



12(1): 1-11, 2016, Article no.BMRJ.20136 ISSN: 2231-0886, NLM ID: 101608140

British Microbiology Research Journal

SCIENCEDOMAIN international www.sciencedomain.org

Microbiological and Physico-chemical Analyses of Borehole Water Samples from Private Schools in Umuahia Metropolis, Abia State, Nigeria

C. N. Obi^{1*}, A. L. Onyegbulam¹, F. Ejukonemu² and M. Ubogu³

¹Department of Microbiology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria. ²School of Applied Sciences, Department of Science Laboratory Technology, Delta State Polytechnic, Oghara, Delta State, Nigeria. ³Department of Biological Sciences, Federal University of Agriculture, Makurdi, Benue State, Nigeria.

Authors' contributions

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

Article Information

DOI: 10.9734/BMRJ/2016/20136 <u>Editor(s)</u>: (1) Kai Zhang, Department of Oral Biology, The State University of New York, Buffalo, USA. (2) Gyanendra Singh, Gene Therapy & Louisiana Vaccine Center, School of Medicine, LSU Health Sciences Center, Louisiana, USA. (3) Hung-Jen Liu, Distinguished professor, Director, Institute of Molecular Biology, National Chung Hsing University, Taiwan. <u>Reviewers</u>: (1) Ashish Kumar, Agra College, Agra, India. (2) Martin E. Ohanu, University of Nigeria Nsukka, Nigeria. (3) N. K. Kortei, University of Ghana, Ghana. (4) Pavlina Simeonova, Bulgarian Academy of Sciences, Bulgaria. Complete Peer review History: <u>http://sciencedomain.org/review-history/12526</u>

> Received 13th July 2015 Accepted 21st November 2015 Published 2nd December 2015

Original Research Article

ABSTRACT

The microbiological and physico-chemical analysis of randomly selected borehole waters used by staffs, students and pupils in twenty (20) private schools in Umuahia Abia State were carried out. Five bacteria: *Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Pseudomonas aeruginosa, Streptococcus faecalis* were isolated together with two fungal species: *Aspergillus niger* and *Mucor racemosus*. The Total Coliform plate count (TCPC) gave a range of 2.40x10⁴-8.61x10³ cfu/ml while the Total Heterotrophic plate count (THPC) was in the range of 3.82x10⁴-9.22x10³ cfu/ml. The faecal coliform was detected in 40.0% of the water samples. The physico-chemical parameters were within acceptable limit except for nitrate whose range fell between 10-53

mg/l above WHO guidelines of 10 mg/l. The results were compared with World Health Organization (WHO). The antimicrobial susceptibility test of the bacterial isolates showed varied responses. The findings showed that 16 of the borehole water samples from the 20 private schools met with WHO standards for drinking water while the water samples from four schools that did not meet with the WHO standards for drinking water and pose serious health threats to the pupils and teachers who drink the water. Thus, there is need to adopt constant treatment, analysis and servicing of these boreholes water sources for them to be safe for drinking.

Keywords: Borehole waters; coliforms; contamination; microbiology; physico-chemical.

1. INTRODUCTION

Water is the most important of all natural resources vital for all living organisms. Accessibility and availability of fresh clean water is the key to sustainable development and an essential element in health, food production and poverty reduction. However, for water to be portable it must be microbiologically safe and in order to achieve this, an approach that will eliminate pathogenic organisms from the source of water must be ensured. [1] defined water as a substance that has a pH value of 7.0, freezing point of 0° and a boiling point of 100° at 760 mmHg. Water is capable of dissolving substances more than any other known solvent and therefore called a universal solvent. Water is useful to man in many ways; for example, it serves as a means of transportation (in bringing goods in and out e.g. Seas, oceans and rivers), Recreation such as sporting activity (swimming, skating). It is also used for generating electricity, cooking, washing, bathing and drinking.

Since the beginning of civilization, water has been recognized as a potential carrier of germs and diseases [1]. An estimated 1.2 billion people around the world lack access to safe water [2]. Every 20 seconds, a child dies from water related diseases [2]. Diarrhoea has been identified as the second leading cause of death among children under five globally. Nearly one in five children deaths each year is due to Diarrhoea. It kills more young children than Acquired Immune Deficiency Syndrome (AIDS), malaria and measles combined [2].

The ensuring of good quality drinking water is a basic a factor in guaranteeing public health, the protection of the environment and probably sustainable economic development [3]. The major problems of safe drinking water are those of availability and quality. The most dangerous form of water pollution occurs when faeces contaminates the water supply. Water from most sources is therefore unfit for immediate consumption without some sort of treatment [4].

Many diseases are perpetrated by the faecaloral-routes of transmission in which the pathogens are shed only in human faeces. The presence of faecal coliform like E. coli is used as an indicator for the presence of water-borne pathogens [5,6]. Conformation with physicochemical and microbiological standards is of special interest because of the capacity of water to spread diseases within a large population. Although standards vary from place to place, the use of normal intestinal flora of human (coliform) as an indicator of faecal pollution is accepted universally for routine monitoring and assessing of microbial safety of water supplies [7]. The objectives is to reduce the possibility of spreading water borne diseases to the barest minimum, in addition to being pleasant to drink which implies that it must be wholesome and potable in all aspects [8]. The objectives of municipal water are the production and distribution of safe water that is fit for human consumption [9]. Borehole water is pumped out with the aid of submersible pumping machine into overhead tanks. A good knowledge of the chemical qualities of borehole water is necessary as to assess its suitability for use.

This work carried out in 2014 aimed at examining different borehole water samples which are sources of drinking water in eight private schools in Umuahia Metropolis, Abia State, Nigeria. This work was necessary because of the increasing population of the area due to the location of private schools in Umuahia metropolis. There is need to safe guard the lives of pupils and staff of these private schools because they depend on the boreholes for drinking water as pipe borne water is largely absent in the area.

2. MATERIALS AND METHODS

2.1 Sample Collection

Twenty functional borehole water samples were collected from twenty private schools in Umuahia Metropolis, Abia State. The water samples were collected into twenty different screw capped sterile 400 ml plastic containers which were labeled appropriately. Cotton wool soaked in 70% ethanol was used to sterilize the nozzles of the borehole from which samples were aseptically collected after the water had run for two minutes into the sterilized screw capped plastic bottles. The water samples were taken to the laboratory in a cooler containing ice cubes for analyses within 2 hrs of collection. Physicochemical and microbiological quality of water samples were determined in triplicates by method of [10].

2.2 Microbiological Analysis

2.2.1 Membrane filtration technique

The sterile membrane filtration apparatus was placed in position and was connected to a source of vacuum pump with the stopcock turned off [11,12]. The funnel of the membrane filter was removed and a filter paper composed of cellulose acetate with pore size 0.45 μ m was placed on the base of the porous disc of the filter paper with the aid of a sterile forceps. 100 ml of the sample was filtered through the membrane such that the organisms to be enumerated were retained on the surface of the membrane which was placed with the grid lines facing upward on MacConkey agar for total coliform plate count. The plates were incubated at 30°C for 18-24

Obi et al.; BMRJ, 12(1): 1-11, 2016; Article no.BMRJ.20136

hours after which the bacterial colonies were counted and recorded [13].

2.2.2 Determination of Total Heterotrophic Plate Count (THPC)

Total THPC of the water samples were obtained using the spread plate method. Dilutions of 10^{-1} to 10^{-4} of the samples were prepared in 0.1% buffered peptone water (Oxoid) in duplicate and 0.1 ml aliquots of each dilution was inoculated on Nutrient agar and Sabourand Dextrose agars (for total fungal plate count, TFPC) previously prepared and spread with a sterile bent glass rod. The Nutrient agar was incubated at 30°C for 24 hrs while the Sabourand Dextrose agar (SDA) was incubated at 22°C for 7 days [14].

2.2.3 Identification bacterial isolates

Cultural, microscopic examinations as well as biochemical and sugar fermentation tests were used to identify the pure isolates [15].

2.2.4 Identification of fungal isolates

A drop of 70% (v/v) alcohol was placed on the clean microscope slide and the test organism then placed on the drop of alcohol. Two drops of the lactophenol cotton blue mountant were added using a rubber Pasteur pipette before the alcohol dried out. A cover slip was placed on the edge of the mountant to avoid getting air bubble before viewing under the microscope [16].

Sample no	Codes	Name of schools	Source
1	SCA	St. Cecilia's Academy	Borehole
2	RA	Resonance Academy	Borehole
3	BA	Bright-tak Academy	Borehole
4	PSC	Pius Secondary School	Borehole
5	CA	Classical Academy	Borehole
6	UDS	University Demonstration Schools	Borehole
7	JS	Jomeg Schools	Borehole
8	GMA	Gleaming Moon Academy	Borehole
9	MA	May Fair Academy	Borehole
10	NPS	Nekkin Private School	Borehole
11	LMA	Living Word Magnet Academy	Borehole
12	CLA	Christ Land Academy	Borehole
13	AIS	Angelics International School	Borehole
14	MVCS	Master Vessel Christian Academy	Borehole
15	MCS	Marvelous Christian Academy	Borehole
16	TLFS	The Lord favour School	Borehole
17	STGS	St. Theresa's Girls School	Borehole
18	DM	Dominion Montessori	Borehole
19	BNMS	Bishop Nwedo Memorial Secondary School	Borehole
20	SPA	St. Patrick's Academy	Borehole

Table 1a. Coding of samples and sources of water

2.2.5 Antibiotic susceptibility test

The bacterial isolates were subjected to antibiotic susceptibility test by inoculating them into peptone broth over night until the turbidity is equivalent to 0.5 Mcfarland standards and allowed for few minutes at room temperature. The test was performed on Mueller-Hinton agar by the standard disk diffusion method. Sterile swab sticks were used to spread the overnight peptone broth carefully on the entire surface of Mueller-Hinton agar plates. The plates were allowed to stay for 15 minutes before the antibiotic multidisc (Oxoid) was placed on the surface of the inoculate plate and gently pressed. The plates were incubated at 37°C for 18-24 hours. The diameter of zone of inhibition was measured in millimeters and isolates were scored as sensitive or resistant by comparing the values recommended on standard charts. The antibiotic used against the test bacteria were Amoxicillin, Augmetin, Gentamicin, Nalidixic acid, Nitrofurantoin, Ofloxacin, Tetracycline and Cotrimoxazole.

2.3 Determination of Physico-chemical Properties of Water Samples

The pH of the water samples was determined after calibrating the JMP kit (WGpH scan 3) instrument with pH 4, 7 and 10 in accordance with the manufactures instruction manual by dipping the electrode in 100 mls beaker containing the test sample [13]. Electrical Conductivity was determined using the Waytech H198311 water proof EC/TDS meter calibrated in accordance with the manufacturers instruction manual using Waytech H17031 calibration solution (14413M S/cm) [13]. The Total Suspended solid was determined using standard method [13]. The values of potassium, calcium and magnesium hardness in the samples were calculated by reading the sample concentration from the calibration curve and multiplying it by the dilution factor [17] while the Total hardness was determined using EDTA Trimetric method. Calcium and magnesium hardness was determined by titration with EDTA disodium salt solution (0.01m) [13,8].

2.4 Statistical Analyses

The results were analyzed by determining their standard deviation at P=0.5

3. RESULTS AND DISCUSSION

Five bacteria (E. coli, Streptococcus faecalis, Enterobacter aerogenes, Pseudomonas aeruginosa and Staphylococcus aureus) and two fungi (Aspergillus niger and Mucor racemosus) were isolated from the twenty selected borehole water samples analyzed in this research (Tables 1 and 2). The results obtained from the water analysis carried out were compared with that of [18] for drinking water and it shows clearly that all the value obtained fell within the Nigeria Industrial Standard and WHO except samples UDS, JS, PS, RA and GMA. [19] isolated E. coli, Salmonella, Klebsiella and Serriatia species from boreholes of female hostels and opined that the occurrence of these microorganisms may be due to the nature of soil or process of handling. This is in accordance with the findings of [20] that pathogenic bacteria gram negative are extensively found in underground water system where they constitute about 6% to 7% of the isolates recovered. The presence of E. coli in water is of significance which may be as an indication of faecal contamination (pollution) or environmental changes. [21] made similar observation and stated that E. coli is the most frequently used indicator organisms of faecal pollution of water. [22] also reported that coliforms are frequently used as microbial indicators, because their presence in water is solely the consequence of faecal pollution. [21] indicated that coliforms do not always represent faecal pollution because the organism may persist in soil and water for long periods of time and occasionally multiply outside the animal body.

The Total Heterotrophic plate count (THPC) was the range of 3.82 x 10^4 to 9.22 x 10^4 cfu/ml (P=0.5) with boreholes PC, AIS and DM having the highest values and borehole CA having the least value. The total coliform plate count (TCPC) was in the range of 2.41 x 10^3 to 8.61 x 10^3 cfu/ml (P=0.5) with boreholes AIS and DM having the highest and boreholes CA and BA having the least values respectively. [23] gave a Total viable count (TVC) of 6.83 x 10⁵, 5.83 x 10^5 , 3.93 x 10^4 and 4.0 x 10^4 cfu/ml from four boreholes analyzed and these values are higher than the THPC of this work $(3.82 \times 10^4 - 9.22 \times 10^3)$ cfu/ml). The differences in the values could be due to the locations of the four boreholes they analysed. [8] stated that the presence of bushes and shrubs around water bodies makes it likely and possible that some individuals may have been coming around to drink water hereby passing out faeces into the stream water.

The distribution of the microbial groups enumerated in the 20 randomly selected

boreholes shows that Aspergillus niger, Mucor racemosus and Staphylococcus aureus have 100% occurrences in all the boreholes analyzed while Streptococcus faecalis has 60% and Pseudomonas aeruginosa E. coli and Enterobacter aerogenes, had (40.0%) respectively.

Result also shows that some of the borehole water samples from eight private schools were contaminated with both faecal and non-faecal coliform bacteria. *Staphylococcus aureus* was isolated from the 20 water samples while *E. coli, Streptococcus faecalis, Enterobacter aeurogenes, Pseudomonas aeroginosa* were isolated from some of the water samples

analyzed. E. coli is known to cause many enteric diseases such as traveler's diarrhoea and other forms of diarrhoea [24]. Pseudomonas aeruginosa is an opportunistic pathogen responsible for many hospital acquired infection (Nosocomial). The presence of these organisms in water sources therefore does not always represent faecal contamination [25]. Boreholes can be contaminated through flood water which forms after rainfall depending on the borehole location and topography. Their depth. contamination could also be through broken underground pipes when the pressure within the pipes becoming lower than the outside. Under this condition, the surrounding flood water flows into the pipes through the cracks [26].

Tabl	le '	lb.	Morp	holog	ical a	and	bioc	hemic	al io	denti	ficati	on of	isol	ates
------	------	-----	------	-------	--------	-----	------	-------	-------	-------	--------	-------	------	------

Colonial Mormphology	Microscopy	Gram Stain	Motility	Oxidase	Coagulase	Catalase	Glucose	Lactose	Maltose	lsolated organism
Colony smooth & small with convex elevation and opaque	Cocci in chain	+	-	+	+	-	A/G	-	A-	Streptococcus faecalis
Smooth and circular	Short rods	-	+	-	-	+	A/G	A/G	A/G	Escherichia coli
Translucent on nutrient agar	Single and separate short rod	-	+	-	-	+	A/G	A/G	A/G	Enterobacter aerogenes
Smooth, large, circular and creamy colonies with outline edge	Cocci in cluster	+	-	-	+	+	A/G	A-	A-	Staphylococcus aureus
Colonies, round and smooth, creamy to white colour	Small scattered rods	+	-	-	+	+	A/G	A-	A-	Pseudomonas aeruginosa

Key: + = Positive, - = Negative, A= Acid production, A/G= Acid and Gas Production

Та	ble	2.	Mor	pho	logy	of	ide	entifie	ed f	unga	liso	lates
----	-----	----	-----	-----	------	----	-----	---------	------	------	------	-------

Macroscopic characteristics	Microscopic characteristics	Reproduction type	Isolates
White colonies later turns reverse side is brown.	Septate hyhea, unbranched of variable length, double strerigmata covers the vesicle and forms a radiate head.	Conidiophores	Aspergillus niger
White surface with brown reverse	Filamentation and non- sepatate without colia thick wall is formed.	Sporangiospore	Mucor racemosus





Table 3. Distribution of bacteria and fungi in the twenty (20) selected borehole water samples

Isolates	No of boreholes examined	No of boreholes contaminated	Occurrence (%)
Esherichia coli	20	8	40
Enterobacter aerogenes	20	8	40
Pseudomonas aeruginosa	20	8	40
Staphylococcus aureus	20	20	100
Streptococcus faecalis	20	12	60
Mucor racemosus	20	20	100
Aspergillus niger	20	20	100

The fungi identified in this research: Aspergillus niger and Mucor racemosus are normal flora of the soil, terrestrial habitat and fruit plants. The presence of these moulds the in water samples can be attributed to the possibility of leakages in the borehole water which could result from routine maintenance and repairs carried out more often on these boreholes. Water samples from boreholes SCA, BA, CA, MVCA and JS are fit for drinking and domestic uses because they have zero 0/100 ml coliform which is in conformity to the set of standard of [18] which says no water sample should contain faecal coliform in any 100 ml water sample. The other water sample analysed were contaminated with faecal coliform, hence the need for them to be treated before consumption.

Codes	Temperature	Colour	рН	Turbidity	Salinity	Dissolved solids	Dissolved oxygen	Conductivity
SCA	26.01 ^c ±0.01	6.01 [°] ±0.01	6.51 ^f ±0.01	0.01 ^{bcd} ±0.00	0.21 ^b ±0.01	0.06 ^f ±0.00	4.05 ^d ±0.07	200.50 ^d ±0.71
CA	26.02 ^c ±0.02	8.02 ^a ±0.03	7.01 ^b ±0.01	0.02 ^{bcd} ±0.00	0.03 ^{cd} ±0.00	0.61 ^b ±0.01	5.10 [°] ±0.14	241.00 ^b ±1.41
JS	26.02 ^c ±0.03	6.06 ^c ±0.08	6.71 ^d ±0.01	$0.00^{d} \pm 0.00$	0.02 ^{de} ±0.00	0.51 ^c ±0.01	0.61 ^f ±0.01	229.50 ^c ±0.71
GMA	27.03 ^b ±0.04	7.06 ^b ±0.08	7.01 ^b ±0.01	0.02 ^{bcd} ±0.00	0.41 ^a ±0.01	0.71 ^ª ±0.01	7.10 ^a ±0.14	241.50 ^b ±2.12
RA	26.05 ^c ±0.07	6.07 ^c ±0.09	6.81 [°] ±0.01	0.04 ^a ±0.05	0.01 ^e ±0.00	0.31 ^e ±0.01	3.05 ^e ±0.07	240.50 ^b ±0.71
BA	28.02 ^a ±0.03	7.03 ^b ±0.04	7.31 ^ª ±0.01	0.21 ^ª ±0.01	0.03 ^{cd} ±0.00	0.61 ^b ±0.01	7.10 ^a ±0.14	231.00 ^c ±1.41
PCS	26.01 ^c ±0.01	8.06 ^a ±0.08	7.07 ^b ±0.09	0.04 ^{bc} ±0.00	0.04 ^c ±0.00	0.41 ^d ±0.01	6.15 ^b ±0.21	241.50 ^b ±2.12
UDS	27.05 ^b ±0.07	6.01 ^c ±0.01	6.61 ^e ±0.01	0.01 ^{cd} ±0.00	0.03 ^{cd} ±0.00	$0.03^{g} \pm 0.00$	5.10 [°] ±0.14	250.50 ^a ±0.71
GMS	26.06 ^c ±0.08	6.02 ^c ±0.03	6.52 ^f ±0.02	0.01 ^{bcd} ±0.00	0.21 ^b ±0.01	0.61 ^b ±0.01	5.05 ^c ±0.07	240.50 ^b ±0.71
MA	26.07 ^c ±0.09	8.03 ^a ±0.04	7.02 ^b ±0.02	0.02 ^{bcd} ±0.00	0.03 ^{cd} ±0.00	0.51 [°] ±0.01	0.61 ^f ±0.01	229.00 ^c ±0.00
NDS	26.04 ^c ±0.05	6.02 ^c ±0.02	6.72 ^d ±0.02	$0.00^{d} \pm 0.00$	0.02 ^{de} ±0.00	0.71 ^ª ±0.01	7.02 ^a ±0.02	241.00 ^b ±1.41
LMA	27.06 ^b ±0.08	7.07 ^b ±0.10	7.01 ^b ±0.01	0.02 ^{bcd} ±0.00	0.41 ^ª ±0.01	0.31 ^e ±0.01	3.06 ^e ±0.08	240.50 ^b ±0.71
CLA	26.06 ^c ±0.08	6.03 ^c 0.04	6.81 [°] ±0.01	0.01 ^d ±0.00	0.01 ^e ±0.00	0.61 ^b ±0.01	7.06 ^a ±0.08	231.00 ^c ±1.41
AIS	28.07 ^a ±0.09	7.02 ^b ±±0.03	7.31 ^ª ±0.01	0.21 ^ª ±0.01	0.03 ^{cd} ±0.00	0.41 ^d ±0.01	6.07 ^b ±0.09	241.50 ^b ±2.12
MVCS	26.07 ^c ±0.10	8.06 ^a 0.08	7.06 ^b ±0.08	0.04 ^{bc} ±0.00	0.04 ^c ±0.00	0.03 ^g ±0.00	5.06 ^c ±0.08	251.00 ^a ±1.41
MCS	27.06 ^b ±0.08	6.01 [°] ±0.01	6.62 ^e ±0.02	0.01 ^{cd} ±0.00	0.03 ^{cd} ±0.00	0.71 ^ª ±0.01	3.01 [°] ±0.01	240.50 ^b ±0.71
TLFS	27.01 ^b ±0.01	6.07 ^c ±0.10	6.61 ^e ±0.01	0.01 ^d ±0.00	0.03 ^{cd} ±0.00	0.31 ^e ±0.01	7.06 ^a ±0.08	241.50 ^b ±2.12
STGS	26.06 ^c ±0.08	7.07 ^b ±0.09	6.51 ^f ±0.01	0.21 ^{bc} ±0.01	0.02 ^{de} ±0.00	0.61 ^b ±0.01	6.06 ^b ±0.08	230.50 ^c ±0.71
DM	28.11 ^ª ±0.15	8.01 ^ª ±0.01	7.06 ^b ±0.08	0.04 ^{bc} ±0.00	0.41 ^ª ±0.01	0.41 ^d ±0.01	5.06 ^c ±0.08	241.00 ^b ±1.41
BNMS	26.05 ^c ±0.07	6.01 [°] ±0.01	6.71 ^d ±0.01	0.01 ^{cd} ±0.00	0.01 ^e ±0.00	0.03 ⁹ ±0.00	5.07 ^c ±0.09	250.50 ^a ±0.71

Table 4a. Physico-chemical parameters of twenty water samples

^{a-1} Means with the same superscripts in the same column are not significantly different (P<0.05) ^{a-1} Means with different superscripts in the same column are significantly different (P<0.05)

Codes	Total solids	Total acidity	Sodium	Sulphate	Nitrate	Magnesium	Chloride	Calcium
SCA	590.50 ^c ±0.71	1.55 ^c ±0.07	171.00 ^d ±1.41	330.50 ^a ±0.71	20.50 ⁱ ±0.71	25.50 ^c ±0.71	200.50 ^e ±0.71	85.50 ^f ±0.71
CA	581.50 [°] ±2.12	1.85 ^b ±0.07	190.50 ^b ±0.71	291.00 ^c ±1.41	40.00 ^d ±0.00	20.50 ^d ±0.71	230.50 ^c ±0.71	90.50 ^d ±0.71
JS	601.00 ^{ab} ±1.41	1.50 ^c ±0.00	200.50 ^a ±0.71	281.50 ^d ±2.12	30.50 ^f ±0.71	15.50 ^e ±0.71	221.00 ^d ±1.41	88.50 ^e ±0.71
GMA	585.50 ^d ±0.71	2.05 ^a ±0.07	181.00 ^c ±1.41	301.50 ^b ±2.12	20.50 ⁱ ±0.71	10.50 ^f ±0.71	240.50 ^b ±0.71	70.50 ⁱ ±0.71
RA	600.50 ^b ±0.71	0.65 ^e ±0.07	191.00 ^b ±1.41	271.00 ^e ±1.41	35.50 [°] ±0.71	30.50 ^b ±0.71	220.50 ^d ±0.71	78.50 ^h ±0.71
BA	600.50 ^b ±0.71	1.05 ^d ±0.07	170.50 ^d ±0.71	261.00 ^f ±1.41	40.50 ^d ±0.71	35.50 ^a ±0.71	240.00 ^b ±0.00	87.50 ^e ±0.71
PCS	603.00 ^{ab} ±1.41	2.10 ^ª ±0.14	191.50 ^b ±2.12	250.50 ⁹ ±0.71	53.10 ^a ±0.14	25.00 ^c ±0.00	245.50 ^a ±0.71	92.50 ^c ±0.71
UDS	603.50 ^a ±0.71	2.05 ^a ±0.07	191.00 ^b ±1.41	301.50 ^b ±2.12	25.50 ^h ±0.71	20.50 ^d ±0.71	230.50 ^c ±0.71	95.50 ^a ±0.71
GMS	581.00 ^e ±1.41	1.52 ^c ±0.03	201.00 ^a ±1.41	330.50 ^a ±0.71	27.50 ⁹ ±0.71	15.50 ^e ±0.71	220.50 ^d ±0.71	80.50 ^g ±0.71
MA	601.00 ^{ab} ±1.41	1.81 ^b ±0.01	181.00 ^e ±1.41	291.00 ^c ±1.41	40.50 ^d ±0.71	10.50 ^f ±0.71	240.00 ^b ±0.00	88.50 ^e ±0.71
NDS	586.00 ^d ±1.41	1.51 [°] ±0.01	191.50 ^b ±2.12	280.50 ^d ±0.71	30.50 ^f ±0.71	30.50 ^b ±0.71	221.00 ^d ±1.41	70.00 ⁱ ±0.00
LMA	600.50 ^b ±0.71	2.01 ^a ±0.01	170.50 ^d ±0.71	301.00 ^b ±1.41	25.00 ^h ±0.00	35.50 ^a ±0.71	240.50 ^b ±0.71	77.00 ^h ±1.41
CLA	601.00 ^{ab} ±1.41	0.61 ^e ±0.01	190.50 ^b ±0.71	270.50 ^e ±0.71	35.50 [°] ±0.71	15.50 [°] ±0.71	245.50 [°] ±0.71	87.50 ^e ±0.71
AIS	602.50 ^{ab} ±0.71	1.01 ^d ±0.01	200.50 ^ª ±0.71	261.00 ^f ±1.41	51.16 ^b ±0.23	10.00 ^f ±0.00	220.50 ^d ±0.71	93.50 ^{bc} ±0.71
MVCS	603.50 ^a ±0.71	2.01 ^a ±0.01	181.00 ^c ±1.41	251.00 ^g ±1.41	25.50 ^h ±0.71	30.50 ^b ±0.71	241.00 ^b ±1.41	95.00 ^{ab} ±0.00
MCS	585.50 ^d ±0.71	2.01 ^a ±0.01	190.50 ^b ±0.71	301.00 ^b ±1.41	30.50 ^f ±0.71	35.50 ^a ±0.71	220.50 ^d ±0.71	70.50 ⁱ ±0.71
TLFS	600.50 ^b ±0.71	1.81 ^b ±0.01	171.00 ^d ±1.41	280.50 ^d ±0.71	47.50 ^c ±0.71	25.50 ^c ±0.71	240.50 ^b ±0.71	78.50 ^h ±0.71
STGS	601.00 ^{ab} ±1.41	1.52 ^c ±0.02	171.50 ^d ±2.12	300.50 ^b ±0.71	25.50 ^h ±0.71	20.50 ^d ±0.71	220.50 ^d ±0.71	87.50 ^e ±0.71
DM	602.50 ^{ab} ±0.71	2.02 ^a ±0.02	190.50 ^b ±0.71	270.50 ^e ±0.71	52.07 ^{ab} ±0.10	15.50 ^e ±0.71	230.50 ^c ±0.71	93.50 ^{bc} ±0.71
BNMS	603.50 ^a ±0.71	0.61 ^e ±0.01	200.50 ^a ±0.71	261.00 ^f ±1.41	40.50 ^d ±0.71	10.50 ^f ±0.71	220.50 ^d ±0.71	95.50 ^a ±0.71

Table 4b. Physico-chemical parameters of twenty water samples

^{a-1} Means with the same superscripts in the same column are not significantly different (P<0.05) ^{a-1} Means with different superscripts in the same column are significantly different (P<0.05)

Bacterial isolates	Amoxicillin (25μg)	Augumetin (10µg)	Gentamicin (10μg)	Nalidixic acid (30µg)	Nitrofurantoin (30μg)	Ofloxacin (30µg)	Tetracycline (30μg)	Cotrimoxazole (25μg)	
E. coli	S (19)	S(19)	R(12)	S(20)	R(10)	S(19)	S(19)	S(19)	
Pseudomonas aeruginosa	R(11)	R(11)	S(19)	S(19)	R(11)	S(19)	R(10)	l(14)	
Staphylococcus aureus	R(11)	S(19)	S(19)	R(11)	S(19)	S(19)	S(19)	S(19)	
Streptococcus faecalis	R(11)	S(19)	S(19)	R(11)	S(19)	S(19)	S(19)	S(18)	
Enterobacter aerogenes	S(19)	S(19)	R(12)	S(19)	R(10)	S(19)	S(19)	S(18)	

Table 5. Antibiotic susceptibility pattern of bacterial isolates (mm)

R= Resistance, S= Sensitivity, I = Intermediate

Interpretative reference range

	Sensitive	Intermediate	Resistant
Amoxicillin	≥18	14-17	≤13
Augmetin	≥15	13-14	≤13
Gentamicin	15	13-14	≤12
Nalidixic acid	19	14-18	≤13
Nitrofurantoin	17	15-16	≤14
Ofloxacin	22	14-21	≤13
Tetracycline	19	15-18	≤14
Cotrimoxazole	10	11-15	≤19

The THPC of the water samples was in the range 2.40 x 10^4 -8.61 x 10^3 cfu/ml (P=0.5) which is higher than the stipulated guideline of 1.000 cfu/ml [18]. The THPC is significantly high for DM $(8.61 \times 10^3 \text{ cfu/ml})$. This could be due to the fact that the borehole has not been functional or in use in recent time and possibly too because the distance between the borehole and the soak away in the school compound did not meet with the WHO's standard of 30 metres distance between soak away pit and a borehole. A high contamination of the reservoir tanks and distribution system or several repairs in the drill holes and may have accounted for high count of aerobic bacteria in the water sample. The Standards for domestic and portable water state that the THPC should not exceed 100 cfu/100 ml [27]. Although the USEPA standard does not consider THPC to be an important parameter for potable water quality, these water sources with are hiah bacteria counts considered unacceptable for drinking purposes [18]. The proximity of these boreholes to refuse dumps is against the stipulated minimum distances of 30 m.

The physico-chemical parameters of the 20 boreholes were compared with regards to WHO

guidelines for drinking water and NIS. The pH of all water samples fell within the acceptable limits of 6.5 to 8.5 [27]. However, high pH values are not desirable since they impact a bitter taste to the water [7]. The fact that the TDS for all the water were within WHO permissible limits shows that the water samples were not polluted by derived substances. The conductivity is the ability of water to conduct electric current and it is directly related to the total dissolved solid [28]. The TDS gives conformity to the regulatory standards. This explains the reason why the water was odourless. The total solid (TS) and total soluble solid (TSS) were in conformity to both NIS (local) and WHO (international) standards.

Zinc and Magnesium levels were also at the recommended standard by [18]. Zinc is needed in man's food. In pregnancy, zinc deficiency may cause growth retardation in the foetus. But high levels of zinc may cause adverse health effects like anaemia and injury to the pancreas and kidney, disturb protein metabolism and cause arteriosclerosis [29]. Nitrates levels in borehole PCS and AIS did not conform to the WHO standards. Nitrates are known to occur in ground water in high amounts. Its potential toxic effects in infants have been demonstrated. The right potential anthropogenic activity aiding the high concentration of nitrate in water is not clear since there is no active agricultural activity which could supply nitrogen compounds that might be washed into the boreholes. High concentration of nitrates in underground water of shallow aquifer beneath areas of extensive development could be a possible explanation for high concentration level of nitrates in the analyzed boreholes [30].

It is good to know that water samples from SCA, BA, CA, JS, BA, JS, LMA, NPS, TLFS, STGS, SPA, MVCS are of good microbiological and physico-chemical qualities than all other analyzed boreholes. They do not have waste refuse bins around it and do not also meet with [31] stipulated minimum distance of 30 m apart from the soak away pit.

Antibiotic susceptibility test showed that all the bacterial isolates were sensitive to Ofloxacin while *E. coli, Streptococcus faecalis, Enterobacter aerogenes* and *Staphylococcus aureus* were sensitive to six out of the eight antibiotics. *P. aeruginosa* showed highest resistance and that to three antibiotics. The bacteria were most resistant to Amoxycillin and nitrofurantoin respectively.

4. CONCLUSION

It has been established that among the 20 different borehole water analyzed that water samples from boreholes SCA, BA, CA, JS, BA, JS, LMA, NPS, TLFS, STGS, SPA and MVCS met with the internationally recommended microbiological standards for potable water while others do not conform to the standard both nationally and internationally. The sites of boreholes are very important since clean and hygienic environment promote safety of water. The geologist drilling boreholes need to be educated on the microbiological importance of ensuring that dump sites and similar places are not used for drilling of boreholes as this could constitute sources of microbial contaminants. Moreover, the populace needs to be educated on the importance of maintaining clean and hygienic environment around the borehole and well waters to ensure the safety of water from such sources.

5. RECOMMENDATION

Since these boreholes serve as the major source of drinking water for the staff, students and pupils of these private schools, it is recommended that

microbiological and physicochemical examination of these boreholes should be carried out periodically so as to access the suitability of the water for consumption. Regular cleaning of the water reservoir with appropriate cleaning reagent is also recommended. Constant maintenance of the water quality stands as a good means of detecting earlier deviation of drinking water from recommended standard. Boiling of water before drinking would also go a long way in reducing the incidence of contracting pathogenic organisms and their diseases.

ACKNOWLEDGEMENTS

The authors are grateful to the staff of the Central Laboratory Services Unit of National Root Crops Research Institute, Umudike, Umuahia, Abia State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- 1. WHO. Diarrhoea: Why children are still dying and what can be done; 2009.
- 2. Retra D. International Water Resources Association. Water International Journal Germany. 2002;27:3.
- 3. Ranjini K, Roland P, John C, Vincent R. Microbiological and physicochemical analysis of drinking water in George Town. Nature and Science. 2010;8(8):261-265.
- Okonko IO, Adejoje OD, Ogunnusi TA, Fajobi E, Shittu OB. Microbiological and physicochemical analysis of different water samples used for domestic purposes in drinking water in Abeokuta and Ojota, Lagos, Nigeria. African Journal of Biotechnology. 2008;7(5):617-621.
- 5. Chukwura El. Aquatic microbiology. Otoba Press Limited, Onitsha, Nigeria; 2001.
- Okpokwasili GBC, Akojubi TC. Bacteriological indicators of tropical water quality. Environ. Toxicol. Water Qual. 1996;11:77-82.
- Dissanyayake SA, Dias MSSV, Pervea MDC, Iddamalgoda IAVP. Microbial quality assurance of drinking water supplies through surveillance, Water professionals' symposium, Colombo, Sri Lanka. 2004;141-147.
- 8. Edama MD, Omemu AM, Fapetu OM. Microbiological and physicochemical analysis of different sources of drinking

water in Abeokuta, Nigeria. Nigerian Journal Microbial. 2001;15(1):57-61.

- Lamikanra A. Essential microbiological for students and practitioners of pharmacy: medicine and microbiology, 2nd edition. Lagos: Amkra Books. 1999;406.
- Kingsley ES, Andrews J. The chemistry of water, 2nd edition, California: University of Science Books. 1998;13-162.
- Villari P, Pucino A, Santagata N, Torie IA. Comparison of different culture media for the membrane filters quantification of Aeromonas in water. Journal of Applied Microbiology. 1999;29:253-257.
- Anon E. Use of membrane filteration for water analysis. In: Microbiology Laboratory and Field Procedures Manual. Millipore Corporation Bedford UK. 2000;2-7.
- Apha. Standard method for examination of water and waste water. 19th Edition, Published by E and F. N Poan, Washington D.C. 1995;2-56.
- 14. Prescott LM, Harley JP, Klein DA. Microbiology 6th edn. MacGraw Hills Company Inc. New-York. 2008;554-555.
- 15. Collee JG, Duguid JP, Fraser AG, Marmion BP. Salmonella, Shigella, Pseudomonas Vibrio isolation and identification. In: Mackie and McCartney Practical Medical Microbiology, Churchill Livingstone UK. 1989;456-571.
- 16. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press. 2000;143-154.
- Adams VG. Water and waste water examination manual. Lewis Publisher Co. Washington D.C. 1995;67-63.
- WHO. Guideline for drinking water quality, Vol.2; health Criteria and other supporting information, 2nd Edition, World Health Organization, Geneva; 1996
- Muhammad A, Saidu BT, Ahmed Salihu B, Mohammed SA. Bacteriological and physico-chemical properties of borehole water in Niger State Polytechnic, Zungeru Campus. Indian Journal of Sci Res. 2003; 4(1):1-6.

- Chapelle F. Ground water microbiology and geo chemistry. John Wiley and Sons Publihsers. New York. 1992;152.
- 21. Tortora G. Microbiology an Introduction (6th Edition) McGraw Hill, NewYork. 2003;210.
- 22. Greenberg AE. Standard Methods for the Examination of Waste Water, (16 edition). American Public Health Association, Washington, D.C. 1985;108.
- Eze SO, Madumere IC. Physicochemical and microbiological analysis of water bodies in Uturu, Abia State, Nigeria. Asian Journal of Natural and Applied Sciences. 2014;1(4):158-165.
- Pandey A, Sharma E, Palni L. Influence of bacterial inoculation on maize in upland farming systems of the Sikkim Himalaya. Soil Biology and Biochemistry. 1998;3:379-384.
- Balance N, Bartram J. Water quality monitoring. E and F.N, Spon, London, U.K. 1996;30-32.
- Nwachukwu CI, Otokunefor TV. Bacteriological quality of drinking water supplies in the University of Port Harcourt, Nigeria, Nigerian. Journal of Microbial. 2006;20(3):1382-1388.
- WHO. Guidelines for drinking water quality. World Health Organisation, Geneva, Switzerland; 1993.
- Aydin A. The microbiological and physiology quality of water in west thrace, Turkey. Polish Journals of Environmental Studies. 2007;16(3):377-383.
- Noakes TD, Godwin N, Rayner BL, Branken T, Taylor RK. Water intoxication: A possible complication during endurance exercise. Wilderness Environ. Med. 2008; 16(4):221-227.
- Obi CN, Okocha CO. Microbiological and physicochemical analysis of selected borehole waters in World Bank Housing Estate, Umuahia, Abia State, Nigeria. Journal of Engineering and Applied Sciences. 2007;2(5):920-929.
- WHO. Guideline for drinking water quality.3rd edition, Switzerland: WHO Press. 2004;16:89.

© 2016 Obi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history: The peer review history for this paper can be accessed here: http://sciencedomain.org/review-history/12526