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Water Quality and Distribution of Pathogenic Microorganisms along Kabianga River in Kericho County, Kenya

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Authors' contributions

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

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ABSTRACT

Background: Safe and clean water is of major concern to mankind because it is directly linked with the health and their wellbeing. The disposal of different kinds of pollutants and nutrients through sewage, agricultural runoff and industrial effluents into rivers and water bodies effect a sequence of changes in its physicochemical characteristics. Kabianga River is known for provision of clean drinking water, a habitat for many aquatic plants and living organisms and irrigation water to farmlands. Excessive industrialization and consequent urbanization has led to several problems of water quality management of the river.
Aim: This research was aimed at assessing the quality of Kabianga river by ascertaining the levels of microorganisms and if it meets the local and international microbiological standards.
Study Design: The study used experimental design.
Methodology: The water samples were collected during rainy and dry seasons. Standard bacteriological techniques was used to describe bacteria content from water samples.

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Results: The results indicates that water is highly contaminated with human pathogenic bacteria. The most dominant bacteria in both seasons were *Escherichia coli, Salmonella, Shigella, Proteus* and *Pseudomonas aeruginosa*.
Conclusion: From the findings it can be concluded that the water from this river is not fit for human consumption or for domestic uses. Therefore regular monitoring of the water microbiological quality and public health education should be carried out to sensitize the residence on the dangers of using the polluted water.

Keywords: Water quality; pathogens; microbiological; coliforms; contamination.

ABBREVIATIONS

MPN: Most Probable; EMB: Eosin Methylene Blue; XLD: Xylose Lysine Deoxy cholate.

1. INTRODUCTION

Microbiological pollution of domestic water has long been a concern and a threat to public health. Such microorganisms' causes water pollutions, according to WHO report on safety of water [1]. Some of such microorganisms are bacteria while others are viruses or protozoa. Coliforms bacteria for example enters water supplies from the direct dumping of waste into rivers, streams or lakes, or from surface runoff, pastures, feedlots, septic tanks, and sewage plants into streams or groundwater [2]. They affect the quality of fresh water without which sustainable development will not be possible [1, 3]. They can also get their way into individual homes through backflow of water from a polluted source, carbon filters, or leaking well caps that allow dirt and dead organisms to fall into the water. The greatest risk from microbes in water is associated with consumption of drinking-water that is contaminated with human and animal excreta, although other sources and routes of exposure may also be significant. In fact according to WHO, 2004, infectious diseases caused by pathogenic bacteria, viruses and parasites are the most common and widespread health risk associated with drinking-water. The public health burden is determined by the harshness of the infection related with pathogens, their contagion and the population unprotected. Failure in water supply systems may result to large-scale pollution and possibly to determinate disease incidences. Other breaks and low-level, potentially repeated pollution may cause a noteworthy periodic disease, but is unlikely to be associated with the drinking-water source by public health observation. Many of such pollutions have also been described to be lethal, mutagenic, and carcinogenic and tumorigenic which means a more new tactic needs to be followed [2,3].

The guidelines stipulates, that fecal coliforms (FC) should not exceed 103 per 100 mL of water to be used in irrigation of crops that are eaten uncooked ,sports fields and public parks in unrestricted regions (WHO,2004). Environmental Protection Agency (EPA) is stricter and requires zero (0) FC/100 mL of water to be used in irrigation of crops not commercially processed including crops eaten raw while Kenya Bureau of Standard limits the concentrations of *Escherichia coli, Shigella, Pseudomonas aeruginosa* or coliforms to be detectable in 250 mL of drinking water. Several water sources in Kabianga especially Kabianga River has been polluted to the extent that the water usage from this source pose a threat to people's health. It has also been affected by several changes in response to variables such as increases in human and animal populations, intensifying use of wastewater, changes in lifestyles and medical mediations, population measure and travel and careful pressures for new pathogens and mutants or recombination of existing pathogens. The protection and immunity of individuals also differs greatly, whether acquired by contact with a pathogen or influenced by such factors as age, sex, state of health and living conditions which is a major boost in treatment of such ailments. However managements of such problems do exist for microbial pollution, but, it is important to understand and assess what is present before treatment is begun. Microbial water contamination and its quality may contrast swiftly and extensively depending on such major factors mentioned. Short-term peaks in pathogen concentration may increase disease risks considerably and may also prompt occurrences of waterborne disease [4,5]. Such outbreaks of

disease may affect large numbers of persons. Thus the first priority in developing and applying quality controls on drinking water should be the control of such sources of contaminations. Available evidence also suggests that domestic drinking water can contribute to background rates of disease in non-outbreak situations, and control of drinking water quality should therefore also address waterborne disease in the general community. This has led to attention being concentrated on end product. However, such tactics are progressively observed as inadequate and not sufficient to prevent occurrences of sickness among many people. Research has further shown that more attention has been paid to preventing illness from source to tap rather than the causal agents themselves [4, 5]. Besides, it has been shown that poor bacteriological water quality is not safe for aquatic life and other ecosystem function [5, 6, and 1]. It can therefore be deduced that people's health and environmental health are obliquely related, mostly if pathogenic risk are assessed along the source-to-tap spectrum [7, 8]. An inclusive thoughtful and well calculated understanding of the dangers and risks of both existing and possible dangers of polluted water versus its quality should be assessed. This can be achieved through assessment of the entire water supply system. The concept 'source-to-tap', is where a high value source, in combination with most effective treatments and distribution can yield water which is safer for consumption. Therefore as a milestone of water quality risk assessment [9,10], the source-to-tap basis has been mobilized for use with a focus on human health. These interventions which include the entire source-to-tap gradient are most probable to successfully report not only point-source contamination of water but also the more composite influence of non-point sources of water pollutants within a given watershed [11]. Complete risk valuation and administration is critical for both drinking water provision [4,12, and 13] as well as larger environment protection [7]. Thus risk managing in a source to tap framework is believed to be serious given the appreciation that hazards are countless and resources to deal with them are inadequate [14,15,12 and 9]. This research was therefore aimed at characterization and determination of microbial concentration in Kabianga River during the rainy and dry seasons to ascertain its quality for domestic uses. This will help both the policy makers and implementers to ascertain the safety of the water and hence come up with a better solution or recommendations which can alleviate or reduce human death due to water contamination and microorganisms pollutions.

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples were collected in 250 mL sterilized bottles from Kabianga River in Kericho West sub-county, Kenya. They were then analyzed in the microbiology laboratory.

2.2 MPN Test

The number of coliforms in water were determined by statistical estimation. Most probable number (MPN) test was used. This test involved a multiple series of fermentation tubes which were divided into three parts: The presumptive, Confirmed and Completed test. In presumptive test, water samples were added to lactose broth fermentation tubes and then topped up to a volume of 10 mL. The tubes were incubated at 35ºC and then observed after 48 hours for evidence of gas production. In the confirmed test samples that showed growth and gas production were streaked onto Eosin methylene blue (EMB) agar plates which prevents growth of Gram-positive organisms. Coliforms which are Gram- negative produced acid. Under acidic conditions the Eosin and methylene blue dyes were absorbed by the organisms of a colony. In the completed test, the colonies formed were inoculated into lactose broth and agar slants. The production of acid and gas in the lactose broth confirmed the identity of Gram negative while non-spore-forming rods from slants constituted a positive completed test.

2.3 Isolation and Identification of Coliforms

The media was first prepared and dispensed in the plates. Samples from the positive completed test were then cultured on the EMB and further sub-cultured in MacConkey, *Salmonella, Shigella* media and Xylose Lysine Deoxycholate agar (XLD). Observations were then made.

2.4 Motility

The colonies from EMB, were streaked onto the media and growth was monitored. If growth occurred on the stabs, they were considered as Non -motile colonies, but if growth occurred along the stab and were washed out from the streaked area, they were considered motile.

2.5 Morphological and Biochemical Tests

Microorganisms' morphologies were classified by direct examination using alight microscope and the staining characteristics by gram stain. This test classified microorganisms into Gram negative and morphologically as *Bacilli.*

2.6 Oxidase Test

These was used to classify microorganisms into Gram negative or Gram positive bacteria. The oxidase discs were moistened using distilled water. A colony was then picked from EMB plate and morphologically characterized as either cocci or rods.

3. RESULTS AND DISCUSSION

3.1 Presumptive Test

The results of the presumptive tests are tabulated in Table 1. They reveal that, there was growth in almost all the tubes. This was a positive presumptive test with higher MPN being 460. This corresponds to MPN of lactose fermentation. This bacteria were presumed to be fecal coliform. During the rainy seasons, all the tubes had higher MPN with highest being 1100 (Table 2).

3.2 Confirmed Test

In the confirmed tests coliforms which were Gram- negative produced acid. Under acidic conditions the Eosin and methylene blue dyes were absorbed by the organisms of a colony.

3.3 Completed Test

In this test, the colonies formed were inoculated into lactose broth and agar slants. These resulted into production of acid and gas in the lactose broth indicating the presence of Gram – negative. Non-spore-forming rods from slants constituted a positive completed test.

3.4 Eosin Methylene Blue Agar (EMB)

There was growth of different colonies in the media .The colonies were pink with dark center (Fig. 1) and mucoid with entire margin greenish. Since EMB is a selective media for gram negative bacteria the bacteria which fermented

Table 1. Presumptive test dry season

Sample points	Number of positive tubes	3 tubes BSLB 10 mL	3 tubes SSLB 1 mL	3 tubes SSLB 0.1 mL	MPN
Sample1	T1	3	3	1	460
	T2	3	3	1	460
	T3	3	2	1	150
Sample 2	T1	3	3	0	240
	T2	3	2	0	93
	T3	3	2	1	150
Sample 3	3	3	3	1	460
	3	3	3	1	460
	3	3	2	1	150

Table 2. Presumptive test rainy season

Sample points	Number of positive tubes	3 tubes BSLB 10 mL	3 tubes SSLB 1 mL	3 tubes SSLB 0.1 mL	MPN
Sample1	T1	3	2	2	210
	T2	3	3	2	1100
	T3	3	2	2	150
Sample 2	T1	3	3	2	1100
	T2	3	2	0	93
	T3	3	2	2	210
Sample 3	3	3	3	2	1100
	3	3	3	2	1100
	3	3	3	2	1100

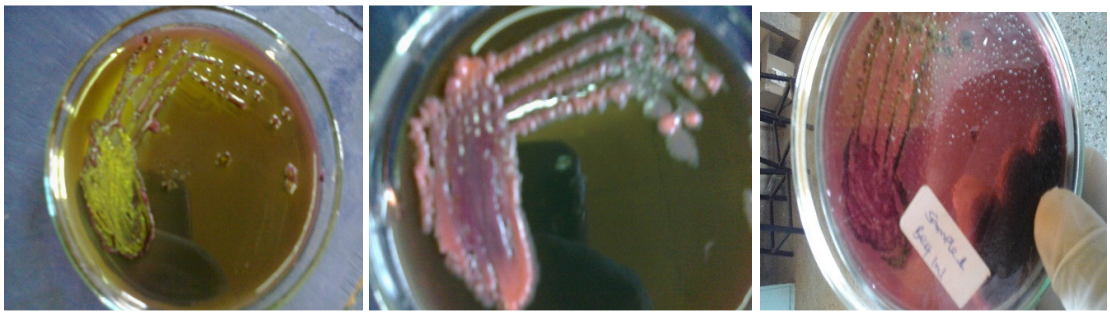

Fig. 1. Growth of *E. coli* on different media

lactose in the medium formed colored colonies, while those that did not ferment glucose appeared as colorless colonies. EMB agar was used to distinguish coliforms and fecal coliforms that signal the possible pathogenic microorganism contamination in water samples. EMB was also used to differentiate in the colon-typhoid - dysentery group*: Escherichia coli* colonies grew like a metallic sheen with dark center while non-lactose fermenting gram negative bacteria appeared pink.

3.5 Mac Conkey Results

The colonies that were transferred from EMB were grayish small mucoid with entire margin appearing pink. Large mucoid had entire margin dry with rough pink colonies. However in another plate the pink colonies (both small and large) had the same characteristics. MacConkey is a selective media for isolating gram-negative bacteria and other enteric bacilli. The agar was used distinguish those Gram negative bacteria that could ferment glucose from those that could not. Lac bacteria such as *Escherichia coli* and *Klebsiella* produced acid which lowered the pH of the agar to 6.8 and resulted in appearance of pink colonies. Non-lactose fermenting bacteria *Salmonella, Proteus* species, *Pseudomonas aeruginosa* and *Shigella* did not use lactose but used peptone water. This formed ammonia, which raised the pH of the agar and lead to formation of white/colorless colonies on the plate. Some organisms, especially *Klebsiella* produced mucoid colonies.

3.6 Xylose Lysine Deoxycholate Results

The pink dark centered colonies from EMB plate, appeared white mucoid. The media turned yellow. XLD is a selective medium used in the isolation of *Salmonella* and *Shigella* species in water .It had a pH of 7.4 leaving it with a bright pink or red appearance due to the indicator phenol red, sugar fermentation. *Salmonella* fermented the sugar xylose to produce acid *Shigella* colonies which remained red. *Salmonella* metabolized Thiosulphate to produce hydrogen sulphide which lead to the formation of colonies with black centers. This differentiated them from similarly colored *Shigella* colonies.

3.7 *Salmonella shigella* Results

The colonies from XLD plate that appeared white were transparently large in SS media. Dark centered pink colonies in XLD plate appeared transparent mucoid colonies (Fig. 2).

Fig. 2. *Salmonella shigella* plates showing growth of microorganisms

The colonies that appeared pink in EMB, turned black mucoid colonies indicating the presence of *Salmonella.* The pink colonies indicated the presence of *Shigella*. The colonies in MacConkey were pink with a characteristic smell. Inhibition of Gram positive microorganisms was obtained by bile salt mixture. Members of the genus *Salmonella* did not ferment lactose but produced hydrogen sulphide gas. The resulting bacteria colonies appeared colorless with black centers. *Shigella* did not ferment lactose or produced hydrogen sulphide gas (Fig. 3).

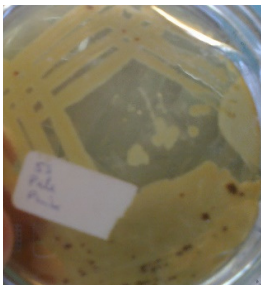

Fig. 3. Plates with growth of *Salmonella*

3.8 Motility

The samples that tested positive in the presumptive test, were cultured in motility media. The samples from points 1and 3 showed positive results for motility. Motile organisms extended from the stub line and produced turbidity throughout the media. Non-motile organism from sample point 2 grew only along the stub line and the surrounding media was clear. The motile organisms were from sample point 1 and 3 during rainy season. This was attributed to the fact that more runoffs enter the river at these points and that the population around these point are high compared to sample point 2. The isolated organism was identified as *Proteus* spp.

3.9 Morphological and Biochemical Tests

Microorganisms displayed diversity of cell arrangements. Microorganisms' morphologies were classified by direct examination with the light microscope and the staining characteristics by gram stain. They were classified as Gram negative and morphologically as *Bacilli.*

3.10 Biochemical Tests

The tubes which were inoculated with colonies from SS had a blue slant. The small mucoid colonies was obtained from XLD plate. The only organism that utilized the citrate and not the sugar in TSI was *Pseudomonas aeruginosa.* Simmons citrate blue was a positive citrate test while green was a negative (no growth) (Figs. 4 and 5). There was increase in pH causing the color change in the bromothymol blue indicator to blue .Under neutral conditions the medium remained green color, the color change to blue is useful because the growth on Simmons citrate agar is often limited and would be hard to observe if it were not for the color change. Bacteria that develops in such media are *Salmonella* and P*rovidencia.*

Fig. 4. Positive tube

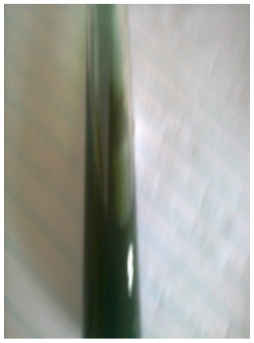

Fig. 5. Negative tubes

3.11 Triple Sugar Iron Tests

The colonies that were transferred from XLD plate appeared red indicating that there was no utilization of the sugars. The only organism that do not utilize sugars is *Pseudomonas aeruginosa.* Others were able to utilize either glucose or sucrose to appear as red slant and yellow butt or yellow slant with yellow butt. Triple sugar iron test (tests the ability of microorganisms to ferment sugar and produce hydrogen sulfide) was used as selective identification of enteric bacteria including *Salmonella* and *Shigella.* Blackening of the butt was due to the production of hydrogen sulfide resulting from utilization of glucose only. Carbon dioxide production was recognized as bubbles of gas between the agar and the wall of the tube or within agar itself. The carbon dioxide production was sufficient to split the agar into two or more sections. Out of all the microorganisms isolated, none of them produced hydrogen sulphide.

3.12 Oxidase Test

The small dry pink colonies from SS plate indicated oxidase positive test. There was a color change to dark blue/purple. This means the bacterium contains cytochrome producing intracellular oxidase enzyme. This oxidase enzyme catalyzes the oxidation of cytochrome c turning reagent color to blue/purple. Organisms lacking cytochrome as part of their respiratory chain do not oxidize the reagent, leaving it colorless within the limit of the test, and are oxidase negative.

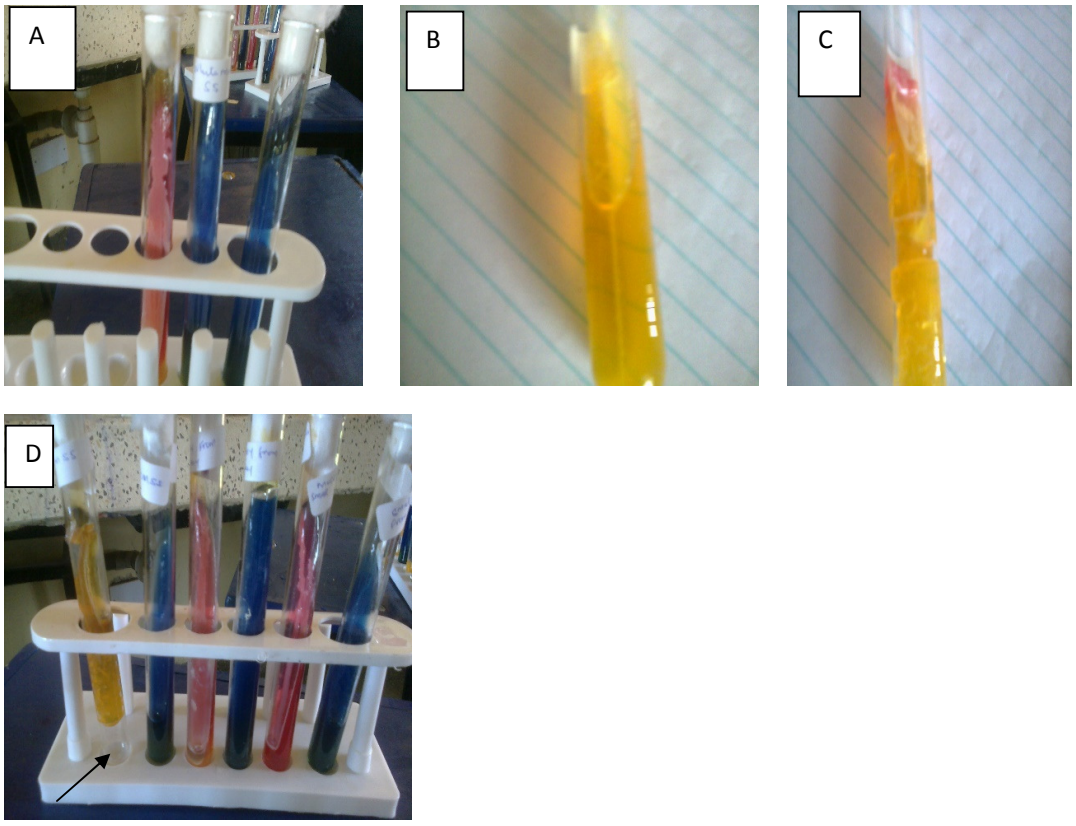

Fig. 6. Oxidase tests

KEY: *A- Tube 1 from left red slant yellow butt, no gas production only glucose utilized -organisms Salmolla and Shigella.*
B-Yellow slant yellow but no gas production all sugars utilized organism-Serratia
C-Yellow butt red slant there was gas production only glucose utilized organism-Escherichia,Shigella
D-Tube 1 from left Yellow butt yellow slant gas production-organism Enterobacter, Klebsiella.
D-Tubes 3 and 5 from left- No sugar was utilized entire tube red that is red slant red butt-organism Pseudomonas aeruginosa.

Table 3. Bacterial types in Kabianga River during dry season

Bacterial type	Upstream	Midstream	Downstream
Escherichia coli	$6.8x10^3 \pm 1.2^x\ 10^3$	$5.8x10^3 \pm 1.2^x\ 10^3$	$6.8x10^3 \pm 1.2^x\ 10^3$
Salmonella spp	$1.3x10^3 \pm 1.2^x\ 10^3$	$1.3x10^3 \pm 1.1^x\ 10^3$	$1.3x10^3 \pm 1.1^x\ 10^3$
Shigella spp	$1.3x10^2 \pm 1.2x10^2$	$1.1x10^2 \pm 1.1x10^2$	$1.3x10^2 \pm 1.1x10^2$
Pseudomona aeruginosa	$1.2x10^2 \pm 1.1x10$^2	$1.1x10$^2 $\pm 1.1x10^2$	$1.2x10^2 \pm 1.1x10^2$
Proteus mirabilis	$1.1x10^2 \pm 1.1x10^2$	$0.9x10^2 \pm 1.1x10^2$	$1.1x10^2 \pm 1.1x10^2$
Klebsiella spp	$1.1x10^2 \pm 1.1x10^2$	$0.9x10^2 \pm 1.1x10^2$	$1.1x10^2 \pm 1.1x10^2$
Enterobacter spp	$1.1x10^2 \pm 1.1x10^2$	$0.9x10^2 \pm 1.1x10^2$	$1.1x10^2 \pm 1.1x10^2$

There was high concentration of *E.coli* upstream and downstream than midstream during the dry season while there was low concentration of *Klebsiella* during the same season (Table 3). The concentration of microorganisms increased substantially during the rainy season (Table 4). This could be attributed to the surface run offs during the rainy seasons which carries many of the pathogens downstream.

Table 4. Bacterial types in Kabianga River during Rainy season

Bacterial type	Upstream	Midstream	Downstream
Escherichia coli	$9.8x10^3 \pm 1.2^x\ 10^3$	$5.8x10^3 \pm 1.2^x\ 10^3$	$9.8x10^3 \pm 1.2^x\ 10^3$
Salmonella spp	$2.3x10^3 \pm 1.1^x\ 10^3$	$1.3x10^3 \pm 1.1^x\ 10^3$	$2.3x10^3 \pm 1.1^x\ 10^3$
Shigella spp	$2.3x10^2 \pm 1.1x10^2$	$1.1x10^2 \pm 1.1x10^2$	$2.3x10^2 \pm 1.1x10^2$
Pseudomona aeruginosa	$2.2x10^2 \pm 1.1x10^2$	$1.1x10^2 \pm 1.1x10^2$	$2.2x10^2 \pm 1.1x10^2$
Proteus mirabilis	$1.1x10^2 \pm 1.1x10^2$	$0.9x10^2 \pm 1.1x10^2$	$1.1x10^2 \pm 1.1x10^2$
Klebsiella spp	$1.1x10^2 \pm 1.1x10^2$	$0.9x10^2 \pm 1.1x10^2$	$1.1x10^2 \pm 1.1x10^2$
Enterobacter spp	$1.1x10^2 \pm 1.1x10^2$	$0.9x10^2 \pm 1.1x10^2$	$1.1x10^2 \pm 1.1x10^2$

4. CONCLUSSION

From the results it can be concluded that the microbiological quality of Kabianga river water was high above the compliance level of National standards and the World Health Organization (WHO) guidelines for drinking water. The water from this river is not potable, and poses a health risk to communities that rely on the river as the primary source of domestic use. This is indicated by higher concentration of bacteria during dry and rainy seasons. There was high concentration of *E. coli* upstream and downstream than midstream during the dry season while there was low concentration of *Klebsiella spp* during the same season. Controlling or eliminating contamination or pollution of public water by microbial sources, goes a long way toward simplifying treatment and reducing costs associated with a contaminated supply. Therefore an effective protection program must be put in place to address a variety of sources of microbial contamination along rivers and other sources of water or advice people on the dangers of using the water.

CONSENT

It is not applicable.

ETHICAL CONSIDERATION

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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