An Assessment of the Bacteriological Quality of Commercial Sachet Packaged Water Brands in Awka, Anambra State, Nigeria

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Abstract Some Sachet water brands sold in Awka, Nigeria were assessed bacteriologically to confirm their compliance with the World Health Organization standard for drinking water. The temperature, pH, total bacterial count, total coliform count, faecal coliform count, faecal Enterococci count and Clostridium perfringens count were determined using standard techniques. The temperature values were 27-29°C; pH, 6.2-7.0; total bacterial count, 35-50cfu/ml; total coliform count, 4-20MPN/100ml; faecal coliform count, 0-10MPN/100ml; faecal Enterococci count, 0-5MPN/100ml while Clostridium perfringens were absent in all the brands assessed. The bacterial isolates were Escherichia coli (9.7%), Staphylococcus aureus (18.0%), Enterobacter aerogenes (17.5%), Bacillus cereus (14.6%), pseudomonas aeruginosa (20.1%), Proteus mirabilis (12.1%) and Enterococcus faecalis (8.0%). Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Bacillus cereus, Pseudomonas aeruginosa, Proteus mirabilis and Enterococcus faecalis were isolated from 66.67%, 46.67%, 40.00%, 33.33%, 53.33%, 26.67% and 20.00% respectively of the brands. The coliforms (27.2%) were the predominant of all the bacterial isolates in the samples. Most of the brands assessed were of poor bacteriological quality and are therefore unfit for drinking without adequate treatment. Routine monitoring of sachet water production and handling by regulatory bodies is commended to handling by regulatory bodies is recommended to safeguard the health of the consuming public.

Keywords Assessment, Bacteriological, Quality, Commercial, Sachet and Water

1. Introduction

Air, water and food are the three basic necessities of life. Humans can survive for about a month without food, a week

without water and less than five minutes without air [1]. Water can exist as liquid, gas and solid. It occupies about 70% of the earth's surface and is an essential commodity needed for the survival of humans, animals, plants and microorganisms. However, good and safe drinking water is unavailable and over 1.1 billion persons (one sixth of the world's population) lack access to clean water [2].

The growing population of most developing countries occur in urban areas thus making it possible to increase the water supply infrastructure as well as improve water supplies [3]. Water is also a most important medium of illness and infant mortality in many developing countries and even in technologically more advanced countries [4]. Although water-related diseases have been largely eliminated in the developed countries, they remain a major concern in most of the developing nations. The high prevalence of diarrhoea among children and infants can be traced to the use of unsafe water and unhygienic practices [5].

Drinking water regulations require that potable water for human consumption must be free from disease-causing bacteria and specific indicator bacteria that are indicative of the presence of these pathogens. Microorganisms that are concerned with water-borne disease include *Salmonella sp*, *Shigella sp*, *Escherichia coli*, *Vibrio cholerae*, [6], *Entamoeba histolytica*, *Giardia lamblia*, *Balantidium coli*, *Cryptococcus parvum* and *Enteroviruses of various clinical ailments which include Poliovirus, Rotavirus and Hepatitis E virus*.

A reliable supply of safe water is often beyond the reach most people in the developing countries. Many of these people in the rural and urban communities depend on sachet water as their drinking water. Sachet packaged water is cheap, popular, easily available and sold in restaurants, and other public places in Nigeria [7]. The sachets are electrically heated and sealed at both ends. There are many different brands of sachet drinking water which are beautifully packages, I properly labeled and advertised in Nigeria [8]. These products are however not free of

microbial contaminants [9-11].

Awka, the Capital City of Anambra State of Nigeria has a high demand for potable water. The public water supplies are not enough to serve the teeming population. This situation has led to the proliferation of private water supplies in the form of boreholes and wells and the sale of the water emanating from them to the public. Some of these water are packaged in 50-60cl sachets in questionable hygiene environments and sold to the consumers without regards to their safety. A knowledge of the bacteriological quality of these sachet packaged water is therefore imperative thus in this work, the bacteriological quality of some commercial sachet packaged water in Awka, Nigeria was assessed. It is hoped that the result of the work will further educate the consumers of such water on their bacteriological quality and the dangers the consumption of bacterially-polluted sachet packaged water poses to them.

2. Material and Methods

2.1. Samples Collection

Fifteen different commercial sachet packaged water brands in Awka, Nigeria were purchased from their retail outlets and labeled A-O. The temperature and pH of the samples were measured at the point of purchase in the morning. They were thereafter transported to the microbiology laboratory of Nnamdi Azikiwe University Awka in an ice box and analyzed bacteriologically within twenty-four hours of collection. The assessment of the bacteriological quality was carried out from July 6 – July 20, 2016.

2.2. Bacteriological Analyses

2.2.1. Presumptive Coliform Test

The most probable number (MPN) technique [12] was used. Ten millilitres (10ml) of each of the water samples were introduced into each of a first set of five tubes each containing 10ml double strength bile salt-lactose broth. One millilitre (1ml) of each of the samples was also introduced into each of a second set of five test tubes each containing 5ml single strength bile salt-lactose broth. 0.1 millilitre of each of the samples was also introduced into each of a third set of test tubes each containing 5ml single strength bile salt-lactose broth. Durham tubes were inserted into each of the test tubes. All the test tubes were incubated at 37°C for 48 hours and examined for the presence of acid and gas. Acid production was indicated by a change of colour of the broth to yellow while gas production was indicated by the presence of sufficient gas space at the top of the Durham tubes. The potable number of coliforms per 100ml of each water sample was obtained using the McCrady's Probability Table.

2.2.2. Confirmatory *E. Coli* Test

This test was ed on the fact that only typical *E. coli* will produce gas and indole in Brilliant Green Bile lactose broth at 44°C. A loopful of culture from each positive tube from the presumptive test was inoculated into each of two tubes, one containing tryptone water and the other Brilliant Green Bile lactose broth durham tubes were also introduced into the tubes. The tubes were incubated at 44°C for 24 hours after which they were examined for gas and indole production. A few drops of kovac's reagent were added to the brilliant green bile lactose broth after incubation at 44°C for 24 hours. A deep red colouration at the non-aqueous layer indicated indole production. The presence of *E. coli* was further confirmed by plating out a loopful of culture from the positive presumptive test tubes on eosin methylene blue agar. The plates were incubated at 37°C for 24 hours. Colonies of *E. coli* exhibited a green metallic sheen.

2.2.3. Completed Coliform Test

Isolated colonies from the eosin methylene blue agar plates were inoculated into test tubes of lactose broth containing durham tubes and were also streaked on nutrient agar slants. Incubation was carried out at 37°C for 24 hours. The presence of gas in the lactose broth as well as the presence of gram negative, non-sporing bacilli on the agar slants indicated the presence of coliform bacteria in the water samples.

2.2.4. Faecal *Enterococci* Count

This was done using the most probable number technique [13]. Ten millilitres (10ml) of each of the water samples were inoculated into each of first set of five tubes each containing 10ml of double strength azide dextrose broth. One millilitre (1ml) of each of the water samples was added to each of a second set of five tubes containing 5.0ml of single strength azide dextrose broth. 0.1ml of each of the water samples was also added to each of another set of five test tubes each containing 5ml of single strength azide dextrose broth. The test tubes were incubated at 35°C for 48 hours and examined for growth which was indicated by the turbidity of the broth. The number of tubes that showed turbidity were noted and the most probable number of the faecal *Enterococci* per 100ml of each sample was determined using the McCrady's Probability Table. The organism was confirmed by transferring a loopful from the tubes that showed turbidity to plates of bile esculin azide agar and incubating at 35°C for 24 hours. The plates were observed for the development of blackish brown colonies with brown halos.

2.2.5. *Clostridium Perfringens* Count

This was also carried out using the most probable number technique [14]. 10ml of each of the water samples were inoculated into a set of five test tubes each containing 10ml of differential reinforced double strength clostridial medium. 1 ml of each of the samples was also inoculated into a second

set of five test tubes each containing 5.0ml of single strength differential reinforced clostridial medium while 0.1ml of each of the samples was inoculated into a third set of five test tubes each containing 5ml of single strength differential reinforced clostridial medium. Incubation of the tubes was carried out at 37°C for 48 hours after which the tubes were observed for the change of colour to black which is a positive reaction.

2.2.6. Total Bacterial Count

One millilitre of each of the water samples was introduced into a petri-dish containing ketoconazole at a concentration of 0.05mg/ml to inhibit fungal growth. Sterile nutrient agar (NA) was added to the petri-dish and the mixture was gently swirled and left to solidify. Duplicate plates from each sample were prepared and one incubated at 37°C for 24 hours and the other incubated at 22°C for 24 hours after which the colonies that developed were counted using a colony counter. Only plates that showed between 30 and 300 colonies were counted and the values expressed as colony forming unit per millilitre of the sample. Each colony was purified by repeated sub-culturing on sterile nutrient agar plates and later stored on sterile NA slants for characterization and identification.

2.2.7. Measurement of Temperature

The temperature of the water samples was measured by using a thermometer which was inserted into a beaker containing each of the samples. The temperature values were taken when the reading stabilized.

2.2.8. Measurement of pH

This was carried out by using a pH meter (JENWAY) that

was standardized with pH buffers 4.0 and 7.0. The electrode of the pH meter was inserted into each of the samples and the pH values were taken when the meter reading stabilized.

2.2.9. Characterization and Identification of the Bacterial Isolates

The isolates were characterized morphologically and biochemically using standard methods and identified according to the scheme of Cheesbrough [15]. The tests carried out were gram staining, catalase test, coagulase test, oxidase test, motility test, spore test, methyl red test, voges proskauer test, indole test, citrate utilization test and sugar (glucose, sucrose and lactose) fermentation test.

3. Results

The bacterial count of the sachet packaged water samples are shown in Table 1. The total bacterial count ranged from 35cfu/ml to 50cfu/ml. Sample H had the highest count of 50cfu/ml while Sample A had the lowest count of 35cfu/ml. The total coliform count was from 4MPN/100ml to 20MPN/100ml with Sample M having the highest count of 20MPN/100ml and Sample D having the lowest count of 4MPN/100ml. The faecal coliform count was between 0MPN/100ml and 10MPN/100ml. Sample K had the highest count of 10MPN/100ml while Sample A had the lowest count of 0MPN/100ml. Faecal coliforms were not detected in samples B, D, G, I, and O. *Faecal Enterococci* counts (MPN/100ml) ranged between 0 and 5. In addition, *Clostridium perfringens* were not detected in any of the samples assessed.

Table 1. Bacterial Count of the Sachet Packaged Water Samples

Sample	Total bacterial count (cfu/ml)	Total coliform count (MPN/100ml)	Faecal coliform count (MPN/100ml)	Faecal <i>Enterococci</i> count (MPN/100ml)	<i>Clostridium perfringens</i> count (MPN/100ml)
A	35	6	2	ND	ND
B	41	10	ND	ND	ND
C	40	9	5	ND	ND
D	37	4	ND	ND	ND
E	42	8	4	ND	ND
F	45	13	9	5	ND
G	43	7	ND	ND	ND
H	50	14	8	ND	ND
I	38	5	ND	ND	ND
J	39	11	6	ND	ND
K	37	15	10	4	ND
L	42	11	7	2	ND
M	44	20	9	ND	ND
N	40	17	8	ND	ND
O	49	12	ND	ND	ND
WHO Standard	100	10	0	0	0

Table 2. Morphological and Biochemical Characteristics Of The Bacterial Isolates From The Sachet Packaged Water Samples

Isolate	form	Gram Staining	Motility test	Catalase test	Indole test	Methyl red test	Voges proskaeur test	Coagulase test	Oxidase test	Spor e test	Citrate Utilization test	Glucose fermentation test	Sucrose fermentation test	Lactose fermentation test	Identity
1	Rod	-	+	+	+	+	-	-	-	-	-	+	-	+	<i>Escherichia coli</i>
2	Coccus	+	+	-	+	-	-	+	-	-	-	+	+	+	<i>Staphylococcus aureus</i>
3	Rod	-	-	-	-	-	-	-	-	-	+	+	+	-	<i>Enterobacter aerogenes</i>
4	Rod	+	+	-	-	-	-	-	-	+	-	+	+	-	<i>Bacillus cereus</i>
5	Rod	-	+	-	-	-	-	-	+	-	+	-	-	-	<i>Pseudomonas aeruginosa</i>
6	Rod	-	+	+	+	+	-	-	-	-	+	+	+	+	<i>Proteus mirabilis</i>
7	Coccus	-	-	-	-	-	+	-	-	-	+	+	+	+	<i>Enterococcus faecalis</i>

+ = Positive reaction

- = Negative reaction

The morphological and biochemical characteristics of the bacterial isolates from the sachet packaged water samples are presented in Table 2. They were identified as *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Enterococcus faecalis*.

The occurrence of the bacteria in the sachet packaged water samples is presented in Table 3. Ten of the samples (66.67%) had *Escherichia coli* while only three (20.00%) had *Enterococcus faecalis* in them. The coliforms had the highest occurrence of 27.2% while *Enterococcus faecalis* had the lowest occurrence of 8.0% in the sachet packaged water samples assessed. All the samples also had coliforms in them.

Table 3. Occurrence of the Bacteria in the Sachet Packaged Water Samples

Bacteria	Number of samples with the isolates (%)	Number of Isolates (%)
<i>Escherichia coli</i>	10 (66.67)	60 (9.7)
<i>Staphylococcus aureus</i>	7(46.67)	112 (18.0)
<i>Enterobacter aerogenes</i>	6 (40.00)	109 (17.5)
<i>Bacillus cereus</i>	5 (33.33)	91 (14.6)
<i>Pseudomonas aeruginosa</i>	8 (53.33)	125 (20.1)
<i>Proteus mirabilis</i>	4 (26.67)	75 (12.1)
<i>Enterococcus faecalis</i>	3 (20.00)	50 (8.0)

The temperature and pH values of the sachet packaged water samples are presented in Table 4. The temperature ranged between 27°C and 29°C. Samples C, G, K and O each had the highest temperature of 29°C while Samples A, D, and H each had the lowest temperature of 27°C. The pH of the water samples were from 6.2 to 7.0. Sample L had the highest pH of 7.0 while Sample K had the lowest pH of 6.2.

Table 4. Temperature and pH Values of the Sachet Packaged Water Samples

Sample	Temperature (°C)	pH
A	27	6.5
B	28	6.9
C	29	6.8
D	27	6.8
E	28	6.7
F	28	6.6
G	29	6.5
H	27	6.7
I	28	6.6
J	28	6.8
K	29	6.2
L	28	7.0
M	28	6.7
N	28	6.3
O	29	6.5
WHO Standard	25-40°C	6.5-8.5

4. Discussion

Total bacterial count represents the total bacterial load in the water samples. It also reflects the general hygiene condition of the samples. The result of this study showed the presence of bacteria in all the samples examined (Table 1). However they complied with the WHO standard of less than 100 heterotrophic bacteria per 100ml of water. This result agreed with the studies carried out by Adekunle et al; [16], Ezeugwunne et al; [10] and Oladipo et al; [11] which showed the presence of bacteria in the sachet water samples they examined in Ibadan, Newi and Ogbomosho respectively, all in Nigeria.

The presence of bacteria in the samples assessed may be attributed to the poor quality of the water sources, improper sterilization of the packaging materials, poor hygienic condition of the producing and packaging environments, contamination from the air, soil, filling and sealing machines, packaging tables, packaging personnel, improper handling during loading and distribution to consumers as well as the time between production and use by the public.

Coliform bacteria are organisms that are present in the environment and in the faeces of all warm-blooded animals and humans and are gram negative, facultative anaerobes and non-sporing rods that may be motile or not and ferment lactose with the production of acid and or gas when incubated at 37°C [17]. Their presence in drinking water indicates that disease-causing organisms could be in the water system. Total coliforms are indicators of drinking water quality and are commonly found in the soil and vegetation. They include *Escherichia coli*, *Klebsiella species*, *Citrobacter species* and *Enterobacter species* [18].

Forty percent of the samples met the WHO permissible limit of 10 coliforms per 100ml of water (Table 1). Olaoeye and Onilude [19] assessed the microbiological quality of sachet-packaged drinking water in Western Nigeria and its public health significance and detected total coliforms in some of them. Kassenga [20] also detected total coliforms in 4.6% of the plastic-bagged drinking water he examined in Dares Salaam, Tanzania. Obiri-Danso et al; [9] also detected total coliforms in 4.5% of the factory-bagged sachet water they examined in Kumasi, Ghana.

Faecal coliforms are a group within the total coliform family which are found in great quantities in the intestines and faeces of humans and animals [17]. They are considered more accurate indicators of faecal contamination of animal or human wastes than the total coliforms. Their presence in drinking water often indicates recent faecal contamination showing that there is a greater risk that pathogens are present than if only total coliforms are detected. 33.33% of the sachet packaged water samples assessed met the WHO standard of zero faecal coliforms per 100ml of water sample.

Addo et al [21] studied the bacteriological quality of the sachet water produced and sold in Teshie-Nungua Suburbs of Accra, Ghana and detected faecal coliforms in them. Obiri-Danso et al; [9] carried out the microbiological evaluation of sachet water in Ghana and reported that 2.3%

of the factory-bagged sachet water contained faecal coliforms. Olaoye and Onilude [19] however detected faecal coliforms in 2.2% of the sachet –packaged water samples they examined in Western Nigeria while Edema et al [7] isolated *Escherichia coli* from 87% of the samples they examined in Nigeria.

Faecal Enterococci can be found within stomachs and intestines of humans and animals. They are also applied as indicators of water pollution. The presence of faecal *Enterococci* indicates the presence of faecal pathogens in water. Faecal *Enterococci* were detected in 20.0% of the water samples assessed. However, Olaoye and Onilude [19] detected faecal *Enterococci* in 5% of the factory-bagged sachet water samples they assessed in Western Nigeria. The high incidence of coliforms and faecal *Enterococci* in the samples examined may be attributed to the contamination of the water sources by human and or animal excreta.

Clostridium perfringens were not isolated from any of the water samples examined. This indicated that the pollution of the samples was as a result of faecal contamination of some recent times.

Escherichia coli, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Enterococcus faecalis* were isolated from the samples assessed (Table 2). The gram negative rods were the predominant bacteria isolated. Olaoye and Onilude [19] isolated *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus mirabilis* and *Staphylococcus aureus* in the water samples they examined in Western Nigeria. Obiri-Danso et al; [9] isolated *Escherichia coli*, *Enterobacter aerogenes* and *Enterococcus* spp from the water samples sold on the streets of Kumasi, Ghana while Edema et al; [7] isolated *Escherichia coli* and *Enterococcus faecalis* from the sachet packaged water they examined in Nigeria.

Escherichia coli occurred in 66.67%, *Staphylococcus aureus* in 46.67%, *Enterobacter aerogenes* in 40.00%, *Bacillus cereus* in 33.33%, *Pseudomonas aeruginosa* in 53.33%, *Proteus vulgaris* in 26.67% while *Enterococcus faecalis* was detected in 20.00% of the water samples assessed (Table 3). The majority of the samples examined contained total coliforms. *Escherichia coli* (9.7%), *Staphylococcus aureus* (18.0%), *Enterobacter aerogenes* (17.5%), *Bacillus cereus* (14.6%), *Pseudomonas aeruginosa* (20.1%), *Proteus mirabilis* (12.1%) and *Enterococcus faecalis* (8.0%) were isolated from the samples (Table 3). The result showed that the total coliforms (27.2%) were the dominant bacteria in the samples indicating that many of the sachet packaged water samples were contaminated with faeces. *Escherichia coli* were isolated from majority of the samples assessed in low numbers. This may be attributed to the variation in the response of organisms to different sterilization methods.

The temperatures of the water samples assessed were within the mesophilic range (Table 4) and were probably suitable for the growth of the bacteria in the water samples.

The temperature values were within the World Health Organization permissible limit (25-40°C). The temperature of any water body affects the rate of proliferation of microorganisms. The pH values of most of the samples (Table 4) were within the permissible limit of 6.5-8.5 established by regulatory bodies [22]. The lower the pH values the more acidic the water while the higher the pH values the more alkaline the water becomes. Low pH may induce the corrosion of metals and pipe works and their leaching into the water thereby leading to serious health problems when their concentrations exceed the permissible limit.

The bacterial isolated in this study have been known to cause diseases of humans. *Escherichia coli* is an intestinal flora and is the best indicator of faecal pollution of water [21]. Its presence indicates faecal contamination of the water which may be of human or animal origin. The presence of *Escherichia coli* also indicates the possible presence of pathogenic bacteria, viruses and protozoans [23]. The bacterium has been linked to diarrhoeal diseases, urethrocystitis, prostatitis and pyelonephritis. *Enterobacter aerogenes* are gram-negative bacteria and opportunistic pathogens that are ubiquitous in nature. Their presence in the intestinal tracts of animals results in their wide distribution in soil, water, sewage, plants and humans. The bacteria cause a variety of conditions including eye and skin infections, meningitis, pneumonia, bacteremia and urinary tract infections [24].

Enterococcus faecalis is a bacterium that is found in human faeces and in the intestines of many-warm blooded animals. It is a leading cause of nosocomial infections and is also responsible for urinary tract infections, bacteremia, endocarditis, meningitis and can be found in wound infections along with many other bacteria [25]. *Proteus mirabilis* is a gram-negative, rod-shaped bacterium that can be found as a free living microbe in soil and water. It is also normally found in the gastrointestinal tracts of human and causes urinary tract infections. It can also lead to the production of kidney and bladder stones [26].

Staphylococcus aureus is a gram-positive coccus and is frequently found in the nose, respiratory tract and on the skin [27]. It can cause a range of illnesses such as pimples, impetigo, boils, cellulitis, carbuncles, abscesses, pneumonia, meningitis, endocarditis, bacteremia, sepsis and osteomyelitis [28]. The presence of this bacterium in some of the water samples is an indication of contamination arising from humans and their activities.

Bacillus cereus is an opportunistic human pathogen that produces toxins and is commonly found in the soil and gastrointestinal tract of ruminants and humans. The toxins can cause severe nausea, vomiting and diarrhoea. Immunocompromised patients are susceptible to bacteremia, endocarditis, septic meningitis, pneumonia, gangrene, cellulitis, infant death, lung abscesses and endophthalmitis [29]. The presence of the bacterium in the water samples may be because of the easy dissemination of their spores which can withstand adverse environmental conditions.

Pseudomonas aeruginosa is a gram negative, rod-shaped, monoflagellated bacterium that has been found in environments such as the soil, water, humans, animals, plants, sewage and hospitals. It is an opportunistic human pathogen that often colonizes immunocompromised patients such as those with cystic fibrosis, cancer and AIDS and may cause life-threatening infections in humans, including nosocomial pneumonia, urinary tract infections, sepsis, folliculitis, malignant otitis externa and skin infections in patients who have suffered burns [30].

In view of the health risk the consumers of bacterially-polluted sachet packaged water are exposed to, it is imperative that adequate hygienic conditions must be maintained before, during and after production of the sachet water to reduce the ingestion of these organisms which may adversely affect human health.

5. Conclusions

This study showed that most of the sachet packaged water samples assessed did not comply with the World Health Organization Standard for drinking water with regards to the coliforms and faecal *Enterococci* and therefore unsafe for human consumption. Routine monitoring of the sachet water producing factories, the producing environment, water source, packaging materials and production machines by regulatory agencies as well as the observance of good manufacturing practices and regular bacteriological analysis of the samples are recommended.

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