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Research Paper

Contaminated Water and an Indication of Risk: Examining Microbial Contamination in the Water Used by Consumers and Commercial Growers in Fresh Produce Systems in Nepal

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A R T I C L E I N F O

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A B S T R A C T

There has been limited research and understanding of the water quality in developing countries. Fresh produce consumed raw is nutrient-dense but is more susceptible to causing foodborne illness when contaminated water is used in production and consumption. There have been increasing reported incidences of foodborne outbreaks in Nepal linked to fresh produce contamination. However, water used in washing fresh produce by consumers and water used by growers or vendors is rarely tested. This research examines the source water used by consumers and growers in fresh produce systems in Nepal. To examine *Escherichia coli (E. coli)* detection as an indicator of contamination risk in water, we selected five major metropolitan cities for consumer households and ten districts representing commercial growers of vegetable growing areas of all seven provinces of Nepal. Altogether, we collected 394 water samples from randomly selected individual households: 156 from consumer households and 238 from growers or vendors. Results suggest that 59% of the water used in fresh produce systems is contaminated with *E. coli* in Nepal. On the water source used by consumers to wash fresh produce before consumption, we found that the dominant sources are the stored water in tanks or containers (46%) and municipal or communal supply water (39%)—which have *E. coli* prevalence rate of 66% and 57%, respectively. On the dominant sources of water used in fresh produce by growers or vendors, we found up to 88% *of E. coli* prevalence in the water they use. We also discussed the location or regional differences in contamination risks. This nationally represented study has implications for intervention policies and programs for safer food production and consumption practices in countries like Nepal where food safety is an emerging priority.

Consumption of fresh produce contributes to dietary diversity and well-nourished individuals, households, and communities. Inadequate food consumption and low dietary diversity contribute to undernutrition in the households of developing countries like Nepal (Singh et al., 2020). Increasing access to nutrient-dense foods, including salad vegetables typically consumed raw, can mitigate this issue— but only if the high risks of foodborne illness through these are eliminated. However, contamination with the organism that causes foodborne illness can undermine their contributions to reaching nutritional targets. Fresh produce is increasingly recognized as a source of foodborne outbreaks in many parts of the world (Lynch et al., 2009; Olmez, 2016).

The increased consumption of fresh produce has further heightened concerns regarding its food safety (Feng & Reddy, 2014). A rising number of foodborne outbreaks are linked to the consumption of fresh produce when the produce is contaminated with pathogenic bacteria (Callejon et al., 2015). In Nepal, the consumption of fruits and vegetables without proper washing, particularly those consumed raw, was found to be one of the predisposing factors to public health issues (Bhandari et al., 2015). Kavre-Panchkhal's foodborne outbreak in September 2018 in Nepal was one of the largest cases in Nepal where 452 cases were identified with gastrointestinal illness, vomiting, and diarrhea. All the affected cases appear to have food eaten from the same place during a mass gathering for a cultural festival (Adhikari et al., 2021).

Water is a primary source of contamination from the production of fresh produce to its consumption. Rivers, wells, hand-pump or tube

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wells, municipal water supplied, and stored water in tanks and containers are regularly used water sources in Nepal for washing fresh produce. The use of unclean water can lead to cross-contamination which could act as a medium for the spread of pathogens while washing fresh produce (Perez-Rodriguez et al., 2014). Shrestha et al. (2017) demonstrated that the consumers of raw vegetables in metropolitan areas (specifically the capital city, Kathmandu Valley) are at significant risk of developing diarrhea, largely because farmers and vendors wash their produce with unsafe water. Ghimire et al. (2020) found a notable presence of pathogenic bacteria in the salad vegetable samples from the markets in Nepal. The researchers also documented instances of inappropriate or inadequate water usage in salad vegetables. Providing clear guidance for producers and consumers on the quality of the water use during production and consumption is important to ensure safety and health (Banach & Van Der Fels-Klerx, 2020). Water is used in many stages of fresh produce growing, marketing, and consumption. However, water used in the fresh produce systems by growers, vendors, and consumers is rarely tested in Nepal.

Finding the current barriers to fresh produce safety and the awareness level among consumers and growers can facilitate strategic policies and investment designs on fresh produce systems in Nepal (Khanal, Gurung, Timilsina, & Poudel, 2023). An evaluation of water contamination is a crucial step to address the contamination risks and to increase awareness. Assessment of the source of contamination in fresh produce would help to improve the adoption of food safety practices by both producers and consumers. Therefore, this study has examined the contamination of location-specific water sources used in fresh produce systems as a part of a research project in Nepal from the Food Safety Innovation Lab (FSIL) and funding support from the US Agency for International Development (USAID).

Specifically, this research focuses on *E. coli* prevalence in the water used in fresh produce because fresh produce is frequently contaminated with foodborne pathogens (Tambekar & Mundhada, 2006). Moreover, the *E. coli* prevalence test has served as an indicator of contamination in food and water (Ashbolt et al., 2001) and the possibility of the presence of pathogenic microorganisms and food-borne bacteria (Benjamin et al., 2013; McEgan et al., 2013). In addition, *E. coli.* is frequently used as an indicator organism to detect levels of fecal contamination in food and water samples that may be intolerable (Atlas, 1998).

We tested water used by consumers to wash their fresh produce before consumption and the water used by commercial growers or vendors in fresh produce systems, including irrigation and wash water before marketing. Following the general understanding of Nepal, we define commercial growers as those who have market connections and sell their fresh produce. To the best of our knowledge, no previous studies had systematically tested consumer and grower/vendor water using nationally represented samples capturing all seven provinces and five regions from east to west Nepal.

Materials and methods

Sample selection method for water collection. To examine consumer's source water in fresh produce systems, we collected water samples from consumer households from five major metropolitan areas of Nepal—namely, Kathmandu, Pokhara, Bharatpur, Butwal, and Hetauda, from June to August of 2022. By Kathmandu, we denote the Kathmandu Valley metropolitan area in this study as it encompasses a larger metropolitan area involving proximity and close commercial, market, and location ties of three districts within this (the Valley captures metropolitan areas of Kathmandu, Lalitpur, and Bhaktapur districts). Moreover, it is common to denote Kathmandu referring to "Kathmandu Valley" as a common larger metro area in studies and communication.

Three to four wards from each metropolitan area (a ward is the smallest unit of local government in Nepal within a metropolitan area or municipality) were selected using stratified random sampling methods without replacement. After preparing the sample frame from the selected ward of each metropolitan area, in-person interviews were conducted with one adult representative member of the household to record the complete information regarding the water source used by the consumer to wash their fresh produce. From the randomly selected ward of five metro cities, we collected 156 water samples, the consumers use to wash the fresh produce before consumption, from randomly selected individual households representing all major Tole clusters within the selected wards. Toles represent the clusters or neighborhoods within a ward of a municipality.

To examine commercial fresh produce growers or vendor's source water, we selected 10 districts representing major vegetable pocket areas of all seven provinces in Nepal. We administered purposive sampling for the selection of municipalities within the districts to include municipalities and villages with major commercial producers of vegetables. A list of commercial vegetable growers was prepared in each selected municipality or village by consulting agriculture institutions and local producer groups. From the list of fresh producers, in-person interviews were conducted to know their source water in fresh produce systems, specifically irrigation water and the water used to wash their fresh produce before marketing. From the growers and vendors who wash their fresh produce before marketing, we collected altogether 238 water samples from randomly selecting individual growers or vendor households representing each district and cluster. Figure 1 shows the water collection districts. As shown in Figure 1, our collected water includes the following districts from east to west Nepal representing all seven provinces: Morang (Koshi Province/ Province 1), Sarlahi (Madhesh Province/Province 2), Kathmandu, Lalitpur, Chitwan, Makwanpur (Bagmati Province), Kaski (Gandaki Province), Rupandehi, Banke, and Palpa (Lumbini Province), Surkhet (Karnali Province), and Kailali (Sudoorpaschim Province).

Water sample collection and testing procedure. Before conducting the water sample collection from the randomly selected households, the project team designed a detailed protocol for water collection and testing. The water collection protocol and the project activities including this water collection and sampling were reviewed and approved by ethical committees of the project partner institutions who are leading and implementing the activities. Prior to the water collection and testing, the Institutional Biosafety Committee (IBC) of the institution leading the water collection and testing process reviewed the process and protocol and approved it.

Collection of fresh produce washing water samples from the sampled consumer households was done between June and August of 2022 while water samples from grower or vendor households were collected between October 2022 and February 2023, in combination with the household survey activities of the project. The Aquagenx CBT EC + TC Kit (Aquagenx LLC) was used to test the collected water sample (detail information on Aquagenx and kit: https://www.aquagenx.com/cbt-ectc/). Aquagenx CBT EC + TC Kit has been widely used in water quality monitoring in developing countries and is well documented and compared with traditional and latest available market products (Pichel et al., 2023). Water from the individual household or farm was collected with the help of a syringe (separate syringe for each household). A total of 100 mL of water was poured from the syringe into a water sampling Whirl-Pak Thio-Bag containing sodium thiosulfate. The places where collection from a syringe was impractical, particularly in deep tanks or containers with narrow necks, a sterile water cup was used as the initial receptacle for the water sample. As per the instruction in the kit package, sodium thiosulfate in bags was used to neutralize residual chlorine if presented in collected samples. Sample details were recorded. Aquagenx EC + TC growth medium was added to the sample in the Thio-bag. The growth medium was completely dissolved in the sample before incubation. The samples

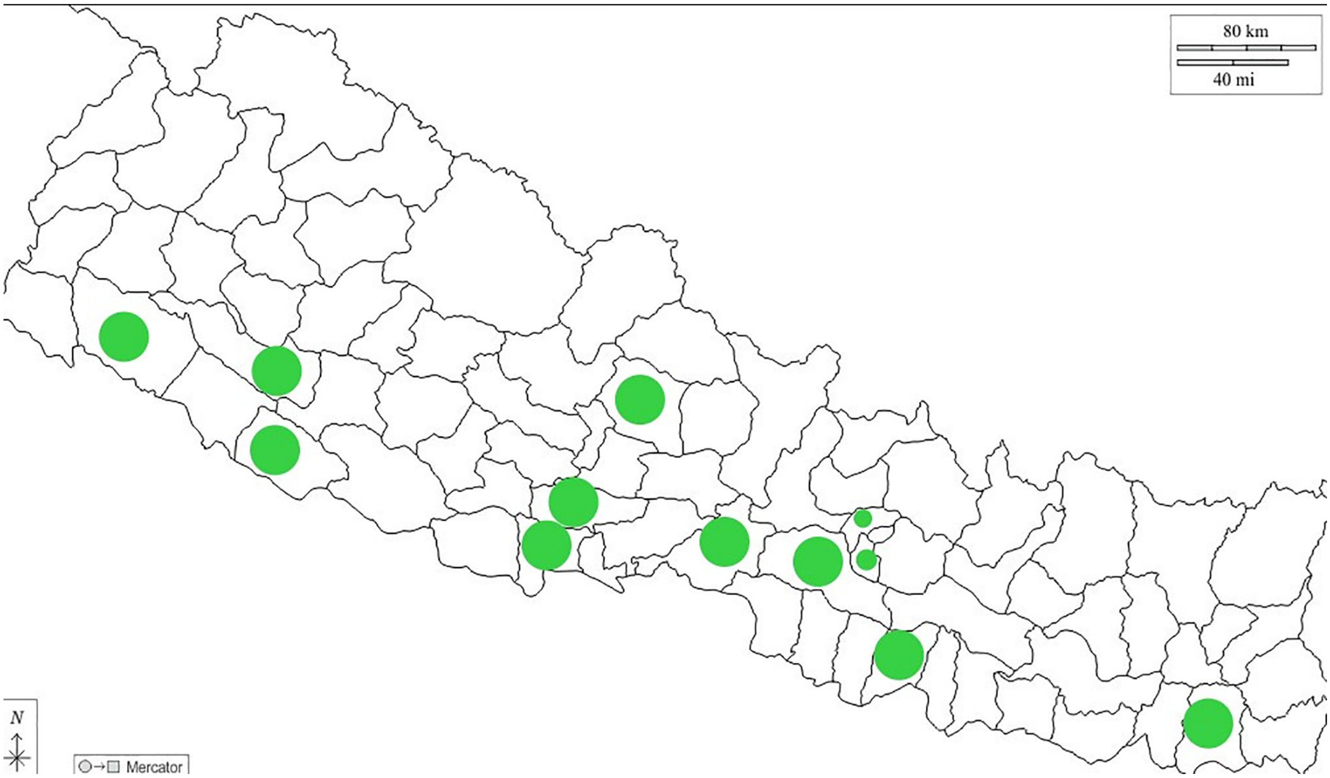

Figure 1. Geographical distribution of water sample collection locations across different regions in Nepal (total samples $N = 394$).

were incubated in a portable incubator at 33°C. Results were recorded after 28 h.

Following the protocol of the test kit, a change in color from yellow to blue or blue-green confirmed the presence of *E. coli.* As per the protocol of the test kit described, contaminated samples were treated with sodium hypochlorite before disposal to prevent further risk of contamination.

Statistical analysis. From the test results data recorded and the information about the sample, data were statistically analyzed to test the significance differences and associations by locations and sources using the statistical package SPSS. The chi-square test was used to test the differences by location and sources. A descriptive method was applied to tabulate the frequency of the variables.

Results and discussion

This section presents the results of the water sample tests collected from different sources and locations and discusses the findings.

Prevalence of *E. coli* in the water of consumer households in different metropolitan cities. Table 1 shows the prevalence rate of *E. coli* in the water samples of consumer households in metropolitan cities. Recall that we collected the water samples used to wash the fresh produce before consumption. Among the five metropolitan cities selected for this study to test the water sample, Pokhara has the highest prevalence rate of *E. coli* (92%), followed by Butwal (83%) and Makwanpur (71%). The association between metropolitan cities and the prevalence rate of *E. coli* in fresh produce wash water was analyzed using a Chi-square test. Findings show that there is a significant difference in contamination of wash water with respect to the locations. Our findings on the association of location and risks are consistent with Uprety et al. (2020) who indicated the spatial differences in water quality risks in Nepal.

Water sources used by the consumers to wash fresh produce and *E. coli* prevalence. Table 2 presents the water sources used by consumers to wash their fresh produce and their *E. coli* prevalence rate. Among the 156 water samples, 46% of consumer households used stored water to wash their fresh produce. Similarly, 40% of households used tap water to wash fresh produce, and 16% of consumer households used other sources of water than tap water and stored water to wash their fresh produce.

Table 1
Prevalence of *E. coli* at consumer households' fresh produce washing water in different metropolitan cities of Nepal

Metropolitan cities	Total water samples	Result of detection test (number)		Prevalence rate (%)	Chi-square statistics	p value[a]
	($n = 156$)	Positive	Negative			
Kathmandu	61	26	35	43	$X_4^2 = 24.92$	0.001***
Bharatpur	23	13	10	57		
Hetauda	23	16	7	70		
Butwal	24	20	4	83		
Pokhara	25	23	2	92		

[a] the significance denoted in p value, ***, indicates significance at 1% level or higher indicating location-wise differences, the prevalence of *E. coli* is contingent upon location.

Table 2
Water sources used by consumer households to wash their fresh produce and *E. coli* prevalence

Water sources	Number of households (n = 156)	Prevalence rate of *E. coli* (%)	Chi-square statistics	p value[a]
Tap Water	61 (39)[b]	57	1.272	0.529
Stored water	71 (46)[b]	66		
Others	24 (15)[b]	67		

[a] the test in this table shows whether the differences in prevalence are statistically significant across sources used by consumers.
[b] numbers in the parenthesis indicate the percentage of this source used.

The prevalence of *E. coli* in different water sources is also illustrated in Table 2. The prevalence rate of *E. coli* in stored water and tap water was 66% and 57%, respectively. We found about 67% of other water sources (other than tap water and stored water) were contaminated with *E. coli*. Other water sources include pump water, tank suppliers, drinking water, and overground or underground tanks. The association between wash water sources and the prevalence rate of *E. coli* was analyzed using the Chi-square test. The p value of 0.529 suggests no significant differences in contamination of fresh produce washing water across water sources used by the consumer to wash fresh produce before consumption.

Prevalence of *E. coli* in the water used by growers or vendors in fresh produce systems. Figure 2 and Table 3 show the prevalence of *E. coli* in the water used by fresh produce growers or vendors to wash their fresh produce before marketing. The prevalence rate of *E. coli* in the water used in marketable fresh produce is alarming. Specifically, among 10 sampled districts, the highest level of the problem seems to be in Surkhet and Makwanpur, where we found all sampled water had the presence of *E. coli*. Additionally, a high-level prevalence was found in Palpa (86%) and Kaski (82%), followed by Kailali (48%), and Kathmandu (45%). On the lowest prevalence range of *E. Coli*, among the 10 sampled districts, we found Chitwan (19%), Banke (24%), Sarlahi (25%), and Morang (27%), respectively. Looking at the geographical distribution, this may imply that the water and water sources used by growers in the mid-hills (Surkhet, Makwanpur, Palpa, Kaski) have a higher prevalence rate of *E. coli* as compared to Terai (Chitwan, Banke, Sarlhai, Morang, Kailali) region of Nepal. Our finding is consistent with findings from some previous studies. For example, Upreti et al. (2020) found a higher coliform count in the samples from the hilly regions of Nepal than Terai. It may be worthwhile mentioning that there was a cholera outbreak in 2009 with 12,500 reported diarrheal incidences and 128 deaths in Jajarkot district, which is one of the mid-hill districts in mid-western Nepal (Bhandari et al., 2009).

Now turning our attention to the sources of water used by growers and vendors, Table 4 discusses the different water sources used by growers or vendors to wash their fresh produce before marketing and *E. coli* prevalence rates. Among the 238 fresh produce growers or vendors selected for water samples, 42% used hand pump/tubewell water to wash their fresh produce, 22% used municipally supplied tap water, and 18% used the river/stream/*khola* water to wash their fresh produce before marketing. Furthermore, the remaining 18% of the growers or vendors used other sources of water. Other sources include well water, public or community tap water, and tank water stored in over- or under-ground tanks.

The prevalence of *E. coli* by the water source shown in Table 4 suggests that the river/stream/*khola* water has the highest (88%) *E. coli* presence rate. Consistently, a high rate of contamination of irrigation source water has been reported in Nepal in previous studies—for example, a study conducted by Shrestha et al. (2016) found that irrigation water has a 100% *E. coli* contamination rate in Nepal. Our test results across sources presented in Table 4 further show that the prevalence rate of *E. coli* in stored water and tap water was 66% and 58%, respectively. Also, note that the least prevalence of *E. coli* was found in hand-pump water (29%). Hand-pump water is fundamentally the underground water pumped directly from the soil water table. This finding is in line with Warner et al. (2008) who suggest that bacterial contamination decreases as the depth of the water sources increases. Finally, about 68% of water sources, marked as 'other' in Table 4, were contaminated.

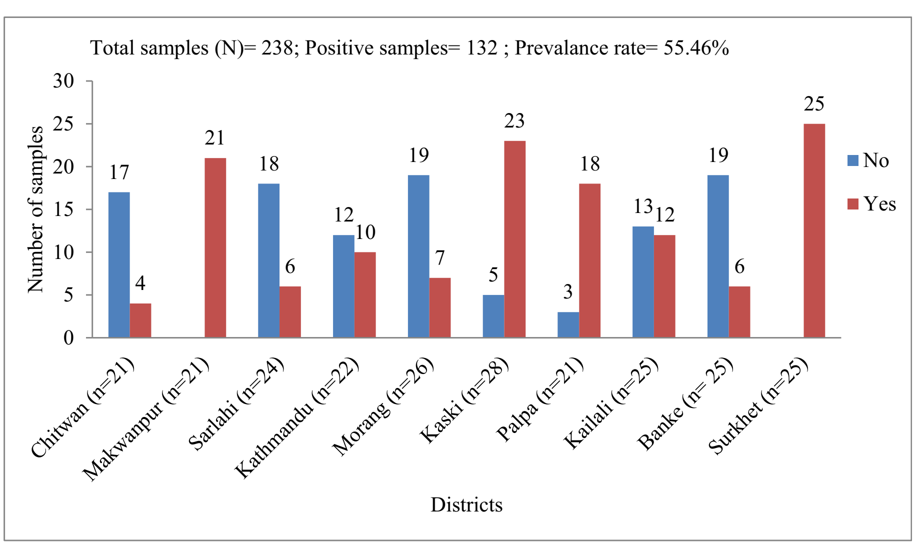

Figure 2. Prevalence of *E. coli* ("Yes" indicates presence, "No" indicates absence) in the wash water used in fresh produce by growers/vendors in Nepal by sampled districts.

Table 3
Prevalence of *E. coli* at producer households in different districts of Nepal

Districts	Total number of water samples (n = 238)	Result of the detection test (number)		Prevalence rate *E. coli* (%)	Chi-square statistics	p value[a]
		Positive	Negative			
Chitwan	21	4	17	19	X_9^2 = 93.13	0.001***
Makwanpur	21	21	0	100		
Sarlahi	24	6	18	25		
Kathmandu	22	10	12	45		
Morang	26	7	19	27		
Kaski	28	23	5	82		
Palpa	21	18	3	86		
Kailali	25	12	13	48		
Banke	25	6	19	24		
Surkhet	25	25	0	100		

[a] the significance denoted in p value, ***, indicates significance at 1% level or higher indicating location-wise differences, the prevalence of *E. coli* is contingent upon location.

Table 4
Water sources used by producers to wash fresh produce and *E. coli* prevalence rate

Water sources	Total number of samples[a] n = 238	Prevalence rate of *E. coli* (%)	Chi-square statistics	p value[b]
Hand pump/tube well	100 (42)	29	54.67	0.001***
Municipal Tap Water	52(22)	73		
River/Stream/*Khola*[c]	43(18)	88		
Others	43(18)	63		

[a] numbers in the parentheses in this column indicate the percentage of that source used;
[b] the significance denoted in p value, ***, indicates significance at 1% level or higher indicating location-wise differences, the prevalence of *E. coli* is contingent upon location.
[c] *Khola* refers to a small stream with stagnant or moving water.

The association between wash water sources and the prevalence rate of *E. coli* was analyzed using a Chi-square test. The p value of 0.001 shows that there is a significant difference, a significant level of 1% or even higher, in contamination of water with respect to water sources used by the growers or vendors to wash their fresh produce before marketing.

Finally, we would like to provide a comparison of our findings between wash water by consumers and growers/ vendors but with a cautionary note that the collection seasons for consumer and grower water were different. Figure 2 and Table 1 depict that the consumer water samples had a slightly higher overall prevalence rate (63%) of *E. coli* than the grower's (56%). As described in the sample collection section under the methodology, consumer water samples were collected during the rainy season. Xue et al. (2018) and Huang et al. (2016) inferred that the rainy season has a higher risk of microbial contamination as compared to the winter season. However, Shrestha et al (2016) in Nepal found that *E. coli* concentration in the irrigation water samples in the rainy season was comparatively lower than that of the dry season.

Ensuring safe water in the food production and marketing systems is critical to maintaining food safety. Particularly in developing countries like Nepal, this is an important but challenging issue. With very limited prior research, Nepal needs more studies in multiple sectors addressing food safety. Food safety is an emerging emphasis in Nepal as the Nepal government is prioritizing the discussions of agricultural food policies. Our findings indicate that due to the elevated contamination rate of water employed in fresh produce systems, particularly the water used for washing, a considerable portion of the fresh produce in Nepal is deemed unsafe for raw consumption. We suggest interventions on multiple sectors and levels to initiate the solution. First, appropriate feasible methods should be sought to decontaminate water used in fresh produce systems—which requires attention from both private and public sectors and relevant research and outreach. Second, agricultural and food-related water testing guidelines, safety measures in washing and cleaning, and regulations should be developed to prevent foodborne disease outbreaks. Third, educational and awareness efforts should be increased on multiple levels of supply chain stages and actors to enhance the awareness of growers, consumers, vendors, and processors as safer production is linked with producer and consumer behavior. Agents should be aware of the water quality issues and adverse health effects of foodborne illness it could bring. Awareness campaigns on multiple levels may also help to reduce moral hazard problems like a separate behavior on fresh produce for own consumption vs. market. Fourth, the government and sectors involved should emphasize the ways to incentivize the adoption of safer food and water use practices in the food supply chains as trade-offs exist among safer food systems, prices, convenience, access, and preference. Finally, there should be strong monitoring to enforce proper implementation of the intended regulations for safer water use in food systems.

Ethics statement

This project was reviewed and approved by the institutional review board/ethics committee of the partner institutions: Tennessee State University (TSU) and Agriculture and Forestry University (AFU); the protocol involving water collection and microbial test was reviewed and approved by the Institutional Biosafety Committee of AFU. The activities were performed in accordance with ethical standards with a consent statement prior to the survey and water collection. The researcher/ trained enumerator read the consent statement and took verbal consent from each participant before the survey and water collection.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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