the various physicochemical parameters that showed some variations from the world health organization (WHO) standards, suggests that the underground waters harbour microorganisms of public health importance. More disturbing, it revealed that the isolates exhibited drug resistance to commonly used antibiotics tested. Generally, the findings from this study suggests a contamination of the underground water sources and the occurrence of antibiotics resistant bacteria which greatly impacts the public health of the residents.

CONCLUSION

The results of these study have shown that the underground water samples analyzed had physicochemical properties that were under the acceptable drinking water standard limit. The bacteriological analysis showed that several bacteria of significance to public health were present in underground water samples in Ohaukwu Local Government Area, Ebonyi State. These bacterial species, which included *Salmonella* spp., *Pseudomonas* spp., *Escherichia coli*, *Klebsiella* spp., and *Staphylococcus aureus*, may contribute to the spread of potentially harmful organisms to consumers, particularly in light of the antibiotic resistance that some of these organisms have shown. According to our study, the isolates were resistant to Bacitracin, Clindamycin, Gentamycin and Mupirocin, but were mostly sensitive to Ofloxacin, Ceftazidime, and Amikacin.

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