Evaluation of the Bacteriological Quality of Sachet and Bottled Water Consumed in A South-eastern University in Nigeria

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Abstract- ***The safety of water is an essential concern. Water is essential to sustain life, and a satisfactory supply must be made available to consumers. It is a critical requirement in the maintenance of metabolic functions and homeostasis (the ability to maintain stable body conditions) in living cells. The aim of this study was to evaluate the Bacteriological quality of sachet and bottled water consumed by undergraduates of Federal University of Technology, Owerri. A total of ten (10) water samples comprising five (5) sachet water and five (5) bottled water samples were collected from different locations. Standard microbiological methods were adopted in the isolation and identification of the bacterial isolates. Total viable bacterial counts ranged from 4.0 x 10^3 cfu/ml to 4.2 x 10^4 cfu/ml for sachet water samples and 8.0 x 10^3 cfu/ml to 1.2 x 10^4 cfu/ml for bottled water samples. There was no coliform growth recorded in all the water samples used in this study. Bacterial isolates from the water samples were; Corynebacterium species, Bacillus species, Proteus species and Pseudomonas species. There is therefore need to ensure good manufacturing practices by producers of sachet and bottled water samples so as to ensure potable water for the students in the study area.***

Indexed Terms- ***Bacteria, Bacteriological Water Analysis, Bacteriological Quality, Potable Water, Sachet Water, Bottled Water***

I. INTRODUCTION

Water is one of the indispensable resources for the continued existence of all living things including man hence adequate supply of fresh, clean and safe drinking water is a basic need for all human beings (Cheesbrough, 2018). Water is defined as a liquid substance that is colourless, odourless, clear and capable of existing in a liquid, solid or gaseous state (Othman, 2015). It is a chemical substance with two atoms of hydrogen and one atom of oxygen in each of its molecules; hence the molecular formula H_2O (Ahmed & Yusuf, 2013). Water is essential to sustain life, and a satisfactory supply must be made available to consumers. It is a critical requirement in the maintenance of metabolic functions and homeostasis (the ability to maintain stable body conditions) in living cells. The human body is composed of about 60% water by weight in adult males, 50% in females and 70% in new born infants (Svagzdiene, Lau, & Page, 2010). Portable water is water pure enough to be consumed or used with low risk of immediate or long term harm. Therefore, adequate and safe water supply should be available to humans, plants, and animals in all parts of the world. The regular intake of adequate

amounts of water is essential in the maintenance of good health and well-being. The approximate human dietary requirement of water is estimated to be two litres per day for an average adult (EFSA, 2010).

According to the World Health Organization (WHO) every effort should be made to achieve a safe drinking water supply in every community of the world because it is known that improving access to safe drinking-water can result in significant benefits to health (WHO, 2010). The most important attribute of drinking water that has to be assured and maintained is its safety and quality for human consumption (Codex, 2001).Drinking water must be free of harmful contaminants, such as pathogenic microorganisms, toxic substances, physical and chemical residues, undesirable organoleptic properties like odour, colour, and taste (Codex, 2009).

The issue of access to portable and safe water is very important in Nigeria, where 48% of Nigerians depend on surface water for domestic use, 57% use hand dug wells, 20% harvest rain, 14% have access to pipe borne water, and 14% have access to borehole water sources (FGN, 2007).The quality and quantity of pipe borne water for drinking is 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 system (Aderibigbe, Awoyemi, & Osagbemi, 2008).It has been estimated that the mortality of water associated diseases exceeds 5 million people per year around the world (Gleick, 2002). Of these, there are reports that more than 50% of these deaths are associated with microbial intestinal infections, particularly with cholera and typhoid.

Microorganisms of concern in contaminated water include bacterial agents of diarrhoea and gastroenteritis namely Salmonella spp., Shigella spp., Escherichia coli and Vibrio cholera (Birmingham, Lea, Ndayiminje, Nkurikiye, Harsh, Well, & Ijeming, 2007). Protozoal agents of diarrhoea include *Entamoeba histolytica, Giardia lamblia, Balantidium coli* (Adeyinka, Wasiu, &Akintayo, 2014). Presence of faecal coliforms or Escherichia coli is used as an indicator for the presence of any of these water borne pathogens (Adeyinka *et. al,* 2014). It is recommended that good quality water should be colourless, odourless, tasteless, and free of faecal contamination and chemicals in harmful amounts (Birmingham *et. al*, 2007).

Hence the need for well treated water for consumption has led majority of the populace to resort to sachet and bottled water. With insufficient government supply of water, private sector participation has evolved and the idea of packaged drinking water popularly referred to as `pure water' is now a common phenomenon in the country. Drinking water is now commercially packed in easy-to-open 50 – 60 ml polyethylene sacs and is referred to as "sachet or pure water". This packaged water is cheap and convenient and have increasingly become popular. Arising from the popularity of the packaged drinking water is the abuse of its production leading to a situation whereby the pure water is everything but pure. Although there is dearth of documented data on incidence rates of water-borne diseases directly associated with consumption of pure water, it has been widely observed that with its advent, the cases of salmonellosis and typhoid fever have significantly increased in recent years (NAFDAC, 2003).

There are several rules and regulations for the production of safe drinking water. In Nigeria, such regulations are monitored by the National Agency for Food and Drug Administration and Control (NAFDAC), which was established as a parastatal of the Federal Ministry of Health by Decree No. 15 of 1993. Surveillance carried out by NAFDAC between 2004, 2005 till date revealed that some producers of packaged water indulge in sharp practices such as packaging of untreated water, production under unhygienic conditions, illegal production of unregistered water in unapproved premises, use of non-food grade sachets and release of packaged water for distribution and sale without date marking. These malpractices compelled the agency to formulate guidelines for the production of wholesome safe packaged water. However, despite the standards formulated by NAFDAC to address this problem, the situation has remained bad. In order to effectively solve the problem, there is a need to fully assess the extent of the problem and its causes. Drinking water regulations require that portable water for human consumption be free from human-disease-causing bacteria and specific indicator bacteria that are

indicative of the presence of these pathogens (Lisle, 2003). Bacteriological quality assessment is of principal concern because of the acute risk to health posed by viruses, bacteria and helminths in packaged drinking-water. Therefore, assessment of drinking-water is primarily a health-based activity which emphasizes the protection of public health through ensuring that the water supplied is of a good quality.
The safety of drinking water is a worldwide public health concern. The World Health Organization (WHO) estimated that 1.1 billion of the world's population does not have access to safe water. In addition, 80 percent of diseases and one-third of deaths in developing countries are due to consumption of contaminated water (WHO, 2011).In Nigeria, it is reported that undergraduates suffer a great deal in the hands of malaria, typhoid and other water borne diseases as a result of poor access to safe water (United Nations, 2012).

Due to the scarcity of safe drinking water in Nigeria, undergraduates obtain their portable water in form of sachet water, popularly referred to as "pure water" and bottled water for those who can afford it. Sachet water is the most common source of drinking water in Nigeria, even among undergraduates given that it is relatively cheap, accessible and generally perceived to be of better quality (Stoler, 2012). Sachet and bottled water industries in Nigeria, mostly owned by private institutions obtain water from surface or underground sources and since both types of water can become contaminated by biological and chemical pollutants originating from point and nonpoint sources.

Significant levels of pesticides like organochloride compounds (lindane, DDT, and endosulfan) and organophosphorus compounds (malathion and chlorpyrifos) have been reported in fresh water systems, sachet water and in the bottled water samples collected from some major cities in Nigeria (Okojokwu & Inabo, 2012). These observations imply that due processes for water treatment are not followed to ensure safe water for consumption (Okojokwu *et. al,* 2012). The bacteriological quality of drinking water is of paramount importance and monitoring must be given highest priority. This is so because studies have attributed several disease outbreaks to untreated or poorly treated water containing bacteria pathogens that have been isolated from sachet water (Shear, Hussein, Chowdhury and. Mamun , 2005). This study was therefore carried out to assess the bacteriological quality of sachet and bottled water consumed by undergraduates of Federal university of Technology, Owerri.

II. METHODS

- Study Setting

This research work was carried out in Federal University of Technology, Owerri. Federal University of Technology, Owerri situated in Owerri West Local Government Area of Imo State.

- Design

A descriptive cross-sectional research design was employed in the study of Bacteriological quality of sachet and bottled water consumed by undergraduates of Federal University of Technology Owerri

- Population of Study

A total of 17 different brands of sachet and bottled water is being sold in Federal University of Technology, Owerri.

- Sample Size

Five bottled and five sachet drinking water brands; making a total of 10 samples out of 17 different manufacturers were purchased from stores and shops in the school hostels, A,B C,D and E respectively. The water samples were labeled according to their various names: Eva, Pax E_dey work, Mr V,Cway, Mangero, Willinelly, Chilec Royal, saint lewis and prephil. The samples were taken to the laboratory for immediate analysis.

- Sampling method

Purposive sampling method was employed to select the sample size used for the study. The researcher used her description to select the most popular and commonly sold and consumed brands of sachet and bottled water respectively.

- Instrument for Data Collection

The instrument used for data collection was experiment. Conical flask, beaker, labeling tape, water samples, autoclave, petri-dishes,70% ethanol, non-

absorbent cotton wool, aluminium foil, test tubes, wireloops, incubators, microscope and distilled water.

- Data Collection Procedures

Five bottled and five sachet water brands from different manufacturers were purchased randomly from local shops, stores from the school hostels of Federal University of Technology, Owerri. All samples were clearly labelled for easy identification and transported to the laboratory for immediate analysis. Each product was carefully opened to avoid contamination. The case of sachet water, an edge of the package was cut with a steriized scissors and carefully placed in a sterilized beaker. The bacteriological parameters were determined by taking water directly from the original package and tested. Ten water samples were analysed in total.

- Preparation of media

The media were dispensed in sterile petri dishes using the spread plate count method and were used for the isolation and subsequent identification of the microorganisms. The media that were used are Nutrient agar, MacConkey agar. Each medium was prepared according to the manufacturer's specifications.

- Sterilization of Glasswares and Media

All the glasswares used in this study were sterilized using laboratory hot air oven at temperature of $160^{O}C$ for 1 hour and media (nutrient agar and macCkonkey agar) used in this study were sterilized using the autoclave at a temperature of $121^{0}C$ at 15 psi for 15 minutes. After the sterilization, the media were brought out together with the glassware and kept on a clean laboratory bench. The media were poured into the Petri-dishes when cooled to $45^{O}C$ and were allowed to solidify (Cheesbrough, 2008).

- Total Heterotrophic Count

An aliquot (0.1ml) of the serial dilutions of 10^4 of each sample was inoculated into well labelled nutrient and macCkonkey agar plates. Hockey stick which has been sterilized by dipping alcohol and flaming was allowed to cool. Each sample was spread evenly onto Nutrient agar, MacConkey agar surfaces respectively (Cheesbrough, 2008).

- Incubation of Cultured Plates

All the plates were incubated for 24 hours at 37ºC for bacterial growth

- Colonial Morphology Identification

The method described by Cheesbrough (2008) was adopted in the colonial morphology identification. Presumptive identification of the colonies was done by observing their individual shape, colour, elevation, edge, surface, consistency and appearance on the media used for isolation. Colonies with characteristic metallic sheen on EMB agar and lactose fermenters on MacConkey agar were noted.

- Purification and Preservation of Isolates

After the various Colony counts, bacterial isolates were picked with a wireloop based on their cultural and morphological characteristics. The picked colonies were sub-cultured onto freshly prepared nutrient agar plates to obtain pure cultures. They were further incubated for 24 hrs at $37^{0}C$. After incubation pure cultures were stored in McCartney Bottle slants and stored in a refrigerator (Cheesbrough, 2008).

- Gram Staining of Isolates

The Gram staining techniques described by Cheesbrough (2008) was adopted. The procedure was as following: A smear of the colony from pure culture was made on a clean grease-free glass slides to be stained. The smears were allowed to air dry and later heat fixed. Crystal violet was added to the slide and allowed for 1 minute. The slide was rinsed with a gentle stream of water for a maximum of 5 seconds. Lugol's iodine was added for 1 minute, then the slide was rinsed again with water. The slide will be rinsed with acid alcohol for 3 seconds and with water. The secondary stain, safranin, was added to the slide and allowed for 1 minute. The slide was rinsed with gentle stream of water for a maximum of 5 seconds. The stained slides were allowed to air dry and were viewed under a microscope using x40 and x100 objective lenses.

Gram positive bacteria retained the primary stain (Crystal violet) and appear purple under the microscope. Gram negative, lost the primary stain and take the secondary stain, causing it to appear pink when viewed under a microscope.

- BIOCHEMICAL TESTS
- Motility Test

A smear of each of the bacteria isolates were made on glass slides and covered with cover slips (wet mount preparation). These were then viewed under the microscope for bacterial motility (Fawole and Oso, 2004).

- Catalase Test

This test is used to differentiate those bacteria that produce the enzyme catalase such as staphylococci from non-catalase producing bacteria such as streptococci. 3 ml of hydrogen peroxide was purred in a test tube. A colony of test organism was taken with sterile wooden or glass rod and immersed it into hydrogen peroxide solution. Generation of bubbles indicated oxygen production. If bubbles were produced, the organism was catalase positive, if bubbles were not produced, the organism was catalase negative.

- Coagulase Test

A drop of distilled water was placed on each end of a slide for each of the test organisms. Thereafter a colony of each of the test organism was emulsified in each of the drops to make two thick suspensions. A loopful of plasma was then-added to one of the suspension and mixed gently for each of the test organism. Clumping within 10 seconds is an indication of positive test while none is an indication of a negative test (Cheesbrough, 2010).

- Indole Test

Some microorganisms are capable of hydrolyzing the amino acid Tryptophan and one of the end products is indole. The ability of a microbe to carry out this reaction can be used for biochemical characterization. The test organisms were suspended in sterile peptone (about 3ml) preparation in sterile test. Tubes and incubated at 37°C for 48 hours after which 0.5ml of Kovac's reagent was added and shaken gently. A red coloration in the surface layer within 10 minutes is an indication of a positive test while none is an indication of a negative test (Cheesbrough, 2010).

- Oxidase Test

The method of Cheesbrough (2010) was adopted for this test. A piece of filter paper was placed in a clean Petri-dish and three drops of fleshly prepared oxidase reagent was added in each case of the test organism. With a sterile piece of stick, each colony of the test organism was removed and smeared on each oxidase reagent drop on the filter paper. The development of a blue-purple coloration is an indication of a positive test while none is an indication of a negative test.

- Sugar Fermentation Test

Each colony of the different test organisms were inoculated onto sterile agar slopes of triple sugar iron agar using stab inoculation. After this, the inoculated, agar slopes were incubated at 37°C for 24 hours. The different colors of the slopes and butts in addition to the presence of gas production and hydrogen sulphide (H_2S) blackening is indicative of the type of bacteria present (Cheesbrough, 2010).

- Citrate utilization test

A bacterial colony was inoculated in Simmons citrate agar and incubated at 35°C to 37°C for 18 to 24 h. Thereafter, development of blue color was observed. Citrate positive showed that growth was visible on the slant surface and the medium became an intense blue while Citrate negative showed trace or no growth was visible and no color change occurred.

III. RESULTS

- Microbial load of the water samples

Table 1 showed the microbial load of the water samples used in this study. Total viable bacterial counts ranged from 4.0×10^3 cfu/ml to 4.2×10^4 cfu/ml for sachet water samples and 8.0×10^3 cfu/ml to 1.2×10^4 cfu/ml for bottled water samples. There was no coliform growth recorded in all the water samples used in this study.

Table 1: Microbial load of the water samples

Samples	Total viable count	Total coliform count (cfu/ml)
Willinelly	4.2×10^4	NG
E_dey work	6.2×10^4	NG
Prephil	8.0×10^3	NG
Saint lewis	1.2×10^4	NG
chilec Royal	4.0×10^3	NG
Mangero	1.2×10^4	NG
Eva	8.0×10^3	NG

Pax	8.0×10^3	NG
C way	1.0×10^4	NG
Mr.V	8.0×10^3	NG

Key: cfu/ml = colony forming unit per millilitre NG = No growth
S1- S5 = Sachet water samples BH1- BH5 = Bottle water samples

- Colonial morphology and biochemical characteristics of the water samples

Table 2 above shows the bacterial isolates from the treated water samples used in this study. They were; *Corynebacterium* species, *Bacillus* species, *Proteus* species and *Pseudomonas* species.

Table 2: Cultural morphology and biochemical characteristics of the bacterial isolates from the water samples

Morphological Characteristics	Gram reaction	Oxidase test	Indole test	Spore test	Catalase test	Citrate test	Coaguase test	Motility test	S	FT	
S	B	G	H_2S								Possible bacteria
Bluish-green, flat,											Gram negative rods
+	-	-		+		-		-	+		
-	+		R	R		-		-			
Pseudomonas species											
non-mucoid colonies											in diploids
Milkish, flat, rhizoid-like											Gram positive rods
-	-	+		+		-		-	+		
-	-		Y	Y		+		-			
Bacillus species											
dry-surface colonies											in short chains
Pale, flat, non-mucoid											Gram negative rods
-	+	-		-		+		-	+		
-	+		R	Y		+		+			
Proteus species											elongated colonies
in short chains											
Milkish, raised, non-mucoid											Gram positive rods
-	-	-		+		-		-	-		
-	-		No Reaction			-		-			
Corynebacterium species											
regular shaped colonies											

KEY: -= Negative += Positive S= color of slope B= color of butt G= Gas production H2S= Hydrogen sulphide production (blackening) R= Reddish coloration (alkaline production) Y= Yellow coloration (Acidic production) SFT= Sugar fermentation test

- Frequency and Percentage Occurrence of the Bacterial Isolates from the Water Samples

The table 3 below shows the frequency and percentage occurrence of the bacterial isolates from the water samples. *Bacillus* species 5(38.5%) was the most frequently isolated bacterial isolate from the sachet water samples while *Proteus* species 1(7.7%) was the least isolated bacteria species. *Corynebacterium* species 3(60.0%) was the most frequently isolated bacterial isolate from the bottled samples used in this study while *Bacillus* and *Proteus* species 1(20.0%) were the least occurring bacteria.

Table 3: Frequency and percentage occurrence of bacterial isolates from the water samples

Bacteria species	Sachet water	Frequency %	Bottled water	(%)
Bacillus species	5	38.5	1	20.0
Proteus species	1	7.7	1	20.0
Pseudomonas species	3	23.1	0	0.0
Corynebacterium sp	4	30.7	3	60.0
Total	13	100.0	5	100.0

Key: % = Percentage

IV. DISCUSSION

Water is an essential resource required for human survival on earth since many activities of man require the use of water. The study demonstrated the microbial

load of the water samples used in this study. Total viable bacterial counts ranged from 4.0 x 10^3 cfu/ml to 4.2 x 10^4 cfu/ml for sachet water samples and 8.0 x 10^3 cfu/ml to 1.2 x 10^4 cfu/ml for bottled water samples. There was no coliform growth recorded in all the water samples used in this study. Omalu *et al.* (2010) in their study on contamination of sachet water reported microbial load of 1.51 x 10^2 to 1.54 x 10^2 with sachet water sold within Lagos metropolis, Lagos State, Nigeria. Bello *et al.* (2013) reported total bacterial count in borehole waters and well water samples from Ijebu-Ode, Ogun State to range from 1.2 x 10^2 cfu/ml to 2.5 x 10^2 cfu/ml. Table 2 shows the bacterial isolates from the water samples used in this study. They were; *Corynebacterium* species, *Bacillus* species, *Proteus* species and *Pseudomonas* species. In Anambra, Nigeria Ezeugwunne *et al.* (2009) isolated bacteria *Escherichia coli* (36%), *Streptococcus faecalis* (19.4%), *Klebsiella pneumonia* (19.4%) and *Staphylococcus aureus* (25%) in sachet water samples analysed. Oladipo *et al*. (2009) worked on the microbial analysis of some vended sachet water in Ogbomoso, Nigeria. The isolates characterized were identified as *Bacillus subtilis, Bacillus alvei, Pseudomonas putida, Pseudomonas fluorecens, Bacillus cereus, Enterobacter aerogens and Proteus mirabilis.* Assessment of quality of packaged water sold in Ibadan, Nigeria showed that 5% of 78 samples (Type A), and 28% of 30 samples (Type B) tested positive for coliform counts. The dominant bacteria were *Klebsella* species, *Streptococcus faecalis* and *Pseudomonas aeroginosa*. Adegoke *et al.* (2012) reported the isolation of *Staphylococcus, Klebsiella*, *Pseudomonas*, *Proteus Enterobacter* species from sachet water samples. Aroh *et al.* (2013) reported the isolation of *Staphylococcus* species, *Enterobacter* species, *Salmonella* species, *Klebsiella* species, *Micrococcus* species from sachet water. The results of this study are similar to the report. The study showed that the frequency and percentage occurrence of the bacterial isolates from the water samples. *Bacillus* species 5(38.5%) was the most frequently isolated bacterial isolate from the sachet water samples while *Proteus* species 1(7.7%) was the least isolated bacteria species. *Corynebacterium* species 3(60.0%) was the most frequently isolated bacterial isolate from the bottled water samples used in this study while *Bacillus* and *Proteus* species 1(20.0%) were the least occurring bacteria. Out of the four (4) bacterial isolates, three (3) were isolated from bottled water while four (4) were isolated from sachet water. Sachet water samples had the highest bacterial isolates compared to the bottled water samples. This could be as a result of non-treatment of water used in filling sachet water. There is therefore need to ensure good manufacturing practices by producers of sachet and bottled water samples so as to ensure potable water for the students in the study area.

CONCLUSION

Assessment of the bacteriological quality of the water samples used in this study showed that sachet water harboured the highest microbial load and bacterial isolates than bottled water samples. Some of the sachet water samples however contained high microbial load which indicates poor manufacturing practices by the manufacturers.

RECOMMENDATIONS

- Assessment of water quality at some important stages of production; pre-production, production and post production stages at the factories is therefore suggested in order to ensure their quality and safety.
- Standard Organization of Nigeria (SON) should be actively involved in the regulation of the quality of packages used in packaging water.
- NAFDAC and the Ministry of Health need to get the producers of 'packaged water' to comply with the national drinking water guidelines. All water that fails NAFDAC and WHO regulations should be withdrawn from the market.

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