



BIOBURDEN (QUALITY) OF DIFFERENT DRINKING WATER SAMPLES

O. Obire^{1*}, Ramesh.R.Putheti^{2*} and Igoni.O.Abigail¹

¹Department of Applied and Environmental Biology,
Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt, Nigeria

^{2*}Member, American Association of Pharmaceutical Scientists, 10314-E, Malcolm
circle, Cockeysville, Maryland, 21030. USA.

E-mail: rutwikusa@yahoo.com

ABSTRACT

The bacteriological quality of various drinking water samples of treated pipe-borne (tap) water, borehole water and well water collected from five (5) locations within "Town" Area of Port Harcourt in Nigeria was evaluated using the standard plate count method and the most probable number (MPN) technique. The temperature and pH mean values recorded ranged from $22.2^{\circ}\text{C} \pm 1.48^{\circ}\text{C}$ to $23.4^{\circ}\text{C} \pm 1.95^{\circ}\text{C}$ and from 7.16 ± 0.055 to 7.36 ± 0.055 respectively. Total aerobic heterotrophic bacterial counts ranged from $7.72 \times 10^3 \text{cfu/ml} \pm 6.42 \times 10^3 \text{cfu/ml}$ to $12.2 \times 10^3 \text{cfu/ml} \pm 7.29 \times 10^3 \text{cfu/ml}$. While the total coliform and faecal coliform MPN index/100ml ranged from 0 to 1800+ and from 0 to 900 respectively. Analysis of variance (ANOVA) using F-test showed that there was significant difference at $p \leq 0.01$ in pH and in faecal coliform MPN and at $p \leq 0.05$ in the other parameters determined. The bacteria isolated include *Chromobacterium* spp., *Corynebacterium* spp., *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Streptococcus* spp., and their percentages ranged from 5% to 15%. The order of decreasing bacteriological quality of samples is treated tap water > borehole water > well water. The presence of *E. coli* and enteric pathogens such as *Shigella*, *Enterobacter* etc., indicated the contamination of the various water sources with faecal matter implying that they are not suitable for drinking.

Key Words: drinking water, bacteriological quality, coliforms, *E. coli*, faecal indicator.

INTRODUCTION

There are various sources of water for drinking and household use. These include rainwater, surface water (streams, rivers, springs, lakes etc.) and underground water (shallow wells and deep wells and springs). Surface water is easily polluted, either by direct contamination by man and animals, or indirectly when rain washes faeces and other pollutants from the banks into the water body. Shallow wells are liable to pollution by seepage from surface water¹⁰.

Port Harcourt City lies within Longitude $7^{\circ} 10'$ E and latitude $4^{\circ} 50'$ N in the south eastern part of the Niger Delta which is fluvial in origin and consists of unconsolidated, massive and porous sands. There is no properly organized potable water supply system in the City. The majority of the populations in Port Harcourt are not supplied with potable water and they are compelled to use unsafe water for drinking and domestic purposes. Many shallow boreholes have been drilled and wells dug into the formation for drinking and domestic water supply. In majority of cases, the water is usually consumed raw without any form of treatment.

Improper and indiscriminate dumping of untreated wastes of various kinds around residential areas is common sight in Port Harcourt City⁸. Sources of water pollution include effluents of untreated sewage that are dumped directly into water bodies, runoffs containing faecal materials, leaking pipes run in gutters or drainages, domestic effluents containing large microbial populations that are involved in degradative processes, and hospital effluents, etc. A suitable water source should provide a supply that is adequate and safe. It should be free from chemical and biological hazards contaminants, and acceptable in terms of its taste, colour and softness⁷.

Faecal pollution of drinking water may introduce various forms of intestinal pathogens which may cause mild diseases like mild gastroenteritis to severe and sometimes fatal dysentery, diarrhoea, cholera, typhoid and hepatitis A etc⁶. In order to protect public health and to ensure that water is safe for public use, any water intended for drinking, treated or untreated, piped or un-piped must meet certain microbiological standards. A violation of set standards warrants treatment of the present source or the need for an alternative water supply^{12,9}

The microbiological analysis of water is determined by the use of indicator organisms since the specific disease organism present in water are readily not identifiable and the techniques are complex and time consuming⁹. The relative degree of contamination in terms of single easily performed test is normally used and the two most popular methods are the Most Probable Number (MPN) Technique and Membrane Filter Technique.

The broad objective of this research is to determine the Total aerobic heterotrophic bacteria counts, Total coliform bacteria count, Total thermotolerant coliform bacteria count using the MPN technique, and types of bacteria present in various drinking water sources such as treated pipe-borne water (Tap water), borehole water and well water in 'Town' area of Port Harcourt. The aim is to determine the efficiency of the treatment process of the 'tap' water, the bacteriological quality of the various water samples with the view of ascertaining the potability or health hazards posed by the levels of contamination of these various sources of drinking water in 'Town' area of Port Harcourt.

EXPERIMENTAL

Description of study area

Various drinking water samples from treated tap water, boreholes and wells were collected from five (5) different study areas as follows; The Nigeria Prison Service Staff Quarters, the Nigeria Custom Services Staff Quarters, I.B Johnson Street, Aggrey Road, and the Nigeria Ports Authority Staff Quarters, all located in *Bundu* within 'Town' area of Port Harcourt, Nigeria. These study areas are designated as locations A, B, C, D, and E respectively. The study areas were chosen because of the large population density and poor infrastructural amenities. The only form of treatment given to the treated tap water is chlorination after collection from a borehole, while the borehole water and well water so referred to in this study did not receive any form of treatment.

Collection of water samples, temperature and pH determinations

From each location, three samples comprising tap water, borehole water and well water were aseptically collected into separate sterile containers and the temperature reading was read off with the aid of Mercury in glass thermometer and covered immediately to avoid contamination. Three readings were recorded and the average temperature calculated and recorded for each sample. The containers were appropriately labeled and taken to the laboratory. All the water samples were processed immediately for analysis within one hour of collection. The pH of each water sample was measured by use of automatic digital pH meter (Model METTLE DELTA-340) made in England.

Cultivation, enumeration and isolation of aerobic heterotrophic bacteria

One milliliter (ml) of each water sample was transferred into 9ml of normal saline and further dilutions were made up to $\times 10^{-2}$. Serially diluted samples were then inoculated onto nutrient agar plates in duplicates by plating out 0.1ml aliquots of 10^{-1} and 10^{-2} dilutions. The spread plate method using a sterile bent glass rod was used. Cultured plates were incubated at 37°C for 24 hours. Discrete colonies that developed on each plate (overnight culture) were counted, their average calculated and recorded as Total Heterotrophic Counts of Aerobic Bacteria. Pure cultures were collected aseptically, inoculated onto freshly prepared agar plates and incubated at 37°C overnight to further purify the isolates. Purified isolates were inoculated onto nutrient agar slants and incubated at 37°C overnight and stored in the refrigerator as stock cultures for further biochemical tests. A total of forty (40) isolates were stored as stock cultures.

Analysis of water samples for coliform and thermotolerant or faecal coliforms

The Most Probable Number (MPN) technique was used for the water analysis. The procedure for the MPN method for total coliform and faecal coliform in which three dilutions (10 ml, 1ml, and 0.1 ml) of each sample were used was adapted from APHA^{1,2}.

Characterization and identification of coliforms

A total of forty (40) isolates stored as Stock cultures in the fridge for further identification were plated onto freshly prepared nutrient agar plates and incubated at 37°C to obtain discrete colonies. Twenty-four (24) hours cultures were used for morphological and physiological or biochemical characterization. The identification of the isolates was accomplished by comparison of their characteristics with those of known taxa of Cowan and Steel⁴.

RESULTS AND DISCUSSION

The values reported in the results section are means of replicate samples. The temperature values recorded for the various water samples ranged from 20 to 26°C while the pH values ranged from 7.1 to 7.4 (slightly alkaline) The mean values of temperature recorded for the tap water, borehole water and well water samples in 'Town' area were 22.2°C ± 1.48°C, 23.4°C ± 1.82°C, and 23.4°C ± 1.95°C respectively; while their pH mean values were 7.16 ± 0.055, 7.2 ± 0.14, and 7.36 ± 0.055 respectively. Analysis of variance (ANOVA) using F-test on the data obtained showed that there was significant difference in temperature between the various water samples at $p \geq 0.05$ and in their P^H at $p \geq 0.01$. The result of the evaluation of total aerobic heterotrophic bacteria count is as shown in Table 3. From the samples analyzed, the results indicated very high microbial counts for the various drinking waters.

Table-1: Population count (cfu/ml) of total aerobic heterotrophic bacteria in the various drinking water samples in "Town" area of Port Harcourt City

Location	Treated Tap water	Borehole water	Well water
A	0.72 x 10 ⁴	0.43 x 10 ⁴	2.32 x 10 ⁴
B	0.18 x 10 ⁴	1.68 x 10 ⁴	1.50 x 10 ⁴
C	0.60 x 10 ⁴	0.34 x 10 ⁴	0.42 x 10 ⁴
D	0.25 x 10 ⁴	1.21 x 10 ⁴	0.82 x 10 ⁴
E	2.30 x 10 ⁴	0.20 x 10 ⁴	1.02 x 10 ⁴
Total	4.05 x 10 ⁴	3.86 x 10 ⁴	6.08 x 10 ⁴
Mean	0.81 x 10 ⁴	0.772 x 10 ⁴	1.216 x 10 ⁴
Standard Deviation	±0.86353	±0.64163	±0.729417

Analysis of variance (ANOVA) using F-test on the data obtained showed that there was significant difference in total aerobic heterotrophic bacterial count between the various water samples at $p \leq 0.05$. Estimation of total coliform bacteria and thermotolerant coliform bacteria counts in water using the MPN technique for water is as shown in Table 2.

Table-2: Total coliform bacteria (TCB) and Thermotolerant coliform bacteria (TtCB) MPN Index/100ml of the various drinking water samples in "Town" area of Port Harcourt

Location	Treated Tap water		Borehole water		Well water	
	TCB	TtCB	TCB	TtCB	TCB	TtCB
A	14	10	1800+	550	140	150
B	25	6	550	550	1600	900
C	2	9	70	50	80	25
D	0	0	0	0	0	0
E	45	20	0	0	110	55
Total	86	45	2420	1150	1930	1130
Mean	17.2	9	484+	230	386	226
Standard Deviation	±18.51215	7.280	±770.7334	292.831	±680.6467	381.04133

Analysis of variance (ANOVA) using F-test on the data obtained showed that there is significant difference between the various drinking water samples in the total coliform MPN at $p \leq 0.05$ and in faecal or thermotolerant coliform bacteria MPN at $p \leq 0.01$. The fifteen (15) bacteria genera isolated

and percentages of occurrence were; *Acinetobacter* spp. (5%), *Bacillus* spp.(15%), *Chromobacterium* spp.(7.5%), *Citrobacter* spp.(2.5%), *Corynebacterium* spp. (7.5%), *Escherichia coli*(12.5%), *Enterobacter* spp. (7.5%), *Flavobacterium* sp. (2.5%), *Klebsiella* spp.(5%), *Proteus* spp. (5%), *Pseudomonas* spp. (2.5%), *Salmonella* spp.(5%), *Shigella* spp.(12.5%), *Staphylococcus* spp.(5%), *Streptococcus* spp.(5%). All these bacteria with the exception of *Proteus* were isolated from the well water samples; nine (9) including *E. coli*, *Salmonella* and *Shigella* were isolated from the borehole water while only four (4):– *E. coli*, *Proteus*, *Staphylococcus* and *Streptococcus* were isolated from the treated tap water.

The present study has shown the presence of aerobic heterotrophic bacteria, coliform and faecal coliform or thermotolerant bacteria (*E. coli*) and the various types of bacteria in the various drinking water samples. The study has therefore revealed the bacteriological quality or status of drinking water in the 'Town' area of Port Harcourt. The pH of the drinking water samples was about neutral or slightly alkaline.

Counts of total aerobic heterotrophs in the untreated water samples (Borehole and well water) appeared very high. It is therefore desirable to disinfect all supplies of drinking water before consumption or use. The presence of *Pseudomonas* was common and their presence is of significant value in determining the extent of water pollution. Environmental bacteria such as *Acinetobacter* and *Bacillus* sp., which are mostly saprophytic in origin were isolated from borehole and well waters. It was also found that 80% of the various water samples were positive for coliform MPN showing high contamination and risk to public health. The count for faecal coliform obtained were high and far above recommended standards. The detection of faecal coliform indicates faecal pollution of the drinking water. The presence of faecal indicators such as *E. coli* and *Shigella* sp and enteric pathogens such as *Chromobacterium*, *Enterobacter*, indicated that the various water sources are polluted with faecal matter. Pathogens such as *Klebsiella*, *Staphylococcus*, and *Streptococcus* were also isolated.

The sanitary conditions and standard of living of inhabitants in the various locations in decreasing order was D>C>B>E>A. Many of the pathogens isolated in this study which are of public health concern were isolated from locations E and A. The various sources of drinking water seems to contain a high microbial load as revealed in this study. The international standards for drinking water states that potable water should not contain 100cells of heterotrophic bacteria per 100ml of water, but unfortunately, the bacteria counts obtained in this study superceded the standard. Therefore, water used directly for drinking in 'Town' poses threat to public health.

For the Presumptive Coliform test, the World Health Organization^{12,3} guidelines for both treated and untreated water samples is 0/100ml, but in occasional untreated water samples, 3/100ml are allowed on the condition that these would not be found in consecutive water samples. The coliform group is an indicator bacteria that is used to evaluate the quality of drinking water and any presence of coliforms indicates the contact of water with sewage or inadequate treatment or post treatment contamination. In un-piped (well) water supplies, sometimes up to 10 coliforms/100ml are allowed for WHO standards for tropical countries, but this should not occur repeatedly; if occurrence is frequent and sanitary condition cannot be improved, an alternative source must be found if possible¹¹. In order to protect public health and to ensure that water is safe for public use, any water intended for drinking, treated or untreated, piped or un-piped must meet certain microbiological standards. A violation of set standards as shown in the results of the various drinking water samples in Port Harcourt warrants treatment of the present source or the need for an alternative water supply^{12,5}.

There were indications in the study that there was lesser contamination in treated (tap water) sample than in borehole and well water (untreated/non-chlorinated water samples). However, the faecal indicator *E. coli* was also isolated from the treated tap water. Contamination must have occurred during the course of distribution through leaky pipes. Thus, the direct consumption of water from these various sources could contribute to the spread of many infectious diseases and may be the cause of serious epidemic rampaging the Port Harcourt city. The results suggest that efficient and proper sanitary check in drinking water

supplies has to be executed regularly in view of its great public health significance and at the same time good observation of personal and household hygiene has to be emphasized.

CONCLUSION

The bacteriological quality of various source of water in 'Town' area of Port Harcourt showed failure to meet the zero faecal and non-faecal coliform WHO standards. The greatest threat posed to water resources arises from microbiological contamination which has long been a concern to public health. Water contamination with potential pathogenic microorganisms represents an obvious health risks for inhabitants living in 'Town'. Inhabitants with low personal and household hygiene are greatly affected by a wide range of microbial contamination. Contamination of these water sources will continue unless effort is put into pollution prevention. Pollution control strategies should include; Public health training, awareness of methods of transmission of pathogens, and organized waste disposal system, along with practical steps at Community and Government levels in addressing the issue must not be ignored. Introduction of easy-to-handle water treatment techniques in the 'Town' or 'Bundu' area of Port Harcourt will ensure drastic reduction in the magnitude of occurrence of faecal coliforms and enteric pathogens in drinking water. Unless the situation is rectified on this basis with particular reference to adequate adoption of sanitary measures for provision of potable water, the problem will not be over in the near future as desired.

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REFERENCES

1. APHA. Standard methods for the examination of water and wastewater. 20th edn. American Public Health Association, American Water Works Association and Water Environment Federation. USA. ISBN 0-87553-235-7, ISSN 55-1979(1998)..
2. H. J. Benson, Bacteriological examination of water: Qualitative tests. In: Microbiological Applications. Laboratory Manual in General Microbiology. 7th Edn. McGraw-Hill, New York. Pp. 208 –211, (1998)
3. S. T. Cowan and K. J. Steel, Manual for the identification of medical bacteria. Cambridge University Press. London(1965)..
4. EPA, Report of Task Force on Guide standard and Protocol for testing Microbiological water purifiers. United States Environmental Protection Agency, Vol. 1, pp 1 – 50(2000)..
5. E. E. Geldreich, Sanitary Significance of Faecal Coliforms in the Environment. (Water Pollution Control Research Series, publ. Wp-20-3) FWPCA, USDI, Cincinnati, Ohio(1966)..
6. G. Lucas, and R. R. Gilles, *Journal of Clinical Pathology*, **9**, 368(1990)..
7. O. Obire, O. Nwaubeta, and S. B. N. Adué, *Journal of Applied Science and Environmental Management*, **6(1)**, 78 – 83, (2002).
8. S. Sloat, C. Zeal, and C. Jay, HACH Technical Center for Applied Analytical Chemistry. Colorado, U. S. A(1991)..
9. C. Van Nosatrand. and A. Wilson, *Journal of General Microbiology*, **13**, 155(1983)..
10. World Health Organization, Drinking Water and Sanitation, 1981 – 1990: A way to Health. pp 1-56(1981)..
11. World Health Organisation, Guidelines for Drinking Water Quality. Vol. 2. pp 1 – 29(1993)..

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