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MICROBIOLOGICAL ASSESSMENT OF BOREHOLES, SACHET AND BOTTLE WATER IN AYOBO, LAGOS

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ABSTRACT

The physicochemical and bacteriological quality of borehole, bottle and sachet water sold within Ayobo community Lagos state, Nigeria was investigated. Microbiological analysis was carried out using standard microbial procedure to ensure that the water is microbiologically safe. It was screened for the presence of coliforms and other pathogenic microorganisms. The total heterotrophic bacterial count for bottle, sachet and borehole water are 16.50-123.50 x 10^3CFU/ml, 65.00-73.00 x 10^3CFU/ml and 0.00-72.00 x 10^3CFU/ml respectively while the total heterotrophic fungal count for bottle, sachet and borehole water are 5.00-54.50 x 10^3CFU/ml, 11.00-27.50 x 10^3CFU/ml and 6.00-16.16.00 x 10^3CFU/ml respectively. Most probable number was determined using membrane filtration method and it ranged from 15MPN/100ml, 22MPN/100ml, and 27MPN/100 ml for bottle, sachet and borehole water respectively. The mean total coliform per 100ml ranged from 22-30 x 10^3CFU/ml while fecal coliform ranged from 4-11 x 10^3CFU/ml. The isolated organisms were *Salmonella paratyphi, Shigella flexneri, Proteus vulgaris, Pseudomonas aeroginosa, Escherichia coli, Citrobacter freudii, Salmonella paratyphi, Aspergillus candidus, Aspergillus niger, Aspergillus sulphureus and Penicillium corylophilum*. Most of the bacteria isolated showed multidrug resistance to Augmentin, Gentamycin, Pefloxacin, Tarivid, Streptomycin, Septrin, Chloramphenicol and Amoxacillin and showed susceptibility to Ciprofloxacin. The study therefore concludes that these water samples do not meet the WHO standards for potable water; hence they can be potential sources of waterborne diseases.

Keywords: Antibiotic resistance, microbiological, pathogenic, membrane filtration

INTRODUCTION

Water is essential to sustain life and a number of activities, therefore a satisfactory supply of potable water must be made available to consumers (**Ababiaka & Sule, 2013**). Potable water therefore is a water pure enough to be consumed or used with low risk of immediate or long term harm (**Bello *et al*., 2013**). Like other developing countries, including Nigeria, the issue of access to potable water is very important. The quality and quantity of pipe borne water for drinking is gradually deteriorating in the country due to inadequacy of treatment plants, direct discharge of untreated sewage into rivers and streams, and inefficient management of piped water distribution systems (**Chukwu, 2008; Adekunle *et al*., 2007; Akatah *et al*., 2018**). Contaminated water can carry pathogenic microbes and heavy metals. Globally, about 80% of all diseases and death in developing countries are water-related as a result of polluted water (**Adetunde *et al*., 2011, Adekunle *et al*., 2007**).

The danger that unsafe drinking water poses to health is enormous (**Obi & Okocha, 2007; Addo *et al*., 2009**). Unsatisfactory water supplies and unwholesome sanitary conditions can result in poor human health (**Chukwu, 2008**). Good quality drinking water is a basic factor in guaranteeing public health, the protection of the environment and sustainable development. Thus, many infectious diseases are transmitted by water through fecal oral contamination. Diseases due to drinking of contaminated water leads to the death of five million children annually and make 1/6 of the world population sick (**Bedane, 2008; Isa *et al*., 2013**). Safe drinking water is a basic need for human development, health and well-being; it is an internationally accepted human right (**Bedane, 2008**). The consumption of unsafe water has been implicated as one of the major causes of diarrhea and deaths. The Federal Ministry of Health and various State Ministries of Health in Nigeria are reporting increased number of cases of gastroenteritis, diarrhea, typhoid and cholera which are indicative of poor drinking water quality (**Isa *et al*., 2013**). The gradual deterioration of water quality is a result of the increase in human populations and urbanization (**Akatah *et al*., 2018; Onifade & Ilori, 2008**). As an alternative, private sector participation has led to the idea of packaged drinking water popularly referred to as `pure water. ". This product is 500ml of water in clear nylon square sachets which have been electrically heated and sealed at both ends and widely patronized by both low and middle income earners (**Addo *et al*., 2009**). Sachet-water or pure water is classified as food and is regulated and screened by National Agency for Food and Drug Administration and control in Nigeria, whose bacteriological standards are as recommended by World Health Organization (**Bello *et al*., 2013**). Many people in rural and urban communities rely on sachet water as the source(s) of their drinking water supply. The quality of these sachet waters is doubtful, in fact, unconfirmed report abounds that most of the vendors do not treat their sachet waters before selling to the public (**Onifade & Ilori, 2008**). Although there is lack of documented data on incidence rates, it has been clearly observed that the appearance of pure water has tremendously increased the case of gastroenteritis such as salmonellosis and typhoid fever in recent years (**Bello *et al*., 2013**). Water pollution has continued to generate unpleasant implications for health and economic development in Nigeria (**Bello *et al*., 2013).** Earlier investigation conducted on the safety of drinking water has shown that water on the market is of good microbiological quality while the quality of some factory bagged sachet and bottled and drinking water was noted to be doubtful (**Duressa *et al*., 2019; Addo *et al*., 2009**).

Also, surface water such as streams, rivers and lakes, which are sources of drinking water, are mostly untreated and associated with various health risks (Bedane, 2008). The groundwater is believed to be comparatively much clean and free from pollution than surface water but over exploitation of resources, prolonged discharge of industrial effluents, domestic sewage and solid waste dump causes the groundwater to become polluted and create serious health problems. Other contaminants find their way into groundwater through activities of seepage of municipal landfills, and septic tank effluent. Also, promiscuous waste disposal which is becoming serious in many Nigerian cities that lack efficient waste disposal system or treatment plants also contribute to contamination (Akatah *et al*., 2018).

Ground water is a major source of drinking water in Anchor University and Ayobo community in Lagos state, Nigeria. The state has experience a tremendous increase in population hence an increase in establishment of industries which may act as source of water pollution (**Adetunde *et al*., 2011**). Boreholes and taps located here are exposed to pathogenic microorganisms which can affect public health (**Duressa *et al*., 2019; Bello *et al*., 2013**). Therefore bacteriological assessment is necessary so that the health of the citizens will be secured.

Potable drinking water serve as an important factor for primary prevention of diseases and it continues to be the pillar for the prevention and control of water borne diseases (Isa *et al*., 2013). The importance of potable water supply in the social and economic life of communities and the country at large cannot be overemphasized. Usually, source and portability of water supply reflects on the health conditions of communities as microbiological contamination of water is the primary cause of disease outbreaks in many communities particularly in many developing countries (**Obi & Okocha, 2007**). The transmission of disease through drinking water is, therefore, one of the primary concerns for safe water supply (**Bedane, 2008; Chukwu, 2008**).

In developing countries, including Nigeria, the cases resulting from water borne diseases is becoming alarming, due to the increase in the pollution of water bodies and spread of water borne diseases such as typhoid fever caused by *Salmonella typhi*, shigellosis caused by *Shigella* sp, cholera caused by *Vibrio cholerea*, giardiasis caused by *Giardia* and a host of many others. Hence this study is aimed at investigating the microbiological qualities of borehole, sachet and bottle water in Ayobo community.

MATERIALS AND METHODS

Study area

Ayobo is a suburb and densely populated area in the Ipaja axis of Lagos State. Ayobo is home to a good number of the working population in Lagos who live on the Mainland and work on the Island. Anchor University is also located in Ayobo. Water samples was collected from Anchor University and Ayobo community which is located in Lagos state. Ayobo has the region font code of Africa/Middle East. It is located at an elevation of 42 meters above sea level with coordinates of 6°36'0" N and 3°13'60" E in DMS (Degrees Minutes Seconds).

Sample collection

A total of 12 samples were collected in duplicates from Anchor University and within Ayobo community. The samples were collected in sterile containers from borehole, bottle and sachet water, and transported to the laboratory. These samples were collected in the month of October, 2020.

Microbiological analyses

Pour plate method was used to isolate total heterotrophic bacteria and fungi count. Membrane filtration method was used to determine the number of total and faecal coliforms. Nutrient agar, Simmon citrate agar, MacConkey agar and Eosin methylene blue agar, was used to determine the total bacteria count, while potato dextrose agar was used to evaluate total fungal growth (**Cheesbrough, 2006**).

Characterization and identification of bacterial isolates

The isolates were characterized and identified mainly on the basis of their colony appearance, cellular morphology and biochemical reactions. The colony characteristics of all the isolates (shape, size, consistency, pigments and type of growth on media) were observed and recorded. Gram staining was carried out on the isolated bacteria colony to determine if they were negative or positive bacteria (**Cheesbrough, 2006**).

Isolation and identification of fungi

0.1 ml of the serially diluted aliquot for water sample was inoculated using the pour plate technique on potato dextrose agar supplemented with streptomycin and incubated at 28^0C for 5days. After incubation, there were fungal growth on the plates and the colonies were counted and expressed in CFU/ml, the fungi were characterized and identified by their macroscopic and microscopic features. Lactophenol cotton blue was used for the microscopic identification of the fungal colonies; a small portion of the colony was picked using a sterile loop and smeared on a grease free slide, then a drop of lactophenol was added to the slide and viewed under the microscope with x100 objective lens (**Onifade & Ilori, 2008**).

Antimicrobial susceptibility test

The antibiotics susceptibility test of the isolates was carried out using the Kirby-Bauer disk diffusion technique according to the methods recommended by Clinical Laboratory and Standards Institute (**CLSI, 2018**). Discrete colonies of the bacterial isolates were inoculated into 5ml of normal saline standardized with 0.5 McFarland standard suspensions. Sterile cotton wool swab was used for the inoculation of the bacterial suspension to freshly prepared Mueller-Hinton agar plates prepared according to manufacturer's instructions. The antibiotic sensitivity discs were aseptically and spaciously placed (20mm away from each other) on the inoculated Mueller-Hinton agar plates. The antibiotic discs used were: SXT; Septrin (30µg), R; Rocephin (25µg), AM; Amoxacillin (36µg); CN; Gentamycin (10µg), PEF; Pefloxacin (10µg), APX; Ampiclox (30µg), S; Streptomycin (30µg), E; Erythromycin (10µg) for Gram negative isolates. while SXT; Septrin (30µg), CH; Chloramphenicol (30µg), SP; Sparfloxacin (10µg), CPX; Ciprofloxacin (30µg), AM; Amoxacillin (30µg); AU; Augmentin (10µg), PEF; Pefloxacin (30µg), OFX; Tarivid (10µg) for Gram positive isolates. After incubation, the test plates were examined for confluent growth and zone of inhibition. The diameter of each zone of inhibition was measured in millimeter (mm) using a ruler on the underside of the plate. The interpretation of the measurement as sensitive, intermediate and resistant were made according to CLSI manual (**CLSI, 2018**).

Biochemical tests

The following biochemical tests were carried out on the isolates: Indole test, Catalase test, Methyl red test, Vorges-Proskauer tests, Urease production, Oxidase test, Citrate utilization, and glucose fermentation tests (**Cheesbrough, 2006**).

Statistical analyses

Data were analyzed using SPSS version 25.0. Prevalence of the bacterial isolates was expressed in simple descriptive statistics such as means and standard deviations. For CFU/mL-1 values, one-way analysis of variance (ANOVA) was used, where the levels of significance were set at $P<0.05$, and the means between the samples were separated.

RESULTS

Table I Descriptive Statistics of pathogenic microorganisms isolated from drinking water samples.

Sample	Location	Mean (10^3) ± Standard Deviation (10^3)	
		THBC	THFC
Bottle Water	Anchor	16.50±4.95	5.00 ±5.66
	Ayobo Community	123.50± 4.95	54.50 ±13.44
	Total	70.00 ± 61.91	29.75 ± 29.79
Sachet water	Anchor	65.00 ± 21.21	11.00 ± 9.90
	Ayobo Community	73.00 ± 1.41	27.50 ±19.09
	Total	69.00 ± 13.11	19.25 ±15.65
Borehole Water	Anchor	0.00 ± 0.00	6.00 ± 5.66
	Ayobo Community	72.00 ± 26.87	16.00 ± 5.66
	Total	36.00 ± 44.37	11.00 ± 7.39
Total	Anchor	27.17 ± 31.75	7.33 ± 6.38
	Ayobo Community	89.50 ± 29.04	32.67 ± 20.68
	Total	58.33 ± 43.60	20.00 ±19.70

THBC- total heterotrophic bacteria count, THFC- total heterotrophic fungi count

Table II Total Heterotrophic Bacteria Count

Samples	Location	Nutrient Agar CFU/ml x10^3	EMB Agar CFU/ml x10^3	MacConkey Agar CFU/ml x10^3
BHW_1	Ayobo	2.5	9.1	2.2
BHW_2	Anchor	2.3	Non detected	Non detected
SW_1	Ayobo	4.2	3.64	4.0
SW_2	Anchor	4.15	2.9	Non detected
BW_1	Ayobo	3.1	6.06	Non detected
BW_2	Anchor	1.15	7.5	Non detected

BHW: borehole water, SW: sachet water, BW: bottle water

Table III Mean Total Heterotrophic bacteria count for drinking water sample compared to WHO limit

Sample	THBC (10^3)	WHO standard
Bottle Water	70.00 *10^3	<500cfu/ml
Sachet Water	69.00*10^3	<500cfu/ml
Tap Water	36.00*10^3	<500cfu/ml

Table IV Mean fungal count of the sampled water

	Mean±SD	95% Confidence Interval for Mean	
		Lower Bound	Upper Bound
Bottle Water	29.75±29.79	-17.66	77.16
Sachet Water	19.25±15.65	-5.65	44.15
Tap water	11.00 ±7.39	-0.77	22.77

In determining if there is a difference in the fungal count, the confidence interval was used. (If the confidence interval range is between a negative and a positive value, then there is no difference). The values were all between the range values indicating that there is no difference in the fungi counts in the water samples obtained.

Table V Microscopic and morphological characteristics of fungi present in water samples

Organisms	Morphology	Microscopy
Aspergillus candidus	*Aspergillus*is exclusively an asexually reproducing species. They are rapid growing colonies, flat and filamentous, surface color is greyish green while the reverse is pale yellow, powdery texture.	*Aspergillus candidus* forms typical branching, septate hyphae which produce conidial head at maturity. Sporulation of the spores were moderate, vesicles are spherical
Penicillium corylophilum	It appears dark green with a reverse colouration of colourless to creamy.	It has a shallow center and radially furrowed raised margin. Sporulation is moderate.
Aspergillus sulphureus	Dirty white with yellow spores at the center with a reverse colouration of orange to chocolate.	It has moderation sporulation with septate hyphae
Aspergillus niger	It appears white with typical black spores. A medium size filamentous mould with white margin and black spores seen at 120 hours	Presence of double walled conidiospres which was smooth and hyaline. Sporulation of the spores were heavy

Table VI Bacterial identification with biochemical tests.

Organisms	H_2S	Gas	Urease	VP	Catalase	Coagulase	Indole	Citrate	Methyl red
Shigella flexneri	+	-	-	-	-	-	variable	+	+
Salmonella paratyphi	+	+	-	-	+		-	-	+
Pseudomonas aeruginosa	-	-	_	-	+	-	-	+	-
Escherichia coli	-	+	-	-	-	+	+	-	+
Proteus vulgaris	+	-	+	-	+		-	+	+
Citrobacter freudii	+	+	Variable	-	+		-	+	+

+ = positive; - = negative.

Table VII Antimicrobial susceptibility test

Organisms	**AU**	**CN**	**PEF**	**AM**	**OFX**	**S**	**SXT**	**CH**	**SP**	**CPX**
Salmonella typhi	R	R	R	R	R	S	S	S	S	S
Shigella flexneri	S	S	S	S	S	S	S	S	S	S
Proteus vulgaris	R	R	S	S	R	R	R	S	S	R
Pseudomonas aeroginosa	R	S	S	R	S	S	S	S	S	S
Escherichia coli	S	S	S	S	S	S	S	S	S	S
Citrobacter freudii	R	S	S	S	R	R	S	S	S	S
Salmonella paratyphi	R	R	S	S	R	R	R	S	S	R

Key: R- Resistance, S- susceptibility; AU- Augmentin, CN- Gentamycin, PEF- Pefloxacin, AM- Amoxacilin, OFX- Tarivid, S-Streptomycin, SXT- Septrin, CH- Chlorophenicol, SP- Septrin, CPX- Ciprofloxacin.

DISCUSSION

Water is considered safe when it is free from *Escherichia coli* according to **Ababiaka& Sule, (2013**). The result of this study revealed high contamination of the drinking water collected from Ayobo community and Anchor University Lagos which suggests that the public health risk to water borne diseases is high. According to guidelines for drinking water, the result of this study indicated that all the water samples tested did not meet the required standard, this indicates high level of contamination of drinking water sold in Ayobo community, and similar report was obtained in **Bello *et al.*, (2013); Addo *et al.*, (2009)** and **Isa *et al.*, (2013**). Antibiotics susceptibility of the bacterial isolate showed high degree of resistance, this is in agreement with the report of **Chukwu, (2008)** and **Akatah *et al.*, (2018).** In this study, most species of common pathogenic microorganisms isolated from the drinking water showed resistance to fluoroquinolones and beta-lactamase antimicrobials. These findings shows the possible role of these water in the spread of antibiotics resistant bacteria in the community. **Bello *et al* (2013**); carried out a study on borehole water to determine the bacteriological qualities of the water and the result showed that the presence of pathogenic microorganisms was higher than the WHO standard, this report is similar with the findings in this study. **Addo *et al.*, (2009**); carried out bacteriological analysis on sachet water consumed by students in the Ghana University and it was observed that the coliforms present in the water samples violated the guidelines and standard of WHO for drinking water quality and this was also seen in this report. According to **Isa *et al.*, (2013**), the bacteriological analysis carried out on the sachet water sold in Maiduguri metropolis, the total bacterial count for the water samples were generally high, exceeding the limit recommended by the Environmental Protection Agency (EPA) and World Health Organization (WHO), similar result was also observed in this work.

*Shigella flexneri*a, a Gram-negative bacterium which causes bacterial dysenteries and shigellosis was isolated in this study. This bacterium has the ability to invade and replicate within the colonic epithelium of its host, which results in severe inflammation and epithelial destruction. *S. flexneri* is highly infectious, requiring as little as 100 cells to cause disease in adult (**Duressa *et al.*, 2019**). This organism has the ability to survive under low acidity hence it is very easy for it to survive in the host's stomach. (**Bedane, 2008**). Once *Shigella* reach the colon, they begin to invade the mucosa, penetrating, replicating within and spreading between the mucosal epithelial cells this leads to the symptoms of shigellosis. *Salmonella paratyphi* causes parathyphoid fever (**Bello *et al.*, 2013**). They are

usually spread by eating or drinking food or water contaminated with the feces of an infected person. This can be prevented by drinking clean water, eating warm food, living in a clean environment. After ingestion of the organism, if the immune system of the host is weak, the bacteria multiply and spreads to the bloodstream. It further penetrates in to bone marrow, liver and bile ducts then it further spreads to the immune tissue of the small intestine (**Ababiaka & Sule, 2013**). *Pseudomonas aeroginosa***,** a type of bacteria that is found commonly in the soil and/ water can cause infections in the blood, lungs (pneumonia), or other parts of the body after surgery, and these bacteria are often associated with antibiotic resistance (**Isa *et al.*, 2013**). *Escherichia coli*, is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia (**Adekunle *et al*.,2007**). *Proteus vulgaris* is an opportunistic human pathogen and is known to cause wound infection and other species are known to cause urinary tract infection. *Aspergillus* sp causes aspergillosis which is a respiratory infection and *Pencillium* sp causes penicillosis also known as skin lesions. With the presence of these organisms in these water samples, it is evident that the water is not potable, hence it is not good for public health consumption.

CONCLUSION

The result of the study showed that the borehole, bottle and sachet water sold in the study area has high contamination of pathogenic microorganisms such as *Salmonella paratyphi, Shigella flexneri, Pseudomonas aeroginosa, Escherichia coli* and *Proteus vulgaris*, hence the consumers of the water, used in this study are at risk of water borne disease. Therefore, utmost effort must be channelled towards controlling the contamination of drinking water and this can be achieved by adequate and efficient water treatment. Water is life and it has various health importance to humans. Therefore the need for the consumption of safe water cannot be overemphasized in order to prevent waterborne diseases.

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