

## Comparative Quality Assurance Studies of Sachet Packaged Drinking Water from Three States of South West Nigeria

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### Abstract

Physicochemical and microbiological analysis of fifteen (15) samples of sachet-packaged drinking water procured from three states (Oyo, Lagos and Ogun) of South West of Nigeria were carried out to evaluate their suitability for drinking. Physicochemical analysis for metals concentration, hardness, pH, acidity, alkalinity and conductivity determined showed that some of the samples were not within the recommended limits. Generally, the metal content of the samples was high as shown by iron in excess of 0.3mg/L in all the samples and lead in excess of 0.05mg/L in two of the samples. Microbiological examination for total counts of bacteria and fungi, the presence of faecal coliform, *Escherichia coli*, *Vibrio sp.*, *Salmonella sp.*, *Shigella sp.*, streptococci and *Staphylococcus aureus* was performed. Coliforms counts 10-200cfu/ml; *E. coli* 0.2-8.0cfu/ml; *Staph. aureus* 10-2000 cfu/ml and total plate count >100cfu/ml, were detected in 11, 4, 8 and 10 samples respectively out of the 15 tested. Using the standards supplied by National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria and as specified by World Health Organisation (WHO), only 13% of the water samples were suitable for drinking. The research confirms the speculations about the impure state of the sachet packaged drinking water sold in Nigeria as "pure water", about 50% of which had NAFDAC registration numbers.

### Introduction

Due to the outbreaks of water-borne diseases like dysentery, gastroenteritis, typhoid fever, cholera and so on, reported some years back, national health authorities, in conjunction with World Health Organization (WHO) have set processing and quality standards for drinking water. Further, occasional chemical poisonings have been reported as contributing to morbidity and mortality worldwide as a result of drinking contaminated water (Medwick, 1990; WHO, 1996; Hunter, 1997). In Nigeria, there has been a shortage in public piped water supplies that made the populace to depend on open dug wells, streams and bore-holes as sources of portable water. However, an increased outbreak of typhoid, cholera and other water-borne diseases has led to the emergence of sachet-packed water popularly called 'pure water' – so called to give an assurance of safety.

As the regulatory body in Nigeria, National Agency for Food and Drug Administration and Control (NAFDAC) has set up limits of physicochemical and microbiological standards as guidelines on the quality of water suitable for drinking purposes (NAFDAC, 2000). The metallic composition of water is important because of the potential health hazards of chemical contaminants like copper, arsenic, phenol and aluminium, and loss of aesthetic quality due to altered physical characteristics. However, more emphasis is laid on the microbiological standards because of serious health implications of ingesting microbial pathogens in water.

WHO and NAFDAC permit  $\leq 100$ cfu/ml as total count and no faecal coliform contaminants (WHO, 1996; NAFDAC, 2000). As *E. coli* is the most numerous facultative organism in the gut which can be detected at very low dilution, this makes it a sensitive indicator of faecal contamination. The *Enterobacteriaceae*: *Salmonella*, *Shigella* and *Escherichia*, along with other organisms like *Vibrio cholerae*, faecal streptococci, *Staph. aureus* and fungi represent the major pathogens causing water-borne diseases worldwide (Lewis, 1991; Richards and Batram, 1993; WHO, 1996; Hunter, 1997; Kirkwood, 1999). For the purpose of this work the 15 samples of drinking water from Oyo, Lagos and

Ogun States of Nigeria were procured randomly and subjected to laboratory examination to verify their extent of purity and suitability for drinking.

## Materials and Methods

### Water Samples:

Fifteen samples of sachet-packaged drinking water were procured randomly from different locations in Oyo, Lagos and Ogun States of Nigeria. Five samples from each state were labeled according to the commercial names on the water sachets, namely: Oyo State (DL, RB, RT, GY, RX), Lagos (VF, PT, KN, YS, GD) and Ogun (BR, AS, BO, PZ, GO).

### Media:

For microbiology assay, MacConkey agar (MA), Salt nutrient agar (SNA), Nutrient agar (NA), Alkaline peptone water (APW), Sabouraud dextrose agar (SDA), Salmonella-Shigella agar (SSA), MacConkey broth (MB) and Cetrimide nutrient agar (CNA) were the media used.

### Physicochemical Analysis

Total hardness was determined by complexometric method of Disodium EDTA to measure the calcium and magnesium ions. Acidity, alkalinity and carbon dioxide were determined by titrimetric methods using 0.1M NaOH, 0.01M HCl and 0.02M Na<sub>2</sub>CO<sub>3</sub> respectively. The pH was measured using a digital pH meter standardized with buffers 4 and 9, while the conductivity was measured with a conductometer (Cambridge). Metal analysis was done with a flame photometer (Jenway) with values for Na, K and Ca determined at their characteristic wavelengths using the meter readings multiplied by average gradient as part per million (ppm) for each metal. An Atomic absorption spectrophotometer (AAS) model Buck 200 was used to test each water samples for Fe, Zn, Mg, Cu, Mn, and Pb measuring the absorbance wavelengths directly in ppm at the correspondent cathode lamp of the metal inserted. All determinations were carried out following standards procedures (Olaniyi and Ogungbamila, 1991; Reynolds *et al*, 1996; B.P. 1998).

### Microbiological Analysis

#### Total viable counts:

Each sample was diluted 1 in 10<sup>6</sup> in Nutrient broth (Oxoid) for bacterial counts and Sabouraud dextrose broth (Oxoid) for fungal counts. From the dilution, duplicate pour-plates were prepared in NA and SDA for bacteria and fungi respectively. The bacterial cultures were incubated at 37°C for 24 to 48 hrs and the fungal cultures at 30°C for 2 to 5 days, both under aerobic condition. Bacteria and fungi were estimated as colony forming units per milliliter (cfu/ml). The samples were also cultured using four differential/selective culture media namely: MCA, SSA, CAN and SNA. The plates were incubated at 37°C for 24 to 48 hrs, and bacteria were estimated as colony forming units per ml (cfu/ml.) (Harrigan, 1976; Adeleke *et al*, 2000).

#### Bacterial and fungal isolates:

Representative bacterial colonies on the plates for total viable count were Gram stained and later subcultured into sterile Nutrient broth of 5ml per test-tube. Isolated colonies resulting from plate cultures on NA were subjected to conventional biochemical tests specifically to identify *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The fungal isolates observed on the total viable count plates were identified by their cultural characteristics such as colour, consistency and growth pattern of mycelia (Cowan, 1974 and Harrigan, 1976).

#### Presumptive coliform test (PCT):

This test gave an estimate of most probable number (MPN) of coliform bacilli per 100ml. of each water sample. Each sample was diluted 1 in 100ml. of sterile distilled water and subjected to multiple tube technique of PCT. The bile-based medium used was MacConkey broth (DIFCO) in double and single-strength concentrations appropriately. Tests that showed acid and gas production after 48 hrs of incubation at 37°C were considered positive for coliform bacilli (Lewis, 1991).

#### Faecal coliforms and Detection of *E. coli*

All the positive tests in the PCT and an overnight broth culture of *E. coli* NCTC 97001, were subcultured in loop-full, each into MacConkey broth and incubated at 44°C to 45°C in an electrothermal water-bath for 48 hr. The subcultures that produced acid and gas as evidence of faecal coliforms were streaked each on MacConkey agar plate and incubated at 37°C for 24 hr. Reddish pink colonies that stained Gram-negative rods as the *E. coli* control culture were subjected to Indole, Methyl Red, Voges-Proskauer and Citrate (IMViC) tests for differentiating the enteric coliforms. *E. coli* was detected as indole and methyl-red positive but Voges-Proskauer and citrates test negative (Harrigan, 1976; Richards and Bartram, 1993).

#### Results and Discussion

All the aesthetic requirements of good appearance, colour, odour and taste were satisfactory in all the water samples as stipulated by WHO. The actual volume of content of each water sample was in excess of the 500ml printed on the sachets. None of the sample had a batch number, which means one cannot be sure of the processing period and data in case of a need for product recall. Only eight of the fifteen samples had NAFDAC numbers, which is an indication that only 53% of the processing facilities were NAFDAC approved. Since NAFDAC is the regulatory body responsible for ensuring the safety of commercial drinking water in Nigeria, lack of NAFDAC number may be implicating.

Based on the result of physicochemical analysis obtained (Table 1), electrical conductivity of all the water samples were higher than normal ( $>5 \times 10^{-6} \mu\text{ohm/cm}$ ), which is an indication of presence of high metallic content. Iron levels (0.4 – 3.1 mg/L) were found to be generally high (0.4 – 3.1 mg/L), in all the samples WHO limits 0.3mg/L (4.6). However, the values were lower than that implicated in iron poisoning, but may enhance redox reactions in the water samples which may lead to discoloration. Toxic metals like copper and lead which may constitute a danger to health if present in excess of the recommended concentrations were found to be within limits in all the samples except two that had 0.06mg/L of lead instead of WHO limit of 0.05mg/L. This however presents no special health implication (WHO, 1993; Reynolds *et al* 1996). Total alkalinity was in excess of 200mg/L in six of the samples while free CO<sub>2</sub> of two samples was in excess of 10mg/L.

The results of microbiological analysis (Table 2) showed that *E. coli* was detected in 4 samples whereas total coliforms were detected in 11 samples out of a total of 15 samples, and this cut across the three states. *Shigella* and *Vibro sp.* were absent in all the samples, but the presence of *Salmonella* in samples RT, GY and VF from Oyo State is a serious indictment. *Salmonella sp.* has been implicated in gastroenteritis, typhoid and paratyphoid fevers. Similarly, the plate counts for *Staph. aureus* showed that the pathogen was present in 8 samples while total plate count revealed excess of microbes (120-2000cfu/ml) in 10 samples.

In conclusion, it is evident, based on the tested samples that most sachet-packaged drinking water, so called "pure water" in Nigeria are below standards as per purity. Therefore compliance monitoring for approved standards of drinking water must be enforced for sachet packaged drinking water samples.

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Table 1: Physicochemical Analysis of the Sachet Packaged Water Samples from Three State in Western Nigeria

Samples of pure water	Oyo State					Lagos State					Ogun State					NAFDAC Limits
	DL	RB	RT	GY	RX	VF	PT	KN	YS	GD	BR	AS	BO	PZ	GO	
Declared content(Cl)	50	50	-	50	60	60	60	-	50	-	55	50	-	-	-	
Net Content, Cl	52	60	64	62	54	62	63	65	58	65	60	67	62	61	59	
NAFDAC No.	Y	Y	N	Y	N	Y	N	Y	Y	N	N	Y	N	N	N	
Batch No.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Conductivity $\mu\text{ohm/cm}$	350	245	270	280	170	120	190	160	520	170	120	81.5	58	47	120	$3-5 \times 10^5$
pH	7.96	7.72	7.49	7.35	6.76	7.7	7.4	6.9	7.4	5.6	7.17	7.12	7.34	7.45	6.91	6.5-9.2
Free Co <sub>2</sub>	12	13	11	19	9	5	7	8	5	9	7	6	5	4	4	10
Sulphate mg/L	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	200
Total hardness	7.0	2.9	2.2	2.9	2.1	1.1	2.4	1.6	0.8	2.0	1.0	3.0	1.4	1.9	1.1	100
Total alkalinity	276	252	144	384	252	72	204	132	132	216	228	180	120	156	132	200
Total Acidity	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sodium mg/L	16.5	39.6	13.68	39.6	15.48	12.2	11.7	12.6	7.9	12.2	10.6	5.2	5.0	5.0	13.9	100
Manganese mg/L	0.03	0.05	0.03	0.04	0.03	0.05	0.02	0.04	0.05	0.03	0.01	0.06	0.09	0.03	0.09	150
Calcium mg/L	29.4	6.86	16.68	12.74	5.88	3.9	7.8	5.9	0.9	6.7	3.9	3.9	1.9	5.9	7.8	70
Potassium mg/L	7.8	5.8	16.4	7.2	5.4	3.4	3.2	3.6	0.8	3.2	2.8	0.4	1.0	3.0	3.2	100
Copper mg/L	0.02	0.62	0.35	0.26	1.02	0.07	0.08	0.06	0.25	0.24	0.7	0.4	0.2	0.7	0.3	1.0
Iron mg/L	0.40	0.80	0.70	1.80	0.80	0.8	1.9	1.2	1.5	0.7	2.4	2.2	1.2	3.1	1.5	0.3
Lead mg/L	0.06	0.00	0.00	0.02	0.06	0.00	0.01	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.05
Zinc mg/L	0.18	0.16	0.26	0.16	0.14	0.18	0.19	0.16	0.13	0.2	0.2	0.2	0.2	0.3	0.2	15
Magnesium mg/L	0.81	0.73	0.87	0.81	0.72	0.3	0.6	0.7	0.7	0.6	0.23	0.60	0.18	0.92	0.32	30
Phosphate mg/L	9.15	27.12	27.12	35.54	22.98	18.3	19.3	20.9	15.7	12.0	3.6	9.4	11.8	18.5	8.9	
	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	

UNS: Unsatisfactory; Y: Yes; N: No.

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Table 2: Microbiological Examination of the Sachet Packaged Water Samples from Three State in Western Nigeria.

Samples of pure water	OYO STATE					LAGOS STATE					OGUN STATE					NAFDAC Limits
	DL	RB	RT	GY	RX	VF	PT	KN	YS	GD	BR	AS	BO	PZ	GO	
Coliforms (cfu/ml.)	NII	NII	50	NII	10	50	2000	800	20	1200	NII	20	24	10	30	
E coli (cfu/ml.)	NII	NII	NII	NII	0.2	8	NII	NII	NII	NII	NII	4	2	NII	NII	
Faecal streptococci (cfu/ml.)	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	
Staph aureus (cfu/ml.)	NII	NII	NII	100	10	60	2000	1000	NII	500	40	10	NII	NII	NII	
Salmonella in 25ml.	NII	NII	+	+	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	
Shigella in 25ml.	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	
Vibrio (cfu/ml.)	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	NII	
Yeasts/moulds (cfu/ml.)	NII	NII	200/0	NII	20/20	500/20	15000	NII/40	NII	NII	NII	NII	NII	NII	NII	
Total plate count (cfu/ml.)	20	20	240	120	40	760	2000	1000	400	2000	NII/10	NII/12	10/NII	NII/16	NII/65	
REMARKS	S	S	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	UNS	

(NAFDAC, 2000).

Recorded values are average of triplicate experiments.

S = Satisfactory

UNS = Unsatisfactory

cfu/ml. = Colony Forming Unit per milliliter.

+ = Present

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