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Research Article

Assessment of the microbiological quality of bottled water and protected spring water in Bushenyi district, Uganda

Lisa Nkatha Micheni[1]*, Peter Nsiko[2], Emmanuel Eilu[1], Isaac Echoru[3], Josephat Maniga Nyabayo[1]
[1]Department of Microbiology and Immunology, Faculty of Biomedical Sciences, Kampala International University-Western Campus, Kampala, Uganda
[2]Department of Public Health, Faculty of Biomedical Sciences, School of Allied Health Sciences, Kampala International University-Western Campus, Kampala, Uganda
[3]Department of Anatomy, Faculty of Biomedical Sciences, Kampala International University Western Campus, Kampala, Uganda

***Corresponding author**
Micheni Nkatha Lisa
Email: lisamicheni@yahoo.com

Abstract: Whereas microbiological quality of bottled drinking water is routinely monitored for reasons of legal compliance, microbial numbers in spring water are grossly understudied. Safety of water is a major public concern. However, the portability status of each type of water is a major concern to the public health. This study was carried out to assess of the microbiological quality of bottled and protected spring water in Ishaka-Bushenyi District. A total of 33 samples of bottled water and 8 samples of protected spring water were analysed using the Membrane Filtration Method. This method involved filtering the samples through a membrane filter (size 25um), culturing on lactose (TTC) with sodium heptadecylsulfate media and performing biochemical tests (oxidase and Indole) for confirmation of coli forms and *E. coli* respectively. Four (50%) of the protected springs showed growth of typical yellow colonies on lactose agar media. The presence of *E. coli* signified that protected springs were being contaminated with fresh faecal matter. However, no bottled water showed any signs of contamination (0%) with total coli forms or *E. coli.* There was a strong association (*P*= 0.01) between water source and water quality. Bottled water sold in Ishaka, Bushenyi District of Uganda showed high quality standards while protected spring water showed significant level of contamination with E. *coli* which could potentially cause disease to the consumers. Therefore all stakeholders in public health should come together to look into the matter of increasing access cheap and safe water other than the unsafe water from the so called "protected springs".
Keywords: Bottled water Uganda, water testing Uganda, Microbiological water assement Uganda.

INTRODUCTION

There has been an increasingly high demand for bottled water during the last decade due to the fact that people living in both developed and some developing countries have no suitable water supply around their homes [1]. This has made bottled water the fastest growing type of the non-alcoholic drink that is consumed in the market worldwide [2].

Between 2000 and 2006 Consumption of bottled water in the UK increased from 1415 to 2275 million litres and in the year of 2003, it was noticed that UK consumers had spent £1 billion on bottled water alone [3]. In a matter of fact, many of these consumers have taken bottled water as a substitute for tap water [4].

Water quality can have a major impact on both individuals and communities health. According to the World Health Organization, about 1.4 million children die from diarrhea due unsafe drinking water [5]. Therefore, for good human health, it is important to ensure that drinking water is well treated before consumption [6].

There are many other uses of water besides drinking alone; these include domestic, recreational and industrial purposes which are; washing, cooking, bathing, food processing, brewing, beverage bottling and for sporting activities [7]. This therefore necessitates a constant supply of potable water to all human communities but for areas that are lacking good improved water supply. However, there is a lot of time, effort, and money that is required or invested so as to find a suitable source of water supply [7]. Unfortunately, such water sources are scarce and even when they are available; they are seldom safe for consumption. There is therefore a great need for water to be treated effectively in order to make it potable and safe for humans.

Consumption of contaminated drinking water was associated with 80 percent of disease and one third of death in developing countries [8, 9]. Therefore, an essential basic requirement for health protection is to provide the public with safe and adequate drinking water [5].

In all urban areas in the developed countries, supply of adequately treated water is done by municipal authorities [10]. For the case of developing countries, there is little or no access to such treated water and therefore it is often very difficult or even impossible to get portable water. This is because of inadequate funds available to the municipal for appropriate treatment and supply of water and this being so, a large percentage of people in developing countries have to rely on their own personal efforts in order to get safe drinking water [10].

Advances in water treatment however have significantly increased the quality of water safety in various communities. However, the quality of drinking water can deteriorate by microbial and toxic chemicals during transportation, storage, packaging and handling before reaching the final consumer. Distribution systems, service lines and home devices could influence the quality of drinking water [11].

Biofilms (an aggregate of microorganisms in which cells adhere to each other on a surface) affect water quality and they are widely spread in the nature. These biofilms are a major problem in many environmental, industrial and medical settings [12]. The existence of dissolved organic compounds in finished processed drinking water is vital for growth of bacteria and its water surface colonization [13]. Biofilm formation is a public health concern because it enables bacteria to persist in water systems. It also protects the bacteria from severe and adverse environmental conditions, including chemicals such as [14]. Biofilms may also contribute to the spread of waterborne diseases because they harbor pathogenic bacteria and support their proliferation in nature [15]. The heterotrophic plate count is a parameter that could be used to reflect the biofilm formation in water systems. It is the basic standard technique for microbiological testing of drinking water [16].

Water for consumption is supposed to be free from microbial contamination but it's not obvious that all microorganisms present in water are harmful. The most dangerous microorganisms are the coliforms which are part of the normal flora of the gut of warm blooded animals. The presence of contamination with any of these organisms and others can be tested using various indicators. However the development of such indicators has never been such an easy job and up to date, there still remains considerable arguments about the best indicators for testing microbial contamination [17]. A study conducted in Bangladesh, showed that most of the bottled water in Dakar the capital of Bangladesh were contaminated by microorganisms [18]. Therefore, compounding the suspicion of most consumers that bottled water may not after all be the clean and safe alternative they were looking for this reason. The study tried to bridge the knowledge gap that existed on the quality of water in Ishaka-Bushenyi. The knowledge generated shall be used to influence policies aimed at enhancing public health.

MATERIAL AND METHODS

Study design

This was a descriptive laboratory based case study that was conducted to determine the microbiological quality of water in Ishaka- Bushenyi using bottled and spring water.

Location of the Study

Water samples for the study were collected around Ishaka-Bushenyi municipality. Ishaka-Bushenyi is found in western Uganda and it is situated approximately 75 kilometers, by road, North West of Mbarara town and 12 kilometers west of Bushenyi district headquarters and within the coordinates 0^0 34 53 s, 30^0 22 48 E. The samples were analyzed in Uganda National Bureau of Standards (UNBS) laboratories in Nakawa industrial in Kampala (Capital city of Uganda) in the month of January 2014.

Sample Size Determination

Sample size was determined by single population proportion formula; the prevalence of coliforms was 36.15% a margin error of 5% and confidence interval of 95% was anticipated. The sample size was 138. From the formula, 33 samples of bottled water were collected (average 3 bottles per brand of the estimated 45 brands in Uganda) and 8 samples from protected spring water by random selection with in Ishaka- Bushenyi municipality during the study period.

Inclusion and Exclusion Criteria

This present study included all bottled water and spring water in Ishaka Bushenyi Municipality Respectively. Any spring water which was not protected and bottled water which had expired was excluded from this study.

Sample Collection

Bottled water samples were randomly collected from selected shops and supermarkets situated around Ishaka-Bushenyi municipality. The number of bottles collected for the study was dependent on the number of bottled water brands available in the market at the time of sample collection. Three medium sized (500 or 650 ml) bottles of water were picked for each brand. Spring water samples were collected from randomly selected protected springs by random sampling. A total of eight were selected within the study area. The samples were collected during mid-morning and afternoon time. The samples were

collected during the dry spell in mid-January 2015. The samples were collected in 500-mL Whirl-Pak bags (Oxoid, UK). 100 mL of the water sample were then mixed thoroughly together with Colilert analysis reagent, which were later incubated for 24 hours at 35°C for further analysis[19]. The springs where these samples were collected supply an estimated $^1/_4$ of the total population of Ishaka Bushenyi municipality.

Sample Analysis

The collected water samples were taken for analysis at UNBS laboratories. The samples were analyzed using the membrane filtration method with some slight modifications to suit conditions and biochemical testing according to International Standards Organization and UNBS standards for bottled water (Iso, 2005) and [20]. The parameters tested were, coli forms and fecal coli forms. Total coli forms were isolated and enumerated using the membrane filtration method and growth on lactose 2,3,5-Triphenyltetrazolium chloride agar (TTC) and sodium heptadecylsulfate media [21, 22]. The samples were filtered and each membrane was placed on a selective lactose agar medium and it was incubated at 36^0C for 24 hours. All typical yellow colonies appearing on the membrane were counted with the aid of a magnifying lens, colony counter and recorded as presumptive total coli forms. For confirmation, the yellow colonies were sub-cultured onto the lactose agar media and incubated at 37°C for 48 hours. After which the sub-cultured colonies were then subjected to an oxidase test, to confirm total coli forms [23]. The results were then expressed as the number of colonies in 100 ml of original sample as described by UNBS and ISO. This was compared to the WHO water indicators which states that one is at high risk if the *E. coli* MPN is > 10–100/100 mL, and at very high risk when the *E. coli* MPN is > 100/100 mL. Fecal coli forms were enumerated using the membrane filtration technique. The plates were incubated at 44°C. Sub-cultures for confirmation were incubated at 44°C for 24 hours. Yellow colonies of various sizes appearing on the membrane were counted with the aid of a magnifying lens and taken to be thermo-tolerant coli forms (presumptive Test). A positive Indole test on a negative oxidase test confirmed the presence of thermo-tolerant coli forms [24].

Quality Control

Standard operating procedures were formulated including positive and negative controls and they were followed to the latter to ensure the highest standards of credibility. Additionally an experienced staff of UNBS was provided to guide the researcher during the work in order to ensure accuracy of results and reporting. The laboratory used is a standard laboratory and is accredited by the South African National Accreditation System (SANAS).

Data Analysis

Data from Microsoft windows excel was exported to SPSS for analysis. A chi- square test was used to check the level of significance by comparing the dependent and independent variables.

Ethical Consideration

Approval to conduct this present study was sought from Uganda National Bureau of Standards and Kampala International University, Faculty of Biomedical Sciences Research and Ethics Committee before commencement of the study. Bottled water brands were kept confidential by giving each brand an appropriate corded initial to conceal its identity. All other ethical issues pertaining to maintaining of confidentiality were strictly adhered to and observed during the study.

RESULTS

All the samples of bottled water showed no growth of typical yellow colonies however, there was bacterial contamination. Among the protected spring water samples, 4(50%) showed growth of typical yellow colonies while the other 4(50%) did not show any typical colony growth. There was a strong correlation (P= 0.01) between water source and water quality.

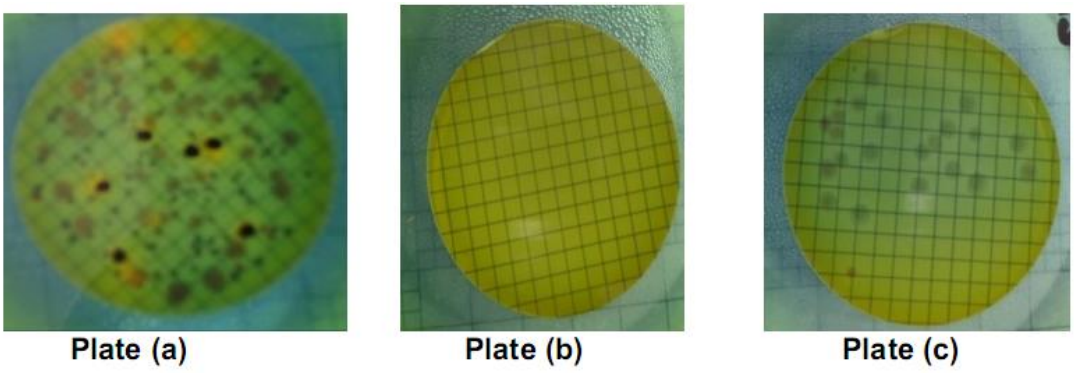

Fig.1 Images of culture plates of the water samples
Sample with typical yellow colonies-Plate (a); Sample with no typical yellow colonies-Plate (b); Sample with bacteria contamination-Plate (c)

Table 1; **Below indicates that there were high percentage values of positive results on spring water sources (50%) and no positive results on bottled water**.

		Water type	
Test	**Result**	**Bottled water (%)**	**Spring water (%)**
Typical colony growth	Positive Negative	0 (0) 33 (100)	4 (50) 4 (50)
SUB-TOTAL		33	8
Oxidase test	Positive Negative	0 (0) 33 (100)	4 (50) 4 (50)
SUB-TOTAL		33	8
Indole test	Positive Negative	0 (0) 33 (100)	4 (50) 4 (50)
SUB-TOTAL		33	8

DISCUSSION

This study revealed that bottled water brands available in Ishaka-Bushenyi were free from contamination with coli form bacteria. None of the bottles tested showed any presence of coli form, as can be seen in the results. This could possibly have been due to the following factors; good manufacturing practices on the part of the manufacturers, good handling during storage and transportation and during the retailing of these products. The other factors could be due to the use of appropriate packaging materials which maintained or stopped leaking or compromising the standard of the environment in the bottles after sealing with the outside environment. This study revealed that the standard of water from the protected springs in Ishaka-Bushenyi was low as 50% of these springs tested positive for all the three parameters used, including; presence of typical yellow colonies, oxidase and Indole test. The positive results meant that the springs are contaminated and therefore unsuitable for use as drinking water. The possible reasons for this could not be ascertained by this study, though a few reasons could be advanced, like the possible contamination of ground water by sewage lines which run in the ground, the pit latrines which in many cases are dug and constructed by people who may not understand the link between streams and underground wells and also the proximity of grazing lands to these springs, which could be aided by surface run off thus affecting the water source [25]. The study found out that for the three parameters used to test the presence of coli forms and *E-coli* and thus the quality of water of both protected spring and bottled water showed that there was a 50% chance of fetching contaminated water in Ishaka from a protected spring, while there was (0 %) chance for bottled water. There was also a strong correlation of significance of 0.01 using the chi- square values and level of significance between, quality of water using these parameters; (presence of typical yellow colonies, oxidase and Indole tests) and water source. This could possibly have been due to several factors such as uncontrolled weather interferences, possible contamination with fecal matter from nearby sewer lines and pit latrines, dead carcasses of animals and the excrement of grazing animals. These factors and many other more could have led to the observance of this result. However if such factors had been controlled, then the results may have looked different. However, a similar study carried out in Katwe and Kisenyi in Kampala showed that most of the springs 90% showed contamination with coli forms and other microorganisms beyond the WHO water quality standards in these areas. However this present study was limited due to the fact that not all bottled water brands were evaluated.

CONCLUSION

From this present study it can be concluded that protected Springs of Ishaka- Bushenyi municipality showed a high level of contamination with Coli form bacteria and of these, 50% also showed contamination with *E-coli*. Bottled water sold in Ishaka-Bushenyi Municipality did not show contamination with Coli forms bacteria. Thus protected spring water should not be consumed by human beings unless treated to eliminate the presence of microbial contaminants. Since this study showed that there was a possible strong correlation between protected spring water and water contamination, this present study therefore recommends that all stakeholders in public health come together to look into the matter of increasing access to cheap and safe water like piped water to solve the problem of shortage of water in Ishaka Bushenyi which has driven the people to unsafe water from the so called “protected springs”. This research is not conclusive because the factors leading to high levels of contamination of protected spring water were not carried out. More research is needed to establish the factors responsible for high levels of contamination.

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