

Potability of some selected sachet water sold in Bwari area Council Federal Capital Territory, Nigeria along the production and distribution chain

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Abstract

Potability of water is the degree to which water is safe and clean. This study focuses on the potability of some selected sachet water sold in Bwari area council of FCT Nigeria. Samples of sachet water of 4 different brands were collected at random, and then subjected to bacterial assessment and antibiotics susceptibility. Most probable number technique were employed for the detection of coliforms and spread plate technique for bacteria loads. A total of 138 isolates were obtained from this study. Seventy-three (73) isolates were obtained from the source of water while 10 isolates were obtained from the sachet water collected from the factories just after production process. Hand swab of the factory workers showed a total of 24 isolates while 15 and 16 isolates were obtained from the distributors. This shows microbial recontamination after treatment processes along the distribution chain. The highest number of bacteria isolated was 44 isolates and this was obtained from brand D. Brand A and B both had 33 isolates while brand C had 28 isolates which represented the least number of bacteria isolated. The microorganism isolated from the four brands analyzed were not significantly difference ($p \geq 0.05$). *Klebsiella pneumoniae* and *Staphylococcus spp* was the most frequently isolated bacteria. All the brands of sachet water analyzed were below the drinking water standards set by WHO and are of doubtful quality. Care must be taken as these product moves from production sites to consumers. There is also need for regular monitoring of the production process by the National Agency for Food and Drug Administration and Control.

Keywords: Potability; Bacteria; Coliforms; Sachet water; Production process; Distribution chain

1. Introduction

The World Health Organization (WHO) classifies sources of water supplies as either improved or unimproved [1-2]. Improved water sources include public standpipes, household connections, borehole, protected dug wells, protected springs etc. while unimproved water sources include unprotected wells, unprotected springs, rivers as well as tanker truck provision of water [1-2].

The WHO estimates that about 1.9 million people in 2001 suffer from diarrheal disease globally by drinking unsafe water contaminated with faeces [3] and the vast majority of diarrheal disease in the world (88%) is attributable to unsafe water, sanitation, and hygiene [4]. Looking at the 20 leading risks factors for health burden in developing regions, unsafe water, sanitation and poor hygiene is third, behind underweight or practicing unsafe sex [4]. Improving and managing universal services of water and sanitation in a holistic manner is critical to achieving the Sustainable Development Goals and addressing the needs of millions of people around the world [5].

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Priority should be given to bacteriological quality of drinking water because studies have shown that several disease outbreaks associated with untreated or poorly treated water containing bacteria pathogen have been isolated from sachet water [6]. The human pathogens that present serious risk of disease whenever present in drinking water include *Escherichia coli*, *Vibrio cholerae*, *Salmonella* species and parasites such as *Entamoeba histolytica* and *Giardia* species and so on [7]. The presence of *Escherichia coli* in water gives an indication of the possible existence of faecal-borne microorganism such as *Salmonella* and hepatitis [8]. Ideally, drinking-water should not contain any microorganisms known to be pathogenic-capable of causing disease-or any bacteria indicative of faecal pollution. [9].

In Nigeria the packaged water industry started in the early 1990s. Vendors would source Government water or water from ground sources, perform minor treatment, cool, and sell in hand filled, hand tied polyethene bags otherwise called ice water [10]. In the late 1990s, there was a gradual decline in the production and consumption of ice water [11] perhaps, due to the realization of the obvious health risk. This gave way to the present factory filled polyethene sachet and bottled water consumed mostly by the lower and middle socio-economic class [12]. But their inadequacies particularly in terms of water quality, poor aesthetic environments and microbial analysis are a cause for concern. In Nigeria, water quality is regulated by the National Agency for Food and Drug Administration and Control (NAFDAC). NAFDAC ensures that all packaged water is of highest quality and complies with the Nigerian industrial standard for portable water NIS 306:2008. The NIS 306:2008 is a standard developed for packaged water in Nigeria in order to ensure that all packaged water is free from substances that are hazardous to health [11]. This study is aimed at assessing the potability and bacterial load of some selected sachet water sold in Bwari area council Federal Capital Territory, Nigeria.

2. Material and methods

2.1. Preparation and Sterilization of Media

The media used in this study include: Nutrient agar (Himedia), Lauryl Tryptose Broth (oxid CM0451), Brilliant green lactose bile broth (oxid), MacConkey (Himedia M001-500G), Eosin Methylene Blue agar (Himedia M001-500G) and Mueller Hinton agar (Himedia M001-500G). All the media were prepared according to the manufacturers' specifications.

2.2. Antibiotic Disc

Antibiotic disc used include; Septrin (30 µg), Chloranphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Amoxicillin (30 µg), Augmentin (30 µg), Gentamycin (10 µg), Perfloxacin (30 µg), Tarivid (10 µg) and Streptomycin ((30 µg).

2.3. Sample Collection

A total of forty (40) water samples were collected from four (4) different sachet water production factories in Bwari Area Council. Two (2) samples each were collected from the sources of water, final product (sachet water). Source of water sample were aseptically collected using sterile universal containers from the factory locations. The samples were transported to the laboratory in ice-cold container and analyzed.

2.4. Bacteriological Analysis of the Water Samples

Tenfold serial dilutions of the sample were made using sterile water as diluents. 0.1 ml of 10^{-3} were inoculated on the Nutrient agar (10^{-3}), Mannitol Salt agar (10^{-3}), *Salmonella-Shigella* agar (10^{-3}) and Mac Conkey Agar (10^{-3}) respectively using the spread plate method. The plates were allowed to stand undisturbed for about 15 minutes and then incubated at 37 °C for 24 hours and the number of colonies on nutrient agar were counted using colony counter. The colonial density was calculated as the colony forming unit (CFU) multiplied by the dilution factor. The mean total count obtained were recorded and expressed in colony forming units per milliliter (Cfu/ml) of the sample.

2.5. Coliform test

Presumptive, confirmatory, and completed test for detection of the presence of coliforms were carried out according to the methods described by [13].

2.5.1. Presumptive Test

Inverted Durham tubes were inserted into the McCartney bottles. fifty (50 ml) of already prepared double strength MacConkey broth were added to one (1) McCartney bottle containing inverted Durham tubes while ten (10) ml of also double strength broth was added to five (5) tubes, which were then inoculated with 50ml and 10ml of water sample.

The McCartney bottles were incubated by placing in an oven at 37°C for 24 hours. This was done to determine the presence of coliform bacteria in the water samples and also to obtain some index as to the possible number of organisms present in the samples under analysis. The bottles were examined for the production of both gas and acid, which indicates positive bottles.

2.5.2. Confirmatory Test

After the incubation of the cultures, two drops from each positive tube from the presumptive test were transferred into a separate tube of Brilliant green lactose bile broth (BGLB) with Durham tubes. The tubes were incubated at 37 °C for 24 hours. Formation of gas in any of the inverted Durham tubes indicated positive test.

2.5.3. Completion Test

The completed test was carried out in order to confirm the presence of coliform bacteria in the water samples. It is necessary to confirm a suspicious but doubtful result of the previous test. In this process, a loopful of sample from each positive BGLB tubes was streaked onto selective medium such as Eosin Methylene Blue agar (EMB), MacConkey agar and incubated at 37 °C for 24 hours for fecal coliforms *E. coli* detection. EMB plates were examined for colonies with greenish metallic sheen.

2.6. Identification of Isolated Bacteria

The bacteria isolated were identified based on the biochemical tests outlined in the Bergey's Manual of determinative bacteriology.

2.7. Antibiotic Susceptibility Test

Antibiotics susceptibility test was done on Mueller Hinton agar (Himedia) using disk diffusion (Kirby Bauer's) technique. This method was carried out according to the procedures described in Clinical and Laboratory Standards Institute [14] guidelines to determine the susceptibility of bacteria isolated to commonly used antibiotics.

3. Results and discussion

The coliform count is shown in Table 1. All brands analyzed had total coliform count ranging from 2MPN/100ml to 18MPN/100ml. Fecal indicator organism *Escherichia coli* was not observed in all the brands. Table 2 shows the morphological characteristics and biochemical features of isolated bacteria from the sachet water. Isolates obtained were identified on the basis of microscopy, biochemical tests, and morphological characteristics through macroscopic features.

Table 1 Result of the MPN index of Different Water Samples

Samples	Presumptive	MPN 100ML	Confirm BGLB	Completed macConkey EMB	SS Agar
Brand A1	1-3	9	Gas production indicating the presence of coliform	Mucoid pink colonies for Enterobacter and Klebsiella spp, colourless for Proteus and green sheen for <i>E. coli</i>	Transparent with black centre for <i>salmonella spp</i>
Brand A2	1-4	16			
Brand B1	0-5	7			
Brand B2	1-2	6			
Brand C1	1-0	2			
Brand C2	1-1	3			
Brand D1	1-4	16			
Brand D2	1-5	18+			

BGLB= Brilliant green lactose broth. SS Agar= Salmonella-shigella agar. EMB= Eosin methylene blue. Keys: Brand = Codes representing the trade names of sachet water from location A, B, C and D.

Table 2 Biochemical Characteristics of Bacteria Isolated from sachet water Sold in Bwari Area Council, FCT Nigeria

Shape	Surface	GR	IN	CL	OX	CA	UR	MR	VP	CO	PROBABLE ORGANISM
Rod	Mucoid	-	-	+	-	+	-	-	+	-	<i>Klebsiella spp</i>
Cocci	Raised	+	-	-	-	-	-	-	-	-	<i>Staphylococcus spp</i>
Rod	Mucoid	-	-	+	-	+	-	-	-	-	<i>Salmonella spp</i>
Cocci	Raised	+	-	-	-	-	-	-	-	-	<i>Streptococcus spp</i>
Rod	Raised	-	+	-	-	+	-	+	-	-	<i>Escherichia spp</i>
Rod	Raised	-	-	+	-	+	-	-	+	-	<i>Enterobacter spp</i>
Rod	Flat	+	-	+	-	+	-	-	-	-	<i>Bacillus spp</i>
Rod	Flat	-	-	+	-	+	+	+	-	-	<i>Proteus</i>

Key: GR=Gram reaction, IN= Indole, CI= Citrate, OX= Oxidase, CA= Catalase test, UR=Urease, MR=Methyl red, VP=Voges-Proskauer, CO= Coagulase t

Table 3 Bacteria Associated with Sachet Water Sold in Bwari Area Council, FCT-Abuja

Isolates	Frequency	Percentage (%)
<i>Klebsiella pneumoniae</i>	36	25.35
<i>Staphylococcus aureus</i>	36	25.35
<i>Salmonella typhi</i>	23	16.19
<i>Streptococcus spp</i>	15	10.56
<i>Escherichia coli</i>	10	7.04
<i>Enterobacter cloacae</i>	7	4.92
<i>Bacillus subtilis</i>	6	4.22
<i>Proteus mirabilis</i>	5	3.52
Total	138	100

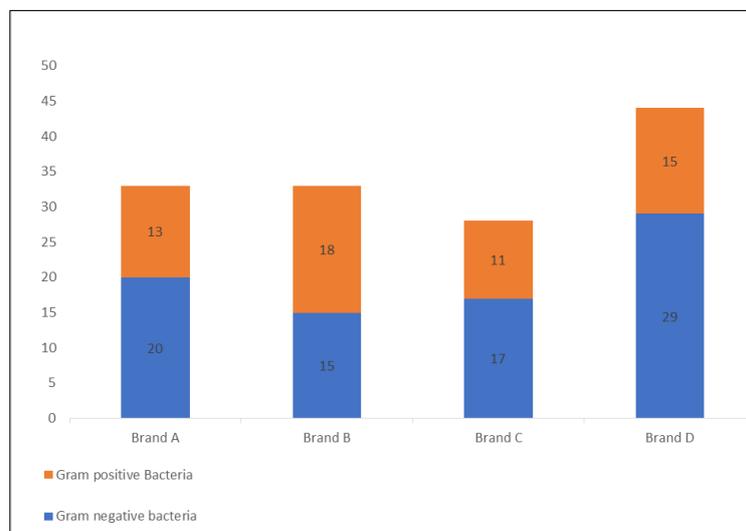


Figure 1 Distribution of bacteria across the four (4) brands of sachet water analyzed. Brand = Codes representing the trade names of sachet water from location A, B, C and D

Eight (8) bacteria species were isolated from sachet water analyzed in this study. Table 3 shows *Klebsiella pneumoniae* and *Staphylococcus spp* as the most frequently isolated bacteria which represented 25.35%. Figure 1 showed the distributions of the bacteria across the four (4) brands of sachet water. Forty-four (44) represented the highest number of microorganisms isolated from brand D. However, the distributions of the microorganisms across the production line and distribution chain as represented in Figure 2 showed the source of water having the highest number of bacteria.

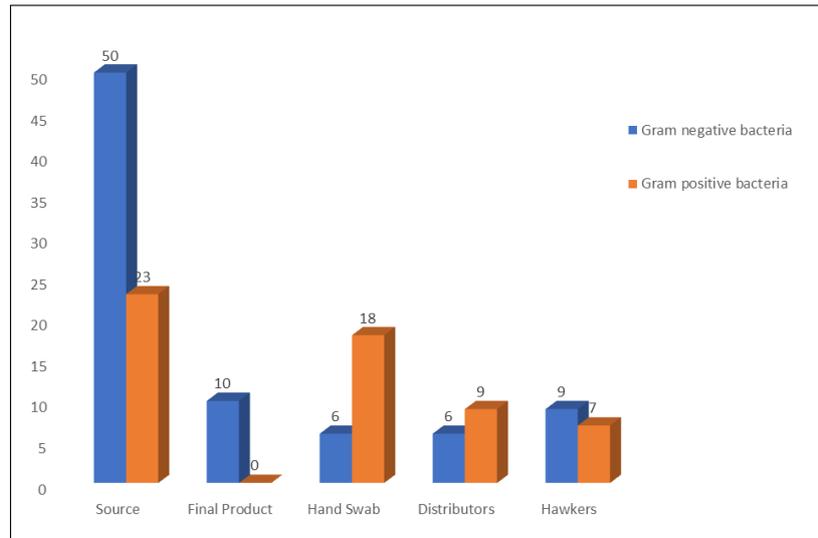


Figure 2 Distribution of bacteria across the production line and distribution chain. Brand = Codes representing the trade names of sachet water from location A, B, C and D

3.1. Antibiotics susceptibility

The result of the antibiotics susceptibility study of the bacteria isolated from the sachet water samples against conventional antibiotics is shown in table 4. Ciprofloxacin showed the most pronounced activity against all the bacteria, while nalidixic acid had the least activity against all the bacteria.

Potable water must meet internationally acceptable standards and be in line with guidelines stipulated by the world health organization. The samples from the source of water used by the production factories in this study had very high microbial count. This could be attributed to contaminated source (underground or surface water). WHO reported that 5 - 25% of the population of Nigeria still practice open defecation [15] which could easily contaminate our underground or surface waters and could also be attributed to high coliform count recorded in this study. The most probably number (MPN) is the degree of contamination and the microbiological quality of drinking water. The standard of drinking water set by WHO permits zero (0) coliform/100ml of water [9]. Going by the zero tolerance levels stipulated by regulatory agency for coliforms in drinking water, none of the brands met the existing standards. The coliforms values recorded in this research ranged from 2MPN/100ml to 18MPN/100ml. No fecal coliforms were found in the final product of all the sachet water, which agrees with the report of [16-17]. Although indicator organism *Escherichia coli* were found as product moved down the distribution chain. *Klebsiella pneumoniae*, *Proteus mirabilis* and *Enterobacter cloacae* were the coliforms isolated from the water samples which were also reported by [18]. The emergence of coliforms at the final product stage could indicates the use of unsterilized equipment and unhygienic practices during production. Samples collected from the distributors had the highest colony count for coliforms.

From this study, a total of one hundred and forty-two (142) isolates belonging to five (5) Gram positive, three (3) Gram negative bacteria genera were isolated. The Gram-negative bacteria isolated were *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Enterobacter cloacae* while the Gram-positive bacteria isolated include *Staphylococcus aureus*, *Streptococcus spp* and *Bacillus subtilis*. Most of the bacteria species isolated have been reported in previous studies [19-20]. All the sachet water were however contaminated with varied number of bacteria species even though their distribution varied among the brands. There is no significant difference between the bacteria isolated ($P \leq 0.05$).

Table 4 Susceptibility Study of Bacterial Isolates against the Tested Antibiotics

Zone Diameter of Inhibition (mm)					
Antibiotics	<i>E. coli</i>	<i>Proteus mirabilis</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella typhi</i>	<i>Enterobacter cloacae</i>
CPX	24.50±0.25	26.00±0.40	22.50±0.50	28.00±0.20	28.00±0.00
OFX	24.00±0.21	21.0±0.22	22.00±0.20	27.00±0.00	28.00±0.00
PEF	25.00±0.00	27.00±0.20	24.00±0.10	25.00±0.50	28.00±0.50
CN	20.00±0.50	23.50±0.50	19.50±0.00	22.00±0.20	24.00±0.20
PN	19.00±0.22	NZ	21.50±0.50	20.00±0.10	22.00±0.50
CEP	19.50±0.11	24.50±0.50	22.00±0.00	NZ	24.00±0.30
S	19.00±0.24	18.00±0.10	NZ	20.00±0.00	23.00±0.25
SXT	17.00±0.25	21.00±0.00	14.00±0.20	21.00±0.00	26.00±0.00
NA	NZ	21.50±0.50	NZ	NZ	20.00±0.10
AU	22.50±0.50	20.50±0.50	18.50±0.00	19.50±0.25	24.00±0.050

OFX = Tarivid, NA = Nalidixic acid, AU = Augmentin, S = Streptomycin, CEP = Ceporex, CN = Gentamicin, PEF= Reflacin, CPX = Ciprofloxacin, PN = Ampicilin, SXT = Septrin, NZ= No zone of inhibition.

However, *Klebsiella pneumoniae* and *Staphylococcus aureus* were the most frequently isolated bacteria represented 25.35%. The frequent occurrence of the bacteria species could be attributed to poor hygiene practices and use of unsterilized equipment during production. Some of the bacteria such as *Staphylococcus aureus* is a normal flora and when the hands of the factory workers are not frequently disinfected especially during packaging, the bacteria may find it way to the final product. In this study, distribution of bacteria down the production line and across the distribution chain showed that the source of water used in the production of the sachet water had seventy three (73) isolates, final product of the sachet water collected from the factories had ten (10) isolates, twenty four (24) isolates were gotten from the hand swabs of the factor workers, sachet water collected from the distributors recorded fifteen (15) isolates while sixteen (16) microorganisms were recorded from the sachet water collected from the hawkers. The bacteria isolated from the sachet water in this study as product moves across the production process and down the distribution chain were not significantly different ($P \leq 0.05$) among the four brands of sachet water analyzed.

The distribution of the microorganisms down the production line and across the distribution chain shows that proper care, techniques, and practices might not have been followed during production and as a result most of the organism seen in the source of water reappeared in the final product after undergoing purification stages. The guide for good hygienic practices for packaged water in European states that every person working in a food handling area is to maintain a high degree of personal cleanliness and is to wear suitable, clean and, where necessary, protective clothing [21]. This was not the case in this study as twenty-four (24) bacteria isolates were recorded from the hand swabs of factory workers, and this could easily have ended up in the final product during packaging. At all stages of production, processing and distribution, food and water meant for human consumption is to be protected against any contamination likely to render the food unfit for human consumption, injurious to health or contaminated in such a way that it would be unreasonable to expect it to be consumed in that state [21]. The potential health effects that may be caused by this microbial contamination include abscesses, ulcers, food poisoning, inflammation of breast and conjunctivitis in new born, nausea, vomiting, diarrhea, urinary tract infections, appendicitis, meningitis, abdominal pain, pneumonia and bacteremia [13,22].

The antibiotic susceptibility result of our study indicated resistance frequencies for all bacteria isolated from the sachet water samples against conventional antibiotics.

Ciprofloxacin, Tarivid, Reflacin, Gentamicine, Septrin and Augmentin all had pronounced activity against all the bacteria tested with zone of inhibition ranging from 14-28 mm in diameter. Ciprofloxacin exhibited the most pronounced activity against *Enterobacter cloacae* and *Salmonella typhi* with inhibitory zone diameters of 28 mm each. This was similar to the study of [23], that showed the bacteria isolated from the sachet water samples were reasonably sensitive to ciprofloxacin (85.2%). *Proteus mirabilis*, *Escherichia coli* and *Klebsiella pneumoniae* had inhibitory zone diameters of 26 mm, 24.50 mm, and 22.50 mm respectively. This study shows that nalidixic acid had no activity against

E. coli, *K. pneumoniae* and *Salmonella typhi*, this observation is consistent with previous studies of [24] who reported significant levels of antimicrobial resistant from similar microorganism. The presence of antibiotics resistant bacteria in sachet water is highly significant in today's world. This resistance develops when potentially harmful bacteria change in a way that reduces or eliminates the effectiveness of antibiotics [25]. Since 2015, FDA approved new antibiotics that can treat certain resistant bacteria. Health care professional are encouraged to use the new antibiotics appropriately and for some antibiotics, use only in patients who have limited or no other treatment options [25]. There was no significant different ($P \leq 0.05$), between the antimicrobial resistant of the bacteria isolated across the distribution chain.

4. Conclusion

The potability of the sachet water samples analyzed in this research shows that a lot of work still needs to be done in terms of compliance to the standard of drinking water by the production factories. A more effective treatment options needs to be developed and adopted by this water companies. microbial isolates found in the final product could be attributed to contamination and recontamination during the production and packaging stages. Proper washing, cleaning and sterilization of these machines needs to be done as and when due. From this study it is recommended that Packaging materials need to be inspected and monitored regularly to ensure that they are sterile and properly handled before use. Furthermore, to safeguard the health of the people there is need for regular monitoring of the quality of the water and the environment they are produced by regulatory agency-NAFDAC.

Compliance with ethical standards

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Disclosure of conflict of interest

There was no conflict of interest /competing interest

References

- [1] World Health Organization- WHO (2000). The World Health Report: Health Systems, improving performance. Geneva, Switzerland. 1-10.
- [2] Gundry S, Wright J, Conroy R. Systematic review of the health outcomes related to household water quality in developing countries. *Journal of water and Health*. 2004; 2: 1-13.
- [3] Kindhauser Kay, M., World Health Organization. (2003) Global defence against the infectious disease threat. *Communicable Diseases 2002*. Geneva. 1-12
- [4] World Health Organization- WHO (2002). Quantifying selected major risks to health. The World Health Report 2002. World Health Organization, Geneva Switzerland. 47-97
- [5] Masinde K. Rouse M. Jepkirui M. Cross K. (2021) .Guidance on Preparing Water Service Delivery Plans: A manual for small to medium-sized water utilities in Africa and similar settings. IWA Publishing. 1-22
- [6] Oladipo IC, Onyenike IC, Adebisi AO. Microbiological analysis of some vended sachet water in Ogbomoso, Nigeria. *African Journal of Food Science*. 2009; 3(12): 406-412.
- [7] Joklik, WK, Willett HP, Amos DB. *Zusser Microbiology*. 20th Edition. Norwalk; Appleton and Lange. 1992; 393-400.
- [8] Pric RG, and Wildeboer D (2017) *E. coli* as an indicator of contamination and health risk in environmental waters, Recent Advances in Physiology, Pathogenesis and Biotechnological Applications. In *TechOpen*, 125-139
- [9] World Health Organisation (1997). Guidelines for drinking-water quality Volume 3 2nded. Surveillance and control of community supplies Geneva, Switzerland, 1-15
- [10] Babatunde MA, Biala IM. Externality effects of sachet water consumption and the choice of policy instruments in Nigeria: Evidence from Kwara State. *Journal of Economics*. 2010; 1(2): 113-131.

- [11] Epundu UU, Adinma ED, Ezeama NN, Emelumadu OF, Ogbonna BO. A Review on packaged drinking water, quality regulations and public health: exploring potability and safety gap implications for public health in Nigeria. *International Journal of Tropical Disease & Health*. 2017; 25(3): 1-10.
- [12] Dada AC. Packaged water: Optimizing local processes for sustainable water delivery in developing nations. 2011; (1): 24.
- [13] Cheesbrough M (2006). *District Laboratory Practice in Tropical Countries Part Two*. Cambridge University Press, 23-140.
- [14] Clinical Laboratory Standard Institute (2012). *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition*. Clinical and Laboratory Standards Institute, 1-12.
- [15] World Health Organization. WHO (2008). *Progress on Drinking Water and Sanitation 2014 Update*. World Health Organization, Geneva, Switzerland. 1-223.
- [16] Halage A, Ssemugabo C, Ssemwanga D, Musoke D, Mugambe R, Guwatudde D, Ssempebwa J. (2015) Bacteriological and Physical Quality of Locally Packaged Drinking Water in Kampala, Uganda. *Journal of Environmental and Public Health*, 1-6.
- [17] Bukar A, Isa M, Adam M, Kyari M, Ibrahim F, Muhammad M, Bello A, Gulani H. Bacteriological Analysis of Sachet Water in Maiduguri Metropolis. *The Journal of Applied Sciences Research*. 2015; 2(1): 20-25.
- [18] Narasimhan B, Himabindu M. Enumeration of microbial contaminants in sachet water: A public health challenge. *Health*. 2010; 2(6): 582-588.
- [19] Waziri M. Assesment of the Microbial Quality of Sachet Water in Damaturu-Yobe State, Nigeria. *Journal of Asian Scientific Research*. 2012; 2(2): 76-80.
- [20] Ayoade F, Fayemi SO, Daramola GG, Asho A, Oyejide NE, Adenodi SA, Anazodo KO. Effectiveness of Storage as a Point of Use Means of Improving the Bacteriological Quality of Drinking Water. *International Journal of Biological and Chemical Sciences*. 2013; 7(1): 96-106.
- [21] European Federation of Bottled Waters EFBW (2012). *Guide to Good Hygienic Practices for Packaged Water in Europe*. Belgium Brussels, 1-12.
- [22] World Health Organisation WHO (2011). *Guidelines for drinking-water quality. Fourth Edition*. World Health Organization. Geneva, Switzerland, 117-124
- [23] Anyanwu CU. Studies on Antibiotic Resistance of Some Bacterial Isolates from Sachet Water Samples in Nsukka, Nigeria *Bio-Research*. 2009; 7(2): 509 – 513.
- [24] Umoessien US, Antia UE, Christopher M, Etanguno EO. Antibiotic Susceptibility Patterns of Bacteria Isolated from Sachet-packaged Water Sold in Uyo Metropolis, Akwa Ibom State, Nigeria. *Nigeria International Journal of Pathogen Research*. 2019; 3(1): 1-11.
- [25] Food Drug and Administration FDA (2019) *Combating Antibiotic Resistance*. White Oak Maryland USA.GOV. [cited 2022] Availble from [Combating Antibiotic Resistance | FDA](#).