Bacteriological Analysis of Qualities of Sachet and Bottle Water Sold in the Streets of Abuja, Nigeria.

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ABSTRACT

Access to good quality drinking water is a challenge in most towns and cities in Nigeria and households have for years depended on other sources of water to supplement their activities. The introduction of sachet and bottled water to consumers was to provide safe, hygienic and affordable instant drinking water to the public. Although this is a laudable idea, current trends seem to suggest that sachet drinking water could be a route of transmission of diseases. The objective of this study was to determine the c of sachet water popularly known as "pure water" and bottled produced and sold in the municipal area of Abuja, Nigeria. Using simple random sampling procedures, 16 samples from 5 brands of sachet water and 3 brands of bottled water were collected from hawkers/vendors (2 samples per brand). The samples were analyzed using multiple tube method and biochemical assays. Results were recorded as Most Probable Number (MPN) of coliform per 100ml of water. The bacteriological quality of the samples was assessed based on the World Health Organization (WHO) classification system for drinking water. Four (25%) of the samples were Excellent, two (12.5%) were Satisfactory, seven (43.75%) were Suspicious and 3 (18.75%) were Unsatisfactory using the MPN values recorded. Seven sachet and two bottled water samples were contaminated with faecal coliform. Escherichia coli, Enterobacteraerogenes, enterococcus faecalis, Klebsiellasp, Staphylococcus aureus, shiqella, and *Pseudomonas aeruginosa*, were present in the sachet water sample while *Citrobacter* spp., Salmonella spp. and *klebsiella*spp was present in the bottled water sample. The level of contamination could be due to inadequate treatment of water samples by the producers, improper use of filters or post-production contamination. The findings shows that even though Nigeria has national guidelines and regulations, and the regulatory agencies, the monitoring of the packaged water quality is poor as shown in this study where a product that has NAFDAC certification still fail to meet standard for portable water. There is, therefore, a need to monitor all those involved in water business to comply with the guidelines to avert possible outbreak of water-borne diseases as a result of consumption of contaminated water.

Keywords: bacteriological quality, pure water, Excellent, Satisfactory, Unsatisfactory, multiple tube method and biochemical assays

INTRODUCTION

The importance of water to human existence and ecological sustainability cannot be overrated as it is essential for life. It is found in virtually all living cells and is paramount to life. Although a human can do without food for up to twenty eight days, man cannot go without water for three days [1]. This is a reason for which water should be given adequate attention at all times. The supply of safe drinking water to all has therefore

engaged the attention of manv individuals, groups, governmental and private organizations [2], considering the inability of the governments to provide adequate pipe borne water to the populace. Well-packaged water in bottles or food grade polyethylene sachets designed for food processing stands is a alternative for readv the growing population of over 140,000,000 people. However, safe drinking water is very scarce. Sachet water in Nigeria is popularly known as 'pure water', normally sold at the rate of ten naira/sachet. Potable water is any packaged water that has been processed, sealed and released into the market under sealed food grade material or other appropriate containers for human consumption [3]. In view of the above, this feasibility relates to the production and packaging of purified water to World Health Organization (WHO) and National Agency for Food and Drug Administration and Control (NAFDAC) standard requirements for human consumptions. Continuous increase in the sale and indiscriminate consumption of packaged drinking waters in Nigeria is of public health significance. 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. Sachet water like any other food product must be processed and packaged under aseptic conditions free from every possible source of contamination. The portability of this water is found uncertain being collected from almost every available water source ranging from rainwater to tanker-borne water most of which are rusty and unwashed [4]. Adherence to production and analytical standards are doubtful as most of the factories are observed to lack the appropriate technology for achieving these standards. The standards of hygiene in the various stages of production of bottled and sachet water vary among some various manufacturers. While employ sophisticated techniques such as ozonization and reverse osmosis, most use ordinary boiling of well water sources

Study Area

The study area is the municipal area of Abuja, which is made up of six local government councils. The city is the Federal Capital Territory of Nigeria with a population of over twelve million and also houses the Diplomatic community in Nigeria and all Federal Ministries and Parastatals and a lot of Universities www.iaajournals.org

and exclusion of particles by use of unsterilized filtration materials. However, contaminant may get introduced during collection, packaging and/or consumer handling [5].

6] reported that several studies on the microbial quality of bottled and sachet have reported violations water of international quality standards. The most widely used indicator for microbial water contaminant is the coliforms group of micro-organisms. Coliforms are used as indicator of water contamination because many of them inhibit the intestinal tract of human and other animals in large number, thus, their presence in water indicates fecal contamination.

Drinking water standard in the United State are specified under the Safe Drinking Water Act which provides a framework for the development of drinking water standards in many other nations also. Minimum standards are generally accepted for the coliforms bacteria. Several brands of bottled and sachet water are vended to the public. An understanding of their microbiological quality and safety are, therefore, imperative [7].

It is in line with the mentioned criterions of NAFDAC and WHO that the present work was carried out to determine the microbial standards of locally produced sachet water with resultant safety and portability indices. The study aims to provide information about the safety of packaged drinking water marketed in selected area of Bwari area council, Abuja, Nigeria bv 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 WHO and NAFDAC.

MATERIALS AND METHODS

owned by both Nigerians and expatriate community alike. However, the city is characterized by high and low level of environmental sanitation, no slums but scattered poor housing in some localities with lack of potable water and improper management of wastes especially in the indigenous core areas characterized by

high density and low income populations mostly the Indigenes.

Sampling of Waters

From the different brands of water sold in the city at the time of study, 118 samples were selected by simple random sampling method from various vendors. The distribution of samples was as follows: 2 samples each of 3 brands of bottled waters and 5 brands of sachet water. It was assumed that the bottled water quality is satisfactory as the quality control is very high in the large scale industries. Therefore bottled water samples from the 3 brands were collected from different sellers. These were purchased directly from water vendors in the markets, food serving areas (Bukhas) and motor parks. The number collected on a day were immediately processed for

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physico-chemical and bacteriological analysis as described in Standard Methods (APHA 1998). For bacteriological analysis, the bottles and sachets were opened under aseptic conditions.

Materials

Autoclave, Bunsen burner, Bijou bottles, Colony counter, Coverslip, Conical flasks, Glass slides, Incubator, Inoculating loop, Microscope, Measuring cylinders, Pipettes(various volumes), Non absorbent cotton wool, Refrigerator, Racks, Weighing Balance, Test tubes.

Media Preparation

All the culture media used in the work were prepared according to the manufacturer's instructions and specifications.

METHODOLOGY Physical Analysis

Determination of Colour, Odour and Taste

A 20 mL volume of each water sample was poured into a clean beaker. The sample was then shaken vigorously to check for any frothing and allowed to settle. Colour was determined through visual examination and then odours were determined through the sensation of Determination of r

The pH of each water sample collected was taken using a calibrated hand held pH meter and recorded for further analysis.

Measurement of total dissolved solids (TDS)

Wash filter paper. Dry evaporating dish & weigh. Stir sample and Pipette 50 ml while stirring. Filter and wash three times. Transfer filtrate to evaporating dish & dry. Cool & weigh. Calculate in mg/ L

Dissolved solid $mg/l = A - B \times 1000/ml$ sample

where:

A=weight of filter + dried residue, mg B =weight of filter, mg

Total Suspended Solids:

Wash filter paper and dry, Cool and weigh filter paper. Assemble filtration apparatus. Wet filter paper with distilled water. Stir sample and Pipette 50ml while stirring. Filter and wash three times. Transfer filter to evaporating dish & dry.

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through visual
en odours weresmell. Taste tests for consumer
acceptability were performed on the water
samples. Small volumes of each sample
was tasted with the tongue and then
immediately rinsed with distilled water
(taste free) after each sample, and the
results were recorded accordingly.Determination of pH of the Water Sample

Cool & weigh. Calculate in mg/ L, Repeat steps 1 to 10 using 10 ml aliquot

Suspended solid mg/l = A - B X 1000/ ml sample

where:A=weight of filter + dried residue, mg

B =weight of filter, mg

Total solids

Weigh evaporating dish, Stir sample. Pipette 50ml into evaporating dish & dry. Cool & weigh evaporating dish. Calculate in mg/ L

solid mg/l = A - B X 1000/ ml sample

where:A=weight of filter + dried residue, mg

B =weight of filter, mg

Microbiological Analysis

(Total and faecal coliforms), multiple tube method was used. The culture media used were MacConkey Broth (MB). The MB cultures were incubated at 37°C for 18 hours. After the incubation period, the cultures were inspected for changes in

colour and gas production. Those showing growth with or without gas production were noted. Those showing no changes in colour were re-incubated for additional 24 hours. The tubes showing changes in colour were counted and the MPN count was expressed per 100 ml of sample as per the MacGrady's Probability Table. The cultures that showed growth were also sub-cultured on MacConkey agar plates to obtain discreet colonies to facilitate easy isolation and identification of the predominant organisms. Colonies on MacConkey agar were further

Determination of bacterial load of the water sample was carried out using nutrient agar by pour plate technique. The total heterotrophic bacteria count were determined by inoculating 0.1ml of each of the water sample on a nutrient aseptically, the plates agar were 37oC incubated at for 24h. After incubation, the different culture plates were observed for microbial growth and the total colonies were counted and expressed as colony forming unit per

Table 1 shows that all the water samples were odourless and clear in appearance. The pH readings of the water samples ranged between 5.7 and 7.3. Nine water samples SA1, 2, SE1,2, BF2, BG1,2 and BH1,2 were within the W.H.O stipulated range of 6.5 - 8.5 while the other six water samples fell below the range. The Total Dissolved Solids (TDS) of water are the particles that are smaller than 2µ found in the water column. The total dissolve solids of the sachet water ranged from 128 - 210 mg/L and 178 - 219 in bottled water. This implies that the total dissolved solid of the water in the area is within the range of the Standard Organization of Nigeria (SON), the total suspended solids (TSS) for the sachet water ranged from 62 - 85 mg/L and 62 -79 in bottled water. The total solids (TS) for the sachet water sampled ranged from 227 - 288 mg/L. This indicates that the (TS) and (TSS) of the sampled water from the study area is within the permissible limit of the Standard Organization of Nigeria [9].

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identified using cultural characteristics, morphology and biochemical tests (Gram stained and the IMVIC (Indole test, methyl red, vogesproskeur and citrate) s tests to identify the colonies as E. coli. Other coliform colonies were also identified using these tests. Quality control and Assurance were Ouality ascertained Standard Methods appropriately. for water analysis as described by the American Public Health Association were employed. The coliform count is expressed as cfu/ 100 ml [8].

Enumeration of Total Heterotrophic Bacteria Count

millilitre and result (cfu/ml)were recorded (APHA, 1985).

Biochemical Characterization of the Isolates

Biochemical tests were carried out and all results obtained with reference to were noted [8].

Staining, Indole test, Citrate Gram utilization Methyl test, Red Test. VogesProskauer Test, Catalase Test. Oxidase, Coagulase.

RESULTS

The brands of sachet water were rated based on the mean MPN values of the two samples as shown in Table 1. Only two brands (SC, BF and BG) met the World Health Organization (WHO) criteria which states that not more than 1 out of 10 analytical units should have an MPN value of >2.2 and that sample should have an MPN value not exceeding 9.2; four of the brands (SA, SB, SD, SE and BH) did not meet the criteria because they had MPN values greater than 2.2.

Faecal coliform was detected in 10 samples (30%) belonging to the SA1, SA2, SB2, SD1, SD2, SE1, SE2, BH1 and BH2 brands. Using the MPN values, the quality of individual sachet water samples was classified as Excellent (<2MPN/100ml), Satisfactory (1-3 MPN/100ml), Suspicious (4-10MPN/100ml)and Unsatisfactory (>10MPN/100ml) (Table 3), based on a WHO classification system for drinking water [7].

A small percentage of the individual samples was found to be Excellent (25%) and Satisfactory (12.5%) but a greater

number (43.75) and (18.75%) were considered to be of suspicious and unsatisfactory quality, respectively (Table 3). Improper handling might be a reason why coliform bacteria were detected in water. bottled WHO some (2011)coliform recommended that faecal bacteria must not be detectable in a 100mL sample of drinking water. Faecal coliform was present in 7 samples of sachet water (SA1, SA2, SB2, SD1, SD2, SE1 and SE2) and two samples of bottle water (BH1 and BH2) 1. More than 60% of all brands of sachet water were contaminated by faecal coliform bacteria. No faecal coliform was detected in bottled water samples (BF1, BF2, BG1 and BG2) and sachet water samples (SB1, SC1 and SC2). In general, most of the tested bottled water samples possessed good bacteriological characteristics; the bacteriological quality of the sachet water samples was poor. The total Bacteria Count cfu/100ml of those samples of sachet water gave moderate growth from 0 - 1.9 x 101. While samples that gave heavy growths are 3.4 x 101 and 8.9 x 101 respectively. The entire bottled water sample analyzed showed no bacterial growth and moderate growth of 1.9 x 191.

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The bacteriological quality of sachet and bottled water commercially sold in the municipal area of Abuja was examined in this study. The results obtained show that the bottled and sachet drinking waters sold in various parts of the municipal area of Abuja exhibited variable characteristics of in terms their microbiological quality. Nine out of the ten samples of sachet water studied were contaminated with at least one type of coliform bacteria. making them for human unsuitable consumption. However, there are non-coliform bacteria present in the brands of sachet water. Escherichia Bacteria such as coli. Enterobacteraerogenes, enterococcus faecalis, Klebsiellasp, *Staphylococcus* Pseudomonas aureus. shiaella. and aeruginosa, were isolated from the samples. 90% of the sachet water brands failed to meet the WHO drinking water standard of zero coliform per 100 ml water. Citrobacter spp., Salmonella spp. and *klebsiella* spp. are present in three out of six samples of the bottle water. The absence of coliform bacteria in most brands of bottled drinking water was attributed to better hygienic practices observed in the industry compared to the sachet water producing industry.

Physio-chemical	colour	odour	taste	PH	TDS	TSS	TS	
parameters					_		-	
Sachet water								
SA1	colourless	none	none	6.7	172	71	277	
SA2	colourless	none	none	6.8	165	68	279	
SB1	colourless	none	none	5.8	181	62	279	
SB2	colourless	none	none	5.7	189	70	280	
SC1	colourless	none	none	6.0	128	81	283	
SC2	colourless	none	none	5.8	129	85	285	
SD1	colourless	none	none	5.9	150	73	284	
SD2	colourless	none	none	6.0	165	78	286	
SE1	colourless	none	none	7.2	200	68	288	
SE2	colourless	none	none	7.3	210	69	286	
Bottled water								
BF1	colourless	none	none	5.9	195	66	278	
BF2	colourless	none	none	6.6	205	74	277	
BG1	colourless	none	none	6.9	219	79	281	
BG2	colourless	none	none	6.7	215	77	280	
BH1	Colourless	none	none	6.2	178	64	285	
BH2	Colourless	none	none	6.0	181	62	283	

Table 1: Concentrations of Some Physic-chemical Parameters in the water samples

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Key words: SA1 =sachet water A sample 1, BF1= bottle water F sample 1, TDS= total dissolved solid, TSS= total suspended solids, TS = total solids.

Table 2: Coliform Count Using the Most Probable Number (MPN) Based on The Presumptive Positive Bottles, feacal coliform and total bacterial count.

Brands of sachet water	In 1 bottle of 50ml broth/50ml of water	In 5 bottles of 10ml broth/10m	MPN/100ml	Faecal coliform count	Total Bacterial count	
	of water	i oi watei			CFU\ML	
SA1	1	4	16	Present	6.0 X10 ¹	
SA2	1	3	9	Present	5.8 x 10 ¹	
SB1	0	3	4	ND	0.3 x 10 ¹	
SB2	1	2	6	Present	1.9 x 10	
SC1	0	0	0	ND	0	
SC2	0	2	2	ND	1.2 x 10 ¹	
SD1	1	4	16	Present	7.1 x 10 ¹	
SD2	1	3	9	Present	4.2 x 10 ¹	
SE1	1	3	9	Present	3.4 x 10 ¹	
SE2	1	5	18	Present	8.9 x 10 ¹	
Bottled water						
BF1	0 0	0		ND	0	
BF2	0 1	1		ND	0.1 x 10 ¹	
BG1	0 0	0		ND	0	
BG2	0 0	0		ND	0	
BH1	1 3	9		Present	1.9 x 10 ¹	
BH2	1 2	6		Present	1.7 x 10 ¹	

www.iaajournals.org Table 3: Mean MPN values of the 5 brands of sachet water and 3 brands of bottled water MPN index of sample

Brands of sachet	FIRST(1)	SECOND(2)	MEAN
SA	16	9	12.5
SB	4	6	5
SC	0	2	1
SD	16	9	12.5
SE	9	18	13.5
Bottled water			
BF	0	1	0.5
BG	0	0	0
BH	9	6	7.5

Table 4: Classification of the samples according to WHO criteria for drinking water

Grade Percentage (100%)	Presumptive count (Per 100ml)	Number of Samples (n=30)	Percentage (100%)
Excellent	0	4	25%
Satisfactory	1-3	2	12.5%
Suspicious	4-10	7	43.75%
Unsatisfactory	>10	3	18.75%

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	I able 5: Biochemical characteristics of isolate obtained from the water samples										
Bioche	Pigment	Gra	Cell	Indo	Citr	Met	Vog	catal	Oxidas	coagu	Probable
mical	ation	m	sha	le	ate	hyl	es p	ase	е	lase	organism
charact		stai	ре			red					
eristics		n									
SA1	Red/pink	-Ve	Rod	+ve	-ve	+ve	-ve	+ve	-ve	-ve	E.coli
SA2	Pink	-ve	Rod	-ve	+ve	-ve	+ve	+ve	-ve	-ve	Enterobacteraer
											ogenes
SB1		+ve	Cocc	-ve	+ ve	+ve	+ve	+ve	-ve	+ve	Staphylococci
			i								aureus
SB2	Pink	-ve	Rod	-ve	+ve	-ve	+ve	+ve	-ve	NA	Klebsiellapneum
											oniae
SC2	Brown	-ve	Rod	-ve	+ve	-ve	-ve	+ve	+ve	-ve	Pseudomonas
											aeruginosa
SD1	Colourles	-ve	Rod		-ve	+ve	-ve	+ve	-ve		Shigella
	s/										
	Transpar										
	ent										
SD2	Green	-ve	Rod	-ve	+ve	-ve	-ve	+ve	+ve	-ve	Pseudomonas
											aeruginosa
SE1	Red	+ve	сосс	-ve	-ve	-ve	+ve	-ve	-ve	-ve	Enterococcus
			i								faecalis
SE2	Red/pink	-ve	Rod	+ve	-ve	+ve	-ve	+ve	-ve	-ve	E .coli
			S								
BF2	Red	-ve	Rod	-ve	+ve	+ve	-ve	+ve	-ve	NA	<i>Citrobacter</i> spp
BH1	Colourles	-ve	Rod	-ve	-ve	+ve	-ve	+ve	-ve	NA	Salmonella spp
	S										
BH2	Pink	-ve	Rod	-ve	+ve	-ve	+ve	+ve	-ve	NA	Klebsiellapneum
											onia

Key: -ve = negative , +ve = positive

Natural water, either from surface or underground sources is subjected to different types of treatment based on the available resources and technologies to meet the criteria for portable water. However, water meant for dinking in developing countries manv are improperly treated, hence, still fall below WHO standard the as far as physicochemical and microbiological qualities are concerned [10]. Sachet water is widely taken by all sections of the population in Nigeria. Six out of the ten sachet-water samples analyzed in this study have pHs that fell below the WHO standard for drinking water [12]. However, they were all odourless and clear, in line with WHO requirements [12]. The pHs of respective sachet-water samples is а reflection of the environmental sources of the water and the anthropogenic activities associated with such environment. The inability of the treatment method employed to bring the pHs within the required standard may be due to process failure. The Total Dissolved Solids (TDS) of water are the particles that are smaller than 2µ found in the water column. The total dissolve solids of the sachet water ranged from 128 - 219 mg/L. This implies that even though the total dissolved solid of the water in the area is within the range of the Standard Organization of Nigeria (SON), sachet water sampled the underwent certain purification methods. An increase in TSS may result to increase in TDS. The total suspended solids (TSS) of water are the particles that are larger than 2µ found in the water column. These are solids in water that can be trapped by a filter. TSS can include a wide variety of material, such as silt, decaying plant and animal matter, industrial waste and sewage. High concentration of suspended solids in drinking water can cause many problems. The total suspended solids for the sachet water ranged from 62 - 85 mg/L. The total solid of water refers to all solid matter that are either suspended or dissolve in water. The total solids for the sachet water sampled ranged from 227 -288 mg/L and 277 - 285 mg/l in bottled

water. This indicates that the total solids of the sampled water from the study area are within the permissible limit of the Standard Organization of Nigeria [9].

Occurrence of bacteria was recorded in eleven of the sachet-water samples. The total bacterial counts in most samples were higher than what is stipulated to be acceptable for portable water (1.0×101) cfu/ml) (NAFDAC, 2004). This study also showed the presence of pathogenic bacteria in 70% of the sachet water samples that were analyzed. High load of coliforms was found in some of the sachet-waters. WHO [12], standard stated that, there must be no pathogenic bacteria in portable water This is an indication that the sachet-water samples are contaminated, especially with feacal materials and unsafe for drinking. The presence of relative heavy load of bacteria in water packaged for drinking purposes has been previously documented in literature [13, 14, and 15]. Microbial contamination of treated and packaged water is due to several factors that contribute to failure of treatment process. They include poor hygienic conditions of the handlers, presence of biofilms on processing surfaces due to poor cleaning and sanitation and packaging of treated water under unhygienic conditions [16].

The bacteria characterized and identified from the sachet water samples were found mostly to be opportunistic pathogens which are usually isolated from unhygienic environments or materials. The predominant once include Staphylococcus aureus, Pseudomonassp, [13] encountered Escherichia coli and Staphylococcus aureus in sachet water samples vended in Ondo State. These microorganisms are versatile in their nutrient requirements and can survive with limited nutrient availability. Most of these bacteria are indigenous to aquatic environments. Pseudomonas is a wellknown indicator of potential bacterial regrowth in nutrient limited environments such as channels of water distribution systems [17]. The ingestion of these bacteria with contaminated water constitutes public health risks to the

immune-compromised members of the population, especially newborn babies, and sick people [18]. elderly This supports the earlier views of that the sachet water being produced is of quality. questionable The possible contamination of sachet water at the point of production has been confirmed by [19] in which he reported that pure water vending machine may not be so

The results obtained so far highlights the fact that communities in urban areas 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 result quality control has been а 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 RECOMMENDATION

Packaged water consumers should be aware of а possible danger of consumption of poorly packaged water especially the sachet water and the potential health risk associated with such. Also NAFDAC should apart from educating the consuming public on the dangers of patronizing sachet pure water that does approved NAFDAC numbers: have producers should also be educated on how to maintain Good Manufacturing Practice (GMP) and companies that fail to maintain the standard should be properly sanctioned either by stipulating adequate fines to be paid or out rite withdrawal of their production Licenses.

Even though Nigeria has national guidelines and regulations, and the regulatory agencies, the monitoring of the

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is,

pure, after all, because investigations found bacteria like Escherichia coli in the machine. However, it was gratifying to note that half the bottle water analyzed in study was free from bacterial this contamination which possibly showed that some of the manufacturers adhered strictly to the guidelines set up by NAFDAC and SON.

CONCLUSION

of better quality than the popular sachet water. Even though Nigeria has national guidelines and regulations, and the regulatory agencies, the monitoring of the packaged water quality is poor as shown in this study where a product that has NAFDAC certification still fail to meet standard for portable water. There is, therefore, a need to monitor all those involved in water business to comply with the guidelines to avert possible outbreak of water-borne diseases as a result of consumption of contaminated water.

packaged water quality is poor as shown in this study where a product that has NAFDAC certification still fail to meet portable water standard. There therefore, a need to monitor all those involved in water manufacturing business to comply with the guidelines. The

national regulatory bodies and Ministries of Health, Water Resources as well as those of Trades and Industries should stringent surveillance exercise more programmes and educate the producers and the consumers alike on the need to look for water quality, proper labeling and certification. To achieve this goal the manufacturers, the consumers and government should work together to achieve this common goal for the betterment of all.

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APPENDIX

MPN values per 100 ml of sample and 95% confidence limits for various combinations of positive and negative results (when one 50-ml and five 10-ml test portions are used)

No. of tubes giving a positive reaction		MPN (per 100ml)	95% confidence limits		
1 of 50 ml	5 of 10 ml		Lower	Upper	
0	0	<1	8 <u></u> 9	<u>11</u> 4	
0	1	1	<1	4	
0	2	2	<1	6	
0	3	4	<1	11	
0	4	5	1	13	
0	5	7	2	17	
1	0	2	<1	6	
1	1	3	<1	9	
1	2	6	1	15	
1	3	9	2	21	
1	4	16	4	40	
1	5	>18	8 <u>—</u> 8	<u>19</u> 3	