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MICROBIOLOGICAL QUALITY OF SACHET AND BOTTLED WATER PRODUCED AND MARKETED AROUND FEDERAL UNIVERSITY OF TECHNOLOGY OWERRI (FUTO), IMO STATE, NIGERIA

Mgbemena Ifeyinwa Celestina

Federal University Technology, Nigeria

Three methods of bacteriological analysis of the water samples, namely total viable count, multiple tube (most probable number) method and membrane filtration analysis, were used for this study. The bacterial organisms isolated were *Staphylococcus aureus*, *Eschericia coli, Enterobacter spp* and *Salmonella spp*. The total heterotrophic bacterial counts in sachet water sample ranged between 15 and 190 ml^{-1} and the Geometric Mean total heterotrophic counts varied from the lowest in Divine water (7. 45 ml^{-1}) to the highest in Deogratias pure water (1.95×10^{-2} ml^{-1}). Two brands of bottled water were found to contain total coliform that ranged between 1 and 120 100 ml^{-1}. Geometric mean coliform counts varied from the lowest in Crystal water (4.23, 100 ml^{-1}) to the highest count in Vriis water (2.04×10^{-2}, 100 ml^{-1}). These levels particularly those of sachet water samples far exceeded the limits of EPA Maximum Contaminant Levels(MCLs) of $<1.0\times10^{2}$ of heterotrophic count in drinking water as stipulated by USEPA. The two groups of water samples (sachet and bottled waters) were found not to be satisfactory. It is therefore recommended that the enforcement agencies in the country (NAFDAC) and the Ministry of Health need to get the producers of packaged water to comply with the national drinking water guidelines while communities on their part should be educated and enlightened on the ill effects of patronizing fake vendors.

Keywords: Bottled water, Quality, Microbiological, Owerri, Sachet, Coliform, Heterotrophic count.

Introduction

Water is vital for man's existence, without water there could be no life on earth. The body of human beings consists of 65 percent water. The basic requirement of water is that it should be adequate and safe for consumption (Brain and Allian, 1984). Water should be supplied only after proper treatment because contaminated water can cause disease known as water borne diseases (Al – layia and Anis, 1978). Pollution poses the greatest danger associated with drinking water in most tropical and developing countries (Ihekoronye and Ngoddy, 1985).

The sale and consumption of sachet and bottled water continues to grow rapidly in most country of the world. In Nigeria particularly, there is an astronomical increase in the consumption of packaged waters especially bottled and sachet water. The increase demand for these drinking water products is attributed largely to factors such as inadequate or non-availability of reliable, safe municipal water in urban areas; impression that high quality natural spring water and drinking water offer a healthy, refreshing and great tasting alternative to high calorie soft drinks and ordinary tap water, and;

convenience which has made the products meet the requirement of any lifestyle when needed (Gardener, 2004). The sachet water was introduce into the Nigeria market as a less expensive means of assessing drinking water than bottled water(Ogundipe, 2008). It also acts as an improvement over the former types of drinking water packaged for sale to consumers in band filled hand tied polythene bags. Today the easy accessibility to drinking water in packaged forms has resulted in a big and thriving water industry with several hundreds of millions liters of these water products consumed every year by Nigerians (Ogundipe, 2008).

Most bottled water manufacturers in Nigeria also engage in sachet water packaging and obtain their raw water from local, municipal piped water or well water. Adherence to production and analytical standards are doubtful as most of the factories are observed to lack the appropriate technology for achieving these. These standards of hygiene in the various stages of production of bottled and sachet water vary among various manufacturers while some employ sophisticated technologies such as ozonization and reverse osmosis, most use ordinary boiling of well water sources and exclusion of particles by use of unsterilized filtration materials. Several studies on the microbial quality of bottled and sachet water have reported violations of international quality standards. In Canadian study, screening of bottled water for indicator bacteria revealed that 3.7% of the sample had total coliforms and 23.3% of the 3460 samples have more than 100 colonies of heterotrophic bacteria per 1of the sample (Warburton *et a*l, 1998). A similar study of brands of bottled water in Trinidad showed that 18 out of the 344 samples checked revealed the presence of total coliforms while five of the samples had *Eschericia coli* and colonies of *Enterococcus faecalis* were occasionally detected in the sample (Bharath *et al*, 2003). The quality monitoring of sachet water in Nigeria have been documented (Adekunle *et al*, 2004; Dada, 2009). In Nigeria, the National agency for Food and Drug Administration and Control (NAFDAC) is the parastatal under the Federal Ministry of Health charged with the responsibility for the regulation and control of imported and locally processed foods and water products (Omotayo *et al*, 2001). To ensure strict adherence to international standards, NAFDAC's regulation for bottled and sachet packaged water in Nigeria has been put at the standards established by the World Health Organization. According to these standards, portable water for human consumption must be free of microbial indicators of faecal contamination and coliform count per 100 ml of drinking water must be zero (WHO, 2010). Members of the faecal coliform group especially *E. coli* are used as indicators of possible sewage contamination because they are commonly found in human and animal faeces. Other microbial indicators of possible faecal contamination are Faecal enterococci especially *E. faecalis* and *Clostridium perfrigens* spores. Coliform bacteria describe a group of enteric bacteria that include *E. coli*, *Klebsiella species* and *Enterbacter species*(Crant *et al*, 2004).

Water borne disease continues to be one of the major health problems especially in developing nations. The high prevalence of disease such as diarrhea, typhoid fever, chlorea and bacillary dysentery among the populace has been traced to the consumption of unsafe water and unhygienic drinking water production practices (Mead *et al*, 1999). The study therefore is aimed at providing information as to the safety of sachet and bottled water produced and marketed in Owerri by determining microbiological quality of several of the brands.

Materials and Methods

Sampling of Water Samples

Ten brands of water samples (sachet and bottle) were selected by simple random sampling method from various vendors around Federal University of Technology, Owerri. The distribution of the samples is as follows: 5 samples of bottled water of different brands and 5 samples of sachet water from different brands (Table 1). The samples were stored in a cool box and transported to the Microbiology laboratory of Federal University of Technology Owerri, for bacteriological analysis as described by Cheesebrough (2005) and APHA (1998). In addition, field trips were taken to some of the production sites of these

water brands to get acquainted with first-hand information on their sources of water and treatment(if any) given before packaging.

Table 1. Bottled and sachet drinking water brands marketed around Federal University of Technology used in this study

Water Type	Brand Name
Sachet Water	Divine water Deogratias water Haojin water Mr Ben water Futo pure water
Bottled Water	Apex water Crystal water Vriis water Divine water Blessed water

The three methods that were adopted for the bacteriological analysis of the water samples are: total viable count, multiple tube (most probable number) method and membrane filtration analysis. The microorganisms isolated were characterized by Gram staining and biochemical tests.

Media Preparation

The media used for the analysis were prepared according to the manufacturers´ specification.

MacConkey agar and nutrient agar were prepared by mixing 250g in 100ml of peptone water and were sterilized using an autoclave at 121psi for 15minutes.The media were allowed to cool a bit, then poured into sterile petri dish and allowed to set.

Total Viable Count

The different samples of sachet and bottled water were collected using test tubes and were diluted using 10 fold serial dilution. The procedure of transferring 1ml of the content of the previous tube into the 9ml of sterile water present in the tube and subsequent tube was continued until the tenth tube. After the serial dilution, O.1ml aliquote of the 10^{-2} of each diluted water samples were each inoculated into sterile Nutrient Agar plates and spread with the aid of a glass rod. The inoculated plates were incubated at 37°C for 24 hours after which the bacterial load of water samples were determined (Fawole and Oso, 2004).

The Presumptive Coliform Test (Multiple Tube Fermentation)

The medium used for isolation of the coliform organisms was lactose broth. Three rows of 3 tubes, each arranged in a test-tube rack. The tube in the first row held 10ml of double strength lactose broth while the tubes in the second and third row contained 5ml of single – strength lactose broth, with a sterile pipette of 10ml of the sample added to each of the three tubes in row 1, 1ml of sample was added to each of the test tubes in row 2, and 0.1ml was added to the test tubes in row 3.

After gentle shaking of the tube to mix the inoculums, the test tubes were incubated at 37^0C for 24 hours. Each of these tubes contained a sterile Durham tubes for indicating gas formation and was tightly

plugged with sterile non-absorbent cotton wool. Tube showing acid and gas formation were recorded as positive. Other tubes that did not show positive result were incubated and examined after another 24 hrs.

After this time, tubes that did not show acid and gas formation were discarded. From the same water sample another inoculums were made on nutrient agar plate to know other micro-organisms present in the water sample.

Confirmed Test

All the positive presumptive tubes that showed gas at the end of 24hrs incubation at 37^0C were utilized in the confirmed test for coliform organisms. The positive tubes were sub cultured on plates of eosin methylene blue agar and were incubated for 24 hrs at 37^0C for confirmation of the presence of coliform organisms. The plates were examined for typical coliform colonies of *Escherichia coli*.

Complete Test

The complete test was performed with the colonies gotten from the confirmed test that showed the characteristics of *Escherichia coli*. Each selected colony was inoculated into tubes of lactose broth and also streaked on nutrient agar slant and the tubes were then incubated for 24 hours to 48 hours at 35^0C-37^0C.

Membrane Filtration Method

The method of Cheesbrough(2005) was adopted in this test. 100ml of each of the water samples were introduced into the funnel of a membrane filtration apparatus, when the set – up had already been put in place. The suction was then connected to mains and the filtration process started immediately and lasted about 30 minutes in each of the water samples. After the filtration, the membrane filter placed on the funnel was aseptically inoculated into sterile plate of MacConkey agar with the aid of a sterile forceps. The plates were incubated for 24 hours at 37°C after which the coliform counts were determined in each of the water samples.

Identification of Bacterial Isolates

A smear of each of the bacterial isolate was made and fixed by air drying. The smears were then covered with crystal violet stain for 60 seconds and rapidly washed off thereafter. The smears were then covered with Lugol's iodine for 60 seconds and washed off with clean water. Then the smear were decolorized with acetone alcohol and washed off after 10 seconds. The smear were finally flooded with safranin for 2 minutes and washed off with clean water. The back of the slides were then wiped and placed in a draining rack for the smear to dry before they were viewed with ×10 on immersion objective lens (Cheesbrough, 2005). Gram positive bacteria gave purple coloration while gram negative bacteria gave pinkish colour.

Total Heterotrophic Bacteria Plate Counts (THBC)

The total heterotrophic bacteria plate counts (THBC) in the water samples were obtained using the pour plate technique according to Anon (1994). Dilutions of water samples in buffered peptone water were inoculated in 1 ml aliquots into each of 10 ml molten standard plate count agar in MacCartney bottles.

After thorough mixing, these were poured into sterile Petri dishes and incubated for 48 hours at 22°C. Petri dishes from dilutions containing discrete colonies were counted and the results expressed as the numbers of bacterial colonies per millilitre.

Determination of pH

The pH of the water sample was determined by using universal indicator. 10ml of the sample was pipetted into a test tube and about 2ml of the indicator was added to the sample in the test tube. The mixture was thoroughly shaken and colour of the solution was compared with the colour chart indicator.

Statistical Analysis

The total coliform and THBC counts of triplicate batches of the various brands of bottled and sachet-packed drinking waters were evaluated with the statistical program for the social sciences (SPSS) version 16.

The average geometric mean of coliform bacteria per 100 ml, average geometric mean of total heterotrophic bacteria plate counts per ml and multiplicative standard deviation (S.D.) were used to summarize the microbial quality of the packaged waters in this study.

Results

Colony Count

Colony count was obtained by counting the number of colonies that grow on the nutrient agar plate

Table 2. Colony Count Result for Bottled Water

Various source of water sample	**Numbers of colony**
1. Apex water	No growth
2. Crystal water	No growth
3. Vriis water	1 growth
4. Divine water	1 growth
5. Blessed water	No growth

Table 3. Colony Count Result for Sachet Water

Various source of water sample	**Numbers of colony**
1. Divine water	1 growth
2. Deogratias water	3 growth
3. Haojin water	3 growth
4. Mr Ben water	1 growth
5. Futo pure water	1 growth

The tables above show the total number of bacterial colonies after 24 hours of incubation at 37^0C and it was found that there was one growth in Vriis and Divine bottled water while no growth occurred in Apex, Blessed and Crystal (Table 2). The number of colonies in Deogratias water and Haojin water (3) was higher than that of Mr Ben, Divine and Futo pure water (1).

Table 4. Most probable number result

Water Type	Brand Name	pH	NEGATIVE TUBE	POSITIVE TUBE
Sachet Water	Divine water	7.0		+ve
	Deogratias water	9.5		+ve
	Haojin water	7.7		+ve
	Mr Ben	9.5	-ve	
	Futo pure water	7.5	-ve	
Bottled Water	Apex water	6.2		+ve
	Crystal water	7.0		+ve
	Vriis water	6.3		+ve
	Divine water	6.0	-ve	
	Blessed water	7.2	-ve	

Table above shows the result of most probable number of bacteria based on the presumptive test and the pH of the water sample. It was found out that some tubes were negative while some were positive which shows that there are biological contaminants in the water which may be caused by substandard equipments, dirty environment, dirty tank and also dirty staffs operating the machines.

Table 5. Geometric means total heterotrophic bacteria plate counts (THBC) in bottled and sachet water brands marketed around Federal University of Technology, Owerri

Water brand BOTTLED WATER	Geometric mean (ml^{-1})	Multiplicative S.D.	Range
Crystal water	4.23	1.48	1 – 25
Apex water	4.43	1.50	2 – 35
Vriis water	2.04×10^2	2.26	4 – 40
Divine water	1.26×10^2	1.32	15 – 45
Blessed water	1.07×10^1	1.34	48 – 120

Water brand SACHET WATER	Geometric mean (ml^{-1})	Multiplicative S.D.	Range
Divine water	7.45	1.30	15 – 22
Futo pure water	9.10×10^1	1.84	35 – 120
Haojin table water	1.10×10^2	1.59	65 – 135
Mr Ben	1.25×10^2	1.28	90 – 170
Deogratias water	1.95×10^2	1.62	110 – 185

The results of the heterotrophic bacteria plate counts (THBC) in the various brands of bottled and sachet-packed drinking waters are shown in Table 5. THBC ranged between 15 and 190 ml^{-1}. Geometric Mean total heterotrophic counts varied from the lowest in Divine water (7. 45 ml^{-1}) to the highest in Deogratias pure water (1.95×10^{-2} ml^{-1}). Only two brands of bottled water were found to contain total coliform that ranged between 1 and 120 100 ml^{-1}. Geometric mean coliform counts varied from the lowest in Crystal water (4.23, 100 ml^{-1}) to the highest count in Vriis water (2.04×10^{-2}, 100 ml^{-1}). *E. coli* and *Staphylococcus aureus* were detected in two brands of bottled water

Table 6. Morphological/Biochemical Test for identifying the organisms

Samples	Isolates	Morphological characteristics	Gram reaction	Oxidase	Coagulase	Catalase	Indole	Voges proskaue	Glucose	Lactose	Methyl red	Probable organism
					Bottled water							
Apex	NG											
Crystal	NG											
Vriis	1	Smooth raised creamy colonies	-ve rods	-	-	+	+	-	+	+	+	*Eschericia coli*
Divine	1	Clustered creamy	+ve cocci	-	+	+	-	-	+	+	+	*Staphylococcus aureus*
Blessed	NG											
					Sachet water							
Divine	1	Clustered creamy	+ve cocci	-	+	+	-	-	+	+	+	*Staphylococcus aureus*
Deogratias	3	Round creamy colonies	-ve rods	-	-	+	+	+	+	+	-	*Enterobacter spp*
		Smooth raised creamy colonies	-ve rods	-	-	+	+	-	+	+	+	*Eschericia coli*
		Raised mucoid colonies	--ve rods	-	-	+	+	-	+	-	+	*Salmonella spp*
Haojin	3	Round creamy colonies	-ve rods	-	-	+	+	+	+	+	-	*Enterobacter spp*
		Clustered creamy	+ve cocci	-	+	+	-	-	+	+	+	*Staphylococcus aureus*
		Smooth raised creamy colonies	-ve rods	-	-	+	+	-	+	+	+	*Eschericia coli*
Mr Ben	1	Raised mucoid colonies	--ve rods	-	-	+	+	-	+	-	+	*Salmonella spp*
Futo water	1	Smooth raised creamy colonies	-ve rods	-	-	+	+	-	+	+	+	*Eschericia coli*

Key: +ve = Positive, -ve = Negative, NG = No Observable Growth

Table 6 above presents the result of the biochemical test carried out on for identification of the organisms isolated from the bottled and sachet water samples sold around Federal University of Technology Owerri, which shows that three bacterial isolates are Gram negative and one Gram positive. *Eschericia coli* and *Staphylococcus aureus* were the dominant organisms found in the two water samples. *Eschericia coli* had a higher colonial growth in sachet water and there was one growth of *Eschericia coli* and *Staphylococcus aureus* in the bottled water samples.

Discussion

The result of this study showed the presence of bacteria in the water samples studied. Four species were found in both sachet water and bottled water samples and the isolates include: *E. coli*, *Enterobacter spp*, *Staphylococcus aureus* and *Salmonella spp* (Table 5). Their presence could be as a result of poor environmental conditions, poor handling by distributors and sellers or insufficient sterilization of the sachets used in packaging the water or contamination by the vending machine use in packaging sachet water or the duration of the sachet water. This was also confirmed from field visits where the surroundings were found filthy and the packaging is done by illiterate or ignorant people, often young children with no visible hygienic equipment. Consumption of contaminated water has far reaching public health impact causing water-borne diseases which include: diarrhoea, typhoid fever, nausea, cholera as well as viral infections (Bergey and Holt, 1994).The presence of these organisms in water is of public health concern because some of these organisms are considered pathogenic. The public health importance of these organisms is highlighted below: *Escherichia coli* are a large group of bacteria that can infect someone via ingestion of contaminated water. Most strains of *E. coli* are harmless, however some strains such as *E. coli* 0157:H7 produce a toxin that can cause diseases like diarrhea (often with blood) and stomach cramps. Serious complication of *E. coli* 0157:H7 infection is hemolytic uremic syndrome (kidney failure) (Ryan and Ray, 2004; Vogt and Dippold, 2005). Gram negative bacteria such as *E. coli* are known to cause urinary tract infection and diarrhea in young children(Antai, 1978). *Enterobacter spp such as Enterobacter aerogenes* are called opportunistic pathogens, they can cause numerous infections to humans such as cerebral abscesses, pneumonia, meningitis and septicaemia. This bacterium can infect someone who drinks water contaminated by it **(**Hart, 2006; Ryan and Ray, 2004**).** *Staphylococcus aureus* is a common member of the human micro flora, it can however, produce diseases of adverse health effect (Cheesebrough, 2005; Antai, 1978**)** such as skin sepsis, post-operative wound infections, enteric infections and many more (Bergey and Holt, 1994). It is relatively spread in the environment, but found mainly on the skin and mucus membranes of animals. It has also been detected in sewage and in drinking water supplies (Antai, 1978). *Salmonella* spp are responsible for two types of salmonellosis: (1) Typhoid and paratyphoid fever; (2) gastroenteritis (Le Minor, 2003).

Some of the water samples tested showed coliform counts indicating inadequacy of treatment for microbiological safety for drinking purposes. The bacteriological analysis showed that four bacterial organisms present from the 10 samples showed coliform growths. Highest colonial growths (3) were obtained in sachet water samples as in Deogratia and Haojin and 1 in Mr Ben, Divine and Futo water respectively. Only 1 growth was observed in Vriis and Divine water for the bottled water samples (Table 2). THBC ranged between 15 and 190 ml^{-1} in sachet water and only two brands of bottled water were found to contain total coliform that ranged between 1 and 110 100 ml^{-1}(Table 5). These levels especially those of sachet water samples far exceeded the limits of EPA Maximum Contaminant Levels(MCLs) of <1.0×102 of heterotrophic count in drinking water as stipulated by USEPA (2003). The coliform test is a reliable indicator of the possible presence of fecal contamination and is, consequently, correlated with pathogens. Dezuane (1990) says that water with counts under 100cfu/ml should be considered “potable” and values 100-500/ml “questionable”.

The high prevalence of diseases such as typhoid fever, diarrheal diseases such as cholera and bacillary dysentery among the populace has been traced to the consumption of unsafe water and unhygienic drinking water production practices (Mead *et al*., 1999). In general terms, the greatest

microbial risks are associated with ingestion of water that is contaminated with human or animal feces (Cabral, 2010). In fact the Federal Ministry of Health and various State Ministries of Health in Nigeria are reporting increased number of cases of gastroenteritis, diarrhea, typhoid and cholera which are indicative of poor drinking water quality. The bottled waters are unaffordable to many middle and low income populations. The sachet waters come handy with their low price tag. In Imo State, the price difference among these brands is very high. The bottled water is sold for ₦50 to 70 for 1.5 litres. But the sachet waters are available at least 10 to 15 times cheaper for 250 or 300ml, this depending on economic level; everyone has a brand to choose to meet their drinking water needs. Another feature with sachet water is that they can be bought at any time, cool and convenient. In spite of the relative cheap price of these packaged water products howevder, there is a dire need for quality control in their manufacturing processes. Earlier investigation conducted on safety of drinking water has shown that water in the market is of good microbiological quality while the quality of some factory bagged sachet and hand filled polythene bagged drinking water was noted to be doubtful (Obiri-Danso*et al.,*2003). Egwari *et al* (2005) in their study on bacteriology of sachet water sold in Lagos reported that enteric pathogens and *Escherichia coli* were not isolated from any samples and brands of sachet water but formed significant part of the isolates on the sachet surfaces of samples collected from the cooling receptacles (pail, wheelbarrow and refrigerator).

Conclusion

The sachet and bottled water which seem to be a source of drinking water in the Federal University of Technology, Owerri, Imo State was found to be contaminated by bacteria which have been implicated for water borne diseases like gastroenteritis, diarrhea, sore throat etc, the levels of these bacteria in the packaged water samples examined is of public health concern. The bacteria isolated include *Eschericia coli*, *Staphylococcus aureus* and *Enterobacter spp* and *Salmonella spp*. Public health education is therefore needed to avoid continual contamination of these products. The results obtained so far highlights the fact that communities around federal university of technology Owerri suffer from acute portable water shortages. To augment this situation, many entrepreneurs took to packaged water business – production and vending. There is a rush to get into business and as a result quality control has been compromised. Therefore, packaged water other than those in company sealed bottles could pose as a source of waterborne infection as this study has shown that the bottle water is obviously of better quality than the popular sachet water.

Recommendation

The national regulatory bodies and Ministries should exercise more stringent surveillance in monitoring the quality of packaged water distributed in Owerri especially around higher institutions and also educate the producers and the consumers alike on the importance of proper labeling and certification as well as water quality. The government should ensure that sewage treatment and disposal should be enhanced to avoid contamination of packaged water.

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