African Journal of Applied Zoology & Environmental Biology. 2005. Vol. 7, 116 – 119
ISSN 1119-023X
www.uniportjournals.com/ajazeb

BACTERIOLOGICAL ANALYSIS OF BOREHOLE WATER IN ULI, NIGERIA

[1]S. N. Ibe* and [2]J. I. Okplenye
[1]DEPARTMENT OF MICROBIOLOGY,
UNIVERSITY OF PORT-HARCOURT, PORT-HARCOURT, NIGERIA.
[2]DEPARTMENT OF MICROBIOLOGY,
ANAMBRA STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY,
ULI, ANAMBRA STATE, NIGERIA.

ABSTRACT

Water samples from four different boreholes within Uli, Anambra State, Nigeria, were collected for five consecutive weeks for bacteriological analysis to assess the potability.The range of the means was from $1.5x10^2$ to $5.9x10^4$ cfu ml^{-1} for total aerobic bacterial load, 9 to 136 MPN per 100ml for total coliforms and 4 to 74 MPN per 100ml for faecal coliforms. The highest counts were consistently found in the sample from Cagramento Lodge, where the borehole was located in an unsanitary environment, near a pit laterine. *Escherichia coli, Klebsiella sp., Proteus sp., Enterobacter sp., Pseudomonas sp.,* and *Staphylococcus aureus* were isolated from the samples. The findings show that the water from all the boreholes did not meet the World Health Organization Standards for Drinking Water and should be treated or boiled and filtered before drinking.

Key words: Faecal coliforms, borehole water, Uli, Nigeria

INTRODUCTION

In developing countries including Nigeria, where the majority of people live in rural areas, rivers, streams, wells and more recently boreholes, serve as the main sources of water for drinking and domestic use. The underground water supplies are usually considered safe provided they are properly located, constructed and operated according to the World Health Organization Guidelines for Drinking Water (WHO, 1971).

Main origins of pollution of wells and boreholes are industrial, domestic and agricultural and pollution can be continuous or accidental. Industrial pollution may involve seepage of used water containing chemicals such as metals and radioactive compounds, or contaminated water from damaged pipelines infiltrating into the borehole. Domestic pollution may involve seepage from broken septic tanks, pit latrines, cesspools and privies. Agricultural pollution is from irrigation water and run off water after rains, carrying fertilizers, pesticides, herbicides and faecal matter. Environmental pollution is mainly from sea water intrusion into coastal aquifer. The WHO recommends that boreholes should be located at least 30m away from latrines and 17m from septic tanks (Chukwurah, 2001; Wagner and Lanoix, 1969).

Microorganisms of concern in contaminated water include the following bacterial agents of diarrhea and gastroenteritis namely *Salmonella sp., Shigella sp., Escherichia coli and Vibrio cholerae (*Birmingham *et al.,* 1997). Protozooal agents of diarrhea include *Entamoeba histolytica, Giardia lamblia, Balantidium coli* (Jawetz *et al.* 1991) *and Cryptococcus pervum* (Kelly *et al.* 1997). Enteroviruses causing various clinical ailments, not necessarily diarrhea, but are transmitted by water include Poliovirus, Rotavirus, Hepatitis A virus (Hejkal *et al.* 1982) and Hepatitis E virus (Benjelloun *et al.* 1997).

Presence of faecal coliforms or *Escherichia coli* is used as an indicator for the presence of any of these water borne pathogens (Chukwurah, 2001; Okafor, 1985; Okpokwasili and Akujobi, 1996). WHO recommends that no faecal coliform be present in 100ml of drinking water. Good quality water is odourless, colourless, tasteless and free of faecal contamination and chemicals in harmful amounts. This study was therefore carried out to determine the bacteriological quality of water from boreholes located in the vicinity of the Anambra State University of Science and Technology, Uli. Students and other members of the community pay for the water and it was important to find out if the water was safe for drinking and domestic use and to recommend treatment if necessary or suggest measures to be taken to eliminate the source of pollution.

***Correspondence Author: S. N. IBE**
E-mail: **sibe_ahs@yahoo.com**

MATERIALS AND METHODS

SAMPLING LOCATIONS

Water samples were collected from boreholes used as sources of domestic water by students and other members of the community in Uli town in Ihiala Local Government Area of Anambra State, Nigeria. The samples were collected at weekly intervals for 5 weeks from the following students' hostels: (A) Victory Lodge, (B) Doggy Hostel, (C) Cagramento Lodge and (D) Global Hostel.

COLLECTION OF SAMPLES

Cotton wool soaked in 70% (v/v) ethanol was used to sterilize the nozzle of the borehole from which samples were collected. The tap was allowed to run for two minutes before sterile 250ml screw capped glass bottles were carefully uncapped and filled with the water and recapped. Water samples were transported to the laboratory in a cooler with ice for bacteriological analysis within two hours of collection.

TOTAL HETEROTROPHIC BACTERIAL COUNT

The spread plate method was used. Water samples were serially diluted using sterile 1ml pipettes and 9ml sterile physiological saline as diluent. Aliquots of 0.1ml of undiluted water sample and water at dilutions of 10^{-1} and 10^{-2} were plated on Nutrient agar (Oxoid) plates in duplicates. The plates were incubated at 37^0C for 24h, before enumeration.

TOTAL COLIFORM AND FAECAL COLIFORM COUNT

PRESUMPTIVE TEST

Coliform count was obtained using the three tube assay of the Most Probable Number (MPN) technique (Speck, 1976). Presumptive coliform test was performed using MacConkey broth (Oxoid). The first set of three tubes had sterile 10ml double strength broth and the second and third sets had 10ml single strength broth. All the tubes contained Durham tubes before sterilization. The three sets of tubes received 10ml, 1ml and 0.1ml quantities of water samples using sterile pipettes. The tubes were incubated at 37^0C for 24-48h for estimation of total coliforms and at 44.5^0C for faecal coliforms for 24-48h and examined for acid and gas production. Acid production was determined by color change of the broth from reddish purple to yellow and gas production was checked for by entrapment of gas in the Durham tube.

The MPN was then estimated from the MPN table for three tube test.

CONFIRMED TEST

Confirmed test was carried out by transferring a loopful of culture from a positive tube from the presumptive test into a tube of Brilliant Green Lactose Bile (BGLB) broth (Oxoid) with Durham tubes. The tubes were incubated at 37^0C for 24-48h for total coliforms and 44.5^0C for faecal coliforms and observed for gas production.

COMPLETED TEST

Completed test was carried out by streaking a loopful of broth from a positive tube onto Eosine Methylene Blue (EMB) agar plate for pure colonies. The plates were incubated at 37^0C for 24-48h.Colonies developing on EMB agar, were further identified as coliforms or faecal coliforms (*Escherichia coli*) using cultural characteristics, morphology and biochemical tests. For faecal coliforms, colonies with green metallic sheen were Gram stained and the IMViC test was carried out on Nutrient agar stock cultures and used to identify the colony as *E. coli*. The MPN per 100ml water was calculated using the completed test.

IDENTIFICATION OF BACTERIAL ISOLATES

Stock cultures of the isolates with different cultural characteristics were made on nutrient agar slants. Gram staining was used to check for morphology and biochemical tests were performed to aid in identification. Various tests performed and used in probable identification of isolates included the oxidase test, motility test, catalase test, urease test, coagulase test, indole test, methyl red test, Voges-Proskauer test and citrate utilization test (Treagan and Pulliam, 1982).

RESULTS

Table 1 shows the range and mean values of total bacterial counts, total coliform counts and faecal coliform counts of water samples collected at weekly intervals over a period of 5 weeks. Total heterotrophic bacterial counts of the four boreholes (A, B, C and D) were relatively low except for sample C, the Cagramento Lodge borehole. The counts for sample A ranged from 4.0 x 10 -2.5 x 10^2 cfu ml^{-1} with a mean value of 1.4 x 10^2 cfu ml^{-1}, sample B ranged from 2.0 x 10 – 1.6 x 10^3 cfu ml^{-1} with a mean value of 4.8 x 10^2 cfuml^{-1}, sample C which had the highest values ranged from 2.5 x 10^3 – 1.5 x 10^5cfu ml^{-1} with a mean value of 5.9 x 10^4 cfu ml^{-1} and sample D ranged from 5.0 x 10 – 2.5 x 10^2 cfu $ml^{-1}$with a mean value of 1.5 x 10^2 cfu ml^{-1}.

TABLE 1 THE RANGE AND MEAN VALUES OF TOTAL BACTERIAL COUNTS, TOTAL COLIFORM COUNTS, AND FAECAL COLIFORM COUNTS

Water sources	Total heterotrophic bacteria count (cfu ml^{-1})	Total coliform counts (MPN/100ml)			Faecal coliform count (MPN/100ml)	
	Range	Mean	Range	Mean	Range	Mean
A	$4.0 \times 10 - 2.5 \times 10^2$	14×10^2	9-11	10	3-7	5
B	$2.0 \times 10 - 1.6 \times 10^3$	4.8×10^2	7 –11	9	3 –7	4
C	$2.3 \times 10^3 - 1.5 \times 10^5$	5.9×10^4	64-240	136	64-93	74
D	$5.0 \times 10 - 2.5 \times 10^2$	1.5×10^2	11	11	7	7

Total coliform counts for the samples were also highest sample C, with a mean count of 136 MPN per 100ml while samples A, B and D had mean counts of 10, 9 and 11 MPN per 100ml respectively.

Faecal coliform count was highest for sample C, with a mean value of 74 MPN per 100ml. Samples A, B and D had values of 5, 4 and 7 MPN per 100ml respectively.

Based on the cultural characteristics, morphology and the results of biochemical tests, six isolates were identified as *Proteus sp., Escherichia coli, Pseudomonas sp., Enterobacter sp., Staphylococcus sp. and Klebsiella sp.* as shown in Table 2.

TABLE 2 CHARACTERIZATIONS AND POSSIBLE IDENTIFICATION OF ISOLATES FROM BOREHOLE WATER

Isolates	Morphology	Gram Stain	Urease	Methyl Red	Indole	VP	Citrate	Catalase	Oxidase	Coagulase	Motility	Glucose	Maltose	Probable Identification
1	Rods	-	+	+	+	-	-	+	-	-	+	A	A	*Proteus sp*
2	Rods	-	-	+	+	-	-	+	-	-	+	A/G	-	*Escherichia coli*
3	Rods	-	-	-	-	-	-	+	+	-	+	A	-	*Pseudomonas sp*
4	Rods	-	-	-	-	+	+	+	-	-	+	A/G	A/G	*Enterobacter sp*
5	Cocci	+	-	+	-	-	-	+	-	+	-	A	A	*Staphyiococcus sp*
6	Rods	-	+	-	-	+	+	+	-	-	-	A/G	A/G	*Klesiella sp*

DISCUSSION

Water suitable for human consumption (potable water) should be free from disease producing organisms or large numbers of non-pathogenic organisms.

The borehole water from three locations (Victory Lodge, Doggy Lodge, Global Allen) had considerably lower heterotrophic bacterial counts and total coliform counts and could be concluded to be of better quality for domestic use than the Cagramento Lodge water which had much higher counts of both bacteriological parameters.

Regarding the faecal coliform counts, even though the Cagramento water had much higher mean value of 74 MPN per 100ml compared to counts of 4 to 7 MPN per 100ml for the other 3 boreholes, it can be concluded that water from all the boreholes are not fit for drinking without processing (WHO, 1971; WHO, 1986; USEPA, 2001). WHO and United States Environmental Protection Agency Standard for faecal coliform in drinking water is zero faecal coliform per 100ml. Therefore water from all the boreholes should be boiled and filtered for clarity before drinking.

The observations in this study support the fact that high heterotrophic counts in water reflect high coliform counts and the presence of faecal coliforms. The presence of high faecal coliform count in sample C could be attributed to the proximity of the borehole to a pit latrine located near the borehole at a distance less than the 30m recommended by WHO and the general unhygienic environment surrounding the borehole. It could be that the pipes used for water distribution were rusty thus allowing seepage of microbial contaminants into the borehole. In addition, the bacterial isolates from the water

belong to genera of potential pathogenic bacteria, hence the recommendation that water from all the boreholes need to be boiled before use.

There is need to increase awareness of the community towards the dangers associated with the use of contaminated water; the danger in constructing pit latrines and septic tanks near a water source and *vice versa;* the use of rust-free polyvinyl chloride (PVC) pipes for water distribution and treatment of water by boiling and filtering before use for drinking and cooking.

REFERENCES

Benjelloun, S., B. Bahbouhi, N. Bouchrat, L. Chericaoni, N. Had, J. Mahjour, and A. Bensumane (1997) Seroepidemiological study of an acute Hepatitis E outbreak in Morocco. *Research Virology* **148**: 279-283.

Birmingham, M.E., L. A. Lea, N. Ndayiminje, S. Nkurikiye, B.S. Hersh, J.G.Wells and M.S. Ijeming (1997) Epidemic cholera in Burundi, Patterns of transmission in the Gadat Rift Valley Lake Region. *Lancet* **349**: 981-983.

Chukwurah, E.I. (2001) *Aquatic Microbiology*. Otoba Press Limited, Onitsha, Nigeria.

Hejkal, T.W., B. Keswick, R.L. Labelle, C.P. Gerba, V. Sanchez, G. Dreesman, B. Hafkin and J.L. Melnick (1982) Viruses in a community water supply associated with an outbreak of gastroenteritis and infectious Hepatitis. *Journal of the American Water Works Association* **74**: 317-321.

Jawetz, E., J.L. Melnick and E.A. Adelberg (1991) *Medical Microbiology* (19th ed.) Appleton and Lange, Norwalk. Connecticut.

Kelly, P.B., K. S. Ndubani, P. Nchito, N.A. Luo, R.A. Feldman and M.J. Farthing (1997) Cryptosporidiosis in adults in Lusaka, Zambia and its relationship to oocyst contamination of drinking water. *Journal of Infectious Disease* **176**: 1120-1125.

Okafor, N. (1985) *Aquatic and Water Microbiology*. Fourth DimensionPublishers, Enugu, Nigeria.

Okpokwasili, G.C. and T.C. Akujobi (1996) Bacteriological indicators of tropical water quality. *Environ. Toxicol. Water Quality* **11**: 77-82.

Speck, M.L. (1976) *Compendium of Methods for the Microbiological Examination of Foods*. American Public Health Association, Washington, DC.

Treagan, L. and L. Pulliam (1982) *Medical Microbiology Laboratory Procedures*. W.B. Saunders Co. Philadelphia.

USEPA (2001) Current Drinking Water Standards. United States Environmental Protection Agency, Washington, DC.

Wagner, E.G. and J.N. Lanoix (1969) Water Supply for World Health Organization General Monograph Series No. 42.

WHO (1971) Guideline for Drinking Water Quality. World Health Organization. Geneva.

WHO (1986) International Standards for Drinking Water Quality. World Health Organization. Geneva **1**:99-120.