BACTERIOLOGICAL ANALYSIS OF PACKAGED WATER SOLD IN MAIDUGURI METROPOLIS

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ABSTRACT

INTRODUCTION: Continuous increase in the sale and indiscriminate consumption of packaged drinking water in Nigeria is of public health significance.

OBJECTIVES: This study was designed to investigate the bacteriological quality of packaged water marked in Maiduguri metropolis Borno state, with emphasis on the incidence of pathogenic bacteria in the water sample.

METHODS: Standard microbiological procedures which include;physical parameters of the samples, multiple tube fermentation method (Most Probable Number),Eijkman test, and Biochemical tests were used to investigate the incidence of pathogenic bacteria such as *Escherichia coli, Pseudomonas aeruginosa* and indicator faecal coliforms.

RESULTS: In the study, Fifty Four (54) water samples were examined, Forty-Six (85%) were sachet water while Eight (14.8%) were bottle water. Different species of bacteria isolated from the 46 sachet water samples are; thirteen (13) *Escherichia coli,* Six (6) *Klebsiella* spp. Eight (8) *Proteus* spp. Five (5) Coliform spp, and Three (3) *Pseudomonas aeruginosa.* While bottled water contain one (1) *Pseudomonas aeruginosa.*

CONCLUSION: These findings indicate that 66.6% of packaged water sold in Maiduguri is either improperly treated or are produced under unhygienic condition, making them unsuitable 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 and bottled water produced and sold in Maiduguri.

INTRODUCTION

Water is an essential requirement for all life forms. Satisfactory supply of clean, safe, and hygienic drinking water is imperative for health (Kalpana *etal.*, 2014). In the olden days no settlements could be made in the absence of good and regular source of water (in most cases, rivers, streams, lakes, and springs supplemented with harvested rain water). These are adequately protected against contamination either by man or animals. In towns and cities, wells, bore-holes and pipe-borne water are the major sources of potable water. As the populations increased, the available sources of water became inadequate. This necessitated the source for alternate sources of water among which is the introduction of packaged waters of various brands including bottled and sachets (Kalpana *et al*., 2014).Bottled water is defined as water that is intended for human consumption, packaged in bottles or other similar containers (mostly plastic material) with no added ingredient except some safe quantity of fluorides. Small scale entrepreneurs introduced small nylon sachets which are electrically sealed at both ends to the market, and is popularly called “pure” water (Oluyege *et al.,* 2014). Members of the low socio-economic class are the ones who often patronize this.

Most sachet water manufactures in Maiduguri obtain their raw water mostly from local and municipal piped water, hence adherence to production and analytical standards are doubtful as most of the factories are observed to lack appropriate technology for achieving these (Oyedeji *et al*., 2010).

National Food and Drug Administration and Control (NAFDAC) carried out surveillance between 2004 and 2005 which revealed that some producers of packaged water indulged 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 packaged water for distribution and sale without date marking (Edema and Atayese, 2010).

Because of the metropolitan nature of Maiduguri, several brands of sachet and bottled waters are sold (Muhammad, 2011). The demand for these has increased over the years due to non-availability of reliable safe municipal water. This has given the impression that most bottled waters are safe for human consumption (Gardner, 2004). The continuous proliferation of these packaged water products and their indiscriminate consumption are of concern to public health (Muazu *et al*., 2012).

Unsafe water is a global public health threat, placing persons at risks of diseases as well as chemical intoxication. The high prevalence of diarrhea among children and infants can be traced to the use of unsafe water and unhygienic practices (Hugles and Koplan, 2005). In developing countries, 80% of diseases and over 30% of deaths are related to contaminated water (Onweluzo, and Akuagbazie, 2010). 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 the presence of pathogenic organisms, including; Salmonella, Shigella, Campylobacter and Vibrio cholera.

Water is an essential ingredient of life; therefore it is of paramount importance that it is safe for human consumption. Water borne diseases continue to be a major problem of developing countries as a result of inadequate sanitation and unhygienic practices that account for the major sources of microbial contamination of potable water (Sahota, 2005).

Many people are involved in the production and sale of sachets and bottled waters. Less care is given to the environmental hygienic practices where these waters are prepared which is of great concern to the public health.

This study was therefore designed to investigate the bacterial quality of sachets and bottled water sold in Maiduguri metropolis.The study when completed will provide evidence-based information of bacteriological analysis of sachets and bottled waters sold in Maiduguri. In addition it will attempt to provide information on safety measures to be adopted by producers of packaged waters.

MATERIALS AND METHODS

Sachet and Bottled waters:

Fifty (54) different brands of water (both bottled and sachets), with NAFDAC registration numbers were purchased from outdoor vendors (hawkers) and retail shops, labelled appropriately and transported to the Microbiology Laboratory, University of Maiduguri Teaching Hospital for subsequent analysis.

Methods

Physical Parameters of the Samples

Samples were observed visually; the label, presence of certification number and other product information. Macroscopic examination of the waters was also carried out to check for specific odour and (colour, turbidity and presence of floating particles or extraneous materials.

Multiple Tube Fermentation Method (Most Probable Number)

50ml, 10ml and 1ml of water samples were added to 1, 5 and 5 flasks containing 50ml, 10ml double strength broth, and 5ml single strength broth respectively and incubated at 37 degree Celsius for 24-48 hours. The media receiving one or more of the indicator bacteria shows growth with a characteristic colour change, which are absent in those receiving an inocula of water without indicator bacteria.

From the number and distribution of positive and negative reactions, the most probable number of indicator organisms in the sample is estimated by reference to McCrady's table (Mackie *et al*., 1996)

Procedure

For each sample, a 50ml (x1), 10ml (x5) of MacConkey broth at double strength concentration and 5ml (x5) volumes of single strength concentration, were each put into appropriate glass test tubes containing inverted Durham tubes and sterilized by autoclaving at 121 degree Celsius for 15 minutes.

Bottles containing water sample were inverted severally by shaking to mix

One 50ml volume, five 10ml volumes of the water were aseptically pipette into corresponding bottles of double strength medium and 1ml volumes of water into corresponding single strength medium tubes.

The seeded medium was incubated aerobically at 37 degree Celsius at 24-48 hours.

Bottles showing gas in Durham tubes and acid production (colour change) are considered "presumptive positive" while cultures negative of acid and gas were further re-incubated at 44◦C for further 24-48 hours.

Positive results were read using McCrady's probability table and a loopful of the contents of each tube was sub cultured into MacConkey Agar plates, incubated at 37◦C for 24 hours.

Confirmed coliform count test was carried out for identification of Escherichia coli using the Eijkman's test.

Eijkman Test

This test is usually done to confirm that the coliform bacilli detected in the presumptive coliform test are Escherichia coli as some spore-bearing bacteria give false positive reactions in the presumptive coliform test.

Procedure

Subcultures were made from all tubes showing acid and gas in the presumptive count to fresh tubes of single strength MacConkey broth

The seeded media were incubated at 44◦C and examined after 24 hours.

Positive tubes showing acid gas are those containing Escherichia coli; the number is read from Mac Crady's table.

Confirmation was done by plating on MacConkey agar and testing for indole production and citrate utilization.

Escherichia coli do not utilize citrate as primary source of carbon and is indole positive.

Biochemical Tests

Biochemical tests were carried out to confirm the identity of the different bacterial isolates and results recorded.

RESULTS

In this study, fifty four (54) water samples were analyzed made of eight (8) bottled and forty six (46) sachet waters. The waters were purchased from vendors and hawkers in different areas within Maiduguri metropolis. Macroscopic examinations of these samples were observed and results noted (Tables 1and 2).

Presumptive Coliform counts were carried out on these Water Samples using the Most Probable Number (MPN) Method based on McCrady's probability table.

Out of the 46 Sachet water tested, 13(28.2%) yielded 53 number of coliforms per 100ml, out all of which turned out to be true Escherichia Coli Bottled water yielded no coliform. (Table 3)

In addition to E. coli, other bacterial spp were isolated from both the bottled and sachet waters as shown in Table 4.

Table 1: Results of Physical Examination of Various Brands of Sachet water

Brands	Registration Number	Manufactured Date	Expiry Date	Batch Number	Net volume (CL)	Producer's name and address
HO	Yes	-	-	-	50	+
QA	Yes	-	-	-	50	+
MA	Yes	-	-	-	50	+
BA	Yes	-	-	-	50	+
KA	Yes	-	-	-	50	+
HAF	Yes	-	-	-	50	+
BAH	Yes	-	-	-	50	+
MAS	Yes	-	-	-	50	+
FF	Yes	-	-	-	50	+
LA	Yes	-	-	-	50	+
DF	Yes	-	-	-	50	+
MK	Yes	-	-	-	50	+
MU	Yes	-	-	-	50	+
MW	Yes	-	-	-	50	+
BAK	Yes	-	-	-	50	+
AB	Yes	-	-	-	50	+
RA	Yes	-	-	-	50	+
NU	Yes	-	-	-	50	+
HAS	Yes	-	-	-	50	+
RA	Yes	-	-	-	50	+
FAT	Yes	-	-	-	50	+
EB	Yes	-	-	-	50	+
AI	Yes	-	-	-	50	+
IN	Yes	-	-	-	50	+
AM	Yes	-	-	-	50	+
BAG	Yes	-	-	-	50	+
KU	Yes	-	-	-	50	+
TA	Yes	-	-	-	50	+
SK	Yes	-	-	-	50	+
HAU	Yes	-	-	-	50	+
DAZ	Yes	-	-	-	50	+
SI	Yes	-	-	-	50	+
UM	Yes	-	-	-	50	+
BS	Yes	-	-	-	50	+
AH	Yes	-	-	-	50	+
NG	Yes	-	-	-	50	+
EBA	Yes	-	-	-	50	+
SUN	Yes	-	-	-	50	+
KUB	Yes	-	-	-	50	+
BAL	Yes	-	-	-	50	+
DAM	Yes	-	-	-	50	+
ALM	Yes	-	-	-	50	+
SO	Yes	-	-	-	50	+
ASH	Yes	-	-	-	50	+
S.U	Yes	-	-	-	50	+

KEY -; Negative and +; Positive

Table 2: Results of Physical Examination of Various Brands of Bottled water

Brands	Registration number	Production date	Expiry Date	Batch Number	Net volume (CL)	Producer's name and address
WIK	Yes	+	+	+	75	+
CB	Yes	+	+	+	80	+
MAM	Yes	+	+	+	75	+
FAR	Yes	+	+	+	75	+
SW	Yes	+	+	+	75	+
NES	Yes	+	+	+	60	+
EVA	Yes	+	+	+	75	+
BTH	Yes	+	+	+	50	+

+: present; -: Absent

Table 3: Result of Presumptive Coliform counts of Sachet Water Samples Using the Most Probable Number (MPN) Method

Brands	In 1 bottle of 50mldouble strength broth/ 50ml of water	In5bottles of 10ml broth/ 10ml of water	In 5 bottles of 5ml broth/ 1ml of water	MPN of coliforms in 100mls of water
QA	1	1	1	5
BA	1	2	0	5
MAS	1	2	0	5
DEF	1	1	0	3
DEP	1	2	1	7
AB	1	1	0	3
HAS	1	1	1	5
BAG	1	3	0	8
DAZ	1	1	1	5
DAM	1	2	0	5
HO	0	0	1	1
LA	0	0	1	1
S.U	0	0	0	>1

Table 4: Showing other Bacterial Spp. Other than E. coli Isolated from Both Bottled and Sachet Waters

Brands of water samples	Organism isolated
MA	*Klebsiella* spp
KA	*Pseudomonas .aeruginosa*
HAF	*Proteus* spp
BAH	*Pseudomonas aeruginosa*
DF	Coliform spp
MK	Coliform spp
MU	*Klebsiella* spp
BAK	Coliform spp
RA	*Klebsiella* spp
NU	Coliform spp
RAJ	*Klebsiella* spp
AM	Coliform spp
KU	*Proteus* spp
TA	*Proteus* spp
SK	*Proteus* spp
HAU	*Proteus* spp
SI	*Klebsiella* spp
AH	*Proteus* spp
NG	*Pseudomonas aeruginosa*
EBA	Coliform *spp*
BAL	*Klebsiella* spp
BTH	*Pseudomonas aeruginosa*

DISCUSSION

All the sachet water were registered with appropriate\ regulatory agency but none of them carried manufacturing date, expiry date and batch number on the sachet, therefore not complying with the labeling procedure as stipulated by the WHO for drinking water (WHO, 1997). The implication of this is that the consumer would not have any means of knowing if the water is within the shelf life. However, all the bottled water complied with the labeling guidelines as stipulated by (WHO, 1997).

The most probable number of coliforms was determined in 100ml 0f water samples according to McCrady's probability table. All positive samples were from sachet water. Organisms were confirmed to be E.coli which indicates fecal contamination of the analyzed sachet water. And therefore unsuitable for human consumption,

Based on the results it indicates that the water samples were contaminated by various pathogenic organisms. Of these samples, sachets water is more affected than bottled water. Predominant organisms isolated were 7.4% Pseudomonas aeruginosa, 14% Proteus spp, 9.3% Coliform spp, 24% Escherichia coli, and 11% Klebsiella spp. Presence of these organisms in drinking water is associated with poor environmental hygiene. In the overall study 66.6% of the packaged water was found to be unfit for consumption. This is in agreement with similar findings of a study in Maiduguri metropolis (Muazu et al 2012) which suggested that 90% of sachet water sold in Maiduguri is not fit for human consumption.

CONCLUSION

In conclusion packaged drinking water sold in Maiduguri metropolis is not safe for drinking due to contamination by pathogenic organisms; however sachet water produced in the metropolis are more contaminated than bottle water due to indiscriminate production in poor sanitary conditions.

RECOMMENDATIONS

It is recommended that water for drinking should be free from microorganisms and other harmful chemical substances, therefore a good sanitary conditions and proper water treatment should be carried out for healthy and safe drinking water.

Packaged drinking water factories should be inspected by regulatory authorities to ensure good and safe drinking water production.

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