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SHORT COMMUNICATION
ASSESSMENT OF BACTERIAL LOAD IN DIFFERENT SACHET WATER SOLD WITHIN SULE LAMIDO UNIVERSITY, KAFIN HAUSA

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ABSTRACT
The research was conducted to assess the bacterial load in different sachet water sold at Sule Lamido University, Kafin Hausa. The samples were collected directly from manufacturers and the school shops. Eight water samples (A_1, B_1, C_1, D_1) collected from shop and (A_2, B_2, C_2, D_2) from the manufacturers were analyzed in triplicate using Serial dilution and pour plate methods on nutrient agar respectively. The isolates were identified using gram stain techniques and biochemical tests. It was found that the total bacterial load found in samples A_1, A2, B_1, B_2, C_1, C_2, D_1 and D_2 were 108, 10, 88, 12, 204, 118, 50 and 10cfu respectively. Samples obtained from shops were comparatively observed to have high bacterial loads of 204cfu and this indicates the improper treatment, contamination during handling and distribution of the finished product at the shop. The organisms found were Staphylococcus spp, Streptococcus spp, Enterobacter spp and E.coli.
Key words: Sachet water, Bacterial load, Sule Lamido,

INTRODUCTION

Water is one of the most essential commodities for the survival of all lives. It is abundant in nature and occupies about 70% of the earth's crust (Kalpana *et al*., 2011). Safe and readly available water is important for public health whether it is used for drinking, domestic, food production or recreational purposes. Improved water supply and sanitation as well as management of water resources can boost countries economic growth and greatly contribute to poverty reduction (WHO, 2019).

Contaminated water and poor sanitation are linked to transmission of diseases such as cholera, diarrhoe, dysentry, hepatitis A, typoid and polio (WHO 2019). The report also added that was in 2017, almost 829,000 people were estimated to die from diarrhoea which was mainly as a result of unsafe drinking-water, sanitation and hand hygiene.

Megarsa (2018) quoted WHO report that, about 80% of all the human diseases are cauesd by water and about 780 million people do not have access to a purified water source while an estimated 2.5 billion people lack access to improved sanitation worldwide (CDC, 2003).

Water is considered portable for human consumption or recreational activities when foreign enteric bacteria are not detected in a specific volume (100ml) of water (Abu *et al.,* 2020). The use of intestinal organisms as indicators of feacal contamination is a universally acceptable process for monitoring and assessing the microbiological safety of water supply before distribution. Two of the most widely accepted bacterial indicator organisms are *Escherichia coli* and *Streptococcus* due to their feacal linkages (Harwood *et al*., 2004).

Though a water supply may pass all laboratory tests, we have to bear in mind that hazard may arise from pollution of the water source through cross-connection, back symphonage, leaks in mains and service reservoirs (Philip *et al.,* 2018). The report indicates that there is isolation of some pathogenic organisms like *Salmonella*, *Shigella* and *Escherichia coli* from drinking water and most reported cases in Typhoid fever were not unconnected with drinking of sachet packaged water. Organisms which are well characterized and mostly present can include strains of Salmonella, *Shigella, Leptospira*, Enteropathogenic *Escherichia coli*, *Francisella*, *Vibrio*, *Mycobacteria*, Human enteric viruses, Cysts of *Entamoeba histolytica*, *Giardia lamblia*, or other pathogenic protozoans and larvae of various pathogenic worms. Salmonella strains have been frequently detected in drinking water sources and this have been correlated with outbreaks of Salmonellosis (Philip *et al.,* 2018).

Contaminated water supplies affect the growth and nutrition in young children adversely. Production of quality water product is increasingly difficult, because the demand for water is high and implementing universal standard for drinking water has not being followed to the letter, due to differences in sociological conditions, varying climates and other specific circumstances found all over the world. However, water treatments such as storage in open reservoirs, coagulation, filtration and treatment with chemicals can be used to improve quality of the product from the raw water (Funmilayo *et al*., 2021).

Packaged drinking water like the sachet water could be water from any potable source such as tap, well and rain, which may be subjected to further treatments like decantation, filtration, demineralization and other methods to meet established drinking standard. The aim of this study is therefore, to assess the bacterial load in different sachet water sold at Sule Lamido University, Kafin Hausa for exploitation towards ascertaining its quality.

MATERIALS AND METHODS

Four Different samples of sachet water of different brands were commercially purchased from school shops and labelled as A_1, B_1, C_1 and D_1. 500ml of each brand were also collected from the production site in a water sample bottles labelled A_2, B_2, C_2, D_2. These were transported immediately to Biological Sciences Laboratory, Sule Lamido University, Kafin Hausa for analyses. Each sample was vigorously shaken and observed for turbidity, odor, shelf life, batch number and manufacturing dates.

Bioassay

Total Viable Counts

The analysis of total viable count (TVC) was done using the standard plate count method. The count represents the number of colony-forming units (CFUs) per ml of the sample. The reported count is the number of colonies counted multiplied by the dilution used for the counted plate. A high TVC count indicates a high concentration of microorganisms, which may indicate poor quality for drinking water. The bacteriological test was done within 2 hours of sample collection. The determination of total heterotrophic bacteria was done using serial dilution and pour plate technique. For this, tenfold serial dilution in sterile water was carried out for each water sample brought for testing. One ml from the 5^{th} test tube was aseptically taken on two occasions and placed in two different sterile Petri dishes. Then, 15ml of nutrients agar (which was prepared as described by the manufacturer) cooled to 50°C was added to each plate and mixed thoroughly. The mixtures were allowed to solidify and incubated at 37°C for 24 hours. After incubation, the number of bacterial colonies in both the plates was counted and the average was reported as CFUs per milliliter of the tested sample. The assay was done in triplicate (Uzma *et al*., 2015).

Characterization and Identification of Bacterial Isolates

Pure forms of the bacterial isolates were characterized and identified based on their microscopic morphology and biochemical characterization as described by Cheesbrough (2006).

Gram stain

Gram's stain was used to stain the bacteria for microscopic examination to identify the morphology of bacteria. The colony were prepared on a microscopic slide using a drop of crystal violet solution which was allowed to stand for 1minute and then washed with water, the smear was flooded with iodine and was allowed for 1minute and then washed with water. The smear was flooded again with acid alcohol and it was then quickly washed with water, smear was finally flooded with safranin for 30secs and with water. The slide was then air dried and observed under the microscope using oil immersion (Bello *et al.,* 2014).

Biochemical Test

These tests were used to identify bacteria on the basis of their biochemical characteristics, namely; Catalase Test (James, 2001); Coagulase Test (Chidi *et al.,* 2006); Oxidase Test (James, 2001); Indole Test (Cheesbrough, 2006); Triple Sugar Iron Test (Funmilayo *et al*., 2021).

RESULTS AND DISCUSSION

After physical examination, all the samples were found to be odorless and no visible particles seen and while noting the shell life as well as the batch number clearly stated, no date of production was provided.

Bacterial viable count

From the research conducted, it was found that there is bacterial growth at all the samples analyzed (Table 1). After conducting a bacterial viable count, samples A_1 and A_2 were found to be creamy with 108 and 10cfu respectively. B_1 were golden and creamy with 88cfu while B_2, C_1, C_2 were golden and milky with 12, 204,118cfu respectively. However, D_1 were creamy and golden with 50cfu while D_2 were milky and golden with 10cfu.

It was indicated that sample C has the highest bacterial loadand this quantity was still accepted as it is within the range of maximum value accepted by CDC (2003), which specify that hetetrophic plate count should be less than 500cfu/ml.

Identified organisms

After 24hrs of incubation, the isolated organisms were identified based on their cultural appearance, microscopic appearance and biochemical tests (Table 2). They include *Staphylococcus aureus*, *Streptococcus spp*, *Escherichia coli* and *Enterobacter spp*. The *E.coli* was present in all the samples except sample D_1 and D_2, Staphylococcus aureus was found in sample B_1, B_2, C_1, D_1 and D_2. *Streptococcus* was isolated in samples C_1 and C_2. *Enterobacter spp* was only present in A_1 and A_2

The presence of the *E.coli* in all the samples indicates that the water is not safe to drink because the composition is above the accepted value by CDC (2003), which stated that water should contain lessthan one coliform bacteria per milliliter. Coliform bacteria are organisms whose presence in the distribution system could indicate fecal contamination, in which *E. coli* is member and also it is a potential important human pathogene associated with a variety of infectious diseases such as gastroenteritis and dysentery as stated by Uzoigwe and Agwa (2012).

The presence of coliform bacteria in drinking water generally suggests that the drinking water may have been contaminated with either humans or animals feaces. Coliform bacteria are enteropathogenic that interfere with human health (WHO, 2008). This result agreed with the work of Jumenten *et al.* (2020) where they found that *E.coli* contamination was due to unhygienic environmental conditions.

Staphylococcus aureus was present and this could be attrinuted to its commensal property. This finding is similar to that of funmilayo *et al*. (2021) were they isolated staphylococcus from the bottle water. Previous study according to Falegan *et al*., (2021) reported that satchet water samples collected from five different manufacturers yielded 20.8% S*taphylococcus aureus*.

Some *Streptococcus spp* are associated with feacal origin due to its ability to withstand advance conditions. According to Standard Organization of Nigeria (SON) in Nigeria, thermo tolerant coliform (*E.coli*), faecal *Streptococcus* and *Clustridium perfringes* spore shuld be 0cfu/100ml in drinking water.

The presence of *Enterobacter spp* which exist at higher temperature may be from its contamination due to the poor storage. Ogueri et al. (2020) stated that long storage of sachet water under unfavourable environmental conditions and lack of good manufacturing practices (GMP) contribute to contamination.

Table 1: Bacterial Viable Count

S/N	Sample	Colony count (cfu)
1	A_1	108
2	A_2	10
3	B_1	88
4	B_2	12
5	C_1	204
6	C_2	118
7	D_1	50
8	D_2	10

Table 2: Microscopic and Biochemical Characteristics of the Isolated organisms

Microscopic appearance		Biochemical characteristics							
GR	SH	CAT	COA	CT	BH	IN	TSI	OX	ORG
-ve	Rod	+ve	*	-ve	+ve	+ve	+ve	-ve	*E.coli*
-ve	Rod	*	*	+ve	-ve	-ve	+ve	-ve	*Enterobacter spp*
+ve	Cocci in bunch	+ve	+ve	-ve	+VE	*	*	*	*Staphylococcus aureus*
+ve	Cocci in chain	-ve	-ve	*	+ve	*	*	*	*Streptococcus spp*

KEY: GR: Gram reaction, SH: Shape of the colony, CAT: Catalase test, COA: Coagulase test, CT: Citrate test, BH: Blood hemolysis, IN: Indole test, TSI: Triple sugar iron test, OXI: Oxidase test, ORG: organism identified

CONCLUSION

The result of this study showed that all the water samples were not safe for drinking due to the presence of *E.coli* and *Streptococcus spp*. Which may occur due to the improper treatment and poor hygiene. Therefore, it is suggested that there should be periodic checks and analysis on the method and strategies used in the production and supply of the manufacturers' products. Also when coliforms and other bacteria are found, it is always recommended that an investigation should be carried out to identify the sources of the contamination as this can help to reduce the spread of water borne diseases.

REFERENCES

Abu, G.O., Otokunefor, K. and Dappa, C.D. (2020). Bacteriological Analysis of water Quality in a recreational Park Pond in Rivers State/ Nigeria*. Journal of Applied Sciences and Environmental Management*, **24**(1):23 - 29

Bello, O.O., Bello, T.K., Fashola, M.O. and Oluwadan, A. (2014*).* Microbiological quality of some locally produced fruit juice in Ogun state. *South West Nigeria Journal of Microbiology Research,* **2**(1):001-008.

CDC (2003). *Centers for Disease Control and Prevention. Guidelines for Environmental infection In Health Care Facilities.* CDCs Division of Healthcare quality promotions infection Control. (https://www.cdc.gov/infectioncontrol/guidelines/environment/index.html).

Cheesbrough M. (2006). District Laboratory Practice in Tropical Countries. Low price Edition part 2". *Cambridge press, England.*

Chidi, F., Ezcama, E. and Caroline, I.I. (2006). Microbiological and Sensory evaluation of Fermented rice snack (masa) supplemented with soybeans*. Journal of Food Technology,* **4**(4):354-349.

Falegan, C.R., Odejemi, A.T. and Ognjobi, L.P. (2021). Bacteriological and Physicochemical Studies of Satchet water in Location at Ado-Ekiti, Ekiti State. *Aquatic Biology* **2:**55-61**.**

Funmilayo, A.J., Oyinyechi, A., Oluwapelumi, O.B., Yomi, A.R. and Buru, A.S. (2021). Identification and Comparism of Microbial Load of Different Water Products Available in Ekiti State. *Journal of Infectious Diseases and Epidemiology,* 7**:**193. doi.org/10.23937/2474-3658/3658/1510193.

Harwood, V.J. Gandhi, J.P. and Eright, A.C. (2004). Method for Isolation and Confirmation of *Vibrio vulnicus* from Oysters and Environmental Sources: A Review*. Journal of Microbiological Methods*, **59:**301-316**.**

James, M.J. (2001). *Modern Food Microbiology* 3rd (Edn.), Tafa MC, Graw Hill publishers Co. ltd. Pp 10-125.

Jumenten, S.H., Yenti, P., Sufiah, A.M. and Tien, P.A. (2020). Microbiological and Biochemical Contamination Analysis of Refilled Drinking Water. Abeli, Kenderi, Sourtheast Sulawesi*. Indonesian Biochemical Journal*, **12**(2):124-129

Kalpana S., Cannon, M.J., Hyde, T.B. and Schmid, D.S. (2011). Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. *Reviews Medical Virology*, **21:**240–255**.**

Megersa, O.D. (2018). *Safe Drinking Water. Concepts, Benefits, Principles and Standard Water Challenges of an Urbanizing World*. Matjaz Glavan, Intech Open, DOI: 10.5772.

Ogueri, N., Chiugo, C., Duba, A. and Oluyemisi, E.O. (2020). Bacteriological quality of Borehole and Sachet Water from a Community in Southeastern Nigeria. DOI: 10.5772/intechopen.91812

Philip, I.A., Anthony, C.O. and Edwina, C.O. (2018). A Comparative Bacteriological Study of Bottled Drinking Water in Nigeria. *International Journal of Aquacreature and Fishery Sciences*, **4**(4):45-50

Uzma, H., Abubakar, M., Arfaa, J. and Ikram, U. (2015). Determination of microbial load of drinking water from different area of Lahore*: Biologia (Pakistan)***,** 61**(1)**:151-156**.**

Uzoigwe, C.I. and Agwa, O.K. (2012). Microbiological Quality of Water Collected from Boreholes Sited near Refuse Dumpsites in Port Harcourt, Nigeria*. African Journal Biotechnology,* **11**(13):3135-3139**.**

World Health Organization, WHO (2008). *Guidelines for Drinking Water Quality*: Incorporating 1st and 2nd Addenda, vol 1, Recommendations 3rd edition. Genevas.