

## Microbial Quality of Packaged Sachet Water Marketed in Maiduguri Metropolis, North Eastern Nigeria

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**Abstract:** Continuous increase in the sale and indiscriminate consumption of packaged drinking waters in Nigeria is of public health significance. This study investigated the bacteriological quality of sachet water brands marketed in Maiduguri Metropolis, Borno State. With emphasis on the incidence of pathogenic bacteria in the samples tested. Standard microbiological procedures were used to investigate the incidence of pathogenic bacteria such as *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and indicator *Fecal coliforms*. The results showed that 55% of the brands had *Fecal coliforms*, 25% had *Pseudomonas aeruginosa*, 15% had *Salmonella* sp. while 5% of the sample brand had *E. coli*. Ninety-five percent of the packaged water samples examined were either untreated or are produced under unhygienic conditions. The findings therefore suggest that 95% of these sachet water sold in Maiduguri are not fit for human consumption and are hazardous to health. Hence, there is need for strict attention and routine monitoring by regulatory agencies with the view of raising standards of quality of sachet water produced and sold in Maiduguri.

**Key words:** Bacterial contaminants, microbiological quality, pathogens, sachet water

### INTRODUCTION

The demand for good quality water for drinking and other purposes is no doubt exceeding supply especially in some regions of developing countries where drought has claimed thousand of lives and lifted economic and social damage (Frederiksen, 1996). The provision of an adequate supply of a safe drinking water was one of the eight components of primary health care identified by the international conference on primary health care in 1978 (Dufour *et al.*, 2002). Unsafe water is a global public health threat, placing persons at risk of diarrhea and a host of other diseases as well as chemical intoxication (Hughes and Koplan, 2005).

Studies done by previous authors shows that the bacteriological analysis of sachet water commonly known as "pure water" carried out in some part of the country is heavily contaminated. Maiduguri Metropolis is an urbanized area in the North-Eastern part of Nigeria where several brands of bottled and sachet water are sold to the public. The continuous proliferation of these packaged water products and their indiscriminate consumption are of concern to public health. An understanding of their microbiological quality and safety are therefore pertinent. 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. Some of these indicator organisms include *E. coli*, *Clostridium perfringens*, *E. faecalis* and *Klebsiella* spp. Which serve as an indicator for the presence of pathogenic bacteria such as *Salmonella typhi*, *Vibrio cholerae* and *Pseudomonas aeruginosa*.

The aim of the study is to examine the microbiological quality of sachet water marketed in Maiduguri Metropolis. The study was carried out in the Department of Pharmaceutics and Pharmaceutical Microbiology, University of Maiduguri in 2011.

### MATERIALS AND METHODS

The media and reagents used include MacConkey Broth, MacConkey agar, Nutrient agar, Deoxycholate agar (DCA), Kligler Iron Agar (KIA), Peptone broth, Eosin Methylene Blue Agar (EMB), Citrate agar, Urea agar and Oxidase reagent, Antisera, Crystal violet stain, Lugol's iodine, Acetone-alcohol decolorizer and Neutral red. They were all purchased from commercial source.

**Sample collection:** Ten samples were used for the study. Four samples each of 20 sachet-packed drinking water brands with regulatory registration number were purchased from different manufacturers. Each sample was purchased randomly from markets, shop shelves and

street vendors in Maiduguri Metropolis in Borno State. They were transferred in cool boxes to the Pharmaceutical Microbiology laboratory of the Faculty of Pharmacy, University of Maiduguri, Nigeria for subsequent analysis.

**Test for odour and colour:** A 20 mL volume of each water sample was poured into a clean beaker. The beaker was then shaken vigorously to check for any frothing and allowed to settle. The beaker was then observed under bright light for presence of any particulate matter and then brought close to the nose to test for any odour present (Yakassai, 2009).

**Test for taste:** Small volumes of each sample was tasted with the tongue and then immediately rinsed with taste free distilled water after each sample, the result recorded accordingly.

**Test for pH:** A 40 mL volume of each sample was poured into a beaker; the beaker was placed on the stage of the pH m. The pH m rod was inserted into the beaker containing the sample and the machine was turned on and the reading was noted and recorded.

**Microbiological method:** All the media were prepared according to manufacturer's specifications. The Microbiological procedure used was multiple tube fermentation technique as describe by Mackie and McCartney (Cowan and Steel, 1993).

Primary isolation was first done using MacConkey broth, using a sterile 10 mL syringe and needle 10 mL was withdrawn from the sample sachet water and dispensed aseptically into 5 bottles each containing 10 mL of the media broth and then 50 mL of the sample was dispensed into 1 bottle containing 50 mL of the media broth. Each bottle contained an inverted Durham tube accordingly. The bottles were closed tightly and then shaken to distribute the sample uniformly throughout the medium and to make sure the inverted durham tube is full of broth and there's no air bubble trapped inside it.

The bottles were then incubated at 37°C for 24 h. The procedure was carried out in a clean-lighted flow hood and was repeated for the remaining 10 brands of samples. The chamber was always disinfected with 70% alcohol before and after the analysis. After 24 h the tubes from the presumptive fermentation test showing gas and acid formation by a change in colour from purple to amber yellow and entrapment of gas in the Durham tube were recorded and the corresponding Most Probably Number (MPN) index was determined from the probability table.

**Gram staining:**

**Preparation and fixing of smear:** A colony from the purified subculture was isolated and emulsified in sterile distilled water and a thin preparation was made on the

slide. It was evenly spread to cover an approximated area of about 15-20 mm in diameter on the slide. After the smear was made, it was left on a dry area to dry; making sure it was protected from dust and sunlight. The smear was then fixed using gentle heat, by rapidly passing the slide with the smear uppermost, three times through the flame of a Bunsen burner. The slide was then placed on the back of the hand just to make sure too much heat was not used, which can affect or even kill the micro-organism. The smear was then allowed to cool before staining was done.

**Staining:** The glass slide containing the smear was placed on the staining rack and covered with crystal violet stain and allowed for 60 sec. The stain was then washed off with distilled water. The water was then completely tipped off and the smear was covered with Lugol's iodine for 60 sec. The iodine was also washed off using distilled water. The smear was then decolorized rapidly with acetone and washed immediately with clean water. The smear was covered with neutral red stain for 2 min, and washed off using clean water. The back of the slide was wiped clean, and placed on a draining rack for the smear to air-dry. Using a microscope the smear was examined with 40x objective lens, to check the staining and to see the distribution of the material.

**Biochemical test:**

**Kligler Iron Agar (KIA) test:** Using a sterile wire loop, a colony from the purified subcultures was isolated and stabbed straight down into the slanted agar medium. The wire loop was removed, flame sterilized and the inoculum was streaked on the surface of the slant. The test tube was covered tightly with a screw cap and labeled accordingly before it was placed into the incubator where it was left for 24 h at 37°C. It was then removed and observed for fermentation, shown by a change in colour from bright red to amber yellow.

**Urease test:** Using a sterile wire loop, a colony from the purified subculture was isolated, stabbed and streaked on the surface of the media. The bijou bottle was then covered tightly with a screw cap and labeled accordingly before it was placed into the incubator for 24 h at 37°C. It was then removed and observed for growth.

**Citrate test:** The procedure was the same as that of urea agar.

**Indole test:** Using a sterile wire loop, a colony from the purified subcultures was isolated and inoculated into the bijou bottles containing 3 mL of the sterile peptone water. The mouth of the bijou bottles was then flamed sterilized and covered tightly with a screw cap and labeled accordingly, it was incubated for 24 h at 37°C. The bijou bottles were then removed and 0.5 mL of Kovac's

Table 1: The results of examination of various labels of the sachet water

Brands	Registration number	Manufacture d date	Expiry date	Nutritional information	Batch number	Net volume (CL)	Producer name and contact address
AG	Yes	-	-	-	-	50	+
MF	Yes	-	-	-	-	50	+
AM	Yes	-	-	-	-	50	+
CH	Yes	-	-	-	-	50	+
FD	Yes	-	-	-	-	50	+
GL	Yes	-	-	-	-	50	+
GM	Yes	-	-	-	-	50	+
GB	Yes	-	-	-	-	50	+
GP	Yes	-	-	-	-	50	+
HW	Yes	-	-	-	-	50	+
BB	Yes	-	-	-	-	50	+
KT	Yes	-	-	-	-	50	+
MB	Yes	-	-	-	-	50	+
MD	Yes	-	-	-	-	50	+
NL	Yes	-	-	-	-	50	+
PC	Yes	-	-	-	-	50	+
RH	Yes	-	-	-	-	50	+
U	Yes	-	-	-	-	50	+
VK	Yes	-	-	-	-	50	+
ZM	Yes	-	-	-	-	50	+

+: present; -: Absent

Table 2: The results of physico-chemical properties of sachet water samples

Brands	Colour	Odour	Taste	Presence of particles	pH
AG	Colourless	Odourless	Tasteless	None	6.5
MF	Colourless	Odourless	Tasteless	None	6.8
AM	Colourless	Odourless	Tasteless	None	7.2
CH	Colourless	Odourless	Tasteless	None	7.0
FD	Colourless	Odourless	Tasteless	None	6.7
GL	Colourless	Odourless	Tasteless	None	6.7
GM	Colourless	Odourless	Tasteless	None	6.9
GB	Colourless	Odourless	Tasteless	None	7.1
GP	Colourless	Odourless	Tasteless	None	6.6
HW	Colourless	Odourless	Tasteless	None	6.9
BB	Colourless	Odourless	Tasteless	None	7.2
KT	Colourless	Odourless	Tasteless	None	7.1
MB	Colourless	Odourless	Tasteless	None	6.8
MD	Colourless	Odourless	Tasteless	None	6.6
NL	Colourless	Odourless	Tasteless	None	7.1
PC	Colourless	Odourless	Tasteless	None	6.9
RH	Colourless	Odourless	Tasteless	None	6.8
V	Colourless	Odourless	Tasteless	None	6.6
VK	Colourless	Odourless	Tasteless	None	7.2
ZM	Colourless	Odourless	Tasteless	None	6.9
Standard limit	Colourless	Not offensive	Not offensive	No visible solids	6.5-8.5

reagent was added. The bijou bottles were then shaken gently and left standing for 10 min. Examination for positive result was done by the formation of red colour in form of a ring on the surface layer of the culture media.

**Oxidase test:** The oxidase test is used to in the identification of pseudomonas species, as it produces the enzyme cytochrome oxidase (Chessbrough, 2004). A piece of filter paper was placed in a clean Petri dish and 2 or 3 drops of the oxidase reagent was added to it. Using a sterile wire loop, a colony from the purified subculture nutrient agar was removed and a smear was made on the filter paper. The filter paper was left for 10 sec after which it was observed for the development of a blue purple colour as a positive oxidase test.

**Serotyping for salmonella:** A glass slide was clean and sterilized using 70% ethanol. Two drops of saline

suspension were dropped on the left side of the slide and 2 drops of salmonella antiserum were dropped on the right side of the slide. Using a sterile wire loop, a colony from the purified subculture (Kligler Iron Agar) was isolated and a smear was made on both the saline suspension and the salmonella antiserum. The glass was then brought under an electric fluorescent lamp for observation of agglutination. A positive result is observed when agglutination forms only on the salmonella antisera smear.

## RESULTS AND DISCUSSION

All the sachet water were registered with appropriate regulatory agency (Table 1) but none of the sachet water producers indicated manufacturing date, expiring date and batch number on the sachet, therefore not complying with the labeling compliance as stipulated by the WHO (1997)

Table 3: Coliform count using the Most Probable Number (MPN) based on the presumptive positive bottles

Brands	No of bottles giving true positive reaction		
	In 1 bottle of 50 mL broth/50 mL water	In 5 bottle of 10 mL broth/10 mL water	MPN of coliforms in 100 mls of water
AG	0	2	2
MF	0	0	0
AM	1	0	2
CH	0	1	1
FD	0	2	2
GL	1	0	2
GM	1	4	16
GB	0	4	5
GP	0	3	4
HW	0	2	2
IB	0	1	1
KT	0	2	2
MB	1	4	16
MD	1	0	2
NL	1	2	6
PC	0	2	2
RH	1	0	2
U	0	1	1
VK	0	2	2
ZM	0	1	1

Table 4: Classification of the samples according to WHO (1997) criteria for drinking water

Class	Grade	Presumption count (per 100 mL)	No of sample (n = 100)	Percentage (100%)
1	Excellent	0	2	5
2	Satisfactory	1-3	28	70
3	Suspicious	4-9	6	15
4	Unsatisfactory	10 and above	4	10

for drinking water. It indicates that the consumer would not have any means of knowing if the water is within the shelf life.

The physico-chemical properties of the various sachet drinking water brands as shown in Table 2

indicates that all sample brands were colourless, odourless, tasteless and are devoid of presence of any particles in them with pH range of 6.5-7.2, which is within the standard limit of 6.5-8.5 as stipulated by WHO (1997) as criteria for drinking water. This also conforms to the pH range reported by other authors (Okonko *et al.*, 2008). According to Mead *et al.* (1999), the PH of most natural waters range from 6.5-8.5 while deviation from the neutral 7.0 is as a result of the carbon dioxide/bicarbonate/carbonate equilibrium. The pH of water is extremely important; fluctuation in optimum pH ranges may lead to an increase or decrease in the toxicity of poisons in water (Okonko *et al.*, 2008).

The result of coliform count using the Most Probably Number (MPN) is shown in Table 3 which defined the degree of contamination and the microbiological quality of the selected sachet drinking water sample brands.

Going by the zero tolerance levels stipulated by regulatory agency for coliforms in drinking water, a cumulative figure of 5% meets the standards of quality water and a cumulative figure of 95% (n = 100) of all the identified packaged water did not meet the existing standards as shown in Table 3 and 4 respectively.

Previous studies in other parts of the country reported similar bacterial load indicative of poor water quality (Olayemi, 1999; Itah and Akpan, 2005). Relatively high aerobic colony counts are indicative of poor, unhygienic handling and processing, bacteria growth in water may be unnoticed even in transparent packaged water and the presence of some of these microorganisms may pose a potential risk to consumer (Geldrieck, 1996).

Table 5 shows the results of various biochemical test carried out to determine the type of bacteria present, while

Table 5: Results of biochemical tests of different samples of sachet water

Brands	Fermentation on Mac Conkey agar	Growth on EMB agar	Growth on nutrient agar	Growth on DCA agar	Indole test	Citrate test	Urea test
AG	+ve	-ve	-ve	-ve	-ve	-ve	-ve
MF	+ve	-ve	-ve	-ve	-ve	-ve	-ve
AM	+ve	-ve	-ve	-ve	-ve	+ve	+ve
CH	+ve	-ve	-ve	-ve	-ve	-ve	-ve
FD	+ve	-ve	-ve	-ve	-ve	-ve	-ve
GL	-ve	-ve	+ve	-ve	+ve	+ve	-ve
GM	+ve	+ve	-ve	-ve	-ve	-ve	-ve
GB	+ve	-ve	+ve	-ve	-ve	+ve	+ve
GP	+ve	-ve	-ve	-ve	-ve	-ve	-ve
HW	-ve	-ve	+ve	-ve	+ve	+ve	-ve
IB	-ve	-ve	-ve	+ve	-ve	-ve	-ve
KT	+ve	-ve	-ve	-ve	-ve	+ve	-ve
MB	+ve	+ve	-ve	-ve	+ve	-ve	-ve
MD	-ve	-ve	+ve	-ve	-ve	-ve	-ve
NL	-ve	-ve	-ve	+ve	-ve	-ve	-ve
PC	+ve	-ve	-ve	-ve	-ve	+ve	-ve
RH	+ve	-ve	-ve	-ve	-ve	-ve	-ve
U	+ve	-ve	-ve	-ve	-ve	-ve	-ve
VK	-ve	-ve	-ve	+ve	-ve	-ve	-ve
ZM	-ve	-ve	+ve	-ve	-ve	-ve	-ve

+ve: Positive growth; -ve: Negative growth

Table 6: Result of biochemical test and the identification of isolate present in sachet water

Brands	Oxidase test	Growth in KIA after (24) h	Growth in KIA after 48 h	Serotyping for salmonella	Gram staining	Isolate present
AG	-ve	-ve	-ve	-ve	-ve	Coliform spp.
MF	+ve	-ve	+ve	-ve	-ve	Coliform spp.
AM	-ve	-ve	+ve	-ve	-ve	Coliform spp.
CH	-ve	-ve	-ve	-ve	-ve	Coliform spp.
FD	+ve	+ve	-ve	+ve	-ve	Coliform spp.
GL	+ve	-ve	+ve	-ve	-ve	Pseudomonas spp.
GM	-ve	-ve	-ve	-ve	-ve	Pseudomonas spp.
GB	-ve	-ve	-ve	-ve	-ve	Coliform spp.
GP	-ve	-ve	-ve	-ve	-ve	Coliform spp.
HW	+ve	+ve	-ve	-ve	-ve	Pseudomonas spp.
IB	-ve	+ve	-ve	+ve	-ve	Salmonella spp.
KT	-ve	-ve	-ve	-ve	-ve	Coliform spp.
MB	-ve	-ve	-ve	-ve	-ve	E. coli
MD	+ve	-ve	-ve	-ve	-ve	Pseudomonas spp.
NL-	+ve	+ve	-ve	+ve	-ve	Salmonella spp.
PC	-ve	+ve	-ve	-ve	-ve	Coliform spp.
RH	-ve	-ve	-ve	-ve	-ve	Pseudomonas spp.
U	+ve	-ve	-ve	-ve	-ve	Pseudomonas spp.
VK	-ve	+ve	-ve	+ve	-ve	Salmonella spp.
ZM	+ve	-ve	-ve	-ve	-ve	Pseudomonas spp.

+ve: Positive result; -ve: Negative result; Coliform spp.: 55%; Pseudomonas spp.: 25%; Salmonella spp.: 15%; E. coli: 5%

Table 6 shows the isolated bacteria species present in each sample of the brands.

It can be seen that (11) samples of the water brands where found to contain isolates of coliform species, which is about 55% of the total number of the sachet water brands used. Therefore, the presence of coliform species as well as E.coli which is also a member of the coliform group found in 10% of the sample water brands, suggests that these sample of water brands have been contaminated with feces either of human or animal origin (Okonko et al., 2008).

The presence of Pseudomonas sp. in (25%) of the sample water brands suggest contamination of the water either through decay or improper sanitization or sterilization of the factory equipment or instrument used in the production processes. It can also result from the use of unsterile polythene which is used for the packaging of the water product.

The presence of Salmonella sp. in (15%) of the sample water brands suggest a serious pathogenic water borne threat, liable of causing serious disease to the consumer of those sample water brand. This could be as a result of serious microbial pollution of the factory equipment or from an infected worker, working under unhygienic practices.

High demand for packaged water for various occasions has led to springing up of small scale entrepreneur who engage in production of package waters without due regard to hygienic practices in the production practices, and the implication of this is lack of guarantee that the products will meet set standard for drinking water quality

### CONCLUSION

The microbiological quality of sachet water sold in Maiduguri metropolis revealed the presence of coliforms,

E. coli, Pseudomonas sp. and Ssalmonella sp. Out of the sachet pure water brands examined, it can be concluded that 95% are not fit for human consumption and are hazardous to health. This could be as a result of inadequate sanitation and under hygienic practices. It can also be as a result of ineffectiveness or malfunctioning of the water treatment process employed. Therefore, appropriate treatment process should be utilized for production of quality and safe packaged drinking waters.

Therefore there is need for regulatory agencies to intensity effort in the routine monitoring of quality of sachet water marketed to unsuspecting consumers. They should also ensure that all sachet water contains both manufacturing and expiry dates as well as batch number for easy recall.

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