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Antibiotic Susceptibility of Bacterial Species Isolated From Underground Waters in Abakaliki Metropolis of Ebonyi State, Nigeria

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ABSTRACT: *Groundwater is generally considered as one of the immaculate sources of water in nature and inability of the Government to provide pipe borne water in Abakaliki has led to the exploitation of groundwater for water supply by the populace. This study is designed to ascertain the bacteriological and physicochemical quality of borehole and well water within the Abakaliki metropolis. Twenty water samples were collected from ten boreholes and ten wells for bacteriological quality and physicochemical parameters (pH, total dissolved solid, temperature, turbidity and total hardness) following standard procedures. One hundred and forty nine bacterial isolates (38.9% Staphylococcus aureus, 24.2 % E. coli, 9.4 % P. aeruginosa, 17.4 % Klebsiella species and 10.1 % Salmonella species) were obtained from borehole water sources and one hundred and nineteen bacterial isolates (31.1 % S. aureus, 24.4 % E. coli, 3.4 % S. faecalis, 12.6 % P. aeruginosa, 10.9 % Klebsiella species, 4.2 % P. mirabilis and 13.4 % Salmonella species) were obtained from well water sources. The values of th bacteria counts for borehole water ranged from 1.5 x 102 to 9.1 x 10 cfu/100ml and well water ranged from 1.4 x 102 to 7.0 x 10 cfu/100ml, exceeded the Environmental Protection Agency (EPA) limit of 0 cfu/100ml for drinking water. The total dissolved solid, pH, ambient temperature and total hardness of all water samples (borehole and well) are all within the EPA limit. The high turbidity observed with the water samples PB2, PB7, PB8, PW4, PW6 and PW7 not within the EPA limit for turbidity. Antibiotic susceptibility studies showed that all isolated bacteria pathogens were highly resistant to most of the tested antibiotic tested but were susceptible to Amikacin, Ofloxacin and Gentamycin and are thereby recommended as drugs of choice in the treatment of bacterial isolates from water sources. The organisms obtained in this study are of public health importance, hence water samples require proper treatment before domestic use to eliminate bacterial involved in waterborne disease outbreaks.*

***Keywords**: Groundwater, physicochemical, Amikacin, Ofloxacin and Gentamycin*

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I. INTRODUCTION

Water is the most universally used single necessity of life [1]. Access to potable water has been an age old challenge because of the rising human populations and activities, which are in no small measure impacting negatively on the chemical and microbiological qualities of available water supplies [2]. Groundwater is generally regarded as one of the pristine sources of water [3]. The contamination of groundwater quality has been related to location and construction of wells, proximity of wells to domestic waste dumpsites, abattoir, sanitary systems such as pit-latrines and septic tanks. Moreover, groundwater quality has been shown to vary seasonally [4]. However, As the world turns its attention to the formulation of the post-2015 Sustainable Development Goals (SDGs) much remains to be done particularly in areas of improving more access to potable water, sanitation, improved sewage management systems and public enlightenment especially in developing countries that still have high percentage of water crisis [5].

Borehole water (groundwater) is the predominant source of water by the inhabitants of Abakaliki metropolis and it is generally considered a safe source of drinking water by the populace [6]. Well water is also an important source of drinking and household water in Abakaliki and communities within. The underground water supplies are usually consumed safe provided they are properly located, constructed and operated according to the World Health Organization Guidelines for Drinking Water [7].

Pathogenic bacteria such as Escherichia coli, Aerobacter aerogenes, Klebsiella species, Pseudomonas species, Proteus species, Staphylococcus species and Acaligenes species have been isolated from groundwater [8]; [9]. Some of these isolates, such as Pseudomonas aeruginosa and Klebsiella species can cause nosocomial infections in immunocomprised patients [10].

Uncontrolled uses of antibiotics have led to rapid evolution of antibiotic- resistance bacteria and antibiotic resistance gene transfer, especially in a pool of aquatic system where resistance, intermediate and susceptible bacteria to some antibiotics strive together [11]. The unappealing manifestation of this situation is the increasing persistence of bacterial infections amongst the public [12]. The chemical, physical and microbial constituents of water sources are critical public health issue that needs to be determined from time to time. Hence this present study is designed to ascertain the bacteriological quality of the groundwater sources in Abakaliki metropolis, determine the antibiogram of the isolates and physicochemical parameters from the assessed groundwater samples.

II. MATERIALS AND METHODS

Study Area

Ebonyi state is known for its large mineral deposits including limestone and lead which are heavily mined for commercial purposes. There have been frequent outbreaks of waterborne diseases including cholera in Abakaliki metropolis and some parts of Ebonyi State. The co-ordinates of Ebonyi state are Latitude 06^0 4´N and longitude 08^0 5´E [13] [14].

Sample Collection

A total of 20 water samples were obtained from 10 boreholes and 10 wells. 250ml capacity sterile bottles were pre-sterilized in an autoclave at 121°C for 15 minutes before they were used for collecting water samples. After allowing several litres of water to run for 5 minutes as waste, water samples were obtained from bore by allowing water to flow aseptically from the dispensing tap into the glass bottles. A stone of a suitable size was attached to the sampling bottle using a piece of string. The bottle was opened and lowered into the well; the bottle was completely immersed in the water, without touching the sides of the well and without hitting the bottom or disturbing any sediment. The bottle was filled and then removed by rewinding the string. Approximately 20–30 ml of water was discarded to provide sufficient airspace to allow shaking before the analysis to achieve a homogenous dispersion of the bacteria. The bottles were then corked and transported to the laboratories for microbial and physicochemical analysis. The water samples were obtained from wells and boreholes with minimum distance from possible sources of contamination such as septic tanks, silos, leach fields, livestock yards, and refuse dump.

Microbiological Analysis of the Soil Samples

Fungi and bacteria were isolated from the soil samples by the standard spread plate technique [15]. One gram of each of the composite soil samples was added to 9 ml of sterile normal saline in a flask and shaken very well. The suspension obtained was diluted to 10^{-7} by serial dilution. Using pour plate method as described by [16], one milliliter aliquots of the dilutions were spread on the surface of plates of Sabouraud dextrose agar (SDA) which was added 25mg of chloramphenicol tablets incubated at 25^0C for 72 hours for fungi growth and on nutrient agar (NA) at 27^0C for 48 hours for bacteria growth by pour plate method as described by [17]. Discrete colonies of aerobic bacteria and fungi were subcultured for purification by streaking on fresh solid media (Nutrient agar) for bacteria while fungi colonies were subcultured in sabouraud dextrose agar which chloramphenicol was added. The culture plates were incubated at 27^0C for 48 hrs for bacteria and at 25^0C for 72hrs for fungi.

Physicochemical Analysis of Soil Samples

A number of physicochemical parameters of the contaminated soil and non-contaminated samples were determined. They included pH, temperature, conductivity, nitrate, phosphate and sulphate. Others included oil and grease, total organic carbon, and exchangeable cations. Hach pH meter (Model EC10) was used for pH measurement; conductivity was measured using Hach conductivity meter (Model CO150). Sulphates, nitrates and phosphates were determined using Barium chloride (Turbidimetric method), Cadmium reduction and Ascorbic acid methods respectively. All analyses were in accordance with American Public Health Association (APHA, 2005).

Antibiotic Susceptibility Test

A 0.5McFarland standard solution of the test organism was prepared by inoculating the organism in normal saline solution. From this solution, the organism was streaked on a Mueller-Hinton agar plate using a cotton swab while ensuring that the entire surface of the agar was streaked. Using a pair of sterile forceps, the antibiotic disc which included; Mupirocin (5µg), Ofloxacin (5µg), Gentamicin (10µg), Amikacin (30µg) Ceftazidime (30µg), Bacitracin (10µg) and Clindamycin 2µg (Oxoid, UK) was placed on the inoculated agar and the plate was incubated at 37°C for 24hours. The zones of inhibition of each antibiotic were measured in millimeters (NCCLS, 2017).

Determination of Multiple Antibiotic Resistance (MAR) index

Multiple antibiotic resistance (MAR) index was determined using the formula MAR=x/y, where x is the number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test organism has been evaluated for sensitivity [18].

III. RESULTS

A total of seven (7) bacteria were identified to contaminate underground water sources sampled in Abakaliki metropolis. The bacteria include Escherichia coli, Salmonella species, Klebsiella species, Pseudomonas species, Proteus mirabilis, Streptococcus species, and Staphylococcus aureus as shown in Table 1.

Table 1: Colonial morphology and biochemical characterization of bacteria isolated from borehole and well water in Abakaliki metropolis

Gram staining	Shape	Colour	TSI	Oxidase	Motility	Urease	VP	Methyl red	Indole	Coagualase	Catalase	Citrate	DNase	Glucose	Lactose	Mannitol	Maltose	Sorbitol	Xylose	Probable Organism
-	Rod	Light pink	K/A	-	+	+	-	+	-	-	+	+	+	+	-	-	-	-	+	*Proteus mirabilis*
+	Cocci	Black	-	-	-	-	+	-	-	+	-	-	-	+	+	+	+	+	-	*Streptococcus feacalis*
-	Rod	Black	A	-	+	-	-	+	-	-	+	-	-	+	-	+	+	+	+	*Salmonella* species
-	Rod	Green	A	+	+		-	-	-	-	+	+	-	+	-	+	-	-	+	*Pseudomonas aeruginosa*
-	Rod	Pink	A	-	-	+	+	-	-	-	+	+	-	+	+	+	+	+	+	*Klebsiella* species
-	Rod	Pink	A/G	-	+	-	-	+	+	-	+	-	-	+	+	+	-	+	-	*E. coli*
+	Cocci	Golden -yellow	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	-	+	*Staphylococcus aureus*

Keys: (+) - positive, (-) - negative, A-Acid, K- Alkaline, K/A- Alkaline/Acid, A/G-Acid/Gas, VP-Voges-Proskauer, TSI-Triple Sugar Iron

The public bore with sample code PB8 has the highest colony count of 9.1 x 10 cfu/100ml, followed by PB9 (8.8 x 10 cfu/100ml), PB1 (7.6 x 10 cfu/100ml), PB3 (5.6 x 10 cfu/100ml), PB4 (4.8 x 10 cfu/100ml), PB2 (4.7 x 10 cfu/100ml), while PB6 (1.5 x 10^{2} cfu/100ml) showed the lowest as indicated in Table 2.

Table 2: Bacteria count (CFU/ 100 ml) of Borehole water sample

Sample Sources Code	Colony Count (CFU/100 ml)
PB1	7.6 x 10
PB2	4.7 x 10
PB3	5.6 x 10
PB4	4.8 x 10
PB5	4.4 x 10
PB6	1.5 x 10^{2}
PB7	1.7 x 10^{2}
PB8	9.1 x 10
PB9	8.8 x 10
PB10	1.8 x 10

Keys: PB1 = Public Borehole Azugwu Street, PB2 = Public Bore Nsugbe Street, PB3 = Public Borehole Ogoja Street, PB4 = Public Borehole Onuebonyi road, PB5 = Public Borele Ogbehausa market, PB6 = Public Borehole Omege street, PB7 = Public Borehole Nnodo Boys Playground, PB8 = Public Borehole Jim Metu Street, PB9 = Public Borehole Presco Campus, PB10 = Public Borehole Ofoke Street

Among the well water sources sampled in this study, sample source code PUW2 had the highest colony count of 7.0 x 10 cfu/100ml, followed by PW3 (5.7 x 10 cfu/100ml), PUW1 (5.6 x 10 cfu/100ml), PW5 (5.3 x 10 cfu/100ml), PW6 (4.7 x 10 cfu/100ml), while PUW3 (1.4 x 10^2 cfu/100ml) had the lowest colony count as indicated in Table 3.

Table 3: Bacteria count (CFU/ 100 ml) of Well water sample

Sample Sources Code	Colony count (CFU/100 ml)
PW1	3.0 x 10
PW2	1.3 x 10^2
PW3	5.7 x 10
PW4	1.6 x 10^2
PW5	5.3 x 10
PW6	4.7 x 10
PW7	4.0 x 10
PUW1	5.6 x 10
PUW2	7.0 x 10
PUW3	1.4 x 10^2

Keys: PW1 = Private well Ogboloko street, PW2 = Private well Nnodo boys street, PW3 = Private well Nwele street, PW4 = Private well Aguchi street, PW5 = Private well Ogbonna street, PW6 = Private well Udude street, PW7 = Private well Echefula street, PUW1 = Public well Ofoke street, PUW1 = Public well Azuyiokwu street and PUW3 = Public well Amike-Aba playground

Out of the 149 bacterial isolates (58 Staphylococcus aureus, 36 E. coli, 14 P. aeruginosa, 26 Klebsiella species and 15 Salmonella species) obtained from borehole water sources, PB6 showed the highest bacterial isolates (13.4 %), followed by PB2 (11.4 %), PB10 (11.4 %), PB5 (10.7 %), PB1 (10.7 %), PB4 (10.1 %), PB3 (9.4 %), PB7 (9.4 %) and PB8 (8.1 %), while PB9 (5.4 %) showed the lowest bacterial distribution as shown in Table 4.

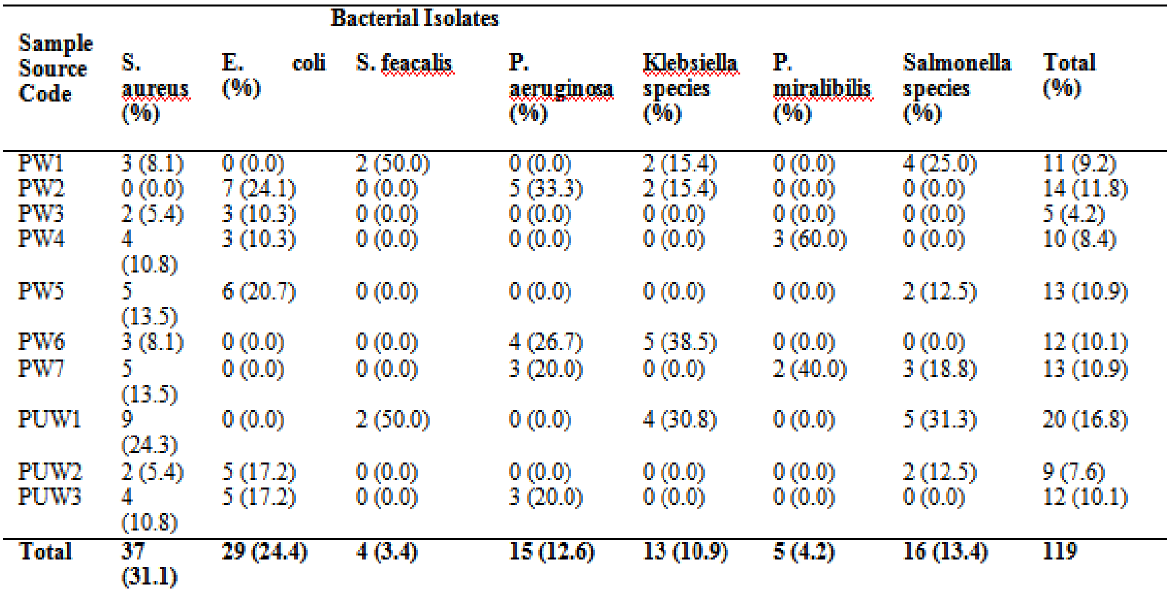
Table 4: Distribution of bacteria isolates from borehole water samples

Key: (%) = Percentage

Sample Source Code	Bacterial Isolates					
	Staphylococcus aureus (%)	**E. coli (%)**	**P. aeruginosa (%)**	**Klebsiella species (%)**	**Salmonella species (%)**	**Total (%)**
PB1	5 (8.6)	3 (8.3)	0 (0.0)	8 (30.8)	0 (0.0)	**16 (10.7)**
PB2	7 (12.1)	0 (0.0)	0 (0.0)	4 (15.4)	6 (40.0)	**17 (11.4)**
PB3	11 (19.0)	0 (0.0)	0 (0.0)	3 (11.5)	0 (0.0)	**14 (9.4)**
PB4	3 (5.2)	3 (8.3)	9 (64.3)	0 (0.0)	0 (0.0)	**15 (10.1)**
PB5	2 (3.4)	5 (13.9)	0 (0.0)	9 (34.6)	0 (0.0)	**16 (10.7)**
PB6	10 (17.2)	8 (22.2)	0 (0.0)	2 (7.7)	0 (0.0)	**20 (13.4)**
PB7	4 (6.9)	10 (27.8)	0 (0.0)	0 (0.0)	0 (0.0)	**14 (9.4)**
PB8	9 (15.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (20.0)	**12 (8.1)**
PB9	3 (5.2)	0 (0.0)	5 (35.7)	0 (0.0)	0 (0.0)	**8 (5.4)**
PB10	4 (6.9)	7 (19.4)	0 (0.0)	0 (0.0)	6 (40.0)	**17 (11.4)**
Total	**58 (38.9)**	**36 (24.2)**	**14 (9.4)**	**26 (17.4)**	**15 (10.1)**	**149**

A total of 119 bacteria were obtained from well water sources which comprises of 37 S. aureus, 29 E. coli, 4 S. feacalis, 15 P. aeruginosa, 13 Klebsiella species, 5 P. mirabilis and 16 Salmonella species (Table 5). PUW1 showed the highest bacterial isolates (16.8 %), followed by PW2 (11.8 %), PW5 (10.9 %), PW7 (10.9 %), PW6 (10.1 %), PUW3 (10.1 %), PW1 (9.2 %), PW4 (8.4 %) and PUW2 (7.6 %), while PW 3 showed the lowest bacterial isolates as shown in Table 5.

Table 5: Distribution of bacteria isolates from well water samples



Sample Source Code	**Bacterial Isolates**							
	S. aureus (%)	**E. coli (%)**	**S. feacalis**	**P. aeruginosa (%)**	**Klebsiella species (%)**	**P. miralibilis (%)**	**Salmonella species (%)**	**Total (%)**
PW1	3 (8.1)	0 (0.0)	2 (50.0)	0 (0.0)	2 (15.4)	0 (0.0)	4 (25.0)	11 (9.2)
PW2	0 (0.0)	7 (24.1)	0 (0.0)	5 (33.3)	2 (15.4)	0 (0.0)	0 (0.0)	14 (11.8)
PW3	2 (5.4)	3 (10.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (4.2)
PW4	4 (10.8)	3 (10.3)	0 (0.0)	0 (0.0)	0 (0.0)	3 (60.0)	0 (0.0)	10 (8.4)
PW5	5 (13.5)	6 (20.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (12.5)	13 (10.9)
PW6	3 (8.1)	0 (0.0)	0 (0.0)	4 (26.7)	5 (38.5)	0 (0.0)	0 (0.0)	12 (10.1)
PW7	5 (13.5)	0 (0.0)	0 (0.0)	3 (20.0)	0 (0.0)	2 (40.0)	3 (18.8)	13 (10.9)
PUW1	9 (24.3)	0 (0.0)	2 (50.0)	0 (0.0)	4 (30.8)	0 (0.0)	5 (31.3)	20 (16.8)
PUW2	2 (5.4)	5 (17.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (12.5)	9 (7.6)
PUW3	4 (10.8)	5 (17.2)	0 (0.0)	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	12 (10.1)
Total	**37 (31.1)**	**29 (24.4)**	**4 (3.4)**	**15 (12.6)**	**13 (10.9)**	**5 (4.2)**	**16 (13.4)**	**119**

The Physicochemical analysis result of the borehole water sources is shown in Table 6. The water temperature of the borehole water sampled ranged from 27 – 32 ^{0}C, pH ranged from 6.6 to 8.3, total dissolved solid ranged from 141 – 213 ppm, the turbidity ranged from 0.1 – 0.8 NTU and the total hardness ranged from 295 – 379 mg/L.

Table 6: Physicochemical parameters of borehole water samples

Physicochemical parameters	**PB1**	**PB2**	**PB3**	**PB4**	**PB5**	**PB6**	**PB7**	**PB8**	**PB9**	**PB10**
Water temperature (°C)	30	27	28	32	29	32	29	27	31	28
pH	6.6	6.7	6.5	7.3	7.1	6.8	7.5	8.3	7.5	7.7
Total dissolved solid (ppm or mg/L)	156	192	173	162	141	169	205	213	168	163
Turbidity (NTU)	0.2	0.6	0.4	0.3	0. 1	0.3	0.7	0.8	0.3	0.3
Total hardness (mg/L)	355	300	379	295	335	310	300	340	300	392

The water temperature of the well water sampled ranged from 27 – 31 ^{0}C, pH ranged from 6.5 to 8.9, total dissolved solid ranged from 142 – 218 ppm, the turbidity ranged from 0.1 – 0.8 NTU and the total hardness ranged from 198 – 378 mg/L (Table 7).

Table 7: Physicochemical parameters of well water samples

Physicochemical parameters	**PW1**	**PW2**	**PW3**	**PW4**	**PW5**	**PW6**	**PW7**	**PUW1**	**PUW2**	**PUW3**
Water temperature (°C)	28	27	30	28	31	29	29	27	29	30
pH	7.8	8.6	7.9	6.9	7. 1	8.6	8.2	7.3	8.9	6.5
Total dissolved solid (ppm or mg/L)	154	180	142	209	157	218	195	153	149	170
Turbidity (NTU)	0.3	0.5	0.1	0.7	0. 2	0.8	0.6	0.2	0.1	0.4
Total hardness (mg/L)	251	298	325	229	327	310	247	198	378	283

Table 8: Antibiotic sensitivity pattern of bacterial isolates from borehole water in Abakaliki
Key: S = Sensitive, R = Resistance

	S. aureus (n = 58)		**E. coli (n = 36)**		**P. aeruginosa (n =14)**		**Klebsiella species (n =26)**		**Salmonella species (n = 15)**	
Antibiotics	S	R	S	R	S	R	S	R	S	R
Amikacin (30µg)	100 %	0.0 %	94.4 %	5.6 %	64.3	35.7 %	80.8 %	19.2 %	80.0 %	20.0 %
Bacitracin (10µg)	12.1 %	87.9 %	27.8 %	72.2 %	14.3 %	85.7 %	15.4 %	84.6 %	40.0 %	60.0 %
Ceftazidime (30µg)	74.1 %	25.9 %	36.1 %	63.9 %	92.9 %	7.1 %	50.0 %	50.0 %	40.0 %	60.0 %
Clindamycin (15µg)	46.6 %	53.4 %	36.1 %	63.9 %	21.4 %	78.6 %	46.2 %	53.8 %	53.3 %	46.7 %
Gentamicin (30µg)	89.7 %	10.3 %	91.7 %	8.3 %	7.1 %	92.9 %	57.7 %	42.3 %	26.7 %	73.3 %
Muprocin (30µg)	17.2 %	82.8 %	33.3 %	66.7 %	28.6 %	71.4 %	23.1 %	76.9 %	33.3 %	66.7 %
Ofloxacin (15µg)	94.8 %	5.2 %	77.8 %	22.2 %	100 %	0.0 %	69.2 %	30.8 %	93.3 %	6.7 %

Table 9: Antibiotic sensitivity pattern of bacterial isolates from borehole water in Abakaliki
Key: S = Sensitive, R = Resistance

	S. aureus (n = 37)		**E. coli (n = 29)**		**S. facialis (n =4)**		**P. aeruginosa (n =15)**		**Klebsiella species (n =13)**		**P. mirabilis (n =5)**		**Salmonella species (n = 16)**	
Antibiotics	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Amikacin (30µg)	100%	0.0%	89.7%	10.3%	100%	0.0%	33.3%	66.7%	0.0%	100%	40.0%	60.0%	87.5%	12.5%
Bacitracin (10µg)	32.4%	67.6%	17.2%	82.8%	0.0%	100%	60.0%	40.0%	23.1%	76.9%	40.0%	60.0%	50.0%	50.0%
Ceftazidime (30µg)	48.6%	51.4%	44.8%	55.2%	50.0%	50.0%	53.3%	46.7%	15.4%	84.6%	0.0	100%	50.0%	50.0%
Clindamycin (15µg)	32.4%	67.6%	34.5%	65.5%	25.0%	75.0%	66.7%	33.3%	30.8%	69.2%	40.0%	60.0%	43.7%	56.3%
Gentamicin (30µg)	81.1%	18.9%	72.4%	27.6%	100%	0.0%	66.7%	33.3%	84.6%	15.4%	100%	0.0%	18.7%	81.3%
Muprocin (30µg)	18.9%	81.1%	17.2%	82.8%	0.0%	100%	26.7%	73.3%	15.4%	84.6%	75.0%	25.0%	0.0%	100%
Ofloxacin (15µg)	91.9%	8.1%	75.9%	24.1%	100%	0.0%	86.7%	13.3	61.5%	38.5%	100%	0.0%	6.2	93.8%

The multiple antibiotic resistance index (MARI) of bacteria isolates from borehole water samples range from 0.4-0.6, while the MARI of bacteria from well water range from 0.3 – 0.7 (Figure 1).

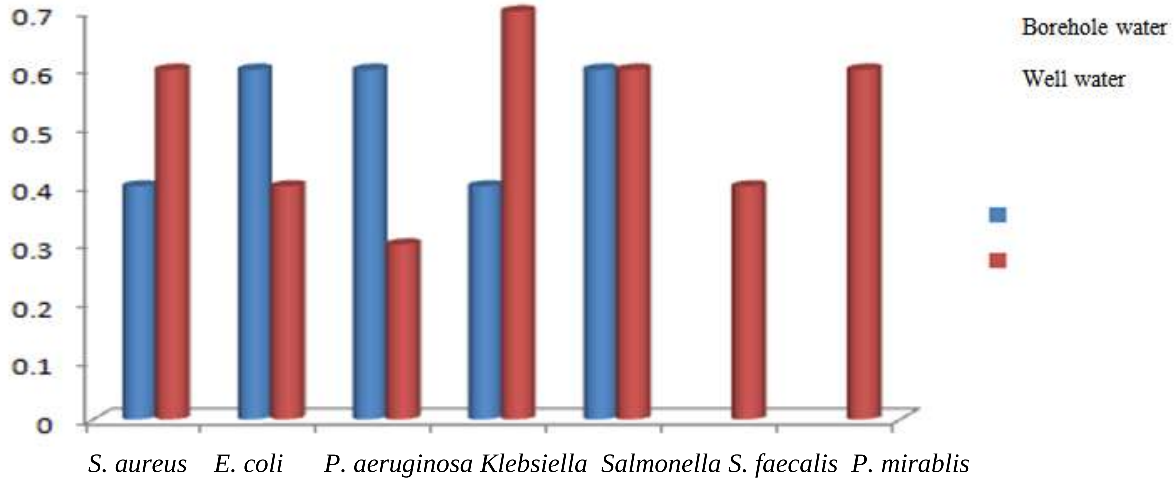


Figure 1: Multiple Antibiotic Resistance Index (MARI) of bacteria isolates from borehole and well water samples

IV. DISCUSSION

In Abakaliki, the inability of the Government to provide pipe borne water has led to the exploitation of groundwater for water supply by indigenes. Groundwater is generally regarded as one of the pristine sources of water. However, studies have shown that the quality of groundwater in most urban areas in Nigeria is deteriorating fast [20]; [21]; [22]. Groundwater may harbor potential pathogens and the presence of these pathogenic organisms can pose severe health risks to consumers in general and immune compromised individuals in particular [23]. In view of the prevailing problem of portable water in Abakaliki metropolis, the study was conducted to evaluate the resistance profile of bacteria to some commonly used antibiotics within the study area. Seven bacteria (Escherichia coli, Salmonella species, Klebsiella species, Pseudomonas species, Proteus mirabilis, Streptococcus species, and Staphylococcus aureus) were identified to contaminate underground water sources (borehole and well) sampled in Abakaliki metropolis as shown in Table 1. The bacterial pathogens isolated from the various water samples in this study are of public health importance because these microbes are implicated in a plethora of human infections. The result of this study is also in line with the report of [24] who revealed the presence [25]; of enteric and non-enteric bacteria in borehole and well water within Benin Metropolis. Similarly, bacteria of public health importance have been isolated from underground water sources [26]; [27]; [28]; [29]; [30]; [31] [32]. The presence of Klebsiella species, Micrococcus species and Enterobacteraerogenes in borehole water samples is unacceptable from the public health point of view [33].

The presence of microbes in water (especially above the acceptable limits) also signifies that water sources are not potable and thus could not be used for drinking purposes [34]. The result of the total bacterial count from the water sampled showed very high counts. The bacteriological count ranged from 4.4 x 10 - 1.7 x 10^2 cfu/100 ml and 3.0 x 10 - 1.4 x 10^2 CFU/100 ml from the borehole and well water sources as indicated in Table 2 and 3. These counts are higher than the acceptable counts of 0 cfu/ml for drinking (APHA, 2005). Results obtained in this study also corroborates results obtained from similar studies on the microbial quality of borehole and well water in Ijebu-Ode and Ago-Iwoye communities in South Western Nigeria, which also reported bacterial count greater than 0 CFU/100ml in the samples examined [35]; [36] while 4.03 X 10^3 has been reported from Groundwater in South Delhi [37]. Contamination could also have originated from the environment through dust particles transported into the tank. WHO report on protecting groundwater for health 2006, identifies leakage from sanitary systems such as septic tanks as the major source of feacal contamination of groundwater [38]. The contamination of groundwater quality has been related to location and construction of wells, proximity of wells to domestic waste dumpsites, abattoir, sanitary systems such as pit-latrines and septic tanks. Moreover, groundwater quality has been shown to vary seasonally [39]. Arguably, the distance between borehole and septic tank can influence the contamination of the groundwater by the septic tanks. Although, there have been reports to indicate a weak/negative correlation between the distance of a pit latrine and/or septic tanks and boreholes on the groundwater quality the distance between a borehole and septic tank remains an important factor in determining groundwater contamination [40]; [41] [42]. According to the WHO Fact sheet on septic tanks, the minimum acceptable distance between borehole and septic tanks should be 30 meters [43]. Some selected site of sample collection in this study did not meet the 30m limit set by the WHO indicating that the observed feacal contamination in borehole and well water samples most likely contaminated from seepage from septic tanks in the study locations. This study revealed the presence of Staphylococcus aureus in almost all the well and borehole water samples analyzed, with high prevalence rate of 38.9 % and 31.1 % borehole and well water sources respectively as shown in Table 4 and 5. High and low prevalence rate of 75 % and 23.1 % Staphylococcus aureus have been reported [44] [45]. Staphylococcus aureus is a common member of the human micro flora. It can produce diseases through two different mechanisms. One is based on the ability of the organisms to multiply and spread widely in tissues, and the other is based on the ability of the organisms to produce extracellular polysaccharides and toxins. Multiplication in tissues can result in manifestations such as boils, skin sepsis, post-operative wound infections, enteric infections, septicemia, endocarditis, osteomyelitis and pneumonia [46]. Gastrointestinal diseases (enterocolitis or food poisoning) is caused by a heat-stable Staphylococcal enterotoxin and characterized by projectile vomiting, diarrhoea, fever, abdominal cramps, electrolyte imbalance and loss of fluids [47].

Klebsiella species occurrence rate of 17.4 % in borehole water and 10.9 % in well water from in this present study (Table 4 and 5) is lower than 25 % occurrence rate of Klebsiella pneumonia isolated from pipe–borne chlorinated (Treated) water and untreated water in Ilorin [8]. Klebsiella organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, and soft tissue infections [13]. Borehole water recorded occurrence rate 24.2 % and well water recorded 24.4 for Escherichia coli in this study (Table 4 and 5). This result is almost similar with 33.3 % occurrence rate of Escherichia coli in Ngwogo and Okpu well water in Afikpo reported by [14]. [41] reported 58.3 % prevalence of E. coli in water obtained from Akure. [11] had also reported that 75 % of unprotected well and spring water sources from North Gondar, Ethiopia, were contaminated by faecal coliforms, especially E. coli.

The presence of faecal coliform like E. coli and Klebsiella pneumoniae in some samples is an indication of recent pollution by sewage. This is not surprising since their presence in the water sample suggests faecal pollution, hence falls short of the standard of safe drinking water. Their presence in groundwater is not unlikely because the environment in which the majority of the waters are located predisposed them to constant pollution from human and animal waste materials. Severe health effects such as gastroenteritis and hemolytic uraemic Syndrome have been associated with consuming Escherichia coli from groundwater [6]. Additionally, Klebsiella species poses health risk to patients with impaired immune systems, such as the elderly or very young, patients with burns or excessive wounds, and those undergoing immunosuppressive therapy, thus their presence in ground water is of significant public concern. Pseudomonas aeruginosa is a recognized cause of hospital-acquired infections with potentially serious complications and has been isolated from a range of moist environments such as sinks, water baths, hot water systems, showers and pools [14]. Although the prevalence rate of Pseudomonas is not high compare to other bacteria isolated in this study, the presence of P. aeruginosa in drinking water in high volumes may be associated with complaints about taste, odour and turbidity according to [25]. The presence of Salmonella species was observed from of the water sources sampled with prevalence rate of 10.1 % and 13.4 % for borehole and well water respectively as observed in Table 4 and 5. Salmonella species is not particularly stable in water environments; their presence in drinking water indicates recent human faecal pollution [4]. Salmonella species are enteric pathogens predominantly transmitted by the faecal-oral route through person-to-person contact, contaminated food and water [34].

Streptococcus faecalis is heterogeneous group of Gram positive bacteria. In this group, the organism Streptococcus faecalis is now considered an opportunistic infection i.e. it is contacted from one’s normal micro flora. However, disease usually occurs in individual with predisposing factors such as viral infection of the respiratory tract, physical injury to the fat, alcohol or diabetes [9]. Undoubtedly, Streptococcus faecalis was the least prevalence bacteria recording 3.4 % (Table 5). This observation is in agreement with the findings of [37]; [38]; [39] who reported that Streptococcus faecalis as the least prevalence bacteria with 3.8 %, 12 % and 2 5 % respectively. The quality of ground water depends on various chemical constituents and their concentration, which are mostly derived from the geological data of the particular region [39]. The pH of all the water samples analyzed in this study are all in agreement with EPA standard, pH of water which ranges from 6.5 – 8.5 for borehole water and 6.5 – 8.9 for well water sources (EPA, 2002). The high turbidity observed with the water samples PB 2, PB7, PB8, PW4, PW6 and PW7 is not in agreement with the EPA standards on turbidity. High turbidity is usually attributed to the presence of higher levels of disease causing microorganism such as bacteria and other parasites in water samples. Low turbidity level observed in sample PB1, PB3, PB4, PB5, PB 6, PB9, PB10, PW1, PW2, PW3, PW5, PUW1. PUW2 and PUW3 may be attributed to the presence of fewer number of disease causing microorganisms. The cloudiness of water (turbidity) fit for consumption is not suppose to go above 5 nephelometric units (NTU) as recommended by EPA (2002). Total dissolved solid in drinking water has been associated with natural sources, sewage, urban runoff, industrial waste water and chemical used in the water treatment process (EPA, 2002), though of aesthetic rather than health hazards [7]; [8]. The total dissolved solid of all water samples (borehole and well) are all in agreement with the environmental protection agency standard of 500 mg/l. The total hardness is an important parameter of water quality whether it is to be used for domestic, industrial or agricultural purposes. Hardness of the water is the property attributed to the presence of alkaline earths. It is the property of water by which it prevents lather formation with soap and increases the boiling point of water [16]. Water can be classified into soft (75 mg/L), moderately hard (75–150 mg/L), hard (150–300 mg/L) and very hard (300 mg/L) based on hardness (Sawyer and McCarty 1967). From the result obtained, the analyzed borehole groundwater samples (PB1, PB2, PB3, PB5, PB6, PB7, PB8, PB9, PB10) are very hard water, except PB4 (295 mg/L) which is hard water as shown in Table 6. It is also observed that most the well water samples (PW1, PW2, PW4, PW5, PW7, PUW1, PUW3) are hard water, except for PW3 (325 mg/L), PW6 (310 mg/L) and PUW2 (378 mg/L) are very hard water (Table 7).The high hardness may cause encrustation on water supply distribution systems. Longterm consumption of extremely hard water might lead to an increased incidence of urolithiasis, anencephaly, prenatal mortality, some types of cancer and cardiovascular disorders [31].

Antibiotics play very important role in decreasing diseases, illness and/or death associated with bacterial infections in humans and animals [40]. The results of antibiotic study revealed marked differences among bacterial isolates in their susceptibility and resistance patterns to antibiotics. The high susceptibility of most bacterial isolates was observed with Amikacin, Ofloxacin and Gentamycin observed in this study might be due to the less use of these antibiotics in clinical practice and/or veterinary medicine. This data is in support with 91.75 % susceptibility profile of E. coli isolates from the various water sources to some prescribed antibiotics [43]. Amikacin is an aminoglycoside which blocks protein synthesis in bacteria and are used presently to treat severe infections as a last resort when other drugs fail [32]. Ofloxacin is a floroquinole that inhibits cell division [23].

Notably, higher levels of resistance to Gentamycin, Bacitracin, Clindamycin and Muprocin were observed in E. coli isolates, S. aureus, Klebsiella species, Pseudomonas aeruginosa, P. mirabilis, S. faecalis and Salmonella species isolates as shown in Table 8 and 9. This is similar to a previous work carried out in Abakaliki were high levels of resistance amongst bacteria isolated from water samples [36]. Multi-antibiotic resistant water bacteria isolates have also been reported by [13] [14]. The findings from this study, suggests that the isolates may have acquired resistant genes to the tested antibiotics (Bacitracin, Mupirocin and Clindamycin), probably due to exposure to sub-lethal doses in the environment or possession of intrinsic genes by the isolates.

Hence, this study clearly establishes how the resistance to different antibiotics is prevalent and is spreading around us in our water sources even in drinking water samples. The differences in resistance profiles in this environmental study clearly reflect the differences in the selection procedure pressure in the investigated sites/areas. The rising trend of resistance in most of the isolates in this study from ground water source affirms the fact that disposed antibiotics may have been washed down the water sources and accumulated downstream especially during the rainy season accounting for the high resistance.

Multiple antibiotic resistance (MAR) analysis has been used to differentiate bacteria from different sources using antibiotics that are commonly used for human therapy [46]. The monitoring of both antibiotic consumption and multiple antibiotic resistance index (MARI) especially in nosocomial infections is critically necessary to setting up of effective containment programs and audit of such programs [2] [3]. The MARI of bacterial isolates from borehole water is S. aureus (0.4), Klebsiella species (0.4), E. coli (0.6), P. aeruginosa (0.6) and Salmonella species (0.6), while well water is P. aeruginosa (0.3), S. feacalis (0.4), S. aureus (0.6), E. coli (0.6), P. mirabilis (0.6), Salmonella species (0.6) and Klebsiella species (0.7) as shown in Figure 1. Escherichia coli in this study showed multiple antibiotic resistance index of 0.83, followed by Enterobacter species (0.83) and S. saprophyticus (0.83) showed the highest followed by S. epidermidids (0.75), Enterococcus species (0.75) and Staphylococcus aureus (0.67). However, samples that yielded MAR indexing above 0.2 indicated high risk of contamination. The difference in MAR indexing in the different water sources indicated the impact of urbanization on antibiotic resistance levels. Resistance to multiple antibiotics can lead to occurrence of newly emerging resistant bacteria which may be transmitted to consumers causing infections that are difficult to treat. The observed high frequency of bacterial resistance may not only result in the therapeutic failure in the groundwater fauna population, but also endangers the health of the people who are at risk of infection with pathogens from these reservoir coupled with the possibility of plasmid transfer of resistance gene to human pathogenic bacteria [47].

V. CONCLUSION

This study show that borehole and well water sampled in this study require proper treatment before domestic use to eliminate bacterial involved in waterborne disease outbreaks. Bacterial isolates from borehole and well water sources exhibited varying resistances to antibiotics and is of public health importance. Considering susceptibility pattern of antibiotic agents (Amikacin, Ofloxacin and Gentamycin) against most of the bacteria isolates, they are thereby recommended as drugs of choice in the treatment of bacterial isolates from water sources.

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