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Bacteriological and Physico-chemical Analysis of Borehole Water in Auta Balefi Community, Nasarawa State, Nigeria

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Authors' contributions

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

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ABSTRACT

Aim: To determine the bacteriological and physico-chemical qualities of borehole water in Auta Balefi, community.
Place and Duration of Study: Auta Balefi, Community, Karu LGA, Nasarawa State; Department of Biological Sciences, Bingham University, Karu, between April 2015 and June 2015.
Materials and Methods: Five water samples from different sources (boreholes) was collected randomly within the community. The total bacterial count was determined by pour plate technique. Total coliform count was determined using 3-3-3 regimen (3-tube assay). Identifications of isolates was carried out using standard methods.
Results: Six genera of bacteria which include *Escherichia* spp*, Klebsiella* spp*, Staphylococcus* spp*, Salmonella* spp*, Pseudomonas* spp and *Proteus* spp was isolated from the water samples. Total

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Heterotrophic bacterial count in the borehole water sampled ranged from 1.03 x 10^{-2} cfu/ml to 2.01 x 10^{-2} cfu/ml, respectively. The total coliform count of the borehole water analyzed ranged from 19 Most Probable Number (MPN) index of coliform/100 ml to 26 MPN index of coliform/100 ml. The fecal coliform count of the water analyzed ranged from $2x10^2$ cfu/ml to $6x10^2$ cfu/ml. Important physico-chemical parameters such as Color, Salinity, Turbidity, Nitrate concentration, Total hardness, Chloride and Calcium levels were within the World Health Organization (WHO) standard for potable water though some parameters such as TDS and pH had values which were beyond these standards.

Conclusion: This study revealed that the borehole water analyzed is not safe for consumption. However, improvisation of safe drinking water by individuals will reduce the spread of the water borne diseases and this can be achieved either by boiling or chlorination. The addition of sodium aluminate (alum), or 'water guard' which contains 1.0% of sodium hypochlorate to water will reduce water contaminants.

Keywords: Borehole water; physicochemical; bacteriological; coliforms.

1. INTRODUCTION

Water is one of the most important and most valuable natural resources. It is essential in the life of all living organisms from the simplest plant and microorganisms to the most complex living system known as human body [1]. Water is significant due to its unique chemical and physical properties and is known to be the most abundant compound (70%) on earth [2,3]. Water in its pure form has a pH value of 7.0, freezing point of 0℃ and boiling point of 100℃ at 760 mmHg [4]. It is also a colorless, transparent, odorless and tasteless liquid.

Access to safe drinking water has improved over the last decades in almost every part of the world especially Nigeria, but approximately 1.1 billion people still lack access to safe water and over 2.6 billion worldwide lack access to adequate sanitation which causes water illnesses such as Cholera, diarrheal disease, Botulism, *E. coli* infection, Dysentery, Legionellosis, Leptospirosis, Salmonellosis, Typhoid fever, and Vibrio illness [5]. The presence of nitrate compounds, heavy metals, pesticides e.t.c in our drinking water can also constitute undesirable pollutant when they are not within World Health Organization (WHO) guidelines for drinking water [6].

Drinking water has always been a major issue in many countries like Nigeria [7] and majority of the rural populace in Nigeria do not have access to potable water. Only few people can afford and rely on purified and treated bottled water particularly for consumption therefore, borehole water serve as the major source of both drinking and domestic water used in the local population of Nigeria [8].

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Auta-balefi Karu, Nasarawa State. This community is located in the middle belt of Nigeria at longitude 8º32'N 8º18'E and Latitude 8.533ºN 8.300ºE and is characterized by a tropical sub-humid climate with two distinct seasons; wet and dry seasons. Monthly temperature ranges from 20℃ to 34℃ and annual rainfall ranges from 1100mm to about 2000 m [9].

2.2 Sample Collection

Water samples was collected from five (5) different water sources designated as location A, B, C, D and E. Water samples from these location were collected into sterile glass bottles (250 ml) which were labeled appropriately. Cotton wool soaked in 70% acetone-alcohol was used to sterilize the nozzle of the borehole from which the water samples were collected. The tap was allowed to run for two minutes after which the 250 ml capped glass bottles were carefully uncapped and filled with water. The pH readings of the water samples were taken using pH meter Wag WT 3020. The pH meter was standardized with buffer 4, 7 and 9 before being used [10]. Conductivity was measured using the electrical conductivity meter. Temperature of each sample was determined using mercury-bulb thermometer and this was recorded at the point of collecting the water before the bottle was recapped and transported to the laboratory for bacteriological and physico-chemical analysis.

2.3 Physicochemical Analysis

The water samples from each source (borehole) were examined in terms of physical and chemical

properties such as colour, temperature, turbidity, dissolved Oxygen, Salinity, total dissolved solids, alkalinity, conductivity, pH, Calcium, Total Hardness, Nitrate, Suspended solids, Magnesium and Chloride [10].

2.4 Bacteriological Quality Determination

2.4.1 Total heterotrophic bacteria count

The spread plate method was used. Ten-fold serial dilution of each water sample was prepared aseptically in physiological saline of 10^{-1} up to 10^{-4} and 0.1 ml aliquot of each dilution was plated on Nutrient agar plates in triplicate. All incubations were conducted at 37℃ for 24 hrs under aerobic conditions and plates containing 30 to 300 colonies were selected and counted. The number of colony-forming units per ml (cfu/ml) was calculated by multiplying the number of colonies by the dilution factor. Also, sub-culture was carried on MacConkey agar and Mannitol Salt agar for identification of bacteria species.

2.4.2 Total coliform count

This was determined by Most Probable Number (MPN) index technique using the three tube assay (3-3-3 regimen). Ten-fold serial dilution of 10^{-1} to 10^{-5} was prepared. The first set of five tubes had 10 ml of double strength broth (MacConkey broth), the second and third set had 10ml single strength broth (Lactose broth). All the tubes contained Durham tubes. The three set of tubes received 10 ml, 1 ml and 0.1 ml of water samples. They were carefully labeled and incubated at 37℃ for 24 hrs for estimation of total coliform. Acid production was determined by color change in tubes from reddish purple to yellow and gas production was checked for by entrapment of gas in the durham tubes [11].

2.4.3 Faecal coliform count

Faecal coliform count was determined using Eosin Methylene Blue medium employing the streaking culture technique. A loopful of broth from positive tubes was streaked onto EMB agar plate for pure cultures. The plates were incubated at 37℃ for 24 hrs. Colonies on EMB agar plate were further identified as fecal coliforms. On Eosin Methylene Blue (EMB) agar, *E. coli* strains appeared as greenish metallic sheen colonies [12].

2.4.4 Identification of isolates

The cultural, morphological and biochemical characteristics of the respective isolates were compared with the criteria in District Laboratory Practice for Tropical Countries, Part 2 [13]. The biochemical tests used in the identification and characterization of the isolates include: Gram-staining, Motility, Indole production, Methyl red-Voges Proskauer, Citrate utilization, Oxidase, Catalase, Coagulase and Sugar fermentation tests. Biochemical reactions were confirmed using microgen test kits for enterobacteriaceae.

2.4.5 Microgen tests

Rapid test was used to confirm the isolated bacteria- Microgen™ GNA-ID System for Enterobacteriaceae (*E. coli, Salmonella, Staphylococcus, Pseudomonas, Proteus and Klebsiella*) and Microgen™ STAPH-ID test for *Staphylococcus.* The Microgen™ tests were carried out as described by Anyanwu and John [14].

3. RESULTS

Table 1 shows Total Heterotrophic Bacteria (THB) from the water samples obtained from the five (5) water sources shows that the THB counts ranged from 1.03×10^2 cfu/ml in sample C to 2.01×10^2 cfu/ml in sample E. The lowest total coliform and fecal coliform counts (19 MPN/100 ml and $2x10^2$ cfu/ml) were observed in sample D and C respectively. The highest counts of 26 MPN/100 ml and 6 $x10^2$ cfu/ml total coliform and fecal coliform was obtained from samples B and E.

The physico-chemical parameters of the water samples revealed that the pH of the water sources ranged from 8.5 to 8.6. Temperature value ranged from 21-25℃. The result of the Colour, Turbidity, Salinity, Dissolved oxygen, Total dissolved solids, Alkalinity, Chloride, Hardness, Calcium, Magnesium, Salinity, Suspended solids, Nitrate, and Conductivity levels of the water samples from all the water sources and the corresponding WHO guideline values for drinking water are displayed in Table 2.

Based on the Cultural and Morphological characteristics and the biochemical tests, Six (6) genera or isolates were identified to include *Salmonella typhi, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumonia* among the numerous common pathogenic bacteria present in water bodies. Their morphological and biochemical characteristics are shown in Table 3.

Table 1. Profile of Total Heterotrophic Bacteria (THB), total coliform and fecal coliform counts obtained from the water samples

Sample	THB (Cfu/ml)	Total coliform (MPN/100 ml)	Fecal coliform ($x10^2$ cfu/ml)
A	1.80×10^2	23	4
B	1.91×10^2	20	6
C	1.03×10^2	22	2
D	1.41×10^2	19	3
E	2.01×10^2	26	4

Table 2. Physicochemical parameters of the water samples

Parameters	Sample A	Sample B	Sample C	Sample D	Sample E	WHO limit
Temperature (℃)	23	21	25	24	23	25
Colour (TCU)	<5.0	<5.0	<5.0	<5.0	<5.0	15
Dissolved oxygen (DO)	4.77	3.16	4.61	4.04	5.95	14
Turbidity (NTU)	0.1	0.4	0.3	0.2	0.4	5
Salinity (Mg/L)	0.02	0.01	0.01	0.03	0.04	5
TDS (mg/L)	283	422	452	762	6391	500
Chloride (Mol/dm^3)	0.0071	0.0042	0.0038	0.0043	0.0027	0.05
Alkalinity (mg/L)	17.5	18.5	10.5	11.5	12.0	50
Conductivity (µS/cm)	0.74	0.79	0.75	0.79	0.77	5.0
P^H	8.58	8.54	8.49	8.52	8.55	6.5-8.5
Calcium (Mg/L)	6.12	7.22	6.59	7.10	8.77	7.5
Hardness (ppm mg/L)	2.75	3.25	3.50	5.76	2.25	5.0
Magnesium (Mg/L)	2.71	5.44	5.20	5.60	3.49	30
Nitrate (Mg/L)	2.71	3.66	4.40	4.33	5.40	50
Suspended solids (Mg/L)	0.006	0.002	0.003	0.002	0.013	0.01

**Keys: Nephelometric turbidity units (NTU); Total dissolved solids (TDS); True colour unit (TCU)*

Table 3. Characterization and identification of isolates from borehole water

Isolates	Morphology	Gram staining	Motility	Indole	MR-VP	Citrate	Oxidase	Catalase	Coagulase	Glucose	Maltose	Bacteria specie
1	Short rods	-	+	-	-	-	-	-	-	+	+	*Salmonella* spp
2	Cocci in clusters	+	-	-	+	-	-	+	+	+	+	*Staphylococcus* spp
3	Short rods	-	+	-	+	-	-	+	-	+	-	*Escherichia* spp
4	Short rods	-	+	-	-	-	+	+	-	+	-	*Pseudomonas* spp
5	Short rods	-	+	+	+	-	+	+	-	+	+	*Proteus* spp
6	Short rods	-	-	-	+	+	-	+	-	+	+	*Klebsiella* spp

Key= (-) Negative, (+) Positive

Table 4a. Environmental assessment of the sample collection sites/ locations

Sample	Physical appearance	Proximity to toilet	Refuse/ solids dump	Domestic discharge	Stagnant water
A	Fairly clean	Close	Close	Present	Present
B	"	Far	Far	Absent	"
C	Clean	"	Close	Present	"
D	Fairly clean	"	Far	Absent	"
E	Clean	Far	Close	Present	Absent

Table 4b. Microgen test results for some isolated microorganisms

(a)

Isolate no.	Lys	Orn	H_2S	Glu	Man	Xyl	ONPG	Ind	Ure	VP	Cit	TDA	Identification
1	+	-	-	+	+	+	+	-	+	+	+	-	*Klebsiella*
2	-	+	+	+	-	+	-	-	+	-	+	+	*Proteus*
3	-	-	-	+	+	+	+	+	-	-	-	-	*E. coli*

Key: Lys-Lysine; Orn-Ornithine; H_2S-Hydrogen sulphide; Glu-Glucose; Man-Mannitol; Xyl-Xylose; ONPG-Ortho-nitrophenol- galactosidase; Ind-Indole; Ure-Urease; VP-Voges Proskauer; Cit-Citrate; TDA-Tryptophan deaminase acid

(b)

Isolate no.	LAT	CPG	NIT	SUC	TRE	MAN	NAG	MNS	TUR	PHO	βGL	βGN	URE	ARG	PYR	Identification
4	+	-	-	+	+	+	+	+	+	+	+	-	+	+	-	*S. aureus*

Key: LAT- Latex Agglutination Test, CPG- Colony Pigmentation, NIT- Nitrate, SUC- Sucrose, TRE- Trehalose, MAN- Mannitol, NAG- N-Acetyl Glucosamine, MNS- Mannose, TUR- Turanose, PHO- Alkaline Phosphate, bGL- bGlucosidase, bGN- bGlucuronidase, URE- Urease, ARG- Arginine, PYR- Pyrrolidonyl Arylamidase

Table 4 above shows the environmental assessment of the sample collection locations which revealed that some of the boreholes locations were situated close to refuse/ solid waste or dump and stagnant water. Some locations were littered with faeces from domestic animals and polythene bags. The boreholes in sites A, B and C were located far away from toilet facilities.

4. DISCUSSION

The analysis of the THB count in the water samples revealed the presence of heterotrophic bacteria in all the water sources (Table 1). The WHO standard for heterotrophic bacteria in potable water states that the total heterotrophic bacteria count should not be more than 100 cfu/ml [15]. The presence of bacteria counts exceeding the WHO limits indicated that the water samples contain bacteria that could make the water unsafe for drinking and domestic purposes. The Heterotrophic Bacteria count from this study exceeded WHO limits. The result from this study agrees with the separate findings of [16,17]. The high values obtained could be due to poor environmental conditions and the presence of stagnant water around the borehole which provide an excellent breeding ground for bacteria. Table 2 shows the total coliform count ranged from 19 MPN/100 ml to 26 MPN/100 ml. Feacal coliform count, ranged from $2x10^2$ cfu/ml to $6x10^2$ cfu/ml (Table 3). This is unacceptable because WHO standard of potable water states that no coliform should be present in any drinking water.

The presence of these bacteria organisms (*Salmonella, Staphylococcus, Escherichia, Pseudomonas, Proteus* and *Klebsiella*) suggests fecal contamination. It could probably be that the pipes used for water distribution were rusty thus allowing seepages of microbial contaminants into the borehole.

Total Dissolved Solids (TDS) of the samples were within the WHO permissible limits except for sample D and E which exceeded the WHO standards with values of 762 mg/L and 6391 mg/L respectively. These high values could probably be attributed to all the disassociated electrolytes that make up salinity concentrations such as Calcium and Sodium, as well as other compounds such as dissolved organic matter. Chloride concentration of the water samples were within the desirable limit (limit of 0.005 mg/l) except sample (A) which had the highest value of 0.0071 mg/l. The pH of all the samples analyzed ranged from 8.5 to 8.6 which exceeded the WHO permissible limit of 6.5 - 8.5 indicating that the water was slightly alkaline. This increased alkalinity could be attributed to the presence of alkaline metabolites such as Carbonate (CO_3^{-2}) ions which react with and neutralize Hydrogen ($H^{+)}$ ions and bicarbonate (HCO_3) ions which neutralize hydroxide ions (OH-) present in water.

Results obtained for the Calcium varied respectively from locations. The quantity of calcium in samples A, B, C and D were within the WHO permissible limit of 7.5 mg/l except for Sample (E) which was beyond the WHO required standard with value of 8.77 mg/l. This could probably be attributed to its abundance naturally in the earth crust. Result obtained for the Total Hardness revealed that the values were within the WHO permissible limits of 5.1 ppm, mg/l in Samples A, B,C and E except for sample D which is beyond the required standard with the value of 5.76 ppm mg/l. This high value could probably be attributed to the concentration of multivalent cations (metal complexes) which enters the water by leaching from minerals within an aquifer. The analysis of other physico-chemical parameters revealed that parameters such as colour, temperature, Dissolved oxygen, Turbidity, Salinity, Conductivity, magnesium, Alkalinity, Nitrate and Suspended solids were within WHO guideline values for drinking water (Table 3).

5. CONCLUSION

The bacteriological analysis of the borehole water (A to E) indicates the presence of bacteria in the water which suggests the water is not fit for drinking without proper processing. Also due to the high concentration of some physico-chemical parameters in some of the locations, the water should be well treated before consumption.

6. RECOMMENDATION

Public awareness on the dangers associated with the consumption of contaminated water should be increased. The construction of pit latrines near water sources should be avoided.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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