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Bacteriological Quality of Drinking Water of Boreholes in Benue State Campus, Makurdi

Diana Adar Dabo[1], Musa Nanre Naomi[2], Mandau Lazarus Yusuf[2], Chali Nwanan Macham[2], and Peter Sesugh Adaaku[3]

[1]*School of General Sciences, Plateau State College of Health Technology, Zawan, Plateau State, Nigeria*
[2]*Department of Community Health, Plateau State College of Health Technology, Zawan, Plateau State, Nigeria*
[3]*Department of Medical Laboratory Science, Plateau State College of Health Technology, Zawan, Plateau State, Nigeria*

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ABSTRACT

The bacterial quality of borehole waters in Benue State University was examined. Presumptive tests were carried out using 15 sets of test tubes containing lactose fermentation broth. Each test tube contained 10ml of fermentation broth and inoculated with the water sample in a sequential order of 10 ml in five of each 2x lactose fermentation broth, 1ml in five of each 1x lactose fermentation broth and lastly 0.1 ml in five of each 10 ml 1x lactose fermentation broth and incubated with durham tubes for 48hours. Confirmatory tests were carried out by inoculating positive test tubes on EMB and Nutrient agar and subjecting the resulting growth to standard biochemical tests. Results obtained revealed that total coliform counts in boreholes ranged from 1.2-5.9x10^4cfu/ml with MPN index ranging from 1.8-12.0 coliform/100ml. Three bacterial species namely *Escherichia coli* (53.33%), *Klebsiella* species (33.33%) and *Pseudomonas* species (13.33%) were isolated from borehole waters. There was no significant difference between bacterial contamination in the various locations sampled (P>0.05). It was concluded that borehole water in Benue State University, Makurdi contains coliform bacteria in amounts exceeding the WHO standard for consumable water and as such is not safe for human consumption. It therefore recommended that borehole waters in Benue State University Makurdi should be treated before consumption in order to prevent water borne diseases.

KEY WORDS: Bacteria, coliform, water, *Escherichia coli*. *Klebsiella* species, *Pseudomonas* species

I. INTRODUCTION

Water is an essential requirement of all life forms. Satisfactory supply of clean, safe, and hygienic drinking water is imperative for health [1]. Access to safe drinking water is a vital agent for human living [2]. Unavailability of good quality drinking water is widespread and this has serious health implications [3].

Water related diseases continue to be one of the major health problems globally. The high prevalence of diarrhea among children and infants can be traced to the use of unsafe water and unhygienic practices [4]. In developing countries, 80% of all diseases and over 30% of deaths are related to drinking water implications [3].

Human welfare and economic development generally depend on the use of water. In Nigeria, water resources management and utilization are crucial to the country's efforts to reduce poverty, grow the economy, ensure food security and maintain the ecological systems [5]. Water is a precious natural resource vital for life and provision of portable water to the rural and urban populations is necessary to prevent health hazards [6]. Water is a basic nutrient of the human body and very essential to living organisms, agricultural production and industrial processes. Population increase over the past century has resulted in increased pressures on water resources of the developed and developing countries. These pressures involve the contamination from domestic, industrial and agricultural wastes, climate change and other ecological disturbances [7]. Pollution of drinking water sources in rural areas may involve seepage from broken septic tanks, pit latrines and runoffs carrying fertilizers, pesticides, herbicides, fungicides and fecal matter. Contaminated water serves as a medium of transmitting infectious diseases such as dysentery, cholera, diarrhea, typhoid, shigellosis, salmonellosis, and varieties of other bacterial as well as fungal, viral, and parasitic infections [8]. In Nigeria, majority of the rural populace do not have access to potable water and therefore, depend on well, stream, boreholes and river water for domestic use. The bacterial qualities of ground water, stream water and other natural water supplies in Nigeria have been reported to be unsatisfactory, with coliform counts far exceeding the level recommendation by W.H.O [9].

Drinking water that does not comply with WHO standards may have become contaminated by human or animal faeces, for example, raw sewage entering a water supply during times of flooding has enormous potentials for spreading microbial diseases [10].[11]. The aim of this research work is to investigate the level of microbial contamination of the Borehole waters in Benue state University, Makurdi, Benue State.

II. MATERIALS AND METHOD

Study Area

Benue State University is located Makurdi which is the capital of Benue State of Nigeria. The city is located in central Nigeria along the Benue River and holds the base for the Nigerian Air force aircraft squadrons. Makurdi has an estimated population of 500,797 persons. The major ethnic groups inhabiting the city are the Tiv, Idoma, Igede and Etulo. It holds the Benue State University and the Federal University of Agriculture. It is located on latitude 7.7306°N and longitude 8.5561°E along the banks of the Benue River which is a major tributary to the Niger River. It is an agricultural catchment area and has a variety of potentials in human capital and material resource

Makurdi LGA has quite a number of modern facilities, a good road network, electricity, telecommunications network, and a modern water system that depends solely on constructed boreholes and wells. Although populations living within the township area of Makurdi LGA depend largely on boreholes for their drinking water, other rural populations living in rural areas have to depend on the surrounding streams.

Sample collection

Fifteen (15) Samples of water were collected by standard methods (WHO, 1995; Cheesbrough, 2000) using heat sterilized bottles with screw caps. The samples were collected from different boreholes by allowing the water to run to waste for 2-3 minutes before allowing it to flow into the 250 ml capacity bottle, and then quickly covered. The bottles were labeled with full details of: source, time, date and numbers.

Methods

The samples were analysed in less than 5 hours after collection: between 9.00 am and 12.00 noon. The Most Probable Number (MPN) method was used to analyse the 15 water samples (comprising 15 Boreholes).

Presumptive coliform test

The water samples were mixed thoroughly and 50 ml amount poured directly into the 50 ml sterile broth (Double strength) bv making up to 100 ml mark previously made on the bottle. A 10 ml sterile hypodermic syringe (with needle, each) was used to inoculate each 10 ml sterile MacConkey's broth (MCB), double strength. While 2 ml and 0.2 ml (immunization syringes and needles) each was used to inoculate the 10 ml (single strength) and 5 ml (single strength) sterile broth respectively.

Incubation

The seeded bottles were incubated aerobically (with the bottles loosely capped) at 37°C for 24 hours and 48 h. At the end of 48 h, the broth cultures that showed production of acid (colour change from purple to yellow) and gas (seen in the Durham tube) were considered positive for "Presumptive coliform count". Bottles that failed to produce gas and acid at the end of 48 h incubation were presumed to contain no coliform bacteria (recorded as 0 or zero).

Determination of Most Probable Number (MPN)

From the various combinations of the sample bottles that were both positive and negative, results were deduced. Any negative sample was recorded as zero (0), while the positive cases based on the number were calculated.

Confirmatory coliform tests

MacConkey Broth (MCB), single strength (S/S) was used in place of Brilliant Green Lactose (bile) Broth (BGLB) for confirmatory Coliform (Eijkman) Test. Using a sterile wire loop, a loopful was subcultured from each presumptive positive broth culture into bottles containing respectively sterile MCB and Tryptone water and incubated for 24 h at 44°C aerobically in water bath (with the bottles loosely capped I. The positive samples (with gas production were recorded as before).

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Isolation of organisms

Sub culturing on solid media

Positive broth cultures were subcultured on the various solid media (BA, CLED, MCA, TSI) for isolation of implicated organisms. These were incubated aerobically and anaerobically (with increased at 37°C overnight. The overnight culture plates were identified morphologically (by colonial appearance), Gram's reactions and Biochemical/ enzyme reaction and sugar fermentation test.

Biochemical test

Rosco Tablets (commercially prepared) were used for most tests by simply adding to a saline suspension of the test organism (0.25 ml + an inoculum in about 0.5 mg tablets) in a small test tube, and the test tubes incubated at 37°C and read overnight.

Catalase test

Hydrogen peroxide, 3% H2O2 (10 volume solution as used according to Cheesbrough (2000).

Other test

Oxidase, Coagulase, Motility, Urease tests were carried out according to Cheesbrough (2000). The Triple sugar iron agar slopes (in tubes) were heavily streaked over the surface of the slope, and stab-inoculated into the butt (Mackie and McCartney, 1996). These were incubated aerobically at37°C overnight. Yellow slope and a yellow butt indicate the fermentation of lactose and possibly glucose due to E. *coli* and *Enterobacter;* while Pink-red (alkaline reaction) and yellow butt (acid reaction) with small blackening due to IHbS production along the stab is typical of *Salmonella Ti/phi.* IndoleTest A drop (about 0.1 ml) of Kovac's reagent was added to each tube of the tryptone water culture (48 h old) and gently mixed.A red colour in the surface layer (indole positive) confirmed *E. coli*.

Data Analysis

The percentage prevalence (%) was calculated in each case. Comparative analysis of the results were done using chi square (χ^2) to determine the relationship between location and occurrence of bacteria associated with borehole water. A p-value less than 0.05 ($p<0.05$) was considered statistically significant.

III. RESULTS

Bacterial quality of water samples was examined in this study by the Most Probable number for microbial determination of coliforms in borehole water. Table 1 presents collection codes from West Wing, Eastern Wing and Medical College of Benue State University, Makurdi, combination of positive codes for bacterial contamination and MPM index. The results show a highest contamination of 7.9 x 10^4cfu/ml and least contamination rate of 1.2 x 10^4cfu/ml. Maximum MPN index/100ml of the water samples also ranged from 1.5-12.0 coliforms/100ml of water.

Three bacteria species were isolated from borehole water samples collected from Benue State University campus. These bacteria species included *E. coli* (53.33%), *Klebsiella species* (33.33%), *Pseudomonas species* (13.33%). Regional distribution of the bacteria species showed that Western wing had an 80.0% occurrence of E. coli, 20.0% occurrence of *Klebsiella* species, and a zero pseudomonas occurrence (0.0%). Eastern wing had a percentage occurrence of 60.0% for E. coli, 40.0% for *Klebsiella* species and zero occurrence for Pseudomonas while Medical school had an occurrence of 20.0% for *E. coli*, 40.0% for *Klebsiella* species and 40.0% for *Pseudomonas* species. This is as presented in table 2 below.

Table 3 shows the biochemical characterization of bacterial isolates obtained from this study with Lactose fermentation positive for *E. coli* and *Klebsiella* species while *Pseudomonas* was a non-lactose fermenter. Oxidase test was used as a confirmatory test for *Pseudomonas* species which is oxidase positive unlike *E. coli* and *Klebsiella* species which were negative. *E. coli* was however indole positive and citrate negative while *Klebsiella* species was indole negative and citrate positive. In order to further distinguish between *E. coli* and *Klebsiella* species which are both lactose fermenters, cultural characterization of *E. coli* and *Klebsiella* on Eosin Methylene Blue culture media were carried out and are presented as shown in Plates 1 and 2.

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Table 1: Total Counts and Most Probable Number of Indicator Bacteria of Borehole Water in Benue State University, Makurdi.

Water sample	Total Count (cfu/ml) (10^4)	Combination of Positives	MPN Index/100ml
WW1	2.6	0-1-0	1.8
WW2	1.6	1-0-0	2.0
WW3	5.1	0-1-1	3.6
WW4	1.8	1-2-4	1.5
WW5	4.6	1-2-1	8.2
EW1	5.9	1-1-0	4.0
EW2	1.2	0-0-2	3.6
EW3	1.6	2-0-1	6.8
EW4	4.7	2-2-1	12.0
EW5	2.6	2-3-0	12.0
MS 1	7.9	0-3-0	5.6
MS 2	2.9	1-2-1	8.2
MS 3	4.1	0-0-1	1.8
MS 4	4.9	0-0-1	1.8
MS 5	3.5	0-0-2	3.6

WW= Western Wing; EW= Eastern Wing; Medical School

Table 2: Distribution of Coliform Bacteria in Water Samples

Location	Bacteria species			
	E. coli	***Klebsiella* species**	***Pseudomonas* species**	**Total**
Western Wing	4 (80.0%)	1(20.0%)	0(0.0%)	5(33.33%)
Eastern Wing	3 (60.0%)	2(40.0%)	0(0.0%)	5(33.33%)
Medical School	1(20.0%)	2(40.0%)	2(40.0%)	5(33.33%)
Total	**8(53.33%)**	**5(33.33%)**	**2(13.33%)**	**15(100.0%)**

χ^2= 4.19; df=2; P=0.47

Table 3: Biochemical Characterization of Isolate Bacteria Species

Biochemical Test	Bacteria species		
	E. coli	***Klebsiella* species**	***Pseudomonas* species**
Lactose Fermentation	+	+	-
Oxidase	-	-	+
Indole	+	-	_
Citrate	-	+	+

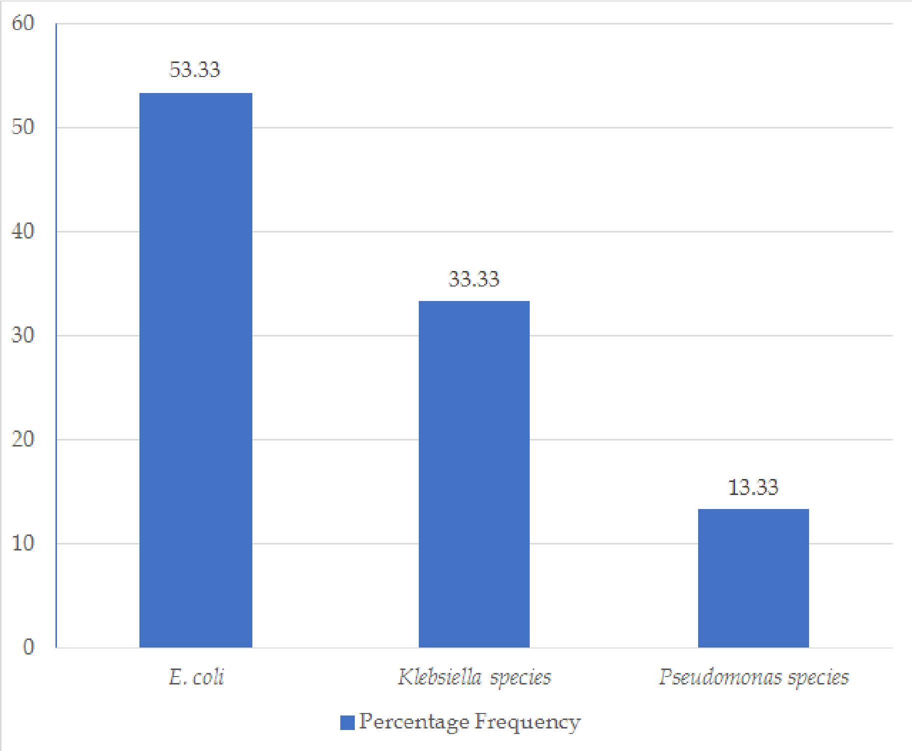

Figure 1: Percentage Frequency of Occurrence of Bacterial Isolates in Water in Benue State University, Makurdi.

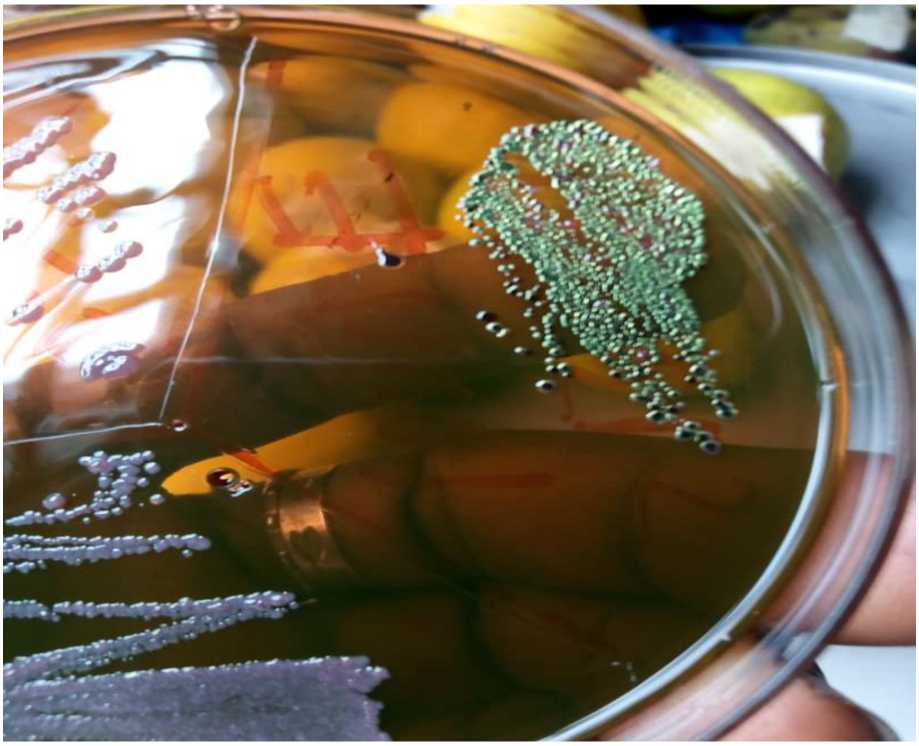

Plate 1: Cultural Growth of *E. Coli*

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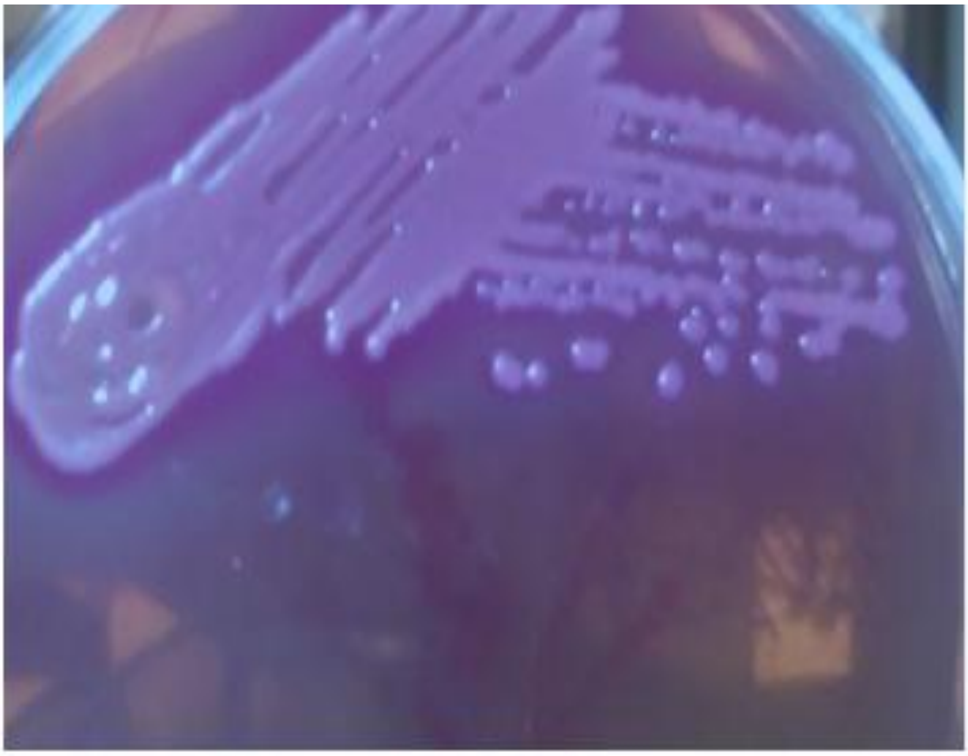

Plate 2: Cultural Growth of *Klebsiella* species

Plate 3: Confirmatory Tests for Coliforms

IV. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Discussion

This study which was conducted to determine coliform bacteria in borehole water in Benue State University, Makurdi showed existence of coliform bacteria in borehole water. The existence of coliforms in water is an indication of contamination of water samples by bacterial samples which renders the water unfit for human consumption. The existence of coliform bacteria in water is an indication of the possibility of water samples to cause bacterial diseases in the future if adequate preventive measures such as water purification are not put in place. This observation correlates with the finding of the World Health Organization who stated that contamination of water samples by coliform bacteria can lead to increase in diarrheal diseases which accounts for an estimated 4.1% of the total daily global burden (WHO, 2005).

Bacterial counts of water samples in this study was high with coliform bacteria ranging from $1.5x\ 10^4$ to $12.0\ x\ 10^4$ cfu/ml. This value is higher than the recommended permissible count of $1.0\ x\ 10^2$ cfu/ml. This deviation from the WHO standard is an indication of water contamination and as such borehole water is unsafe for consumption. Also, the presumptive analysis of water samples showed that borehole water contains as much as 1.5-12.0 coliforms/100ml of water. This value is higher than what is recommended by the World Health Organization as permissible in water samples (0/100ml).

In terms of distribution of bacteria isolated in this study. *E. coli* had the highest abundance in all the regions of Benue State University with a percentage occurrence of 80%. The presence of such bacteria as *E. coli* is of significant value in determining the extent of water pollution. Occuence of this bacteria in water samples is an indication of faecal contamination of water samples. Pathogens such as *Klebsiella* and *Staphylococcus aureus* were also isolated. The presence of *Klebsiella* species in some of the borehole water samples are unacceptable from the public health point of view. These organisms could be pathogenic. Isolation of these bacteria from borehole water in Makurdi is in agreement with the findings of [13] who obtained similar distribution of bacteria for coliform in stored borehole water samples. Despite variation in distribution of bacterial isolates in the various borehole water samples collected, statistical analysis showed that there was no significant difference in contamination across the various locations (P>0.05).

Environmental bacteria such as *Pseudomonas* which are mostly saprophytic in origin were isolated from some of the waters. It was also found that all the water samples were positive for coliform, showing high contamination and risk to public health. High contamination of water samples in this study is similar to the results domestic water supplies reported by [6]

E. *coli* in borehole is an indication of poor hygiene and sanitation, and a general collapse in the provision of safe drinking water. E. *col*, with the other isolated organisms may not actually constitute a public health hazard in a population of healthy individuals, but their presence is a pointer to the presence of or an indication that pathogenic bacteria, viruses, parasites, protozoa, including other aquatic microbes exists. This may cause diarrhoea, typhoid and paratyphoid fevers, cholera and dysentery in the near future.

Poor sanitation caused by coliform contamination in this study is in consonance with the report of [12] who associated contamination of water samples with poor sanitary conditions of the water samples. It has also been observed by [14] that many brands of water sold in Makurdi did not meet WHO microbiological standards. coliform bacteria are an indicator bacterial group that is used to evaluate the quality of drinking water, and any presence of coliforms indicates contact of the water with sewage or inadequate treatment or post treatment contamination.

The World Health Organization Standard for presumptive coliform test for both treated and untreated water samples is 0/100 ml, but in occasional untreated water samples, 3/100 ml are allowed on the condition that these would not be detected in consecutive water samples. The findings of this study obviously was against this standard.

Conclusion

Borehole water in Benue State University, Makurdi contains coliform bacteria in amounts exceeding the World Health Organization Standards for consumable water and is not safe for human consumption except treatment methods such as boiling, dechlorination and addition of alum are imputed.

The high occurrence of bacteria isolates like *E. coli*, *Klebsiella* and *Pseudomonas* is also an indication of faecal contamination of the water from sources unknown. Faecal contamination is basically a pointer to the hygiene and sanitary practices carried out by bore hole water users in Makurdi which is not satisfactory.

Recommendation

From this study, the following is recommended:

- Borehole waters in Benue State University should be treated before consumption in order to prevent water borne diseases.
- Strict hygiene and sanitation practices should be adhered to by parents, workers and residents of Benue State University so as to increase the remediation process of contaminated waters.
- The populace need to be educated on the importance of maintaining clean and hygienic environment around the borehole to ensure the safety of water from such boreholes.

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