

## Examination of two brands of sachet water and tap water for pathogenic microorganisms

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**Abstract:** Water, popularly called 'pure water' in Nigeria is commercially packaged, easy-to-open 50-60 ml polyethylene sacs of water. It is usually sold at the rate of five Naira (5.00) per sachet and is readily available to a large percentage of the population who cannot afford bottled water. It is an important drinking water in Nigeria because safe drinking water is very scarce and there is an ever increasing demand for drinking water. 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. With increase in the sale of packaged drinking water and continuous demand by the consumers, adherence to analytical standards is doubtful as most of the factories operate from residential apartments and lack the appropriate technology for achieving the standards. A total of 36 water samples were collected, 10 samples of Lifespan water, 10 samples of Aquadivine water, 10 samples of Ahmadu Bello University tap water and 2 samples each of the main source of these water. The samples were analysed for microbial contamination using the most Probable Number Method (PNM) and Total Aerobic Mesophilic Count (TAMC). The isolates were characterized using colonial morphology on different culture media and biochemical test. The bacterial colony counts were compared with WHO standard for potable drinking water. The sachet water with the highest bacterial colony count was Lifespan water with  $3.84 \times 10^3$  cfu/ml followed by Tap water with  $2.41 \times 10^3$  cfu/ml and Aquadivine water  $0.36 \times 10^3$  cfu/ml. Bacteria isolated from the water include; *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Salmonella typhi*, *Proteus vulgaris* and *Enterobacter cloacae*. The sachet water and tap water in the University were loaded with a wide spectrum of pathogenic bacteria. This study advocates proper water treatment by water manufacturers and strict monitoring by the regulatory agency.

[Mbah C.E. and Muhammed H. **Examination of two brands of sachet water and tap water for pathogenic microorganisms.** *World Rural Observ* 2015;7(1):1-7]. ISSN: 1944-6543 (Print); ISSN: 1944-6551 (Online). <http://www.sciencepub.net/rural>. 1

**Keywords:** Sachet water, tap water, water-borne pathogen.

### Introduction

Water is an essential part of human nutrition, both directly as drinking water or indirectly as a constituent of food, in addition to various other applications in daily life. Water is not only essential for life; it is also remains an important source of disease transmission (Botkin and Keller, 1998), and infant mortality in many developing countries. It is also a key parameter influencing survival and growth of microorganisms in foods and other microbial environments (Edema *et al.*, 2011).

Water-borne pathogens are found in different water bodies these include; streams, rivers, lakes, springs, wells, and every known source of water for human consumption including surface and underground waters. Prominent among these pathogens are: *Salmonella typhi* responsible for typhoid fever, *Shigella dysenteriae* implicated as the causative agent of bacillary dysentery, *Escherichia coli* which causes gastroenteritis, *Klebsiella pneumoniae* which causes pneumonia and *Enterobacter cloacae* which causes urinary tract and respiratory tract infections, to mention a few of them (Okafor, 1985).

The provision of adequate supply of safe drinking water was one of the eight components of Primary Health Care identified by the international conference on Primary Health Care in 1978. Increasing human population has exerted an enormous pressure on the provision of safe drinking water especially in developing countries (Edema *et al.*, 2011).

The demand for safe drinking water in Nigeria cannot be overemphasized, considering the inability of the governments to provide adequate pipe-borne water to the populace. Packaged water in bottles or food grade polythene sachets designed for food processing is a ready alternative for the ever-growing population of over 140 million people in Nigeria (National Census 2006). However, safe drinking water is very scarce. The ever-increasing demand for readily available drinking water has led to the concept of sachet water. It is a general perception that packaged water is safe for human consumption. Sachet water in Nigeria is popularly known as 'pure water', normally sold at the rate of five naira (₦5.00) per 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 (Food Drug Administration FDA, 2002).

In view of the above, this study relates to the production and packaging of purified water to meet the World Health Organization (WHO) and National Agency for Food and Drug Administration and Control (NAFDAC) standard requirement for human consumption. Increase in the sale and indiscriminate consumption of packaged drinking water in Nigeria is of public health significance.

Sachet water like any other food product must be processed and packaged under aseptic condition, free from every possible source of contamination. This water is collected from all available water sources ranging from rainwater to tank water most of which are rusty and unwashed (Dibua *et al.*, 2007). Adherence to production and analytical standards are doubtful as most of the factories operate from residential one room apartments lacking space and appropriate technology for achieving these standards (Dibua *et al.*, 2007).

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 of the producers use ordinary boiling of well water sources and exclusion of particles by use of unsterilized filtration materials. Contamination may be introduced during collection, packaging and/or consumer handling (Warburton *et al.*, 1997).

Drinking water is now commercially packaged in easy-to-open 50-60ml polyethylene sacs and is referred to as sachet or "pure water". This packaged water is cheap and convenient and have increasingly become popular. Arising from the popularity of the packaged drinking water is the abuse of its production leading to a situation where the *pure water* is everything but pure (Adekunle *et al.*, 2004). Although there is dearth of documented data on incidence rates of water borne diseases directly associated with consumption of pure water. It has been widely observed (Olowe *et al.*, 2005), with its advent, that cases of salmonellosis and typhoid fever have significantly increased in recent years (Kalpana *et al.*, 2011). Between January and August 2010, over 20 deaths and more than 200 hospitalizations were reported in several parts of Nigeria as a result of cholera outbreaks (Kalpana *et al.*, 2011). Water pollution has continued to create negative impacts on health and economic development in Nigeria (Kalpana *et al.*, 2011).

There are several rules and regulations for the production of drinking water in Nigeria; such regulations are monitored by the National Agency for

Food and Drug Administration and Control (NAFDAC). Surveillance carried out by NAFDAC between 2004 and 2005 revealed that some producers of packaged water indulge in sharp practices such as packaging of untreated water, production of sachet water under unhygienic conditions, illegal production of unregistered water in unauthorized premises, use of non-food grade sachets and release of packaged water for distribution and sale without date-marking. These malpractices compelled the agency to formulate guidelines for the production of wholesome packaged water (Kalpana *et al.*, 2011). Despite the standards formulated by the NAFDAC to address this problem, the situation has remained bad (Kalpana *et al.*, 2011).

Most of the sachet water brands fell below WHO drinking water standards (<2 coliform/100ml) and are therefore of doubtful quality. Efforts need to be intensified in the monitoring activities in this rapidly expanding industry with a view to raising standards (Kalpana *et al.*, 2011).

Sachet water and tap water (even after treatment) by vendors may contain some contaminants and pathogens (Kwakyie-Nuako *et al.*, 2007). There is need to examine this water at the point of distribution and their original sources to ascertain whether or not they meet NAFDAC/WHO minimum standards for safe drinking water.

This study aimed at determining the prevalence of water-borne pathogens in water samples, producing a baseline data for the assessment of water-borne pathogens in sachet and tap water and their source within Zaria and comparing the bacteriological quality of these water samples to ascertain whether or not they meet National Agency for Food and Drugs Administration and Control (NAFDAC) and World Health Organization (WHO) minimum standards for safe drinking water.

## Materials And Methods

A total of 36 water samples were collected, 10 samples of Lifespan water, 10 samples of Aquadivine water were obtained from Ahmadu Bello University Samaru Zaria shops, 10 samples of A.B.U tap water and also, 2 samples each of the main sources of water which are: Shika reservoir water for Aquadivine, A.B.U reservoir water for Tap water and Borehole water for Lifespan all were analysed using different batches at one-week interval. The samples were processed within one hour after collection at room temperature and analysed using the procedure outlined by Cheesbrough (2000), Singh and McFeters (1992) and Pelczar and Chan (1996) for detection of water-borne pathogens.

### Total Aerobic Mesophilic Count

Total aerobic bacteria count was done using the pour plate technique as modified by Cheesbrough (2000). Firstly, Nutrient Agar was prepared, autoclaved and kept in a water bath of room temperature. Using peptone water (9ml), serial dilutions were made and pipetted into sterile petridishes, at the same time 1.0ml of the water sample was added also to the petridishes, 15ml of liquefied nutrient agar was added to each plate, thoroughly mixed by tilting and rotating the petridish and allowed to solidify. The petridishes were then incubated at 35°C for 24 hours.

### Enumeration of Microorganism

The most probable number (MPN) method was used to analyse the samples for the presence of microorganisms. In this method, 15 set of bottles containing sterile MacConkey broth, with 5 of the bottles containing double strength and 10 bottles containing single strength broth. All the bottles had Durham tubes in them. These bottles were inoculated with different volumes of water from the samples.

10ml of water was added to 5 tubes of 10ml double strength MacConkey broth (Plate VI), 1ml of water was added in 5 tubes of single strength MacConkey broth and another 0.1ml was inoculated into the last 5 tubes of single strength MacConkey broth (Plate VIII). All the tubes were then incubated at 37°C for 24 hours. After 24 hours of incubation, all the tubes were observed for gas production and colour change and the counts were recorded using the standard table.

After this, the presence of coliform was further confirmed by the inoculation of the positive samples into Eosin methylene blue agar plates and was

incubated for 24 hours at 37°C. The plates were later observed and the results were noted and recorded.

The complete test was carried out by re-inoculation of suspected coliform colonies into MacConkey broth for confirmation of gas production. The colonies taken from the Eosin methylene blue agar plate were gram stained to observe their gram reactions and morphology.

### Biochemical test

The isolates were characterized using biochemical tests. These tests include Indole test, methyl red-Voges Proskauer test, Motility test, Triple sugar Iron test (TSI) and Citrate Utilization test. The tests were performed using standard methods and observing all standard operating procedures.

### Simmon Citrate Test

A simmon citrate agar slant in bijou bottle was inoculated with the colony isolated and incubated at 37°C for 24-72hours; the development of a deep blue colour indicates a positive reaction.

### Statistical Analysis

Analysis of Variance (ANOVA) was used to calculate the level of significance of all the water samples and multiple comparisons was used to compare between the water samples.

### Results

The average bacterial count for Lifespan water is  $3.84 \times 10^3$  colony forming unit per milliliter (cfu/ml) and this is greater than the bacterial count for Tap water  $2.41 \times 10^3$  cfu/ml, while the lowest is from Aquadivine water  $0.36 \times 10^3$  cfu/ml. They are therefore in the following order of CFU count: Lifespan > Tap water > Aquadivine water.

Table 1: Total Bacterial count for Lifespan water.

Sample code	1st Dilution factor( $10^2$ )	2nd Dilution factor( $10^2$ )	Average count	Cfu/100ml
A	26	28	27	$2.7 \times 10^3$
B	13	17	15	$1.5 \times 10^3$
C	22	21	21.5	$2.2 \times 10^3$
D	30	41	35.5	$3.6 \times 10^3$
E	39	40	39.5	$4.0 \times 10^3$
F	152	128	140	$14.0 \times 10^3$
G	24	27	25.5	$2.6 \times 10^3$
H	19	29	24	$2.4 \times 10^3$
I	35	49	42	$4.2 \times 10^3$
J	12	11	11.5	$1.2 \times 10^3$

Key: A-J = Lifespan water samples

Table 2: Total Bacterial Count for ABU Tap water

Sample code	1 <sup>st</sup> Dilution factor	2 <sup>nd</sup> Dilution factor	Average Count	cfu/ml
T1	14	22	18	$1.8 \times 10^3$
T2	25	30	27.5	$2.8 \times 10^3$
T3	14	25	19.5	$2.0 \times 10^3$
T4	23	23	23	$2.3 \times 10^3$
T5	16	24	20	$2.0 \times 10^3$
T6	25	33	29	$2.9 \times 10^3$
T7	18	26	22	$2.2 \times 10^3$
T8	30	25	27.5	$2.8 \times 10^3$
T9	32	20	26	$2.6 \times 10^3$
T10	28	26	27	$2.7 \times 10^3$

Key: T1-T10= A.B.U Tap water.

Table 3: Total Bacterial Count for Aquadivine water

Sample Code	1 <sup>st</sup> Dilution factor ( $10^2$ )	2 <sup>nd</sup> Dilution factor ( $10^2$ )	Average Count	cfu/ml
A	3	1	2	$0.2 \times 10^3$
B	3	6	5	$0.5 \times 10^3$
C	4	6	5	$0.5 \times 10^3$
D	4	2	3	$0.3 \times 10^3$
E	2	4	3	$0.3 \times 10^3$
F	3	2	3	$0.3 \times 10^3$
G	4	2	3	$0.3 \times 10^3$
H	2	6	4	$0.4 \times 10^3$
I	4	4	4	$0.4 \times 10^3$
J	5	3	4	$0.4 \times 10^3$

Key: A-J= Aquadivine water samples.

Biochemical test indicates that tap water harbored more pathogenic microorganisms (55.5%) namely: *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Salmonella typhi*, and *Enterobacter cloacae* compared with Lifespan water which had 4(44.4%) pathogenic microorganisms namely: *Shigella dysenteriae*, *Salmonella typhi*, *Proteus vulgaris* and *Enterobacter cloacae*.

No pathogens were detected in Aquadivine water.

Raw water which were the sources of Lifespan, Aquadivine and Tap water were also subjected to biochemical characterization and it was found that Borehole water where Lifespan gets its supply had 3 pathogenic microorganisms namely: *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*. While Shika reservoir which is the source of water for Aquadivine had 5 pathogenic microorganisms namely: *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus vulgaris* and *Enterobacter cloacae* and ABU reservoir harbored 4 pathogenic microorganisms

namely: *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Enterobacter cloacae*.

Micro-organisms from water source decreased in the following order: Shika reservoir > ABU reservoir > Borehole water.

For confirmation, the 2 brands of water samples were again analysed and no pathogens were detected in both the Lifespan and Aquadivine water. This signifies that the pathogens present in the first analysis for Lifespan water are either due to re-growth or contamination.

Table 6: Pathogens present in the water samples.

Lifespan water	Pathogens	ABU Tap water	Pathogens	Aquadivine water	AQUA-DIVINE
A	<i>Sh.dysenteriae</i>	T1	<i>E.coli, Sh.dysenteriae</i>	Q1	
B	-	T2	<i>Ent.cloacae</i>	Q2	
C	<i>Sh.dysenteriae</i>	T3	<i>E. coli</i>	Q3	
D	<i>Pr. vulgaris</i>	T4	<i>K.pneumoniae</i>	Q4	
E	-	T5	<i>E.coli</i>	Q5	
F	<i>Sh.dysenteriae</i>	T6	<i>E.coli, Sh.dysenteriae</i>	Q6	
G	<i>Sh.dysenteriae</i>	T7	<i>S.typhi</i>	Q7	
H	<i>Ent. cloacae</i>	T8	<i>K.pneumoniae</i>	Q8	
I	<i>S. typhi</i>	T9	<i>K.pneumoniae</i>	Q9	
J	<i>Sh.dysenteriae</i>	T10	<i>Sh.dysenteriae, Ent.cloacae</i>	Q10	

Table 7: Pathogens isolated from raw water sources

SOURCE	PATHOGENS
Borehole, source of lifespan water	<i>Escherichia coli, Klebsiella pneumoniae, Salmonella typhi.</i>
ABU Reservoir, source of Tap water	<i>E.coli, S.typhi, K.pneumoniae, Enterobacter cloacae</i>
Shika Reservoir, source of Aquadivine	<i>E.coli, K.pneumoniae, S.typhi, P.vulgaris and Ent.cloacae.</i>

### Discussion

Examination of two brands of sachet water and tap water for the presence of micro-organisms indicated that four micro-organisms were detected in Lifespan water, namely *Proteus vulgaris*, *Enterobacter cloacae*, *Shigella dysenteriae* and *Salmonella typhi*. In contrast, no micro-organism was detected in Aquadivine sachet water. This is probably because Aquadivine water was properly treated and screened for pathogenic microorganisms before packaging for consumption.

Biochemical characterization of isolates (Barrow and Feltham, 1993) from Tap water which also serve as a major source of drinking water in Ahmadu Bello University, Zaria (Main Campus) revealed the presence of five micro-organisms, namely: *Enterobacter cloacae*, *Shigella dysenteriae*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Escherichia coli*.

The detection of pathogenic micro-organisms in the 'pure water' and tap water consumed on campus is a source of worry to the University community. This is because these micro-organisms have jointly and severally been incriminated as causative agents of many medically important water-borne diseases.

*Proteus vulgaris* found in lifespan water is known to cause nosocomial infections of the urinary tract, lower respiratory tract and less frequently, bacteraemia especially in elderly patients (Emori and Gaynes, 1993).

Lifespan water and tap water were contaminated with *Enterobacter cloacae* which causes infections among burn victims, immunocompromised patients and victims with malignancy. These infections are also manifested as nosocomial urinary tract or pulmonary infections which have been associated with *E.cloacae* colonization of certain surgical equipments and operative solutions (Wolma *et al.*, 1973).

Another micro-organism detected in both Lifespan water and tap water is *Shigella dysenteriae* incriminated as the causative agent of bacillary dysentery. It is the leading cause of diarrhoea worldwide. *Shigella* is frequently found in water polluted with human faeces, and is transmitted through the faecal-oral route (Kalpana *et al.*, 2011).

*Salmonella typhi* which occurred in Lifespan water and tap water is the notorious cause of typhoid fever which is a serious health problem worldwide accounting for more than 25,000 deaths annually and millions of hospitalizations (Kathleen and Talaro, 2004). Salmonellosis and typhoid fever have increased in recent years probably due to the large scale consumption of unhygienically processed pure water (Kalpana *et al.*, 2011).

*Klebsiella pneumoniae* was detected only in tap water. This is worrisome because this micro-organism accounts for a significant proportion of hospital acquired urinary tract infections, pneumonia, septicemias and soft-tissue Kathleen and Talaro, 2004).

The presence of *E.coli* in tap water in ABU Main campus is an evidence of faecal contamination of drinking water which was not properly treated and screened before water was pumped for consumption. *Escherichia coli* causes intestinal tract infections, uncomplicated urinary tract infections and neonatal meningitis. The range of micro-organisms detected in this study is a cause for concern because of the wide spectrum of diseases which they cause (Olowe *et al.*, 2005). They pose continuous health risk to the University community who patronize these pure water vendors or drink the tap water directly without boiling or further treatment.

When processed pure water (Lifespan and Aquadivine water) and tap water were compared with

their raw water sources, it was discovered that out of four pathogenic microorganisms detected in Lifespan water, only one, *S. typhi* was found in the Borehole source; *E. coli* and *K. pneumoniae* were screened out but unfortunately in the course of storage, production and packaging three more pathogens were introduced.

Average permissible bacteria count recommended from the World Health Organization is <2 MPN/100ml (WHO, 1985) and National Agency for Food and Drugs Administration and Control, (NAFDAC) is 0 MPN/100ml (NAFDAC, 2001). The only water that meets this standard is Aquadivine. The variety of pathogenic micro-organisms found in the three water samples is greater in tap water.

The tap water source is ABU reservoir. The treatment to screen out pathogenic micro-organisms seems to be ineffective because all four micro-organisms present in the raw water were detected in the 'treated' tap water.

Aquadivine water was the only sachet water that was free from pathogenic micro-organisms detected from the raw water source. Aquadivine water can be said to be 'pure' as at the time of this test. It does not call for celebration because many manufacturers do not adhere strictly to laid down standards and tend to relax their production rules when they obtain NAFDAC registration or license to operate.

Disinfection of drinking water is important for human health (Matsunaga *et al.*, 1992). Chlorine has generally been used for this purpose, and although this method is effective and cheap, it is unstable, and leaves disagreeable taste and impurities in water. Chlorine added before the water leaves water treatment plant is meant not only to disinfect (King *et al.*, 1998) but also to provide a level sufficient to help prevent future proliferation of pathogens.

In addition to chemicals, proper boiling of water kills micro-organisms. Temperature is very important in controlling microbial growth in drinking water (King *et al.*, 1998). Furthermore, microbial quality of water may deteriorate during storage on reservoirs in particular at the user sites in developing countries, where the water is often believed to be handled under unhygienic conditions (Dahi and Thogerson, 1996).

The most significant factor responsible for contamination is non-adherence of manufacturers to GMP. GMP is defined as part of quality assurance which ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by products specifications (NAFDAC, 2001).

Other factors include poor state of the manufacturing environment, dirty filling equipment, contaminated packaging materials, unhygienic handling of the products and lack of microbiological in-house controls. The failure of the various tiers of

government to provide clean, hygienic and portable water for the populace has led to the proliferation of commercial pure water producers who try to fill the vacuum (Oni Okanlawon and Olayeni, 2003).

### Conclusion

Biochemical examination of pure water and tap water in ABU indicated that they were loaded with wide-spectrum of pathogenic micro-organisms responsible for the spread of serious ailments on campus. Proper water treatment by sachet water manufacturers and close monitoring by NAFDAC will go a long way to ensure that minimum requirements for safe drinking water are maintained to minimize health hazards posed by unscrupulous 'pure water' merchants.

### Recommendations

Expiry date of sachet water produced in Nigeria should not exceed four weeks from the date of production. The public should be sensitized not to drink sachet water that has exceeded four weeks from the date of manufacture. The regulatory body should promulgate standardized method of storage of sachet water in order to increase its shelf life. Periodic sanitary inspection of sachet water factories by the regulatory body is absolutely necessary to ensure conformity.

Regulation of packaged water is therefore a government intervention in the private sector for public good as it assures quality. This is where NAFDAC comes in by ensuring access to only safe and good quality packaged water to the public (Akunyili, 2003).

When people drink water from the main sources directly and not treated (A.B.U reservoir water, Shika reservoir water and bore-hole water), they stand a greater risk of contracting water-borne diseases. Some villages close to the water source have no other means of drinking water, so they have to be enlightened on how to boil, cool and filter the water to avoid water-borne diseases. In some traditional communities *Moringa olifera* (Zogoli seeds) were usually added to water for purification.

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