

## Original Research Article

# Determination of microbial contamination of water used in the household for domestic purposes in Mombasa County, Kenya

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### ABSTRACT

**Background:** Globally, 45% of the global population, showing that the compliance level is very low in most developing countries. In Kenya, 10% of all deaths caused by waterborne illnesses are due to water scarcity and poor sanitation. Mombasa County is facing a major problem in the provision of domestic water to its residents, thus causing a water shortage.

**Methods:** Descriptive cross-sectional study was conducted in Mombasa County between November 2020-March 2021. 55 water samples were randomly collected for analysis of microbial contamination. Using stata for analysis, t-test was calculated to determine the relationship with  $p < 0.05$ .

**Results:** TC mean for boreholes was  $\pm 761.68$  CFU and tap water was  $\pm 712.23$  CFU. There was a significant difference in means between the two groups for TC ( $t=7.38$ ,  $df=41.94$ ,  $p=0.000$ ). Faecal coliforms (FC) for borehole and tap water was  $\pm 739.52$  CFU and  $\pm 115.42$  CFU respectively. FC showed a significant difference between the two groups ( $t=3.74$ ,  $df=36.84$  and  $p=0.0003$ ). HPC means for borehole and tap water of water were  $\pm 7730.62$  CFU and  $\pm 4092.12$  CFU respectively. There was no significant difference in means for HPC for the two groups ( $t=1.73$ ,  $df=53$  and  $p=0.0445$ ). 34.3% ( $n=12$ ) and 20% ( $n=4$ ) of boreholes and tap water were contaminated with salmonella respectively. None of the water samples collected had *Shigella*.

**Conclusions:** All borehole water samples stored in the household storage containers were more contaminated than tap water, hence not fit to be consumed in the household.

**Keywords:** Microbial contamination, Household storage containers, Water quality

### INTRODUCTION

Globally, 2.2 billion people face challenges accessing potable water, while 4.2 billion people face challenges accessing improved sanitation.<sup>18</sup> For drinking water to be regarded as potable it should be free from microbial pathogens. Several developing countries face chronic shortages of freshwater accessibility or heavily polluted water resources.<sup>15</sup> The 88 % of deaths in the developing world have been attributed to the use of unsafe drinking water.<sup>2</sup> In most deprived communities, underground water is preferred as the main source of drinking water supply

used in the household.<sup>3</sup> The presence of faecal coliforms indicates faecal contamination, thus showing the presence of harmful bacteria.<sup>4</sup> *E. coli*, *Shigella* specie (spp), and *Salmonella* spp. are the most common enteric bacteria microorganisms in waterborne diseases.<sup>10</sup> *Salmonella* is one of the critical global causes of diarrheal diseases.<sup>17</sup> The evaluation of water consumed in the household for coliform and other thermotolerant bacteria is important in determining water quality. The three sub counties namely Mvita, Kisauni and Nyalı, have the highest diarrheal cases recorded in the county, hence the need to determine water quality. There are no studies that have been

conducted on water quality in the households in these three sub counties hence the need for this study.

The objective of the study was to determine the microbiological quality of water collected in 55 households (HHs), where total coliforms, faecal coliforms, HPC, *Salmonella* and *Shigella* were isolated.

## METHODS

This was a descriptive cross-sectional study that was conducted in Nyali, Mvita and Kisauni sub county. The sub-counties were purposively selected because of the high cases of diarrheal reported in the DHIS 2014, shortage of water, the extensive use of borehole water in households by the residents and the high dependency on unimproved sources of water for drinking and domestic purposes. A simple random method was adopted to sample HHs where water samples were collected. 55 water sample were collected (35 borehole and 20 tap water) were collected in the household storage container. The water samples from storage containers were collected using clean and closed sterile 500 ml polyethylene glass bottles. While filling the bottle, one inch was left from the top to allow air to occupy space. The bottle was capped immediately and placed in a cooler box after labelling. The samples collected were labeled with the date of collection, location and coded for identification, then transported in a cooler box at low temperatures (4°C or lower) to the Kenya government chemistry lab for analysis. Samples analyzed within 24 hours of sampling.

### *Selection criteria*

Households that stored water in the household storage containers were the only ones considered in the study. They should have been the residents of the study area for more than two months.

### *Statistical analysis*

Stata was used for analysis. T test was used to find the mean values, standard deviation (SD) and p values of water quality and determine if there are significant relationship.

Percentages was used to determine the concentration of *Salmonella-Shigella* in the water sampled.

### *Analysis of microbial parameters*

The microbial quality was determined by analysing for the presence of total coliforms, faecal coliforms, heterotrophic bacteria. Most probable Number method and presence of faecal coliforms, pour plate count for heterotrophic bacteria and *Salmonella/ Shigella* species using selenite F method. The 55 samples of water collected in the household water containers that were randomly collected were analysed in this research.

### *Multiple tube fermentation method was used to isolate total coliforms and faecal coliforms*

The tests were conducted in 3 stages namely presumptive, confirmed and completed tests which used different media and temperatures and the results are interpreted in terms of most probable number (MPN).

#### *Presumptive test (Preliminary test)*

MacConkey broth (40 gm of MacConkey 1L of distilled water) was prepared. The 9 ml of MacConkey broth was dispensed into nine Mactney bottles for each dilution ( $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$ ). Nine ml of distilled water was also dispensed in the Mactney bottled to be used for serial dilution. An inverted durham was inserted to each of the Mactney bottles. Sterilization was done by auto-claving the MacConkey media the dilution water and the tips at a temperature of 121°C for fifteen minutes, cooling and labelling was then done. A serial dilution was prepared by transferring 1ml of the sample to the dilution  $10^{-1}$  bottle, then 1ml from the dilution of  $10^{-1}$  was transferred to bottle of dilution  $10^{-2}$ , 1 ml of the sample was inoculated to the first three actney bottles containing the broth making dilution to  $10^{-1}$ , 1 ml of the diluted sample from the dilution  $10^{-1}$  bottle to the second three Mactney bottles containing the broth to make  $10^{-2}$  was inoculated. Lastly, 1 ml of the diluted sample from the dilution  $10^{-2}$  bottles to the last three Mactney bottles containing the broth to make dilution  $10^{-3}$  was inoculated. The samples with broth were incubated at temperature of 37°C for 48 hours. The color changes were observed from purple to yellow as shown Figure 1 and gas accumulation in the durham tube. MPN table was used to estimate the number of colonies that were formed.

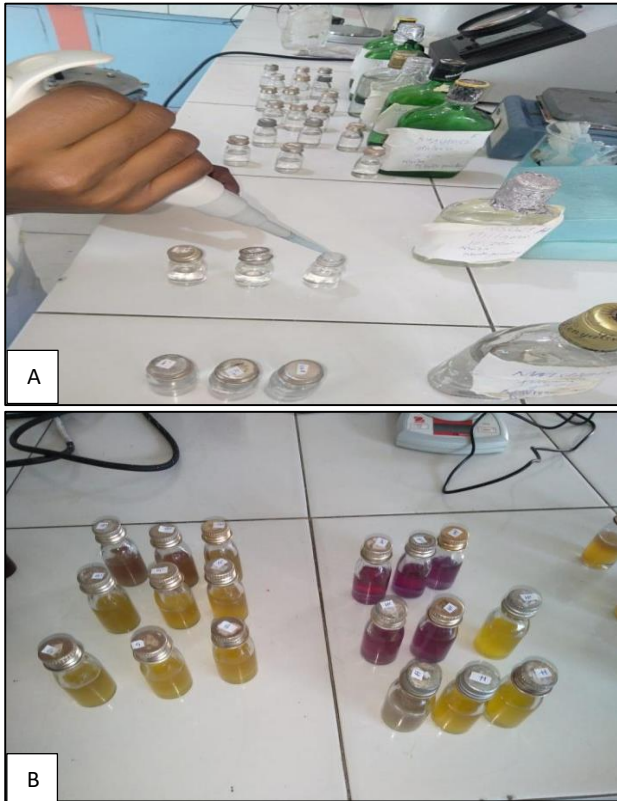
#### *The confirmation tests*

Only the positive bottles for total coliforms are confirmed. Bacteriological peptone (10 gm of peptone and 5 gm of sodium chloride in 1L of distilled water) was prepared. 10ml of the medial was dispensed into each of the Mactney bottles representing total positive for total coliforms. The inoculation loop was sterilized by autoclaving and cooled down. 0.1 ml of the culture from the positive bottles was inoculated. Incubation was done at temperatures of 44.5°C for 24 hours. 2-3 drops of Kovacs reagent were added and mixed gently. The presence indole was indicated by presence of red-violet color in the Kovacs reagent, forming a film over the aqueous phase of the medium. The MPN table was used to estimate the number of faecal coliforms. Figure 2 shows samples with presence of faecal coliforms.

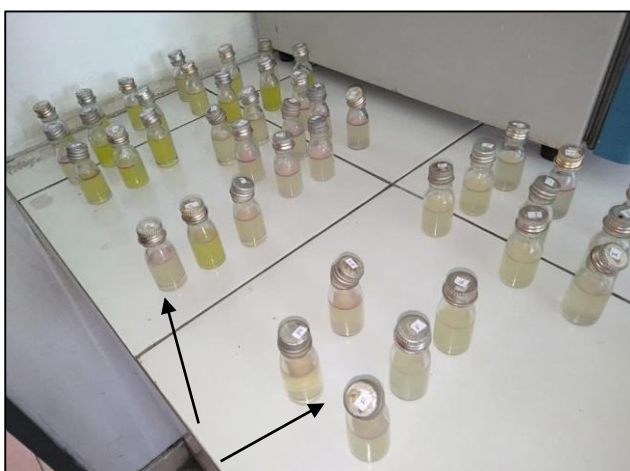
#### *The completed test*

The test aims to identify coliforms through various biochemical means. This was conducted by streaking a loopful of culture from a positive tube of presumptive diagnosis on eosine methylene blue (EMB) agar. The

colonies were then incubated at 37°C for 24-48hours. Colonies with a green metallic sheen after incubation was taken as a confirmation of faecal coliforms *E. coli*. Nucleated colonies with or without metallic sheen colonies were marked as typical colonies and transferred to sterile lauryl tryptose (LT broth) and nutrient agar slants. Gas production was observed. Gas production on LT broth indicated completed test.



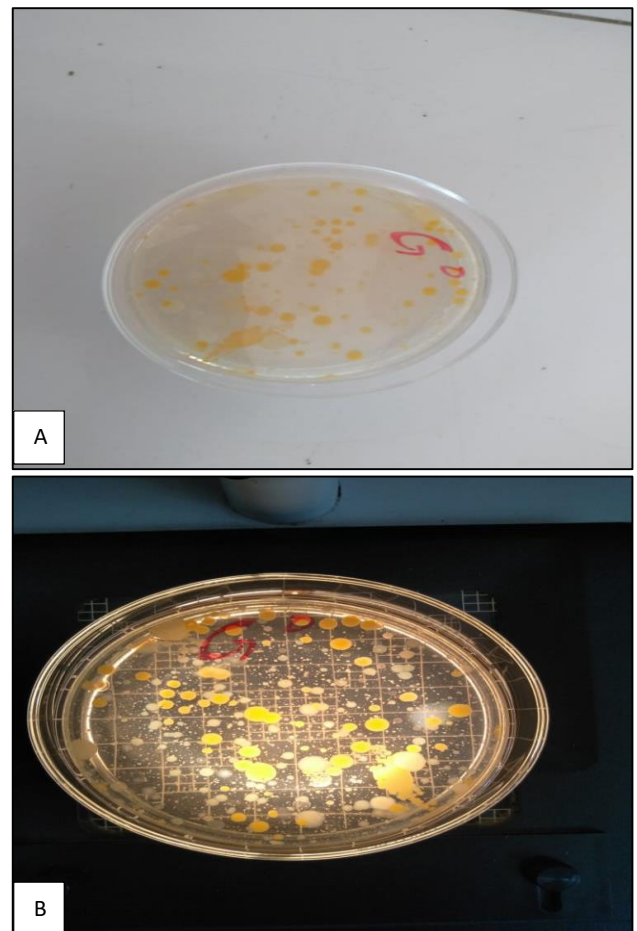
**Figure 1 (A and B): Testing of the samples in the lab using multiple tube fermentation method (Yellow are positive with coliform and purple are the negative results).**



**Figure 2: Pink film formed (indole) showing presence of faecal coliform.**

**Pour plate count**

The working bench was sterilized with 70% ethanol. The Bunsen burner was lit and set on the right and the sterile petri dish was kept on the left. A serial dilution was prepared ( $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$ ). 1ml off diluted sample was inoculated on a well labeled petri dish. The sterile molten agar from the water bath (45° C) was picked and the neck of the conical flask was flamed. The sterile molten agar was poured into the petri dish. Gently the petri dish was swirled to mix the culture and ensuring the agar doesn't slip over the edges of the petri dish. The plate was then sealed and incubated in inverted position to prevent condensation. The samples were incubated at temperature of 37°C for 48 hours. The colonies were then counted. The colonies were calculated as CFU/ml using (colony forming unit). Dilution factor used was 1/ aliquot (volume of diluted specimen). Figure 3 shows the colonies that were formed.



**Figure 3 (A and B): Colonies formed after incubation.**

***Salmonella and Shigella spp.***

Aseptically 1 ml of each of the water samples were transferred to 10 ml of buffered peptone water. The samples were incubated at 37°C for 16 hours. After inoculation 0.5 ml of the sample was enriched to selenite

F-broth then incubated at 42°C for 22 hours. The broth was then streaked in a petri dish with *Salmonella-Shigella* agar (SSA) at 37°C for 24 hours. The presence of salmonella and *Shigella* was confirmed by transferring the suspected colonies onto an agar with triple sugar iron (TSI). Confirmatory tests were conducted by inoculating the colonies onto motility indole urease agar test and incubated at 37°C for 18-24 hours. *Salmonella* are motile urease positive, shown by blackening in part of butt (hydrogen sulphide producing microorganisms while *Shigella* are non-motile. Urease negative and non-hydrogen sulphide producing microorganisms.

## RESULTS

### Total coliforms in water stored in household storage containers

50 samples (15 controls and 35 cases) had presence of total coliforms. Results in Table 1 below shows the combined means for the two groups were equal to 1365.66 ±1039.98 CFU. Mean for case (borehole) was ±761.68 CFU and for controls was ± 712.23 CFU. The results showed that there was difference in means of ±1510.54 CFU between the two groups. Total coliform (TC) (t=7.38, df=41.94, p=0.000). With the p-value of less than 0.05 it showed that there was statistically significant difference on total coliform counts for the two groups. Water samples from HHs using borehole water was on average had a higher volume of total coliforms.

### Faecal coliforms in household storage containers

The 44 samples (10 controls and 34case) were found to be positive with faecal coliforms. The Table 2 shows the combined means for the two groups 363.33 ±634.74CFU.

The mean for borehole water was ±739.52 CFU and that of control was ±115.42 CFU. The mean difference for the two groups is ±478.07 CFU. The results show there was difference in mean for faecal coliform (FC) for the two groups (t=3.75, df=36.84 and p=0.0006). The p value for the two groups is <0.05, this showed that there was significant difference in means between borehole water and other sources.

### Heterotrophic plate count (HPC) coliforms in household storage containers

In the Table 3, 55 samples that were analysed (35 boreholes and 20 other sources). Five samples of controls had HPC at acceptable range (<100) while 50 samples (15 controls and 35 cases) had HPC above unacceptable range. The combined mean for the two groups were 4519.66 ±6781.15 CFU. The borehole water was ±7730.62 CFU and that of other sources was ±4092.12 CFU. The results show difference in mean for heterotrophic place count (HPC) for the two groups (t=1.73, df=53 and p=0.0891). With the p value of greater 0.05 this means there was no significant difference on heterotrophic plate count between the two groups collected at the household.

The table 4 below shows, all the water samples collected at the household (n=55). *Shigella* was not detected in all the samples that were analysed. *Salmonella* was detected in 29.1% (n=16) of the samples collected at the household. The 34.3% (n=14) of the *Salmonella* detected was from samples collected from stored borehole water and 20% (n=4) was water collected from other sources of water stored at the household. In this analysis borehole water recorded the highest proportion.

**Table 1: Total coliforms in the water stored in the household storage containers.**

Group	Obs	Mean (CFU/ 100ml)	±SD	95% CI	
				Lower limit	Upper limit
Case (borehole water)	35	1914.94	±761.68	1653.3	2176.59
Control (tap water)	20	404.4	±712.23	71.06	737.74
Combined	55	1365.66	±1039.98	1084.51	1646.8
Diff		1510.54			

Diff= mean-(Case)- Mean (Control), Ha: diff !=0, Pr (T)> (t)=0.0000.

**Table 2: Faecal coliforms in water stored in the HH storage container.**

Groups	Obs	Mean (CFU)	±SD	95% CI	
				Lower limit	Upper limit
Case (borehole)	35	537.17	±739.52	283.14	791.21
Control (tap water)	20	59.1	±115.42	5.08	113.12
Combined	55	363.33	±634.74	191.73	534.92
Diff		478.07		219.41	736.73
<b>Diff= mean-(Case)- Mean (Control)</b>					

Ha: diff !=0, Pr (T)> (t)=0.0006, t=3.75, df=36.84

**Table 3: HPC coliforms in water stored in the household storage containers.**

Groups	Obs	Mean (CFU)	±SD	95% CI	
				Lower limit	Upper limit
Case (borehole)	35	5695.37	±7730.62	3039.81	8350.93
Control (tap water)	20	2462.16	±4092.12	546.98	4377.33
Combined	55	4519.66	±6781.15	2686.45	6352.86
Diff		3233.22		-510.58	6977.02
<b>Diff=mean-(Case)-mean (Control)</b>					

Ha: diff !=0, Pr (T)> (t)=0.0891, t=1.73, df=53.

**Table 4: Proportion of Salmonella and Shigella in water stored in the storage containers.**

Type of sample	No. of samples collected	No. of sample detected with Salmonella	Salmonella (%)	Shigella (%)
Case (borehole)	35	12	34.3	Nil
Control (tap water)	20	4	20	Nil
Combined	55	16	29.1	Nil

**DISCUSSION**

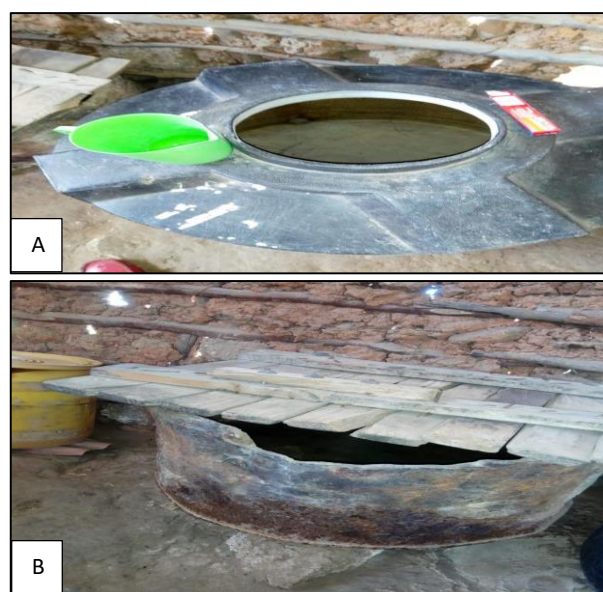
**Total and faecal coliforms stored in household storage containers**

In this study, borehole water showed higher levels of total coliform contamination compared to tap water when analyzed. The presence of pathogens shows there may be pathogenic organisms in the water. This shows that there is a poor sanitary condition of borehole water stored in the HH. It also suggests that there is poor protection, i.e., the use of uncovered household containers. This causes vectors to easily contaminate water, as shown in Figure 4 below. Poor sanitation and undesirable handling practices can be the reasons for contamination with coliforms. Sometimes groundwater sources are not adequately protected or maybe very close to a pit latrine, which causes contamination. Similar findings by who high coliform in the household water which they associated with poor water handling practices.<sup>9,14</sup>

Borehole water samples collected at the household were contaminated with faecal coliforms more than tap water. The faecal coliforms exceeded the acceptable maximum limits, hence the borehole water that was stored in the HH was not suitable for consumption. High numbers of *E. coli* detected in the water samples collected at the HHs indicate that there might be higher human involvement in the contamination of water sources. The presence of coliforms in water shows that there was recent faecal contamination, hence the risk of the presence of harmful pathogens in water. The presence of faecal indicators might indicate inadequate or no water treatment. The use of dirty storage containers can cause contamination of water in the household. Poor hygiene practices like failure to practice handwashing with soap and poor water handling practices can cause water to be contaminated with *E. coli*. Contamination in the household can also arise from defecated materials and discharge from sanitary waste. Water seepage from latrines into the borehole water may also cause contamination of borehole

water at the source, which is later consumed in the household. The percentage of positive samples may increase after water collected from safe sources because of recontamination through hands, unwashed containers, and water seepage where the boreholes where water has been collected have been dug near latrines and dippers.

Where basic sanitation is lacking, there is a greater likelihood of indicator bacteria from faeces being introduced into stored water. The result of the presence of *E. coli* in this study was in agreement with a study that found that the quality of water declines because of recontamination in the home, and further suggested that efforts to improve source water quality and sanitation be maintained at all levels of water provision.<sup>8</sup>The findings were similar where majority of water samples taken from household storage containers were not in compliance with the WHO guideline value of zero CFU/100 ml.<sup>7,12</sup>



**Figure 4 (A and B): Containers utilized by the residents to store water.**

### **Heterotrophic plate count coliforms stored in household storage containers**

This research found that both borehole and other sources of water stored in the storage containers were found to have a high heterotrophic plate count. The high levels of heterotrophic plate count show that the microbial quality of drinking water deteriorated from the point-of-collection through the household containers. Hence, the water in the containers was of poor quality. Although it might not be a health risk, it can cause regrowth, which affects aesthetic problems like tastes, smells, and discoloration of water. Similarly, it may indicate the ineffectiveness of water treatment. This also shows the possibility of regrowth of organisms that may have a sanitary significance and which may have been caused by the poor condition of storage containers. Detection of coliforms in water indicates pathogenic bacterial contamination.<sup>5</sup> This also shows that water has been affected by surface effects. Elevated levels of heterotrophic plate count may indicate the presence of nutrients and biofilms which could easily harbor pathogens.<sup>13</sup> who had similar findings and he found that both municipal water and borehole water had high HPC.

### **Salmonella and Shigella in water**

In this study, prevalence of *Salmonella* was 29.1%. Borehole water had the highest number of *Salmonella* (34.3%), while tap water had 20%. Borehole water consumed in the household was found to be of poor quality. This may have been contributed by contamination from different sources around like wastewater and septic tanks at the source. Poor personal hygiene at the household level and poor household water handling practices. The findings were similar to where groundwater samples collected were more to be contaminated with salmonella and unfit for human consumption.<sup>11</sup>

All the water samples collected were absent with *Shigella* spp. This is because *Shigella* is difficult to culture from water, and isolation from water is unusual. They also do not survive for a long period of time in the water. This may also be due to the presence of a low number of viable but not non-culturable, or some combination of the bacteria in these water sources.<sup>16</sup> Their survival time in the water is limited unless it is grossly contaminated with sewage.<sup>1</sup> Similar findings by who found that *Shigella* was more prevalent in surface water such as rivers, lakes, and shallow wells, than in groundwater sources.<sup>4</sup>

### **Limitations**

Water samples were only collected at the household level and not at the point of collection, making it impossible to determine the source of contamination. The study did not consider the seasonal variations i.e., rainy and hot seasons.

### **CONCLUSION**

The study was able to demonstrated that, borehole water stored in the household storage containers was more contaminated than tap water. All borehole water samples (35 samples) collected in the household containers were found to have faecal coliforms while 12 of the samples were detected with salmonella hence found to be of poor water quality. Both water samples i.e., borehole water (35 samples) and tap water (14 samples) were found to have high heterotrophic plate count and total coliforms 35 samples of borehole water and 15 samples of tap water. High levels of microbial contamination may be caused by inadequate water treatment, poor water handling and poor sanitation practices, and failure to consistently clean household containers which provide sediments and becomes a habitat for bacteria.

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