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# Bacterial Quality of Spring Water in Ihitte/Uboma LGA of Imo State, Nigeria

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## Abstract

Water-borne diseases are a common cause of epidemics and deaths. It is common knowledge that spring water is consumed raw without any form of treatment in many communities in Nigeria. Water intended for drinking if not properly treated and monitored could be a vehicle of some bacteria disease of public health importance. The aim of this study therefore, is to determine the microbiological quality of spring water samples collected from three communities in Ihitte/Uboma Local Government Area. The objective is to carry out bacteriological examination to ascertain the quality of these spring water for potability and compare results obtained with water quality standards of WHO. Standard microbiological methods were used for the isolation, enumeration, characterization and identification of bacterial isolates. Results showed that, temperature values ranged from 19$^{o}$C to 23$^{o}$C while pH was 7.1 in all the spring water samples. The total aerobic heterotrophic bacterial counts ranged from (1.9 to 5.64) $\times$ $10^{7}$ cfu/ml. Total Coliforms ranged from 4 to 17 (MPN index 100 per ml) while Thermotolerant Coliforms ranged from 6 to 20 (MPN index 100 per ml). The bacteria isolated were *Escherichia coli*, which occurred in the three spring water samples. *Salmonella* sp. occurred in Oturugo spring water and Inyenta spring water. *Shigella* sp occurred in Oturugo and Inyenta spring waters, *Klebsiella* sp, occurred in Wogba and Inyenta spring waters and *Bacillus* sp, occurred in Wogba and Inyenta spring waters. Analysis of variance (ANOVA) using F-Test showed that there was no significant difference at $p \geq 0.05$ in the incidence of bacteria between the different spring water samples. The presence of these organisms revealed microbial and faecal contamination of the spring waters. This study therefore emphasizes the need for treatment of spring water before human consumption.

Keywords: Spring water, human activities, *Escherichia coli*, faecal contamination.

## Introduction

Spring is a water of a natural situation where water flows from an underground layer of water bearing permeable rocks and rock fractures (aquifer) to the earth's surface or emerges as a spring. Most of the water that emerges as springs originally fell as rain or snow on the surface of the earth (Zimmerman, 1996; Thurman *et al.,* 1998).

Spring water is used for a variety of human needs primarily as a source of drinking water. Spring water is the subject of many popular misconceptions. For example, many people believe that spring water is actually "pure" water. On the contrary, spring water contains many of the same impurities found in drilled wells (Johnson, 1976).

Most water resources face a host of serious threats, all of which are caused primarily by human activities and these results to pollution (Obire and Aguda, 2015). In many locations the contamination of surface and subsurface waters by domestic human activities causes poor health and sickness to persons who use this water for drinking (Obire and Aguda, 2015).

Several springs are located in Ihitte/Uboma Local Government Area of Imo state Nigeria. These locations are hilly and mountainous. The communities in Ihitte/ Uboma LGA do not have access to public water supply. The inhabitants solely depend on these spring water source for drinking and domestic use and believe the spring water is totally "pure". They therefore consume these spring water without any form of treatment. There has also been no report on bacterial quality of these spring waters. Human activities that can pollute spring water in Ihitte/Uboma LGA includes: Improper collection of the spring water when humans go to collect water for consumption, without putting off their footwear. This could result to fecal contamination and any other possible contaminants attached to the foot wear (Benka-Coker and Ojior, 1995). Furthermore, when the containers used by humans is unclean and carried to the spring water location, it is possible that the water can get contaminated by these vessels. Most of the families also carry out activities such as clothes washing close to the spring. Chemical fertilizers and pesticides are used by farmers in Ihitte/Uboma LGA to protect their crops. These chemical fertilizers and pesticides get mixed with the water due to rainfall because the rain washes off these chemicals down to the spring water thereby causing pollution (Johnson, 1976). Drinking water quality management has been the foundation for the prevention and control of water borne diseases. Hence there is need to ascertain the water quality of spring water in these rural communities.

## Materials and Methods

### *Study Area*

The spring water samples were collected from different locations within Ihitte/ Uboma Local Government Area in Imo state Nigeria. These locations are hilly and mountainous. First sample was from Wogba spring in Amainyi community, the other was Oturugo spring in Umuihi community and the third was Inyenta spring in Umuoma community. Imo State lies in the South East of Nigeria with Owerri as its capital and largest city (Vanguard, 2015). It is Located in the south-eastern region of Nigeria and occupies the area between the lower River Niger and the upper and middle Imo River. Imo State is bordered by Abia State on the East, River Niger and Del-

ta State to the West, Anambra State on the North and Rivers State to the South (Vanguard, 2015). The State lies within latitudes 4°45'N and 7°15'N, and longitude 6°50'E and 7°25'E with an area of around 5,100 sq km (Imo, 2010).

### *Collection of Spring Water Samples*

Sterile glass bottles were used in the collection of water samples from each spring source. The spring water was allowed to run for a while and then the bottles were used in collecting them aseptically and covered immediately to avoid contamination. Samples were collected from three (3) different spring water locations. The bottles were properly labeled as (A, B, and C) respectively. Temperature readings of the water samples were taken using a mercury thermometer and recorded. The samples were then transferred into a cool box containing ice cubes to reduce the metabolic activity of microorganisms in the samples. The cool box containing the water samples was immediately taken to the laboratory for further analysis within 3 hours. All the spring water samples were processed immediately on arrival.

### *Sample Processing*

The spring water samples were processed by testing and recording some physiochemical properties before conducting the microbiological analysis. The temperatures of all the water samples were recorded using a mercury thermometer. Temperature readings were recorded at the spring site before transportation to the laboratory. The pH of all the water samples was recorded using Hanna pH meter.

### *Microbiological Analysis of Spring Water Samples*
### *Isolation and Enumeration of Aerobic Heterotrophic Bacteria*

Serial dilution was done on each water sample. The dilution factor was up to $\times 10^{-5}$. The process was carried out so as to obtain discrete colonies when plated on the medium. One millimeter (ml) of each water sample was transferred into 9ml of normal saline and further dilutions were made up to $x10^{-5}$. This was then inoculated on a nutrient agar plate by plating out 0.1ml aliquots of various dilution factor (i.e $\times 10^{-3}$, $\times 10^{-4}$ and $\times 10^{-5}$) respectively. A sterile bent glass rod was used to perform the spread plate method. It was incubated at 37°C for 24hours. Discrete colonies grown on the plate (overnight) were counted; the average was recorded as Total heterotrophic counts of Aerobic bacteria. Pure cultures were collected aseptically, inoculated on agar slants and incubated at 37°C overnight and stored in the fridge as stock cultures for further biochemical tests. A total of Nine (9) stock cultures were stored.

### *Estimation of Coliform and Faecal Coliform Bacteria in the Spring Water Samples*

Estimation of the coliform bacteria in the spring water samples was conducted using the most probable number (MPN) technique. Reaction to MPN technique and thermotolerant coliform bacteria MPN index/100ml of each water sample was done using double strength MacConkey broth for 10ml of sample and single strength Mac-

Conkey broth for 0.1ml and 1ml of the sample. The test for the estimation of coliforms involves the following steps: presumptive, confirmatory and completed test. It was performed as described by Verma *et al*., (1999).

### *Characterization and Identification of Bacteria in Spring Water Samples*

Pure cultures of bacteria were subjected to the following characterization tests performed in duplicates. Gram staining, catalase test, coagulase test, urease test sugar fermentation test, methyl red test, indole test and acid gas test were carried out as described by Cappuccino and Macfaddin (2005) and Kirk *et al*., (2005). The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics in accordance with methods described by Cruikshank *et al.,* (1975) and with reference to Holt (1977).

## Results

The mean values of temperature of the spring water samples ranged from 19 to 23$^{o}$C, while the pH value was 7.1 and all three springs recorded the same pH value. Wogba spring recorded the highest temperature value while Inyenta spring water sample recorded the lowest temperature value. Oturugo spring recorded a mean temperature value of 21$^{o}$C

The mean values of the total aerobic heterotrophic bacteria counts indicated high microbial counts and microbial pollution of the spring waters. the values obtained ranged from 1.9 x $10^7$ cfu/ml to 5.64 x $10^7$ cfu/ml. Wogba spring recorded the highest mean count while Inyenta spring recorded the lowest count. On the other hand, Oturugo spring recorded 2.40 x $10^7$cfu/ml.

The reaction of the spring water samples to MPN techniques to total coliform count showed that, Wogba spring recorded the highest coliform count of 17 MPN Index/100ml and Oturugo spring recorded the lowest count of 4 MPN Index/100ml, while Inyenta spring recorded a coliform count of 9 MPN Index/100ml.

The reactions of the spring water samples to the MPN technique of Thermotolerant Coliform showed that Wogba spring recorded the highest Thermotolerant Coliform count of 20 MPN Index/100ml and Oturugo spring recorded the lowest count of 6 MPN Index/100ml, while Inyenta spring recorded a Thermotolerant Coliform count of 9 MPN Index/100ml.

The bacteria that were isolated from the spring water samples and their incidence (%) were *Escherichia coli* (39%) which occurred in all the spring water samples while *Salmonella* (18%) and *Shigella* (14%) occurred in Oturugo and Inyenta spring water samples. On the other hand, *Klebsiella* (19%) and *Bacillus* species (10%) occurred both in Wogba spring and Inyenta spring water samples. All the bacteria isolated during the study occurred in the Inyenta spring water.

The incidence (%) of bacteria showing the error bars with percentage in each of the spring water samples is shown in Figure 1. Analysis of variance (ANOVA) using F-Test on the data obtained showed that, the calculated F value (0.8276) was less than the tabular F value at 5%. There was therefore no significant difference at $p \geq 0.05$ in the incidence of bacteria between the different spring water samples.

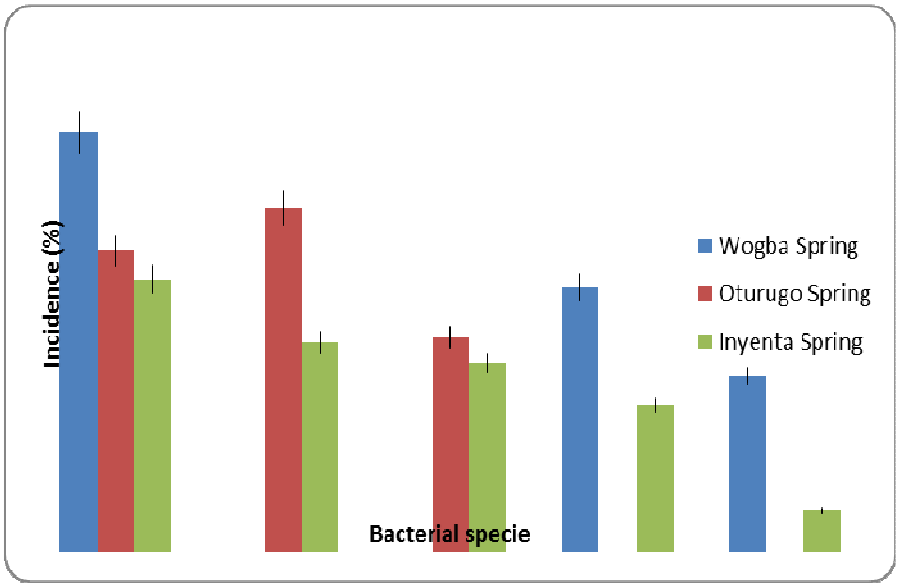


Fig. 1: Incidence (%) of bacteria in the different spring water samples

## Discussion

The present study showed the presence of Aerobic Heterotrophic bacteria, Total coliform and Thermotolerant coliform bacteria in the different spring water samples in Ihitte/Uboma Local Government Area of Imo state. The investigation of each spring water sample showed that Wogba spring water has the worst sanitary surrounding when compared with Oturugo spring water and Inyenta spring water and this affected the results obtained.

In the course of this study Wogba spring water has more bacteria count as well as more Total coliform and Thermotolerant coliform counts. In relation to counts of bacteria Inyenta spring water is of better quality while Wogba spring water is of worse quality. In relation to coliform counts Otorugo spring water is of better quality while Wogba spring water is of worse quality. Wogba spring water recorded the highest count of *Esherichia coli* than Oturugo spring water and Inyenta spring water. The presence of *Esherichia coli* indicates faecal contamination of water and this result reflected the unsanitary condition of the surroundings of Wogba spring water. The bacterial quality of springs in Chinthapali Mandal has also been reported to be unsatisfactory with coliforms count far exceeding the recommendation levels by WHO (Naraina *et al*., 1995). Due to lack of sanitation, improper waste disposal 40% or more of the disease outbreaks were attributed to consumption of polluted ground water (Naraina *et al*., 1995). The analysis of spring water in Ebonyi south zone in Nigeria were also revealed to be unsafe for human consumption with regards to high acidity and total coliform counts, here some level of treatment such as liming is required to rid the water of high acidity (Chesbrough,2010). In many developing countries, availability of water has become a critical and urgent problem and it is a

matter of great concern of families and communities depending on non-public water supply system. Confirmation with microbial standards is of special interest because of the capacity of water to spread diseses within a large population. The objective anywhere is to reduce the possibility of spreading water borne diseases to the minimum in addition to being pleasant to drink and must be wholesome and potable (Edema *et al*., 2011).

Accesses to safe water are universal needs and indeed basic human rights; however, many of the world's population lack access to adequate and safe water. Use of improved sanitation facilities is low especially in Sub-Saharan Africa and South Asia. Eight hundred eighty four million people in the world still do not get their spring drinking-water from improved sources; Sub-Saharan Africa accounts for over a third of that number. Fresh water has become a scarce commodity due to over exploitation and pollution. Increasing population and its necessities have led to the deterioration of surface and sub surface water (WHO/UNICEF, 2010).

The greatest risk to public health from microbes in water is associated with consumption of drinking-water that is contaminated with human and animal excreta. Human faeces can contain a variety of intestinal pathogens which cause diseases ranging from mild gastro-enteritis to the serious dysentery, cholera and typhoid. The most predominant waterborne disease, diarrhea, has an estimated annual incidence of 4.6 billion episodes and causes 2.2 million deaths every year (Shyamala, 2008). Children are the main victims of diarrhea and other faecal-oral disease, and also the most likely source of infection (WHO, 2011). Total and fecal coliforms have been enumerated from various water sources. The presence of fecal coliforms indicates the contamination of water with fecal material than may contain pathogenic organisms. These indicator bacterial were found to be more common in unprotected water sources than protected water sources (Opisa, 2012).

From the results obtained, in as much as all the isolates are potential pathogens Wogba spring water is of better bacteriological quality because of the absence of *Salmonella* sp and *Shigella* sp which are enteric organisms. Benker-coker and Ojior (1995) stated that any form of human activity obviously will influence the indication of these microorganisms in spring water since it has been believed in the 70's that the water from this source is considered pure. Despite this belief one has to be careful while consuming spring water (Benker-coker and Ojior, 1995). Water can support the growth of many types of microorganisms. However, the presence of other disease causing microbes in water is unhealthy and even life threatening (Rheinheimer, 1991).

Comparing the data of the bacteriology of all the spring water samples to the guidelines of drinking water quality (WHO, 1996), the spring water sources are not potable and are not fit for direct human consumption without treatment. This study has therefore revealed that, spring water is not 100% pure and is not safe for human consumption without treatment due to the fact that contamination is inevitable.

## Conclusion

The bacterial quality of the different spring water samples from different communities in Ihitte/Uboma local Government area of Imo state showed the presence of

pathogenic microorganisms. The different spring water sources which serve primarily as drinking water in these rural communities contains microbial load which revealed microbial contamination as showed in this study.

Spring is a place where natural outflow of ground water occurs. Therefore a spring source should be protected from human activities and spring water should be treated properly before human consumption. This should be taken seriously as microbial contamination poses great threat to our water resources which results in waterborne diseases caused by pathogenic microorganisms found in water. Awareness on spring water contamination should be made known to inhabitants in various rural areas. The public should also be enlightened on the fact that spring water is not 100% pure as its contamination is inevitable.

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