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Seasonal Variety on Bacteriological Evaluation of Borehole Waters in Orumba South Local Government Area in Anambra State

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Authors' contributions

This work was carried out in collaboration between all authors. Author AME designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author ENC managed the analyses of the study. Author CCE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was carried out to determine the seasonal variety on physico-chemical and bacteriological evaluation of Borehole waters.
Place and Duration of Study: 10 selected borehole tanks in 5 different towns were collected from five towns from (ORS) Orumba South L. G. A; (Umunze, Onneh, Nawfija, Isulo and Ezira) in Anambra State, with one hundred and twenty samples each in both dry season (December, January, February) as January (peak of dry season) and rainy season (May, June, July) as June (peak of rainy season) within 2018.
Methodology: A total of one hundred and twenty water samples were analyzed. (1) ml of water sample was added to 10 ml single strength of the Lactose fermentation broth and serially diluted. The isolated bacterial isolates were identified using their morphological characteristics, biochemical tests, microscopical and molecular characteristics. The DNA was extracted from the identified isolates and analyzed by 16S rRNA. The bacteria isolated from the studied water samples were identified to be *Bacillus subtilis* (BTC), *Escherichia coli* (RSS), *klebsiella aerogenes* (TSS) and *staphylococcus aureus* (GY), PC – *Providencia stuartii*, BCD – *Bacillus toyonensis*, FY- *Bacillus*

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spp and ANT – *Proteus* spp. Frequency rate of occurrence in percentage for the isolates in dry and rainy seasons in ORS L. G. A were determined and for removal of all the isolates were within the WHO limit of 0.08ct (mg.min L^{-1}) by the use of chlorine solution. Confirmatory profile on bacteriological in chlorine treated borehole water samples was determined.

Results: The highest TCC was recorded for sample 10 which was 27 MPN/100ml estimated during rainy season against (TCC) recorded for samples 10 which was 22 MPN/100ml during dry season. Out of eight isolates, *Escherichia coli* had the highest rate frequency of occurrence of 70% in rainy season against dry season. All the isolates 1 to 8 range from (0.01ct) (mg.min L^{-1}) to (0.06ct (mg.min L^{-1}) all the values were within the WHO recommended limit for water purity. The confirmatory test on sample locations form ORS 1 to ORS 10 for total coliform count in chlorine treated borehole water samples by most probable number estimation revealed that all the samples were within the WHO permissible limit of 0.00MPN/100ml.

Conclusions: Chlorine solution may have proven to be the cheaper and better solution for achieving water purity in boreholes water.

Keywords: waters; bacteria; seasons; borehole.

1. INTRODUCTION

Water is life and quality management of water resources is an integral constituent of the new paradigm for sustainable development i.e. one that permit the readily improvement in living standards without getting the rid of the weak natural resources of rivers, marine and groundwater system [1]. Groundwater is made up of 97 percent of the world's readily accessible freshwater and support the rural, urban, industrial and irrigation water supply needs of 2 billion people all over the world [2]. As the more readily accessed surface water resources are before expected time being overused and densely polluted, pressure on groundwater is increasing [3]. The World Health Organization (WHO) estimated that 1.8million people in developing countries die every year from diseases related to unsafe water and inadequate sanitation [4]. Nigeria is a developing country; drinking polluted water has undesirable results for Nigeria and other developing countries since the availability of good quality drinking water is vital for the wellbeing of all people and the socio-economic development of a nation [5,6]. Due to the failure of governments to meet the ever increasing water demand via the public water works, people devise to alternative source such as boreholes. There is an approach that groundwater is an infinite resource [7]. Groundwater pollution occurs when surface water makes its way into the ground surface water contains various contaminants such as organic chemicals, inorganic chemicals that occur naturally in the soils, sediments and potential pathogens which may degrade the groundwater quality [8]. It is reported that 80% of all illness in developing countries is associated to water and sanitation [4]. Problems of water scarcity and poor sanitation issues account for approximate 10% of all deaths relating to water-borne or sanitation-related diseases in Nigeria [4] Orumba South Local Government Areas of Anambra State is an upcoming L.G.A which, in recent pass have experienced rise in population due to Federal Technical College Umunze in Orumba South L.G.A in Anambra State. Currently, Orumba South L.G.A lacks an adequate solid waste management system and this has resulted to in Umunze and Ezira dumpsites located in these areas [9]. These conditions pose a threat on the groundwater quality. These areas are also known as agricultural bases, dominated by cassava, yam, rice and corn farms which use pesticides, fertilizers and herbicides in the farms; may contaminate the groundwater through infiltration. However people have continued to use this water without testing, mainly due to ignorance or lack of awareness to all these risks surrounding the resources. Bashir et al. [10] studied a total of three water samples which were collected from available boreholes within the major sites of Arkilla area namely; Arkilla layout, Arkilla Federal low cost and Arkilla state low cost. They were analysed for the total bacterial, *coli*form and faecal *coli*form counts, using the standard plate count and most probable number (MPN) assays. Obtained results were compared with (WHO) standards for drinking water sources. The mean total bacterial count ranged from 5.4 x 10^4 to 3.7 10^6 cells/ ml, whereas, the total *coli*form counts of the water samples ranged from 12 – 16 MPN/100 ml. The faecal *coli*form count ranged from 0 – 1 MPN ml. General bacterial genera encountered were *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. The bacterial load recovered from the studied borehole water samples were

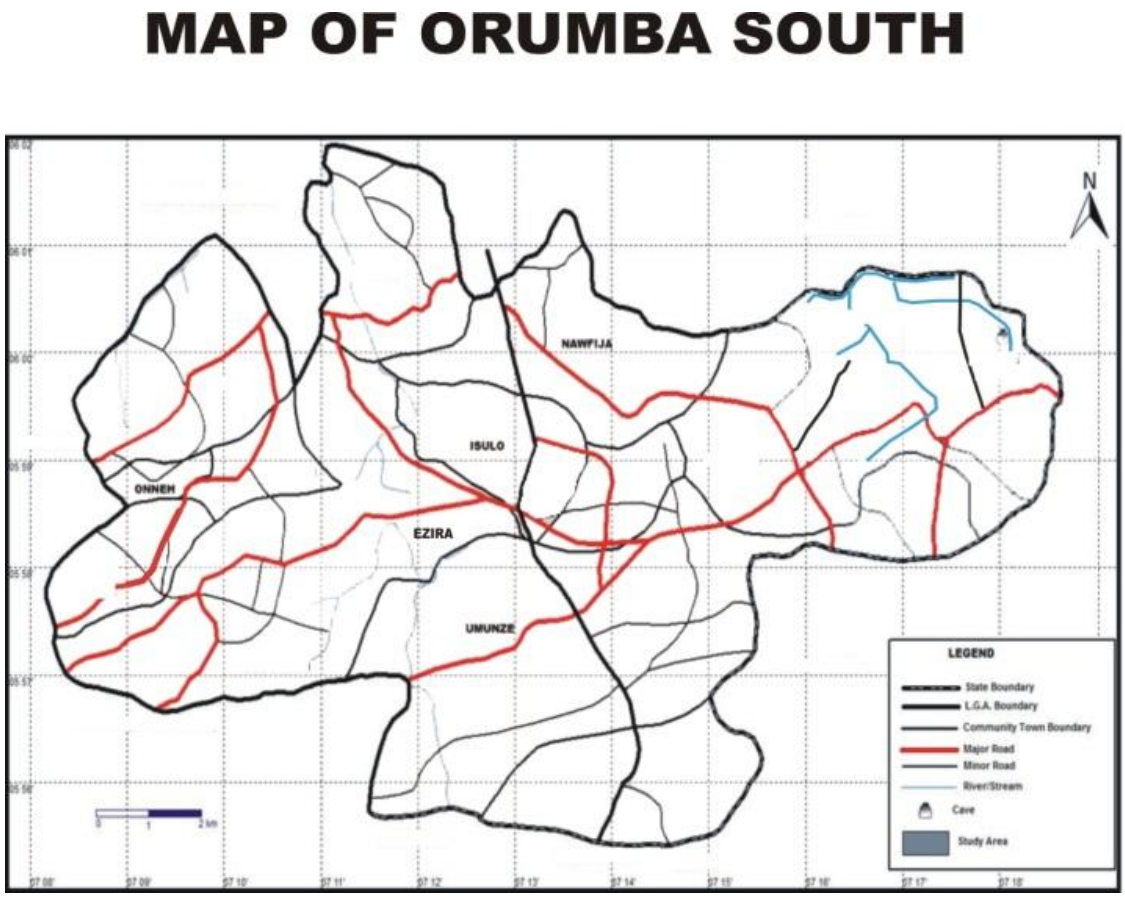

Fig. 1. Map of Orumba South showing distribution of sampled locations in the study Area. source [7]

above the WHO standard for bacterial loads, and *coli*form content. Therefore, current results suggested that some of the borehole waters in Wamakko local government area, Nigeria; were no safe for drinking. The presence of contaminants that deviate from World health organization (WHO) guideline values has been associated with the occurrence of different kinds of waterborne diseases such as typhoid fever, dysentery, gastro intestinal and infectious hepatitis etc. Access to adequate urban water supply and water Corporation are serious problems facing the inhabitants in Orumba South L.G.A of Anambra State. Dumping of solid wastes, siting of pit latrines close to boreholes, disposal of untreated sewage are common practices in the Orumba South Metropolis which may indirectly affect groundwater quality. More so, there are no policies or strategies to guide the management of groundwater resources [4].

2. MATERIALS AND METHODS

2.1 Collection of Water Samples for Bacteriological and Treatment Analysis

Drinking water samples selected from ten boreholes tanks in South Local Government Area in Anambra State were collected aseptically from each site with the help of new high-density PET screw-capped containers of 1 L capacity and they were labeled according and sterilized with alcohol overnight placed in ice pack coolers. Water from the borehole was allowed to run for 5 minutes, immediately followed by reduction in the water flow in order to avoid splashing during filling of bottles. Gases were removed from the bottles by filling and emptying the bottles before the collection of actual samples. At each site 8 bottles were filled with water, one each for bacteriological and chlorine treatments. And then

transported to the laboratory for bacteriological and treatment Analysis and stored at 4^{o}c in refrigerator. A total of 80 water samples were collected from 10 selected borehole tanks in 5 different towns; five town from ORS (Umunze, Onneh, Nawfija, Isulo and Ezira) with 40 samples each in both rainy season (May, June, July) as June (peak of rainy season) and dry season (December, January, February) as January (peak of dry season) and when the temperatures were warmer, in order to establish the seasonal variety on bacteriological parameters present in the water.

2.2 Source of the Water Guard and Hth Samples

The water guard and HTH in this study were manufactured by Tuyil Pharmaceutical Industries Limited and bought at Bridge Head, Onitsha in Onitsha South Local Government Area in Anambra State, Nigeria.

2.3 Bacteriological Analysis

2.3.1 Most probable number (MPN)

As stated by [11], in this method, the three basic tests to detect *coli*form bacteria in water were presumptive, confirmatory and completed tests. The tests were determined using Lactose Fermentation Broth (LFB) and Levine's Eosine Methylene Blue agar (EMB). They detected the presence of *coli*form bacteria (indicators of faecal contamination) that fermented lactose with the production of acid and gas that was detectable following a 24 h incubation period at 37ºC.

2.3.2 The presumptive test

This was done by adding 10 ml of each water sample onto 10 ml of double strength (two times the normal quantity of agar to the same volume of distilled water during the preparation of the medium). Lactose fermentation broth in each three set of five test tubes, 1 ml of sample to 10 ml single strength of the broth and 0.1 ml of sample to 10 ml single strength of the broth. Inverted Durham tube was put into each of these test tubes before the sample and both was added. This set up was left for 24h at a temperature of 37ºC; after which positive tube evidenced by acid (change of colour of broth from purple to yellow).

2.3.3 The confirmatory test

This was carried out by subjecting gas produced to confirmatory test while the negative ones were left for another 24 h. using an inoculating loop; an aliquot from each of the first set up was transferred to a new set up with the same number of tubes and left for 24 h.

2.3.4 The completed test

This was carried out by inoculating freshly prepared and cooled Levine's EMB agar with cultures from the positive tubes in the confirmatory test for 24 h at the same temperature. The presence of nucleated colonies with dark center was evidence of the presence of *coli*forms. The MPN of coliform bacteria in 100 ml of water was determined using the MPN statistical probability table based on the number positive tubes in the presumptive test.

2.4 Isolation and Purification of the Isolates

Tenfold serial dilution was carried out in which 9 ml of distilled water (diluent) was dispensed into each test tubes in order to reduce the microbial load and allow for picking of distinct colonies,. A dilution factor of 10^{-3} was used and inoculation was carried out by aseptically inoculating 1.0 ml of the sample on Nutrient agar using pour plate method and the incubation was carried out inverted at 37ºC for 24h. After 24 h incubation, the grown colonies were sub-cultured by aseptically streaked a single colony on sterile poured plate, and this was incubated at 37ºC for [12].

2.5 Characterization and Identification of Isolates

Pure cultures of the isolates were examined for colonial appearances such as colony colour, surface, optic characteristics, size, elevation and edges and of colonies [13].

2.6 Molecular Identification of Isolates

Identification of the isolates was done phenotypically and molecularly.

2.7 Microscopic Characterization

2.7.1 Gram staining

The isolated bacteria were stained using gram staining technique to differentiate between gram positive bacteria and gram negative bacteria.

2.8 Treatment of Water Samples

As stated by the standard method of [14]. The Water samples from the different sites were poured into clean measuring glass cylinders to ascertain their volumes. The standard recommended dosage of 2 drops of 8.25% chlorine solution was added in each 1 L or quart of water. Based on the volume obtained, the corresponding volume of water guard and HTH were weighed out on a measuring balance and added to the cylinder and thoroughly mixed for 2 minutes and shaken. The water guard and HTH solutions treated water were allowed to stand for 30 minutes before samples were taken for analysis. Temperature and pH at contact time (ct) 1 mg each of both water guard and HTH solutions were made up to each of 10 mg of deionized water in two different graduated test tubes which were compared to each blank control.

2.9 Determinations of Chlorine Treated Water Samples

The chlorine treated water samples were determined following the analytical standard methods of APHA, [14].

2.10 Statistical Analysis

The prevalence rate of the bacterial isolates was ascertained using the T – test with the level of significance set at $p < 0.05$.

3. RESULTS

3.1 Isolated Organisms

The microscopic, morphological and biochemical characteristics of the isolates; The organisms were identified as *Bacillus subtilis*, *Escherichia coli*, *Providencia stuartii*, *klebsiella aerogenes, Bacillus toyonensis, Staphylococcus aureus, Bacillus s*pp. and *Proteus* spp.

4. DISCUSSION

This study has proven that borehole water is also a repertoire of some micro-organisms. It contains some members of the enteric bacteria such as *E. coli*, *Klebsiella aerogenes* and *Proteus* spp. The organisms were identified as *Bacillus subtilis*, *Eschericha coli*, *Providencia stuartii*, *klebsiella aerogenes*, *Bacillus toyonensis*, *staphylococcus aureus*, *Bacillus* spp. and *Proteus* spp. This finding differs from that of [15] who did not find *E. coli* or any other enteric bacteria samples from groundwater. However, the works of [16] identified *Shigella* and *Salmonella* spp. as enteric organisms found in borehole water. According to the authors, the presence of enteric organisms in water could be as a result of proximity of sewage systems to the groundwater source. Thus, contamination could be as a result of leaching. [16] isolated *Klebsiella aerogenes* and *Proteus mirabilis* at a frequency of occurrence 38% each higher in rainy season while this research work as shown in (Fig. 2 & 3) isolated *Escherichi coli* at a highest occurrence of frequency 70% in rainy season and *Providencia stuartii*, *Bacillus toyonensis* and *Proteus* spp. recorded least value of 5% in dry season both in Orumba South L.G.A. Total *coli*form counts (TCC) across the samples for rainy and dry seasons are shown in (Table 1 and 2). The highest TCC was recorded for sample 10 which was 27 MPN/100ml estimated during rainy season while the lowest (TCC) was recorded for samples 10 which was 22 MPN/100ml during dry season. TCC was more during rainy season in Orumba South L.G.A. This finding is similar with the work of Otieno et al. [17] explained that this could be as a result of more volume of water during rainy season, that carry along dirt prevalent in this L.G.A which could be of faecal origin and leach them into the groundwater source from where bore-hole water is obtained. However, dry season is with less volume of water and thus, possibilities of top soil and its components leaching into underground water is grossly reduced. The World Health organization (WHO) standard for *coli*form count of safe drinking water is 0.00 MPN/ml. These parameters were significantly ($p<0.05$) seen more in rainy season than in the dry season at their respective depths. From the above discussion, it could be deduced that sample locations ORS 10 and 5 remarked highest in this bacteriological parameter (total *coli*form count) respectively of water samples which significantly ($p<0.05$) increased in rainy season than in dry season of some samples from ORS L.G.A, could be that drinking water from groundwater sources were sited close to sewage septic tanks, pit latrines, agricultural land. [18], recorded the similar work on the use of chlorine solution in killing microbial contaminants (e.g. Bacteria) and removal of 99% of physical and chemical contaminants in boreholes water in order to achieve purity in borehole water, who revealed that disinfection treatment applied to borehole water samples minimized micro biological

properties of the treated water samples. The result of treatment profile values of the samples collected from sampling borehole water in ORS L.G.A in Anambra State as shown in (Table 3). WHO standard for achievability by the use of chlorine solution in water samples is 0.08ct mg.min L^{-1}. From isolate 1 to 8 ranges from (0.01ct) (mg.min L^{-1}) to (0.06ct (mg.min L^{-1}) all the values were within the WHO recommended limit for water purity. The result of the confirmatory test on sample locations form ORS 1 to ORS 10 for total *coli*form count in chlorine treated borehole water samples by most probable number estimation in (Table 4). revealed that all the samples were within the WHO permissible limit of 0.00MPN/100ml. This finding was similar to the work of [19] revealed that disinfection treatment applied to borehole water samples minimized the microbiological properties.

Table 1. Determination of *coli*form count of borehole water in dry season by Most Probable Number estimation in Orumba south

Sample locations	LB2X-10 Tubes					LB1X-1 Tubes					LB1X-0.1 Tubes					Reading	MPN /100ml	Range 95% Probability
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5			
ORS1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	2	<1.0 -7.0
ORS2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	2	<1.0 -7.0
ORS3	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	010	2	<1.0 -7.0
ORS4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	000	<2	<1.0 -7.0
ORS5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	000	<2	<1.0 -7.0
ORS6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	000	<2	<1.0 -7.0
ORS7	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	110	4	<1.0-11.0
ORS8	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	010	2	<1.0 -7.0
ORS9	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	2	<1.0 -7.0
ORS10	+	+	+	+	-	-	+	+	-	-	-	-	-	-	-	420	22	7.0 -67.0
WHO																		

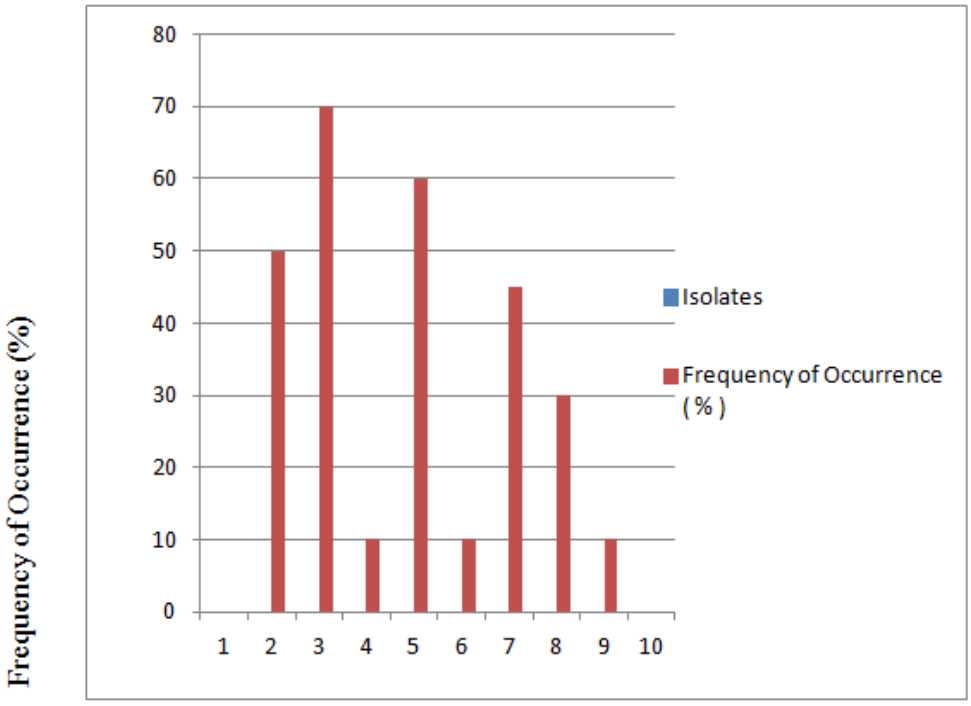

Fig. 2. Frequency rate of occurrence in percentage for bacterial isolates in water samples in rainy season in Orumba South Local Government Area

Table 2. *Coli*form counts of borehole water in rainy season by Most Probable Number estimation In Orumba South

Sample locations	LB2X-10 Tubes					LB1X-1 Tubes					LB1X-0.1 Tubes					Reading	MPN /100ml	Range 95% Probability
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5			
ORS1	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	2	<1.0 -7.0
ORS2	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	2	<1.0 -7.0
ORS3	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	010	2	<1.0 -7.0
ORS4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	000	<2	<1.0 -7.0
ORS5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	000	<2	<1.0 -7.0
ORS6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	000	<2	<1.0 -7.0
ORS7	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	110	4	<1.0-11.0
ORS8	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	010	2	<1.0 -7.0
ORS9	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	2	<1.0 -7.0
ORS10	+	+	+	+	-	-	+	+	+	-	-	-	-	-	-	430	27	9-78
WHO																	**0.00**	

Table 3. Treatment Profile

S/N	Isolates	Chlorine Ct (mg-min L^{-1})	pH	T (^{o}c)
1	*Bacillus subtilis*	0.03	7.00	<2
2	*E. coli*	0.05	7.00	<2
3	*Klebsiella aerogenes*	0.04	7.00	<2
4	*Providencia stuartii*	0.02	7.00	<2
5	*Bacillus toyonensis*	0.03	7.00	<2
6	*Staphylococcus aureus*	0.06	7.00	<2
7	*Bacillus spp*	0.02	7.00	<2
8	*Proteus spp*	0.01	7.00	<2
	WHO standard	0.08		

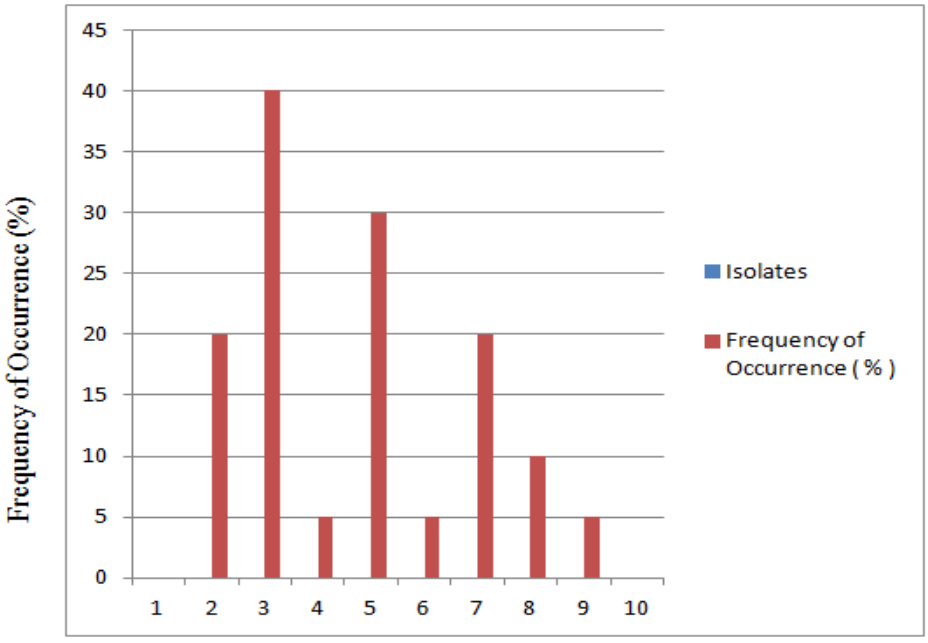

Fig. 3. Frequency rate of occurrence in percentage for bacterial isolates in water samples in dry season in Orumba South Local Government Area

Table 4. Confirmatory profile on total *coli*form count for chlorine treated borehole water samples by Most Probable Number estimation

Sample locations	MPN/100 ml
ORS1	0.00
ORS2	0.00
ORS3	0.00
ORS4	0.00
ORS5	0.00
ORS6	0.00
ORS7	0.00
ORS8	0.00
ORS9	0.00
ORS10	0.00
WHO	0.00

5. CONCLUSION

This research work has been able to show that borehole water sources can be pure to bacteriological, contamination which eventually turns to a health hazard to man. Therefore it is imperative that such water be tested and treated before use by man either for direct drinking or for industrial purposes in order to curb cases of intoxication or illness from untreated water consumption. Contamination of groundwater is more pronounced during the rainy season. Lastly boreholes should not be sited close to sewages, dump sites or related environment to avoid presence of leachates in the groundwater. Chlorine solution may have proven to be the cheaper and better solution for achieving water purity in boreholes water.

CONTRIBUTION TO KNOWLEDGE

Having discovered bacteria contaminant in some of these borehole waters and the use of cheap, safe and environmental friendly chlorine treatment for absolute eradication of them in water samples, subsequent measures should be carried out to ascertain whether fungi; moulds and yeasts may be found and also use the same treatment strategies for them.

ETHICAL APPROVAL

There were no ethical issues in the collection of the samples.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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