Bacteriological and Physico-Chemical Analysis of Drinking Water Quality in Uli

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Abstract: The bacteriological and physicochemical examination of drinking water quality was carried out in Uli to determine their levels of contamination. The sources of water examined were borehole water, well water and stream water. This analysis was carried out in the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University, Uli. Water samples where obtained from the three different sources named above using properly labeled sterile universal containers. The samples were analyzed for their physical properties. Identification of coliform, feacal coliforms and *Vibrio* species where done using Eosin Methylene Blue agar for differentiation of coliform and feacal coliforms, MacConkey agar was used for total coliform count, thiosulfate citrate bile salt agar for isolation of *Vibrio cholera*.

Triple Sugar Iron agar was used for differentiating enteric organisms based on their ability to reduce sulphur and ferment carbohydrate. The Most Probable Number technique was also used to detect the presence of coliforms and were carried out in three stages which are the presumptive test, the confirmatory test and the completed test.

The presumptive and confirmatory tests were carried out using the Lactose broth and Brilliant Green Lactose Bile broth. The completed test was carried out using the Eosin Methylene Blue agar. The results showed that the well and stream water samples S6–S15, were grossly contaminated, containing *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Staphylococcus* species, *Bacillus* species, *Streptococcus* species, *Pseudomonas* species and *Vibrio* species. Borehole water samples could serve as better alternative but still needs further treatment to improve the drinking water quality.

Keywords: Borehole, well, water, coliform, presumptive, contaminants.

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1.0 Introduction

Most of our water supplies are from surface water which includes; rivers, streams, lakes, oceans and seas. These water bodies are likely to be polluted with domestic and industrial as well as agriculture waste. As populations increase, the problem becomes more serious and as such, water can endanger the health and life of human beings because when polluted by fecal materials it becomes potential carrier of pathogenic organism (Carpenter, 2008).

Water is of course absolutely essential to life, not only human life but all life, animal and plants alike. Most of the biochemical reaction that occur in metabolism and growth of living cells involve water (Camp *et al.*, 2009). Man uses water not only for drinking purposes but also for bathing, washing, laundering, heating, air conditioning, agriculture, stock raising and gardens. Natural water contains not only the natural flora but also microorganisms from soil, animals and sewage. Surface waters in streams or pools and stored waters in lakes and large ponds vary considerably in microbial content (Frazier, 2008).

The generality of bacteria are most commonly found in fresh water, some of which include: *Pseudomonas, Archaebacter,* and *Vibrio.* These are Gram-negative. The Gram-positive bacteria which are found in water include: *Micrococcus, Archaebacter* and Actinomycetes (Gebharal, 2005). Tap water, as one of the water sources is mostly used domestically, it is observed that tap water sometimes do not turn out clear in appearance, bacteriology of water ought to be carried out in order to be sure of its portability (Bonde, 2007). Most drinking water sources are often contaminated with different pollutants like faeces, animal and plant wastes, making such water unfit for drinking if not treated. The pollution of water with pathogenic organisms and other pollutants can only be detected by carrying out microbiological assessment of such water. Most human diseases such as typhoid, paratyphoid, cholera, amoebiasis, gastroenteritis, salmonellosis, shigellosis, diphtheria, giardiasis, etc., are known to be water borne diseases (Ewington *et al.*, 2001).

Water borne diseases are those diseases which have water as their vehicle of transmission. These diseases are capable of destroying a whole community if not checked. Therefore, the quickest ways to prevent outbreak of such diseases and to determine the portability of such water sources is to determine the microbial load or content, if the microbial load is not within acceptable limit, such water sources should be condemned immediately (Fair *et al.*, 2000). This research aims at comparing the water quality of the various drinking water sources available at Uli.

2.0. Materials and Methods

2.1. Method

Water samples were collected from public and private boreholes for physicochemical and bacteriological analysis using standard analytical techniques and instruments such as portable

pH meter to measure pH and other physicochemical parameter like appearance, temperature, colour, odour, taste, turbidity were determined. Pour plate method was used for detection of indicator organisms for water contamination (*Escherichia coli, Streptococcus faecalis, Clostridium perfringens* and total heterotrophic bacteria).

2.2. Sample Collection

Water samples were collected from fifteen different sources comprising; five tap water samples from different compounds, five well water samples from different compounds and five stream water samples in well labeled sterile universal sampling bottles using aseptic techniques. The samples were thereafter brought to the Microbiology Laboratory of Chukwuemeka Odumegwu Ojukwu University Uli campus, Anambra State for analysis in ice pack within two hours of collection.

2.3. Sample Analysis

The water samples were first analyzed for its physicochemical properties such as pH, odor, taste and color, after which the samples were labeled 1-15, and analyzed for its bacteriological quality using modified methods of Duguid and Mitarb (2005).

2.3.1. Physicochemical analysis of the sample

pH determination

This was determined using a digital pH meter. During the determination, the pH meter was calibrated using pH buffer solution ranging from 4–9, the pH meter was then dipped into the various water sample and the results were recorded respectively.

2.4. Serial Dilution

A tenfold serial dilution was carried out for all 15 samples from their representative stock samples. One milliliter of the sample aseptically transferred into the first test tube (10^{-1}) using a sterile pipette. From the first test tube, one millimeter was equally transferred to the test tube labeled 10^{-2} , this was done up to dilution 10^{-5} .

Note: All glassware used were sterilized in a hot air oven at 160°C for one hour.

2.5. Isolation and Identification of Test Isolates

2.5.1. The most probable number technique for fecal coliform count

Fecal coliform were detected using the most probable number technique. This test was carried out in three stages which are presumptive, confirmatory and completed test.

2.5.1.1. Presumptive test

Presumptive test carried out using Lauryl tryptose broth 10ml of the double strength broth was poured into first set of three tubes and 9.9ml of the single strength broth was poured into second and third set of the tubes respectively. Durham's tubes filled with media where inverted in all the tube and autoclaved at 121°C for 15mins.

The three sets of test tube where inoculated with 10 ml of diluted water sample for 10ml of double strength broth tube. 0.1ml of diluted sample where inoculated into 9.9ml single strength broth tube and 1ml of diluted sample was inoculated into 9ml single strength broth tube.

The tubes where inoculated at 37°C for 48hours, after which acid production where detected by color change and gas production checked for entrapment of gas in the Durham tube. The faecal coliform counts for positive tubes where read directly from the MPN table.

2.5.1.2. Confirmed test

Confirmed test was carried out using Brilliant Green Lactose Bile (BGLB) broth according to the modified methods of Shariq *et al.*, (2016). Fermentation tubes containing Brilliant Green Lactose Bile (BGLB) broth and Durham tubes filled with media where inoculated with the positive presumptive test tubes.

The test tubes where then incubated at 44.5°C for 48hours then it was observed for gas production.

2.5.1.3 Completed test

This was carried out using Eosin Methylene Blue (EMB) agar in accordance with (WHO, 2012) by streaking a loopful of broth from the positive confirmatory test on to Eosin Methylene Blue (EMB) agar plates. The plates where incubated at 44.5°C to 24-48hours for visual evidence of growth.

Colonies that grew as pink mucoid with green metallic sheen on EMB agar and MacConkey agar plates where further identified using their cultural, morphological and biochemical characteristics. Grams staining and spore staining also constituted the completed test for fecal coliform.

2.6 Total Coliform Determination

Positive tubes with yellow coloration (acidic) and gas trapped by the Durham tubes were matched using the MacCrady's Statistical table to confirm the presence of coliform present in 100 ml of the water samples.

The positive tubes was also inoculated into MacConkey, triple sugar iron, and nutrient agar plates containing 2 ml of 0.02g nystatin to prevent fungal growth. This was done in duplicate and controls was equally prepared without adding the positive samples.

The plates were labeled, allowed to solidify, inverted and finally incubated at $37^{\circ}C$ for 24 - 48 hours. After incubation, the colonies on each plate were counted using colony counter promptly after incubation.

2.7 Vibrio Species Count

Pour plate technique was used and the culture medium was TCBS Agar. One milliliter of the sample from 10^{-2} test tube was aseptically transferred into sterile Petri dishes using sterile pipette. The TCBS Agar was prepared according to the manufacturer's instruction and allowed to cool.

The culture medium was poured into the Petri dishes and properly mixed with the sample. This was done in triplicates. A control was equally prepared, but without adding the sample. The plates were labeled, allowed to solidify, inverted and finally incubated at 37°C for 24–48 hours.

3.0 Results

Total coliform count of the water samples that grew on the MacConkey agar. It shows the total number of coliform after 3 days incubation at 37°C and it was found that only pipe borne water has none colony but other sources has more colonies. The physical characteristics of the water were accessed and recorded. The fecal coliform was checked for its presence or absence as well as the assessment of *Vibrio cholera*.

Sample number	Source	Odor	Taste	Color
Sample 1	Borehole	-	-	-
Sample 2	Borehole	-	-	-
Sample 3	Borehole	-	-	-
Sample 4	Borehole	-	-	-
Sample 5	Borehole	-	-	-
Sample 6	Stream	-	-	+
Sample 7	Stream	-	-	+
Sample 8	Stream	-	-	+
Sample 9	Stream	-	-	+
Sample 10	Stream	-	-	+
Sample 11	Well	+	+	+
Sample 12	Well	+	+	+
Sample 13	Well	+	+	+
Sample 14	Well	+	+	+
Sample 15	Well	+	+	+
Key: (+) Positive				
(–) Negative				

Table 1. Physical analysis being carried out on the water samples

Table 2. Biochemical and morphological test for identification of microorganisms

	Morphological Test Biochemical Test				Identified				
Colony	Gram	Motility	Citrate	Catalase	Oxidase	Coagulase	TS	Indole	Bacteria
Charact-	Stain	Test	Test	test	test	test	Ι	Test	
eristic									
Mucoid	- Rod	-	-	-	-	-	+	+	Escherichi
slightly									a coli
raised									
Colony									
Pinkish red	- Rod	-	+	+	-	+	-	-	Klebsiella
irregular									spp.
shape, Flat									
and									
butyrous									
Circular,	+Cocci	-	-	+	-	+	-	+	Staphyloc
Raised									occus
Circular,	- Rod	-	-	+	-	+	+	+	Enterobac
Raised									ter spp
Circular	+Rod	+	-	+	-	-	-	-	Bacillus
flat cream									spp
Colony									
with dull									
Feather -									
like edge.									
Circular,	+Cocci	-	-	-	-	-	-	-	Streptococ
flat, white									cus spp.
Smooth									
surface									
Colonies	- Rod	+	-	+	+	-	-	-	Pseudomo
were green,									nas spp.
Flat and									
smooth.									
Keys: (+) Positive									
(-) Negative									

Table 3. Total coliform count of the water samples				
Sample no	Sample Source	Colony Count		
Sample 1	Borehole	5		
Sample 2	Borehole	10		
Sample 3	Borehole	6		
Sample 4	Borehole	None		
Sample 5	Borehole	8		
Sample 6	Stream	19		
Sample 7	Stream	25		
Sample 8	Stream	15		
Sample 9	Stream	22		
Sample 10	Stream	30		
Sample 11	Well	35		
Sample 12	Well	30		
Sample 13	Well	40		
Sample 14	Well	32		
Sample 15	Well	46		

Table 3. Total coliform count of the water samples

Table 4. Isolation of *E. coli* from the water samples

Sample number	Sample Source	E. coli	
Sample 1	Well	+	
Sample 2	Well	+	
Sample 3	Well	+	
Sample 4	Well	+	
Sample 5	Well	+	
Sample 6	Stream	+	
Sample 7	Stream	-	
Sample 8	Stream	-	
Sample 9	Stream	+	
Sample 10	Stream	+	
Sample 11	Borehole	-	
Sample 12	Borehole	-	
Sample 13	Borehole	-	
Sample 14	Borehole	-	
Sample 15	Borehole	-	
Key: (+) Positive, (-) Negative			

Table 5. Isolation of Vibrio species in water samples

Sample no	Sample Source	Vibrio
Sample 1	Well	+
Sample 2	Well	+
Sample 3	Well	+
Sample 4	Well	+
Sample 5	Well	+

Sample 6	Stream	+	
Sample 7	Stream	-	
Sample 8	Stream	-	
Sample 9	Stream	+	
Sample 10	Stream	+	
Sample 11	Borehole	-	
Sample 12	Borehole	-	
Sample 13	Borehole	-	
Sample 14	Borehole	-	
Sample 15	Borehole	-	
Key: (+) Positive, (-) Negative			

Table 6. Prevalence	rate for bacteria	isolate of the	e water samples

Isolates	Average number	Percentage
Escherichia coli	34	21.12
Klebsiella species	23	14.29
Enterobacter species	18	11.18
Staphylococcus species	30	18.63
Bacillus species	21	13.04
Streptococcus species	10	6.21
Pseudomonas species	25	15.53

4.0 Discussion

In this research, on the basis of the results obtained from the analysis of drinking water quality in Uli, Ihiala, Anambra, Nigeria, the mean total bacteria counts were between 0.4×10^8 CFU/ml in well water at sample 13 and 0.46×10^8 CFU/ml in well water at sample 15, indicating not so high level of pollution of the well water due to human and animal activities (Table 6). These counts are higher than the acceptable counts of 0 CFU/ml for drinking water (NIS 2007).

The higher total bacteria counts especially in the two well waters at sample 13 and sample 15, and the two streams at sample 9 and sample 7 is an indication of the presence of high organic matter in the water. The main source of these bacteria in the water samples could be attributed to both human and animal activities (Scott *et al.*, 2003).

These sources of bacterial contamination include surface runoff, animal waste deposition and pasture. Other human activities like swimming, waste disposal, domestic activities and faecal discharge (Egberongb *et al.*, 2012) are also `possible ways of introducing foreign microorganisms in the water thereby making more nutrients available for the microorganisms in the water thus enhancing their growth at all the various water sources.

The mean total fungi counts were between 0.30×10^8 CFU/ml in sample 10 stream water and 0.46×10^8 in well water (Table 3). Human activities are responsible for the high microbial counts in sample 15 well water which results in the disturbance of the already contaminated sediments arising in possible nutrient release (Scott *et al.*, 2003).

The results of the total coliform counts (TCC) (Table 3) exceeded that of the WHO standard for coliform bacteria in water, which is zero total coliform per 100 ml of water.

The result showed that the total coliform counts for all the various water sources where the least coliform counts was recorded at sample 4 borehole water (0 MPN/ml) and the highest total coliform counts was 46.0 MPN/ml in well water at sample 15 which could be attributed to the discharge of sewage into the streams and wells by the surrounding people.

The presence of coliform counts obtained from the samples is an indication of faecal contamination. None of the well and stream samples complied with the WHO standard for coliform in water, and this is in agreement with previous work by Onajite *et al.*, (2018) who had earlier reported high microbial counts on water containing higher organic matter. According to WHO (2004, 2012), any water sample that contains coliform should be investigated for the presence of faecal coliforms.

The result also showed that seven bacteria isolates were isolated from the various water samples, which include *Escherichia coli*, *Staphylococcus* species, *Streptococcus* species, *Klebsiella* species, *Enterobacter* species, *Pseudomonas* species and *Bacillus* species, Table 6 showed the frequency of distribution of the bacteria isolates, *Escherichia coli* and *Staphylococcus* species are the most prevalent isolates, and the least prevalent were Enterococcus luteus. and *Streptococcus* species. *Enterobacter* spp. isolated from the water samples are non-faecal coliforms which can be found in vegetation and soil, which serve as potential source by which microorganisms can enter the water body. The polluted water may be due to water runoff from farm lands carrying manures, pesticides, animal and human waste matter.

It is interesting to note that no growth was recorded at sample 4 borehole water see Table 3. The percentage frequency of bacteria isolated ranged from 21.12% to 6.21% as shown in Table 6. The result shows that *Escherichia coli* had the highest value while *Streptococcus* species had the lowest value. The results from the test for *Vibrio* species on TCBS plates revealed the presence of *Vibrio* species in all well and stream samples but none in borehole samples, see Table 5.

Conclusion

Stream water and well water in Ihiala Local Government Area (Uli locality) has been found to be unsafe for consumption and for industrial uses because of the large number of bacteria that grew on agar plate incubated for 24 hours and also the gas production in the Durham tube. Judging from the result obtained I would like to recommend the following. Personal hygiene should be adopted by everyone using natural water, that is, water obtained from any of the natural sources should be boiled or treated before consumption.

Water purification method that provides safe drinking water should be made available by government in order to avoid out break cause by pathogenic organism found in water. The government should make more sacrifices to provide adequate treatment facilities that purify sewage prior to discharge or disposal, so as to save our drinking water form continuous pollution.

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Plate 1. Salmonella sp. on Brilliant green agar



Plate 2. Escherichia coli on EMB agar



Figure 1. Prevalence percentage for bacteria isolate of the water samples