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EVALUATION OF MICROBIAL QUALITY OF WATER IN KAWEMPE DIVISION, KAMPALA SURBURB
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ABSTRACT

Objective: **The aim of the study was to evaluate the microbial quality of water in Kawempe Division, Kampala district.**

Design**: A cross sectional study was conducted on two major water supplies used in the Kawempe division, Kampala district.**

Setting**: Between June 2015 and April 2016, total coliforms and *Escherichia coli* (*E.coli*) were investigated in two major sources of water during wet and dry seasons. Water samples from tap water and protected spring wells were collected and analyzed for total coliforms and *E. coli*.**

Subjects: **A total of 100 water samples were collected and analyzed for microbial quality. Plate count method was used. 10 ml of water sample was mixed with 90 ml of 1% peptone water saline. 1 ml of the inoculum was transferred to each of the two petri-dishes containing agar and mixed thoroughly. The solidified agar plates for total coliforms and *E. coli* were incubated at 37^0 C and 44.5 respectively and read after 24-48 hours. The results were expressed as Colony Forming Units (CFU) per milliliter (mL).**

Results**: Of the 100 samples analyzed, protected wells were more contaminated with *E. coli* (48%) and total coliforms (56%) than tap water with *E.coli* at (4%) and total coliforms (7%) respectively. Tap water had a significantly higher degree of mean microbial count of 362 CFU/mL ($P<0.05$) while protected spring well had mean microbial count of 83 CFU/mL. The microbial counts ranged between 5.0 x 10^0 to 1.92 x 10^3 CFU/mL in the wet season and 5.0 x 10^0 to 6.4 x 10^2 CFU/mL in the dry season. Protected spring wells had a mean microbial count between 2.0 x 10^0 to 2.6 x 10^2 CFU/mL in the wet season and 2.0 x 10^0 to 2.6 x 10^2 CFU/mL in the dry season. There was a statistical significant difference between counts of total coliforms and *E.coli* in the wet season and dry season ($P<0.05$).**

Conclusions: **Tap water and protected spring wells in Kawempe Division, Kampala district are contaminated with total coliforms and *E.coli*. The presence of total Coliforms and *E.coli* in drinking water is of great public health significance and this may lead to the onset of various enteric diseases. Therefore, this calls for immediate response from health authority to enforce public health intervention measures in the area.**

INTRODUCTION

Over the past decades, there has been marked increase in the consumption of water derived from different sources in many regions of the world [1]. Significant measures have been put in place to improve the quality of water sources through the use of some technologies such as protected spring wells, protected dug wells and tap or piped water [1]. However, concerns have been raised about the quality of these sources due to their potential to cause waterborne disease outbreaks associated with drinking water, particularly among the immuno-compromised populations [1-2].

Thus, the quality and safety of the drinking water continues to be an important public health issue [3], because water contamination has been frequently associated with the transmission of infectious diseases that have caused serious illnesses and associated mortality worldwide [4]. The World Health Organization (WHO) water supply assessment 2000 indicates that approximately four billion cases of diarrhoea each year cause 2.2 x 10^6 million deaths, mostly in children under the age of five. This is equivalent to one child dying every 15 seconds [5]. Water borne infections including diarrhea, schistosomiasis, trachoma, intestinal worms and cholera are public health hazards that directly affect health and economic development [5]. They are transmitted through drinking contaminated water in area where there is poor sanitary conditions, poor hygiene and inadequate excreta disposal facilities [6]. These are frequently seen in areas where fecal materials are deposited without regard to eventual hazardous effect to the general population [7].

Uganda is predominantly a rural country with nearly 80 percent of the population living in rural areas where traditional drinking water sources such as springs wells, open water reservoirs, and open wells are still being used by rural communities [8]. Frequently, water from these sources rarely complies with WHO permissible standard limits for drinking water (9). While up to 60% of the rural population have access to safe water supply, the 40% depend on traditional water sources which are vulnerable to water borne diseases [9]. Kampala, Uganda capital City, is faced with serious public health concerns; overcrowding, inadequate excreta disposal facilities, poor use and maintenance of existing excreta disposal facilities, poor sanitary conditions and deposition of animal feces particularly in the suburbs. These risk factors potentially, contribute to water contamination leading to waterborne infections such as diarrhea [5]. Although various improved water supplies technologies are used as major sources of water in the region, there are limited information about the quality and safety of these water supplies. Therefore, this study was conducted to assess the microbial quality and safety of water from two water sources, Kawempe Division, Kampala district.

MATERIALS AND METHODS

***Design*:** A cross sectional study was done on two major water sources used in Kawempe Division, Kampala district.

Setting: Between June 2015 and April 2016, total coliforms and *E. coli* were investigated in two major sources of water during wet and dry season. Water samples from tap water and protected spring wells were collected and analyzed for total coliforms and *E. coli.*

***Subjects*:** A total of 100 water samples were collected and analyzed for microbial quality. Each protected spring well and tap water was used as a sampling unit. Water samples were randomly collected ensuring no accidental contamination occurred during sampling. The samples were clearly labeled with the site, time and nature of test samples required and were then placed in an insulated double walled container with dry ice-parks to maintain cold storage. The water samples were transported to Faculty of Veterinary Medicine, Makerere University for analysis within six hours. Each sample was analyzed for counts of total coliform, microbial load and *E.coli.*

Determination of microbial load: Plate count method was used for enumeration of bacteria present in the water sample as described by [10]. Briefly, ten milliliters of water sample was mixed with 90 ml of 1% peptone water saline. The mixture was serially diluted to obtain a working dilution (10^{-1} 10^{-2}). The mixture was vortexed to ensure uniform distribution of the organisms. Using a sterile pipette, 1 ml of the inoculum was transferred to each of the two petri-dishes. About 15-20 ml of plate count agar (HiMedia Lab-India) was mixed with the inoculum uniformly. After solidification of the agar, the plates were inverted to prevent condensation of moisture on the agar surfaces. The plates were incubated at 37^0C between 24-48 hours and the colonies were counted. The results were expressed as CFU/mL. The dish containing not more than 150 characteristic colonies at two consecutive dilutions were retained. The number of CFU/mL was calculated by multiplying the colonies formed from particular plate by reciprocal of dilution (CFU/mL = 1/d) where d is the dilution.

Determination of fecal contaminants: Selective media methylumbelliferryl-beta-glucurunideaga, (MUG) (Milano-Italy) was used for isolation of total coliforms. Ten milliliters of water sample was mixed with 90 ml of 1% peptone water saline. The mixture was serially diluted to obtain a working dilution (10^{-1} 10^{-2}). The mixture was vortexed to ensure uniform distribution of the organisms. Using a sterile pipette, 1 ml of the inoculum was transferred to each of the two petri-dishes. About 15-20 ml of MUG agar (Milano-Italy) was mixed with the inoculum uniformly. The plates were incubated at 37^0C between 24-48 hours. The colonies were counted under florescent lamp at 366nm and the results expressed as CFU/mL. The number of CFU/mL was calculated by multiplying the colonies formed from particular plate by reciprocal of dilution (CFU/mL = 1/d) where d is the dilution.

Enumeration of E.coli: The presumptive *E.coli* was detected by its ability to grow and ferment lactose at higher temperature (44-45.5^{0}C in 24 hours. Selective media MUG (Milano-Italy) was used for isolation of *E.coli.* Ten milliliters of water sample was mixed with 90 ml of 1% peptone water saline. The mixture was serially diluted to obtain a working dilution (10^{-1} 10^{-2}). The mixture was vortexed to ensure uniform distribution of the organisms. Using a sterile pipette, 1 ml of the inoculum was transferred to each of the two

petri-dishes. About 15-20 ml of MUG agar (Milano-Italy) was mixed with the inoculum uniformly. The plates were incubated at 44.45.5^{0}C between 24-48 hours. The colonies were counted under florescent lamp at 366nm and the results expressed as CFU/mL. The dish containing not more than 150 characteristic colonies at two consecutive dilutions were retained. The number of CFU/mL was calculated by multiplying the colonies formed from particular plate by reciprocal of dilution (CFU/mL = 1/d) where d is the dilution.

Biochemical tests for identification of E.coli (IMViC tests): All strains of *E. coli* were tested for catalase production as described by Collins and Lyne (1980) [10]. Briefly, this study performed tests on *E.coli* isolates for indole production, methyl red reactive compound tests, VogesProskauer reactive compound tests, and citrate utilization. IMViC pattern of ++-- and -+-- was interpreted as Biotype I and II *E. coli* strains respectively as described by [11].

Statistical analyses: Analyses were performed using the statistical package using SPSS for Windows, version 15. Descriptive statistics were used to describe measures of central tendencies, standard deviation, and interquartile range. Fisher Exact test was used to test for the difference between means of total coliforms and *E. coli* in the two water supplies. The chi square test was used to determine whether there was significant difference in level of contamination between tap water and protected spring wells. The linear regression model was used to determine whether there was statistical significant association between the counts of total coliforms and *E.coli*. Statistical significance was determined when the P-values equal≤ 0.05.

Ethical Approval: This study was reviewed and approved by the institution review board of Makerere University and National council of science technology.

RESULTS

The microbial load: This cross sectional study was conducted on water samples collected from tap water and protected spring wells from June 2015 to April, 2016 in Kawempe Division, Kampala district. Out of 100 samples collected, 25% (25/100) samples were from protected spring wells and 75% (75/100) were from tap water (Table 1). Of the samples analyzed, protected spring wells had a higher number of samples positive for *E.coli* (48%) and total coliforms (56%) than tap water with *E. coli* at (4%) and total coliforms (7%) respectively. There was a statistical significance difference between the number of tap water samples positive for *E.coli* and total coliforms and protected wells (P<0.05). Thus, the number of CFU/mL exceeded the allowable limits of zero CFU/mL of drinking water as per WHO guidelines [13].

Table 1

Proportion of total coliforms and *E.coli* detected in tap water and protected spring wells

Water supplies	Number samples (N)	Total coliforms n (%)	*E. coli* n (%)
Tap water	75	5 (7)	3 (4)
Protected spring wells	25	14(56)	12(48)
Total	100	19	15

N; Total number, n; number of sample positive for total coliforms and E. coli. Protected spring wells were more contaminated with total coliforms and E.coli than tap water (P<0.05).

Tap water had a significantly higher degree of mean microbial count of 362 (95% CI: 335.8-388.2) Colony forming units per milliliters (CFU/mL) (*P*<0.05) with standard deviation 132while protected spring well had mean microbial count of 83(95% CI: 62.4-103.6) CFU/mL with standard deviation of 132.3 (Table 2). The microbial counts ranged between 5.0 x $10^0$to 1.92 x 10^3CFU/mL in wet season and 3.0 x 10^0 to 6.4 x 10^2 CFU/mL in dry season. Protected spring well had a range count between 2.0 x 10^0 to 2.92 x 10^2 CFU/mL in wet season and 2.0 x 10^0 to 2.6 x 10^2 CFU/mL in dry season.

Table 2
Results of microbial load counts in tap water and protected wells

Microbial count, CFU/m[a]					
Water supplies	**Min.**	**Max.**	**Mean**	**SD**	**95%CI**
Tap water (n=75)[b]					
Wet Seasons	5.0 X 10^0	1.92 x 10^2	3.62 X 10^2	132.2	(335.8-388.2)
Dry seasons	3.0 X 10^0	6.4 X 10^2	2.4 X 10^2	43	(231.5-248.5)
Protected wells(n=25)[c]					
Wet season	2.0 X 10^1	2.92 X 10^2	8.3 X 10^1	132.3	(62.4-103.6)
Dry season	2.0 X 10^0	2.6 X 10^2	5.4 X 10^1	182	(45.5-62.5)

Abbreviations: N; total number, X; Mean, Min.; Minimum; Max.; Maximum CI; Confidence interval, SD; Standard deviation, CFU; colony forming units.

a: Microbial counts in tap and protected spring wells

b: Mean microbial counts in tap water during wet and dry seasons

c: Mean microbial counts in protected spring well during wet and dry seasons

Total coliforms and E.coli: Protected spring wells had a mean count CFU/mL of total coliforms and *E.coli* at 4.3 X $10^1$and 8.3 X 10^1 respectively during wet season (Table 3). The range counts CFU/mL of total coliforms and *E.coli*were2.0 X 10^0 to 2.6 X 10^1 and 2.0 X 10^0 to 9.2 X 10^1respectively.The mean count CFU/mL of total coliforms and *E.coli* during dry seasons were 2.2 X 10^1 and 5.4 X 10^1respectively. The range count CFU/mL of total coliforms and *E.coli* were 2.0 X 10^1 to 4.4 X 10^1 and 2.0 X 10^1 to 2.6 X 10^1. Statistical significance difference was observed between *E.coli* and total coliforms mean counts (P<0.05) during wet and dry seasons. Tap waters had a mean count CFU/mL of total coliforms and *E.coli* at 4.0 X 10^1 and 2.0 X 10^1during wet season respectively. The range count CFU/mL of total coliforms and *E.coli* count were 1.0 X 10^0 to 2.5 X 10^1 and 1.0 X 10^0 and 4.0 X 10^0 respectively. The mean count CFU/mL of total coliforms and *E.coli* during dry season were 1.0 X 10^0 and 2.0 X 10^0respectively. The range count CFU/mL of total coliforms and *E.coli* were 1.0 x 10^0 and 2.0 X 10^0 and 1.0 X 10^0 and 2.0 X 10^0. There was no statistical significance difference observed between the mean count CFU/mL of total coliforms and *E. coli* in tap water during dry seasons (P>0.05).

Table 3
Results of total coliforms and *E.coli* isolates from water supplies

Indicator organism count, CFU/mL[a]

Water supplies	Min.	Max.	Mean[b]	SD	95%CI
Protected spring wells					
Total coliforms					
Wet Season	2.0 X 10^0	2.6 x 10^1	4.3 X 10^1	16.9	(330.6-393.4)
Dry season	2.0 X 10^0	4.4 X 10^1	2.2 X 10^1	29.7	(18.1-25.9)
E.coli					
Wet season	2.0 X 10^1	9.2 X 10^1	8.3 X 10^1	63.6	(70.4-95.6)
Dry season	2.0 X 10^1	2.6 X 10^1	2.4 X 10^1	16.9	(45.5-62.5)
Tap water					
Total coliforms					
Wet season	1.0 X 10^0	2.5 X 10^1	4.0 X 10^0	16.9	(20.6-27.4)
Dry season	1.0 X 10^0	2.0 X 10^0	1.0 X 10^0	0.7	(0.86-1.14)
E.coli					
Wet season	1.0 x 10^0	4.0 x 10^0	2.0 x 10^0	2.1	(1.68-2.58)
Dry season	1.0 X 10^0	2.0 X 10^0	1.0 X 10^0	0.7	(0.86-1.14)

X: Mean, CFU: Colony forming units, SD: standard deviation

a : indicator organism counts CFU/mL in protected spring wells and tap water
b: Mean total coliforms and *E.coli* counts in protected spring wells during wet and dry seasons

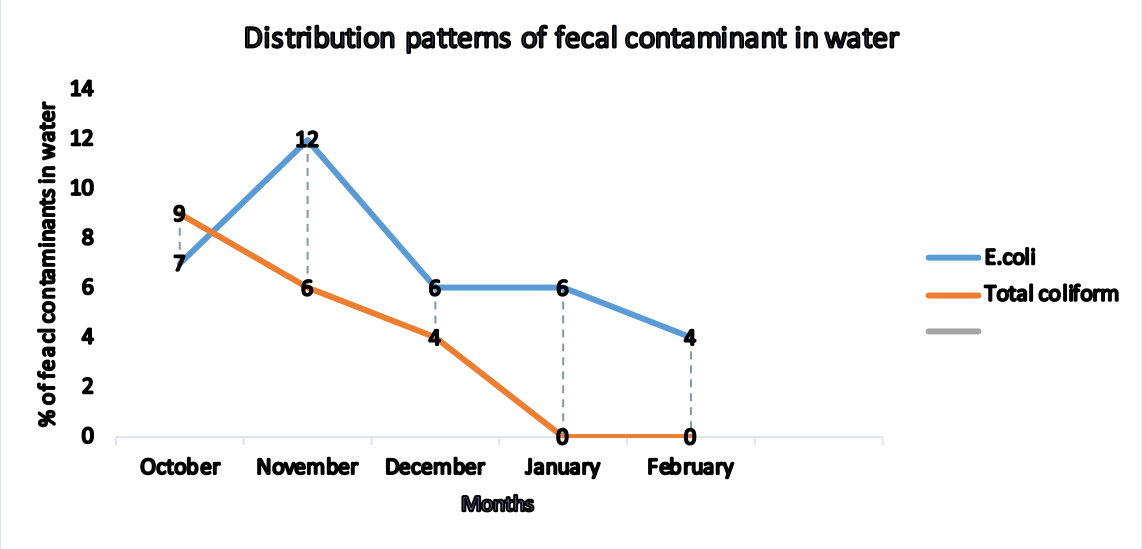
Relationship between Total coliforms and E. coli: The number of samples positive for total coliforms were significantly higher than those of *E.coli* in the two water sources (P<0.05). While there was positive correlation between total coliforms and *E.coli* counts (r = 0.612, P<0.05), the results of linear regression analysis showed that no statistically significant associations between counts of total coliforms and *E.coli* in protected spring wells and tap water (P<0.05). Total coliforms mean counts were significantly higher than those of *E.coli* (P<0.05) in protected spring wells. Additionally, total coliform mean counts were significantly higher than those of *E.coli* in tap waters. However, protected spring wells were more contaminated with fecal contaminants than tap water. Microbial counts showed statistical significance difference between the number of total coliforms and *E.co*li in the two water sources (P<0.05).

Seasonal variations on fecal contaminants: Seasonal variations had profound effects on the distribution of fecal contaminants. Whereas the level of fecal contaminants increased during wet seasons, in the month of October, November and December, the patterns of the distribution of *E.coli* and total coliforms isolates in protected spring wells decreased from January, February and March (Figure 1). During wet season, the *E.coli* had a higher mean count CFU/mL of 8.3 X 10^1than those of total coliforms which was at 4.4 x 10^1. *E. coli* counts

showed significant difference between the dry and the wet season for the two water sources (Table 3). The lowest *E.coli* count was detected in tap water. The overall prevalence of *E. coli* contamination in water samples was 48% (n=25). The combined data on wet and dry season showed that 4% (n=75) water samples of the tap water and 48% (n=25) of protected spring wells were contaminated with *E. coli*. It should be noted that, higher prevalence of *E. coli* was generally observed during the wet season compared to the dry season (Table 2). Rank's correlation of the fecal contaminants with seasonal effects showed positive associations with the two water sources. However, there was statistical significant association between fecal contaminants during wet and dry seasons ($P<0.05$). This means that relative distribution of the total coliforms, microbial load and *E. coli* varies across the seasons.

Figure 1

Figure 1 shows the distribution patterns of fecal contaminants during wet and dry seasons. There is gradual increase of total coliforms and E.coli during wet season and a decrease during dry



DISCUSSION

The presence of fecal indicator organisms in drinking water is an indication that the water is not fit for human consumption [11]. According to the WHO 2008, water quality guidelines, there should not be any detectable indicator organisms per 100mL of water (5). The results from the present study indicates that the two major water sources in Kawempe Division, Kampala district namely; tap water and protected spring wells are potentially a source of waterborne disease outbreaks because they are contaminated with total coliforms and *E.coli*. In the present study, 15% samples from protected spring wells and 19% of samples from tap waters failed the WHO drinking water regulation. The 48% contamination by *E.coli* in the water supplies were due to some of the wells having pit

latrines located only few distances from the water sources. Additionally, some protected spring wells have tunnels on the slope and these allow water contaminated with fecal deposits to flow downwards [3].

Some of these protected spring wells have no constructed barrier walls and thus allow dirty water to find their ways into the wells. The most contaminated spring wells are those located near the residences where deep pit or stage-constructed latrines are found and those with tunnels on the slope of the hills [3]. Such tunnels allowed infiltration of waste/dirty water containing fecal deposits into the water sources. In the present study, protected spring wells had significant levels of microbial loads. The overall count of *E.coli* during the study was 48% ($P<0.05$). These indicated that some protected spring wells are contaminated with fecal indicator organisms. The 56% of wells which passed the WHO guidelines are either located far away from residences, well-constructed and properly maintained or had no tunnels on the slopes.

The results in the present study are similar with other previous studies done elsewhere. Gwimbi, 2011 [2] while conducting a similar study in Masero district, Manonyane Community, in Lesotho found out that total coliform were detected in 97% and *E.coli* in 71% of the water samples. Protected well sources had significantly less number of colony forming units (CFU) per 100 ml of water sample compared to unprotected sources. In Ethiopia, the quality of water investigated in both dry and wet season was analyzed for *E. coli* contamination [4]. The overall prevalence of *E. coli* in water samples was 54.9% and the higher prevalence was recorded during the wet compared to the dry season [5]. In Amuria district, North eastern Uganda, a similar study done to assess the microbiological quality of water indicated that 40% of protected spring wells were contaminated with total coliforms and 25 % by *E.coli* respectively [13]. This implies that the quality of water from these sources were a function of the number of total coliforms and *E.coli* per 100ml of water. Whereas number varies in each country, being less than 50 per ml of water in Uganda and zero in South Africa [13], some countries are yet to establish their individual set limits.

Seasonal variations of fecal indicator organisms: Seasonal variations had significant profound effects in the distribution of total coliforms and *E.coli* over the study period. Since this study was conducted in both wet and dry season, it had significant variation in the microbial loads, total coliforms and *E.coli*. Additionally, the high contamination by fecal indicator organisms were mainly significant during wet seasons ($P<0.05$) as compared with the dry season. In the present study, the mean counts of total coliforms and *E.coli* were higher especially during wet season and relatively lower during dry season for both water supplies. There was statistical significant difference between mean counts of total coliforms and *E.coli* for the two seasons ($P<0.05$). Therefore, this shows that the high level of contamination of water supplies were mainly influenced by seasonal practices such as grazing of livestock in some areas and digging of tunnels on the slope of hilly areas to divert water flows away from residence [3]. More so, heavy rainfalls had significant effects in increasing the deterioration of the quality of drinking water in the two main sources. This may be either through direct washing of more fecal materials into sources of water or by reducing efficiency of treatment through organic and particulate material into supplies or indirectly [3]. Previous studies have reported similar results.

Sanyu, 1996 [14] reported that microbiological quality of water supplies deteriorated with increase in rainfalls but improved during dry seasons. A study done to recover coliforms on tap waters found that 30% had fecal coliforms [15]. A similar study conducted in United States of America showed that pipe water was less contaminated with fecal indicator bacteria and the decline in the level of contamination in the dry seasons was attributed to few animals present in the area, absence of wells near residence and proper maintenance of the existing spring wells. In Brazil, total coliform and *E.coli* counts were seasonally influenced in spring wells and tap water, with the highest counts seen during the months of September to March and falling from April through August [9].

CONCLUSION AND RECOMMENDATIONS

This study indicates that the two major water sources; tap water and protected wells water in Kawempe Division, Kampala district is contaminated with fecal indicator organisms. The presence of total coliforms and *E.coli* in drinking water is of great public health significance and this may lead to the onset of various enteric pathogens. Therefore, the health authority needs to institute intervention measures for improvement of the quality of drinking water in the area. Further studies needs to conduct research on other water sources such as bore holes.

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