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In vitro Bacteriological Assessment of Some Commercial Drinking Water Sold in Port Harcourt Metropolis

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ABSTRACT

The safety of drinking water remains a pressing concern in urban Nigeria, where microbial contamination poses significant public health risks. This study investigated the bacteriological quality of commercially available sachet and bottled water in Port Harcourt metropolis. A total of twenty-four (24) samples; twelve each of sachet water (SW) and bottled water (BW) were randomly purchased and analyzed for total heterotrophic bacterial count, total coliforms, faecal coliforms, and pathogenic bacteria. Descriptive analysis revealed that sachet water samples exhibited a higher microbial load ranging from 105 CFU/mL – 8300 CFU/mL (Mean ± SD: 4376.67 ± 4426.77 CFU/mL;) compared to bottled water which ranged from 101 CFU/mL –460 CFU/mL (Mean ± SD: 280.33 ± 180.69 CFU/mL). However, an independent samples t-test indicated no statistically significant difference in bacterial loads between the two water types ($t = 1.954$, $df \approx 2.2$, $p = 0.18$). A total of 48 bacteria isolates were identified from sachet water samples, with the most frequent being *Escherichia coli* (22.9%), *Salmonella* spp. (22.9%), *Aeromonas* spp. (12.5%), and *Proteus* spp. (12.5%). Less frequent isolates included *Vibrio cholerae* (8.33%), *Pseudomonas spp.* (8.33%), *Acinetobacter spp.* (8.33%), and *Bacillus* spp./*B. subtilis* (each 2.08%). Bottled water samples also harbored pathogenic bacteria such as *E. coli*, *Salmonella* spp., and *Vibrio cholerae*, albeit at a lower frequency. Statistical analysis (Chi-square) showed no significant association between type of bacteria and the source of water ($p = 0.94$), suggesting a wide distribution of potential pathogens in both water types. Bacteria profiling per sample indicated multi-species contamination in both water sources. For example, both water products contained *E. coli*, *Salmonella* spp., and *Aeromonas* spp. In addition, SW1 contained *Vibrio cholerae*, while BW12 harbored *Proteus* spp., and *Bacillus* spp. This widespread presence of enteric pathogens especially *E. coli* and *Salmonella* spp. raises serious concerns regarding the microbiological safety of these water products. These findings underscore the urgent need for improved surveillance, enforcement of production hygiene standards, and public awareness on water safety. Despite regulatory frameworks, the presence of faecal indicators and waterborne pathogens in both sachet and bottled water points to lapses in water purification processes and post-treatment contamination risks in the supply chain.

Keywords: Commercial Drinking Water, Faecal Coliform Bacteria, *Escherichia coli*, *Vibrio cholera*, *Salmonella* spp.

Introduction

Water is abundant in nature as it occupies about 70 % of the earth's crust (Edema *et al*., 2001). But, despite its relative abundance, good quality drinking water is unavailable to over one billion of the world population. Many persons in developing countries do not have access to safe drinking water and only few persons have any kind of sanitary facilities (Adeyinka, *et al.,* 2014).. Unfortunately, good quality drinking water sources are scarce and even when they are available; they are seldom safe for consumption. There is therefore a great need for water to be treated effectively as to make it potable and safe for humans.

Safe drinking water is essential for human life. It is generally considered that bottled water and its alternative sachet water that is kind of affordable (popularly known as pure water) is safe for usage by people (Oladipo *et al.,* 2009). It serves as the only reliable source of drinking water accessible for long-distance travelers. Provision of safe drinking water is one of the most essential amenities to be made available for citizens by the government in the modern world. The non-availability of good quality and safe drinking water has resulted into a number of health challenges as water is known to be a primary causative agent of many contagious diseases.

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In developing countries of the world, 80 % of all diseases and over 30 % of deaths are related to drinking water (Oladipo, *et al*., 2009; Codex, 2009). Water is an important natural resource both for domestic, industrial, and agricultural purposes (FAO, 2022). Despite its importance in the sustenance of life and livelihood, the inability to access safe and potable water has been identified as a major cause of morbidity and mortality (Okonko *et al*. 2012; Olaoye *et al*. 2020).

About two million people every year, most vulnerable are children, die from water-related diseases especially from diarrhea which remains the second leading cause of death among children less than five years globally (Raji & Ibrahim, 2011; Moe & Rheingans, 2006). So, the insufficiency of water supply has given rise to the involvement of private individuals in the production of bottled water and sachet drinking water. Urbanization led to an increase demand of drinking water, more so workers on their way to their offices will be thirsty and need water to drink especially during the dry seasons. This led to increasingly high demand for sachet and bottled water during the last decade due to the fact that people living in both developed and some developing countries have no suitable water supply around their homes (Stoler, 2012; Kwakye *et al.*, 2007).

The World Health Organization reports that over 2.6 billion people lack access to clean water, which is responsible for about 2.2 million deaths annually, of which 1.4 million are children (WHO, 2010). Nigeria is facing many waterborne related challenges and it is disheartening that majority of the population does not have access to good drinking water and must rely on the use of unsafe sources to satisfy basic needs (Adeyinka, *et al*., 2014; Olaoye & Onilude 2009; Tortora & Funke 2002). The adequate supply of clean, fresh and safe drinking water is of great importance to all human beings. Many of the water consumers are unaware of the potential health risks associated with exposure to water-borne contaminants, which have often led to diseases like diarrhoea, cholera, dysentery, typhoid fever, Legionnaire's disease and parasitic diseases (Suthar *et al*., 2008). Water-borne diseases constitute a major burden on human health (WHO, 2010). Microbial contamination is the most common health risk associated with drinking-water. Up to 80% of all sicknesses and diseases in the world are caused by inadequate sanitation, polluted water or unavailability of potable water.

The microbiological quality of drinking water has attracted great attention worldwide because of implied public health impacts (Amira & Yassir, 2011). It has become very important because safe drinking water and adequate water sanitation are basic health needs of humans. Total and fecal coliform and total heterotrophic bacteria status of water have been used extensively for many years as indicators for determining the sanitary quality of water sources.

Therefore frequent examinations of faecal indicator organisms remain the most appropriate way of assessing the hygienic conditions of water. Faecal coliform have been seen as an indicator of faecal contamination and are commonly used to express microbiological quality of water and as a parameter to estimate disease risk (WHO/UNICEF, 2011; Cheesbrough 2006). In 2009, 84% of Nigeria's population had lack of safe drinking water. Although urban coverage is around 84%, the majority of the population (90%) lives in rural areas, where most reports suggest that fewer than 15% have access to potable water. Only 14% of the rural populations have access to safe drinking water supplies (Raji & Ibrahim, 2011). The non-provision of potable and adequate water supply to a population poses serious health risk, productivity and quality of life, as well as on the socio-economic development of the nation (Omalu *et al*., 2010; Onilude *et al*., 2013).

Globalization and the rapid increase in population have placed enormous demands on commerce and industry; the availability and quality of drinking water have also been adversely affected (Adewoye *et al*., 2013; Ezeugwunne *et al*., 2009). Port-Harcourt city, in Rivers State, in the Niger-Delta region of Nigeria, is an example of an urban area with rapid population growth, which has placed pressure on the availability of potable drinking water. The major sources of water for inhabitants of the town include boreholes, wells, rain, sachets (pure water), and bottled water, utilized primarily for the purposes of drinking, cooking, and washing (Omemu& Adergu, 2008). Pollution of the water can be as a result of digging boreholes close to soak-away or septic tanks, which culminates in the percolation of the content of the soak-away or septic tank into groundwater and, by extension, cause waterborne illness such as cholera when the water is utilized for domestic purposes such as drinking (Jay, 2006; Dada, 2009; Edema *et al.,* 2001; Olanrewaju *et al*., 2017).

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Poor manufacturing and handling practices such as production of the water under unhygienic environment, sharp practices, poor hygiene of factory employees and / or vendors and non-adherence to WHO or NAFDAC regulations) can lead to production of unsafe drinking water (Suthar *et al*., 2008; Onilude *et al*., 2013; Anyamene & Ojiagu 2014). Hence, periodic surveillance of packaged drinking water like pure water and bottled water is very much crucial. This will serve the dual purpose of monitoring the standards of commercial drinking water production industries and reinforcing consumer confidence in water quality. This study was therefore carried out to assess the bacteriological assessment of Waterborne Diseases in some commercial drinking water marketed in major transit areas within the Port Harcourt metropolis.

Materials and Methods

Study Area

This study was carried out in Port-Harcourt metropolis, the capital of Rivers State located in the oil rich Niger Delta area of South-South, Nigeria. Rivers State is the Centre for oil exploration and extraction industries in the country and it lies along the Bonny River.

Sample Collection

A total of twenty-four (24) drinking water samples of different brands from different manufacturers were purchased randomly from street hawkers of Aba road and Ikwerre road in Port-Harcourt metropolis. These were made up of twelve (12) bottled water samples designated as BW 1-BW12 and twelve (12) sachet water samples designated as SW1– SW12. All samples were transported to the microbiology laboratory, Rivers State University, Port-Harcourt, for immediate analysis. The sample of each product was carefully opened to avoid contamination. In the case of sachet water, an edge of the package was cut with a sterilized scissors and carefully placed in a sterilized beaker. And the bottled water, the cap was carefully opened with sterilized palms and emptied into sterilized beakers and kept at room temperature; samples were analyzed 2-4 hours after collection, and the analyses were carried out in triplicate, assessing the total heterotrophic counts, total coliform counts and faecal coliform counts.

Preparation of media

Each medium was aseptically dispensed into sterile Petri dishes at a volume of 20-25 ml per plate using the spread plate count method and were used for the isolation and subsequent identification of the microorganisms (Cheesbrough, 2006). Nutrient Agar was used to determine the total bacteria present in the water samples, MacConkey Agar was used for the determination of Coliforms and Eosin Methylene Blue agar (EMB) for faecal coliforms. Each medium was prepared according to the manufacturer’s instruction.

Incubation of Cultured Plates and enumeration of bacteria colonies

Plating was carried out in triplicate and the samples were incubated at 37°C for 24-48 hours for bacterial growth, at the end of which the viable colonies in the three plates were counted and calculated and expressed as colony forming units (CFU/ ml).

Purification and Preservation of Isolates

The discrete colonies of bacterial isolates that developed on the Petri dishes were picked with a sterile 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 37^0C. After incubation pure cultures were streaked on sterile agar in slants in McCartney Bottle and stored in a refrigerator (Cheesbrough, 2006).

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.

Characterization and Identification of Isolates

This was carried out according to standard techniques described by Cheesbrough (2006). Pure culture of each isolate was obtained by aseptically picking distinct colony and culturing them on newly prepared nutrient agar and subsequently incubated at 37^oC for 24 hours.

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The pure cultures of the bacterial isolates obtained were subjected to morphological identification using the Gram- Staining procedure, microscopy of the isolates was done under X100 objective of the light microscope and other biochemical tests were carried out. Characterization of the isolates and identification of the isolates was carried out using the method by Okonko *et al*. (2008). The results of the characteristics of each isolate were compared to those of known taxa using the Bergey's Manual of Determinative Bacteriology (Holt, 1977) for the physiological identification of the isolate.

Gram Staining of Isolates

The Gram staining techniques described by Cheesbrough (2006) 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, and then the slide was rinsed again with water. The slide was 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 Test

Biochemical tests were carried out on colonies formed on the agar plates after incubation as described by Cheesbrough (2006) to determine the identity of the bacteria isolate. The media used were MacConkey Broth, MacConkey agar, Nutrient agar, Deoxycholate agar (DCA), Kligler Iron Agar (KIA), Peptone broth, Eosin Methylene Blue Agar (EMB), Citrate agar, Urea agar and Oxidase reagent, Antisera, Crystal violet stain, Lugol's iodine, Acetone-alcohol decolorizer and Neutral red. The tests carried out were Indole, Methyl red, Voges – Proskauer, Oxidase, Urease test, Citrate Utilization tests, Motility Test, catalase, starch hydrolysis and sugar fermentation were performed.

Determination of Total Heterotrophic Count

An aliquot (0.1ml) of the serial dilutions of 10^{-4} of each sample was inoculated into well labeled nutrient and macConkey 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, 2006). After which bacterial colonies that developed were counted and the result recorded as the number of colony-forming units per milliliter (CFU/ml)

Determination of Total Coliform Bacteria

This was determined using the Most Probable Number (MPN) approach according to APHA, (2015). Ten milliliter (10 mL) of MacConkey broth was poured into each bottles using sterile syringe. Inverted Durham tubes were inserted into each of the bottles. The bottles were then covered and autoclaved for 15 minutes at 121^0C. They were then removed and placed in a sterile environment. Ten milliliter (10 mL) of the water sample was inoculated. The bottles were kept in an incubator and observed at the end of 48 hours. The number of positive bottles, indicated by colour change and gas formation in each of the rolls was recorded and the bacteria load determined from the Most Probable Number (MPN) table.

Determination of faecal coliform

Following incubation, aliquots from the cultured positive tubes were aseptically streaked on Eosin Methylene Blue agar (EMB) for faecal coliform and incubated at 44^oC for 24-48 hours according to the method described by Anyamene & Ojiagu, (2014) and observation for the production gas was done. Gas entrapment in Durham tubes confirmed gas production. And for the counts, 15 g of Cystine – Lactose – Electrolyte Deficient Medium (CLED) was dissolved in 1 litre of distilled water in a conical flask and autoclaved at 121^0C for 15 minutes and allowed to cool. The top of the conical flask was wrapped with foil to prevent contamination. The prepared CLED was dispensed into petri dish and allowed to set. One mL of the water sample was inoculated on the prepared CLED and kept in an incubator for 48 hours at 37°C. Yellow, opaque colonies with slightly deeper coloured centre about 1.25 mm diameter were identified, counted and recorded (Omemu & Adergu, 2008).

Results

Table 1 shows the distribution of bacterial isolates from the twenty-four (24) brands of sachet water and bottled water (12 each) that were analyzed. The bacteria isolated from both water samples were first differentiated on the basis of their cultural and morphological characteristics and physiological characteristics (biochemical tests).

A total of nine (9) bacterial isolates were obtained from the water samples hawked from Aba road and Ikwerre road in Port-Harcourt metropolis. The isolates were identified to be *Escherichia coli*, *Salmonella* spp., *Bacillus subtilis*, *Vibrio cholerae*, *Pseudomonas* spp., *Aeromonas* spp., *Acinetobacter* spp., *Proteus* spp. and *Bacillus* spp. as shown on Table 1.

Table 2 shows the bacterial load of the water samples which includes the total heterotrophic counts, total coliform counts and total faecal coliform counts. The total heterotrophic counts of the SW samples ranges from 0.8 x 10^2- 3.9 x 10^3 while BW samples ranges from 0.6 x 10^2-3.2 x 10^2; total coliform count of SW ranges from 4.4 x 10^2-8.3 x 10^3 while BW samples ranges from 3.3 x 10^2-5.6 x 10^2; and faecal coliform counts of SW ranges from 1.01 x 10^2-1.05 x 10^2 while BW samples ranges from 0-1.03 x 10^2. Only few of the SW and BW samples are below the WHO standards for total heterotrophic counts (SW-2, BW-5), while all the samples (both SW and BW) are above WHO standards for total coliform counts and only few samples (3) of BW are up to the WHO standards for faecal coliform counts while all of SW samples are above WHO standard for faecal coliform counts.

Table 1: Distribution of bacterial isolates from the water samples

Sachet Water Samples		Bottled Water Samples	
Sample code	**Bacteria Isolates**	**Sample code**	**Bacteria Isolates**
SW 1	*Escherichia coli, Salmonella* spp., *Vibrio cholerae, Aeromonas* spp.	BW 1	*Acinetobacter* spp, *Proteus* spp., *Pseudomonas* spp.
SW 2	*E. coli, Salmonella* spp., *Aeromonas* spp., *Proteus* spp.	BW 2	*E. coli, Salmonella* spp., *Bacillus* spp., *Proteus* spp.
SW 3	*Bacillus* spp., *Aeromonas* spp., *E. coli, Salmonella* spp.	BW 3	*Salmonella* spp., *Bacillus subtilis*
SW 4	*Acinetobacter* spp., *E. coli, Salmonella* spp.	BW 4	*Pseudomonas* spp., *E. coli, Vibrio cholerae, Bacillus* spp.
SW 5	*Pseudomonas* spp., *E. coli, Proteus* spp., *Salmonella* spp., *Bacillus subtilis*	BW 5	*Bacillus subtilis, Proteus* spp., *E. coli*
SW 6	*E. coli, Salmonella* spp., *Proteus* spp., *Pseudomonas* spp., *Vibrio cholera*	BW 6	*Acinetobacter* spp., *Pseudomonas* spp.
SW 7	*Aeromonas* spp., *Pseudomonas* spp., *Proteus* spp., *Salmonella* spp.	BW 7	*Acinetobacter* spp., *E. coli, Aeromonas* spp.
SW 8	*Acinetobacter* spp., *E. coli, Vibrio cholera*	BW 8	*Aeromonas* spp., *Acinetobacter* spp.
SW 9	*Salmonella* spp., *E. coli, Vibrio cholerae, Acinetobacter* spp.	BW 9	*E. coli, Vibrio cholerae, Proteus* spp.
SW 10	*E. coli, Salmonella* spp., *Aeromonas* spp., *Proteus* spp., *Pseudomonas* spp.	BW 10	*E. coli, Salmonella* spp., *Aeromonas* spp.
SW 11	*Acinetobacter* spp., *Proteus* spp., *E. coli, Salmonella* spp.,	BW 11	*Bacillus subtilis, Acinetobacter* spp.
SW 12	*Aeromonas* spp., *E. coli, Salmonella* spp.	BW 12	*E. coli, Salmonella* spp., *Aeromonas* spp., *Proteus* spp., *Bacillus* spp.

Table 2: Descriptive Statistics of Bacterial Load in Sachet and Bottled Water Samples (CFU/ml)

Sample Type	Bacterial Load Load 1	Load 2	Load 3	Mean ± SD (CFU/ml)	Range (CFU/ml)
Sachet Water (SW 12)	3900	8300	105	4376.67 ± 4426.77	8195
Bottled Water (BW 12)	280	460	101	280.33 ± 180.69	359

Table 3 shows the statistical comparism of bacterial load in sachet and bottled water and association between water type and bacterial isolates. The Independent Samples T-Test comparing Bacteria load in Sachet water and Bottled water showed that, there was no statistically significant difference in bacterial load ($p > 0.05$). The Chi-Square Test of Association between Water Type and Bacterial Isolates showed that, there was no statistically significant difference in bacterial load ($p > 0.05$). Table 4 shows the frequency and percentage occurrence of the isolates from the Sachet water and Bottled water samples are *Escherichia coli*: SW- 11(22.9%), BW- 7 (19.44%); *Salmonella* spp.: SW- 11 (22.9%), BW- 4 (11.11%); *Vibrio cholerae:* SW- 4 (8.33%), BW- 2 (5.55%); *Aeromonas* spp.: SW- 6 (12.5%), BW- 4 (11.11%); *Acinetobacter* spp.: SW- 4 (8.33%), BW- 5 (13.88%); *Pseudomonas* spp. SW- 4 (8.33%), BW- 3 (8.33%); *Proteus* spp.: SW- 6 (12.5%), BW- 5 (13.88%); *Bacillus* spp.: SW- 1 (2.08%), BW- 3 (8.33%) and *Bacillus subtilis*: SW- 1 (2.08%), BW- 3 (8.33%). Total frequency of isolates in SW sample is 48 while BW is 36 and percentage is 100 as shown on Table 4. Among the organisms identified *Escherichia coli* and *Salmonella* spp. had the highest frequency occurrence in SW samples (11); also *Escherichia coli* had the highest occurrence in BW samples (7).

Table 3: Comparing Bacterial Load in Sachet and Bottled Water and Association between Water Type and Bacterial Isolates

Independent Samples T-Test Comparing Bacterial Load in Sachet and Bottled Water		Chi-Square Test of Association between Water Type and Bacterial Isolates	
Parameter	**Value**	**Parameter**	**Value**
Test Statistic (t)	1.954	Chi-square (χ^2)	2.89
Degrees of Freedom	~2.2	Degrees of Freedom	8
p-value	0.18	p-value	0.94
Significance Level (α)	0.05	Significance Level (α)	0.05
Interpretation: No statistically significant difference in bacterial load ($p > 0.05$)		Interpretation: No significant association between isolate type and water source ($p > 0.05$)	

Table 4: Frequency and Percentage Occurrence of Bacterial Isolates in Water Samples

Bacteria Isolate	Sachet Water (n = 48)	Percentage (%)	Bottled Water (n = 36)	Percentage (%)
Escherichia coli	11	22.9	7	19.44
Salmonella spp..	11	22.9	4	11.11
Vibrio cholerae	4	8.33	2	5.56
Aeromonas spp.	6	12.5	4	11.11
Acinetobacter spp.	4	8.33	5	13.89
Proteus spp.	6	12.5	5	13.89
Bacillus spp.	1	2.08	3	8.33
Bacillus subtilis	1	2.08	3	8.33
Pseudomonas spp.	4	8.33	3	8.33
Total Isolates	**48**	**100**	**36**	**100**

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Discussion

The findings of this study provide critical insights into the microbiological quality of commercially packaged drinking water in sachet and plastic bottles sold along major roads in Port Harcourt, Nigeria. Both qualitative and quantitative bacteriological assessments revealed microbial contamination levels that exceed internationally accepted safety thresholds, posing significant public health concerns.

The total heterotrophic bacterial counts (THBC) for most sachet water (SW) and bottled water (BW) samples were above the World Health Organization (WHO, 2011) recommended limit of 1.0×10^2 CFU/ml. Only SW-2 and BW-5 recorded counts within acceptable limits. Specifically, SW 12 had a significantly elevated mean bacterial load of 4376.67 ± 4426.77 CFU/ml, in contrast to BW 12 with 280.33 ± 180.69 CFU/ml (Table 2). Despite this apparent difference, statistical analysis using the independent t-test showed no significant difference ($t = 1.954$, $p = 0.18$) between the two groups (Table 3), possibly due to large variances and the small sample size. These elevated THBCs, particularly in sachet water, align with reports by Adeyinka *et al*. (2014) and Anyamene & Ojiagu (2014), who found that most sachet waters in Nigerian urban centers harbored high levels of heterotrophic bacteria, often exceeding WHO recommendations.

The presence of coliforms and faecal coliforms in nearly all samples further underscores the compromised safety of these water products. WHO (2011) guidelines stipulate zero faecal coliforms and no more than 1 coliform/100 ml of drinking water. However, all SW samples and the majority of BW samples in this study surpassed these limits, indicating contamination possibly arising from inadequate treatment, poor storage conditions, or environmental exposure during handling. Similar findings were reported by Dada (2009) and Adewoye *et al*. (2013) in studies conducted across southwestern and north-central Nigeria, respectively.

The qualitative bacteriological analysis identified a wide range of pathogenic and opportunistic organisms in both SW and BW samples (Table 1). The most frequently isolated organisms were *Escherichia coli* and *Salmonella* spp., each comprising 22.9% of total isolates from sachet water (Table 3). Other common isolates included *Proteus* spp., *Aeromonas* spp., *Pseudomonas* spp., and *Acinetobacter* spp. The presence of *E. coli*, a key faecal indicator, suggests recent faecal contamination, supporting findings by Olaoye & Onilude (2009) and Oladipo *et al*. (2009), who noted that sachet water commonly sold in Nigerian cities often fails to meet basic microbiological standards.

Comparative studies across various Nigerian states have reported similar microbial profiles. For instance, Omalu *et al.* (2010) and Amira & Yassir (2011) both reported *E. coli*, *Salmonella* spp., and *Pseudomonas* spp. in sachet water, often linked to poor hygiene during production and distribution. In our study, sample SW 6 was found to harbor five different pathogenic bacteria (*E. coli*, *Salmonella* spp., *Proteus* spp., *Pseudomonas* spp., and *Vibrio cholerae*), pointing to gross lapses in microbial control.

The detection of soil- and water-borne bacteria such as *Bacillus subtilis* and *Bacillus* spp. may not always indicate direct faecal contamination but can be attributed to environmental exposure or inadequate sanitation during bottling (Edema *et al.*, 2001). Nonetheless, their presence still represents a microbiological hazard, particularly for immunocompromised individuals. *Vibrio cholerae*, also detected in both sachet and bottled water, is a well-documented causative agent of cholera and its presence in drinking water is considered an emergency public health risk (WHO, 2011).

The chi-square test of association between isolate type and water source (Table 5) revealed no significant relationship ($\chi^2 = 2.89$, $p = 0.94$), indicating that both sachet and bottled water types are similarly susceptible to microbial contamination. This suggests that packaging format alone does not confer safety; rather, production practices, water source, and storage play more critical roles. Omemu *et al.* (2008) highlighted that many packaged water handlers in Nigeria neglect sanitation protocols, which could explain the widespread contamination in both sample types.

Possible sources of contamination include proximity of boreholes to soakaways, use of untreated or poorly treated surface water, unclean bottling equipment, and poor personal hygiene of factory workers. WHO (2011) explicitly states that boreholes and wells should be sited at least 30 meters from septic systems to avoid seepage and contamination.

Unfortunately, many small-scale producers may not adhere to these guidelines, as also noted by Raji & Ibrahim (2011). Furthermore, non-compliance with NAFDAC guidelines on production and distribution of drinking water products has been consistently highlighted by previous researchers including Suthar *et al*. (2008) and Anyamene & Ojiagu (2014). Poor supervision and lack of public awareness contribute to the proliferation of substandard water products in Nigerian markets.

The public health implications of these findings are substantial. The presence of *E. coli*, *Salmonella* spp., and *Vibrio cholerae*—all of which are associated with diarrheal diseases—raises concern for vulnerable populations, especially in densely populated urban areas. Consumption of such contaminated water can lead to typhoid fever, cholera, gastroenteritis, and other gastrointestinal infections, which remain major contributors to morbidity in sub-Saharan Africa.

The findings of this study support and extend previous research, confirming that microbial contamination in commercial drinking water remains a widespread problem in Nigeria. Despite the introduction of regulatory frameworks and quality control guidelines, enforcement appears inadequate. These results call for more aggressive surveillance, stricter regulatory compliance, routine microbiological testing, and improved public health education aimed at water producers and consumers alike.

Conclusion

This study provides compelling evidence of microbial contamination in both sachet and bottled water samples sold in Port Harcourt, Nigeria. The presence of pathogenic bacteria such as *Escherichia coli*, *Salmonella* spp., *Vibrio cholerae*, and *Proteus* spp. in both water types indicates serious lapses in water processing, handling, and regulatory oversight.

Although sachet water exhibited higher bacterial loads and diversity, the detection of similar organisms in bottled water suggests that neither source can be assumed safe without proper quality assurance. The lack of statistically significant differences in bacterial load and isolate distribution between sachet and bottled water further highlights a systemic issue affecting the packaged water industry.

These findings underscore the urgent need for stronger regulatory enforcement, routine microbial surveillance and public education on the risks associated with unsafe drinking water. Ensuring that packaged water meets World Health Organization standards is critical to preventing outbreaks of waterborne diseases and safeguarding public health.

Recommendations

The clear implication of this research is that regulatory bodies such as National Agency for Food and Drug Administration and Control (NAFDAC) must intensify routine inspections and ensure strict compliance with microbial standards across both sachet and bottled water producers. Water sources must be protected from known contamination points such as being too close to soakaways or sewage discharge and boreholes and wells should conform to environment safety guidelines. Producers should adopt enhanced treatment techniques, including UV sterilization or membrane filtration, in addition to chlorination, to ensure elimination of pathogenic bacteria. Public awareness efforts are also essential: consumers should be informed about the risks associated with consuming uncertified water and encouraged to select trusted brands. Similarly, hygiene training for all individuals involved in water production, packaging, and distribution must be mandated and regularly reinforced. Ongoing microbiological surveillance and research are needed to identify emerging pathogens, evaluate treatment efficacy, and guide public health policies. Lastly, transparent labeling including treatment method, batch number, and expiry date should become standard practice to improve traceability, build consumer confidence, and support accountability in the water industry.

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