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Bacterial Quality Assessment of Sachet Water Sold in Some Parts of Kaduna South Local Government Area, Kaduna State

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Abstract

Sachet water is any water commercially treated packaged and distributed for sale in sealed nylon bags intended for human consumption. This study was carried out to assess the Bacterial Quality of sachet water from 3 different locations in Kaduna South Local Government Area of Kaduna State. A total of 60 samples of sachet water were collected and analyzed for physicochemical parameters, bacterial counts and the types of bacteria present using standard methods. The result of physicochemical parameters were in accordance with the standards set by the World Health Organization. The results also revealed significant variations in bacterial counts among the sampled locations. The highest bacterial count (4.5×10^4 cfu/ml) was obtained from Unguwan Sunusi while the least bacterial count (1.8×10^3 cfu/ml) was obtained from Sabon Gari. Of the 60 samples analyzed 3 where positive (in MPN values). *Enterobacter* species was the most frequently occurring contaminant (50%) while *Salmonella* species had the least frequency of occurrence (10%). The presence of these organisms constitute public health significance.

Key words: Bacterial quality, assessment, sachet water, Kaduna State.

Introduction

Water is a clear, colorless liquid that forms the natural rivers, streams, wells and other water sources. The quality of natural water is influenced by human economic growth, and human anthropogenic activities (Olanrewaju, *et al*., 2017). Availability of good quality drinking water has resulted into a number of health challenges (Onweluzo *et al.,*2018). Sachet drinking water popularly called "pure water in Nigeria is a lucrative business, with many people involved in the production and marketing of the product (Mosi *et al*., 2019). Constant and periodic assessment of packaged drinking water is needed to satisfactorily enlighten the consumers about quality. Researches have shown that when clean water and needed hygiene conditions are provided, the chances of occurrence of diarrhoea, sleeping sickness and guinea worm can be eliminated or prevented by 50, 80 100 % respectively (Alhassan *et al*., 2015). Bacteriological quality assessment of water involves testing for total coliform, faecal coliform and total heterotrophic status of water. This will provide a reliable means of assessing the extent of pollution as their presence in water exceeding both national and international standards is an indication of a possible presence of enteric pathogens in such water (Alhassan *et al*., 2015).

The conformation of water with the microbiological standards is of special interest because of the capacity of water to spread diseases within a very large population. Although the drinking water standard varies, the objective is to reduce the possibility of spreading waterborne or water-related illness to the barest minimum and also to be pleasant for a drink (Olanrewaju *et al*., 2017). The principal objectives of municipal wastes are to produce and distribute safe water for human consumption. Water contamination happens when undesirable materials with possibilities to undermine human and other common frameworks find their ways into waterways, lakes, wells, streams, boreholes or even held new water in homes and industries. Thus both the quantity and quality of water are affected by an increase in anthropogenic activities (Shar *et al*., 2021).

Contaminated water serves as a mechanism to transmit communicable diseases such as cholera, dysentery, typhoid, and parasitic infections. About 1.8 million deaths per year are attributed to unsafe water supply due to inadequate sanitation and hygiene. Children under five are the most affected group. Waterborne pathogens cause diseases in approximately 250 million people each year, resulting in 10 to 20 million deaths around the globe. The bacteriological quality of 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 bacterial pathogen that have been isolated from sachet water (Ojedapo *et al*., 2023). Assessing the bacterial quality of sachet water sold in some parts of Kaduna State will provide valuable information about the quality of drinking water in the study area.

Materials and Methods

Sachet water sample packaged in transparent nylon bags were purchased from different vendors in Unguwan Sunusi, Tudun Wada and Sabon Gari In Kaduna South Kaduna. Exactly 20 sachets of water were collected from three different locations in kaduna south amounting to a total of 60 samples. The water samples collected were transported to Kaduna State University Laboratory for bacterial analysis.

Media Preparation

Differential media, macConkey, nutrient, eosin methylene blue and salmonella shigella agar were prepared according to manufacturers instructions and used to analyze bacteria in sachet water.

Determination of Physico-Chemical Parameters

The conventional parameters used in assessing the quality and portability of water for drinking are; odor, taste, color, pH, temperature, turbidity, electrical conductivity,total dissolved solid and total suspended solids

Detection of Coliforms

The most probable number (MPN) technique was used to detect the presence of coliform organisms. It involves the presumptive, confirmatory and the completed tests.

Presumptive Test

The sample was shaked vigorously, fifteen (15) test tubes each containing 10ml of macConkey broth were used. The tubes were grouped into three sets of five tubes, thoroughly and covered with foil paper.1ml was transferred from set I tubes were inoculated with 10ml of the water sample, set 2 and set 3 were inoculated with Iml and O.1ml of the sample respectively.Durham tubes were inverted into each of the tube.All these were arrange in the autoclave. The inoculated tubes were then incubated at 36°c for 24-48 hours. Evidence of gas production in the inverted Durham tubes and color change in the broth were noted as positive presumptive test. While absence of gas and no color change were indicated as negative presumptive test.

Confirmatory Test

A confirmatory test was also done to further confirm the presence of coliform. An inoculum from the positive presumptive test was taken and then inoculated into just tubes containing macConkey broth and was incubated at 36°c for 24-48 hours. The presence of any amount of gas constitutes a positive confirmed test for coliform. For feacal coliform, the tubes were incubated at 44-45° for 24-48hours. Gas production indicated a positive result.

Completed Test

For the completed test, Eosine methylene blue agar plates were used. The inoculum from the positive fermentation tubes were streaked on the agar and incubated at 36°c for 24hours. Determination of the Gram-stain characteristics of the organism isolated concluded the completed test for coliform organism.

Isolation of Bacteria

Serial Dilution

For each sample 3 test tubes were labelled each and kept in a test tube rack. Using a sterile syringe 1ml of water sample was transferred to the first tube (10:1) containing 9ml of distilled water. It was mixed thoroughly to ensure complete mixing before adding to the next tube.1ml was transferred from the first dilution tube to the second tube(10:2) containing the same volume of sterile diluent it was mixed and the second dilution tube to the last tube(10:3) it was mixed thoroughly and it contain more liquid than the previous dilutions. 1ml was discarded from the last tube at the end of the serial dilution. The second (10:2) and last (10:3) tubes were used for the pour plating.

Pour Plate Techniques

Two petri dishes were labelled with the water samples name and number (10:2) and (10:3). The first (10:2) petri dish was opened and 1ml of the diluted sample was poured on it. A sterilized nutrient agar was heated a little and 15-18ml of it was poured into the sample making sure that the sample wasn't too hot or too cold. The lid of the dish was closed before it was swirled slowly. Same was done to the second plate (10:3) before they were allowed to solidify. The plates were then incubated at an optimal temperature (usually 37°C) for 24-48 hours. After incubation, the colonies formed were counted and expressed in colony forming unit per millilitre (cfu/ ml).

From the nutrient agar plates, each colony was picked using a sterilized wireloop and streaked onto a differential medium. (EMB, SSA and MacConkey agar) and incubated for 24h

Identification of the Bacterial Isolate

Discreat colonies were Gram stained and all bacteria were confirmed using standard biochemical tests

Results

The physicochemical parameters of the water samples obtained from Kaduna south. The water samples were observed and found to be void of color, taste and odor (Table 1). Sample S.G4 recordedthe highest pH value of (8.4) and sample U.S4 had the least pH value of (6.5) respectively. Similarly the temperature of the water samples showed that sample T.W3 recorded the highest temperature value of $27^{O}C$ and sample U.S1 and S.G1 were recorded with the least temperature value of ($25^{O}C$) respectively.

Similarly the turbidity of the water samples showed that sample U.S5 recorded with the highest turbidity value (1.900). and sample T.W5 was recorded with the least turbidity value of (0.023) respectively. Similarly the electrical conductivity of the water sample showed that sample S.G2 recorded with the highest (EC) value(94.4). and sample U.S4 was recorded with the least EC value of (0.090) respectively.

Similarly the Total dissolved soilds (TDS) of the water sample showed that sample U.S2 recorded with the highest (TDS) value of (599) and sampleS.G2 was recorded with the least TDS value of (215) respectively. Similarly the total suspended solid of the water samples showed that

sampleU.S1,U.S2,U.S3,U.S4,T.W1,T.W2,T.W3,T.W4,T.W5,S.G1,S.G2,S.G3,S.G4andS.G5 have the same (Tss) value of (o).

Table 2 shows the estimated coliform count of the water samples (MPN). Were only 3 water out of 60 were found positive. Which ranged from 5-34 MPN index/100ml.Sample U.S1 recorded the highest coliform count of (34) and sample S.G1 recorded with the least coliform count of (5) and the mean average is 16 respectively.

Table 3 shows the result of the bacteria count of the water sample. Sample U.S has the highest bacteria count of 4.5×10(4) cfu/ml and followed by sample T.W with bacteria count of 3.1×10(3) and sample S.G was recorded with the lowest bacteria count of 1.8×10(3) respectively.

Table 4 shows the cultural and biochemical characteristics of the bacteria isolate. Were the *E. coli* appears as green metallic sheen colony grown on EMB (Gram negative rod). It has indole (+), MR (+), VP(-), citrate(-) and TSI(-). Enterobacter species appears as purple/pink buff color with dark center colony grown on EMB.Gram negative rod It has indole (-), MR(+), VP(-), citrate(+) and TSI(-). Salmonella specie appears as circular black mucoid, buff color with dark colonies grown on SSA. Gram negative rod It has indole (-), MR (+), VP(-), citrate(+) and TSI(-).

Table 5 shows the percentage occurance of the bacteria isolate. The bacterial isolated from the water include *E. coli*, *Enterobacter* and *Salmonella.* Were *Enterobacter* has the highest percentage occuranc and salmonella has the least percentage.

Table 1: Physicochemical Parameters of Sachet Water Samples from Various Locations

S/N	Sample Code	Color	Odor	Taste	pH	Temperature ºC	Turbidity (NTU)	Electrical Conductivity (µSCM)	Total Dissolved Solid (TDS) (mg/L)	Total dissolved solid (TDS) (mg/L)
1	U.S1	Colorless	odorless	tasteless	7.0	25	0.032	84.9	320	0
2	U.S2	Colorless	odorless	tasteless	6.9	26	0.077	73.6	210	0
3	U.S3	Colorless	odorless	tasteless	7.3	26	0.222	88.6	229	0
4	U.S4	Colorless	odorless	tasteless	6.5	26	0.054	0.090	490	0
5	U.S5	Colorless	odorless	tasteless	6.9	26	1.900	0.132	324	0
6	T.W1	Colorless	odorless	tasteless	6.7	26	0.360	79.2	380	0
7	T.W2	Colorless	odorless	tasteless	6.9	26	0.218	69.1	428	0
8	T.W3	Colorless	odorless	tasteless	8.0	27	0.189	93.1	470	0
9	T.W4	Colorless	odorless	tasteless	7.8	26	0.247	70.1	255	0
10	T.W5	Colorless	odorless	tasteless	7.5	26	0.023	22.8	219	0
11	S.G1	Colorless	odorless	tasteless	7.3	25	0.502	77.3	420	0
12	S.G2	Colorless	odorless	tasteless	7.5	26	0.266	94,4	215	0
13	S.G3	Colorless	odorless	tasteless	6.9	26.2	1.288	0.180	485	0
14	S.G4	Colorless	odorless	tasteless	8.4	26	0.528	40.3	180	0
15	S.G5	Colorless	odorless	tasteless	8.0	26	0.600	51.5	440	0
16	NSD/WHO limit	Colorless	odorless	tasteless	6.5-8.5	25-28	0-5	1000	500	-

Key: U.S = Unguwan Sunusi, T.W= Tudun Wada, S.G= Sabon Gari, SN= serial number.

Table 2: Mean Bacterial Counts/cfu/ml of Sachet Water from Different Locations in Kaduna

Location	Mean Count cfu/ml
Unguwan Sunusi,	4.5×10^4
Tudun Wada	3.1×10^3
Sabon Gari	1.8×10^3

Keys: U.S = Unguwan Sunusi, T.W = Tudun Wada, S.G = Sabon Gari

Table 3: MPN Estimation of Coliform Count (Range 5-34) MPN index/100ml

S/N	Sample Code	Combination of Positive MPN Index			
		10ml	1ml	0.1ml	Per 100ml
1	U.S1	4	4	1	34
2	T.W20	2	2	1	9
3	S.G20	2	0	0	5
4	U.S 2- U.S 19	0	0	0	0
5	T.W 1-T.W 19	0	0	0	0
6	S.G 1-SG 19	0	0	0	0

Keys: U.S = Unguwan Sunusi, T.W = Tudun Wada, S.G = Sabon Gari, MPN = Most Probable Number.

Table 4. Cultural and Biochemical Characteristics of Bacterial Isolated from Water Samples

Probable isolates	Colony morphology	Gram Reaction	Cell Morphology	Indole	MR	V.P	Citrate	TSI
Escherichia coli	Green Metallic Sheen	Negative	Rod shape	+	+	-	-	-
Enterobacter species	Purple/Pink buff color with dark centers	Negative	Rod shape	-	+	-	+	-
Salmonella species	circular black mucoid/ color with dark colonies	Negative	Rod shape	-	+	-	+	-

Key:-=not detected, += present, MR=methyl red, VP= Vogesproskauer

Table 5: Percentage Occurrence of the Bacterial in Sachet Water from Different Locations

Location	Sample size	*Escherichia coli*	*Enterobacter species*	*Salmonella species*
Unguwan Sunusi	20	2(10%)	3(15%)	0
Tudun Wada	20	2(10%)	4(20%)	1(5%)
Sabon Gari	20	1(5%)	3(15%)	1(5%)
Total	60	25%	50%	10%

Discussions

All the samples of water analyzed fell within the WHO/NIS standard value for quality water. The standard temperature of drinking water according to WHO/NIS is 25-28°C. (Yakasai *et al*., 2010). The temperature and pH of the sachet drinking water samples fell within the WHO-recommended levels for drinking water. This correspond to similar study on sachet water conducted in southwestern Nigeria.

The difference in pH could result from the water source for sachet water production (APHA, 2023). Turbidity for example refers to water clarity. Higher turbidity levels are often associated with higher levels of disease causing microorganisms such as viruses, parsites and bacteria. Sample U.S5 has the highest turbidity value

In this study, the presence of *E. coli, Enterobacter species*, and *Salmonella* in some of the water samples can be attributed to bad hygienic practices. The presence of *E.coli* could be as a result of fecal contamination from hands of handlers of the packaged water. However in a similar study conducted in Ghana, fecal coliforms including *E. coli* were found in water packaged in sachets (Mosi *et al*. 2019).

The presence of *Salmonella* and *Enterobacter* in sachet water could be due to contaminated water sources, poor hygiene during water purification process, or inadequate sanitation measures in the production environment (WHO 2023; CDC, 2023).

Conclusions

The result of the physicochemical parameters of Sachet Water sold in Kaduna south Local Government Area in Kaduna State conform with WHO and NIS standards.The samples of water collected from Unguwan Sunusi has the highest bacterial contamination. While sample with the least contamination was the sample obtained from Sabon Gari. The types of bacteria isolated were *Enterobacter*, *Escherichia coli* and *Salmonella* species where *Enterobacter* has the highest percentage

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