Research Paper

Longitudinal study of microbial load of drinking water and seasonal variation of water quality at the point of use in food establishments of Addis Ababa, Ethiopia

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ABSTRACT

The study aimed to determine the status of microbial load of drinking water and seasonal variation of water quality. An institution-based longitudinal study was conducted. 1,141 food establishments were divided into slum and non-slum areas based on their location. Moreover, they were categorized as large and small food establishments. Then, 125 food outlets were selected using a simple random sampling technique. From the selected food outlets, 250 drinking water samples were collected directly from the drinking water storage in the rainy and the dry seasons. Data analysis was conducted using a repeated-measure ANOVA statistical model. The finding indicated that, 26.4% and 10.7% of the food establishments' drinking water was positive for *Escherichia coli* in the wet and the dry season, respectively. Moreover, 3.2% and 1.6% of the food establishments' drinking water had very high health risk to customers during the wet and the dry season, respectively. The drinking water at the point of use was found to be vulnerable to microbiological contamination and had a serious health risk. Therefore, good sanitation and proper handling of drinking water, and effective drinking water treatment, such as disinfection and filtration, should be practiced in all food establishments.

Key words | Addis Ababa, drinking water, food establishment, longitudinal, microbial load, seasonal variation

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HIGHLIGHTS

- It is a new study on seasonal variation of microbial load and it implies degree of health risk of drinking water contamination.
- Food establishments' drinking water was vulnerable to microbiological contamination.
- Statistically significant difference was observed in the microbial load of the drinking water between the dry and the rainy seasons.

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ABBREVIATIONS

AAFMHACA	Addis Ababa Food, Medicine and Health Care Administration and Control Authority
ANOVA	Analysis of variance
EMB	Eosine Methylene Blue
SPSS	Statistical Package for the Social Sciences
CFU	Colony forming unit
E. coli	*Escherichia coli*
POU	Point of use
WHO	World Health Organization
USA	United States of America

INTRODUCTION

Globally, drinking water quality is continuously deteriorating and becoming inappropriate for human use and well-being of society due to high population growth, expansion in industries, discharge of waste water and chemicals into canals and other water sources (Memon *et al.* 2011; Mohsin *et al.* 2013). As a result, access to potable water is one of the major challenges of the 21st century because 20% of the world population does not have access to pure drinking water (Saxena *et al.* 2015; Zameer *et al.* 2015). Over the past several decades, many people in developing countries did not have a safe and sustainable water supply scheme (Hunter *et al.* 2010; World Health Organization 2015). Due to these and other human-made factors, the health burden of poor water quality and poor sanitation practice is considerable (Chauhan *et al.* 2017; Girmay *et al.* 2019). Worldwide, waterborne diseases have been estimated to cause more than two million deaths and four billion cases of diarrhea annually (El-Kowrany *et al.* 2016).

United States of America (USA)-based data indicated that of 9,040 food and waterborne disease outbreaks, approximately 4,675 (52%) of these were attributed to food establishments (Boro *et al.* 2015). Poor handling of drinking water, inadequate water supplies, and inadequate sanitation are responsible for a large proportion of disease transmission in developing countries (Mara 2006; Taylor *et al.* 2015). Moreover, poor environmental sanitation, poor personal hygiene practices, inappropriate storage of drinking water, and poor waste management practice of food establishments may cause water contamination and affect the health of customers (Ifeadike *et al.* 2014; Girmay *et al.* 2020a).

Like many African countries, Ethiopia has a shortage of water, poor sanitation, and a lack of access to clean water sources (Hendrix 2012). Even though access to quality water and good sanitation are major determinants of preventable diseases in developing countries such as Ethiopia, a study conducted by Water.org stated that, only 42 and 11% of Ethiopians have access to a clean water supply and adequate sanitation services, respectively (Seyoum & Graham 2016). This indicated that above half of the population did not have a chance to access clean and safe drinking water. Addis Ababa city gets water from Dire, Gefersa, and Legedadi reservoirs, as well as several boreholes concentrated around Akaki. Moreover, drinking water is supplied via water trucks for low pressure areas. However, nearly half of the drinking water demand of the city is not met. This supply deficit is causing frequent water supply interruptions. The food establishments of Addis Ababa suffer from a shortage of drinking water. As a result, they are suspected to be major sources of water- and foodborne disease which might arise from the inadequate drinking water, poor sanitation, and poor storage practices. Hence, this study aimed to assess the quality of drinking water at the point of use (drinking water storage) in Addis Ababa food establishments.

METHODS

Description of the study area

The study was conducted in Addis Ababa, the capital city of the Federal Democratic Republic of Ethiopia and the seat for the African Union (Girmay *et al.* 2020b). There are 1,141 licensed food establishments in the city. Of the total food establishments, 95 (8%) are high quality hotels with one or more stars, and the majority 1,046 (92%) are small food establishments, which include non-star rated hotels, bars, cafes, restaurants, etc. (Girmay *et al.* 2020c). The Addis Ababa city administration government provides safe water supply to the food establishments. However, the safety of the supplied water handling practice varies from sub-cities to sub-cities. Moreover, the provided water also, in general, is inadequate and frequently interrupted. The location map of Addis Ababa city is depicted in Figure 1.

Study design

An institutional-based longitudinal study was conducted in Addis Ababa city.

Source population

All food establishments were located within Addis Ababa city administration.

Study population

All selected food establishments were located within Addis Ababa city administration.

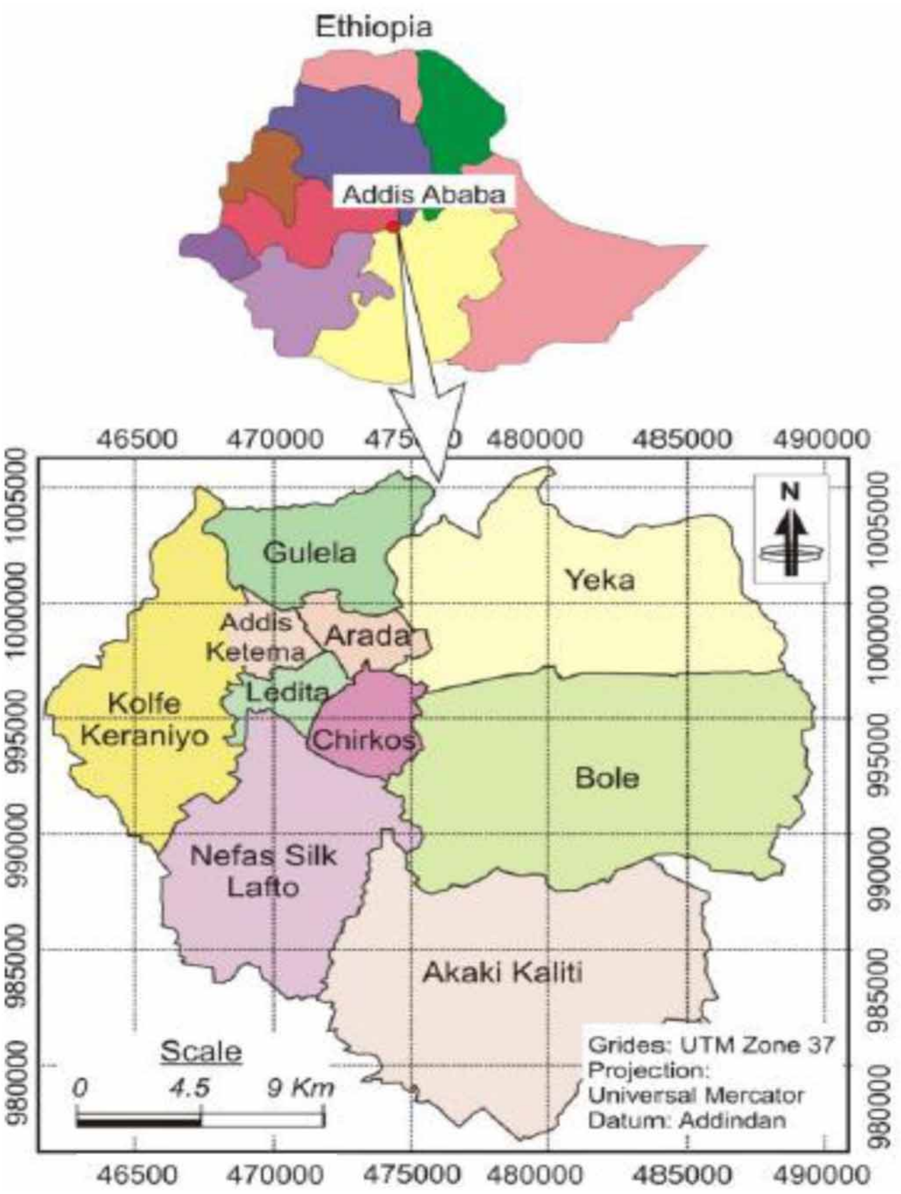

Figure 1 | Map of Addis Ababa city administration.

Inclusion criteria

All food establishments that provided services in the city were included during the first selection.

Exclusion criteria

Establishments that provide only packed drinking water were excluded from the study, because they are less likely to be contaminated.

Sample size determination

The sample size was calculated using an unmatched cohort and cross-sectional study formula (EPI INFO version 7.2.2.6, STATCALC), by considering 95% confidence interval, 80% power to detect if there is a difference, 1:1 ratio and 15.9% occurrence of microbial load or salmonella (outcome in unexposed group), based on a study result undertaken in treated, stored, and drinking water in Nauru north, Kenya (Waithaka *et al.* 2014). Then, using the unmatched cohort and cross-sectional study formula (EPI INFO version 7.2.2.6, STATCALC), the sample size was equal to 114 samples of drinking water. After adding 10% for non-response rate or to involuntary owners, the total sample size for this longitudinal study was equal to 125 samples of drinking water.

Sampling procedure

At the first instance, a listing of the 1,141 licensed food establishments was obtained from Addis Ababa Food, Medicine Health Care Administration and Control Authority (AAFMHACA). These food establishments were divided into slum and non-slum areas based on their location and into large and small food establishments, based on their size. Sample allocation was conducted. Then, the required food establishments were selected using simple random sampling technique. From the non-slum area, 7 large and 44 small food establishments were included. From the slum area, 3 large and 71 small food establishments were selected. In total, 10 samples from the large and 115 samples from the small food establishments (total of 125 samples of food establishments) were included. Addresses of these selected food establishments were registered, to make the next visit simpler. To assess seasonal variation of drinking water quality, 125 samples of drinking water were collected during the dry season (December–March) from water storage facilities and repeated during the wet season (June–September). In summary, the sampling procedure for this study is depicted in Figure 2.

Data collection procedures

Drinking water samples were collected at both seasons of the year from the point of use (POU) in food establishments and were bacteriologically tested in the laboratory. To collect the drinking water samples from the food establishments, heat-sterilized bottles of 100 ml capacity were used and the methods of sampling were adapted from the WHO guidelines for drinking water quality. The bottles were delivered to the laboratory within 6 hours and kept refrigerated at 4 °C until the time of analysis.

Data analysis procedures

All laboratory results were recorded and coded appropriately. Then, data were entered to the SPSS (Statistical Package for the Social Sciences) software version 20. Data analysis was conducted using a repeated-measure ANOVA statistical model.

Microbial load assay

Membrane filtration method was used for microbiological analysis of the drinking water. In this method, a measured volume of the water sample (100 ml) was filtered through a membrane with a pore size small enough to retain the indicator bacteria to be counted. In the presumptive test, the membrane was placed on Eosine Methylene Blue (EMB) agar and incubated at 44.5 °C for 24 hours, so that the indicator bacteria grew into colonies on its surface. According to Osman *et al.* (2018) and Cowan *et al.* (1978), these colonies, which are recognized by their color, morphology and ability to grow on the selective EMB medium, were counted as separate colonies. Moreover, in the confirmatory test, to confirm whether positive samples were fecal coliform or not, they were re-inoculated into peptone broth test tubes

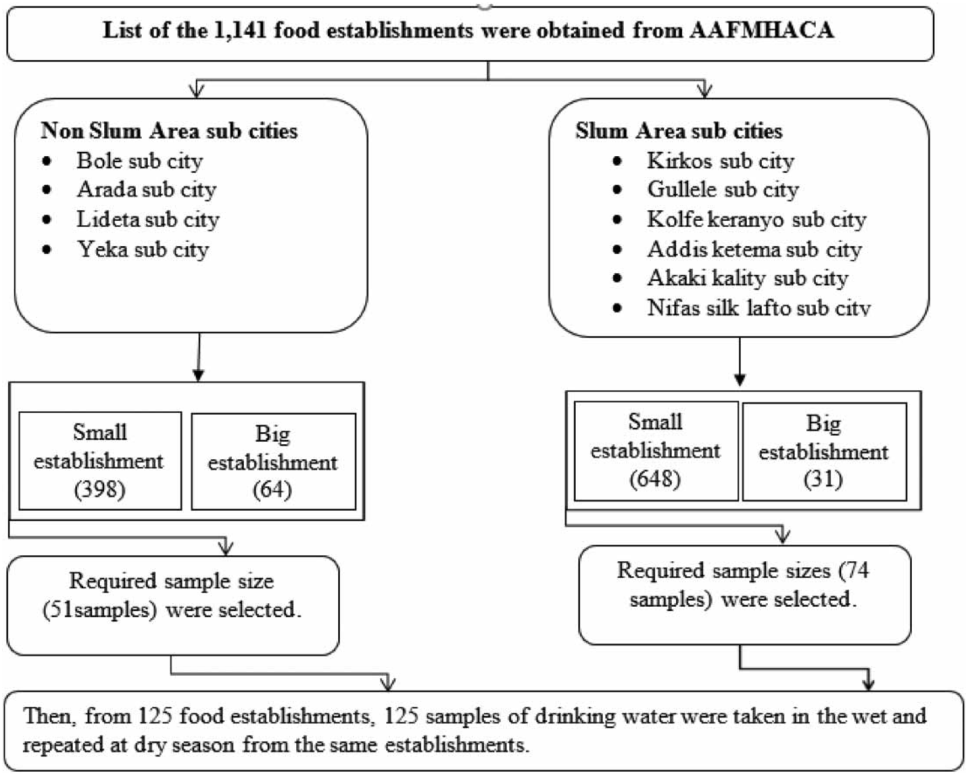

Figure 2 | Systematic structure of the study sampling procedure.

for 24 hours at 44.5 °C. Then, drops of Kovac's reagent were added to the re-incubated peptone broth test tubes. Finally, test tubes which indicated reddish color at the top were identified as positive for fecal coliforms and/or *E. coli*.

Operational definitions

According to WHO drinking water standard 2004, water samples with <1 CFU/100 ml were considered to be uncontaminated and samples with ≥1 CFU/100 ml to be contaminated (World Health Organization 2004). Moreover, based on previous studies, the fecal coliform count contamination levels in the drinking water samples were categorized into 0 (no health risk), 1–10 (low health risk), 11–100 (high health risk) and >100 CFU per 100 ml (very high health risk) (Lloyd & Bartram 1991).

Food establishments: Institutions that provide food and drinks for selling to customers.

Food: A material consisting of nutritious substances that people eat or drink in order to maintain life and growth.

Large food establishment: Hotels ranked with one or more stars.

Small food establishment: Small vendors, non-star ranked hotels, bars, restaurants, cafes.

Slum area: Area with poorer sanitation infrastructure.

Non-slum area: Area with better sanitation infrastructure.

Study variables

Independent or predictor or explanatory variables

In this study, the independent study variables are defined as a factor or phenomenon that causes or influences the dependent variable. The predictor variables of the study are:

- time,
- type of food establishment,
- location of food establishments, either in slum area and non-slum area.

Dependent or outcome or response variables

In this study, dependent variables are defined as phenomena that are changed by the effect of independent variables. The outcome variable of this study is:

- microbiological quality of drinking water (fecal coliforms, *E. coli*).

Ethical consideration

The ethical procedure followed a series of three stage-based validation processes that incorporate (1) research approval, (2) technical clearance, and (3) collaborative or consultative approaches. First, a research letter of support was obtained from the Ethiopian Institute of Water Resources, Addis Ababa University. Second, an ethical clearance and approval technical letter was collected from the Ethiopian Public Health Institute, Scientific and Ethical Review Board with reference number EPHI 613/138 in June 2019. Third, in collecting the research data, written consent of a collaborative letter was obtained from the owner of each food establishment.

RESULTS

Microbial load of drinking water and seasonal variation of water quality

The majority (98.4%) of the food establishments' drinking water source was municipal. In this study, the drinking water in 73.6% and 89.3% of the food establishments was found to be clean, safe, and free from *E. coli* in the rainy and dry season, respectively. In the presumptive test, 32.8% and 16.4% of the samples had thermo-tolerant/fecal coliforms during the wet and the dry season, respectively. However, in the confirmatory test, 26.4% and 10.7% of the food establishments' drinking water was positive for *E. coli* in the wet and the dry season, respectively. Out of the total of 247 drinking water samples, 46 (18.6%) had fecal coliforms and/or *E. coli*. The mean scores of thermo-tolerant/fecal coliforms count per 100 ml were found to be 7.59 and 3.12 in the rainy and dry season, respectively (Table 1).

Microbial load, seasonal variation, and impact of health risk implications

The finding of the study revealed that 3.2% of the food establishments had drinking water with very high health risk to customers during the wet season and 1.6% of them during the dry season. Moreover, 12% and 2.5% of the food establishments had high health risk to customers due to their drinking water in the wet and the dry season, respectively (Table 2).

Tests of within-subjects effects of time on the number of fecal coliforms' count

Having fulfilled the assumption of the 'Mauchly's test of sphericity' (non-significant *P*-value), 'tests of within-subjects effects' were conducted. Then, as observed in Table 3, the

Table 1 | Microbial load and seasonal variation of drinking water quality at the POU in food establishments of Addis Ababa ($n = 250$)

Presence of indicator of contaminants (Cfu/100 ml) at 44.5 °C	Rainy season ($n = 125$)		Dry season ($n = 122$)	WHO permissible level in number of micro-organisms (n)
Thermo-tolerant/fecal coliforms	Present	41 (32.8%)	20 (16.4%)	0
	Absent	84 (67.2%)	102 (83.6%)	
E. coli	Present	33 (26.4%)	13 (10.7%)	0
Mean of thermo-tolerant or fecal coliforms/colony count per 100 ml		7.59	3.12	0
Sources of the food establishments drinking water			Frequency	Percent(%)
	Municipality		123	98.4
	Spring and others		2	1.6

Table 2 | Microbial load and seasonal variation of drinking water quality at the POU and impact of health risk in food establishments of Adds Ababa ($n = 250$)

Health risk categories of fecal coliforms in drinking waters samples (CFU/100 ml) based on	Rainy season ($n = 125$)		Dry season ($n = 122$)	
	Frequency	%	Frequency	%
No health risk (0)	84	67.2	102	83.6
Low health risk (1–10)	22	17.6	15	12.3
High health risk (11–100)	15	12	3	2.5
Very high health risk (>100)	4	3.2	2	1.6

Table 3 | Tests of within-subjects effects of time on the number of colony counts in drinking water at the POU in food establishments of Addis Ababa ($n = 250$)

Tests of within-subjects effects

Source		Df	F	P-value
Time	Sphericity assumed	1	5.631	0.019
	Greenhouse–Geisser	1.000	5.631	0.019
	Huynh–Feldt	1.000	5.631	0.019
	Lower bound	1.000	5.631	0.019
Error (time)	Sphericity assumed	121		
	Greenhouse–Geisser	121.000		
	Huynh–Feldt	121.000		
	Lower bound	121.000		

value of F is 5.631, which reached significance with a *P*-value of 0.019 (less than the 0.05 alpha levels). This indicated that there was a statistically significant difference between the means of the number of fecal coliforms' count per 100 ml between the dry and the rainy seasons (Table 3).

Pairwise comparisons to observe the effect of time on the number of colony counts

The study showed that the mean of thermo-tolerant/fecal coliforms difference reached significance in the different seasons (dry and rainy seasons). Although there was a statistically significant difference in the thermo-tolerant or fecal coliforms means and season of a year, it was not yet known in which season of the year the mean difference was statistically significant. A repeated-measure ANOVA determined that the mean of thermo-tolerant/fecal coliforms count per 100 ml scores had the same significance across the two time points or seasons ($F\ (1,121) = 5.631$, $P = 0.019$. Therefore, to see this difference, pairwise comparison results were needed. A post hoc pairwise comparison using Bonferroni correction showed increased mean scores of the thermo-tolerant or fecal coliform count per 100 ml between the dry and the rainy season (3.123 vs 7.5902, respectively), and reached a statistically significance. Therefore, the results for the repeated-measure ANOVA indicated that there was a significant time effect for the growth of thermo-tolerant or fecal coliforms or number of colony counts per 100 ml (Table 4).

Tests of within-subjects effects of time on *E. coli in* drinking water

Similar to the above stated results, after fulfilling the assumption of the 'Mauchly's test of sphericity' (non-significant *P*-value), 'tests of within-subjects effects' was conducted. As indicated in Table 5, the value of F was 16.244, which researched significance with a *P*-value of 0.000. This indicated that there is a statistically significant difference between the means of *E. coli* per 100 ml between the dry and rainy seasons (Table 5).

Pairwise comparisons to observe effect of time on the occurrence of *E. coli*

As seen in Table 6, there was a statistical significance in the occurrence of *E. coli* and season of the year although it was not yet known in which season the mean difference was significant. A repeated-measure ANOVA determined that the mean of occurrence of *E. coli* had the same statistical

Table 4 | Pairwise comparisons to observe effects of time on the number of colony counts in drinking water at the POU in food establishments of Addis Ababa ($n = 250$)

Pairwise comparisons

(I) time	(J) time	Mean difference (I–J)	Std. error	P-value	95% Confidence interval for difference[a] Lower bound	Upper bound
1	2	−4.467*	1.883	0.019	−8.194	−0.740
2	1	4.467*	1.883	0.019	0.740	8.194

Based on estimated marginal means.
[a]Adjustment for multiple comparisons: Bonferroni.
*The mean difference is significant at the 0.05 level.

Table 5 | Tests of within-subjects effects of time on *E. coli* in drinking water at the POU in food establishments of Addis Ababa ($n = 250$)

Tests of within-subjects effects

Source		Df	F	*P*-value
Time	Sphericity assumed	1	16.244	**0.000**
	Greenhouse–Geisser	1.000	16.244	**0.000**
	Huynh–Feldt	1.000	16.244	**0.000**
	Lower bound	1.000	16.244	**0.000**
Error (Time)	Sphericity assumed	121		
	Greenhouse–Geisser	121.000		
	Huynh–Feldt	121.000		
	Lower bound	121.000		

Table 6 | Pairwise comparisons to observe effects of time on fecal coli form counts in drinking water at the POU in food establishments of Addis Ababa ($n = 250$)

Pairwise comparisons

					95% Confidence interval for difference[a]	
(I) Time	(J) Time	Mean difference (I–J)	Std. error	*P*-value	Lower bound	Upper bound
1	2	−0.156*	0.039	0.000	−0.232	**− 0.079**
2	1	0.156*	0.039	0.000	0.079	**0.232**

Based on estimated marginal means.
*The mean difference is significant at the 0.05 level.
[a]Adjustment for multiple comparisons: Bonferroni.

significance across the two time points or seasons F (1,121) = 16.244, $P = 0.000$. A post hoc pairwise comparison using Bonferroni correction showed an increased mean of occurrence of *E. coli* per 100 ml scores between the dry and the rainy seasons (0.1066 vs 0.2623, respectively) and statistical significance with *P*-value <0.00. Therefore, the results for the repeated-measure ANOVA indicated that, there was a significant time effect for the occurrence of *E. coli* per 100 ml (Table 6).

DISCUSSION

As many of the food establishments' drinking water source was municipal, the majority of the drinking water was found to be in the acceptable level. This indicated that there might be good drinking water treatment practice. However, even if WHO 2004 drinking water quality standard specifies 0 CFU/100 ml as the limit for fecal coliforms and/or *E. coli* in potable water, the mean values of the fecal coliforms and/or *E. coli* in this study were above the acceptable level for drinking water that is required for human use and consumption. The results of the study showed that the drinking water at the storage/POU was susceptible to microbiological contamination and compelling serious health risks to consumers. The sources of contamination for the water quality in the food establishments could be from the source, the distribution system, or from poor handling of water at food establishments. Leakage of water supply pipelines, and pollution from sewage lines entering into drinking water supplies could be problems associated with the distribution system. This idea is supported by previous studies (Bain *et al.* 2014; AL-Dulaimi & Younes 2017; Kirianki *et al.* 2017).

The fact that *E. coli* was twice as great during the wet season as during the dry season. This could be due to the effects of flooding and effluents of wastes from different toilets that could cause water contamination through leakages. The higher occurrence of *E. coli* could be due to poor washing practice of drinking water reservoirs and low utilization of disinfectants in the wet season. Moreover, utilization of unimproved sources, contamination of drinking water by vendors in their premises, poor storage and handling can also be causes. In addition, the causes could be due to the poor sanitation system installation, absence of drinking water treatment at food establishments, and lack of continuous monitoring and evaluation of drinking water quality.

In addition, the microorganisms might have found their way into the drinking water through anthropogenic actions and cross contamination in the rainy season representing potential threats to human health, causing diseases such as acute gastroenteritis, waterborne diarrheal diseases, and other infections. This is supported by a study carried out by Figueras & Borrego (2010).

CONCLUSION AND RECOMMENDATION

The majority of the food establishments' drinking water was safe, clean, and free from pathogenic microorganisms. However, a large number of food establishments have drinking water with *E. coli*. The levels of selected indicator bacteria (fecal coliforms and/or *E. coli*) exceeded the WHO recommended guidelines for drinking water. The microbial load of the drinking water at the POU in food establishments greatly differs between the dry and the rainy seasons. The occurrence of fecal coliforms and/or *E. coli* was higher during the rainy season. There was a time effect for the presence of fecal coliforms and/or *E. coli* in the drinking water. The findings of the study revealed that a large number of food establishments' drinking water had health risks. Therefore, there must be creation of awareness in food establishments for the appropriate management of drinking water to curb outbreaks of waterborne/foodborne diseases. This includes the practice of effective drinking water treatment such as disinfection, filtration, good sanitation and hygiene at the food establishment level. In addition, all concerned decision-making bodies, in particular, the government, should conduct regular and continuous microbial drinking water monitoring, evaluation and learning (MEL) practices to improve drinking water quality in food establishments.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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