International Journal of Bacteriology Research ISSN: 2392-5690 Vol. 2 (2), pp. 028-031, April, 2014. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

The bacteriological survey of borehole waters in Peri-Urban areas of Abakaliki; Ebonyi State, Nigeria

****Uhuo, C.A, *Uneke B.I, Okereke C.N, Nwele D.E and Ogbanshi M.E**

[1]Department of Applied Biology, Ebonyi State University, Abakaliki.
[2]Department of Biochemistry, Ebonyi State University, Abakaliki.

Accepted 22 April, 2014

The study on the bacteriological examination of borehole water was conducted in peri-urban areas of Abakaliki Ebonyi State. A total of forty samples were assessed for the presence of coliform bacteria using Most Probable Number techniques (MPN). The study revealed that some coliform bacteria which make water unsafe for drinking were implicated from these samples assayed and thus, recorded as follows: *E. coli, (65.5%) klebsiella aerogenes* (8.64%), *Bacillus sp (*6.25 %), *Salmonella sp (*10.5%) and *Bacilla subtilis (8.62%)* were isolated from the water samples assessed, where *E. coli* (65.5%) was recorded higher among others from samples collected at Ukwuachi road and Umuoghara. The statistical analysis ($p<0.05$) showed that Ukwuachi and Umuoghara boreholes showed high bacteria load compared to others hence the quality of the borehole waters for drinking. From the standpoint of bacteriological analysis, all borehole water in these peri-urban areas did not meet the World Health Organization Standards and should be boiled before drinking to reduce or avoid infectious microorganism which constitute the coliform bacterium in water making it unsafe for drinking. Since most households in these peri-urban areas solely rely on borehole water as their main source of drinking and usage for domestic activities but, hence contaminated, the consequences could be fatal due to the public health risk and dangers associated with drinking such contaminated water.

Key words: Bacteriological, examination, borehole, water, Abakaliki.

INTRODUCTION

Water is of fundamental importance to human life, animals and plants. It is of equal importance with the air we breathe in maintaining the vital processes of life and it makes up about 60% of body weight in human. Among the various sources of water, borehole water is known to be more appropriate and often meet the criteria of water quality. The most widely used source of water in most African countries, Nigeria inclusive is borehole water. Water is consumed universally in large quantities and when polluted or contaminated brings about broad spread of infections (Geidreich, 1996). Water contamination is a very dangerous situation health wise because almost all degradation routes follows a chain like system and leads finally to underground water. The ingestion of faecally contaminated water has been a source of worry to health workers and has been associated with high mortality and morbidity rate in developing countries (WHO 2005). This is because of the kinds of disease that is caused by these organisms arising from contaminated water. The quality of borehole water is the resultant of all the processes and reactions that act on the water from the moment it condensed in the atmosphere to the time it is discharged by a well or spring and varies from place to place and with the depth of the water table (Gardon, 2001). Borehole waters have unique features, which renders them suitable for public water supply (Okpokwasili and Akujobi, 1996). Borehole water is particularly important as it accounts for about 88% safe drinking water in rural areas, where population is widely dispersed. The term coliform bacteria describes a group of gram negative enteric bacteria which includes *Escherichia coli, Salmonella, Shigella* and *Klebsiella*. They are facultative anaerobic non-spore forming rods that may or may not be motile.

They are able to ferment lactose to produce acid and gas within 24 hours at 35^{O}C. Although they are generally not harmful themselves, they indicate the possible presence of pathogenic bacteria, viruses and protozoa.

*Corresponding author. E-mail:coscusanas@gmail.com

These organisms are widely distributed in the intestine of humans and warm blooded animals and are the predominant facultative anaerobes in the bowel and parts of the essential intestinal flora that maintains the physiology of a healthy host (APHA, 2000). Borehole water is particularly important as it accounts for about 88% safe drinking water in rural areas, where population is widely dispersed and the infrastructure needed for treatment and transportation of used surface water does not exist.

Microorganisms of concern in contaminated water include the following bacterial agents of diarrhea and gastroenteritis namely *Salmonella sp*, *Klebsiella spp*, *Escherichia coli* and *Vibrio cholerae* (Gardon,2001). Protozoal agents of diarrhea include *Entamoeba histolytica*, *Giardia lamblia, Balantidium coli* and *Cryptosporidium pervum* (Kelly *et al,* 1997). Entero-viruses causing various clinical ailments, not necessarily diarrhea, but are transmitted by water include poliovirus, Rotavirus, Hepatitis A virus and Hepatitis E virus (Hagedorn *et al,* 2003). Good quality water is odourless, colourless, tasteless and free of faecal contamination and chemicals in harmful amounts. The number of outbreaks that have been reported throughout the world demonstrats that transmission of pathogens by drinking water remains a significant cause of illness (Narayan and Tao,1981). WHO recommends that boreholes should be located at least 30m away from latrines and 17m from septic tanks

This study was therefore carried out to determine the bacteriological contamination of the borehole waster used by rural dwellers and the general public who pay to buy the water for their general uses. This borehole water is regarded as the only source of water mostly during dry seasons when there is extreme scarcity of water. This will direct if the water is safe for drinking and other domestic uses and thus recommend treatment if necessary or suggest measures to be taken in eliminating the source(s) of contamination.

MATERIALS AND METHODS

Sampling Locations

One liter of water samples were collected from various boreholes used for domestic activities by rural dwellers and general public in peri-urban areas of Abakaliki Ebonyi State. The samples were collected once weekly from the following places (A) Ezza Road (b) Umuoghara, (C) Ukwuachi, (D) Oriuzor (E) Ishieke (F) Amike Aba.

Collection of Samples

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

Media Preparation

Medias used were prepared in Applied Biology laboratory of Ebonyi State University Abakaliki. Samples and materials used were sterilized with an autoclave, which provided moist heat to kill the organisms present.

Preparation of Mac-Conkey Broth, Single and Double Strength used

For each sample of water, 15 test tubes of MacConkey broth were needed. 5 test tubes contained 10ml double strength of broth, 5 test tubes contained 10ml single strength of broth and 5 test tubes contained 5ml single strength broth; all the tubes were fitted in Durham tubes. About 100ml of double strength broth 70kg of the MacConkey broth powder was weighed out and dissolved in 100ml of distilled water 10mls of the broth was dispensed and autoclaved at 121^0C for 15min. While for single strength, 35g of MacConkey was dissolved in distilled water and dispensed into fermentation tubes fitted with Durham vials and lastly, autoclaved at 121^0C for 15min.

Preparation of Eosine Methylene Blue Agar used

Eosine methylene blue agar was prepared by dissolving 44g of the powder in 150ml of distilled water, heated to dissolve and autoclaved at 121^0C for 15minutes. It was allowed to cool to 45^0C and then dispensed into 10 sterile disposable Petri dishes, allowed to gel and tested for sterility. Sterile plates were then used for culturing.

Preparation of Brilliant Green.

Brilliant green was prepared by dissolving 60g of the powder in 200ml of distilled water, heated to dissolve and autoclaved at 121^0C for 15minutes. It was allowed to cool to 45^0C and then dispensed into 10 sterile disposable Petri dishes, allowed to gel and tested for sterility. Sterile plates were then used for culturing.

Gram Staining

This technique involved making a thin smear of the culture from each bottle and stained with different dyes to

Table 1. Presumptive Coliform Tests Result. No: of tubes inoculated.

Sample sources	No of samples collected	10ml SS	10ml DS	5ml SS	No of tubes positive	MPN of Coliforms in 100ml of Water (CFU/ml).
Ezza Rd	10	55	55	55	3 1 1	10
Umuoghara	5	25	25	25	5 0 0	25
Ukwuachi	5	25	25	25	2 2 1	15
Oriuzor	13	70	70	70	2 0 1	7
Ishieke	5	25	25	25	2 2 0	9
Amike Aba	6	30	30	30	3 0 1	11

Key note:
10ml DS = 10ml double strength MacConkey broth
5ml SS = 5ml single strength MacConkey broth.
CFU = Colony forming units.

Table 2. Confirmatory Result.

Sample sources	Colony colours that determines the microorganisms presence	Organism(s) identified
Ezza Rd	Whitish mucoid with smooth surface and another margin formation showing white colonies with small black at center.	*Bacillus sp* and *Salmonella*
Umuoghara	Dirty white creamy, with smooth surface and rough edge colonies	*Bacillus subtilis, E.coli*
Ukwuachi	Yellow creamy colour with smooth surface and edge and greenish metallic sheen on Eosine methylene blue Agar.	*E.coli*
Oriuzor	White colonies with small black at center.	*Salmonella*.
Ishieke	White, creamy flat surface colonies	*Bacillus sp.*
Amike Aba	White mucoidal rough surface colonies and Greenish metallic sheen.	*Klebsiella aerogenes.*

distinguish them into either Gram- positive or Gram negative organisms. This also helped to identified the various shapes of the organism present. The technique is carried out thus; the organism from the agar slant was collected using a flame sterilized wire loop and smeared on a clean glass slide with a drop of distilled water. It was allowed to dry then the slide was passed over the flame to heat fix the organism. The smear was covered with slow running clean water. Lugol's iodine was used to cover the smear for another 30seconds and rinsed. Then the smear was decolorized with alcohol, rinsed and covered with safaranin red, which was allowed for 30seconds and then rinsed. The slides were allowed to drain on a draining rack after which it was viewed under the microscope, first with 40x objective lens and then with 100x oil immersion lens. The color and shapes of the organisms were noted.

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. 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 tubes.

Confirmed Test

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 carried out confirmed test. 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 into Eosine Methylene Blue (EMB)

Table 3. Characterizations and Possible Identification of Isolates from Borehole Waters.

Isolates	Morpho-logy	Gram Stain	Catalase	Oxidase	Citrate	Methyl Red	Indole	Voges Proskauer	Probable Identificat-ion
1	Short rods	+	+	-	+	-	-	+	*Bacillus sp*
2	Short rods	+	+	-	+	+	+	-	*Bacillus subtilis*
3	Short rods	-	+	-	+	+	+	-	*E. coli*
4	Short rods	-	+	-	+	-	-	-	*Salmonella sp*
5	Short rods	+	+	-	+	-	-	-	*Bacillus sp*
6	Short rods	-	+	-	+	+	-	-	*Klebsiella aerogenes*

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 slant. 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 include the oxidase test, catalase test, indole test, methyl red test, Voges- Proskauer and citrate utilization test (Cheesbrough, 2000).

RESULTS

Presumptive Coliform Tests Result

Positive tubes turned from purple to yellow with gas produced at the Durham vials. The numbers of tubes showing acid and gas were noted and recorded.

The colonies formed were examined under the microscope and some were found to contain greenish metallic sheen colour, while some had white mucoid colours and some colonies were yellow creamy in colour. Since colonies were formed, completed test was carried out**.**

DISCUSSION

Water suitable for human consumption should be free from disease producing organisms or large numbers of non- pathogenic organisms. Access to good quality drinking water is a challenge in most towns and cities in Nigeria and households have for years depended on other sources of water to supplement their activities. The introduction of borehole waters to consumers was to provide safe, hygienic and affordable drinking water to the public. Although this is a laudable idea, current trends seem to suggest that site or location of bore-hole drinking water sources could be a route of transmission of diseases.

The borehole water from Ezza road, Oriuzor, Ishieke and Amike Aba has considerably low faecal bacteria counts and could be deduced to be of better quality for domestic use and drinking than the Umuoghara and Ukwuachi waters which had high counts of coliform bacteria. This could be that samples from this areas had a very low environmental hygiene and that the pipe from which the borehole emerge from is either rusted and form some growth like that of algae, spirogyra and colours of cynobacteria which may actually give rise to the bacteria growth.Regarding the faecal coliform counts, even though the Umuoghara and Ukwuachi waters had much higher values of 25MPN and 15MPN per 100ml respectively compared to counts of 7 to 11MPN per 100ml for the other four boreholes, it can be concluded that all the boreholes are not fit for drinking without processing (WHO, 2005; WHO 2011).

World Health Organization standard for faecal colifom in drinking water is zero faecal coliform per 100ml. Therefore water from the boreholes should be boiled and filtered for purity before drinking. The findings in this work support the fact that high bacteria count in water indicates high coliform count and the presence of faecal coliforms. The presence of high faecal coliform counts in sample Umuoghara and Ukwuachi could also be attributed to the nearnes of the boreholes to a pit latrine located near the boreholes at a distance less than the 30m recommended by WHO. There is also leaf cover in the environment of the borehole which is brought about by some overgrown plants shedding their leaves within the borehole sources. In addition, the bacterial isolates from the water belong to the genera of potential pathogenic bacteria, hence the recommendation that water from all the boreholes need to be boiled before use. The construction of septic pits where feaces pass

through is seen most closely to the water pipes as seen in the areas of this work.

The physical examination of borehole waters for taste, colour and odour showed that all water samples tasted salty and therefore contrary to the WHO standard. The statistical analysis using chi-square ($p<0.05$) showed that Umuoghara and Ukwuachi boreholes have high bacteria load with normal levels of chemical constituents compare. 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 others; 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 may help towards bacterial infection reduction.

CONCLUSION

The presence of faecal coliforms in all the water samples assessed shows that users and consumers of borehole waters are vulnerable to the risk of infection. Thes risk of infection is further enhanced by the presence of *E. coli* in bacteriological parameters examined thus increasing water quality and increasing health risk simultaneously. Therefore, the quality of the borehole waters for drinking has to be ensured by boiling and filtering the water for purity before drinking and other uses.

REFERENCE

Cheesbrough M (2000). Medical laboratory manual for tropical countries 4thed, Cambridge University Press, Cambridge pp: 143-167.

Gardon DM (2001). Geographical structure and host specificity in bacteria and the implications for tracing the source of coliform contamination. *Microbiology* 147: 1079-1085.

Geldrich EE (1996). Sanitary significance of fecal coliforms in the environment. Publication No WP -20-3. Federal water pollution control administration, Cincinnati, Ohio, *British J. Pharmacol. Toxicol. 3***(1):** 33-38.

Hagedorn C, Drozier JB, Mentz KA, Booth AM, Graves NJ, and Reneau RB. (2003). Carbon source utilization profiles as a method to identify sources of faecal pollution in water. *J. Appl*. *Microbiol*. 95: 792-799.

Kelly PB, Ndubani KS, Nchito P, Luo NA, Feldman RA, Farthing MJ (1997). Cryptosporidiosis in adults in Lusaka, Zambia and its relationship to oocyst contamination of drinking water. *J. infect. Dis.* 176: 1120 –1125.

Narayan KL, Tao PS (1981). Warrangal borehole waters bacteriological quality. *Indian J. Environ. Health* 23:149-151.

Okpokwasili GC, Akujobi TC (1996). Bacteriological indicators of Tropical Water Quality*. Environ. Toxicol Water Quality* 11: 77-82.

Public health association ADHA (2005). Standard methods for the examination of water and waste water 20th ed. American Public Health Association. Washington D.C. USA.

WHO (2005). Water borne disease is world's leading killer. World Health Organization.

WHO (2011). Guidelines for drinking water quality World Health Organization,Geneva, Switzerland. WHO OE CD. pp. 47-55.