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Bacteriological Analysis of Well Water Obtained from Onueke Metropolis, Ezza South Local Government Arae, Ebonyi State, Nigeria

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ABSTRACT

Many households in Nigeria are dependent on alternative sources of water supply and are exposed to preventable water-borne diseases. This study was carried out to examine the bacterial quality of well water obtained from Onueke metropolis in Ebonyi State. The physicochemical parameters of water samples collected from 25 closed and 25 opened wells were analyzed following standard procedures while bacteriological quality of the water samples including coliform count and bacterial isolate identification were determined using serial dilution and multiple fermentation techniques. Results of the physiochemical parameters showed that pH, Electrical Conductivity, Total Hardness, Turbidity and nitrite were within the WHO standard limit for drinking water. The bacterial count ranged from 0.8 – 3.1 x 10^4 CFU /100ml involving *E. coli* (25%), *Shigella* spp (12.5%), *Citrobacter* spp (4.2%), *Klebsiella* spp (29.2%), *Enterobacter* spp (12.5%) and *Salmonella* spp (16.7%) as the dominant isolates and exceeded the WHO guidelines limit of 0 cfu/100ml for drinking water. Generally, the uncovered wells were more highly contaminated with bacteria pathogens than the covered wells. All samples were above the most probable number (MPN) per 100 ml permissible limit of WHO guidelines for untreated drinking water. In conclusion sampled well water from Onueke metropolis contained bacterial contaminants making the water not safe for drinking devoid of any further water treatment process.

Keywords: Well water, Bacteria pathogen, Physiochemical, Bacteriological analysis, Waterborne

INTRODUCTION

Water is the most valuable natural resource, accounting for 70% of the earth's crust and all life forms, including humans, rely on it for survival (Oyedum *et al*., 2016). Well water is basically water in its natural state and can be affected in a number of ways depending on location, depth, geology which may result to changes in the odour, taste, colour, mineral content and presence of microbes such as bacteria, cysts, etc. Underground water supplies are normally potable and safe to drink if properly located, built, and operated in accordance with the World Health Organization guidelines for drinking Water (Ballester and Sunyer, 2000; Oyedum *et al*., 2016). The availability of drinkable water is critical for human survival and has a direct impact on human life quality. However, readily available potable water remains a challenge in many third-world countries including Nigeria. Many people, particularly in the developing countries, rely on untreated surface and ground water sources for their daily water needs, and the water from these sources is frequently contaminated (Oyedum *et al*., 2016). The growing awareness for quality water supply has led to an unregulated increase in the rate at which boreholes are drilled for domestic, agricultural and industrial use (Palamulen and Akoth, 2015). These water bodies unfortunately get polluted as a result of uncontrolled waste disposal, poor citing of drainages and extensive use of contaminating chemicals in the agricultural and industrial sectors (Bello *et al*., 2013; Palamulen and Akoth., 2015; Amoo *et al*., 2018; Hassan *et al*., 2018).

Faecal pollution of water is one of the primary causes of waterborne diseases around the world. In many developing countries, much of the water accessible for drinking is not only scarce, but also liable to contamination as a result of poor personal sanitation (Gimba, 2011). The presence of faecal contaminants in drinking water is a potential health risk for those who are exposed to it. A high incidence of typhoid fever, cholera, dracunculiasis, viral and bacterial gastroenteritis, hepatitis and other water and food- borne diseases have been reported in different parts of the Nigeria (Adeyinka *et al*., 2014 and Nwabor *et al*., 2015) and the Federal Ministry of Health ranked diarrhea second behind malaria as a disease with a high prevalence, accounting for 16 percent of death among children under the age of five. On a global scale approximately 525,000 children under the age of five die yearly from diarrhea (WHO, 2017). In Nigeria where 63 million people lack access to quality drinking water (Ogundipe *et al*., 2017), this illustrates the need for improved potable water supply (Aderigbe *et al*., 2019). From the public health angle, the importance of periodic water supply surveillance to ascertain the portability of water supplied cannot be over emphasized (WHO, 1996). In view thereof, this study was carried out to determine bacteriological quality of well water in Onueke metropolis which constitute the major source of drinking water for residents.

MATERIALS AND METHODS

Study Area and Sampling

Onueke is the headquarters of Ezza South LGA, Ebonyi State, Nigeria with GPS coordinates of Latitude: 6.11667, Longitude: 8.001116° 7' 0" North, 8° 0' 4" East. Fifty (50) well water samples were randomly collected from ten different locations described as follows: Covered well cites included Amaoloanya (A), off sacred heart Parish Onueke (B), Ekka junction (C), NdufuAmeka (D), Ndufu Ezzama (E), while uncovered well cites included; New Ikwo road (F), Old Ikwo road (G), Rice mill (H), Eke market square (I) and Uburu 14 (J). The wells are privately owned and freely accessible for public use. Water was fetched from these wells by the use of 4-5 liter containers mostly brought along by well users.

Collection of Water Samples

Water samples were collected from identified covered and open wells between the month of February and May, 2008. Sterile McCartney bottle was tied to 20 meter rope and lowered to a depth of 5-8 meters and when no air bubbles rose to the surface, the bottle was pulled out and covered with a screw cap (Cheesebrough, 2000). The samples were properly labeled and transported under ice to microbiology research laboratory of Ebonyi State University, Abakaliki for bacteriological analyses within 24 hours.

Physicochemical Analysis

All water samples collected from each sampling point was subjected to the following physicochemical analyses: pH (pocket pro pH tester) turbidity (2100Q portable turbid meter), electrical conductivity (EC) (HQ430D laboratory single input multi-parameter meter) total hardness (SP510 hardness analyzer) and nitrite measured (EZ7750 nitrite analyzer). These parameters were analyzed following the standard analytical methods outlined by APHA (2005).

Bacteriological Analysis

Total Bacterial Count

After preparing 1:100 sample dilution, 1.0 mL of diluted sample was inoculated into 19 mL of nutrient agar, mixed properly and poured into a sterile Petri dish. The agar was allowed to set, and incubated at 37 °C for 24 hrs. The colony forming units were counted and result expressed as cfu /mL (Cheesebrough, 2000).

Presumptive Enumeration of Coliforms

Coliform enumeration in 100 mL water sample was conducted using the Most Probable Number (MPN) and multiple tube (MTT) standard techniques (SMEWW, 1992). Briefly, the collected water sample were serially diluted in 1:10, and 0.1 mL and the diluted samples was inoculated into five (5) test tubes containing 50 mL of sterile single strength lactose broth with an inverted Durham's tube for the collection of gas produced after 24 hrs of incubation at 37 °C. The procedure was repeated with 10 mL of single strength lactose broth and 1.0 mL of water sample. The presence of coliform in the samples was determined using Mc Crady's Table (SMEWW, 1992).

Identification of Isolates

Tubes that were positive in the presumptive tests were sub cultured on Mac-Conkey agar and Triple Sugar Iron agar (TSI) for the enumeration of bacteria, all media were incubated for 24 hrs at 37 °C. Colonial and morphological features on solid media as well as standard biochemical tests were used to characterize the isolates (Cowan and Steel, 1993).

Confirmed Test

The confirmed test was carried out in accordance with (Majula *et al*., 2011). This was accomplished by aseptically transferring a Loopful of culture from test tubes with positive results in the presumptive test into test tubes with sterile peptone water and plates with sterilized violet red blue agar (VRBA). After that, the plates and test tubes were incubated for total and faecal coliforms, respectively. While the formation of pink colonies with a metallic sheen and bleaching in the center on VRBA proved the presence of coliforms, the production of gas and the emergence of red color indicating indole production in the peptone water indicated the presence of *E. coli*.

Completed Test

Completed test was performed in order to ascertain distinct colonies in accordance with WHO guidelines (2012). This was accomplished by streaking positive test findings on sterilized Eosin Methylene Blue (EMB) agar, and Salmonella-Shigella agar. The plates were then incubated for 48 hours at 37 °C. Production of green metallic sheen colonies on EMB was ascertained as positive test for further method of coliforms or faecal coliforms (*Escherichia coli*) (Adetunde and Glover, 2010).

All of the bacterial isolates were gram stained thereafter for morphological identification according to Olutiola *et al*. (1991). The following biochemical test catalase, indole, methyl red, and Vogesproskauer (Cheesebrough, 2006); citrate, oxidase, urease (Ochei and Kolhatkar, 2008); mannitol, lactose, and triple sugar iron (Hemraj *et al*., 2013); and glucose (Aryal, 2018) was performed to identify bacterial isolates

Data Analysis

Results were expressed as the mean values ± standard deviation by measuring three independent replicates per location. One way Analysis of variance (ANOVA) was done and Duncan's post hoc test was performed to identify difference between means at 5% level of significance using statistical package for social sciences SPSS software for windows (version 21, IBM SPSS). Differences were considered significant at $p<0.05$.

RESULTS

The physiochemical analyses of the well water sampled showed that pH, total hardness (TH), electrical conductivity (EC), nitrite and turbidity (TU) of the well water samples obtained (Table 1) were all within the WHO permissible limit.

Table 2 reveals the bacterial count of isolates of water samples in ten (10) different sites in Onueke metropolis. The bacterial counts of the water samples differ from each other. Water samples from location H, and I (open well) had highest bacteria count while samples from locations A-E (covered well) showed no difference and were also statistically the same with lower bacteria load.

Table 1: Physicochemical parameters of well water samples obtained from Onueke metropolis

Location	pH	TH (mg/l)	EC (us/cm)	TU (NTU)	Nitrite (mg/l)
A	7.6±0.4[abc]	60.5±0.5[a]	360±0[a]	1.03±0.03[b]	0.03±0[ab]
B	7.3±0.3[ab]	66.6±0.6[b]	416±2[bc]	0.79±0.02[b]	0.02±0[ab]
C	7±0[a]	80.2±0[d]	550±0[d]	0.17±0[a]	0.02±0.02[ab]
D	7.1±0.1[a]	65.4±0.4[b]	431±1[c]	3.1±0[f]	0.01±0[a]
E	8±0.5[bc]	69.7±0.7[c]	430±10[c]	2±0[d]	0.02±0[ab]
F	7.7±0.2[abc]	70.1±0.0[c]	400±10[b]	1.6±0.1[c]	0.05±0.01[b]
G	7.8±0.2[abc]	70.8±0[c]	545±5[d]	2.5±0[e]	0.07±0.01[cd]
H	8.2±0.1[c]	300.9±0.9[g]	700±0[f]	4±0.3[g]	0.98±0.02[e]
I	7.4±0[abc]	234.1±0.1[f]	600±0[e]	2.2±0[de]	0.08±0[cd]
J	7.2±0[ab]	90.3±0.3[e]	346±6[a]	2.2±0.2[de]	0.1±0[d]

Means values represented by different letter along same column are significantly different from each other at $p < 0.05$.
Key: A-E are water samples from covered wells F – J are water samples from uncovered wells Amaoloanya - (Location A), Off sacred heart Parish Onueke- (Location B), Ekka junction - (Location C), NdufuAmeka - (Location D), NdufuEzzama- (Location E), New Ikwo road- (Location F), Old Ikwo road - (Location G), Rice mill- (Location H), Eke market square - (Location I), Uburu 14- (Location J).

Table 2: Total bacteria count of well water samples obtained from Onueke metropolis

Location	Bacterial cfu/mL (X10^4)
A	1.0 ±0.20[a]
B	0.8 ± 0.10[a]
C	1.2 ± 0.10[a]
D	1.3 ± 0.20[a]
E	1.4 ± 0.00[ab]
F	1.9 ± 0.10[bc]
G	2.1 ± 0.40[cd]
H	3.1 ± 0.10[e]
I	2.6 ± 0.20[de]
J	1.42 ± 0.00[c]

Means values represented by different letters along the same column are significantly different from each other at P<0.05
A-E are water samples from covered wells F – J are water samples from uncovered wells Amaoloanya - (Location A), Off sacred heart Parish Onueke- (Location B), Ekka junction - (Location C), Ndufu Ameka - (Location D), Ndufu Ezzama- (Location E), New Ikwo road- (Location F), Old Ikwo road - (Location G), Rice mill- (Location H), Eke market square - (Location I), Uburu 14- (Location J).

The presumptive coliform count measured by the most probable number per 100 ml in the multiple tube fermentation technique of bacterial enumeration from the covered and open well water samples from the different locations are indicated in Table 3. The most probable number MPN per 100ml for the well water sample ranged between 12 and 180. Morphological and biochemical characterization of bacteria isolated from well water samples obtained from this study are presented in Table 4. The result showed that *Klebsiella* spp, *E. coli*, *Salmonella* spp, *Shigella* spp, Enterobacter and Citrobacter were common bacteria present in the well water samples

DISCUSSION

The physiochemical analyses of the well water sampled showed that pH of the well water samples obtained (Table 1) were statistically similar across the locations and fell within the WHO recommended limits of ≥7 to ≤9 (WHO, 2012). Similar findings were reported by Onwa *et al*. (2019) who conducted water quality assessment of selected well and boreholes in a geological location similar to the area under study. Also, the total hardness of the water samples showed that samples in Rice mill (locationH) and Eke market square (Location J) has higher total hardness when compared to other samples but is within the WHO recommended limit of 150-500 mg/L. The EC value of less than 1000 µS/cm as reported in this study is within the WHO recommended limit (WHO, 2004). Generally, all the values of the physical parameters were within the permissible limit approved for drinking water (WHO, 2004).

Table 3. Total coliform count of well water samples obtained from Onueke metropolis

Locations	MPN/100mL
A	14.00 ± 2.00[a]
B	17.00 ± 2.00[a]
C	12.00 ± 3.00[a]
D	20.00 ± 0.00[a]
E	20.00 ± 0.00[a]
F	90.00 ± 0.00[b]
G	95.00 ± 1.00[b]
H	180.00 ± 0.00[d]
I	160.00 ± 10.00[c]
J	14.60 ± 0.00[c]

Means values represented by different letter along same column are significantly different from each other at $p<0.05$ A-E are water samples from covered wells F – J are water samples from uncovered wells Amaoloanya - (Location A), Off sacred heart Parish Onueke- (Location B), Ekka junction - (Location C), Ndufu Ameka - (Location D), Ndufu Ezzama- (Location E), New Ikwo road- (Location F), Old Ikwo road - (Location G), Rice mill- (Location H), Eke market square - (Location I), Uburu 14- (Location J).

The total bacterial count of 0.8 – 3.1 x 10^4 cfu/mL (Table 2) is in excess of the WHO recommended for drinking water. Samples from locations H and J had the highest bacteria load when compared to other locations. This may be attributed to unrestricted access of environmental particles into the well when compared to the closed wells. This is an indication that well water in this metropolis were highly contaminated by microbes thereby unfit for human consumption and at the same time poses danger to public health. Onsite inspection during sample collection revealed these well were less than ten meters away from septic tank; less than 15 meters underground against the 30 meters minimum acceptable distance between borehole and septic tanks as recommended by WHO. The effluent from this sewage system into the wells might have also contributed to the high faecal coliform as reported in this study. This finding is in line with (Adetunde and Glover (2010); Bello *et al*., 2013; Kamanula *et al.*, 2014; Palamulen and Akoth, 2015; Hassan *et al*., 2018; Amoo *et al*., 2018; Adeleye *et al*., 2020;) where they opined that uncontrolled disposal of waste materials, poor siting of drainages, proximity of septic tanks to groundwater source and seepage of environmental contaminating chemicals in the agricultural and industrial sectors also pollute groundwater sources.

The most probable number (MPN) per 100 mL obtained from the well water samples (Table 3), ranged from 1-50mL across the ten sampled locations. Samples from uncovered wells especially Rice mill and Eke market square from location H and J has the highest number while samples from covered wells recorded the lowest number (12-180+) MPN/100mL respectively. This is above the standard limit according to WHO recommendations for drinking water (WHO, 1998). Also, the results obtained in this study corroborates with (Onwa *et al*., 2019; Idowu *et al*., 2011; Oyetayo *et al*., 2007; Orogu *et al*., 2017) obtained from similar studies on the antibiotic susceptibility of bacterial species isolated from underground waters in Abakaliki metropolis of Ebonyi State, Nigeria; bacteriological analysis of well water samples in Sagamu and bacteriological quality of borehole and well water in Ijebu-Ode and Ago-Iwoye communities in South Western Nigeria respectively, reported bacterial count greater than 1 CFU/100ml.

Although total coliform count is not an indicator of faecal contamination, it is used to assess the overall sanitary quality of treated and disinfected drinking water. The World Health Organization (WHO) recommends a zero coliform bacteria per 100ml in either treated water entering the distribution system or in the distribution system itself (WHO, 1998).

Klebsiella spp, *E*. *coli*, *Salmonella* spp, *Shigella* spp, Enterobacter and Citrobacter were microbes isolated in this study (Table 4). The bacterial pathogens isolated from the various water samples in this study are of public health importance because these microbes have been reported to cause a plethora of human infections. This is in agreement with (Iroha *et al*., 2016; Onwa *et al*., 2019) who reported the presence of enteric and non-enteric bacteria in borehole and well water within Abakaliki Metropolis including *Escherichia coli*, *Salmonella* species, *Klebsiella* species, *Pseudomonas* species, *Proteus mirabilis*, *Streptococcus* species, and *Staphylococcus aureus.* The presence of enteric coliforms especially *E. coli* makes the water samples unsuitable for human consumption according to WHO guideline for the evaluation of bacteriological quality of drinking water (Reasoner, 1992; WHO, 1996), a guideline adopted by the national agency for food, drug and administration control (NAFDAC). Previous reports in other parts of the country - Lagos, Ibadan, Afikpo; Ebonyi state as well as Ogun States - on well water quality suggest the alternative source of drinking water and for other domestic purposes were highly polluted with enteric organisms (Adeyemo *et al.*, 2002; Akinyemi *et al.*, 2006; Efuntoye and Apanpa, 2010; Idowu *et al*., 2011).

Table 4: Morphological and biochemical characterization of bacteria isolated from well water samples obtained from Onueke metropolis

Location	Gram stain	TSI	OX	MOT	UR	VP	MR	ID	CAT	CIT	GLU	LA	MAN	Confirmed org
A	-	A	-	+	-	-	+	-	+	-	+	-	+	*Salmonella spp*
	-	A	-	+	+	+	-	-	+	+	+	+	+	*Enterobacter sp*
B	-	A/G	-	+	-	-	+	+	+	-	+	+	+	*E. coli*
	-	A	-	-	+	+	-	-	+	+	+	+	+	*Klebsiella spp*
C	-	A	-	-	+	+	-	-	+	+	+	+	+	*Klebsiella spp*
D	-	K/A	--	-	-	-	+	-	+	-	+	+	-	*Shigella spp*
	-	A/G		+	-	-	+	+	+	-	+	+	+	*E. coli*
E	-	A	-	+	-	-	+	-	+	-	+	-	+	*Salmonella spp*
	-	A	-	-	+	+	-	-	+	+	+	+	+	*Klebsiella spp*
F	-	K/A	-	+	-	-	+	-	+	+	+	+	+	*Citrobacter*
G	-	A	-	+	-	-	+	-	+	-	+	-	+	*Salmonella spp*
H	-	A	-	+	-	-	+	-	+	-	+	-	+	*Salmonella spp*
	-	K/A	-	+	-	-	+	-	+	+	+	+	+	*Citrobacter*
I	-	A	-	-	+	+	-	-	+	+	+	+	+	*Klebsiella spp*
	-	A	-	+	-	-	+	-	+	-	+	-	+	*Salmonella spp*
J	-	A	-	-	+	+	-	-	+	+	+	+	+	*Klebsiella spp*
	-	K/A	-	-	-	-	+	-	+	-		+	-	*Shigella spp*

Key: + = Positive; - = Negative; CA = Catalase; MR = Methyl Red; VP = Voges- Proskauer; LA = Lactose; MA = Mannitol; UR = Urease; CI = Citrate; OX = Oxidase; IN = Indole; TSI = Triple Su gar Iron; GLU = Glucose

A-E are water samples from covered wells F – J are water samples from uncovered wells Amaoloanya - (Location A), Off sacred heart Parish Onueke- (Location B), Ekka junction - (Location C), NdufuAmeka - (Location D), NdufuEzzama- (Location E), New Ikwo road- (Location F), Old Ikwo road - (Location G), Rice mill- (Location H), Eke market square - (Location I), Uburu 14- (Location J).

A situation where enteric bacteria were highly recovered from drinking water sources for human consumption poses a great threat to health concern for government and policy makers as it may lead to possible future outbreak of water borne diseases. The cause for this high microbial pollution of well waters by microbes as reported in this study can be attributed to open nature of wells, low depth profile, proximity to septic tanks as well as lack of personal and environmental hygiene by residents of these areas.

CONCLUSION

The result of this research indicated that *E. coli*, *Salmonella* spp*, Shigella* spp, *Citrobacter, Enterobacter* and *Klebsiella* spp were all recovered in well water sampled hence not fit for human consumption. However, the physiochemical parameters of sampled water in the study area showed the physical parameters pH, EC, TH, TU and nitrite were all within WHO permissible limit for drinking water. It is recommended that underground water drilling should be cited at least 30 meters away from septic tank, pit latrine and sewage sources. Also, environmental sanitation, good and personal hygiene practice must be adhered to within and around the wells. Also, other water treatment processes recommended in the WHO guideline for drinking water should be adopted by users in order to mitigate incidence of water borne diseases.

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