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Diversity of Microorganisms on the Surface of sachet Water Packs: Implication for Water Quality and Consumer's Health

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Abstract
The physicochemical and bacteriological characteristics of sachet water obtained twice over a two-week period from 9 different sachet water companies located in Owerri West Local Government Area in Imo State were analyzed by standard methods. In addition, swabs were taken from the bag handle as well as inside the bag for analysis. Physical examination of the sachets revealed that only two of the nine water samples, AV and MB met the compliance levels set by the regulatory authorities in terms of label requirements such as registration number, manufacturing and expiration date and producers name and address. Data shows that in the first week, water samples AP and FR recorded the highest temperature levels (26.90 °C). The water samples with the lowest temperature were DO and CH (24.90°C) respectively. In the samples obtained in the second week, FR water sample recorded the highest temperature level (28.9 °C), followed by DA (28°C), while CH recorded the lowest temperature (26.9°C). In all cases, temperature levels of the water samples appeared higher in water samples acquired in the second week. The standard range for conductivity acceptable to WHO is from 0-1000µs/cm. The range observed in the present investigation is a range of 7.67-80.3 which is well in the acceptable range. However, the values are too low. The standard pH value recommended by WHO is a pH of 6.5-8.5. The observed pH range was 4.70-7.30, thus the samples were outside the recommended WHO standard. In the first week of sampling 11% of the sachet were contaminated with both coliform (1.0 x 10^3cfu/ml) and total coliform (5.0 x 10^3cfu/ml) above the WHO and Environmental Protection Agency recommended standards of zero coliforms. The total variable count for swabs from the bag handle ranged from 1.10 x 10^5 to 1.10 x 10^6cfu/ml with the lowest value occurring for sample DO. The total variable count for swabs from inside the bag ranged from 1.0 x 10^4 to 1.49 x 10^6 cfu/ml, which was the highest TVC for water sample AP. The TVC for the sachet water samples ranged from 1.08 x 10^2 to 2.48 x 10^4cfu/ml. Total coliform count range for bag handle swabs was from 2.0 x 10^2 to 5.0 x 10^3cfu/ml for sample PG. The swabs from the inside of the bags ranged from 1.5 x 10^2 to 2.50 x 10^4cfu/ml for water sample IB. Sachet water samples CH, AV, AP, MB, FR, and DA had no total coliform counts. The TCC for the water samples ranged from 0 to 8.0 x 10^2cfu/ml for samples AV and FR respectively. Thus two of the water samples were not safe for consumption. The bacterial isolates observed include Staphylococcus aureus, Escherichia coli, Enterobacter asburiae,Klebsilella pneumoniae, Lysinbacilluscapsici, and Salmonella typhi.

Keywords: *Water quality, Physico-chemical properties, micro-organism, sachet water*

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I. Introduction

Water is an essential component of all living things. It is one of the most exploited natural resources and is thus used for industrial, agricultural and household activities. Water is life, only when it is safe and wholesome (Hassan *et al.,* 2016). Drinking water quality is solely dependent on the quality of source water, the treatment in water treatment plants before distribution, the water distribution system and the tanks used for water storage and household filters (Li and Wu, 2019). The availability of a reliable and clean supply of water is one of the important determinants of health (Parsons and Jefferson, 2006). The World Health Organization (2011) estimates that 1.2 billion of the world's population do not have access to safe water which is odorless, colorless, and free from fecal pollution (Addo *et al.,* 2020). In view of this 80% of diseases and one – third of deaths in developing countries are due to consumption of contaminated water.

Potable water is any packaged water that has been processed, sealed and released into the market under sealed food grade material or other appropriate containers for human consumption (FDA, 2002). Various studies carried out on sachet water have revealed the lack of quality and safety of the sachet water sold in Nigeria

(Afiukwa*et al.,* 2010; Onilude*et al.,* 2013). The lack of cheap pipe borne water is what has led to consumption of pure water.The finding is attributed mostly to dishonest practices such as falsifying meter reading, bribing in order to get pipes connected and inflating the cost of infrastructure. Meanwhilepoor hygiene by the vendors and non-adherence to WHO/NAFDAC regulations (Omalu *et al.,* 2010;Eneh and Eneh, 2014) account for the poor microbiological quality of the pure water samples. Contamination by bacteria has also been reported at various stages of production (Dodoo *et al.,* 2006; Dada, 2011 and Semerjian, 2011). However, sales of packaged water which is generally referred to as 'pure water' has exploded all over the world in recent years, largely because of the perception that they are safe, taste better and are of better quality than tap water (Doria*et al.,* 2009; Sultan *et al.,* 2011).Pure water is mostly sold in motor parks, bus stops, in traffic jams, markets, street corners, shops and public gatherings. Although NAFDAC declared a possible gradual nationwide ban on sachet water to allow the manufacturers of sachet water to gradually change to bottle packaging (Dada, 2009), the sachet water market continued to grow tremendously.

The goal of the World Health Organization is that all people, whatever their stage of development and their social and economic conditions, have access to an adequate supply of safe drinking water (WHO, 2004). In developed countries one has just to turn on the tap to get drinking water. The situation is more exasperating in the developing countries (Parsons and Jefferson). One in three people globally endure some form of water scarcity while one – quarter of the world's population lives in areas where water is physically scarce and human, institutional and financial capital are not available to access the water (Yigezu, 2004; Escobar and Schafer, 2010; Mwebaza, 2010). Botswana, Egypt, Mauritius and Tunisia have 91-100% of their population with access to potable water. However, Nigeria along with Chad, Equatorial Guinea, Mozambique and Niger have just 41-50% of their population with access to potable water (Eneh, 2011).

More people die annually from unsafe water than from all forms of violence combined, including war (Eneh and Eneh, 2014). Every year, an estimated one million Africans die from disease related to unsafe drinking water and poor sanitation. The demand for safe drinking water in Nigeria cannot be over stated because of the inherent inability of government to provide adequate pipe borne water to its teaming populace (Sultan *et al.,* 2011). The increasing demand for the availability of potable water is what has precipitated the need for sachet/pure water.

The indiscriminate consumption of sachet water in Nigeria is of public health concern.The bacteriological quality of some pure water samples sold in Edo, Kebbi and Lagos stateswere found wanting. Sultan *et al.*(2011), Daniel *et al.* (2016), and Halage*et al.* (2015) observed organisms such as *Bacillus, E. coli, Staphylococcus aureus*and *Streptococcus*in water samples. Intensive monitoring activities must be put in place to monitor this quickly expanding industry. It is such health concerns that evolved into this present enquiry.

II. Materials And Methods

Sample Collection

Nine bags of sachet water were obtained from 9 different pure water companies located in Owerri Municipal, Owerri North, and Owerri West Local Government Areas in Imo State and transported to a research laboratory in Federal Polytechnic Nekede, Owerri with minimum delay. A swab stick was later used to swab both the bag handle and the inside of the bag. In addition, the water in the sachets were analyzed. The exercise was repeated again a week after.

Media Preparation and Isolation of Microorganisms

Eosin methylene blue broth, Eosin methylene, MacConkey, salmonella-shigella, Mannitol salt, and nutrient agars were prepared according to manufacturer's instructions, sterilized at 121°C for 15 minutes, and dispensed into sterile petri plates.

Determination of Bacteriological Qualities

Swab sticks were rinsed in sterile distilled water and 0.1ml plated. The swabs were pre-enriched before culturing.The media plates were incubated at 37°C for 48 hours. After the incubation period, the colony forming units were counted and the different colonies isolated. Total coliform and fecal coliform organism numbers were determined using Standard Method 9221 B, standard total coliform fermentation technique. Heterotrophic bacteria were enumerated using Standard Method 9215 C, spread plate method according to Standard Methods for the Examination of Water and Wastewater (APHA, 2012). The number of colony forming units was counted manually.Preliminary characterization of the isolates was done based on microscopy, morphology, and cultural characteristics. Further identification was based on biochemical and molecular analysis (Alwakeel, 2017).

Physicochemical Properties of Sachet Water

The physicochemical parameters of the water were determined via standard AOAC (2008) and APHA (2012) methods. Temperature was measured using standard method 2550 B by way of a multipurpose pH meter

adjusted for temperature in degrees Celsius. The pH was measured by dipping the electrode into 10 ml of the water sample, holding in place for 2-3 minutes to stabilize and reading the pH value. Conductivity was determined using Standard Method 2510 B via a conductivity meter in micro-Siemens per centimeter (µs/cm).Dissolved oxygen was measured by the modified Winkler method as described by Unegbu*et al*. (2017). 300ml BOD bottle was filled with the sachet water sample and closed with a stopper. The stopper was removed and 1 ml of manganous sulphate solution was added to the top of the liquid. The stopper was closed, the bottle shaken, and the floc allowed to settle. 1 ml of concentrated acid was added and the content shaken until precipitate dissolved. 200 ml of the sample was poured into a flask and the solution titrated against 0.0250 N sodium thiosulphate until the solution turned pale yellow. The amount of titrant used was recorded, and a small quantity of starch was added until the blue color disappeared. The total number of ml of sodium thiosulphate used was recorded. Calculation was done using the formula: mg/l DO = ml titrant x normality of titrant x 8000/ volume of sample.

Statistical Analysis

Results obtained were subjected to both descriptive and inferential statistic as described by Ojekunle and Adeleke (2017). The statistical tests were run on SPSS statistical tool version 25. The data was subjected to analysis by anindependent sample t-test.

Genomic Characterization

1. DNA Extraction (Boiling Method)

Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 min. The cells were re-suspended in 500µl of normal saline and heated at 95^{o}C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20^{o}C for other downstream reactions.

2. DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

3. 16S rRNA Amplification

The 16s rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5uM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator.

4. Sequencing

Sequencing was done using the BigDye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10µl, the components included 0.25 µl BigDye® terminator v1.1/v3.1, 2.25µl of 5x BigDye sequencing buffer, 10µM Primer PCR primer, and 2-10ng PCR template per 100bp. The sequencing conditions were as follows: 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

5. Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analysed. The evolutionary distances were computed using the Jukes-Cantor method (Jukes and Cantor, 1969).

III. Results And Discussion

Table 1 represents the physical analysis of the sachet water samples. According to the findings all the water bags were machine packed direct from the various factories. Three of the samples had odor (samples AV, AP and MB). Only 2 of the water samples were not turbid (samples DO and CH). Four of the water samples had production dates (samples AV, PG, AP and MB) while 2 had expiration dates (samples AV and MB). All the samples were colorless except for sample DA).

All the water companies had NAFDAC certification numbers. All packaged water is supposed to undergo scrutiny by NAFDAC which results in certification and allocation of an approval number. According to Dada (2009), virtually all pure water sachet have NAFDAC numbers, however, many are fake and even some of those that were registered have been known to reduce their standards once NAFDAC approval had been awarded. There is also a need to focus on the regulatory aspect of the industry while continuing the microbial and physicochemical studies on the sachet water samples. Water samples AV and MB met the compliance levels set by the regulatory authorities in terms of label requirements such as registration number, manufacturing and expiration date and producers name and address (Duwiejuan*et al.,* 2013).

Figure 1 depicts a descriptive data of the temperature levels of the water samples at both the first and second weeks of sampling. Data shows that in the first week, water samples AP and FR recorded the highest temperature levels (26.90 °C). The water samples with the lowest temperature were DO and CH (24.90°C) respectively. In the samples obtained in the second week, FR water sample recorded the highest temperature level (28.9 °C), followed by DA (28°C), while CH recorded the lowest temperature (26.9°C). In all cases, temperature levels of the water samples appeared higher in water samples acquired in the second week.

Temperature is basically a measure of the thermal energy of a substance. The water temperature range observed is just about ambient temperature. Temperatures within this range are favorable for maximum growth of mesophilic bacteria, including disease causing agents. This temperature range has a tendency to promote the development of undesirable taste and odor in water with time (Ibrahim *et al.,* 2015).

Table 1: Physical analysis of sachet water samples

SAMPLE	COLOURLESS	EXPIRATION DATE	PRODUCTION DATE	MACHINE PACKED	TURBIDITY	ODOUR
DO	+	-	-	+	-	-
CH	+	-	-	+	-	-
AV	+	-	+	+	+	+
PG	+	-	+	+	+	-
AP	+	-	+	+	+	+
MB	+	-	+	+	+	+
FR	+	-	-	+	+	-
CL	+	-	-	+	+	-
DA	-	-	-	+	+	-

Keys + = yes, - = no

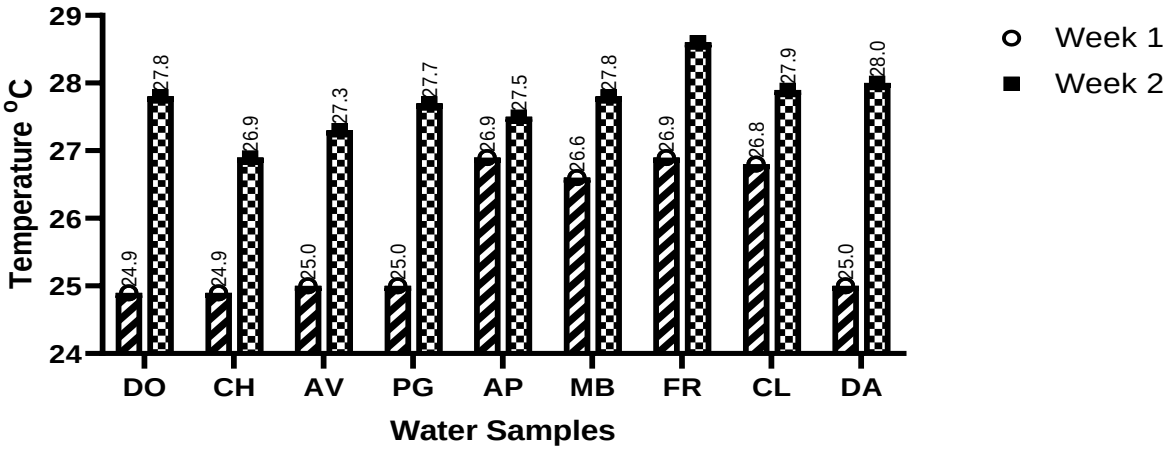

Figure 1: Descriptive data of the temperature levels of the water samples at both the first and second weeks of sampling respectively.

Figure 2 is an illustration of the degree of conductivity of the water samples during the first and second weeks of sampling. It shows that of the sachet water samples taken in the first week, FR water samples recorded the highest conductivity(80.3 µs/cm), followed by CH water sample (63.4µs/cm). The lowest conductivity was observed in MB water samples (7.67 µs/cm). In the samples taken in the second week, AP water sample recorded the highest conductivity (66.2), followed by DO water (64.2µs/cm), while MB water indicated the lowest conductivity (13.79µs/cm). Water samples DO, AV, AP, MB, CL and DA indicated higher conductivity levels in the second week than the first week. On the other hand, water samples from CH, PG and FR appeared higher in the first week compared with the second week. There should not be any variation from week to week. The standards were lowered and the NAFDAC regulations flaunted (Omalu *et al.,* 2010).

The standard range for conductivity acceptable to WHO is from 0-1000µs/cm. The ranged observed in the present investigation is a range of 7.67-80.3 which is well in the acceptable range. However the values are too low. Conductivity is a measure of water's capability to pass electrical flow which is directly related to the concentration of ions in the water (Wetzel, 2001). The more ions that are present, the higher the conductivity of water and vice versa. It therefore follows that the low electrical conductivity denotes the presence of small amounts of dissolved salts in the water (Ndinwa*et al.*, 2012; Opafolaand David, 2020). The results are similar to that observed by Ibrahim *et al.* (2015) and Joshua *et al.* (2019).

Figure 3 represents the pH level of the water samples during the first and second weeks respectively. The data shows that of the sachet water samples examined in the first week, water sample AP indicated the highest pH level (7.23), followed by sample FR (6.74), while sample CL appeared to have the lowest pH (5.52). For sachet water samples obtained in the second week, water sample CH indicated the highest pH (7.30), followed by AV (6.83) while MB water sample had the lowest pH (4.57). Water samples from DO, CH, and AV indicated higher pH levels for samples obtained in the second week than the first week. On the other hand, water samples PG, AP, MB, FR, CL and DA appeared higher in the first week compared with the second week.

The pH is one of the parameters that affect the aesthetic quality of drinking water (WHO, 1996). The pH can control the availability of nutrients, biological functions, microbial activity and the behavior of chemicals. The standard pH value recommended by WHO is a pH of 6.5-8.5. The observed pH range was 4.70-7.30, thus a few samples were outside the recommended WHO standard. Three of the water samples, CL, DA and MB had low pH values and thus were too acidic. The results were not within NAFDAC standards as also observed by Abasiekong*et al.* (2016) and Joshua *et al.* (2019), with their sachet water samples.

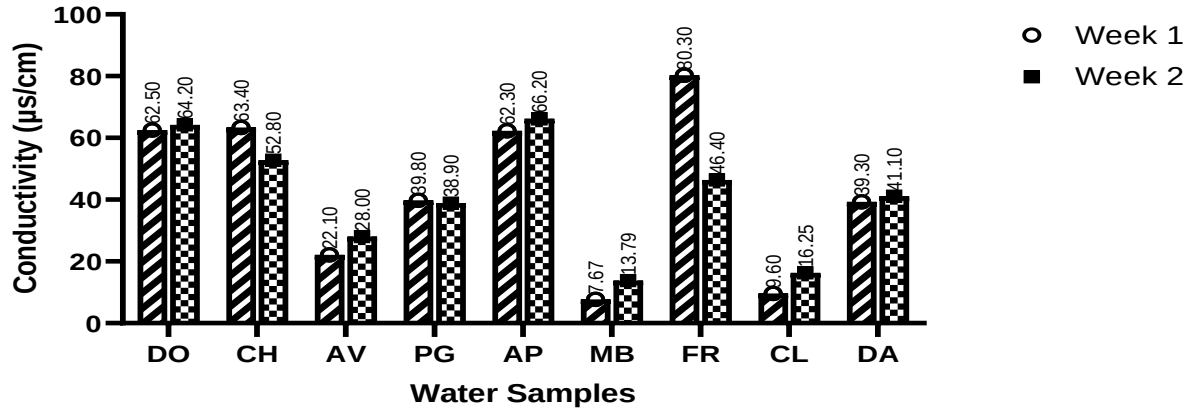

Figure 2: The degree of conductivity of the water samples during the first and second weeks respectively

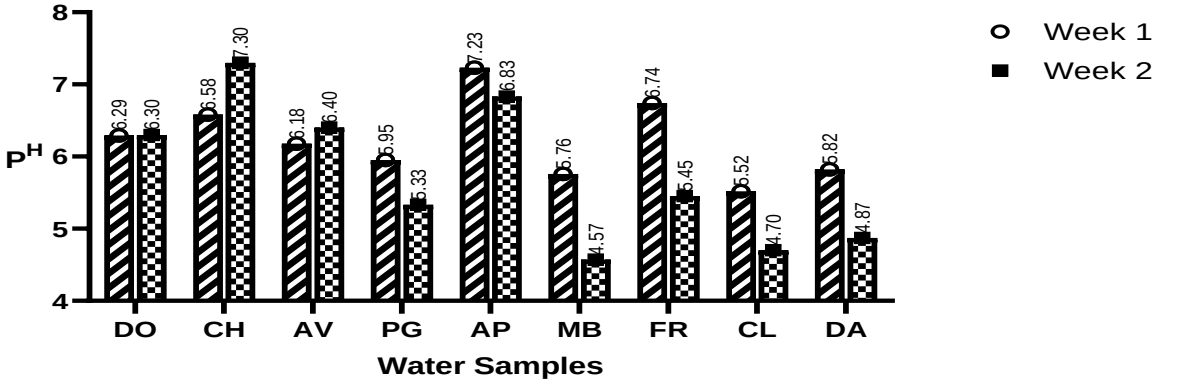

Figure 3: The pH level of the water samples during the first and second weeks of sampling respectively

Figure 4 represents the dissolved oxygen level of the water samples during the first and second weeks of sampling. The figure shows that water sample CL contained a greater amount of dissolved oxygen (4.42 mg/dL) followed by AP (4.41 mg/dL) in the first week. The AV water sample recorded the lowest level of dissolved oxygen (4.27 mg/dL). On the other hand, AV and DA water samples indicated the highest dissolved oxygen levels (7.55 mg/dL), while sample MB recorded the lowest dissolved oxygen level (6.99 mg/dL). In all

cases, the dissolved oxygen levels in the water samples were higher in the second week of sampling when compared with the first week.

The level of dissolved oxygen in a sample of water is one of the most important parameters in determining its quality, because it indirectly indicates whether there is water pollution. The DO depends on water temperature, atmospheric pressure and dissolved salts. The dissolved oxygen value obtained was lower than the WHO limit of 5-13 mg/l for drinking water. The results were in disparity with those reported by Unegbu (2017) but agreed with that of Ojekunle*et al*.(2015).

The mean properties of water samples collected fortwo different samplingweeksare shown in table 2. Independent sample t-test indicated that the mean temperature of all water samples was significantly higher ($p < 0.001$) in the second week (27.72 ± 0.47°C) compared with the first week (25.77 ± 0.97°C). Similarly, the mean dissolved oxygen level was significantly higher ($p < 0.001$) in the second week (7.33 ± 0.20 mg/dL) compared with the first week (4.47 ± 0.40 mg/dL). In contrast, no significant differences were observed in conductivity and pH levels between the first and second weeks of sample collection.

There should not be any variation in the sample readings for the two different weeks. The variation in temperature is permissible due to the prevailing ambient temperature but the significant difference in dissolved oxygen is not good. This may be due to the irregular monitoring of sachet water producers by regulating agencies (Obiri-Dansoe*et al.,* 2003; Adekunle *et al.,* 2004).

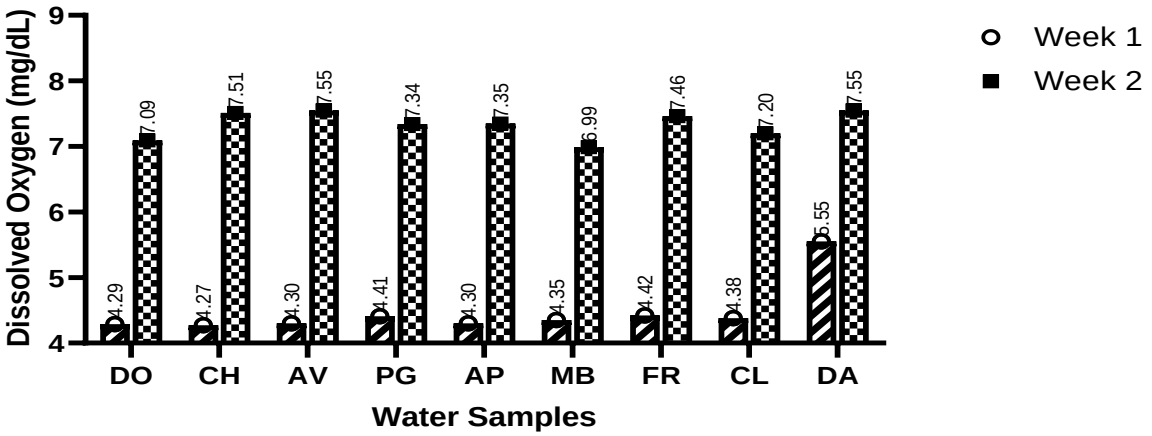

Figure 4: The dissolved oxygen level of the water samples during the first and second weeks respectively.

Table 2: Mean properties of water samples collected from different factories according to duration of storage

Parameters	First Week	Second Week	t-Statistics	P – Value
Temperature (°C)	25.77 ± 0.97	27.72 ± 0.47	-5.38	<0.001
Conductivity (µs/cm)	42.99 ± 25.94	40.84 ± 18.93	0.20	0.844
pH Level	6.23 ± 0.54	5.75 ± 0.98	1.27	0.220
Dissolved Oxygen (mg/dL)	4.47 ± 0.40	7.33 ± 0.20	-18.85	<0.001

Table 3 represents the microbial load of the sachet water, bag handle and inside bag swabs from the first week of sampling. According to the results the total variable count for swabs from the bag handle ranged from 1.10×10^5 to 1.10×10^6cfu/ml with the lowest value occurring for sample DO. The total variable count for swabs from inside the bag ranged from 1.0×10^4 to 1.49×10^6cfu/ml, which was the highest TVC for water sample AP. The TVC for the sachet water samples ranged from 1.08×10^2 to 2.48×10^4cfu/ml. Only the bag handle of sample PG had a TSSC count of 1.0×10^3cfu/ml.

Total coliform count ranged for bag handle swabs ranged from 2.0×10^2 to 5.0×10^3cfu/ml for sample PG. The swabs from the inside of the bags ranged from 1.5×10^2 to 2.50×10^4cfu/ml for water sample IB. Sachet water samples CH, AV, AP, MB, FR, and DA had no total coliform counts.The total *Staphylococcus* count for bag handles ranged from 1.0×10^4 to 3.0×10^5cfu/ml for sample AV. The TSC for swabs from inside the bags ranged from 0 to 5.0×10^5cfu/ml for water sample AP. The TSC for the water samples ranged from 0 to 2.0×10^4cfu/ml for sample CH.

The increase in human population has increased the demand for potable drinking water. The suitability of sachet water for human consumption has been questioned due to the unhygienic practices surrounding its production (Opafolaand David, 2020). In the first week of sampling 11% of the sachet were contaminated with

both coliform (1.0 x 10^3cfu/ml) and total coliform (5.0 x 10^3cfu/ml) above the WHO and Environmental Protection Agency recommended standards of zero coliforms and the values obtained were slightly higher than those observed by Ndinwa*et al.* (2012), Ojo (2015) and Bukar *et al.* (2015).Thus sachet water sample PG had issues.However, Addo *et al.* (2020) observed total coliforms in 30% of the sachet water collected from the production site. The heterotrophic bacteria count was a bit high for most of the samples and was above the 1.0 x 10^2cfu/ml recommended standard set by WHO (2002). A high heterotrophic count is indicative of the presence of high organic and dissolved salts in the water. A high heterotrophic count does not present a risk to human health. However, total heterotrophic bacteria can grow to levels that may be harmful to humans under improper or prolonged storage of packaged water at favorable environmental conditions (Warburton *et al.,* 1992). There were high total staphylococcus counts on swabs taken from both the bag handle and inside the bags which implies human contact as *Staphylococcus* is part of human skin flora (Taylor and Unakal, 2022).

Table 3: Microbial load of the sachet water samples, bag handle and inside bag swabs in the first week (cfu/ml)

SAMPLE		TVC	TSSC	TCC	TSC
DO	**BH**	1.10 x 10^5	------	2.0 x 10^2	2.70 x 10^4
	IB	7.9 x 10^5	------	1.5 x 10^2	7.0 x 10^4
	W	1.25 x 10^2	------	------	------
CH	**BH**	2.80 x 10^5	------	------	4.0 x 10^4
	IB	2.75 x 10^5	------	------	2.0 x 10^5
	W	2.20 x 10^3	------	------	2.0 x 10^4
AV	**BH**	2.50 x 10^5	------	------	3.0 x 10^5
	IB	2.0 x 10^4	------	------	------
	W	2.60 x 10^2	------	------	4.0 x 10^4
PG	**BH**	1.10 x 10^6	1.0 x 10^3	5.0 x 10^3	3.0 x 10^4
	IB	1.0 x 10^4	------	3.0 x 10^3	2.0 x 10^4
	W	2.0 x 10^2	------	4.0 x 10^2	2.50 x 10^2
AP	**BH**	1.44 x 10^5	------	------	2.50 x10^4
	IB	1.49 x 10^6	------	------	5.0 x 10^5
	W	1.78 x 10^2	------	------	1.0 x 10^3
MB	**BH**	1.0 x 10^6	------	------	2.30 x 10^5
	IB	1.0 x 10^4	------	------	4.0 x 10^4
	W	1.86 x 10^3	------	------	2.50 x 10^2
FR	**BH**	9.8 x 10^5	------	------	2.30 x 10^4
	IB	3.0 x 10^4	------	------	2.00 x 10^3
	W	2.48 x 10^4	------	------	2.48 x 10^3
CL	**BH**	8.1 x 10^5	------	------	1.0 x10^4
	IB	8.5 x 10^5	------	2.50 x 10^4	------
	W	1.08 x 10^2	------	------	3.0 x 10^1
DA	**BH**	1.40 x10^6	------	------	6.0 x 10^4
	IB	2.30 x 10^5	------	------	2.0 x 10^4
	W	1.80 x 10^2	------	------	2.0 x 10^3

KEY: TVC = Total Viable Count, **TSSC =** Total Salmonella/Shigella Count,
TCC = Total Coliform Count, **TSC =** Total Staphylococcal Count,
BH = Bag Handle, **IB =** Inside Bag, **W =** Water, **CFU =** Colony Forming Unit.

Table 4 depicts the microbial load of the sachet water samples, bag handle and inside bag swabs for the second week of sampling. According to the results the TVC for the swabs of the bag handles ranged from 2.3 x 10^4 to 2.8 x 10^5cfu/ml for sample MB. TVC for inside bag swabs ranged from 2.0 x 10^4 to 8.0 x 10^5cfu/ml for sample IB. The sachet water samples had TVC ranges from 4.8 x 10^2 to 8.0 x 10^4cfu/ml for sample AV which was highest. The total variable counts presently observed were higher than values observed by Daniel and Daodin (2016). Five of the water samples (DA, AV, PG, AP, and FR) had TSSC values ranging from 2.0 x 10^1 to 1.0 x 10^3cfu/ml. The major issue here is the presence of TSSC counts in four new sachet water samples as opposed to one in the previous sampling week. This implies that the water companies are not taking the standards seriously since they have gotten the NASDAC registration numbers (Omalu *et al.,* 2010). The results made the water unsafe for consumption. Efforts should be made to increase the monitoring exercises (Sultan *et al.,* 2011). These companies must have ineffective or malfunctioning treatment processes (Adekunle *et al.,* 2004).

The TCC for bag handles ranged from 0 to 3.2 x 10^5cfu/ml for sample BH. The TCC for the inside of the bags ranged from 0 to 5.0 x 10^5cfu/ml for sample IB. The TCC for the water samples ranged from 0 to 8.0 x 10^2cfu/ml for samples AV and FR respectively.The coliform counts on the bag handles and inside the bags is from the handlers/workers and the immediate environment inside the plants (Omalu *et al.,* 2010; Addo *et al.,* 2020).

There was no total Staphylococcus count for sample DO. The TSC for the bag handles ranged from 0 to 1.2 x 10^5cfu/ml for sample MB. The values for swab from inside the bags ranged from 0 to 4.0 x 10^4cfu/ml for sample PG. Lastly, the range for the water samples is 0 to 1.0 x 10^4 for sample DA.

Table 4: Microbial load of the sachet water samples, bag handle and inside bag swabs in the 2ND Week (cfu/ml)

SAMPLE		TVC	TSSC	TCC	TSC
DO	**BH**	6.0 x 10^4	------	1.40 x 10^3	------
	IB	3.0 x 10^4	------	1.00 x 10^3	------
	W	4.8 x 10^2	------	2.31 x 10^2	------
CH	**BH**	2.3 x10^4	------	1.2 x 10^3	------
	IB	2.0 x 10^4	------	1.2 x 10^3	------
	W	8.0 x 10^3	------	3.0 x 10^1	3.0 x 10^2
AV	**BH**	2.6 x 10^5	------	2.8 x 10^5	2.0 x 10^4
	IB	1.20 x 10^5	------	5.0 x 10^5	4.0 x 10^4
	W	8.0 x 10^4	8.1 X 10^2	8.0 x 10^2	------
PG	**BH**	1.0 x10^5	1.00 X 10^3	2.4 x10^3	1.0 x 10^4
	IB	4.0 x 10^4	------	5.0 x 10^4	4.0 x 10^4
	W	4.0 x 10^3	2.0 X 10^1	7.0 x 10^1	------
AP	**BH**	1.7 x 10^5	------	4.0 x 10^4	------
	IB	1.4 x 10^5	4.0 X 10^2	4.0 x 10^4	------
	W	1.5 x 10^3	3.0 X 10^1	7.0 x 10^2	1.6 x 10^2
MB	**BH**	2.8 x10^5	------	1.0 x10^4	1.2 x10^5
	IB	1.1 x 10^5	------	2.0 x 10^4	2.0 x 10^4
	W	1.5 x 10^4	------	1.3 x 10^2	3.0 x 10^2
FR	**BH**	2.80 x 10^5	------	3.2 x10^5	1.0 x10^4
	IB	1.10 x 10^5	------	2.5 x 10^5	3.0 x 10^4
	W	7.0 x 10^3	4.0 X 10^1	8.0 x 10^2	1.0 x 10^1
CL	**BH**	8.0 x10^4	------	4.0 x 10^4	1.0 x10^4
	IB	1.4 x 10^5	------	------	------
	W	8.6 x 10^3	------	------	------
DA	**BH**	5.0 x 10^4	------	------	------
	IB	8.0 x 10^5	------	------	1.0 x 10^4
	W	1.4 x 10^4	3.0 x 10^2	4.0 x 10^1	------

KEY: TVC = Total Viable Count, **TSSC =** Total Salmonella/Shigella Count,
TCC = Total Coliform Count, **TSC =** Total Staphylococcal Count,
BH = Bag Handle, **IB =** Inside Bag,

Table 5 represents the bacteria isolated from the bag handle, inside bag and water samples. The isolates were characterized microscopically by morphology and cultural characteristics and confirmed by biochemical and molecular analysis. The bacterial isolates observed include *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter asburiae*,*Klebsilella pneumoniae*,*Lysinbacilluscapsici*, and *Salmonella typhi*. However, *Bacillus, Streptococcus,* and *Pseudomonas* species have also been reported (Sultan *et al.*, 2011; Bukar *et al.*, 2016; Daniel *et al.,* 2016). The present findings are dissimilar to that of Adewoyeand Adewoye (2013) and Adekunle (2013)who reported the finding of *Pseudomonas* isolates from their water samples. The present work, however, is similar to the findings of Kalpana *et al.* (2011), who observed *Staphylococcus aureus and Escherichia coli.*Egwari*et al.* (2005), however, observed *E. coli* only on the surface of the sachet and not in the water samples.

Staphylococcus aureus was found mostly on the bag handle and inside the bag thus implying the source is from human contact as it is found in the nose and on the skin of humans (Taylor and Unakal, 2022). Infections caused by this pathogen are common in both community-acquired and hospital-acquired settings. The treatment remains challenging due to the emergence of multi-drug resistant strains such as Methicillin-Resistant Staphylococcus aureus (MRSA) (Anuj *et al.*, 2019).

*Lysinbacilluscapsici*was identified through genetic analysis.*Lysinbacilluscapsici*is a gram-positive, spore-forming and motile bacteria (Burkett-Cadena *et al.,* 2019). It is well known for its insecticidal activity against insects, including mosquitoes. In addition, some *Lysinbacillus*species have a potential for heavy metal remediation. *Lysinbacillus*species are now attracting attention as plant-promoting and disease control agents that can be used as alternatives to agrochemicals (Ahsan and Shimizu, 2021).

Occurrence of coliforms in finished water in the absence of known breaches of treatment barriers, continues to be a major problem in the drinking water industry.Thus the isolation of *Escherichia*

*coli*and*Salmonella typhi*observed in the water samples is of public health concern. Total coliforms are widely used as indicators of the general sanitary quality of the treated drinking water while fecal coliforms give much closer indication of fecal pollution and consequently, WHO states that none should be detected at all (Wiesenberger, 2004; WHO, 2004;Halage*et al.,* 2015;Ojekunle*et al.,* 2015).The largest public health impact of unsafe drinking water is diarrheal disease and the majority of water-associated outbreaks of disease can be related back to the microbiological quality of drinking water. These include infectious and parasitic diseases such as cholera, typhoid, dysentery, and Guinea worm (Parsons and Jefferson, 2006).Ineffectiveness and malfunctioning of the treatment process employed could result in the presence of coliform bacteria in the water samples (Dada, 2009).

Table 6 represents the percentage occurrence of the bacterial isolates from sachet water samples, bag handle and inside bag swabs. According to the result *Staphylococcus aureus* had the highest rate of occurrence(41.76%), followed by *Escherichia coli* (19.78%), *Klebsiella pneumoniae* (13.18%), *Enterobacter asburiae*(10.98%),and *Salmonella typhi* (8.79%) respectively, with*Lysinbacilluscapsici*having the least rate of occurrence (5.49%). Daniel and Daodin (2016), reported *Staphylococcus aureus* as having the highest percent occurrence of 24% while *E. coli, Bacillus and Corynebacterium species* had the lowest percentage occurrence of 8% each. In addition, they also isolated *Proteus vulgaris* (16%) and *Aeromonas* (13%) species.

Table 5: The bacterial isolates fromsachet water samples, bag handle and inside bag swabs as identified by cultural and molecular characteristics

COLONY CODE	BACTERIAL ISOLATES
1	*Staphylococcus aureus*
2	*Escherichia coli*
3	*Enterobacter asburiae*
4	*Klebsiella pneumonia*
5	*Lysinbacilluscapsici*
6	*Salmonella typhi*

Table 6: Percentage occurrence of the bacterial isolates from the sachet water samples, bag handle and inside bag swabs

S/NO	ISOLATES	NUMBER OF OCCURANCE	PERCENTAGE OF OCCURRENCE (%)
1	*Staphylococcus aureus*	38	41.76
2	*Escherichia coli*	18	19.78
3	*Enterobacter asburiae*	10	10.98
4	*Klebsiella pneumoniae*	12	13.18
5	*Lysinbacilluscapsici*	5	5.49
6	*Salmonella typhi*	8	8.79

Provision of public toilets in motor parks, markets and street corners where pure water is sold is not common place. Consumers in transit often purchase these products and consume them without washing the sachet. The level of microbial contamination of the sachet water from the hands of the factory workers and vendors, factory environment, transport vehicle and even the water used to chill the products is a concern as it affects the quality of the product and has public health implications for consumers.

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