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Microbial Assessment of Water Used by the Residents of Kabianga in Kericho, Kenya from the Different Sources

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Authors' contributions

This work was carried out in collaboration between both authors. Author KD designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors KD and JM managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the research was to assess water quality from different sources used by residents of Kabianga.
Study Design: The research employed experimental design.
Methodology: Techniques used in this research project included test for indicator organism preferable *E. coli*, total viable count, and enumeration of filamentous fungi and yeasts. For bacteriological quality of the water, indicator organisms were used to indicate the presence of pathogenic microorganisms.
Results: The results showed that water obtained from springs and wells were safe for human usage and consumption as it was free from indicators of contamination. The presence of colorless colonies in well water was not considered hazardous because the colonies were well below the

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lethal colony number which is set to be a hundred colonies per plate. However the microbial populations identified showed that the river water was contaminated with *E. coli* an indicator of microbial contamination of water sources.

Conclusion: From the findings it can be concluded that river water is likely to be unsafe for use, especially to people who are immunocompromised as they may suffer from diarrheal related diseases. Spontaneous outbreaks related to water-borne diseases in these area, could also be attributed to increase in the number of indicator organisms. There is need for policy makers and implementers to initiate corrective measures to reduce contamination.

Keywords: Microbial; bacteriological; water quality; coliform.

ABBREVIATIONS

EMB : Eiosin Methylene Blue
TSI : Triple Sugar Iron
MIO : Motility Indole Orthidine
NA : Nutrient Agar
SDA : Sobouard Dextrose Agar

1. INTRODUCTION

The provision of safe and sustainable drinking water supply is one of the hallmarks of a successful society. Without safe drinking water, public health is always at risk and the economic wellbeing of the community cannot be realized. Pure and less polluted water are required for drinking purposes, whereas pure and quality water are needed for other home chores. Safe and clean water is a key need for wellbeing of persons and for their basic right. Clean, fresh and uncontaminated water is already a scarce resource in many rural parts of the world especially in developing nations [1]. In near future, it is projected to be more limited resource due to increasing population, modernization, urbanization, and effects of climate change [2]. Deteriorating quality of drinking water ascends from dumping and disposal of chemical compounds into the water catchment system through seepages of sewage wastes [3]. Lack of pure and clean water to many families and sanitation is an international fear since majority of people are still using contaminated water especially in poverty stricken areas. Nonetheless, third world countries, like Kenya, have agonized from a lack of pure, clean drinking water from improved catchments and sources to adequate sanitation services [4]. Due to this contaminations and pollutions, people are still reliant on unprotected water sources such as underground sources of water, rivers, streams and springs and bore wells. Such sources are open and thus attract lots of contamination through flooding (which carries wastes into them), birds, animals and human pollution [5]. The quality in terms of free from contamination is affected by an increase in microbial load and other anthropogenic activities in addition to other sources of pollution which can either be physical, chemical causes or changes to the quality of the receiving water body [6]. Contamination in drinking water has become a common problem in the world which has endangered human health. Moreover, many sources are near gullies in which open field defecation is common which finds their way in water bodies through flood-washing and this has affected the quality of water [7]. Lack of clean piped water for the residents of Kabianga has prompted them to seek other means to acquire water. The region, being in the rural area is thus characterized by rivers, wells, rain harvesters, and springs, which the residents use as their water sources. The springs and rivers in turn serve as direct water sources for the water vendors who bridge the gap and take water to residential homes. The quality of this water from the different sources is yet to be determined even though they believe that their water sources provide good quality. When it comes to wells especially, due to lack of education, they believe that the ground water is of a better quality than any other source including municipal water. As a result of population increase water scarcity and contamination has become a major problem in this area and especially to urban dwellers where contamination is more pronounced [7,8]. Water resources are key factors considered particularly for planning a sustainable socioeconomic development of an area where there are high populations of people [8]. This contamination has resulted to use of bottled water as it believed to contain fewer impurities. These shows deterioration in the quality of water resources accessible to many people. There is therefore need to initiate appropriate and timely corrective measures which pose a minimal negative impact on public health but increases the quality of water [9]. In the past many nations in arid and semi-arid regions have relied heavily on the desalination of

seawater to meet their growing needs as a way of reducing contaminations. Kenya for example is considered as endowed with freshwater from its fresh rivers and lakes, but many of such sources have been polluted. Thus in many parts of Kenya surface water sources (i.e. dams, lakes, and open water reservoirs) which are considered to be very common resources have been exploited for almost every use. However they are exposed to urban wastewater disposal from both industrial wastes and wastewater stations which has made the surface water resources to be highly contaminated [10]. The recurrent outbreaks of waterborne diseases in this areas and adjacent environments are the result of a direct discharge of such untreated domestic sewage water sources located beside local gutters near the water sources. Water taken from such sources is considered as of better quality than river water or other open water sources. If the water source has the soil which is fine-grained and its bedrocks do not have cracks, crevices, and bedding plants, which permit the free passage of polluted water especially within metropolitan zones means that water is a better source [10,11]. The assumption has been that natural, uncontaminated water from deep wells is clean and healthy, and this is has been usually true with regard to bacterial load composition. However, bacterial pollution of water sources occurs from watershed corrosion as well as drainage from sewage, swamps, or soil with a high humus content [12]. Such hazards are common particularly in limestone areas where underground leakages or fissures allows water to flow in moving streams without extensive purifications. Such supposed water sources cannot be used without carefulness for drinking purposes because of the intrinsic health risks that may occur [13,14]. The main objective of ministry of health in developing quality standards water for urban dwellers is focused on the acknowledgement, listing, identification and valuation of microorganisms related to waterborne diseases that are measured indicators of microbiological parameters and contamination of water. Such pointers are of great significance to evaluate the microbial situation of the observed water sources. The purpose of this research was to evaluate the bacteria load and its effects on the water quality. The geographical relations and location of many major urban water sources in the study area will also be considered. The findings of the research will be measured as a basis for water health policy decisions at the county and national administrative levels in Kenya.

2. MATERIALS AND METHODS

2.1 Sample Collection

About 1000 mL of fresh water samples were collected from households in Kabianga; 4 samples, Kapmaso/Tea farm 8 samples, and Chepnyogaa 8 samples. Random sampling method was used to collect samples from households. Two households were selected based on their source of water. This was achieved by interviewing them on where they drew their water from.

2.2 Determination of the Microbial Load

A serial dilution of 10^{-3} was done for all samples. 1 mL of the sample was transferred into a test tube containing 9 mL of distilled water; from this tube another 1 mL was drawn and transferred to another tube containing 9 mL of distilled water and another 1 mL was drawn and transferred to a tube with 9 mL distilled water. At this point the desired dilution was achieved. 1 mL of each of the final dilutions was drawn using sterile syringe (each sample) and transferred to a Petri plate which was then added to the Nutrient agar media, swirled and left to solidify. The plates were then incubated at 37℃ for 24 hours and observations recorded. Plates containing SDA amended with 2 mL of penicillin were incubated at 37℃ (for yeasts) and another set at 25 ℃ for 24 hrs and observations recorded.

2.3 Determination of the Bacteriological Properties

In the presumptive test, a series of lactose tubes were inoculated with measured amounts of the water sample to be tested. A series of 3 test tubes in a group of 3 were used to establish the presence of fecal coliforms. For double strength, 10 mL of sample was inoculated, then 1 mL of the sample to one set of single strength and lastly 0.1 mL to the last set of tubes. They were all incubated at 37 ℃ for 24 hours.

2.4 Confirmative Tests

MacConkey agar was streaked using a minute of an inoculum drawn from a test tube that would turn positive. A plate of MacConkey agar was used for one each of the inoculums used in the presumptive test and incubated at 37 ℃.

From MacConkey agar the pink colonies that grew were then transferred to a plate EMB agar

for the characteristic identification of *E. coli* the target fecal coliform.

2.5 Biochemical Tests

Using a sterile straight inoculating wire a minute of the sample was picked and used to inoculate TSI agar by first stabbing through the center of the media to the bottom and then streaking on the surface of the agar slant. The slant was then left with a loose cap incubated at 37℃ for 24 hours.

For MIO test; using a sterile straight inoculating wire the picked colony was inoculated into the media through a single stab through the media to the bottom and exited through that line. The tube was then sealed slightly and incubated at 37 ℃.

Simmon citrate agar green in color was also inoculated by streaking the slant using a picked minute colony and the tube incubated at 37 ℃.

3. RESULTS AND DISCUSSION

3.1 Sources of Household Water Used by the Residents of Kabianga

The results (Fig. 1) showed that 55% of the residents get their drinking water from the river. This source is majorly used by residents living in the Kapmaso-tea farm region and the market area of Chepnyogaa. 27% of residents get their water from open deep wells that are mechanically pumped. 11% of the residents obtain their water from the spring found in Kabianga location. 7% use both well water and river water; these are the residents living in the upper regions of Chepnyogaa and a small population in the lower sides of tea farm.

3.2 Microbial Load of Water

3.2.1 Total viable count

This gave a quantitative analysis of the total bacterial load in water samples (Tables 1, 2 and 3). The bacteria use the nutrient agar media components to grow and agar provides a support matrix. Since it is a pour plate technique all sorts of bacteria grow including anaerobic bacteria that appear as small colonies in the media. Anaerobic colonies that grow grew on the surface. This test however, assumed that each colony originated from a single cell that was in the sample. Since dilutions were made prior to inoculation, the diluent factor was put as a factor of the total count using a colony forming unit. The total coliform group has been selected as the primary indicator bacteria for the presence of disease causing organisms in drinking water [15,16]. It is a primary indicator of suitability of water for consumption. If large numbers of coliforms are found in water, there is a high probability that other pathogenic bacteria or organisms exist. The WHO and Kenya drinking water guidelines require the absence of total coliform in public drinking water supplies [17]. In this study, all sampling sites were not detected of fecal coliform bacteria. Tables 1, 2 and 3 shows the mean values of total coliform bacteria (bacterial load) in drinking water collected from the study area. As with many water pollutants, the behavior of fecal coliform and *E. coli* in the environment is complex. Factors affecting bacteria levels include seasonal weather, stream flow, water temperature, and distance from pollution sources, livestock management practices, wildlife activity, age of fecal material, sewage overflows, and rainfall. In addition, bacteria in stream sediments can survive for extended periods and even grow. Despite the potential for growth of bacteria in stream sediments, die-off is still the dominant process. Bacteria levels in lakes generally tend to be lower than those seen in streams and rivers. The types of bacteria sources over which the MPCA has the clearest authority are more likely to discharge directly to a stream or river than a lake. If the stream or river feeds a lake, bacteria levels are often much reduced before reaching the lake. Bacterial contamination in lakes is often associated with designated swimming beaches affected by users (e.g. children in diapers), which typically is dealt with by local health officials [18].

Table 1. River water samples

Sample ID	Total count	CFU/ML
1	37	3.7×10^4
2	21	2.1×10^4
3	55	5.5×10^4
4	52	5.2×10^4
5	53	5.3×10^4
6	51	5.1×10^4
7	61	6.1×10^4
8	48	4.8×10^4
9	69	6.9×10^4
10	51	5.1×10^4
11	40	4.0×10^4

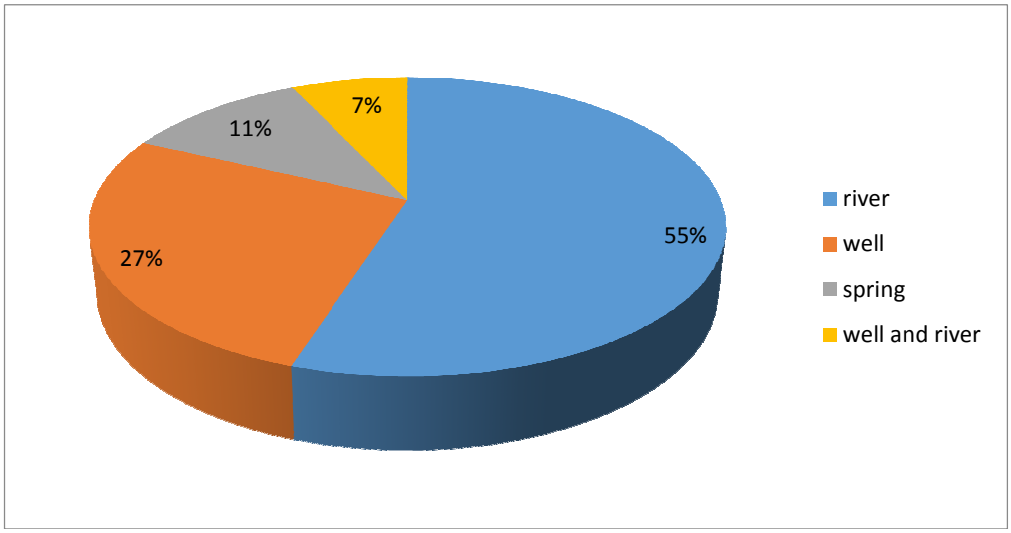

Fig. 1. The percentage population using different water sources

Table 2. Well water samples

Sample ID	Colony count	CFU/ML
1	31	3.1×10^4
2	27	2.7×10^4
3	22	2.2×10^4
4	35	3.5×10^4

Table 3. Spring water samples

Sample ID	Colony count	CFU/ML
1	6	6.0×10^3
2	4	4.0×10^3
3	5	5.0×10^3
4	3	3.0×10^3
5	3	3.0×10^3

3.2.2 Bacteriological analysis of water for portability

The presence of coliforms in the media was indicated by gas production and change in color of the media from yellow to turbid pale. This is because coliforms have the ability to ferment the lactose in the media raising the pH and producing carbon IV oxide which are indicated by the change in color and presence of gas bubbles in the Durham tubes respectively. Out of the twenty samples that were tested for the presence of coliforms, 15 samples showed positive results. 5 samples showed neither gas production nor change in media color [17,18]. Other four showed change in media color but no gas production (false negative/positive (Table 4). Chemical contaminants occur in drinking water throughout the world which could possibly threaten human health and this could explain why water borne diseases have been reported in this area [18].

3.3 Confirmative Tests for Presence of Coliforms

In this test out of the 15 test tubes that were confirmed in this test, 11 showed flat pink colonies and pink Mucoid colonies growing on MacConkey media. The other 5 had pink Mucoid colonies only. MacConkey agar acts as a selective and a differential media. It actively selects Gram negative and inhibits the growth of Gram positive bacteria as a result of bile salts and as a differential media it differentiates between lactose fermenters and non-lactose fermenters. Lactose fermenters are characterized by localized pink colonies due to production of mixed acids byproducts which reduce the pH of the media while none lactose fermenters by colorless colonies, this could be the reason for the observed changes. In the same media *E. coli* the target coliforms grew with a distinctive flat pink colonies while other coliforms like Enterobacter species have Mucoid colonies. The pink colonies were then cultured in EMB agar to confirm the presence of *E. coli* (Plates 1 and 2).

3.4 EMB Agar Test

The 11 samples with pink colonies in MacConkey agar were inoculated in EMB agar to confirm the

presence of *E. coli.* EMB agar is a sensitive media and is both a selective and differential media used to isolate fecal coliforms. The presence of the fecal coliform *E. coli* was indicated by metallic green sheen that tended to spread (not localized) (Fig. 1). The metallic sheen was due to the metachromatic effects of the dyes in the media, flagella movement of the bacteria and an indicator of vigorous lactose/sucrose fermentation. All the 11 samples showed the characteristic metallic green sheen and hence *E. coli* was confirmed to be present. The sheen was then bio-typed in an IMViC series of tests. This was an indication of high level contamination by bacteria since anthropogenic activities affect the quality of water. The current standard is for fecal coliform: A monthly average of 200 colony-forming units per 100 milliliters of water (cfu/mL), and a maximum of 2,000 cfu/100 mL not to exceed 10 percent of samples collected in a month. The proposed rule revision will replace fecal coliform with an *E. coli* standard of 126 cfu/100 mL monthly average, and 1,260 cfu/100 mL maximum [18].

The media used in these tests were: Triple Sugar Iron TSI, Motility media, Indole/motility and Simmon citrate agar. This could be because different strains *of E. coli* react differently in biochemical media and thus possible to identify the number of strains available.

3.4.1 TSI

It was used to differentiate microorganism on their ability to ferment the three sugars or to produce hydrogen sulfide gas. Prior to inoculation, the media was red in both slant and butt. The media changed from red to yellow in both slant and butt upon inoculation, this was inferred as positive. This meant that the microorganism could ferment all the three sugars. A negative slant (red) indicated that the organisms only fermented one sugar i.e. dextrose.

3.4.2 MIO

It was used to test motility, Indole production and ornithine-decarboxylase. Motility was exhibited by growth of the inoculants from the line of inoculation outwards. Tryptophan, present in the basal media, was degraded by organisms that possess the enzyme tryptophinase. Degradation of tryptophan produced indole. The kovac'sreagent was added to the media (a compound called p-dimethyllaminobenzaldehyde) to reacts with indole to form a red quinodial compound which indicated a positive Indole test. This caused an alkaline shift turning the media from purple to dark purple. This happens with *E. coli*. For other coliforms like *Klebsiella* they ferment dextrose

Table 4. Most probable number

Sample ID	Number of tubes showing positive reactions			MPN index
	3 tubes of 10 ml each	3 tubes 01 ml each	3 tubes of 0.1 ml each	
1	3	3	3	1100
2	3	3	3	1100
3	3	3	3	1100
4	3	3	3	1100
5	3	3	3	1100
6	3	3	3	1100
7	3	3	3	1100
8	3	3	3	1100
9	3	3	3	1100
10	3	3	3	1100
11	3	3	3	1100
12	3	3	3	1100
13	3	3	3	1100
14	3	3	3	1100
15	3	3	3	1100
16	0	0	0	3
17	0	0	0	3
18	0	0	0	3
19	0	0	0	3
20	0	0	0	3

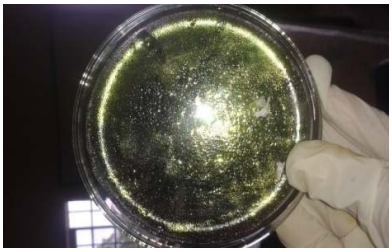

Plate 1. Cultured colonies in EMB agar

Plate 2. Pink colonies in EMB agar

Table 5. Biochemical reactions

Sample ID	Triple sugar iron				MIO			CITRTATE
	BUTT	SLANT	GAS	H_2S	MOT	IND	ORN	
Control	-	-	-	-	-	-	-	-
1	+	+	+	-	+	+	+	-
2	+	+	+	-	+	+	-	-
3	+	+	+	-	+	+	+	-
4	+	+	+	-	+	+	+	-
5	+	+	+	-	+	+	+	-
6	+	+	+	-	+	+	+	-
7	+	+	+	-	+	+	+	-
8	+	+	+	-	+	+	+	-
9	+	+	+	-	+	+	+	-
10	+	+	+	-	+	+	+	-
11	+	+	+	-	+	+	+	-

but do not produce decarboxylase acid hence causes the media to change to yellow throughout or a yellow with a purple top indication that that environment is acidic.

3.4.3 Simmon citrate

This tested the ability of an organism to utilize citrate as the sole carbon source (Table 5). Organisms that can utilize citrate do so by using the enzymes citrase or citrate-permease and the gives out a blue colored slant due to a raise in pH from acidic to alkaline.

4. CONCLUSION

From the findings in this research it can be concluded that water obtained from springs and wells was free from indicators of contamination. There was presence of colorless colonies in well water which were well below the lethal colony number which is set to be a hundred colonies per plate. The water from these sources can therefore be considered relatively safe. However boiling of the water or its treatment should be recommended before use.

River water was found to be contaminated with *E. coli* an indicator which makes it unsafe for use. The concentration of *E. coli* was found to be higher as water disturbance during the day increased. Further studies need to be conducted on the waters in order to ascertain the level of contamination.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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