Bacteriological and Physicochemical Assessment of Selected Brands of Bottled Water in Ilorin, Nigeria

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Abstract

Twenty different brands of bottled water sold in Ilorin metropolis, Kwara State, Nigeria were examined for their bacteriological and physicochemical qualities. Bacteriological analyses were carried out to determine the counts of heterotrophic bacteria, total coliform and faecal coliform. Physicochemical qualities such as pH, chloride content, total hardness and total suspended solids were also examined. The heterotrophic bacterial count ranged from 0 to 121cfu/ml while the total coliform count ranged from 0 to 1100 coliform/100ml of the sample. There was absence of faecal coliform in the bottled water samples. The pH, chloride content, total hardness and total suspended solids ranged from 6.50 to 8.40, 3.55 to 4.79mg/l, 1 to 90mg/l, and 0.001 to 0.003 g/100ml respectively. The bacterial species isolated belong to the genera *Staphylococcus*, *Micrococcus* and *Aeromonas*. In terms of quality, 19 (95%), 18 (90%) and 0 (100%) of the 20 brands of bottled water samples met the standard for heterotrophic bacteria, total coliform, and faecal coliform respectively. All the bottled water samples conformed to the standard for pH, chloride content, total hardness and total suspended solids. It can be concluded from this study that 85% of the bottled waters were suitable for drinking with regard to bacteriological and physicochemical parameters tested. It is recommended that stringent measures should be adhered to in the manufacture of bottled water, the bottles should be properly capped, and information on the manufacturing date, expiry date and batch number should be written on the bottles for easy recall.

Keywords: Packaged water, Potable, Quality, Safety

1.0 Introduction

Safe and potable water supplies in urban areas in Nigeria are still inadequate inspite of decades of independence and several efforts from various governments. The standard model for delivery of safe drinking water through treated pipe borne water at municipal level is grossly inadequate in most the developing countries [1]. The public health significance of water quality cannot be overemphasized. Many infectious diseases are transmitted by water through the faecal-oral route. Diseases contacted through drinking water kill about 5 million children annually and make one-sixth of the world population sick [2]. Going by the renewed global commitments toward the Millennium Development Goals (MDGs) marked for 2015, the importance and contribution of locally sourced low-cost alternative drinking water schemes to achieve sustainable access in rural and urban settings of developing nations cannot be overemphasized.

Bottled water is readily available but not quite affordable to a vast majority of Nigerian population. Bottled water is sold all around the country and it differs in quality due to different manufacturers and this raises concern about the potability of some of these products. National Agency for Food, Drug Administration and Control (NAFDAC) took it upon itself to rid the country off unclean bottled and sachet water by asking the concerns to register with it, a measure aimed at monitoring the manufacturers' activities [3].

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Given the low access to safe drinking water and the hot weather in Nigeria, sachet and bottled water sales remained strong in 2015 [4]. A high percentage of households in Nigeria, in both urban and rural areas, do not have access to safe drinking water. It is estimated that at least 50% of the population purchase water daily [5].

The proliferation of bottled water in Nigeria calls for investigation into the activities of the producers in order to determine the extent of compliance with the laid down standards. Over the past one decade, the Federal Government of Nigeria has been spending so much on primary health care as many health personnels have expressed their concerns over the high incidence of deadly water borne diseases such as typhoid fever, diarrhea and measles [6]. For this reason, a few have adopted preventive measures by properly boiling and filtering drinking water in their homes. However, in some other homes that are fairly buoyant, a safer way of avoiding contact with these diseases is by drinking already bottled water. The product was introduced into the Nigerian market in order to provide safe drinking water devoid of water borne diseases. However, with the proliferation of companies producing bottled water, there is deviation from the intended goal of providing safe drinking water.

In view of high demand for safe and potable drinking water and also the emergence of small scale entrepreneurs in the bottled water production, this study was carried out to investigate the quality of bottled water. Therefore, the aims of this research were to determine the bacteriological and physicochemical qualities of bottled water; characterize and identify the bacterial isolates present in the bottled water; determine the occurrence of bacterial isolates in the bottled water; and suggest possible measures to improve the quality of bottled water sold in Ilorin metropolis, Nigeria.

2.0 Materials and Methods

2.1 Collection of Bottled Water Samples

Twenty different brands of bottled water were purchased from retail outlets in Ilorin and they were coded A to T in order to conceal the identity of the producers.

2.2 Bacteriological counts of bottled water samples

The heterotrophic bacterial count was determined using standard plate count (SPC). Nutrient agar was used for the cultivation of bacteria [7, 8]. The total coliform count was determined using the 3-3-3 regime of multiple tube fermentation. MacConkey broth was used for the cultivation. After incubation, the number of positive tube with acid and gas production were noted and reference was made to MPN index table in order to obtain the most probable number (MPN) of coliform per 100ml of the water sample [8]. Eosin methylene blue (EMB) agar was used to determine the faecal coliform (*E. coli*) of the water samples using spread plate technique. The number of typical colonies of *E. coli* were counted and expressed in cfu/ml [9].

2.3 Characterization and identification of bacterial isolates

The bacterial isolates were identified based on their colonial morphology, cellular characteristics and biochemical reactions [10, 11]. All colonies isolated were sub-cultured from the mixed culture to obtain pure culture of the isolates. The pure isolates were then transferred into sterile nutrient agar slants and stored in the refrigerator as stock cultures [8].

2.4 Determination of physicochemical parameters

2.4.1 Determination of pH and Total Suspended Solids

The pH of the bottled water samples was measured using a Denver pH meter model 20 which was standardized before use [8]. The total suspended solids was determined by taking note of difference in weight of pyrex beaker when a known volume of water was dried in an oven at 105^{o}C for 1 hour [7].

2.4.2 Determination of Total Chloride

The total chloride was determined by argentiometric titration using 0.1 N $AgNO_3$ to titrate 100 ml of the water sample with 2 ml of 5% potassium chromate as an indicator. At end point, the solution turned from yellow to faint pink [12].

2.4.3 Determination of Total Hardness

The total hardness was determined by complexometric titration using 0.1 N EDTA to titrate 100 ml of the water sample with Erichrome black-T as indicator. Ten drops of 25% ammonia was initially added to the water sample before the commencement of titration. The initial purple colour changed to light blue at end point [13, 14].

3.0 Results

3.1 Bacteriological Counts

The total heterotrophic bacterial counts of the bottled water ranged from 0 to 121 cfu/ml while the total coliform counts ranged from 0 to 1100 coliform/100ml. All the bottled water samples were devoid of faecal coliform (Table 1). About 95% of the bottled water samples selected for this study had heterotrophic bacterial counts less than 100 cfu/ml. In actual sense, 80% of the bottled water samples had heterotrophic bacterial counts not more than 10 cfu/ml.

Table 1: Bacteriological Counts of Bottled Water Samples

Bottled Water Sample	Heterotrophic Bacterial Count (cfu/ml)	Total Coliform (MPN/100ml)	Faecal Coliform (cfu/ml)
A	3	0	0
B	0	0	0
C	0	0	0
D	0	0	0
E	61	0	0
F	1	0	0
G	1	0	0
H	3	0	0
I	121	1100	0
J	14	0	0
K	3	0	0
L	2	0	0
M	5	240	0
N	51	3	0
O	0	0	0
P	0	0	0
Q	1	0	0
R	10	0	0
S	1	0	0
T	2	0	0

3.2 Bacteria Isolated from Bottled Water Samples

Four species of bacteria were characterized and identified in the selected bottled water samples namely *Staphylococcus* sp., *Micrococcus varians, Micrococcus* sp. and *Aeromonas* sp. (Table 2). Their occurrence in the bottled water samples is presented in Table 3.

3.3 Physicochemical Properties of Bottled Water Samples

The pH of the bottled water samples ranged from 6.40 to 8.40 while the total hardness ranged from 1 to 90 mg/l. The values of chloride content and total suspended solids ranged from 3.55 to 4.79 mg/l and 0.001 to 0.003 g/100ml respectively (Table 4).

Table 2: Characterization and Identification of Bacterial Isolates

Bacterial isolates	Gram reaction	Cell shape	Cell arrangement	Motility	Oxidase	Catalase	Coagulase	Arabinose	Starch hydrolysis	Citrate	Indole	Urease	Glucose	Lactose	Sucrose	Maltose	Mannitol	Raffinose	VP	Nitrate	Cellobiose	Trehalose	Fructose	OF	Probable identity
1	+	C	S	-	+	+	-	-	+	+	-	-	A	-	-	A	-	A	+	+	A	A	A	F	*Staphylococcus* sp.
2	+	C	CL	-	+	+	-	-	-	+	-	-	A	-	A	A	-	-	-	+	A	A	A	O	*Micrococcus varians*
3	+	C	CL	-	-	+	-	-	-	+	-	+	A	A	-	A	-	-	+	-	-	A	-	O	*Micrococcus* sp.
4	-	R	CH	+	+	+	-	-	+	+	-	-	A	-	-	A	-	-	-	+	A	A	A	F	*Aeromonas* sp.

Key: – = Negative reaction; + = Positive reaction; C= Cocci; R= Rod; S= Single; CL = Cluster; CH = Chain;

F = Fermentative; O = Oxidative; OF = Oxidation-Fermentation; VP = Voges proskauer; A= Acid production

Table 3: Occurrence of the Bacterial Isolates in the Bottled Water Samples

Bottled water samples	B1	B2	B3	B4
A	+	+	-	-
B	-	-	-	-
C	-	-	-	-
D	-	-	-	-
E	+	+	-	-
F	-	+	-	-
G	-	+	-	-
H	-	+	-	-
I	+	+	+	-
J	-	+	+	+
K	-	-	+	-
L	-	+	-	-
M	+	-	+	+
N	+	+	+	-
O	-	-	-	-
P	-	-	-	-
Q	-	-	+	-
R	+	-	-	+
S	+	-	-	-
T	-	+	-	-

Key: + = isolated; – = not isolated; B1 = *Staphylococcus* sp.; B2 = *Micrococcus varians*;

B3 = *Micrococcus* sp.; B4 = *Aeromonas* sp.

Table 4: Physicochemical Qualities of Bottled Water Samples

Bottled water samples	pH	Total Hardness (mg/l)	Chloride content (mg/l)	Total suspended Solid (g/100ml)
A	6.70	35	4.26	0.001
B	6.50	15	4.62	0.001
C	6.70	10	3.83	0.002
D	6.50	13	4.79	0.001
E	6.80	17	3.55	0.003
F	6.50	7	4.26	0.001
G	6.60	15	3.91	0.001
H	7.50	90	4.79	0.002
I	7.50	8	4.79	0.003
J	7.60	1	3.94	0.003
K	8.40	5	4.19	0.001
L	8.30	8	3.91	0.001
M	7.50	4	4.40	0.002
N	7.90	9	3.91	0.002
O	7.5	11	4.62	0.003
P	7.20	15	3.91	0.001
Q	7.90	2	3.87	0.002
R	7.50	4	3.73	0.001
S	7.40	22	4.62	0.002
T	7.60	2	3.91	0.003

4.0 Discussion

The importance of good and safe drinking water cannot be overemphasized as regard the health of the population. The advent of bottled water has greatly reduced the occurrence of some water borne related diseases such as cholera, typhoid and dysentery. All the manufacturers were able to adhere to zero faecal coliform count in their products. Furthermore, 18 brands of the bottled water (90%) met the maximum limit of 10 coliform per 100 ml of water allowed in potable water [15]. However, 17 out of the 20 brands of bottled water representing 85% had zero total coliform count recommended for water to be used under emergency situation [16]. The bacteria presence in the bottled water could have been derived from equipment used in production, improper capping of the bottled water, and post-production contamination during distribution and sales.

The loads of heterotrophic bacteria, total coliform as well as the diversity of bacterial species obtained in this study were far lesser than those reported in sachet water by many researchers. Bukar *et al*. [17] reported heterotrophic bacterial count in the range of 1.54 x 10^4 to 1.96 x 10^4 cfu/ml in 5 brands of sachet water sold in Maiduguri metropolis. These values are higher than the highest heterotrophic bacterial count of 121 cfu/ml obtained in a brand of the bottled water in this study. Aerobic bacterial counts which ranged from 0–800 cfu/ml were obtained in 22 brands of bottled water sold in Jaffna Peninsula [18]. Ugochukwu *et al*. [4] reported the isolation of *Klebsiella*, *Proteus*, *Pseudomonas* sp. and *Chromobacterium violaceum* from sachet water sold in Samaru-Zaria, Kaduna, Nigeria. Similarly, a variety of bacteria including *E. coli, Streptococcus faecalis, Bacillus subtilis, Staphylococcus* spp., *Pseudomonas aeruginosa, Klebsiella* spp. and *Salmonella typhi* were isolated in sachet water from North Central Nigeria [19]. Igbeneghu and Lamikanra [20] isolated coliform, *Staphylococcus* and *Pseudomonas* in different brands of bottled water sold in Ile-Ife, South Western, Nigeria. Furthermore, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa* and *Pasteurella haemolyticus* were isolated from non-carbonated bottled drinking water sold in Sri Lanka [21]. However, in this study, the three genera of bacteria isolated namely *Staphylococcus, Micrococcus* and *Aeromonas* indicate that bottled water is less contaminated when compared with sachet water. *Staphylococci* and *Micrococci* are known to be commensals and they can be found on human skin surface, soil etc. Hence, they dominated the bacterial species isolated from bottled water samples in this study. *Aeromonas* species can be found in water and soils [22].

pH play an important role in the survival of microorganisms in water. The pH values of bottled water samples selected for this study were within the range of 6.5 to 8.5 allowed in potable water [15]. pH values which ranged from 4.11 to 7.58 were obtained in 22 brands of bottled water sold in Jaffna Peninsula [18]. The total suspended solids in the bottled water conformed to the limit of 30 mg/l (which is equivalent to 0.003 g/100ml allowed in domestic water [23]. The acceptable chloride content according to WHO is 250 mg/l [16]. The chloride content of all the samples was quite well below this limit. Furthermore, 19 of the 20 brands of bottled water used in this study representing 95% can be described as being soft water since their total hardness is less than 50 mg/l [13].

5.0 Recommendation and Conclusion

Government should intensify effort in monitoring the rapidly expanding water bottling industries with a view to supplying potable and wholesome water to the public. Manufacturers should strictly adhere to all rules of good manufacturing practices (GMP) during production processes. Manufacturers should also ensure that bottled water is appropriately labeled with valid manufacturing date, expiry date and batch number, and the bottles should be properly capped to prevent contamination. The populace should be adequately informed by the regulatory bodies if any batch of product failed regulatory standards. In such cases, the product should be recalled from the market.

It can be concluded from this study that eighteen out of the twenty brands of the bottled water samples representing 90% conformed to laid down standards for drinking water qualities in term of bacteriological and physicochemical characteristics tested. Hence, majority of bottled water sold in Ilorin metropolis are potable.

References

[1] Dada, A. and Ntukekpo, D.S. (1997). “Pure water”; How safe? Ultimate Water Technology and Environment, Vol. 1, No. 3, pp. 8-11.

[2] WHO (2004). Water Sanitation and Health Programme. Managing Water in the Home: Accelerated Health Gains from Improved Water Sources. Geneva, Switzerland.

[3] NAFDAC Act (2011). National Agency for Food, Drug Administration and Control. Bottled and Sachet Water Quality Regulatory Standards. Cap N1, LFN and CapF33 LFN.

[4] Ugochukwu, S., Giwa, F.I. and Giwa, A. (2017). Bacteriological evaluation of sampled sachet water sold in Samaru-Zaria, Kaduna State, Nigeria. Nigerian Journal of Basic and Clinical Sciences, Vol. 12, No.1, pp. 6-12.

[5] Olayemi, A.B. (1999). Microbial Potability of Bottled and Packaged Drinking Waters Hawked in Ilorin Metropolis. International Journal of Environmental Health Research, Vol. 9, No. 3, pp. 245 - 248.

[6] Moremi, I.R. (2012). Challenges of Infectious Diseases in Africa. Journal of Medical Sciences, Vol. 6, No. 3, pp. 9-12.

[7] American Public Health Association (APHA) (1998). Standard Methods for the Examination of water and Waste Water. 20th edition. Prepared and Jointly published by APHA, AWWA and WPCF, Washington DC, USA.

[8] Fawole, M.O. and Oso, B.A. (2007). Laboratory Manual of Microbiology. 4th ed., Spectrum Books Limited Ibadan, Nigeria. pp. 15-35.

[9] Aneja, K.R. (2014). Laboratory Manual of Microbiology and Biotechnology. 1st ed. Published by MedTech Scientific International PVT. Ltd., New Delhi, India, pp. 120 – 121.

[10] Collins, C.H. and Lyne P.M. (1970). Microbiological Methods. Butterworth London, University Park Press, Baltimore, pp. 164 – 311.

[11] Cowan, S.T. and Steel, K.J. (1985). Manual for the Identification of Medical Bacteria. 4th ed., Cambridge University Press, London, pp. 1– 217.

[12] Sule, I.O., Odebisi-Omokanye, M.B., Gambari-Ambali, R.O. and Okewale, T.A. (2016). Effects of 'Disinfectant A' on the Physicochemical and Bacteriological Quality of some Well Water. Journal of Science, Technology, Mathematics and Education, Vol. 12, No. 1, pp.1 – 8.

[13] De Zuane, J. (1997). Handbook of Drinking Water Quality, 2nd ed., John Wiley and Sons, Inc., New York, pp. 30–35.

[14] Hammer M.J. and Hammer M. Jr. (2003). Water and Wastewater Technology, 4th ed., Prentice Hall, New Delhi, India. pp. 536.

[15] SON (2007). Standards Organization of Nigeria. Nigerian Standards for Drinking Water Quality. Nigerian Industrial Standard, NIS 554: pp. 15 - 21.

[16] WHO (2011). Guidelines for Drinking Water Quality, 4th ed., Geneva, Switzerland. http://www.who.int

[17] Bukar, A.M., Isa, M.A., Mustapha, A., Kyari M.Z. and Ibrahim, F.K. (2015). Bacteriological Analysis of Sachet Water in Maiduguri Metropolis. The Journal of Applied Sciences Research, Vol. 2, No. 1, pp. 20–25.

[18] Sasikaran, S., Sritharan, K., Balakumar, S. and Arasarahman, V. (2012). Physical, Chemical and Microbial Analysis of Bottled Drinking Water. Ceylon Medical Journal, Vol. 57, No. 3, pp. 111-116.

[19] Omalu, I.C.J., Mohammed, A.Z., Olamide, P.I., Ayanwale, V.A., Adeniran, I. and Gbise, S. (2012). Bacteriological and Physicochemical Analysis of Sachet Water in North Central Nigeria. Journal of Pharmaceutical and Biomedical Sciences, Vol. 21, No. 2, pp. 1– 4.

[20] Igbeneghu, O.A. and Lamikanra, A. (2014). The Bacteriological Quality of Different Brands of Bottled Water Available to Consumers in Ile-Ife, South Western, Nigeria. BioMed Central Research Notes, Vol. 7, pp. 847 – 859.

[21] Herath, A.T., Abayasekara, C.L., Chandrajith R. and Adikaram N.K. (2012). Temporal Variation of Microbiological and Chemical Quality of Non-carbonated Bottled Drinking Water Sold in Sri Lanka. Journal of Food Science, Vol. 77, No. 3, pp. 160 – 164.

[22] Willey, J.M., Sherwood, L.M. and Woolverton, C.J. (2008). Prescott, Harley and Kleins, Microbiology, 7th edn., McGraw Hill, New York, pp. 471– 589.

[23] Ojosipe B.A. (2007). Parameters in Water and Wastewater Analysis, 6th Mandatory Training Workshop with Theme: Water. Institute of Public Analysts of Nigeria (IPAN), Lagos, pp. 29.