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Comparative Study of Bacteriological Quality of NAFDAC Registered and Unregistered Sachet Water Sold in Lafia Metropolis

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Authors' contributions

This work was carried out in collaboration between all authors. Author BEAE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CA and DRT managed the analyses of the study. Authors OG and AV managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The bacteriological quality of NAFDAC registered and unregistered sachet water sold in Lafia metropolis was investigated. A total of 150 water samples; 100 samples with NAFDAC registered number and 50 without NAFDAC registered number were obtained and analysed using standard Most Probable Number-MPN method and other standard microbiological tests. Results revealed that the registered sachet water had coliform count of 0/100 ml and Physical parameters such as colour and turbidity within the permissible range as provided by the Federal Ministry of Environment- FME and WHO and therefore safe and fit for consumption. The unregistered sachet water, recorded coliform count ranging from 45/100 ml to 550/100 ml indicating high level of contamination with faecal and non-faecal coliforms. The total bacterial count from the unregistered sachet water ranged from 0.4 x 10^3 cf/ml to 7.9 x 10^3 cfu/ml. Isolates such as *Esherichia coli, Klebsiella spp and Enterobacter* species were identified, and *E. coli* was more prevalent. The

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presence of these organisms which are of faecal origin, revealed that the water sources (unregistered sachet water) are bacteriologically unfit for human consumption. This calls for regular monitoring and assessment of all sachet water sources in Lafia metropolis.

Keywords: Coliforms; faecal; bacteriological; heterotrophic; biochemical; sachet; metropolis; batch number.

1. INTRODUCTION

Water is a fundamental human need, being the most important single requirement of man and ranking only second to air amongst the six basic essentials or necessities of life. It is a known fact that, water makes up more than two thirds of human body weight, about 80%, the human brain is made up of 95% water, blood is 82% and lungs 90% water [1,2]. Despite the overriding importance of water, large numbers of people globally do not have access to adequate supply of water that is safe for drinking and other numerous uses. This informed why the United Nation General Assembly and the UN Human Rights Council in 2010 declared access to safe drinking water and sanitation as a human right [3]. The Assembly recognised the right of every human being to have access to sufficient water for personal and domestic uses (between 50 to 100 litres of water per person per day, which must be safe, acceptable and affordable and physically accessible (the water source has to be within 1,000 metres of the home and collection time should not exceed 30 minutes [4].

Furthermore, the global community through the Millennium Development Goals, MDGs set up a target of reducing to half (50%) the world's population that will have access to safe water by 1990 and 2015. Good enough the target has been met at the international level and presently only 11% of the global population are yet to have access to an improved source of drinking water. [4].

Unsafe water is a global public health threat, placing a large population of human at risk for a host of several waterborne diseases as well as chemical intoxication, [5]. To attain a safe water supply for various communities globally, an understanding of water that is microbiologically and chemically certified is therefore imperative. Above all, to ensure that the microbiological characteristic of drinking water is safe for human consumption, the Nigeria based National Agency for Food and Drugs Administration Control (NAFDAC) in association with the World Health Organization (WHO), recommended that potable water for human consumption should not contain any microorganism that is known to be pathogenic and the coliform number per 100 ml of water must be zero, (0/100 ml) although , it may contain three coliform per 100 ml (3/100 ml) of water sample in occasional samples.

The most reliable source of drinking water is bottled water which is assumed to be of good bacteriological quality [6], but it is expensive and thus only within the means of the affluent in the society. As an alternative, small-scale industries have come up with sachet water, popularly known as "pure water". This product is usually packaged in 50 ml to 60 ml of water in clear nylon square sachets which have been electrically heated and sealed at both ends and widely patronized by both low and middle income earners. The production of sachet water has increased tremendously in Nigeria. The integrity of these sachet waters is doubtful, in fact, unconfirmed report abounds that most of the vendors do not treat their sachet waters before selling to the public. This becomes a concern for public health workers and any right- thinking individual when one considers the fact that, the public including nursing mothers patronize these vendors to procure water for their new born children. Many people in rural and urban communities rely on sachet water and or borehole water as the source(s) of their drinking water supply.

It has been discovered that some sachet water do not bear the stamp of approval of NAFDAC. Even those who have registered do not always meet the standard required of them. Regardless of all these problems, the production of sachet water enjoys a high patronage because apart from affordability, it is considered wholesome for drinking purposes as compared to tap or well water. Surveillance carried out by NAFDAC between 2004 and 2005 revealed that some producers of Sachet water indulge in sharp practices such as packaging of untreated water, production under unhygienic conditions, illegal production of unregistered water in unapproved premises, use of non-food grade sachets and release of Sachet water for distribution and sale

without date marking. These malpractices compelled the agency to formulate guidelines for the production of wholesome Sachet water [7].

However, despite the policies formulated by public and international agencies to address this problem, the situation in Nigeria seems degenerating and therefore demands increased attention. In order to effectively solve the problem, there is a need to fully assess the extent of the problem and its' causes. Drinking water regulations require that potable water for human consumption be free from human-disease-causing bacteria and specific indicator bacteria that are indicative of the presence of these pathogens.

The bacteriological quality of drinking water is of paramount importance and monitoring must be given highest priority, this is so because studies have attributed several disease outbreaks to untreated or poorly treated water containing bacteria pathogen that have been isolated from sachet water. The microbiological quality of drinking water is a concern to consumers, water suppliers, regulators and public health authorities. The potential of drinking water to transmit microbial pathogens to great number of people causing subsequent illness is well documented in many countries at all levels of economic development.

Each year, more than 2 million persons, mostly children less than 5 years of age, die of diarrheal disease [8,9]. For children in this age group, diarrheal disease accounted for 17% of all death from 2000 to 2003 [10], ranking third among causes of death, after neonatal causes and acute respiratory infections. Nearly 90% of diarrheal-related deaths have been attributed to unsafe or inadequate water supplies and sanitation [11] conditions affecting a large part of the world's population [5].

The inability of Government to consistently provide adequate water contributed to the proliferation of the so-called 'pure water' manufacture in Nigeria. The provision of drinking water that is not only safe, but tasteless, odourless and clean in appearance is top priority in any country that cares for good health, and poverty alleviation towards sustainable development. Consumers cannot by themselves ascertain the quality of drinking water, [7].

Regulation of Sachet water is therefore a must through government intervention in the private water sector for the sake of public health as it assures quality in the production and packaging of water. This is where National Agency for Food and Drug administration and Control comes in to safeguard the health of the nation by ensuring access to only safe and good quality pack- aged water to the public [12].

Clean and safe environment is one of the greatest legacies that a nation can bequeath to its unborn generation. Currently, consumers in municipal and urban authorities of our nation are enmeshed in sticking problem of consumption of unsafe packaged sachet drinking water, with the attendant non-existent plastic waste management agency cum environmental pollution- littering and clogging of drainages. There is growing need for the government and producers to provide the consumers with safe drinking water without endangering the society. Social rascality and irresponsibility amongst producers and marketers of packaged sachet drinking water is an unassailable fact. Also evident is governments' insensitivity with respect to environmental management, preservation and protection awareness.

This study is significant in that it would serve as an instrument to evaluate the microbial contamination extent to which production and sale of NAFDAC registered and unregistered sachet water sold in Lafia has impacted on the environment and human health as well as, to generate a re-think of a better way of preventing waterborne diseases. Also, this study will be of immense help to the management of many organization and consumers on how their actions or inactions can help protect and improve their health while drinking the sachet water.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of one hundred and fifty (150) sachet of water, one hundred (100) with NAFDAC number and fifty (50) without NAFDAC number, were purchased from various markets and street vendors in Lafia metropolis.(Lafia is located at the center of the middle belt region on longitude 8°,57' 67''E and latitude 8°,48' 33N''. Each bag in known to contain about 20 sachets of 50 – 60 cl of water. Samples were collected randomly without bias out of the over thirty (30) brands sold in the metropolis, some of which were produced within the metropolis, while others were imported from neighbouring states.

Samples were transferred in their packaging bags to the Microbiology/Biology laboratory of the Faculty of Science, Federal University of Lafia for immediate analysis.

2.2 Sample Identification

Ten samples each were obtained from ten (10) brands of the sachet water with NAFDAC numbers and labelled A,B,C,D, E, F,G ,H, I and J, while each from the five (5) brands without NAFDAC numbers were labelled K,L,M, N, and O. The manufacturing dates were noted as well as the collection and purchase date.

2.3 Materials

Material used were of analytical quality as follows; Autoclave, incubator, test tube rack, cotton wool, weighing balance, inoculating loop, desiccators, strings, surgical blade, slides, cover slip, microscope. Others include; test tubes, Durham's tube, pipette, petri dishes, beakers, measuring cyclinder, conical flask. Media and reagents were also of analytical standards and include; Nutrient agar, MacConkay broth, Eosine methylene blue agar, Brilliant green agar, plate count agar, lactose broth, Lauryl tryptose broth., manitol Salt agar, Crystal violet, lugol's iodine, ethanol, safranin, oil immersion, Hydrogen peroxide, petroleum jelly, kovac's ethanol, distilled water.

2.4 Methods

2.4.1 Sample analysis

2.4.1.1 Observation for physical quality

Sachets were obtained randomly from each brand of water, well mixed and the edge cleaned with 70% ethanol. They were cut open with sterile scissors and the water poured into a sterile measuring cylinder. The water sample in the cylinder was then observed for colour, turbidity, presence of suspended particles and the volume of content. Results were noted and recorded.

2.4.2 Bacteriological analysis

2.4.2.1 Total bacterial count

The sachet was well mixed, edge was cleaned with 70% alcohol, cut open with scissor and one millilitre (1 ml) was extracted for ten-fold serial dilution. One millilitre (Iml) of the 10^{-4} and 10^{-5} dilutions were placed in sterile petri dish in duplicate. Molten nutrient agar medium was poured on the plate (pour plate technique) and the plates were rocked or mixed gently to allow even distribution of colonies in the water. Plates were incubated at 37℃ for 24 hrs. Plates with discrete colonies of 30 – 300 were counted. Colonies were counted as colony forming units per millilitre (cfu/ml) of the water samples. Observed colonies were sub cultured and placed on stock for further biochemical investigations.

2.4.2.2 Total coliform and fecal coliform count

Most Probable Number method. MPN

The method was used for the determination of coliform in the water samples. This consists of three steps including presumptive, confirmed and completed test.

2.4.3 Presumptive test

2.4.3.1 Preparation of medium. Double strength broth

Seventy three point two grams (73.2 g) of MacConkey broth was dissolved in one (1) litre of distilled water. It was shaken and heated to solubilize, then 10 ml was dispensed in test tubes containing inverted Durham's tube. Test tubes with Durham's tube and medium were sterilised by autoclaving at 121℃ for 15 minutes. Tubes were allowed to cool down slowly to prevent bubbles in Durham's tube which could give false result of gas formation. Five test-tubes of double strength were used for each sample of water.

2.4.3.2 Single strength

The manufacturers strength of 36.6 grams of MacConkay broth was dissolved in 1 litre of distilled water and treated as above. Ten millilitres (10 ml) of the single strength medium was dispensed into 10 test tubes, with Durham's tube and treated as above.

2.4.4 Total coliform test

Three sets of the above test tubes were arranged in a row in test tube rack. The first set of 5 contained double strength of test medium while the second and third of 5 tubes each contained single straight of the lauryl tryptose medium. See outlay below.

Set	Strength of medium	No of tubes	Vol. of medium	Vol. of water
1	Double strength	5	10 ml	10 ml
2	Single strength	5	10 ml	1 ml
3	Single strength	5	10 ml	0.1 ml

Using sterile pipette or syringe 10 ml each of sample water was added into the first set of rows of 5 test tubes with Durham's tube. To the second set of 5 test tubes 1ml each of sample water was added while 0.1 ml was added to the third set of 5 test tubes.

The15 tubes of three sets per water sample were incubated at 35 – 37℃ for 24 – 48 hours for total coliform and 44.5 ± 0.5$^{\circ C}$ for faecal coliform.

Test tubes were then observed for turbidity, colour change and production of gas in Durham's tubes (for samples with positive result or coliform content). No colour change or gas production in samples with negative result indicating no coliform in the water sample.

The number of positive test tubes were used to read and state the total coliform count per 100 ml of water sample from the probability table (Macready's table in analysis). Those with negative results were further incubated for 24 hours. The method was used for all samples of water to obtain the presumptive number of coliform in the water samples.

2.4.5 Faecal coliform

For every tube showing fermentation (primary fermentation, presumptive coliforms), one new tube of Lauryl sulphate broth was inoculated, from the tube showing primary fermentation, and incubated at 44.5 ± 0.5℃ after 8 to 24 hours, indole test was carried out by adding Kovac;s reagent. A positive indole test in the broth tube showing gas production at 44.5 ± 0.5C indicates the presence of *E. coli*.

From the number and distribution of positive and negative reactions at 44.5 ± 0.5℃, count of the most probable number (MPN) of indicator organisms in the sample may be estimate by reference to statistical tables. The test gives *E. coli* count equivalent to faecal coliform count.

2.4.5.1 Confirmed test

A loopful of culture from a positive tube from the presumptive test was transferred into a tube of Brilliant Green Lactose Bile (BGLB) broth (Oxoid) with Durham tubes. The tubes were incubated at 37℃ for 24hrs for total coliforms and at 44.5 ± 0.5℃ for 48 h for faecal coliforms and observed for colour change and gas production.

2.4.5.2 Completed test

A loopful of broth from a positive tube was streaked onto Eosine Methylene Blue (EMB) agar plate for pure colonies. The plates were incubated at 37℃ for 24 h. Metallic sheen colonies on EMB agar, were presumptively considered to be *Escherichia coli* and their identities were confirmed using morphological and biochemical tests. For faecal coliforms, colonies with green metallic sheen were gram stained and the indole, methyl red, VogesProskaeur and citrate utilization (IMViC) test was carried out on Nutrient agar stock cultures and used to identity the colony as *E. coli.* The MPN per 100 ml water was calculated using presumptive test.

2.4.5.3 Identification of bacterial isolates

Stock culture of the isolates with different cultural characteristics was made on nutrient agar slants. Gram staining was used to check for morphology and biochemical tests were carried out to aid in identification. Various tests performed and used in probable identification of isolates included the Gram's staining procedure, oxidase test, motility test, catalase test, indole test as described by [13].

3. RESULTS

The result of the inventory as shown in Table 1 revealed over twenty (20) brands of water with NAFDAC registration numbers. Some are made within, while others are imported from neighbouring states.

The result of the physical observation of sachet water as revealed in Table 2 shows that, most brands of the sachet water analysed complied with the regulatory body's (NAFDAC) specification in terms of product name, manufacturers address, NAFDAC number and metric volume. Other requirements such as Batch number, manufacturers date, expiry date.

Table 3 shows that all the branded sachet water with NAFDAC number had zero coliform counts

per hundred ml 0/100 ml coliform while the control from a well water showed the maximum coliform count of 1800/100 ml.

Table 1. Inventory of sachet water sold in LafiaTown

S/No.	Name of brand	NAFDAC No:
1.	Kay Kay	C1 – 2843L
2.	Cimthog	C1 – 0621L
3.	Mejoi	01 – 50611L
4.	Piaans all star	01 – 3322L
5.	Kaura Global	C1 – 3864L
6.	Charlie	01 – 3594L
7.	All Grace	01 – 1426L
8.	Pajo	01 – 2753L
9.	Pasaha	B1 – 1222L
10.	Floxy	C1 – 0665L
11.	Alamari	01 – 2543L
12.	Mosmera	01 – 4286L
13.	Maidunama	B1 – 2363L
14.	Fambel	B1 – 2363L
15.	Afrique	01 – 1308L
16.	Safiy	C1 – 5791L
17.	Omolijo	C1 – 0626L
18.	ALB Gwarzo	A1 – 9603L
19.	Mailafiya	C1 – 0620L
20.	Baba	C1 – 3793L

The result of the Most Probable Number coliform count from the unbranded sachet water ranged from 45/100 ml to 550/100 ml.

The results obtained from the total bacterial count (Table 5) revealed that the unregistered sachet water had more bacterial and coliform count which ranged from 0.4 x 10^3 cfu/ml to 7.9x10^3cfu/ml while the registered water had none. Biochemical analysis as indicated in Table 6 revealed isolates of the coliform group including, *Klebsealla, Proteus, E. coli* and *Enterobacter.*

4. DISCUSSION

There is rapid increase in the population of Lafia Metropolis, arising from increase in insurgency in the far North Eastern part of the Country. This increase has placed pressure on existing water supply thus resulting in influx or proliferation of sachet water brands to meet demand. A research during this study shows that over twenty (20) brands of sachet water with NAFDAC number are sold in Lafia Metropolis as indicated in Table 1. Besides the above security reason, this proliferation may also be due to the weather condition with high temperature. This, thus increases the body temperature of residents with concomitant quest for water for drinking and rehydration as well as bathing regularly [14].

It was also observed that the manufacturers complied with basic standard requirements on provision of basic information such as NAFDAC numbers, manufacturing dates, expiry date and even batch number (Table 2) for quality assurance monitoring in case of any eventuality on quality. This compliance is probably due to proximity of Lafia Metropolis to the Federal Capital Territory Abuja, which carries out effective and regular monitoring.

The result of coliform counts in Table 3, revealed zero counts per hundred millilitre of water, 0/100 ml indicating high quality of the water, potability and safety of all the samples. This reveals that the sachet water brands with NAFDAC numbers are highly recommended for consumption and use for other domestic uses by residents. This fact could be the reason why there is low incidence of water borne diseases in the metropolis especially for those that patronize the sachet water with NAFDAC numbers.

Table 2. Physical examination of registered sachet water

Sample	NAFDAC no	Batch no	Manufacture date	Best before	Volume	Manufacturers address
A	+	+	+	+	50cl	+
B	+	+	+	+	50cl	+
C	+	+	+	+	50cl	+
D	+	+	+	+	50cl	+
E	+	+	+	+	50cl	+
F	+	+	+	+	50cl	+
G	+	+	+	+	50cl	+
H	+	+	+	+	50cl	+
I	+	+	+	+	50cl	+
J	+	+	+	+	50cl	+

Keys;+ =confidential

The results of coliform count of the sachet water without NAFDAC number as shown in Table 4 did not meet acceptable standards by Federal Ministry of Environment and World Health Organisation WHO. The sachet water coded L had the highest coliform count of 550/100 ml while M had the least coliform count. The presence of coliform counts in the unregistered sachet water could be due to poor sanitation habit by manufacturers, and faecal contaminants introduced from the environment. [9,6,1], reported on the implications of poor handling of water sample in diarrhoeal diseases especially among children.

The total heterotrophic bacterial count reveal the presence of bacterial isolates from the unregistered sachet water as against non in the registered. The implication of poor sanitary environment cannot be ruled out as evidenced in most areas within Lafia metropolis. It was also observed that the unregistered sachet water was sold at cheaper rate which attracts patronage from the low income earners and the poor masses who cannot afford the registered brands.

Biochemical analysis as indicated in Table 6 revealed isolates of the coliform group including, *Klebsiella, Proteus, E. coli* and *Enterobacter* which are in line with expected coliforms in contaminated water samples. These organisms have been implicated to be associated with gastrointestinal tract infections in human. Therefore, when consumed along with the contaminated water, they could pose serious health problems and diseases in humans

Table 3. Most probable number (MPN) result for the NAFDAC registered water

	10 ml	1 ml	0.1 ml	MPN form standard table (appendix)
A	0	0	0	0
B	0	0	0	0
C	0	0	0	0
D	0	0	0	0
E	0	0	0	0
F	0	0	0	0
G	0	0	0	0
H	0	0	0	0
I	0	0	0	0
J	0	0	0	0
Control (1) well water	5	5	5	1,800
Control (2) distilled water	0	0	0	0

Table 4. MPN result for the unregistered sachet water

	10 ml	1 ml	0.1 ml	MPN from standard table (appendix)
K	5	4	2	225/100 ml
L	5	5	2	550/100 ml
M	5	1	1	45/100 ml
N	5	4	5	325/100 ml
O	5	3	1	110/100 ml

Table 5. Result of total heterotrophic bacterial count. (THB) from unbranded sachet water

Sample	1st plate	2nd plate	Average cfu/ml	Total Cfu/ml
K	40	70	55×10^2 cfu/ml	5.5×10^3 cfu/ml
L	120	38	79×10^2 cfu/ml	7.9×10^3 cfu/ml
M	7	5	6×10^2 cfu/ml	0.6×10^3 cfu/ml
N	3	7	5×10^2 cfu/ml	0.5×10^3 cfu/ml
O	4	4	4×10^2 cfu/ml	0.4×10^3 cfu/ml

Table 6. Biochemical reaction pattern and characterization

	Gram reaction	Catalase	Methylated	Citrate	Vogues Prosekaver	Indo	Glucose	Lactose	Fructose	Motility	Probable Organisms
K	(-ve) rod	+	+	-	-	+	+	+	+	+	*Escherichia coli*
L	(-ve) rod	+	+	-	-	+	+	+	+	+	*Escherichia coli*
M	(-ve) rod	+	-	+	+	-	+	+	+	-	*Klebsiella species*
N	(-ve) rod	+	-	+	+	-	+	+	+	+	*Enterobacter species*
O	(-ve) rod	+	+	+	-	-	+	+	+	+	*Citrobacter species*

5. SUMMARY AND CONCLUSION

The increasing demand for water by individuals including those living in Lafia, Nasarawa State calls for the regular monitoring and assessment of the quality of water (NAFDAC registered and unregistered sachet water), which provide most of the drinking water for individual homes. Quality of water cannot be determined by mere inspection, hence necessity to combine both physical, chemical and bacteriological methods of analysis to determine the water quality. The result of the total coliform count per 100 ml in this study shows that the unregistered water is contaminated and unfit for consumption while the registered sachet water has zero coliform which conforms to the standard of drinking water by WHO and FME and there by fit for drinking. The reason for the zero coliform could be due to appropriate sources (boreholes), effective and efficient processing procedures as well as proper quality assurance practices. On the other hand the high bacteria count in the unregistered water sources could be due to their poor and unsanitary sources. In view of the outcome of this study, and the overriding importance of water to human's existence we strongly encourage residents of Lafia metropolis to patronise the NAFDAC approved sachet water sources as they have been assessed to meet WHO and FME standards of 0/100 ml coliform and refrain from the use of unregistered sachet water in view of their health implications.

6. RECOMMENDATION

To prevent the outbreak of waterborne disease and other water related diseases in Lafia metropolis we hereby make the following recommendations;

1. There should be regular inspection of drinking water supplies including onsite inspection of production companies and site of all sachet water sources and the frequent and regular bacteriological, physical and chemical testing of water samples.
2. Health education of the public, school children and teachers should be carried out regularly to explain the importance of clean water and the relationship which exist between water, health, sanitation and hygiene nature of activities to protect water supplies from faecal contamination, through television, radio and print media.
3. Residents of rural communities should be well educated and coordinated on their activities and proper management of their waste to protect water supplies from faecal contamination in their communities
4. Government and philanthropic individuals and groups should provide safe alternative sources of water such as boreholes to all communities to reduce patronage of unregistered sources of water.
5. Residents should be encouraged to boil drinking their water and if possible filter them before drinking.
6. Frequent microbiological examination should be carried out on all batches of water before distribution to adequately control the quality of water.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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