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Bacteriological Quality Assessment of Bottled Water Brands Marketed in Kitale Town, Trans- Nzoia County, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Authors IYA, NGJ and PN designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors NGJ and PN managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Consumption of bottled water is increasing rapidly in developing countries as it is generally perceived to be pure, clean and of good quality. This has led to the sale of different brands of bottled water in several markets including Kitale town.
Aim of the Study: This study was conducted to assess the bacteriological quality of bottled water brands consumed in Kitale town.
Study Design: It was a cross-sectional study design which involved getting a snap shot situation of the prevailing bacteriological standards of bottled water sold in the Kitale town area.
Place and Duration of the Study: The current study was conducted in Kitale town area among outlets of bottled water brands.
Methods: This cross-sectional study was conducted to assess the bacteriological quality of bottled water brands consumed in Kitale town. A total of 60 samples of bottled water from 20 different brands were randomly selected from several markets and analyzed for bacteriological

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contamination using multiple tube fermentation method to detect the presence of *Escherichia coli* coliform per 100 ml.
Results: From this study, only 90% of the bottled water brands representing the products of 18 companies had counts within the acceptable limits. *Escherichia coli* coliforms present in 100 ml of water were detected in two of the bottled water brands constituting 10% (6/60) of the samples. The presence of coliform bacteria in drinking water suggests the possible presence of pathogenic enteric microorganisms thus unsafe for drinking. The data presented here clearly raise the concerns regarding the quality of bottled water and highlights the danger posed to the public health.
Conclusion: The results from this study indicate a possibility that not all the bottled water sold in Kitale town is of good quality as perceived by the buyers. Therefore, any contamination may pose a unique hazard because of the widespread distribution of the bottled water. There is also need for continuous bacteriological screening, good manufacturing and sanitation practices that must be employed by the manufacturing companies of bottled water.

Keywords: Bottled water; bacteriological quality; Kitale; Escherichia coli; coliforms.

ABBREVIATIONS

KEBS: Kenya Bureau of Standards; MPN: Most Probable Number; CCP: Critical Control Point; PET: Polyethylene Terephthalate; WHO: World Health Organisation; EBM; Eosin-Methylene Blue; IMViC: Indole Production Methyl Red for Acid Production; TSI: Triple Sugar Iron.

1. INTRODUCTION

Water is an essential requirement of all life forms. It is a crucial requirement in the maintenance of metabolic functions and homeostasis. The human body is composed of about 60% water by weight in male, 50% in females and 70% in newborn infants [1]. The human dietary requirement for water is estimated to be approximately two liters per day for an average adult [2]. This regular intake of water is essential in the maintenance of good health and well-being. Since water is life, the quality of water reflects the quality of life [3]. The production and consumption of bottled water has increased considerably over the years resulting from the concerns about the contamination of public water supplies, disagreeable taste and smell of tap water as well as due to the limited access to safe drinking water supply during journeys [4]. It is reported that over 1 billion people in the world lack access to potable water [5]. Potable water is water of high quality that could be consumed without risk of acute or chronic harm or injury from chemical, biological and physical contaminants. One of World Health Organization primary goals is access to adequate supply of safe drinking water for all. However, this goal is far from being achieved in most developing countries especially in the rural and peri-urban areas as over 5 million people die annually from water-borne diseases such as; cholera, typhoid, diarrhoea, polio and meningitis [6].

Drinking unsafe and unhygienic water can cause high prevalence of waterborne diseases like diarrhoea, gastroenteritis, *giardiasis*, hepatitis, *Salmonellosis,* dysentery, typhoid and cholera [7]. This means that drinking water should not contain any kind of harmful contaminants such as disease causing pathogens, toxic substances, physical or chemical residue [8]. In May 2000, 2,300 people became seriously ill and seven died because of water contamination with *E. coli* in Philippines [9]. Despite the abundance of water covering 71% of the earth surface, people spend billions of dollars per year to buy purified water that is pre-bottled. Most people around the world regard bottled water as safe for consumption. Consumers have various reasons for purchasing bottled drinking water such as taste, convenience or fashion and safety. Although, people consider bottled water safe however, it can be contaminated with chemical and biological agents [10]. Presence of coliform bacteria or *E. coli* in bottled water can pose a great threat to the public health. Infants, young children, debilitated and immuno-compromised people are at high risk of waterborne diseases, even at lower infective doses [8].

According to WHO recommendations, potable water should have <20 colony forming units/mL heterotrophic bacterial count with complete absence of coliform bacteria, fecal coliforms, *E. coli*, *enterococci* and *Pseudomonas aeruginosa*. Although, coliform organisms may not always be considered as indicator of fecal

contamination, their presence in drinking water suggests the potential presence of pathogenic enteric microorganisms such as *Salmonella* spp., *Shigella* spp. and *Vibrio cholera*. Therefore assessment of bacteriological quality of bottled water in this study will accelerate progress towards provision of safe and clean drinking water to the public and prevent/monitor outbreaks of water borne diseases.

2. MATERIALS AND METHODS

2.1 Study Site

This study was conducted in Kitale town, Trans-Nzoia County between June – August 2016. The town is estimated to have a population of 804,000 by 2012 [11]. Kitale is among the most diverse commercial towns in the country. However, with its growth and development comes an increased demand in basic utilities, infrastructure and most importantly safe and clean drinking water. The main source of water is the municipal water supplied by pipes to household by the Nzoia water service located inside the town. Other sources of water are underground water, springs and commercial bottled water. The municipal water is unable to cater for the needs of growing population thus the town is faced with problems related to availability of quality drinking water.

2.2 Study Design

This was a cross – sectional study, which involved getting a snap shot situation of the prevailing bacteriological standards of bottled water sold in the Kitale town area.

2.3 Study Site

The study used bottled water samples that were collected in several commercial centres in the Kitale town. Twenty bottled water brands were used in the study. The samples were acquired from four points: 3 big stores, 3 small stores, 7 shops and 3 street vendors.

2.4 The Inclusion Criteria

The study used bottled water sample purchased from stores based on the following inclusion criteria: Presences of valid business permit by the county government and located within the study area.

2.5 The Exclusion Criteria

Bottled water samples were rejected based on the following criteria: bottles with damaged packaging and bottles with expired shelf life.

2.6 Sample Size Determination

The sample size for the bottle water samples was calculated according to Kassenga [12] study in Tanzania who found that the total coliform and faecal coliform organisms recorded were 4.6% and 3.6%, respectively from bottled water collected from shops, supermarkets and street vendors. Therefore a mean 4.1% prevalence was use:

$$n = \frac{Z^2 pq}{L^2}$$

n= Desired minimum sample size
Z= Standard normal deviation (1.96)
P= Prevalence of condition under study (0.041)
q= 1-p
L= Allowable error at 95% confidence level (0.05)

$$n = \frac{1.96^2 * 0.041 * 0.959}{0.05^2}$$

n= 60

2.7 Sampling Method

Five different batches of 20 brands of bottled water currently available in Kitale town market were collected through a stratified random sampling method. The sub-samples were randomly drawn from samples within different batches that are more or less equal on some characteristic. The method was easy to use and required minimum knowledge of the population.The selected samples were labelled with the collection date, time and place of purchase and then transported in cooler box to the Microbiology laboratory of Medical Laboratory Science, Department at Jomo Kenyatta University of Agriculture and Technology for bacteriological analysis.

2.8 Bacteriological Analysis

In this study the bacteriological parameter assessed was the number of viable *E. coli* coliforms count as indicators for bacteriological quality of water [13]. All Glassware and materials used in this experiment were washed with distilled water and then autoclaved at 121℃ for 15 minutes to ensure sterility. Also the Medias

used were sterilized by autoclaving at 121℃ for 15 minutes before use to prevent cross contamination. The bacteriological and physical quality of the bottled water was determined by comparing it with the national and WHO standards for packaged water which requires water samples of good quality to be with 0 coliforms per 100 mL of water sample.

2.8.1 Viable *E. coli* count

Here, a multiple-tube fermentation method was used and the statistical results expressed as most probable number (MPN) units. This test was carried out in three phases: 1) presumptive, 2) confirmed and 3) completed phase based on the principle that *E. coli* has the ability to ferment lactose hence producing gas and acid after 48 hours of incubation. In the presumptive test, all the samples were cultured primarily in a series of MacConkey broth tubes. Three set of tubes of three dilutions of the water sample to be tested (10 ml, 1 ml and 0.1 ml) were used for the presumptive test. Inverted Durham's tubes were inserted in each tube. The tubes were incubated at 37℃, for 48 hours for presumptive evidence of the presence of coliforms.

In the confirmed phase, all the positive presumptive tubes (tubes that showed turbidity, gas production and a colour change indicating the production of acid) were inoculated in MacConkey and EMB agar by streak plate method and incubated at 37℃, for 24 hours to observe the colony morphology. MacConkey and EMB agar are selective media that enhance the growth of the Gram-negative *E. coli* while inhibiting the growth of Gram positive and spore forming bacteria. A positive EMB test is indicated by the appearance of colonies with a green metallic sheen (*E. coli*) and pink to red colonies on MacConkey. The bacteria isolated were subjected to a number of biochemical tests for identification of *E. coli* known as IMViC tests, which stands for indole production, methyl red for acid production, Voges-Proskauer and citrate utilization. Triple sugar iron (TSI) test was also performed to determine the utilization of glucose and lactose or sucrose fermentatively and production of hydrogen sulphide (H_2S). *E. coli* shows a positive indole and methyl red reactions and a negative Voges-Proskauer and citrate utilization tests [14,15].

2.9 Data Analysis and Presentation

The data obtained was analysed using SPSS version 23. The analysis involved calculation of the mean, standard deviation, frequency distribution and critical value (p value). The results were summarised and presented in form of graphs, charts and tables for better understanding.

2.10 Ethical Considerations

The permission and approval was obtained from Jomo Kenyatta University of Agriculture and Technology Ethics Committee and the public health and sanitation officer Trans-Nzoia County. Samples of bottled water were purchased only from stores. As some information and data from the study were commercially sensitive e.g. poor water quality, all data in this study was purely used for academic purposes and information collected was subjected to strictest level of confidentiality.

3. RESULTS

3.1 Descriptive Characteristics of Bottled Water Brands

The twenty brands tested in this study were the most widely available on the shelves of many stores in the study area. Majority of the brands, 85% (17/20) came outside of the study area while only 15% (3/20) were being produced locally.

In term of the purification method used, 90% (18/20) of the brands used at least two combination methods while 10% (2/20) of the brands used only one method. Consequently, 5% (1/20) were using reverse osmosis and another 5% (1/20) used filtration methods.

Table 1. Percentage source distribution of bottled water in Kitale town

Study areas	Frequency	Percent
Kitale	3	15.0
Nairobi	11	55.0
Nakuru	2	10.0
Nyeri	1	5.0
Ruiru	1	5.0
Sigona	1	5.0
Thika	1	5.0
Total	20	100.0

3.2 Bacteriological Screening

Out of the 60 bottles sampled, 10 samples were positive for *E. coli* coliforms giving the total

number of contaminated tests to be 10 out of 60 (16.67%) samples. The overall positive tests represented 10% of the analysed samples. These samples demonstrated evidence for presence of gas production, growth and acid production on McConkey broth indicating the presence of lactose fermenting coliform. The positive sample belonged to brand H (3 samples) and J (3 samples) with samples from J having higher MPN levels than those from H. On sub culturing in EMB and MacConkey agar, characteristic colonies with green metallic sheen were produced on EMB (Plate 1) and pink to red colonies on MacConkey (Plate 2). Grams stain and biochemical tests (IMVIC) and growth in triple sugar Iron agar.

Table 2. Distribution of purification methods as presented in bottled water samples

Purification methods	Frequency	Percent
Filtration, UV, Ozonation & Reverse osmosis	1	5.0
Filtration	1	5.0
Filtration & UV	2	10.0
Filtration, UV & Ozonation	11	55.0
Filtration, UV & Reverse osmosis	2	10.0
Filtration, UV, Ozonation & Reverse osmosis	1	5.0
Reverse osmosis	1	5.0
Reverse osmosis, UV & Ozonation	1	5.0
Total	20	100.0

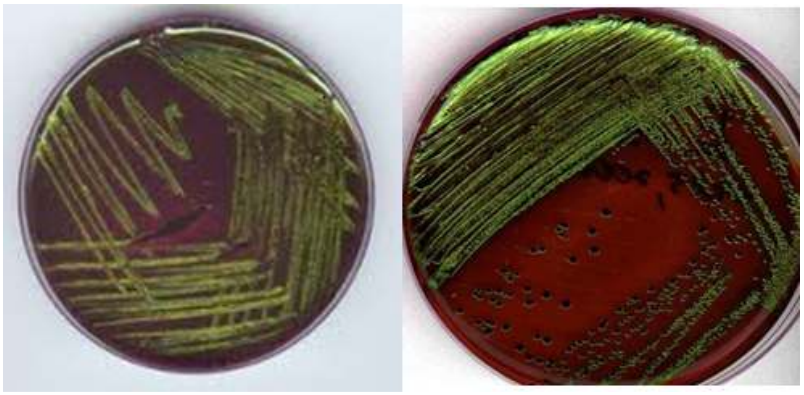

Plate 1. Eosin-methylene blue (EMB) agar plate inoculated with *Escherichia coli* showing good growth of colonies with metallic green sheen indicating vigorous fermentation of lactose and acid production which precipitated the green metallic pigment

Plate 2. Mac Conkey agar plate inoculated with *Escherichia coli* showing pink colonies caused by fermentation of lactose and acid production. Acid production caused a drop in the pH that was detected by neutral red, which was red in colour at pH below 6.8. As the pH drops, neutral red was absorbed by the bacteria, which appear as bright pink colonies on the agar

3.3 Morphological & Cultural Characteristics of *E. coli* Isolated from the Brands of Bottled Water

The Gram stain revealed Gram negative non-spore forming rods, while the biochemical tests were positive for indole and methyl red, negative for Voges-Proskauer and citrate utilization tests and acidic butt and slant with cracks and no hydrogen sulphide produced on TSI. The Coliform organism isolated from the positive samples was *E. coli.*

3.4 MPN Index Distribution for the Bottled Water Samples

The bacteriological results indicated that 6/60 (10%) of the samples were contaminated with *E. coli* bacteria with the majority of the samples 54/60 (90%) having 0 MPN indices. The MPN index for the samples was positively skewed with a mean of 1.6 and a standard deviation of 6.549.

Table 3. Bacteriological results for positive samples (3) from brand H and J

Sample tested	Water brand	MPN Index per 100 ml
22	H	4
23	H	4
24	H	7
28	J	43
29	J	23

As shown in the Table 4. a one sample t test provided a statistically significant evidence to conclude that the contamination of the six samples was significant i.e., the mean MPN index was above the expected levels (n=6, t=2.586, p=0.0245, α = .05).

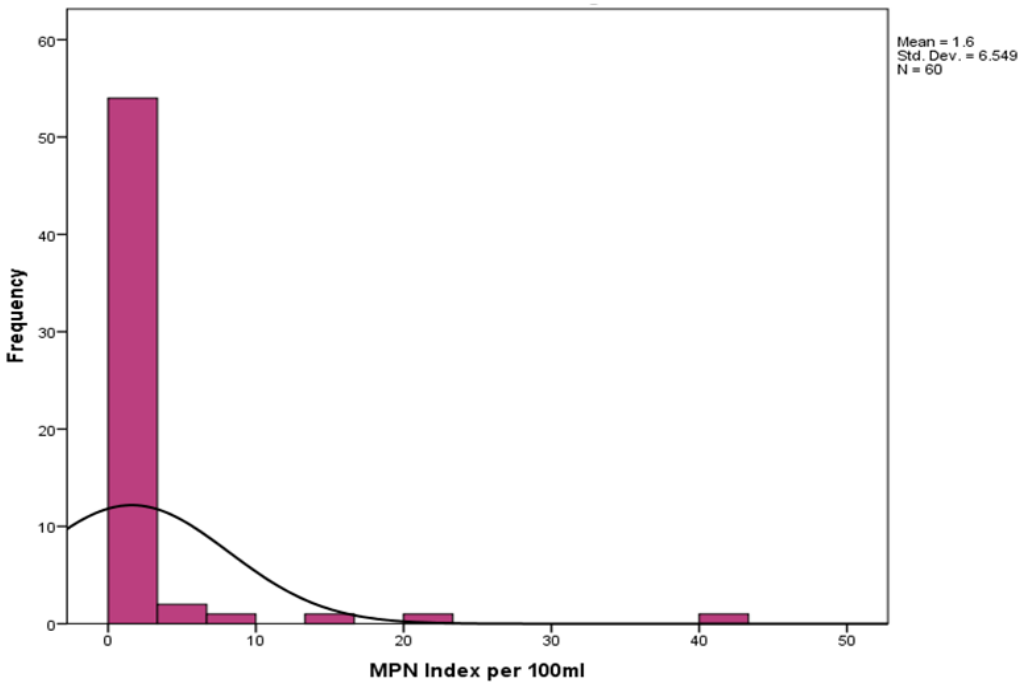

Fig. 1. Frequency and distribution of MPN index for the bottled water samples

Table 4. Results of one-sample t-test statistics

MPN value	t	df	Sig. (1-tailed)	Mean difference	95% confidence interval of the difference	
					Lower	Upper
MPN Index per 100 ml	2.586	5	.0245	16.000	0.10	31.90

Table 5. Results of a paired t-test for MPN

MPN index	t	df	Sig.(1-tailed)	Mean difference	95% confidence interval of the difference	
					Lower	Upper
MPN Index per 100 ml	1.892	59	.9706	1.600	-.09	3.29

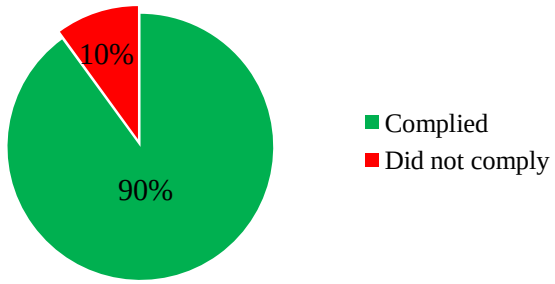

Fig. 2. Compliance to the WHO bacteriological standards

However, a paired t test failed to reveal a statistically significant difference between the mean MPN per 100 mL of brand H (mean=5.0, std. deviation=1.732) and J (mean=27, std. deviation=14.422), $t(4) = 2.623$, $p = .059$, $\alpha = .05$. The mean MPN per 100 ml for all the samples was statistically significant as compared to the normal expected MPN per 100 ml for drinking water (0 MPN per 100 ml) and was found not exceeding the acceptable limits (Z=1.892, p=0.9706, $\alpha = .05$)

3.5 Bacteriological Compliance with the WHO Regulatory Standards

The Fig. 2 shows the level of compliance to WHO bacteriological standards of the sampled bottled water brands. The study revealed that out of the 20 brands sampled, eighteen (90%) of the brands were complying to faecal and total coliform standards of zero MPN /100ml of bottled water, as opposed to two (10%) of the brands that did not comply to the standards.

4. DISCUSSION

The bacteriological state of all bottled water is a very important aspect that should be considered by all the bottling companies. Bottled water is rated to be good quality for drinking, but if it is not properly protected during bottling and transit, it could be contaminated [16]. Majority of the bottled water brands in this study came from the outcast of the study area with Nairobi being the largest external source. However, only three of the tested brands were manufactured locally. The study reveals that although most of the analyzed bottled samples were negative for faecal coliform, six of these were found positive for *E.coli*, an indicator of faecal contamination. The presence of coliforms and *E. coli* in bottled water samples does not only indicate the potential presence of pathogenic enteric microorganisms, but also question the efficiency and integrity of production system. The results of this study are in line with the studies conducted by Kassenga in Tanzania, Nyundu et al. in Zambia and Warburton et al. in Canada respectively [12,17,18,19].

In the current study, there is presence of *E.coli* coliforms in two brands of bottled water. One possible explanation for this observation could be the effect of using only one method of water purification. Majority of the brands in this study employed multiple barrier treatment which mostly included filtration, UV and Ozonation. It was reported by Marquis [20] that polyethylene terephthalate (PET) bottles as used by brands in this study promote greater bacterial growth than glass bottle. Consequently, the contaminated water samples from brand H and J with coliform does not necessarily confirm the presence of pathogens, but the probability of adverse effects occurring depends on the interaction of the organism with the immune system of the host.

Contamination of bottled water can originate from poor quality-water source or ineffectiveness of the treatment method applied. In addition, contamination during processing can occur due to inadequate sanitary facilities and practices, or

improper implementation of quality control programs at the critical control point (CCP). Furthermore, the improper storage of the bottled water provides favourable conditions for the bacteria to grow up to harmful levels. As per the national and WHO standards, bottled water should have total coliform and faecal coliform of 0 MPN per 100 ml. Only eighteen brands in the study complied with this regulation with two brands indicating MPN level >0 per ml of the water sample. Studies conducted by Ilyai et al. [21] and Muriithi [22] indicated that knowledge was associated with compliance to standards. The results indicated that companies, whose workers have low knowledge on standard, had reduced chances of complying with the set standards than those whose workers have high knowledge. The presence of the testing facility can also increase the level of compliance. WHO [23], reported that having a laboratory encourages the water bottling companies to test water regularly resulting to lower levels of contamination as the quality will be determined before it is dispatched for sale.

5. CONCLUSION

Bottled water is live and not sterile product. However, it should be pathogen free to ensure safety for human consumption. The aim of the study was to assess the bacteriological quality of bottled water brands in Kitale town. The results indicate that not all the bottled water sold in Kitale town was of good quality as perceived by the buyers. Although disease outbreaks due to contaminated bottled water are rare or never been reported in Kitale town, any contamination may pose a unique hazard because of the widespread distribution. The overall safety of bottled water is a Multifactorial practices that include effective treatment and continuous bacteriological screening, good manufacturing and sanitation practices that must be employed by the manufactures for quality drinking water.

5.1 Limitations of the Study

The study covered a small area of Kitale and did not consider all the bacteriological parameters that are important in making a comprehensive conclusion of the water quality due to financial limitation. In addition, only water brands that were registered with Kenya Bureau of Standards (KEBS) those were included in the study. Therefore, there is a possibility of leaving out some consumed brands that might not be registered with KEBS.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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