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Original Article

BACTERIOLOGICAL AND PHYSICOCHEMICAL ASSESSMENT OF PACKAGED WATER SOLD IN MINNA, NIGER STATE, NIGERIA

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ABSTRACT

Ten (10) different brands of packaged water samples from different locations in Minna, Niger State were evaluated for their bacteriological and physicochemical properties. The samples were subjected to bacteriological assessment using standard spread plate method and MPN technique. The viable bacteria counts for all the packaged water sampled ranged from 0 - 2.8 x10^8 cfu/mL, while the occurrence of coliform ranged from 0 - 150MPN/100 mL. No organism was detected from one of the brands. The physicochemical assessment showed that there was no significant difference in the levels of biochemical oxygen demand (BOD_5) and temperature of the different brands. However, there was significant difference in the levels of other physicochemical parameters (pH, Dissolved oxygen, Turbidity, Chloride, Sodium, Copper, and Nitrate) tested and in the microbial counts of the different packaged water sampled. The physicochemical analysis revealed that the water were within the acceptable standard for potable drinking water while the microbial counts exceeded the permissible limit. The bacteria isolated were identified as *Klebsiella* sp and *Echerichia coli*. The presence of these organisms in the packaged water could be hazardous to public health. This suggests that the packaged water are unsafe for consumption hence the need for strict monitoring by the appropriate regulatory body.

Key words: Assessment, Bacteriological, Packaged water, Physicochemical

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INTRODUCTION

Quality water is very essential for the well-being of all people, unfortunately in some countries around the world, including Nigeria; some water supplies have become contaminated and have impacted on the health and economic status of the people. Contaminants such as microorganisms, heavy metals, and salts have found their way into water supplies as a result of inadequate treatment and disposal of waste, industrial discharge and over-use of limited water resources (Atlas, 1995).
Water is the most important resource for humans as it is essential for hydration and therefore, for life. Studies have shown that keeping one's self properly hydrated helps reduce the risk of having Type II diabetes (Aramas and Sutherland, 1999). Water forms 50 to 60% in weight of our body and plays an active role in all the vital processes of the body. It allows digestion, food elaboration, and waste elimination. Humans can survive for several weeks without food, but for only a few days without water. The exact amount of water a human need is highly individualistic, as it depends on the condition of the subject, the amount of physical exercise, and on the environmental temperature and humidity (Chetna*et al.,* 2006).

It has been estimated that approximately 17% of the world's population uses water from unprotected and remote sources, 32% from some form of protected source and 51% from some sort of centralized (piped) system to the dwelling or plot; of the latter, a small but increasing proportion applies some form of treatment within the home (Gadgil and Derby, 2003). The chemical quality of water has become an increasing cause of concern globally especially in industrialized nation and urban cities. This is due to environmental pollution from toxic chemicals and wastes from industries, insecticides, smokes from machineries, inadequate sewage, and waste disposals. Consumers cannot by themselves ascertain the quality of water either for drinking purpose or otherwise. Naturally, water that appears dirty, discolored, smelly or with unpleasant taste will be treated with grave suspicion by consumers, thus causing them to find an alternative (Kassenga, 2007).

In Minna, most people rely on package water to meet their daily water needs especially for drinking not minding its chemical constituents whether or not it has microbial contaminants. To this end, this study was carried out with a view to ascertain the microbial load present in some of the packaged water sold in Minna, Niger state, Nigeria.

MATERIALS AND METHODS

Ten (10) brands of packaged water samples were collected from different locations in Minna metropolis

Media used

All media used were of analytical grade and prepared according to the manufacturer's instructions.

Total viable heterotrophic counts

The populations of microorganisms in the packaged water sample were enumerated using standard spread plate method (APHA, 2005). The packaged water

sample was well shaken to homogenized suspension and thereafter, ten-fold (10-fold) serial dilution was made by aseptically transferring one milliliter (1 mL) of the homogenized suspension into a sterile test tubes containing nine milliliter (9 mL) of sterile, distilled water (deionized water). This gave ten times dilution. Subsequent dilutions were made from the aforementioned dilution. Then, using a sterile pipette, 0.1 mL aliquots of the dilutions were aseptically removed with a sterile pipette and separately spread plated with flamed-sterilized glass spreader (bent glass rod) on well-dried nutrient agar (NA), for bacteria in triplicates for the enumeration of viable heterotrophic bacteria counts. The plates were inoculated on the surface using the standard spread plate technique (APHA, 2005). The plates were allowed to remain undisturbed for 25 minutes in the laminar flow before being inverted and incubated. The culture plates were incubated at 37°C for 24-48 hours (APHA, 2005). Three uninoculated plates were used as control. After incubation, plates that contained 30-300 colony forming units (cfu) were selected and counted with the aid of a colony counter. Viable numbers of colonies on each plate were enumerated and expressed or recorded as colony forming units per milliliter (cfu/mL) of the sample. Colonies were purified by repeatedly subcultured aseptically onto fresh NA and incubated at 37°C for 48 hours to obtain discrete pure colonies. Pure colonies were then stored on NA slants at 8°C to maintain viability for subsequent analysis and identification. Gram staining was performed for all the isolates.

Most probable number (MPN) method

The most probable number (MPN) method was used to determine coliform counts of the packaged water samples using three tests: the presumptive, confirmed, and completed test.

Presumptive test

Nine test tubes were set up for each sample both single and double strength. Ten milliliters (10 mL) of lactose broth was transferred to the test tubes and Durham tubes were introduced in an inverted position, sterilized at 121°C for 15 minutes. Ten milliliters (10 mL) of water sample was added to the double strength, 1.0 mL and 0.1 mL of water sample to single strength tubes, follows by incubation at 37°C for 24 to 48 hours. Positive tubes were sub-cultured to Macconkey agar for confirmed test.

Confirmed test

Macconkey agar medium was prepared and sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool and thereafter dispense into sterile Petri dishes and allowed to solidify. Positive tubes of presumptive test were sub cultured on the Macconkey agar individually using streaking method and incubating at 37°C for 24 hours to obtain discrete colonies.

Completed test

Nutrient agar medium was prepared and dispensed into sterile slant bottles based on the number of discrete colonies (pure culture) obtained. The slant were sterilized by autoclaving at 121°C for 15 minutes, nutrient agar slant was made by slanting the bottles at 40°C to gel, using the sterile wireloop, discrete colonies was picked individually (pure culture) and sub cultured unto agar slant bottles. The pure isolates were Gram stain and further biochemical tests were carried out to identify the isolates.

Characterization and identification of the bacterial isolates

The characterization and identification of the bacterial isolates were carried out based on cell morphology, Gram's reaction and other biochemical tests according to the methods described by Nester *et al.* (2007). The isolates were identified by comparing with those of known taxa using the schemes of Cowan and Steel (1973).

Physicochemical properties of the water samples

All physicochemical parameters of the packaged water sample were determined in accordance with the standard methods published by American Public Health Association (APHA, 2005).The basic parameters that were analysed for the packaged water sample are as follows: Temperature, pH, dissolved oxygen, turbidity, chloride, Sodium, biochemical oxygen demand (BOD_5), copper, and nitrate.

RESULTS

The results of the study revealed that all brands analysed had total viable counts ranging from 0 - $2.8x10^8$cfu/mL.(Table 1). There was significant difference in the total viable counts of thevarious packaged water samples analysed.

Table 1 Total viable count for all the different locations

Sample	Cfu/mL
P1	0^h
P2	$2.8x10^{8a}$
P3	$1.48x10^{8c}$
P4	$2.0x10^{8b}$
P5	$8.0x10^{7e}$
P6	$1.2x10^{8d}$
P7	$2.8x10^{7a}$
P8	$1.2x10^{8d}$
P9	$4.0x10^{7f}$
P10	$8.0x10^{7e}$

Values (a,b,c,d,e,f) on the same column with different superscript are significantly different ($p< 0.05$) while those with same superscript are not significantly different ($p>0.05$).

The results of the coliform counts of the different brands are presented in Table 2.It was revealed that all the brands except P1 had coliform present in the water.

Table 2: Occurrence of coliform in various packaged water sample

Sample	MPN/100mL
P1	0^{a}
P2	150^{e}
P3	23^{d}
P4	23^{d}
P5	75^{b}
P6	28^{c}
P7	23^{d}
P8	15^{e}
P9	23^{d}
P10	9^{f}

Values (a,b,c,d,e,f) on the same column with different superscript are significantly different ($p< 0.05$) while those with same superscript are not significantly different ($p>0.05$).
The bacteria isolated were identified as *Klebsiella* sp and *Echerichia coli* from all the various packaged water samples (Table 3).

Table 3: Bacterial isolates from the various samples of packaged water

SAMPLE	ORGANISM ISOLATED
P1	No organism detected
P2	*Escherichia coli and Klebsiella* sp
P3	*Escherichia coli and Klebsiella* sp
P4	*Escherichia coli*
P5	*Escherichia coli*
P6	*Klebsiella* sp
P7	*Escherichia coli*
P8	*Escherichia coli*
P9	*Escherichia coli*
P10	*Klebsiella* sp

The results from the study showed that the temperature, pH, dissolved oxygen, and turbidity from all brands ranged from 25.2-25.4, 2.9-8.7, 2.58-3.35 and 0.32 -1.04 respectively. There was significant difference in the values of pH, dissolved oxygen and turbidity for the sample as shown (Table 4).

Table 4: Physicochemical properties of the various packaged water

Sample	T(°C)	pH	DO (mg/L)	Turbidity (NTU)
P1	25.4^{a}	7.7^{c}	2.64^{b}	1.04^{b}
P2	25.4^{a}	7.8^{b}	2.65^{a}	1.03^{c}
P3	25.4^{a}	2.9^{d}	2.63^{c}	0.60^{d}
P4	25.3^{a}	7.9^{a}	2.59^{e}	0.51^{f}
P5	25.3^{a}	7.8^{b}	2.58^{e}	0.52^{f}
P6	25.3^{a}	8.3^{b}	3.35^{a}	0.40^{e}
P7	25.2^{a}	8.2^{c}	7.80^{f}	0.32^{b}
P8	25.2^{a}	7.8^{d}	2.70^{e}	0.42^{e}
P9	25.2^{a}	7.7^{e}	2.73^{d}	0.40^{e}
P10	25.2^{a}	8.7^{a}	3.09^{b}	0.41^{e}

Values (a,b,c,d,e,f) on the same column with different superscript are significantly different ($p< 0.05$) while those with same superscript are not significantly different ($p>0.05$).

The results from the study shows that the chloride, sodium, BOD_5, copper, and nitrate from all the brands ranged from 8-23, 5.92-51.33, 0, 0-0.21, and 0-21.2 respectively. There was significant difference in the values of chloride, sodium, copper and nitrate for the sample as shown (Table 5).

Table 5: Physicochemical properties of the various packaged water

Sample	Chloride	Sodium	BOD_5	Copper	Nitrate
P1	23^{b}	51.33^{b}	0^{a}	0^{d}	21.2^{b}
P2	12^{c}	6.91^{c}	0^{a}	0.06^{c}	5.7^{d}
P3	8^{a}	51.33^{b}	0^{a}	0^{d}	0^{f}
P4	8^{a}	6.91^{c}	0^{a}	0.10^{b}	5.9^{c}
P5	9^{d}	6.91^{c}	0^{a}	0^{d}	3.0^{e}
P6	10^{d}	5.92^{e}	0^{a}	0.02^{e}	2.0^{d}
P7	14^{b}	14.81^{b}	0^{a}	0.21^{b}	7.1^{b}
P8	10^{d}	7.90^{c}	0^{a}	0^{f}	0^{f}
P9	11^{c}	6.91^{d}	0^{a}	0.20^{c}	1.5^{e}
P10	9^{e}	7.90^{c}	0^{a}	0.09^{d}	5.2^{c}

Values (a,b,c,d,e,f) on the same column with different superscript are significantly different ($p< 0.05$) while those with same superscript are not significantly different ($p>0.05$).
(All values in mg/L)

DISCUSSION

The study revealed that all the packaged water sample collected from different locations in Minna, Niger State, Nigeria were contaminated with bacteria except one (P1). The bacteria identified were *Klebsiella* sp. and *Escherichia c*oli. This agrees with the work of Nwadozie (2000) who reported the presence of *Klebsiella* sp and *E.coli* in water samples. The data obtained were subjected to one-way analysis of variance (ANOVA) ($p< 0.05$) which showed significant difference in the microbial counts of the various water sampled. This agrees with the work of Bala (2006) who reported a similar result on occurrence of coliform in well and tap

water in Jimeta-Yola, Nigeria. Data obtained from physicochemical analysis of the packaged water were subjected to one-way analysis of variance (ANOVA) ($p<0.05$) which showed that there were si gnificant difference in the levels of chloride, sodium, nitrate, copper, dissolved oxygen, pH, turbidity and no significant differences in BOD_5 and temperature. The presence of these organisms is attributed to poor hygienic practices during production as reported by Odukoya (2000). In the present study, the presence of coliform especially *Escherichia coli* in the packaged water samples is an indication of faecal contamination and this is in conformity with the work of Bala (2006). Water contaminated with human waste always contains coliforms and it is also likely to contain pathogens excreted by infected individuals in the community. Coliforms are used as indicator organisms for measuring biological quality of water. The coliform count thus reflects the chance of pathogens being present (Oyeku *et a*l., 2001). The physicochemical qualities of the packaged water samples revealed that the physicochemical properties measured were within the acceptable standard set by the World Health Organization (WHO) (2004) and National Agency for Food and Drug Administration and Control (NAFDAC) (2002), while the microbiological standard for potable water quality state that no organism should be present. This agrees with the present study which was evident only with P1 packaged water that has no organism isolated. The presence of these organisms in the other packaged water could generally be attributed to unhygienic practices during production, packaging, distribution, and they could cause a number of diseases such as diarrhea, cholera, dysentery etc. Several studies have shown that poor hygienic practice and lack of awareness about these organisms among people are major contributing factors to high rate of the diseases (Lamka *et al*., 1980; Mark *et al*., 1996).

CONCLUSION

The result obtained from bacteriological quality of some packaged water sold in Minna, Niger state, Nigeria revealed that the packaged water contains microbes. Therefore the water may not be suitable for human consumption while the physicochemical properties measured were within the acceptable standard. Thus it is recommended that the packaged water should be adequately treated and well processed. Good hygienic practice should be ensured during its production. Bacteriological and physicochemical analysis of packaged water should be carried out as a routine practice during the production process. The inspectorate division of NAFDAC in Niger State should enforce compliance with regulation of the agency in order to safe guard the populace from preventable diseases.

REFERENCES

APHA. (2005). Standard Methods for the Examination of Water and Wastewater. 21st edn, American Public Health Association (APHA), Washington, DC.

Aramas, A.B. and Sutherland, J.P. (1999). Survey of microbiological quality of bottle water sold In UK and changes occurring during storage. *International Journal of Food Microbiology*, 48(1):59-65.

Atlas, R. M. (1995). *Microorganisms in our World.* Mosby St. Louis publishers, United States of America, pp. 752-753.

Bala, J.D. (2006). Occurrence of faecal coliforms in water sources in Jimeta-Yola Nigeria. *Journal of Environmental Sciences,* 10(2):64 – 69.

Chetna, A., Pratima, A. and Rina, C. (2006). Bacteriological and water quality status of rivers in Yamuna, Delhi. *Journal of Environmenatal Biology,* 27(1): 97 – 10.

Cowan, S. T. and Steel, K.J.(1973). *Manual for the identification of medical bacteria*. 1sted, Cambridge university press, London, pp.125-161.

Gadgil, A,J. and Derby, E.A. (2003). Providing safe drinking water to 1.2 billion unserved people. Paper # 70492 presented at the 96th Annual AWMA conference, San Diego, CA June 22-26, 2003.

Kassenga, G.R. (2007). The health related microbiological quality of botted drinking water sold in Dares Salaam, *Tanzania Journal of Water Health*, 5(1): 179 – 185.

Lamka, K.G., lechevallier, M.W. andSeidler, R.J. (1980). Bacterial contamination of drinking water supplies in modern rural neigbourhood. *Applied Environmental Microbiology*, 39:734-738.

Mark, W.L., Welch, J. N. and Smith, D.B. (1996). Full-Scale Studies of Factors Related to Coliform Regrowth in Drinking Water, *Applied and Environmental Microbiology*, 62 (7):2201-2211.

National Agency for Food and Drug Administration and Control (NAFDAC) (2002).Guidelines for registration and production of packaged water in Nigeria. Available online at www.nafdac.com Accessed on 2nd September, 2012.

Nester, W.E., Denise, G.A., Evans, C.R. Nancy, N.P. and David, H. (2007). *Microbiology Human Perspective* Fourth Edition McGraw hill Publishers, New York, pp. 786-796.

Nwadozie, J. (2000). A case study of water effluent parameter of some industries in Kaduna, *Journal of Environmental studies,* 1 (1): 84 – 89.

Odukoya, O.O. (2000). Pollution trend in Ogun river, Abeokuta, Nigeria, *Nigeria Journal of Science*, 34 (2):183 – 186.

Oyeku, O.M., Omowumi, O.J., Kupoluyi, F. and Toye, O. (2001). Wholesomeness of water produced and sold in plastic sachets (pure water) in Lagos metropolis, *Nigerian Food Journal,* 17(1): 63-69.

World Health Organization.(WHO) (2004). Guidelines for drinking water qualities. Available online at *www.who.com* Accessed on 2nd September, 2012.