Occurrence of *Escherichia coli* in Packaged Drinking Water Distributed in Katabi Sub County Uganda. A Cross Sectional Study.

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Abstract

Background:
The world health organization (WHO) estimates that about a 1.1billion people drink unsafe water globally, and over 1.7 million deaths occur annually due to the consumption of water that is of faecal origin. Water in sachets is readily available and affordable but there is no concern about its safety. The purpose of this study was to assess the occurrence of *Escherichia coli* in packaged drinking water distributed in the Katabi sub country using the membrane filtration technique, isolation of organisms, and subjecting them to biochemical tests. The study was conducted in 2017.

Methodology:
A cross-sectional study design was used where Rwenzori, Riham water, Nevana, Highland, Blue wave, and sachet water were purposively selected in the main trading center within the Wakiso district. A total of 5 bottled water and 10 sachets of water were used. Duplicate sample (2) bottles of water from each were used. Therefore, 30 samples of packaged water were used in this study. The bacteria were grown on media and confirmed using biochemical tests.

Results:
Sachet water contained pink lactose fermenting colonies on MacConkey culture media. Bottled water had no growth. The pink colonies were positive with Kligler iron agar (KIA), triple sugar iron agar (TSI), and sulphur and indole motility (SIM) positive.

Conclusion and recommendation[a]
The majority of sachet water was contaminated with *Escherichia coli* an indicator of faecal contamination; and therefore, unsuitable for human consumption. The government of Uganda should carry out surveillance activities to enforce strict hygienic measures on sachet water producers and distributors.

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1 Background:

The world health organization (WHO) estimates that about a 1.1billion of people drink unsafe water globally (WHO, 2014). Worldwide, poor water quality, sanitation, and hygiene account for about 1.7 million deaths a year (Sobsey, 2008). The quality of drinking water is a powerful environmental determinant of health and water is vital to sustaining life and needs to be treated. There is a need for adequate, safe, and accessible water supply to all (Shandas, 2014; Bartram, 2010). Diseases related to contamination of drinking water constitute a major burden on human health, interventions

to improve the quality of drinking water provide significant health benefits (Bain, 2014).

According to (Ugochukwu, 2015), disease-causing micro-organisms transmitted via drinking water are predominantly of faecal origin and are referred to as enteric pathogens. The WHO standards state that drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. Packaged water is any potable water that is manufactured or processed for sale which is sealed in food-grade bottles, sachets, or other containers and intended for human consumption (Halage, 2015).

According to (Halage, 2015), the Sale of packaged water has exploded all over the world in recent years, largely as a result of public perception that it is safe, tastes better, and has a better quality compared to raw tap water (Robinson, 2016). Robinson further said that the increment in consumption globally could also be due to a result of an increase in per capita use as well as population growth. The demand for packaged water is so high, making packaged water the fastest growing product of the nonalcoholic beverage market worldwide (Gleick, 2010). In Wakiso, and all over Uganda, water is packaged in bottles and polythene bags. It has become a common consumer product most especially in the urban centers (Komakech, 2014). The high demand may attract substandard products and counterfeits into the market. Several studies have shown that packaged water can be contaminated with bacteria at various stages of production (Rakhi *et al.,* 2013). Also, improper or prolonged storage of bottled water can result in bacterial growth to levels that may be harmful to human health (Komakech, 2014 & Cabral, 2010). However, the consumption of packaged water has grown rapidly in recent decades, first in high-income countries and more recently in low and middle-income countries (Elbra, 2013). There is increased concern among governments and international organizations on packaged water. Recently concerns have been raised about possible links of packaged sachet water to outbreaks of cholera and other waterborne diseases (Fisher, 2015). There is also a concern about the impact of packaged sachet water production overburdened municipal water supplies as well as the environmental consequences of improperly managed plastic waste from packaged sachet water products.

Over 1.7 million deaths occur annually due to the consumption of water that is of faecal origin (WHO, 2014). According to (Miner &Tagurum (2015), water in sachets is readily available and affordable but there is no concern about its safety. The hygiene of the environment where sachet water is packaged is questioned. Although nationally documented evidence is rare, there are claims of past outbreaks of waterborne illnesses that ensued from the consumption of polluted water in sachets (Ugochukwu & Giwa, 2015). An understanding of their microbiological quality and safety is therefore imperative and should be a cause of concern to consumers, water suppliers, regulators, and public health authorities (Tang, 2013).

Packaged water has been implicated as a source of an outbreak of cholera, typhoid fever, and traveler's disease in countries like Portugal and Spain (Berger, 2017). There is no continuous surveillance of packaged water quality at retail premises in Uganda (Bagume *et al.,* 2010).

2 Materials and Methods

Study design

The study was about the assessment of the occurrence of *E. coli* in packaged drinking water distributed in Katabi Sub County located in Wakiso district. A cross-sectional study design was used where common brands of water and sachet water available on the Market were picked as subjects for this study. A qualitative study design was used.

Study area

Wakiso district is located in Uganda's central region and shares borders with Kampala, Mpigi, Luwero, Nakaseke, Mityana, Muknono and Kalangala district. It is the second populated district with a population of 2,007,700 as per the 2014 census and covers a total area of 2,807.75 square kilometers (UBOS, 2014). Available sources of drinking in this city include spring, piped water, boreholes, and Lake Victoria. It has many sub-counties among which was Katabi which was the area of study.

Sampling strategy

The main trading center within Wakiso district that was Kawuku, Abaita Ababiri, Kitoro were randomly selected for involvement in the study. A purposive sampling technique was used for the above centers since most of the packaged water brands' sales are located in these areas.

Study population

The study population was common brands of packaged water being sold in the above trading centers in Wakiso district. The study population included Rwenzori, Riham, Nevana, Highland, blue wave, and sachet water that were sold in the trading centers of Kisubi, Kawuku, Abaita, and Kitoro.

Sample size

A total of 5 bottled water and 10 sachets of water were used. Duplicate sample (2) bottles of water from each were used. Therefore, 30 samples of packaged water were used in this study.

Sample collection for bacteriology water analysis

Samples were purchased from the selected center in Katabi sub county (Wakiso district). These included Kawuku, Abaita, Kisubi and Kitoro. Water brands that were randomly selected in the study included Rwenzori, Riham water, Nevana, Highland, Blue wave, and sachet water. The samples were then incubated at 44°C for an overnight.

Media preparation

Preparation of the MacConkey

MacConkey culture media was prepared by dissolving 44.4g of the powder in 1.0litre of distilled water, and then autoclaved at 121°C (15psi) for 15 minutes. The media was left to cool before casting the media into culture plates, according to the manufacturer's instructions (Mast Group Ltd., Merseyside, U.K.). Care was taken not to contaminate the media during the casting. Sterility testing was done by incubating it at 37oC overnight.

Filtration of water brands using a membrane filtration technique

A filtration unit and a suction device were used. Using sterile blunt ended-forceps, a sterile membrane filter with a pore size of 0.45μm was placed on the uppermost part of the filter base. The samples of different brands were mixed thoroughly by inverting the bottle several times and 100ml of each sample was filtered. Suction was applied to draw the water through the filter membrane. Using some sterile blunt-ended forceps, the membrane from the filtration unit was removed aseptically and placed onto the MacConkey media.

The procedure was repeated for all the samples selected in the study. The culture plates were labeled with the number that corresponds to the water brands. These were inoculated overnight and lactose fermenting colonies (pink in color) were observed. Pure cultures of the pink lactose fermenting organisms were prepared on MacConkey media and incubated at 37oC overnight.

Confirmation of the organism of interest

Gram staining of the isolates

While using a sterile wire loop an individual colony was tapped in the sides and placed on a labeled glass side containing one drop of distilled water. A smear was made and allowed to air dry. The smear was then heat-fixed and flooded with crystal violet for 1 minute. The slides were rinsed with tap water and flooded with Gram's iodine solution and left to stand for 1 minute. The iodine solution was washed off by using tape water. Ethyl alcohol (95%) was used to decolorize the smear. The slide was rinsed with running water and counterstained using carbolfuchsin solution for 1 minute. The stain was rinsed off with tap water and blot dried, air dried, and mounted with oil immersion, and viewed under a microscope. Pink rods were observed.

Biochemical tests on the organisms

Triple sugar iron agar

This was prepared by suspending 65g in 1.0 liter of distilled, mixed thoroughly, and distributed into test tubes. The test tubes were plugged with cotton wool and aluminum foil and autoclaved at 121°C for 15 minutes. They were allowed to set as slopes. The procedure followed the manufacturer's instructions (OXOID LTD, Basingstoke, and Hampshire, England). Sterility testing was done by incubating the media at 37°C overnight. The samples were inoculated into TSA tubes which were labeled with the respective water brand and were incubated at 37°C overnight. The formation of a yellow butt and a yellow slant, with the production of a colorless gas, was monitored as a positive test for *E. coli* on triple sugar iron agar (Cheesbrough, 2006).

Kligler Iron Agar (KIA)

This was prepared by suspending 53.5g in 1.0 liter of distilled water, and heated until was completely dissolved. It was distributed into test tubes which were later plugged with cotton wool and aluminum foil, and autoclaved at 121°C for 15 minutes, allowing cooling and solidifying as slopes. The procedure followed the manufacturer's instructions (OXOID LTD, Basingstoke, Hampshire, England). Sterility testing was done by incubating the media at 37°C overnight. The samples of water were inoculated into the KIA. The tubes were labeled with respective water brands and incubated at 37°C overnight. The expected positive result

for *E. coli* on KIA would be an acidic butt, an acidic slant with gas production, and hydrogen sulphide production (Cheesbrough, 2006).

Sulphur Indole Motility (SIM) media

Procedure

It was prepared by suspending 36.23g into 1.0litre of distilled water and mixed thoroughly until completely dissolved. It was distributed into test tubes that were plugged with cotton wool and aluminum foil. The medium was autoclaved at 121°C for 15 minutes. It was allowed cooling and solidifying. The medium was prepared following the manufacturer's instructions (TULIP diagnostics (P) LTD, India). Sterility testing was done by incubating it at 37°C overnight. The media were inoculated and labeled with the respective water brand. Growth was observed around the area of inoculation without gas production hence hydrogen sulphide negative. Motility was positive. In addition on three drops of Kovac's reagent turned pink and this is an appositive test for the indole test. This procedure is as stated by Cheesbrough (2006)

3 Results:

In the case of locally packaged sachet water, the following was observed when it was filter membranes were plated on MacConkey agar:

There was no growth was observed for the sachet water purchased from A3 (shop 3 at Abaita Ababiri trading Centre. The rest of the sachet water bought from other shops showed pink colonies, with exception of one shop (K3) that had non-lactose forming organisms. The organisms were considered to be lactose fermenters. These were observed to be Gram-negative rods. With kliger iron agar (KIA), yellow slant, yellow butt, and a colorless gas were observed. With triple sugar iron agar (TSI), a yellow butt with colorless gas production and a yellow slant was observed and hence TSI positive (Cheesbrough, 2006). With sulphur indole motility (SIM) media, growth was observed at the site of inoculation without gas production (hydrogen sulphide gas), it was motility positive. In addition of Kovac's reagent turned pink and thus indole test was positive. Based on the above observations these were considered to be *Escherichia coli.* Other brands of water did not have any growth.

4 DISCUSSION

The physical and bacteriological state of packaged water is important in preparing drinking water for human consumption (Halage *et al.,* 2015). Bottled water is generally of good quality for drinking, but if it is not properly protected during bottling and transit, it could be contaminated (Halage *et al.,* 2015). The Uganda national guidelines for packaged water should be a pH range of 6.5–8.5, and total coliform and faecal coliform of 0.00 per 100 ml. According to this study, the bottled water distributed in Wakiso was free from feacal contamination since there was an absence of *E. coli*. These findings agree with those of (Halage *et al.,* 2015) who conducted a similar study in Uganda. However, other studies in Bangladesh showed feacal contamination of bottled water in that country. The factors that to the findings in this study may include good packaging methods employed by the manufacturers, high-quality standards employed during the manufacturing process, proper hygiene practice during manufacturing, use of protected water sources, proper storage of the water for processing, use of leak-proof bottles that do not allow external protection from feacal contamination (Halage *et al.,* 2015).

Bottled water is very safe for drinking but it is very expensive and hence not easily affordable by the highest percentage of people in Wakiso district. This makes the highest population in the Wakiso district consume sachet water. Most of the sachet water tested in this study was contaminated with *E. coli* as an indicator organism. These results are similar to what other previous researchers have researched about the bacteriology of locally packaged drinking water in sachets. A study carried out in Nigeria showed that all the sachet water had *E. coli* (Ugochuku, 2015). Another study conducted in Ethiopia found some of the packaged water unsuitable for human consumption since it was fecally contaminated (Birhanu, 2016). These findings are also similar to studies conducted in India (Mellor *et al.,* 2016), Tanzania (Kassenga *et al.,* 2007), and Ghana (Ampofo *et al.,* 2007).

Other studies in Pakistan also showed that sachet water bears faecal contamination (Woken, 2014). Other researchers state that the possible causes of faecal contamination in locally packed sachet water include possess hygienic practice by the handler, high temperatures, lack of protective mea-

Table 1. Results

Brand of water	Bacteria growth
Sachet	Growth present
Rwenzori	No growth
Riham water	No growth
Nevana	No growth
Highland	No growth
Blue wave	No growth

Source: This is primary data

sures due to common methods used, use of dirty water source for packagings like lakes, boreholes, springs faecally contaminated, poor manufacturing practices, poor quality standards undertaken during the process, improper storage, poor hygienic fillings and use of feacally contaminated polyethene (Thanh, 2015).

Sachet water is hand-filled, inexpensive, and therefore the majority of the poor people in Wakiso district can afford it as compared to bottled water. It is necessary that Uganda National Bureau of Standards follows its preparation and distribution to ensure safety. If this is not done to properly regulate the making and distribution of locally packaged sachet water, it may lead to outbreaks of cholera, salmonellosis, and typhoid fever (Halage *et al.,* 2015).

5 Conclusions

The majority of sachet water was contaminated with *Escherichia coli* an indicator of faecal contamination; hence it's not being suitable for human consumption. The bottled water tested was free from *Escherichia coli.*

Recommendation

The government of Uganda should carry out surveillance activities to enforce strict hygienic measures on sachet water producers and distributors.

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