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A microbiological and parasitological study of drinking water from point-of-use taps and wastewater from adjacent open ditches in Addis Ketema and Akaki/Kality sub-cities, Addis Ababa, Ethiopia

Helina Mogessie*, Mengistu Legese, Aklilu Feleke, Tilahun Teklehaymanot, Haile Alemayehu and Mogessie Ashenafi

[1.] Department of microbiology, Aklilu Lemma Inistitute of Phatology, Addis Ababa University
[2.] Center for Food security Studies, College of Development Studies, Addis Ababa University. Addis Ababa Ethiopia

Abstract: Wastewater drained through open ditches poses a threat of contamination to households and drinking water distribution lines. This study assessed the bacterial and parasite load of drinking water and wastewater. A total of 205 drinking water and wastewater samples were aseptically collected from three woredas each of Addis Ketema and Akaki/Kality sub-citiesand analyzed for enteric pathogens, total aerobic mesophilic bacteria, enterobacteria, total coliforms,and protozoan and helminth parasites using standard microbiological and parasitological techniques. Wastewater samples from both sub-cities had mean counts of aerobic mesophilic bacteria, enterobacteria and total coliforms above log 6 cfu/ml (CV, <10%). Counts of enterobacteria and total coliform from drinking water in both sub-cities were beyond permissible levels (>log 2 cfu/ml). Mean counts (log cfu/ml) of aerobic mesophilic bacteria, enterobacteria, and coliforms in drinking water showed significant difference at P=0.013 (CI: -0.82722, 0.27937); P<0.001 (CI: -1.797, -3.358) and P<0.001 (CI: -2.289, -0.759] between the two sub-cities, respectively. From surface wastewater samples, only the total coliform counts showed a significant difference with p=0.008 (-1.149, 0.003), however, there was no significant difference in mean counts of aerobic mesophilic bacteria p=0.764 (-0.022, 0.434) and enterobacteria 0.115 (-0.311, 0.363) between sub-cities. No *Salmonella* or *Shigella* were encountered.Various non-lactose fermenting Gram negative bacteria, mainly diminated by *Proteus* Spp., *Pseudomonas aeruginosa,* and *Alcaligenes faecalis* were isolated from wastewater in both sub-cities. Protozoan and helminth parasites in wastewater and drinking water samples were mainly dominated by *Giardia lamblia, Taenia* spp, and *Ascaris lumbricoides*. In conclusion, drinking water in the study sub-cities was contaminated with various opportunistic pathogens and disease causing parasites. Thus, households in the study sub-cities should treat drinking water before consumption. Responsible authorities should check the integrity of drinking water distribution lines periodically.

Keywords/Phrases: Addis Ketema, Akaki/Kality, Addis Ababa, drinking water, wastewater, microbial load, parasites.

INTRODUCTION

All living organisms need water to survive.The human body is about 70% water. Nutrients are transported to cells and wastes are taken away there from by water. All metabolic reactions in the body occur in aqueous solutions. For this reason, the water we drink must be free from disease-causing organisms and poisonous chemicals that are a threat to human health (Nelson and Cox, 2017; Popkin et al., 2010).

The Sustainable Development Goal 6 is to "ensure availability and sustainable management of water and sanitation for all" (UN, 2020). Improving access to safe drinkingwater supplies may involve, among others, constructing or improving water supply systems or services such as the provision of safe piped water at point-of-use. In Addis Ababa, Ethiopia, households secure drinking water from treated municipality lines.

A safe sanitation system is designed and used to separate human excreta from human contact at all steps of the sanitation service chain, from safe toilets and containment through conveyance (in sewers or by emptying and transport), to treatment and final disposal or end use (WHO, 2018).

Water sources could be contaminated by pathogenic bacteria, viruses, parasites, and parasitic worms (Kristani et al., 2022). Source water is, therefore, treated to remove harmful biological contaminants and to improve the taste, smell, and visual appearance of drinking water. Drinking water is not, however, sterile and bacteria can be found in the distribution system and at the tap (Fawel and Nieuwenhuijsen, 2003). Although low levels of microorganisms may persist in the treated water, and drinking water may contain between 10^3 and 10^6 cells/ml at the point of use, the presence of indicator or pathogenic bacteria is unacceptable (Prest et al., 2016).

Various studies showed that, in many developing countries, drinking water at point-of-use was more contaminated than at the source (Sheeba et al., 2017, Ferdous et al., 2021). A report by Gundryet al. (2006) indicated contamination of drinking water between source and point-of-use in South Africa and Zimbabwe. Saima et al. (2023) found that,in Dhaka, Bangladesh, fecal contamination, along with the prevalence of diarrheagenic bacteria, were more frequent in water at point-of-use than in the public domain source water in a low-income community. In Ethiopia, studies by Gemechu Ameya et al. (2018) and Yohanis Alemeshet et al. (2021) revealed that bacteriological quality of drinking water deteriorated from source to point-of-use.

In Addis Ababa, centrally treated municipal water is distributed to households and is believed to be safe to drink. However, while municipal water is believed tbe free of undesirable biological contaminants, particularly bacteria, when it exits central treatment sites, contamination can occur within the distribution system. It is common to see open ditches, serving as sewers along inner roads in residential areas, to convey wastewater and rain water away from residences. Most low-income households use common pit latrines, which may leak their contents into open ditches. During the rainy season, flooding results in the overflow of sewers forming small pools along the surfaces of roads. It has long been known that sanitary sewer overflow can spill raw wastewater onto city streets (USEPA, 1996). According to Calderón et al. (2017), combined wastewater overflow yielded over 1000 species of bacteria belonging to twenty-two classes.

Wastewater drained through open ditches may serve as a source of bacterial and parasite contamination to households through vectors. Moreover, open-ditch sewers are usually blocked at some point along the line resulting in stagnant wastewater close to residences. When it seeps down into the soil and travels underground, it may also pollute municipal water by contact with defective water distribution lines. Since recently, new underground water distribution pipes, made of synthetic material, have been used. Older lines were, however, made from metal pipes. According to Gemechu Ameya et al. (2018), incorrect cross-connection with sewer lines, interconnection with toilets, pipe corrosion, and pipe breakage could lead to the infiltration of bacterial contaminants into water distribution lines. Owa (2014) also reported wastewater leakage into drinking water distribution lines.

Several workers have isolated various bacteria, viruses, and parasites from drinking water. As reported by Fawell and Nieuwenhuijsen (2003), some opportunist pathogens, such as *Pseudomonas aeruginosa* and *Aeromonas* spp., may multiply during distribution given suitable conditions. Similarly, Suthar et al. (2009) also reported isolation of Gram-positive and Gram-negative bacteria from drinking water in India. A review by Kristanti et al. (2022) showed that pathogenic bacteria, viruses, protozoan parasites, and parasitic worms were isolated from drinking water. The study by Amsalu Mekonnen et al. (2020) showed that 10%, 7%, and 3% of municipal water samples in Addis Ababa were positive for bacteria, coliforms, and fecal coliforms, respectively. There is no available information on bacterial safety risks posed by wastewater drainage through open-ditches and possible contamination of drinking water therefrom. The aim of this study was, therefore, to determine the level of bacterial and parasite contamination of drinking water at point-of-use in households and wastewaterin adjacent open ditches in Addis Ketema and Akaki/Kality sub-cities in Addis Ababa, Ethiopia.

MATERIALS AND METHODS

Description of the study areas

This study was conducted in Addis Ketema and Akaki Kality sub-cities from May to July 2023. Addis Ketema is one of the 11 sub-cities of Addis Ababa comprised of 10 woredas. It is in the northwestern part of the city. It is the major commercial area close to the city center. It has a total area of 7.41km^2 and is the most densely populated area in the city (49,616 people/km^2). Akaki/Kality sub-city is located in the southern part of the city, 20 km from the city center. It has an area of 118.08 km² with low population density (2,163 people/km²). The sub-city is the industrial zone of Addis Ababa. About 16% of the population of Addis Ababa live in the two sub-cities.

The two sub-cities were purposively selected because they had accounted for 50% of the total cases during the 2017 and 2019 cholera outbreaks in Addis Ababa. For this study, three woredas were selected, each from the two sub-cities, based on the high cholera incidence in 2017 and 2019. These were woredas Six, Seven, and Eight from Akaki/Kality sub-city and woredas Three, Seven, and Eight from the Addis Ketema sub-city (Fig. 1). A woreda is the lowest administrative unit in Addis Ababa.

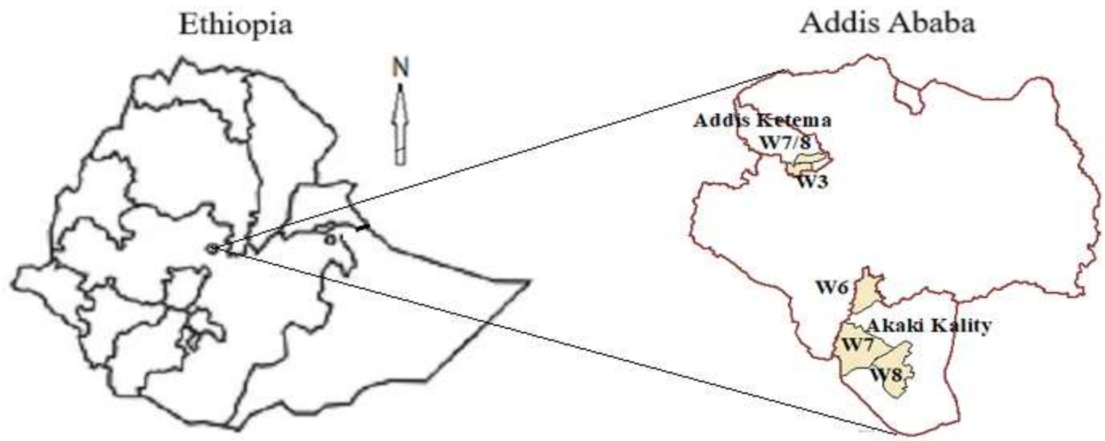

Fig. 1. Map showing the study woredas in Addis Ketema and Akaki/Kality sub-cities, Addis Ababa (modified from Worku Adefris et al., 2013)

Sampling and Sample size determination

The study sub-cities were selected purposively based on their high incidence of cholera cases in the 2019 outbreak in Addis Ababa. The following formula was used to estimate the prevalenve of waterborne pathogens in a given population (drinking water and wastewater samples) as in Yohanis Alemeshet et al. (2021) to determine the sample size.

Sample size of drinking water and wastewater samples for microbiological analysis was determined as follows:

$$n = \frac{Z\alpha_{/2}^2 P(1-P)}{d^2}$$

$$n = \frac{(1.96)^2 \times 0.14(1-0.14)}{(0.05)^2}$$

n= 186

Where: n = sample size

$Z\alpha_{/2}$ (alpha risk expresses in z-score) = 1.96

P (expected prevalence) = 14% (0.14)

D (absolute precision) = 0.05

After taking 10% unwillingness rate to provide water sample at point of use, the final sample size would be 205.

A total of 205 environmental samples consisting of drinking water (n=97) and surface wastewater (n=108) were collected for the study from the two Sub cities proportionally based on the populations in each sub city and woreda. The data of current population and households in each sub cities and woredas were collected from tax and revenue offices of each woreda. Based on the data the total number of samples were allocated proportionally to the study sub cities and woredas.

Study households were selected following the systematic random sampling method. Wastewater samples were collected from open wastewater ditches aroundselected households.

Drinking water collected from point-of-use taps in households and wastewater samples (n=205), collected from adjacent open ditches along inner roads in two sub-cities in Addis Ababa, were considered in this study. A total of 132 samples made up of drinking water (65) and wastewater (67) were collected from Addis Ketema sub-city. Similarly, a total of 73 samples consisting of 32 drinking water and 41 wastewater were collected from Akaki/Kality sub-city.

Sample collection

A volume of 250 ml of drinking water samples wereaseptically collected using 500-ml sterile narrow-necked screw capped bottle from a total of 97 households at point-of-use taps from each selected sub-city. Similarly, 250 ml of wastewater samples were aseptically collected from 108 open ditch sewers, around drinking water collection sites. Open ditch sewers were found along inner roads in residential areas in both sub-cities. Collected samples were immediately transported in an icebox to the microbiology laboratory at Aklilu Lemma Institute of Pathobiology. Samples were processed microbiologically within two hours of collection.

Bacterial and parasitic detection and identification

Aliquotes of 0.1 ml from appropriate dilutions of homogenized samples were surface plated in duplicates on pre-dried surfaces of the following media (Oxoid) for microbial enumeration: Plate Count (PC) agar for aerobic mesophilic bacteria, Violet Red Bile (VRB) agar for total coliforms, and Violet Red Bile Glucose (VRBG) agar for enterobacteria. The plates were incubated under aerobic conditions for 1 to 2 days at 30-32°C. Samples were also directly streaked on Xylose Lactose Deoxycholate (XLD) agar plates after primary enrichment in buffered peptone agar (24 h) and selective enrichment in Rappaport-Vassiliadis broth (24 h) to isolate *Salmonella* and *Shigella*i spp. All non-lactose fermenting colonies were picked from XLD plates, purified by streaking on PC agar plates and screened for *Salmonella* and *Shigella* by biochemical tests using Triple sugar Iron agar, Lysine Iron agar, Urea agar and Citrate agar tubes. For further confirmation, isolates were subjected to Zybio EXS3000 mass spectrometry (MALDI-TOF MS) as in Li et al. (2023).

For the detection of parasites, a well vortexed 100ml sample was filtered by a 47-mm diameter, 0.450-µm pore size membrane filter using a vacuum pump. Organisms on the filter membrane were transferred into distilled water in a 15-ml conical centrifuge tube and centrifuged at 5000 rpm for 10 min. The sediment was examined under the microscope at low- (10X) and high-power (40X) magnification for the presence of protozoan cysts or trophozoites, and helminth ova/eggs and larvae (Amsalu Mekonnen et al., 2020).

Data were analyzed using SPSS version 29 and presented as means and coefficient of variation, Comparison of mean counts among sub cities and within woredas was conducted using independent samples T-test and One-Way ANOVA, respectively.

RESULTS AND DISCUSSION

Microbial counts in Drinking water from the study sub-cities

In Addis Ketema sub-city, mean Aerboci mesophilic bacterial (AMB) count of drinking water in the three woredas of ranged from log 3.87 (C.I. 95%; 3.43, 4.31) to log 4.13 (C.I. 95%; 3.67, 4.60) cfu/ml (Table 1). Variations in counts among samples was not high (C.V. 19-21%). Similarly, mean counts of enterobacteria from log 3.7 (C.I 95%; 3.07, 4.34); to log 3.9 cfu/ml (C.I. 95%; 3.29, 4.46) and mean total coliform counts from log 3.25 (C.I 95%; 2.84, 5.12) to log 3.36 cfu/ml (C.I 95%; 2.38, 4.34) were obtained from drinking water samples in the three woredas. Variations in counts of enterobacteria and total coliforms among samples was acceptable (C.V., 12% - 23%).

In Akaki/Kality sub-city, mean aerobic mesophilic bacterial (AMB) count of drinking water in the three woredas ranged from log 4.34 (C.I. 95%; 3.86, 4.82) to log 4.41 (C.I. 95%; 4.06, 4.75) cfu/ml (Table 2). Variations in counts among samples was acceptable (C.V. 15-19%). Similarly, mean counts of enterobacteria ranged from from log 3.8 (C.I 95%; 2.95, 4.58); to log 4.4 cfu/ml (C.I. 95%; 3.55, 5.20) and mean total coliform counts from log 3.5 (C.I 95%; 2.84, 4.55) to log 4.3 cfu/ml (C.I 95%; 3.67, 4.98) were obtained from drinking water samples in the three woredas. Variations in counts of enterobacteria (C.V., 6.8% - 24.1%) and total coliforms (C.V., 11.2% - 25.7% among samples was noticable.

Independent T-test among mean counts (log cfu/ml) of aerobic mesophilic bacteria, enterobacteria, and coliforms in drinking water showed significant difference at $P=0.013$ (CI: -0.82722, 0.27937); $P<0.001$ (CI: -1.797, -3.358) and $P<0.001$ (CI: -2.289, -0.759] between the two sub-cities, respectively (Annex 5). The independent T-test also exhibited a significant mean Enterobacteriaceae count difference within drinking water samples at $P= 0.017$ (CI: -2.678, -0.943); and coliforms count difference at $P=0.008$ (CI: -1.149, 0.003) among subcities (Annex 6).

One-Way ANOVA showed that there were no significant differences in mean counts of aerobic mesophilic bacteria, enterobacteria, and coliforms among the sampled woredas in Addis Ketema sub-city (P=0.69; CI: 1.70-1.79) and Akaki/Kality sub-city (P=0.63; CI: 0.398-0.369) sub-cities (Annex 7). According to WHO (2017), the minimum allowable limit is 100 to 500 cfu/ml for aerobic bacteria and <1 cfu/100 ml water for coliforms. Thus all drinking water samples considered in this study exceeded the WHO permissible limits for drinking water. Similarly, Studies in Ethiopia by Mohammed Yasin et al. (2015) from Jimma zone and Bayeh Abera et al. (2014) from Bahir Dar city indicated that AMB, total coliform and enterobacteria counts in drinking water exceeded the WHO standard. Other studies from various countries also reported that counts of total bacteria, enterobacteria and total coliform in drinking water were above permissible WHO limits (Pintor-Cora et al., 2021; Tesfaye Legesse et al., 2018; Adesakin, 2020; Diakite, 2019).

Table 1. Mean bacterial load of drinking water and wastewaterin samples from Addis Ketema sub-city.

Woreda	Sample source (No.)		Bacterial load (log cfu/ml)		
			AMB	EB	Coliforms
Seven	Drinking water (21)	Mean	4.13	3.9	3.25
		SD	0.81	0.66	0.75
		%CV	19.6%	16.9%	23.1%
		C.I. (95%)	(3.67, 4.60)	(3.29, 4.46)	(2.84, 5.12)
	wastewater (21)	Mean	6.98	6.85	6.42
		S.D.	0.47	0.60	0.59
		%CV	6.7%	8.8%	9.2%
		C.I. (95%)	(6.70, 7.27)	(6.56, 7.14)	(6.15, 6.79)
Three	Drinking water. (22)	Mean	4.06	3.7	3.3
		S.D.	0.79	0.71	0.57
		%CV	19.5%	19.1%	12.3%
		C.I. (95%)	(3.62, 4.66)	(3.07, 4.34)	(2.58, 3.81)
	wastewater (23)	Mean	6.55	5.89	5.27
		S.D.	0.45	0.88	0.76
		%CV	6.9%	14.9%	14.4%
		C.I. (95%)	(6.30, 6.79)	(5.48, 6.28)	(4.95, 5.64)
Eight	Drinking water. (22)	Mean	3.87	3.81	3.36
		S.D.	0.83	0.88	0.71
		%CV	21%	23.1%	20.1%
		C.I. (95%)	(3.43, 4.31)	(2.89, 4.73)	(2.38, 4.34)
	wastewater (23)	Mean	7.14	6.50	6.62
		S.D.	1.06	0.80	0.72
		%CV	14.8%	12.3%	10.9%
		C.I. (95%)	(6.46, 7.26)	(6.02, 6.99)	(6.28, 7.02)

AMB, aerobic mesophilic bacteria; EB, enterobacteria; SD, Standard deviation;CI, Confidence Interval; CV, Coefficient of variation.

Microbial counts in wastewater from the study sub-cities

Wastewater samples from the three woredas of Addis Ketema sub-city had mean counts of aerobic mesophilic bacteria ranging from log 6.6 (C.I. 95%, 6.30, 6.79) to log 7.1 (C.I. 95%, 6.46, 7.26). Mean counts for enterobacteria ranged grom 5.9 (C.I. 95%, 5.48, 6.28) to 6.9 (C.I. 95%, 6.56, 7.14); and for total coliforms ranged from 5.3 (C.I. 95%, 4.95, 5.64) to 6.4 (C.I. 95%, 6.28, 7.02) (Table 2).

Mean counts of aerobic mesophilic bacteria, enterobacteria and total coliforms in all wastewater samples from the three woredas of Akaki/Kality sub-city were within the range of log 6 cfu/ml. From surface wastewater samples, only the total coliform counts showed a significant difference with p=0.008 (-1.149, 0.003), however, there was no significant difference in mean counts of aerobic mesophilic bacteria p=0.764 (-0.022, 0.434) and enterobacteria 0.115 (-0.311, 0.363) between sub-cities (Annex 6). Bacterial load of drinking water and waste water in samples from Akaki/Kality sub-city

Table 2. Bacterial load of drinking water and waste water in samples from Akaki Kality Subcity.

Woreda	Sample source (No.)	Bacterial load (log cfu/ml)	AMB	EB	Coliforms
Six	Drinking water (12)	Mean	4.34	3.77	3.47
		SD	0.64	0.91	0.89
		%CV	14.7%	24.1%	25.7%
		C.I. (95%)	(3.86, 4.82)	(2.95, 4.58)	(2.84, 4.55)
	wastewater (12)	Mean	6.79	6.48	6.37
		S.D.	0.66	0.68	0.72
		%CV	9.7%	10.5%	11.3
		C.I. (95%)	(6.32, 7.26)	(6.00, 6.96)	(5.83, 6.92)
Seven	Drinking water (11)	Mean	4.38	4.35	4.34
		S.D.	0.72	0.73	0.86
		%CV	16.4%	16.8%	19.8%
		C.I. (95%)	(3.83, 4.92)	(3.90, 5.04)	(3.67, 4.98)
	wastewater (15)	Mean	6.87	6.66	6.61
		S.D.	0.56	0.76	0.74
		%CV	8.15%	11.4%	11.2%
		C.I. (95%)	(6.55, 7.30)	(6.18, 7.12)	(6.17, 7.05)
Eight	Drinking water (9)	Mean	4. 41	4. 38	4.30
		S.D.	0.83	0.30	0.48
		%CV	18.9%	6.8%	11.2%
		C.I. (95%)	(4.06, 4.75)	(3.55. 5.20)	(3.82, 4.77)
	wastewater (15)	Mean	6.47	6.20	6.11
		S.D.	0.55	0.61	0.71
		%CV	8.5%	9.8%	11.6%
		C.I. (95%)	(6.12, 6.81)	(5.84, 6.56)	(5.76, 6.61)

AMB, aerobic mesophilic bacteria, EB, enterobacteria, SD, Standard deviation, CV, Coefficient of variation..

Similar to observations in our study, wastewater is known to harbor total and fecal coliforms as well as pathogenic bacteria (Xie et al., 2022), although domestic wastewater was reported to have a relatively low microbial population (Latrach et al., 2015). In Addis Ababa, drinking water is centrally treated for potability before distribution. Shegaw Fentaye et al. (2024) reported that drinking water from the treatment plant was of low risk when compared to water in the distribution system. Thus, presence of microorganisms in drinking water above permissible limits is indicative of contamination in distribution lines. According to Some et al. (2021), sewage is the prime source of microbial pollution of water. A report by Owa (2014) indicated that bacteria in wastewater could leak into drinking water distribution lines and contaminate potable water. Incorrect cross-connection with sewer lines, interconnection with toilets, pipe corrosion, and pipe breakage could lead to the infiltration of bacterial contaminants into water distribution lines as reported by Gemechu Ameya et al. (2018). In a study by Fikralem Alemu et al. (2023), the proportion of households with contaminated drinking water in Eastern Ethiopia is markedly high. Amsalu Mekonnen et al. 2020 reported that in Addis Ababa, 10% and 7% of municipal water samples contained aerobic mesophilic bacteria and total coliforms, respectively.

Although no *Salmonella* or *Shigella* were encountered in our drinking water samples, . Edessa Negera et al. (2017) isolated *Salmonella* spp.

from drinking water samples from households in Shashemene, Ethiopia.

Identification of non-lactose fermenting rods from drinking water and wastewater from the study sites

None of the drinking water and wastewater samples yielded *Salmonella* or *Shigella* species. A total of six different species (all non-lactose fermenting rods on XLD) isolated from both types of samples in the study sub-cities were encountered in this study (Table 3). *Proteus vulgaris* (n=16) from wastewater and *Pseudomonas aeruginosa* (n=20) from both wastewater and drinking water were the most frequently encountered species. A small number of drinking water samples from Addis Ketema sub-city yielded only *Pseudomonas aeruginosa* (Table 3).

Table 3. Non-lactose fermenter sare frequently isolated from wastewater and drinking water samples in the study sub-cities.

Sub-city	Source	Non-lactose Fermenters	No of positive samples
Akaki/Kality	wastewater (42)	*Proteus vulgaris*	10
		Proteus mirabilis	4
		Alcaligenes faecalis	3
		Morganella morganii	3
		Serratia marcescens	2
		Pseudomonas aeruginosa	2
	Drinking water (33)	-	-
Addis Ketema	wastewater (66)	*Pseudomonas aeruginosa*	14
		Alcaligenes faecalis	6
		Proteus vulgaris	6
	Drinking water (65)	*Pseudomonas aeruginosa*	4

Although the isolates in this study are generally considered as commensal residents in the intestinal tracts of both humans and animals, they are also reported as opportunistic pathogens in people with compromised immunity (Magruder et al., 2020). Some of the bacteria species isolated from wastewater by Okonkwo et al. (2022) include *Escherichia coli, Klebsiella* spp, *Proteus* spp, and *Pseudomonas aeruginosa*, which are known as opportunistic pathogens (Al-Kharousi et al., 2016).

Thus, in additions to polluting drinking water in distribution lines, wastewater,found in open ditches along inner roads in residential areas, remains a constant source of contaminationby opportunistic pathogens to surrounding households through insects and rodents, which are frequent visitors of sewers.

Isolation of protozoan and helminth parasites from open wastewater and drinking water in the study sub-cities

The most frequently encountered protozoan parasite in 57% wastewater and 12% of drinking water samples in Akaki/Kality sub-city was *Giardia lamblia* trophozoites and cysts (Table 4). No helminthic parasites were seen in samples from this sub-city. In Addis Ketema sub-city, however, wastewater samples contained different species of protozoan and ova of helminthic parasites, the most frequently encountered being trophozoits and cysts of *Giardia lamblia* (56%). Drinkin water samples in Addis Ketema sub-city were dominated by eggs of *Taenia* spp.(46%), trophozoits and cysts of *Giardia lamblia* (26%) and eggsof *Ascaris lumbricoides* (22%).

The presence of various protozoan and helminth parasites in drinking water was reported by various workers (Al-Morshidy et al., 2015; Omarova et al., 2018). A recent study indicates that *Giardia* is one of the important causative agents of intestinal parasitic diseases and is ubiquitously distributed in the environment, especially in wastewater and reclaimed water (Hemati et al., 2022).

Table 4. Protozoan and helminth parasites were encountered in wastewaterand drinking water samples in the study sub-cities.

Sub-city	Source	Parasite	No ofpositive samples
Akaki/Kality	Wastewater (41)	*Giardia lamblia*(trophozoits& cysts)	24 (58.5%)
		Ciliates (Rotifers)	10 (24.4%)
		Ciliates (Blogorrhea)	6 (14.6%)
	Drinking water (33)	*Giardia lamblia* (trophozoits)	4 (12%)
		Ciliates (Rotifers)	4 (12%)
		Ciliates (Blogorrhea)	2 (6%)
Addis Ketema	Wastewater (66)	*Giardia lamblia*(trophozoits & cysts)	37(56%)
		Entamoeba histolytica/Entamoeba dispar (trophozoits & cysts)	5 (7.5%)
		Ascaris lumbricoides (eggs)	3 (4.5%)
		Hookworm species (eggs)	4 (6.1%)
		Strongyloides stercoralis (larvae)	5 (7.6%)
		Ciliates (Rotifers)	14 (21.2%)
		Ciliates (Blogorrhea)	4 (6.1%)
	Drinking water (65)	*Taenia* spp. (eggs)	30 (46%)
		Diphlobotrium latum (eggs)	1 (1.5%)
		Giardia lamblia (trophozoits & cysts)	17 (26%)
		Entamoeba histolytica/Entamoeba dispar (cysts)	2 (3.1%)
		Ascaris lumbricoides (eggs)	14 (22%)
		Hookworm species (eggs)	1 (1.5%)
		Strongyloides stercoralis (larvae)	1 (1.5%)
		Ciliates (Rotifers)	8 (12.3%)
		Ciliates (Blogorrhea)	3 (4.6%)

Giardia lamblia cyst has prolonged survival in the environment, and is also highly resistant to common disinfectants, such as chlorine (Winiecka-Krusnell and Linder., 1998). Water contamination can be of human origin (wastewater effluent) or of animal origin (e.g., runoff from contaminated fields (Kistemann et al., 2012). Giardia lamblia is one of the most prevalent intestinal protozoan parasites in Ethiopia (Fasil Kenea et al., 2020).

Moreover, most drinking water samples from the Addis Ketema sub-city had eggs of *Taenia* spp., eggs of *Ascaris lumbricoides. Entamoeba histolytica* (cysts), hookworm (eggs), and *Strongyloides stercoralis* (larvae) (Table 4). In general, wastewater and drinking water samples from this sub-city contained a higher variety of protozoan and helminthic parasites than those from Akaki/Kality sub-city. Considering the fact that drinking water is treated with chemicals for potability, the presence of parasites in drinking water at point-of-use taps is unacceptable.Moreover, the type of parasites in drinking water is similar to those in wastewater. This is indicative of contamination with parasites through faulty distribution lines.

Wastewater in open ditch sewers, particularly when blocked along the line, becomes stagnant and sinks into the soil surrounding old and possibly corroded and perforated water distribution lines. According to Gemechu Ameya et al. (2018), incorrect cross-connection with sewer lines, interconnection with toilets, pipe corrosion, and pipe breakage could lead to the infiltration of bacterial contaminants into water distribution lines. Owa (2014) also reported wastewater leakage into drinking water. Following the frequent interruption of water supply in Addis Ababa, resumption of water supply would create negative pressures in distribution lines resulting in a suction effect inside the pipe. Thus, pathogens in the immediate surrounding would be sucked into the system through pipe leaks as observed by Collins and Boxall et al. (2013). Contaminant intrusion through leaks in the water distribution system by negative pressure was experimentally proved by Fontanazza et al. (2015).

Significance of the study

This study has revealed that wastewater water in open ditches along inner roads in residence areas can serve as a constant source of contamination with undesirable bacteria and parasites to households by flies and rodents. Moreover, seeping of wastewater into soil can result in the contamination of municipal drinking water through defective distribution lines by negative pressure. The presence of opportunistic pathogens and protozoan/helminth parasites in municipal drinking water is indicative of such contaminations.

Limitations of the study

The samples were collected only from a limited number of woredas from two sub-cities which had a high number of cholera cases in the last epidemic. The observation may not be applicable to other sub-cities,

CONCLUSION AND RECOMMENDATION

This study has shown that in Addis Ketema and Akaki/Kality sub-cities, drinking water at point-of-use generally may not be considered safe. Moreover, houseflies and rodents may carry disease-causing organisms from open ditch sewers, found along inner roads in residential areas, into households resulting in the contamination of food items and kitchen utensils, eventually resulting in foodborne hazards to consumers. It is recommended that households treat drinking water from point-of-use taps to make it safe for drinking, hand washing, and cleaning kitchen utensils. Responsible government authorities may have to check the integrity of drinking water distribution lines occasionally so thatdrinking water at point of use is safe to drink. Neighborhoods may need to clean open-ditch sewers frequently and ensure a constant flow of wastewater through them.

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Annex 1: Bacterial counts of drinking water samples collected from three woredas in Addis Ketema Sub-city

	Count (log cfu/ml)								
	WOREDA SEVEN			WOREDA THREE			WOREDA EIGHT		
No.	AMB*	EB	TCF	AMB	EB	TCF	AMB	EB	TCF
1	3.10	<2.0	<2.0	4.29	3.40	2.90	<2.0	<2.0	<2.0
2	5.09	4.48	4.20	<2.0	<2.0	<2.0	3.32	<2.0	<2.0
3	4.94	4.08	3.70	>4.48	4.20	4.14	3.0	<2.0	<2.0
4	>6.48	6.15	5.93	3.40	<2.0	<2.0	4.15	<2.0	<2.0
5	4.72	4.38	4.11	3.34	<2.0	<2.0	<2.0	<2.0	<2.0
6	4.48	4.30	<2.0	>5.48	3.72	3.59	3.0	<2.0	<2.0
7	5.34	4.70	<2.0	3.99	2.90	<2.0	5.09	4.49	4.45
8	<2.0	<2.0	<2.0	4.32	3.95	2.98	<2.0	<2.0	<2.0
9	>7.48	>7.48	7.33	4.04	<2.0	<2.0	4.37	<2.0	<2.0
10	<2.0	<2.0	<2.0	2.85	<2.0	<2.0	4.45	4.08	3.60
11	>4.48	3.30	<2.0	5.30	3.20	3.08	5.05	<2.0	<2.0
12	5.36	3.08	2.0	2.0	<2.0	<2.0	5.42	4.91	<2.0
13	3.46	<2.0	<2.0	4.41	<2.0	<2.0	3.04	<2.0	<2.0
14	2.70	2.95	2.84	4.60	<2.0	<2.0	4.32	<2.0	<2.0
15	3.30	2.70	<2.0	<2.0	<2.0	<2.0	3.54	<2.0	<2.0
16	>4.48	4.08	3.80	4.28	<2.0	<2.0	2.79	<2.0	<2.0
17	4.14	2.95	2.69	4.23	3.04	2.48	4.17	2.78	2.48
18	4.03	3.20	3.20	4.33	<2.0	<2.0	>4.48	3.82	3.58
19	3.51	<2.0	<2.0	4.04	<2.0	<2.0	3.97	2.78	2.70
20	3.83	<2.0	<2.0	3.95	<2.0	<2.0	<2.0	<2.0	<2.0
21	3.99	<2.0	<2.0	>5.48	<2.0	<2.0	2.60	<2.0	<2.0
22	-	-	-	5.31	5.23	<2.0	3.57	<2.0	<2.0

AMB, Aerobic mesophilic bacteria, EB, enterobacteria, TCF, Total Coliforms,

Annex 2: Bacterial counts of drinking water samples collected from three woredas in Akaki/Kality Sub-city

	Count (log cfu/ml)								
	WOREDA SIX			WOREDA SEVEN			WOREDA EIGHT		
No.	AMB*	EB	TCF	AMB	EB	TCF	AMB	EB	TCF
1	4.67	<2.0	<2.0	4.67	<2.0	<2.0	4.43	4.03	3.64
2	5.34	5.25	5.24	5.34	5.25	5.24	4.99	5.00	4.72
3	3.1	2.9	2.6	3.1	2.9	2.6	4.53	4.49	4.32
4	4.93	4.83	4.45	4.93	4.83	4.45	<2.0	<2.0	<2.0
5	<2.0	<2.0	<2.0	<2.0	<2.0	<2.0	5.48	5.48	5.15
6	>5.48	>5.48	5.48	>5.48	>5.48	5.48	4.18	4.19	4.01
7	4.94	3.78	3.47	4.94	3.78	3.47	2.60	<2.0	<2.0
8	4.36	4.20	3.18	4.36	4.20	3.18	4.42	4.39	4.33
9	3.71	2.30	2.30	3.71	2.30	2.30	>4.48	4.33	3.90
10	4.26	3.32	3.23	4.26	3.32	3.23	-	-	-
11	3.78	3.57	3.32	3.78	3.57	3.32	-	-	-
12	4.32	<2.0	<2.0	-	-	-	-	-	-

***AMB, Aerobic mesophilic bacteria, EB, enterobacteria, TCF, Total Coliforms,**

Annex 3: Bacterial counts of Wastewater samples collected from three woredas in Addis Ketema Sub-city

	Count (log cfu/ml)								
	WOREDA SEVEN			WOREDA THREE			WOREDA EIGHT		
No.	AMB*	EB	TCF	AMB	EB	TCF	AMB	EB	TCF
1	7.48	7.33	7.03	>7.48	5.95	5.92	6.94	6.87	6.56
2	7.48	7.36	7.13	5.99	4.04	ND	7.41	7.36	7.14
3	7.23	6.99	6.73	6.08	4.75	4.45	6.99	6.80	6.71
4	7.26	6.97	6.71	6.48	6.48	6.29	6.36	5.58	5.11
5	>7.48	7.35	7.16	>6.48	6.13	5.36	7.39	7.35	7.34
6	6.49	6.36	6.28	6.07	5.57	4.90	6.76	6.38	6.20
7	6.89	6.69	5.30	6.36	6.03	5.57	6.83	6.62	6.43

8	6.90	6.34	6.08	6.18	4.57	4.18	>7.48	>7.48	7.06
9	5.95	5.48	ND	6.87	5.85	5.65	>7.48	>7.48	>7.48
10	6.11	5.90	5.30	6.36	5.63	5.10	>7.48	>7.48	>7.48
11	>7.48	7.42	6.11	6.09	4.76	3.90	>7.48	>7.48	>7.48
12	>7.48	7.34	7.03	7.05	6.26	5.26	7.25	6.86	6.49
13	7.39	7.47	6.85	6.04	5.34	4.78	>7.48	>7.48	>7.48
14	>6.48	5.68	6.11	7.36	4.58	4.95	>7.48	>7.48	>7.48
15	6.99	6.72	6.62	7.12	6.69	6.33	6.71	6.52	6.15
16	>7.48	7.14	5.	>7.48	7.17	6.15	>7.48	>7.48	7.30
17	7.25	7.13	6.25	7.12	6.26	5.70	>7.48	>7.48	7.34
18	>7.48	7.41	6.91	6.76	4.95	4.0	7.45	6.43	7.10
19	>7.48	>7.48	7.45	>7.48	7.07	ND	>6.48	5.0	4.95
20	7.42	7.39	7.05	>7.48	7.09	5.79	>6.48	7.16	6.75
21	6.89	6.52	6.27	6.79	6.30	4.79	>7.48	7.34	7.08
22	-	-	-	>7.48	6.73	6.26	5.40	4.78	ND
23	-	-	-	-	-	-	>7.48	>7.48	7.34

**AMB, Aerobic mesophilic bacteria, EB, enterobacteria, TCF, Total Coliforms, ND, Not detectable*

Annex 4: Bacterial counts of Wastewater samples collected from three woredas in Akaki/Kality Sub-city

	Count (log cfu/ml)								
	WOREDA SIX			WOREDA SEVEN			WOREDA EIGHT		
No.	AMB*	EB	TCF	AMB	EB	TCF	AMB	EB	TCF
1	6.65	6.63	6.56	5.68	4.70	4.70	5.94	5.63	5.48
2	7.31	7.28	7.31	7.24	7.12	7.02	5.97	5.57	5.19
3	>7.47	>7.47	>7.47	>7.48	6.41	6.41	6.93	6.93	6.97
4	7.09	6.56	6.67	6.88	6.00	5.85	6.88	6.72	6.74
5	6.98	6.56	6.23	6.96	6.76	6.74	5.32	5.32	5.18
6	5.40	5.25	5.15	6.46	6.39	6.12	7.30	7.03	7.19
7	7.29	7.04	6.97	7.43	7.20	7.11	6.66	6.59	6.54
8	7.39	6.75	6.71	>7.48	>7.48	7.28	>6.48	6.40	6.18
9	7.20	7.14	7.06	7.15	7.12	6.94	6.59	6.54	6.54
10	7.05	6.26	6.04	5.94	5.79	5.73	5.93	5.79	5.46
11	6.81	6.72	6.63	7.19	7.01	6.91	7.13	6.93	6.83
12	5.52	5.08	5.08	7.48	7.46	7.35	6.79	5.42	5.20
13	-	-	-	7.29	7.19	7.14	6.09	5.51	5.56
14	-	-	-	7.35	7.31	7.25	6.53	6.43	6.43
15	-	-	-	-	-	-	>7.48	>7.48	7.30

**AMB, Aerobic mesophilic bacteria, EB, enterobacteria, TCF, Total Coliforms, ND, Not detectable*

Annex 5: Independent T-test for comparison of Means among Sub cities

Sub city	*AMB count (Log cfu/ml)	P- value (95% C.I.)	Mean EB Count (Log cfu/ml)	P- value (95% C.I.)	TCF (Log cfu/ml)	P- value (95% C.I.)
Addis ketema	5.27[a]	0.013 (-0.82722, 0.27937)	5.55[a]	<0.001 (-1.797, -3.358)	5.44[a]	<0.001 (-2.289, -0.759)
Akaki kality	5.71[b]		5.58[b]		5.47[b]	

**AMB, Aerobic mesophilic bacteria, EB, enterobacteria, TCF, Total Coliforms*
[a] no significant difference with mean counts superscripted with the same letters but has significant differences with mean counts with superscript[b]

Annex 6: Independent T-test for comparison of Means among Sub cities by Sample source.

Sub city	Sample source	*AMB (Log cfu/ml)	P- value (95% C.I.)	EB Count (Log cfu/ml)	P- value (95% C.I.)	TCF (Log cfu/ml)	P- value (95% C.I.)
Addis ketema	Drinking water	4.01[a]	0.154 (-1.150, 0.187)	3.81[b]	0.017 (-2.678, -0.943)	3.61[a]	0.433 (-2.950, -1.381)
Akaki kality		4.36[a]		4.22[c]		4.10[a]	
Addis Ketema	Surface sewage	6.78[a]	0.764 (-0.022, 0.434)	6.37[a]	0.115 (-0.311, 0.363)	6.10[d]	0.008 (-1.149, 0.003)
Akaki kality		6.72[a]		6.44[a]		6.39[e]	

AMB, Aerobic mesophilic bacteria, EB, enterobacteria, TCF, Total Coliforms
[a] no significant difference with mean counts superscripted with the same letters
[b] has significant differences with mean counts with superscript[c]
[d] has significant differences with mean counts with superscript[e]

Annex 7: One-Way ANOVA for comparison of Means within Woredas of Sub cities

Sub city	Woreda	*AMB count (Log cfu/ml)	P-value	EB Count (Log cfu/ml)	P-value	TCF count (Log cfu/ml)	P-value
Addis Ketema	3	5.338[a]	0.691	3.663[a]	0.170	2.898[a]	0.179
	7	5.571[a]		4.817[a]		4.028[a]	
	8	5.191[a]		4.027[a]		3.717[a]	
Akaki kality	6	5.460[a]	0.629	4.765[a]	0.398	4.614[a]	0.369
	7	5.889[a]		5.546[a]		5.433[a]	
	8	5.547[a]		5.258[a]		5.060[a]	

AMB, Aerobic mesophilic bacteria, EB, enterobacteria, TCF, Total Coliforms
[a] no significant difference with mean counts superscripted with the same letters