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Full Length Research Paper

Microbiological quality of packaged drinking water brands marketed in Ibadan metropolis and Ile-Ife city in South Western Nigeria

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Continuous increase in the sale and indiscriminate consumption of packaged drinking waters in Nigeria is of public health significance. One hundred and eight samples comprising 16 bottled and 20 sachet water brands purchased randomly all over Ibadan and Ile-Ife cities in South Western Nigeria were analysed for presence of bacterial indicators of water quality. Total heterotrophic bacteria plate counts (HPC) ranged from 2 to 150 for bottled water and 5 to 200 for sachet water brands. One brand of bottled water and eight brands of sachet water had mean HPC greater than 100 per ml millilitre water and thus fell below the United States Environmental Protection Agency (USEPA) and World Health Organisation (WHO) dinking water standard of 100 HPC per millilitre water. Total coliforms and *Escherichia coli* were detected in only one brand of bottled water. All brands of sachet water (100%) had total coliforms, four brands (20%) had presence of *E. coli* while *Enterococcus faecalis* was recovered from two (10%) of the brands. Most of the sachet water brands fell below WHO drinking water standards and are therefore of doubtful quality. Efforts need to be intensified in the monitoring of activities in this rapidly expanding industry with a view to raising standards.

Key words: Bottled water, sachet water, microbiological quality, drinking water, South Western Nigeria.

INTRODUCTION

The sale and consumption of bottled water continues to grow rapidly in most countries of the world. In Nigeria particularly, there is an astronomical increase in the consumption of packaged waters especially bottled and sachet drinking water. The increased 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 requirements of any lifestyle when needed (Gardner, 2004).

The sachet drinking water was introduced into the Nigerian market as a less expensive means of accessing 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 hand 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 million litres 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 mostly 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. The standards of hygiene in the various stages of production of bottled and sachet water vary among various manufacturers. While some employ sophisticated techniques such as ozonization and reverse osmosis most use ordinary boiling of well water sources and exclusion of particles by

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use of unsterilized filteration materials.

Several studies on the microbial quality of bottled and sachet water have reported violations of international quality standards. In a Canadian study, screening of bottled water for indicator bacteria revealed that 3.7% of the samples had total coliforms and 23.3% of the 3460 samples had more than 100 colonies of heterotrophic bacteria per ml of sample (Warburton et al., 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 *Escherichia coli* and colonies of *Enterococcus faecalis* were occasionally detected in the samples (Bharath et al., 2003). The quality monitoring of sachet water in Nigeria have been documented (Adekunle et al., 2004; Onifade et al., 2008; Dada, 2009). However, there is little information in scientific literatures on the quality of the many brands of bottled water produced and marketed by local and multinational companies.

Water borne diseases continue to be one of the major health problems especially in developing nations. The high prevalence of diseases such as diarrhoea, typhoid fever, 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). The most dangerous form of water pollution occurs when faecal contaminants enter the water supply. Pathogenes such as *Salmonella* species, *Shigella* species, *Vibrio cholerae* and *E. coli* being shed in human and animal faeces ultimately find their way into water supply through seepage of improperly treated sewage into ground water (DiPaola, 1998).

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., 2002). To ensure strict adherence to international standards, NAFDAC's regulation for bottled and sachet-packed water in Nigeria has been put at the standards established by the World Heath Organization (WHO). According to these standards, potable 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 (World Health Organization, 1997; Pierre, 1999).

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 perfringens* spores. Microbial contamination by human or animal excreta is the most common reason for water to be considered unsafe for drinking because of the high probability of presence of pathogenic organisms. Coliform bacteria describe a group of enteric bacteria that includes *E. coli*, *Klebsiella* species and *Enterobacter* species (Chao et al., 2004). They are gram negative, facultatively anaerobic, non sporing rods that may be motile or not. They are able to ferment lactose to produce acid and gas within 48 h at 35°C (Grant et al., 2002). Although they are generally not harmful themselves, they indicate the possible presence of pathogenic bacteria, viruses and protozoans (Kara et al., 2004).

Ibadan metropolis and Ile-Ife city are highly urbanised areas in South Western Nigeria where several brands of bottled and sachet water are vended to the public. The continuous proliferation of these packaged water products and their indiscriminate consumption are of public health significance. An understanding of their microbiological quality and safety are therefore imperative (Drinking Water Research Foundation, 2004).

This study therefore aims to provide information as to the safety of packaged drinking water marketed in selected areas of South Western Nigeria by determining the microbiological quality of several of the brands. This will give an understanding of the extent to which the products meet the standards and recommendations of the World Health Organization (WHO).

MATERIALS AND METHODS

Sampling of water samples

Sixteen bottled and 20 sachet-packed drinking water brands all of different manufacturers were used for this investigation. Triplicate batches of each of the brands were purchased randomly with each batch sampled at monthly intervals, from markets, shop shelves and street vendors in Ibadan metropolis in Oyo State and Ile-Ife city in Osun State of South Western Nigeria (Table 1). They were transported in cool boxes to the Microbiology laboratory of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria for immediate analysis.

Estimation and isolation of total coliforms and *Escherichia coli*

The microbial quality of the drinking water samples was assessed by making use of the multiple tube fermentation test (American Public Health Association, 1998).

Total coliforms were estimated by using the 5-tube most probable number (MPN) method. MacConkey broth was used for the presumptive tests. Inoculated tubes of MacConkey broth were incubated at 44°C for 24 h. Positive presumptive tests were confirmed using eosin methylene blue agar. Colonies with characteristic growth were reinoculated into tubes of MacConkey broth. Growth characteristics in MacConkey broth as well as reactions to indole, methyl red, Voges Proskauer and citrate utilization tests were used as confirmation of presence of *E. coli.*

Detection and isolation of faecal enterococci

A membrane filteration technique was used as the detection and isolation of faecal enterococci (Anon, 1994). Triplicate 100 ml water

Table 1. Bottled and sachet drinking water brands marketed in different parts of Ibadan metropolis and Ile-Ife city in South Western Nigeria used in this study.

Water type		Brand name (Location of vendor)		
Bottled water	1.	Aquadana (Ibadan)	9.	Gossy (Ile-Ife)
	2.	Billeys (Ibadan)	10.	Grandberg (Ibadan)
	3.	Blessed (Ile-Ife)	11.	Henzy (Ibadan)
	4.	Eve (Ibadan)	12.	Ollan (Ibadan)
	5.	Fandi (Ibadan)	13.	Ragolis (Ile-Ife)
	6.	Femut (Ibadan)	14.	Swan (Ile-Ife)
	7.	Feotamy (Ibadan)	15.	Top Victory (Ile-Ife)
	8.	Funduk (Ile-Ife)	16.	Yomfab (Ibadan)
			11.	Nazzofah (Ibadan)
Sachet water	1.	Blessed (Ile-Ife)		
	2.	De Faith (Ile-Ife)	12.	Pharmadox (Ibadan)
	3.	Doyot (Ibadan)	13.	Promise (Ibadan)
	4.	Fandi (Ibadan)	14.	Racamok (Ibadan)
	5.	Faith (Ile-Ife)	15.	Rehoboth (Ile-Ife)
	6.	Gafanik (Ile-Ife)	16.	Ronik (Ile-Ife)
	7.	Kona (Ile-Ife)	17.	Top Victory (Ile-Ife)
	8.	Layabog (Ile-Ife)	18.	Topsey (Ibadan)
	9.	M & B (Ibadan)	19.	Toyox (Ibadan)
	10.	Mercy (Ibadan)	20.	Victory (Ile-Ife)

samples were filtered through 47 mm diameter Corning membrane filter with the grid side upward on Petri dishes of enterococcus selective agar and incubated at 37°C for 4 h before being taken to 44°C for 45 h. Red or pink colonies were counted as presumptive colonies. Hydrolysis on bile aesculin agar, growth in brain heart infusion broth containing 6.5% sodium chloride, negative catalase reaction, reactions to other biochemical tests and microscopic examinations were used for confirmation of presence of *E. faecalis*.

Total heterotrophic bacteria plate counts (HPC)

The total heterotrophic bacteria plate counts (HPC) 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 h at 22°C. Petri dishes from dilutions containing 50 discrete colonies were counted and the results expressed as the numbers of bacteria colonies per millilitre.

Statistical analysis

The total coliforms and HPC 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

Bottled water

Total coliforms and *E. coli* were detected in only one of the sixteen brands of bottled drinking waters investigated in this study. The geometric mean coliform count was estimated to be 70.24, 100 ml^{-1} drinking water. *E. faecalis* was however not found in any of the brands analysed. The HPC concentrations ranged between 2 and 150 ml^{-1} drinking water. Geometric mean HPC counts varied from the lowest in Aquadana (6.46 ml^{-1}) to the highest in Top victory (1.07×10^2 ml^{-1}) (Table 2).

Sachet water

Heterotrophic bacteria plate counts in the various brands of sachet-packed drinking waters are shown in Table 2. HPC ranged between 5 and 200 ml^{-1}. Geometric Mean total heterotrophic counts varied from the lowest in M & B (9.65 ml^{-1}) to the highest in Blessed (1.65×10^2 ml^{-1}). All the twenty brands were found to contain total coliforms that ranged between 2 and 140 100 ml^{-1} (Table 3). Geometric mean coliform counts varied from the lowest in Topsey (3.04, 100 ml^{-1}) to the highest count in Layabog (1.06×10^2 100 ml^{-1}). *E. coli* was also detected in four of the brands (20%) while two of the brands (10%) had *E. faecalis*.

Table 2. Geometric means of total heterotrophic bacteria plate counts (HPC) in bottled and sachet water brands marketed in different parts of Ibadan metropolis and Ile-Ife city in South Western Nigeria.

Water brand	Geometric mean (ml^{-1})	*Multiplicative S.D.	Range
Bottled			
Aquadana	6.46	2.24	3 - 15
Ragolis	6.69	2.90	2 - 15
Ollan	6.84	1.61	4 - 10
Gossy	7.94	3.59	2 - 25
Swan	8.43	1.59	2 - 12
Femut	1.04×10^1	4.57	2 - 40
Yomfab	1.09×10^1	1.74	6 - 18
Grandberg	1.22×10^1	1.23	10 - 15
Henzy	1.45×10^1	1.23	12 - 18
Blessed	2.11×10^1	1.67	13 - 36
Feotamy	2.47×10^1	1.56	16 - 39
Billeys	3.56×10^1	1.66	21 - 58
Funduk	5.11×10^1	1.48	38 - 80
Fandi	5.68×10^1	1.51	37 - 84
Eve	8.40×10^1	1.41	68 - 125
Top Victory	1.07×10^2	1.67	60 - 150
Sachet			
M & B	9.65	1.90	5 - 18
Nazzofah	9.81	1.47	7 - 15
Pharmadox	1.44×10^1	1.62	10 - 25
Doyot	1.90×10^1	1.87	10 - 35
Kona	2.38×10^1	1.77	15 - 45
Toyox	3.74×10^1	1.94	20 - 75
DeFaith	5.81×10^1	1.56	35 - 80
Ronik	6.04×10^1	1.47	40 - 85
Racamok	6.38×10^1	1.58	40 - 100
Promise	6.64×10^1	1.18	58 - 80
Fandi	6.82×10^1	1.35	55 - 96
Topsey	7.10×10^1	1.46	55 - 110
Faith	1.00×10^2	1.19	85 - 120
Layabog	1.15×10^2	1.61	70 - 180
Top Victory	1.15×10^2	1.22	100 - 145
Victory	1.16×10^2	1.40	85 - 165
Gafanik	1.17×10^2	1.35	85 - 155
Mercy	1.21×10^2	1.59	80 - 200
Rehoboth	1.32×10^2	1.41	90 - 175
Blessed	1.65×10^2	1.20	135 - 195

*Multiplicative standard deviation.

DISCUSSION

Results obtained in this study show that the bottled and sachet drinking waters sold in various parts of Ibadan metropolis and Ile-Ife city both in South Western Nigeria exhibited variable characteristics in terms of their micro-biological quality. All twenty brands of sachet drinking water studied were contaminated with coliform bacteria although to varying levels of counts per 100 ml of drinking water. The average geometric mean coliform counts vary from 3.04, 100 ml^{-1} drinking water in the Topsey brand to 1.06×10^2 100 ml^{-1} in the Layabog brand. All the brands failed to meet the WHO drinking water standard of zero coliform per 100 ml water making them unsuitable for human

Table 3. Geometric means of most probable number (MPN) estimations of coliform bacteria in sachet water brands marketed in different parts of Ibadan metropolis and Ile-Ife city in South Western Nigeria.

Water brand	Geometric mean (100 ml^{-1})	*Multiplicative S.D.	Range
Sachet			
Topsey	3.04	2.06	2 - 7
DeFaith	4.82	1.38	4 - 7
Pharmadox	5.24	1.26	4 - 6
M & B	5.52	1.34	4 - 7
Kona	5.65	1.52	4 - 9
Doyot	5.81	1.38	4 - 7
Nazzofah	6.60	1.55	4 - 9
Toyox	8.65	1.42	6 - 12
Fandi	9.11	1.31	7 - 12
Racamok	1.05×10^1	1.26	8 - 12
Ronik	1.50×10^1	1.30	12 - 20
Faith	1.97×10^1	1.78	11 - 35
Blessed	2.39×10^1	1.58	17 - 40
Mercy	2.67×10^1	1.63	17 - 45
Promise	3.40×10^1	1.34	25 - 45
Victory	3.78×10^1	2.81	14 - 110
Rehoboth	4.61×10^1	2.72	20 - 140
Gafanik	4.63×10^1	1.17	40 - 55
Top Victory	5.13×10^1	1.42	35 - 70
Layabog	1.06×10^2	1.44	70 - 140

*Multiplicative standard deviation.

them unsuitable for human consumption. *E. coli* and *E. faecalis* were detected in four (20%) and two (10%) of the brands respectively. The total heterotrophic bacteria plate counts were very high in the various brands with eight of the 20 brands having geometric mean counts equal to or above100 ml^{-1}. The bottled water brands were of relatively better microbiological quality with only one of the 16 brands (6.25%) containing total coliforms and *E. coli.* Geometric mean coliform count was 70.24, 100 ml^{-1} drinking water in this one brand of bottled drinking water which also had total HPC above 100 ml^{-1}. The result of the data obtained for coliform count and HPC underlines the unsuitability of this brand for human consumption. *E. faecalis* was however absent in all the brands of bottled drinking water.

The absence of faecal indicator bacteria in most brands of bottled drinking water could be attributed to better hygienic practices observed in the industry compared to the sachet water producing industry. These include use of protective sealed caps on bottles, improved and hygienic filling system and use of non-returnable plastic containers. Ajayi et al. (2008) had reported an earlier study of packaged drinking waters in Ibadan, Nigeria in which larger proportions of sachet water were found to show positive coliform counts compared to bottled waters. Several workers have implicated coliform bacteria in bottled drinking water. Erginkaya and Var (1997) in a study on microbial quality of bottled spring water in Turkey had stated that coliform bacteria were found in 12 of the 130 bottles of spring water analyzed. Also in 81 samples of bottled and sachet waters hawked in Ilorin metropolis, forty percent failed to meet microbial quality standards (Olayemi, 1999).

According to World Health Organization, 2002 report, a high HPC concentration does not itself present a risk to human health. Nevertheless HPCs are used as good indicators of the overall quality of production (Ferreira et al., 1994; Obiri-Danso et al., 2003). These may therefore be used in assessing the cleanliness of the different brands of bottle and sachet drinking waters sold in the selected areas of study. Fifteen of the 16 brands of bottled drinking water and twelve of the 20 brands of sachet water compared very well with the European Community standards of maximum 100 HPC per millilitre of drinking water. This study shows that the total HPC in bottled drinking water ranged from 2 to 150 ml^{-1} and from 5 to 200 ml^{-1} in sachet drinking water.

The microbial contaminations of packaged drinking water could be influenced by factors such as their raw water source, treatment process employed and hygienic

practices observed in production (Geldreich, 1996). Most sachet water manufacturers are observed to utilize well water or at best shallow, contaminated boreholes and municipal tap water as raw water source. Well water is usually contaminated by surface waters especially during the rainy season and inadequate attention paid to the environmental sanitary qualities of these wells could result in wild animals and birds constituting natural sources of zoonotic pathogens. Though about 25% of bottled waters sold in developed countries such as United States are sourced from municipal tap water such are generally of high quality by being placed under strict regulatory standard of the United States Environmental Protection Agency (National Defence Research Council, 1999). Municipal tap water systems have been implicated in microbial contaminations. In an investigation of the quality of drinking waters of Kalama region of Egypt, 30% of samples from public tap waters were found to be contaminated with coliform bacteria (Ennayat et al., 1988). Another study of the quality of tap drinking water in Quebec City of Canada showed that 36 and 28% of water samples were contaminated by at least one coliform or indicator bacterium and or at least one pathogenic bacteria (Levesque et al., 1994). At best raw water is subjected to ordinary boiling and packaging by most producers, which may not produce the desired quality and safe products. It is advisable that these two sources should therefore be avoided as sources of raw water for production. Ground waters such as boreholes when properly constructed and maintained provide a relatively safer source of raw water in terms of microbial load compared to unprotected water sources such as river, spring and well waters (Howard et al., 2003).

Ineffectiveness or malfunctioning of the treatment process employed could also result in the presence of coliform bacteria in the samples of water. According to Edberg (1996) no treatment process or method used in mass production of drinking water yields a sterile product, it only produces a safe product devoid of pathogenic organisms. Appropriate treatment processes should therefore be utilised for production of quality and safe packaged drinking waters.

Inadequate sanitation and unhygienic practices account for the major source of microbial contamination of any potable water (Sahota, 2005). High demand for packaged water for various occasions has led to springing up of small scale entrepreneurs who engage in production of packaged waters without due regard to hygienic practices in the production processes. The implication of this is lack of guarantee that the products will meet set standards for drinking water quality. In the bottled water industry, some bottlers have their bottles arriving at the factory without caps in cardboard boxes and these were always not properly washed or rinsed before being filled. This results in microbial contamination of the emerging product.

In this study, the microbiological analysis of bottled and sachet drinking water sold in selected areas of South Western Nigeria revealed the presence of total coliforms and *E. coli* in concentrations that make the products unfit for human consumption going by WHO and NAFDAC recommendations and guidelines. There is therefore need for NAFDAC to intensify efforts in the routine monitoring of activities in the packaged drinking water industry. The safety of bottled and sachet drinking water should be ensured through comprehensive regulatory programs at both the federal and state levels. NAFDAC regulations for packaged waters should be protective of public health and there should be continuous adoption of packaged water quality standards. Testing of market samples will be a good way of detecting if the water is actually pure as claimed by these producing companies. High premium should be placed on ascertaining compliance with Good Manufacturing Practice (GMP) with emphasis on management of raw water source to the consumer product point as recommended by the International Bottled Water Association.

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