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Research Article

Assessment of the Microbial Quality of Sachet Water Sold in New Nyanya, Nasarawa State, Nigeria

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ABSTRACT

Sachet water is a widely consumed source of drinking water in Nigeria due to its affordability and accessibility, particularly in urban and semi-urban areas. However, concerns about its microbial safety persist. This study assessed the microbial quality of sachet water sold in New Nyanya area of Karu Local Government, Nasarawa State. 20 sachet water samples were randomly collected from different vendors and analysed for microbial contamination using the Most Probable Number (MPN) method. The pH values of all samples ranged between 7.4 and 8.1, which falls within the World Health Organization's recommended limits for drinking water (6.5 – 8.5). However, microbial analysis revealed coliform contamination in two brands (F and I), with Brand I showing a significantly high coliform count of 43 MPN/100ml – far exceeding the permissible limit of 0 MPN/100ml. Biochemical identification of the isolates confirmed the presence of potentially pathogenic organisms such as *Shigella spp.*, *Klebsiella pneumoniae*, *Salmonella spp.*, and *Pseudomonas aeruginosa*. These findings highlight the potential public health risks associated with the consumption of contaminated sachet water and underscore the need for stricter regulatory oversight, improved quality control during production, and increased public awareness regarding water safety.

Keywords: Coliforms; Microbial Quality; Most Probable Number; Portable Water; Sachet Water

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INTRODUCTION

Water is universally recognized as one of the most vital compounds on Earth, functioning as a universal solvent with several applications in daily life (Fardami *et al*., 2019). It is the main constituent of Earth's hydrosphere and the fluids of all known living organisms. Although it provides no nutrients or calories, water is indispensable for life.

Potable water, also known as drinking water, refers to water that is safe for human consumption, and is vital for hydration, proper body function, and the prevention of dehydration. Global health authorities recommend varying levels of daily intake, typically ranging between 2.0 – 3.7 liters, depending on sex, age, physical activity, and environmental conditions (Agostoni *et al*., 2010).

Despite its importance, lack of safe drinking water remains a major public health issue in developing countries, contributing to around 80% of diseases and more than 30% of deaths (Pal *et al*., 2018). Drinking water quality is generally assessed using physical, chemical, and microbiological parameters. Microbial contamination is however, the most significant contributor to water-related health issues (Phyo *et al*., 2019).

Microbial contamination in portable water is often influenced by factors such as nutrients, disinfectants, pipe materials, packaging, and water chemistry (Zhang *et al*., 2024; Zhao *et al*., 2025). Opportunistic pathogens like *Pseudomonas aeruginosa, Mycobacterium avium,* and *Legionella pneumophila*, thrive within drinking water distribution networks, and pose severe risks to vulnerable populations. Their ability to endure in biofilms and withstand common disinfection techniques

makes their proliferation in water systems worrisome (Falkinham *et al*., 2015).

The World Health Organization reported that unsafe water, sanitation, and hygiene (WASH) were linked to 1.4 million global deaths in 2019, with over 1 million from diarrheal diseases (accounting for 69% of all diarrhea-related deaths in that year) attributed to unsafe WASH practices (Okesanya *et al*., 2024; WHO, 2020). In Nigeria, diarrhea (which largely results from poor WASH conditions) contributes to over 70,000 annual deaths among children under five (UNICEF, 2018).

Sachet water – commonly called "pure water" – has become the most accessible and affordable drinking water source for many Nigerians, particularly in urban and semi-urban communities where there are few alternative sources of potable water (Agbasi *et al*., 2024; Chukwuma *et al*., 2018). Packaged in low-density polyethylene, sachet water is widely distributed in public spaces such as markets, motor parks, and recreational centers. The high demand for sachet water is driven by affordability, ease of access, and the lack of reliable public water supply, with an estimated 60 million units consumed daily in Nigeria (Omole *et al*., 2015).

Although sachet water is widely consumed, concerns about its safety persist. Studies reveal that many sachet water products fail to meet microbiological standards due to poor hygiene during production, inadequate storage, and weak regulatory enforcement (Nwinyi *et al*., 2020). Despite oversight from NAFDAC, numerous producers operate without registration or quality control, leading to frequent contamination. These issues underscore the urgent need for continuous evaluation of sachet water quality. This study therefore aims to investigate the microbial safety of sachet water sold in the New Nyanya area of Karu Local Government, Nasarawa State.

MATERIALS AND METHODS

Study Area

This research was carried out using sachet water samples purchased from New Nyanya, a rapidly growing urban settlement in Karu Local Government Area of Nasarawa State, Nigeria. The town (located at coordinates 8° 34' 13.8000'' N and 8° 18' 31.6800'' E), lies on the outskirts of Abuja, the Federal Capital Territory, and has witnessed rapid population growth due to urban expansion and migration. This demographic pressure has outpaced the development of basic infrastructure, particularly the provision of reliable municipal water supply.

As in many peri-urban Nigerian communities, residents of New Nyanya face inadequate access to safe piped water, making sachet water the most common and affordable source of drinking water. Local entrepreneurs produce and distribute sachet water widely within the community, and its consumption cuts across both low- and middle-income groups. The popularity of sachet water in New Nyanya is therefore directly linked to the settlement's rapid urbanization, limited alternatives for potable water, and the daily demand for affordable, accessible hydration.

Sample Collection

A total of twenty (20) different sachet water brands were purchased from different vendors in New Nyanya, and the samples were coded 'Brand A – Brand T'. The samples were then transported to the Biology Research Laboratory of the National Open University of Nigeria for analysis.

pH of Samples

The pH of each of the samples were determined using a pH meter. The pH meter was calibrated with standard buffer solutions (pH 4, 7 and 10). Distilled water was used to clean the pH electrode to avoid contamination. 100 ml of water sample was transferred carefully into a clean dry beaker, and the pH electrode was dipped into the beaker, ensuring the electrode was fully immersed. The electrode was left in the beaker for a few seconds until a stable pH reading was observed, and the pH value displayed on the pH meter was noted.

Microbial Analysis by Most Probable Number Method

The presence of bacteriological contaminants in the sachet water samples were ascertained through laboratory analysis. The Most Probable Number (MPN), also known as the multiple-tube fermentation technique, was used to detect the presence of coliforms. The MPN procedure was conducted in three stages; presumptive test, confirmed test and completed test as described by (Kumar *et al*., 2013).

Presumptive Test

Single-strength and double-strength lactose broth were prepared according to the manufacturer's instruction. The broth was dispensed into 3 sets of test tubes, comprising 3 test tubes in each set (i.e. 9 test tubes in total); one set of 3 test tubes with 10 ml of double-strength broth, and the other two sets (3 test tubes each) containing 10 ml of single strength lactose broth. Durham tubes were placed in each of the test tubes and the tubes were autoclaved at 121^0C for 15 minutes. 10ml water sample was transferred aseptically into each of the 3 double-strength lactose broth tubes, 1ml was transferred aseptically into each of the 3 tubes of one set of the single-strength lactose broth and 0.1ml was transferred aseptically into each of the 3 tubes of the

last set of single-strength lactose broth. All the inoculated test tubes were incubated at 37^0C for 24 hours. This procedure was followed for all the 20 sachet water samples collected. After incubation, the test tubes were observed for production of gas in the Durham tubes and for color change or turbidity of the media. The number of positive tubes from each set were recorded and compared with the standard chart to give a presumptive coliform count per 100ml water sample.

Confirmed Test

Brilliant Green Lactose Broth (BGLB) was prepared according to the manufacturer's instruction, and dispensed into different test tubes. The tubes were autoclaved at 121^0C for 15 minutes with inverted Durham tubes. A loopful of the suspensions of the positive tubes from the presumptive test were used to inoculate the brilliant green lactose fermentation tubes, and the tubes were incubated at 37^0C for a maximum of 48 hours. After incubation, the tubes were observed for gas production in the Durham tubes and for color change or turbidity of the media. Gas production in BGLB is a confirmed positive result for fecal coliforms.

Complete Test

The completed test is carried out in order to confirm the presence of *E. coli* in water samples. In this process, a loopful of suspension from each positive BGLB tubes was streaked onto Eosin Methylene Blue (EMB) and MacConkey agar plates individually, and the plates were incubated for 37^0C for 24 hours for *E. coli* detection. After incubation, EMB plates were examined for presence of typical colonies with greenish metallic sheen.

Identification of Bacteria Isolates

For further confirmation and satisfactory differentiation between *E. coli* and other coliforms, isolated colonies on EMB and MacConkey agar plates were identified by gram staining, cultural/macroscopic characteristics and biochemical tests according to Bergey's manual.

Cultural Characteristics

The bacterial colonies were observed macroscopically for size, texture, color, shape, colony surface and edges/margin.

Biochemical Tests

Indole, methyl red, Voges-Proskauer, citrate utilization and catalase (IMViC) tests were carried out to identify and differentiate the coliforms.

Indole Test

An 18hr culture of each isolate was inoculated in bijoux bottles containing 3 ml of sterile tryptone water and incubated at 37^0C for 48 hrs. To test for indole, 0.5ml of Kovac's reagent was added and shaken gently to examine for a red ring coloring in the surface layer within 10 minutes. Red surface ring layer indicates positive indole test.

Citrate Utilization Test

This test was carried out by inoculating an agar slant and stabbing the butt of a 5 ml Simmon's citrate agar with the test organism. An uninoculated control was setup in each case. These were incubated at 37^oC for 48hrs, growth indicated that the organism is able to use citrate as a sole carbon source which was accompanied by the medium turning from green to bright blue.

Catalase Test

Three (3) ml of hydrogen peroxide solution was dispensed in a sterile test tube and several colonies of 18 h culture of the test organism was picked and immersed in the hydrogen peroxide solution using a glass rod. It was observed for immediate bubbling which indicated positive result.

Methyl Red Test and Voges-Proskauer test

An 18-hour culture of organism was inoculated in distilled water and incubated for 24 hours. Prepared distilled water plus 0.5 g of D glucose plus 0.5g of potassium palladium was sterilized and then allowed to cool and 2.5 ml of a suspension of the test organism in distilled water was added and incubated for 24 hours. On addition the appearance of red color indicated positive reaction for methyl red. To the remaining 4ml broth in the stock bottle, 5 drops of 40% potassium hydroxide was added followed by 15 drops of 5% α-naphtha in ethanol and shake. The cap of the bottle was loosed and placed in a sleeping position within one hour of standing. No change in coloration indicated negative reaction for Voges-Proskauer test.

RESULTS

The pH values for each water sample were determined, and the results are presented in Table 1. Samples A, F and R had the lowest pH values of 7.4, while samples E and N both had the highest pH values of 8.1. All other samples had their pH values within these two extremes. The 20 sachet water samples were all therefore within the safe pH limits of 6.5 – 8.5 for drinking water.

Bacteriological examination of water for total coliform count was done by Most Probable Number (MPN) method. The presumptive coliform count and 95% confidence limits is shown in Table 2. After 24hrs of incubation, the lactose broth culture tubes were observed for gas production in the Durham tube and cloudiness of the culture broth. It was observed that only 2 out of the 20 samples showed positive results. Brand F had only 1 positive in double strength, with total coliform count of 3.6 MPN/100ml; while Brand I had 3 positives in double strength and 1 positive in single strength tubes, with a total coliform count of 43

MPN/100ml. The other 18 samples had no coliform contamination (<3.0 MPN/100ml).

Some microorganisms other than coliforms also produce acid and gas from lactose fermentation. To confirm fecal coliform presence, the positive tubes from the presumptive test (Brand F and I) were used to inoculate Brilliant Green Lactose Bile (BGLB) in the confirmed test. BGLB contains inhibitors (brilliant green) of Gram-positive organisms and bile which selects for coliforms. After 24hrs of incubation, it was observed that only BGLB tubes from Brand I showed acid and gas production. This confirms the presence of coliform in Brand I. The result for the confirmed test is shown in Table 3.

To detect the presence of the indicator organism (*E. coli*), the positive BGLB tube was used as inoculum to streak onto Eosin Methylene Blue (EMB) and MacConkey agar plates respectively. After an incubation period of 24hrs, the EMB and MacConkey agar plates were examined for distinct colonies, and the results are presented in Table 4. Bacterial growth was observed on both EMB and MacConkey agar plates. However, typical greenish metallic sheen and bright pink colonies which are characteristic morphological features of *E. coli* on EMB and MacConkey agar respectively, were not observed. This is suggestive of the presence of other coliforms besides *E. coli*. For further identification of the isolates, their macroscopic characteristics were examined and biochemical tests (IMViC) were carried out. Table 5 and 6 shows the morphological and biochemical characteristics of the isolates respectively.

Table 1. pH value of Sachet Water Samples

SAMPLES	pH VALUE
Brand A	7.4
Brand B	7.6
Brand C	7.8
Brand D	7.7
Brand E	8.1
Brand F	7.4
Brand G	7.7
Brand H	7.7
Brand I	7.6
Brand J	8.0
Brand K	7.8
Brand L	7.6
Brand M	7.5
Brand N	8.1
Brand O	8.0
Brand P	7.6
Brand Q	7.5
Brand R	7.4
Brand S	7.7
Brand T	7.7

Table 2. Presumptive coliform count per 100ml water sample and 95% confidence limits

Samples	Number of Positive Tubes			MPN	95% confidence limits	
	10ml (DS)	1ml (SS)	0.1ml (SS)	INDEX per 100ml	Low	High
Brand A	0	0	0	<3.0	--	9.5
Brand B	0	0	0	<3.0	--	9.5
Brand C	0	0	0	<3.0	--	9.5
Brand D	0	0	0	<3.0	--	9.5
Brand E	0	0	0	<3.0	--	9.5
Brand F	1	0	0	3.6	0.17	18
Brand G	0	0	0	<3.0	--	9.5
Brand H	0	0	0	<3.0	--	9.5
Brand I	3	1	0	43	9	180
Brand J	0	0	0	<3.0	--	9.5
Brand K	0	0	0	<3.0	--	9.5
Brand L	0	0	0	<3.0	--	9.5
Brand M	0	0	0	<3.0	--	9.5
Brand N	0	0	0	<3.0	--	9.5
Brand O	0	0	0	<3.0	--	9.5
Brand P	0	0	0	<3.0	--	9.5
Brand Q	0	0	0	<3.0	--	9.5
Brand R	0	0	0	<3.0	--	9.5
Brand S	0	0	0	<3.0	--	9.5
Brand T	0	0	0	<3.0	--	9.5

Key: DS = Double Strength; SS = Single Strength

Table 3. Results for confirmed test

Sample	Growth on BGLB
Brand F	-
Brand I	+

Key: - = no growth; + = growth

Table 4. Result for complete test

Sample	Growth on EMB	Growth on MacConkey agar	Greenish metallic sheen on EMB	Bright pink colonies on MacConkey agar
Brand I	**+**	**+**	**-**	**-**

Key: - = no growth; + = growth

Table 5. Morphological characteristics of isolates on EMB and MacConkey agar

Isolates	Shape	Color	Edge	Elevation	Surface	Optical
X1	Rod	Colorless	Entire	Raised	Smooth	Opaque
X2	Rod	Colorless	Entire	Raised	Mucoid	Opaque
X3	Rod	Black	Entire	Raised	Smooth	Opaque
X4	Rod	Blue	Entire	Raised	Smooth	Opaque

Key: X1 = Isolate one; X2 = Isolate two; X3 = Isolate three; X4 = Isolate four

Table 6: Biochemical characteristics of isolates from EMB and MacConkey agar

Isolates	Morphology	Biochemical Tests						Probable organisms
		GR	IN	MR	VP	CI	CA	
X1	Rod	-	-	+	-	-	+	*Shigella spp.*
X2	Rod	-	-	-	+	+	+	*Klebsiella pneumonice*
X3	Rod	-	-	+	-	+	+	*Salmonella spp.*
X4	Rod	-	-	-	-	+	+	*Pseudomonas aeruginosa*

Key: + = Positive, - = Negative, GR = Gram Reaction, IN = Indole Test, MR = Methyl Red Test, VP = Voges Proskauer Test, CI = Citrate Utilization Test, CA = Catalase Test

DISCUSSION

All 20 sachet water samples tested had pH values within the range of 7.4 (Brand A, F and R) and 8.1 (Brand N and E), suggesting that the samples were neither too acidic nor too basic for consumption. This indicates that the sachet water brands meet the acceptable pH standard for drinking water, which is between 6.5 and 8.5 (WHO, 2022). The result also agrees with similar studies by Amoo *et al*. (2025), who analyzed the physicochemical and microbiological quality of sachet water in Dutse urban, Nigeria. According to their report, the pH values of the sachet water samples analyzed was between 6.81 and 7.41. Also, (Adekanmi *et al*., 2020), examined the physicochemical and microbiological analysis of selected sachet water vended in Akure, Ondo State, Nigeria, and reported pH of the samples to be between the range of 7.3 to 8.3.

The bacteriological analysis using the Most Probable Number (MPN) method however revealed that 2 out of the 20 samples (Brand F and Brand I) were contaminated with coliforms. Brand I in particular showed a significantly high coliform count of 43 MPN/100ml, which far exceeds the WHO and Nigerian Standard for Drinking Water Quality (NSDWQ) permissible limit of 0 MPN/100ml for total coliforms in drinking water (NSDWQ, 2015; WHO, 2022). This suggests potential fecal contamination and poor hygienic conditions during production, packaging, or handling.

The confirmed and completed tests showed that the coliforms present in Brand I were not *Escherichia coli*, as no green metallic sheen was observed on EMB agar, nor were typical bright pink colonies observed on MacConkey agar. Nonetheless, further morphological and biochemical tests identified the isolates as *Shigella spp., Klebsiella pneumoniae, Salmonella spp.,* and *Pseudomonas aeruginosa*. This aligns with similar studies conducted across Nigeria. Recent research also reports coliform contamination in sachet water, attributing the issue to inadequate regulation, poor quality control, and unhygienic practices during processing and packaging (Jethro *et al*., 2020). Furthermore, pathogens such as *Klebsiella* and *Pseudomonas* have been isolated from sachet water samples in studies by Anyamene & Ojiagu (2014), reinforcing the findings of this study. Also, a study by

Edema *et al*., (2011) investigated the bacteriological quality of commercial sachet-packed drinking water at point-of-sale in south-western Nigeria, with emphasis on pathogenic bacteria in 108 samples tested. According to their findings, 87% of the sachet-packed water samples examined contained *Salmonella* and/or *Escherichia coli*, indicative of faecal contamination and inadequate water treatment or no treatment at all. These organisms are associated with various waterborne diseases including shigellosis, typhoid fever, diarrhea, pneumonia, and other opportunistic infections (WHO, 2022). Their presence in drinking water is a significant public health concern.

Humans require a consistent, easily available supply of high-quality water since it makes up a sizable portion of the protoplasm and is essential for physiological and biochemical functions (Olusegun *et al*., 2022). Since drinking water is intended for human consumption, it must be devoid of microbial contaminants and other undesirable substances or characteristics, such as color and odor. Because water serves as a conduit for the spread of waterborne pathogens that have the potential to cause illness, customers, water suppliers, regulators, and public health authorities are all concerned about the microbiological quality of drinking water.

CONCLUSION

This study reveals that although the majority of sachet water sold in New Nyanya conforms to acceptable pH levels for drinking water, a portion still falls short in terms of microbiological safety. While pH values ranged between 7.4 and 8.1 (which falls within WHO's recommended range of 6.5 – 8.5), microbial analysis revealed contamination in two samples, especially Brand I, which presented a high coliform count of 43 MPN/100ml. This suggests a breakdown in hygienic protocols during the water's treatment, packaging, or distribution stages.

The presence of harmful bacteria such as *Shigella spp., Klebsiella pneumoniae, Salmonella spp.,* and *Pseudomonas aeruginosa* in Brand I underscores the severity of this issue. These pathogens are known to cause a range of waterborne illnesses, from gastrointestinal infections to life-threatening systemic conditions. The presence of such organisms in consumable water not only violates national and international drinking water standards but also represents a major public health hazard.

Regulators such as NAFDAC and SON should intensify their surveillance and make sure that manufacturers of sachet water adhere to microbiological standards by conducting routine inspections and disciplining noncompliant producers. Public enlightenment campaigns on the dangers of drinking unregulated sachet water and how to recognize certified water products should be conducted.

Good Manufacturing Practices (GMP) should be implemented by producers. These include sterile packing procedures, frequent microbial testing, and appropriate water treatment (chlorination, UV, or ozonation). In order to guarantee continuous compliance, sachet water companies should be required to submit their products for microbial examination to independent labs on a regular basis. To reduce the reliance on sachet water, the government and non-profit organizations should fund community-based potable water projects like boreholes equipped with purifying equipment. To avoid secondary contamination from hands, dust, or sunshine, retailers and distributors should get training on how to handle and store sachet water safely.

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Author Contributions

Conceptualization and Methodology: [Stephen Enyojo Omada and Abraham Gana Yisa]; Formal analysis and investigation: [Ekwo Ogbu Boniface and Marvellous Ukioma Anagwe]; Writing - original draft preparation: [Sadiya Awala Samuel]; Writing - review and editing: [Adaora Nwabiodimma Osegbo].

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