Bacterial Contamination and Antimicrobial Resistance in Drinking Water From Food and Drinking Establishments in Shashemane Town, Ethiopia

Authors: Bonso, Musa, Bedada, Dinaol, and Dires, Simachew

Source: Environmental Health Insights, 17(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/11786302231216864

Bacterial Contamination and Antimicrobial Resistance in Drinking Water From Food and Drinking Establishments in Shashemane Town, Ethiopia

Environmental Health Insights
Volume 17: 1–8

DOI: 10.1177/11786302231216864

Musa Bonso[1], Dinaol Bedada[2] and Simachew Dires[3]

[1]Department of Environmental Health, Bule Hora University, Bule Hora, Ethiopia. [2]College of Health Science, Salale University, Fitche, Ethiopia. [3]College of Health, and Medical Science, Hawassa University, Hawassa, Ethiopia.

ABSTRACT: We investigated the bacteriological quality of drinking water and antimicrobial resistance of bacterial isolates in food and drinking establishments in Shashemane town, Ethiopia. A cross-sectional study was conducted from July to August 2022. One hundred water samples were collected from the tap water and storage containers of 50 selected establishments. All the collected samples were analyzed for bacteriological and antimicrobial susceptibility tests using standard procedures. The study found approximately 80% of water samples from the tap and all water samples (100%) from storage containers were contaminated with total coliforms. *E. coli* was detected in 20% and 26% of water samples from the tap and storage containers, respectively. A total of 68 bacterial isolates were identified, including *E. coli* (33.8%), *Staphylococcus* (25%), *Salmonella* (17.64%), *Klebsiella* (11.76%), *Shigella* (10.29%), and *Pseudomonas* (1.4%). The highest resistance by the isolates was observed against ampicillin (96%), followed by amoxicillin (94%), cotrimoxazole (76.8%), chloramphenicol (36%), gentamycin (23%), ciprofloxacin (23%), and ceftriaxone (12%). The study concluded that drinking water in food and drinking establishments was found vulnerable to microbiological contamination and it is a health risk to consumers. The level of contamination in stored water was found higher than tap water. In addition, antimicrobial-resistant bacteria such as *E. coli, Salmonella, Shigella, Klebsiella*, and *Staphylococcus aureus* were detected in both tap water and stored water. Therefore, awareness should be given to food handlers and owners of the establishments on hygienic water handling practices by the regulatory bodies of Shashemane town and stakeholders.

KEYWORDS: Antimicrobial resistance, bacteriological quality, coliform, food establishments and indicator organisms

RECEIVED: July 31, 2023. **ACCEPTED:** October 23, 2023.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded by ministry of Education, Ethiopia, grant number Capacity Building.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Musa Bonso, Department of Environmental Health, P.O.Box: 144 Bule Hora Univeristy, Bule Hora, Ethiopia. Email: musabonso50@gmail.com

Introduction

Water quality is deteriorating globally due to severe and increasing pollution of fresh water sources.[1] Factors contributing to this deterioration include the inadequacy of treatment plants, high population growth, expansion in industries, and direct discharge of wastewater and chemicals into canals and other water sources without treatment.[2] Lack of safe water, sanitation, and hygiene are responsible for approximately 88% of diarrheal diseases reported worldwide.[3]

In developing countries like Ethiopia, millions suffer from poor water quality and the risk of water-borne diseases such as diarrhea because most people have no access to clean water or sanitation services.[4] Of the 159 million people collecting drinking water directly from surface water sources, the majority (58%) were in Sub-Saharan Africa.[5] Due to inadequate access to improved water supply and sanitation services in developing countries, such as Ethiopia, where only 65% of the population has access to improved water supply and a mere 6% has access to sanitation facilities, a significant number of people are affected by waterborne illnesses. In fact, it is estimated that up to 80% of all illnesses in these countries can be attributed to the consumption of microbial contaminated drinking water.[6]

The deterioration of water quality in food and drinking establishments results in food and waterborne disease outbreaks. In the United States, 9040 food and water-borne disease outbreaks occurred, with food and drinking establishments accountable for 52%.[7] Drinking water samples from these establishments showed that 69.1% and 73.2% were contaminated by bacterial contamination.[8] This may occur due to poor hygiene behaviors such as improper collection, storage, handling, and serving methods. Many foods establishments store drinking water without proper hygienic precautions.[7]

The common waterborne enteric pathogens include *Vibrio cholerae, Salmonella, Shigella, E. coli, Klebsiella, Legionella, Pseudomonas aeruginosa, Campylobacter, Cryptosporidium parvum Enterobacter, and Helicobacter*.[9] Antimicrobial resistance (AMR) is an emerging issue that may occur within any bacterial species. These AMR genes may horizontally transfer between bacteria and present a higher risk due to antimicrobial treatment failures.[10]

Antimicrobial-resistant bacteria have become a global public health threat found in humans, population, foods, livestock, and drinking water.[11] In Ethiopia rising levels of antimicrobial resistance was reported among human patients, food animals, food products, and the environment in Ethiopia.[12] Even though one of the crucial interventions for saving lives in cases of diarrhea and other waterborne diseases is antimicrobial treatment, treatment options become limited

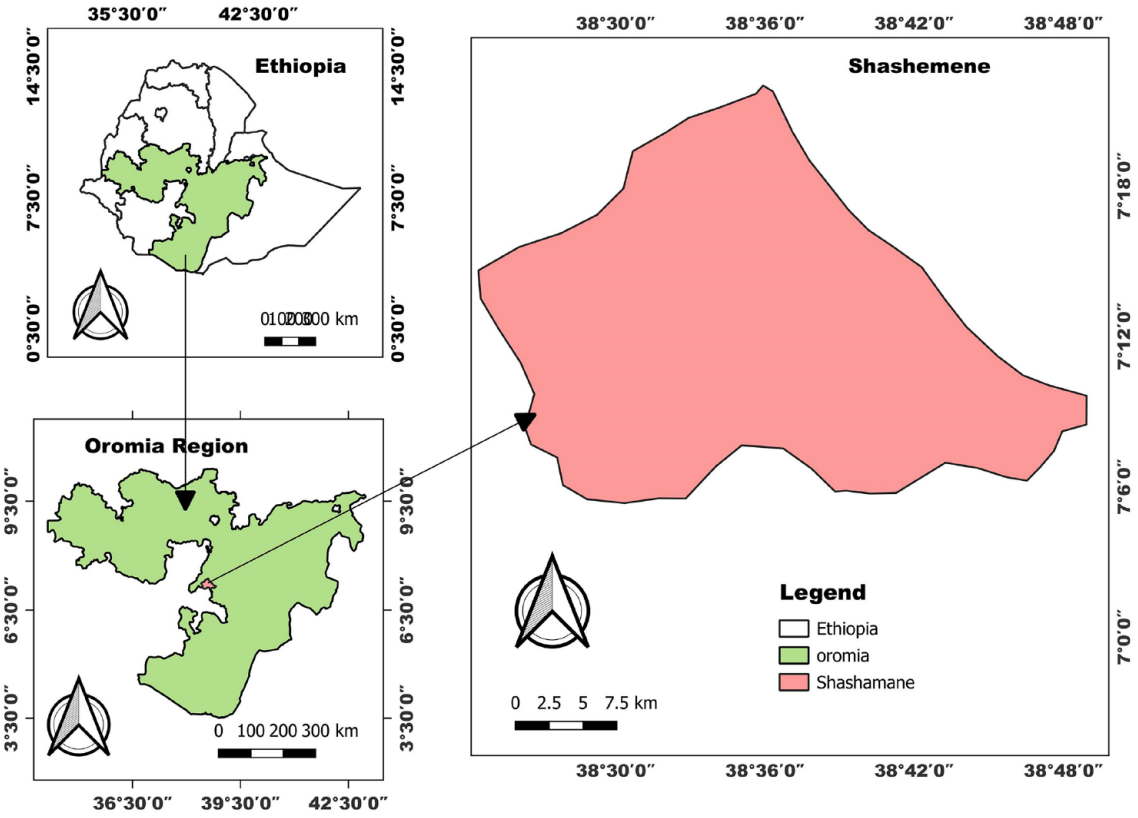

Figure 1. Location map of the Shashemane town.

due to the widespread occurrence of pathogens resistant to commonly prescribed antibiotics.[41]

Diarrheal diseases were consistently ranked among the top leading causes of high diseases burden in Ethiopia.[13] This can be attributable to several factors, including inadequate sanitation, contaminated water sources, poor hand hygiene practices, absence of rotavirus vaccination, improper disposal of feces, and lack of food hygiene.[13] In addition, unhygienic water and food handling practices and weak regulatory systems in food establishments was reported as significant predictors of water and food borne diseases.[14]

Study conducted in Wolaita Zone, Boloso Sore district reported that 20% to 87.5% of water sources were contaminated by fecal coliforms and did not meet the World Health Organization's criteria for drinking water quality standards.[15] Drinking water used in food and drinking establishments in Addis Ababa was found contaminated with fecal coliforms, and posed serious health risks to consumers.[16] Similarly, study conducted on drinking water in food establishments in Bishoftu town revealed heavy contamination by fecal coliforms at the point of use.[17] In Shashemane district, despite continued efforts on water sanitation and hygiene as well as frequent inspection and licensing of food establishments, frequent outbreaks including acute watery diarrhea persist.[18]

Nonetheless, these studies focused on detecting the presence of *E. coli* in drinking water only at the point of use, without detecting at the source. This study aims to assess the bacteriological drinking water quality both at the point of use and at its source, with a focus on identifying potential contamination from hygienic handling of stored water in food establishments. Therefore, this study fills this gap by identifying the bacteriological quality of drinking water and antimicrobial resistance pattern from source to point of use in Shashemane town food and drinking establishments.

Materials and Methods

Study area

The study was conducted in Shashemane town, located in the West Arsi zone of the Oromia region, Ethiopia. Shashemane is 248 km far from Ethiopia's capital city, Addis Ababa. The study was conducted between July and August 2022. The town has a total population of 218 335 based on the 2007 Census and is composed of 8 kebeles, which are the lower-level administration. The town's geographical coordinates are between latitudes of 7°8′50″N and 7°18′17″N and longitudes of 38°32′43″E and 38°40′58″E, with an elevation range of 1826 to 2107 m above mean sea level. The average highest temperature occurs in May at 24.3°C, while the average lowest temperature occurs in December at 7.5°C. The town also experiences an average annual precipitation of 1200 mm. According to data from Shashemane town health office, there are a total of 500 licensed food establishments in the town. Additionally, the town relies on 6 boreholes and one surface water source for its water supply.[19] The location map of the town is illustrated in Figure 1.

Study design

A cross-sectional study design was employed to evaluate the bacteriological quality and antimicrobial susceptibility of bacterial isolates present in the drinking water of food and beverage establishments located in Shashemane town.

Sample size determination and sampling procedure

For water quality analysis, at least 5% to 10% of the total sample size was recommended depending on laboratory capacity, test method availability, and resources.[20] Due to resource limitations in this study, a total of 50 food and drinking establishments, representing 10% of the total food and drinking establishments, were selected for bacteriological analysis. The sample sizes were distributed proportionally among the 8 kebeles. Systematic random sampling was used to select food and drinking establishments, and the selection of the first establishment for the study was determined by a lottery method.

Water sample collection

Before collecting samples, written informed consent was obtained from each food and drinking establishment. Sample bottles were sterilized using an autoclave to avoid contamination and 2.5 ml of sodium thiosulphate was added to each bottle prepared for chlorinated water samples.[21] Water samples were collected from tap water and at the point of use from 8:30 to 11:30 a.m. In total, 100 water samples were collected, with 50 taken from tap water and 50 from stored drinking water, using sterilized 250 ml sterilized glass bottles according to standard methods for the examination of water and wastewater.[21]

When collecting tap water samples, the tap was turned on at maximum flow rate and allowed to flow for 2 minutes. The tap was then disinfected using alcohol (70%) for a minute and allowed to flow for another 2 minutes at a medium flow rate before collecting the sample in a prepared sterilized glass bottle.[21] Water samples from storage containers were collected using cups by food handlers. After collection, samples were transported to Hawassa University Environmental health laboratory within 1 hour of collection using an ice box.

Analysis of water samples

Physicochemical analysis. The temperature of each sample was measured using a thermometer (Bio Abron student mercury thermometer). Before dipping the thermometer into the sample, it was cleaned with distilled water to remove any contaminants that could affect the measurement. The thermometer was then immersed in the sample and the temperature was read to the nearest 0.1°C.

The pH of the sample was determined using Wagtech pH meter (model CP 1000, Singapore). The pH meter was calibrated before use by using buffer solutions of pH 4, 7, and 9. The pH of the sample was then measured by placing the electrode in the sample and reading the pH value on the meter.

To measure the turbidity of the sample, Wagtech turbidity meter (model Wag–WT 302, Singapore) was used. Prior to its use, the turbidity meter was calibrated by employing a blank solution. Subsequently, the sample's turbidity was measured by placing it in a cuvette and reading the turbidity value displayed on the meter. The reported turbidity is expressed in Nephelometric Turbidity Units (NTU).

Microbial Analysis. The analyses of indicator organisms were done in the Hawassa University Environmental Health department laboratory, and the tests of bacteria isolation and susceptibility were done at Hawassa University microbiology department laboratory.

The determination of total and fecal coliform counts was performed using the membrane filtration method.[21] In this process, growth absorbent pads (Millipore EO, USA) were carefully transferred into the base of Petri dishes, utilizing a sterilized pad dispenser. These growth pads were saturated with Hach's new m-ColiBlue24® Broth (HACH company, US), which was used to detect simultaneously *E. coli* and total Coliform within 24 hours.[15] These media were obtained from the Environmental Health department laboratory. A 100 ml water sample was filtered using a 0.45 µm membrane filter (HACH company, USA), and all the filters were transferred to the absorbent pad saturated with the Broth. The Petri dishes were incubated for 24 hours at 35°C, and all the red and blue colonies were counted as total coliforms, whereas all the blue colonies were counted as *E. coli*.[15,22] The duplicate culture was used to perform bacterial counting.

For testing the presence of *Salmonella and Shigella*, 1 ml of each sample was aseptically inoculated into 10 ml of buffered peptone water and incubated at 37°C for 24 hours for recovery and proliferation of cells. After the pre-enrichment, 1 ml of the culture from peptone water was transferred into 10 ml of secondary enrichment broth (selenite F broth) and incubated at 42°C for 48 hours. After secondary enrichment, 0.1 ml of culture from selenite F broth was streaked onto *Salmonella–Shigella* agar and incubated at 37°C for 18 hours.[21] Suspected colonies were selected and inoculated into the respective biochemical media, which included Triple Sugar Iron Agar (TSIA), Lysine Iron Agar (LIA), Urea Agar (UA), Simmons Citrate Agar (SCA), and Sulfide Indole Motility (SIM) medium.[23]

For the isolation and count of *Staphylococcus aures*, 0.1 mL of the diluted sample was streaked on mannitol salt agar and incubated at 37°C for 24 hours. Then colonies showing a yellow zone of fermentation were suspected as *Staphylococcus* species and further confirmed by a catalase test. Gram-positive colonies were counted using a digital colony counter as *Staphylococcus* and reported in colony forming unit per millimeter. First catalase test was conducted for *Staphylococcus* species, and the coagulase test was followed to isolate *Staphylococcus aures* from other species.[24]

Pseudomonas species were tested using a 0.1 ml diluted sample streaked on MacConkey agar and incubated at 37°C for 24 hours. After incubation, non-lactose fermenting typical presumptive colonies from MacConkey agar were selected and assessed for oxidase reaction. Then positive oxidase tests were subjected to the Indole test, methyl red, and Voges–Proskauer test to differentiate *Pseudomonas* from *Aeromonas* species.[25]

Klebsiella species isolation and count were tested using a 0.1 ml diluted sample streaked on MacConkey agar and incubated at 37°C for 24 hours. After incubation, lactose fermenting colonies that appear large, mucoid, and red with diffusing red pigment on MacConkey agar indicating fermentation of glucose and acid production was suspected as *Klebsiella* colonies. Suspected colonies were further analyzed by biochemical test, and colonies that do not produce hydrogen sulfide and are positive on methyl red and Voges-Proskauer tests were identified as *Klebsiella*.[21]

Antimicrobial Sensitivity Test

The susceptibility of the identified bacteria to the common antibiotics was determined using the Kirby-Bauer disk diffusion method.[26] Bacterial isolates were inoculated into a tube containing 5 ml sterile nutrient broth and grown to a 0.5 McFarland turbidity standard within 4 hours. A sterile swab was dipped in a bacterial suspension and streaked onto Mueller-Hinton Agar.[26] The following commonly prescribed drugs were tested on bacterial isolates: ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), cotrimoxazole (25 µg), gentamicin (10 µg), ampicillin (10 µg), and amoxicillin (2 µg). Antibiotic disks were applied using sterile forceps and incubated at 37°C for 18 hours, and the zone of inhibition was measured in millimeters using a ruler. Following 18 hours of incubation at 37°C, the diameters of the inhibition zones were measured in millimeters using a manual caliper to classify them as susceptible and resistant (intermediate considered as resistant in this study).[26]

Quality control

Bacteriological analysis of water samples was performed according to standard operating procedures and laboratory safety rules.[21] The sterility of the used media was checked using 5% of the prepared batch of media incubated overnight and checked for microbial growth in the media. Field blank, positive control, negative control, and internal quality control were used to assure the quality of species identification and the antimicrobial susceptibility test. A reference strain of *Escherichia coli* ATCC 25922 was used to check the performance of biochemical and disk diffusion tests at each stage of species identification and antimicrobial susceptibility test.[26] The water samples were filtered using a 0.45 -µm membrane filter, and the filtrate was then inoculated onto a nutrient agar plate and incubated at 37°C for 24 hours. The colonies that grew on the agar plate were then identified using biochemical tests. The antimicrobial susceptibility of the identified bacteria was determined using the Kirby-Bauer disk diffusion method.[21,26]

Data analysis and interpretation

The investigator entered, coded, recorded, edited, and cleaned the data in Epi data before transforming it into SPSS version 25 for analysis. The results were then presented using tables and graphs, and compared and discussed based on different principles, standards, and other similar findings. A Mann-Whitney U test was used to analyze the data at a $P < .05$ significance level and 95% confidence interval to detect any significant differences in the mean coliforms and *E. coli* between water samples taken from taps and storage containers.

Results

Water storage and hygienic handling practices

During the assessment, water storage and handling procedures in food and beverage establishments were evaluated using an observational checklist. Results showed that during the visit, about 58% and 76% of water storage containers were discovered uncovered and unclean, respectively. Additionally, it was found that 82% of these establishments did not utilize any water treatment methods, and 66% of them fetched water from storage containers by hand, leading to water contamination.

Physico-chemical parameters of water samples

The temperature of the water samples ranged from 20°C to 22°C, which was higher than the WHO standard of <15°.[27] Elevated temperatures in drinking water can lead to taste and odor issues, affecting esthetic value of water for consumption in food and beverage establishments.

The pH of the samples taken from the establishments ranged from 6.9 to 7.1, with the tap water having the lowest pH of 6.9 and water samples from storage containers having the highest pH of 7.1. These pH values fell within the WHO recommended range of 6.5 to 8.5.[27] The turbidity of the water samples ranged from 1 to 3 NTU, with a mean turbidity of 1.6 ± 0.69 for tap water samples and 1.9 ± 1.5 for water samples from storage containers.

Biological Analysis

Total and fecal coliforms count. This study investigated the quality of drinking water from tap water and storage containers in Shashemane town, Oromia, Ethiopia. A total of 100 water samples were collected from 50 food and drinking establishments, including restaurants, hotels, and cafes. The samples were analyzed for total coliforms (TC) and *E. coli*.

Approximately 80% and 48% of the water samples taken from the tap water were contaminated with total coliforms and *E. coli*, respectively, with counts ranging from 17 to 176 colony

Table 1. Indicator bacteria concentration of water samples taken from food and drinking establishments in Shashemane town.

INDICATOR		WATER SAMPLES	
		TAP WATER (N=50)	SC (N=50)
E. coli (CFU/100 ml)	Mean	2.34	11.2
	95% CI for Mean	1.31-3.36	8.64-13.75
TC (CFU/100 ml)	Mean	91.84	116.1
	95% CI for Mean	80.05-103.6	107.41-124.78

Abbreviations: CI- Confidence Interval, SC- Storage Container.

Table 2. Prevalence of pathogenic bacteria detected in drinking water samples taken from food and drinking establishments of Shashemane town.

ISOLATED BACTERIA	WATER SAMPLES	
	TAP WATER (N=50)	STORAGE CONTAINER (N=50)
E. coli	10/50 (20%)	13/50 (26%)
Klebsiella	2/50 (4%)	4/50 (8%)
Salmonella	4/50 (8%)	7/50 (14%)
Shigella	3/50 (6%)	4/50 (8%)
Staphylococcus	3/50 (6%)	7/50 (14%)
Pseudomonas	0	1/50 (2%)

Table 3. The distribution of bacterial isolates of water samples taken from food and drinking establishments of Shashemane town.

BACTERIA ISOLATES	TAP WATER SAMPLES	STORAGE CONTAINER SAMPLES	TOTAL
E. coli	10 (14.7%)	13 (19.1%)	23 (33.8%)
Klebsiella	2 (2.94%)	6 (8.8%)	8 (11.76%)
Salmonella	5 (7.35%)	7 (10.29%)	12 (17.64%)
Shigella	3 (4.4%)	4 (5.88%)	7 (10.29%)
Staphylococcus	7 (10.29%)	10 (14.7%)	17 (25%)
Pseudomonas	0	1 (1.4%)	1 (1.4%)
Total	27 (39.7%)	41 (60.29%)	68 (100%)

forming unit per 100 ml (CFU/100 ml) for TC and 0 to 11 CFU/100 ml for *E. coli*. Analysis of indicator organisms in water samples taken from storage containers revealed that 100% and 18% of the water samples had total coliforms and *E. coli*, respectively (Table 1).

According to WHO's risk classification for thermotolerant coliforms, 12% of tap water samples were categorized as low risk (1-10 CFU/100 ml), while 8% were categorized as intermediate risk (11-100 CFU/100 ml). As for water samples obtained from storage containers, 26% of them tested positive for *E. coli*, with 16% of samples falling into the low-risk category (1-10 CFU/100 ml) and 10% categorized as intermediate risk (11-100 CFU/100 ml).

Isolation of pathogenic bacteria. The predominant species isolated from water samples was *E. coli* which was detected in 10 (20%) and, 13 (26%) of tap water and storage containers, respectively (Table 2). *Staphylococcus aureus* was found in 3 (6%) of tap water and 7 (14%) of the water samples from storage containers. *Klebsiella* species were found in 2 (4%) of tap water samples, while 4 (8%) were detected from storage containers. *Salmonella* species were also detected in 4 (8%) of the tap water and 7 (14%) of storage container water samples. In addition, *Shigella* species were detected in 6% of tap, and 8% of water samples from storage containers.

In this study, a total of 68 bacterial isolates of 6 genus were isolated (Table 3). These include *E. coli, Salmonella, Shigella,*

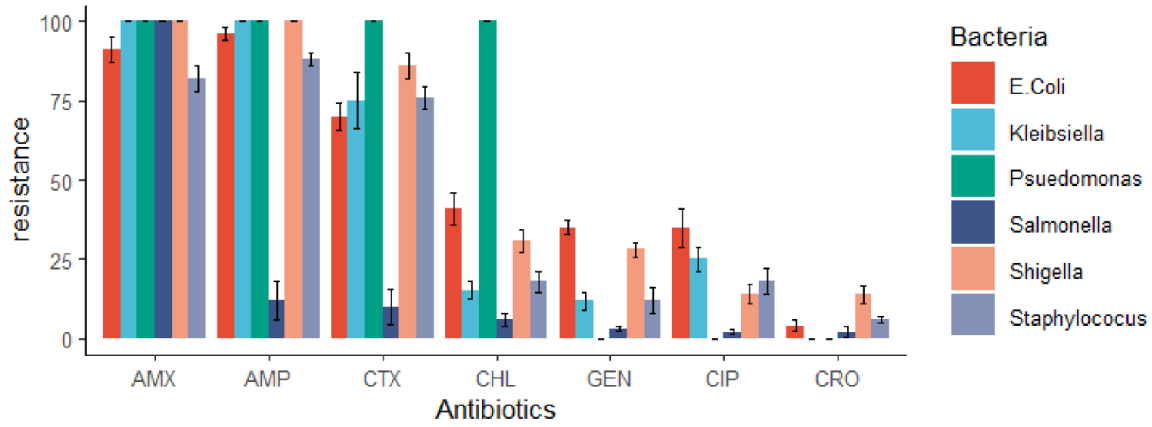

Figure 2. Antimicrobial resistance pattern of bacterial isolates from water samples taken from food and drinking establishments of Shashemane town. Abbreviations: AMX, Amoxicillin; AMP, Ampicillin; CTX, Cotrimoxazole; CHL, Chloramphenicol; GEN, Gentamycin; CIP, Ciprofloxacin; CRO, Ceftriaxone.

Klebsiella, Pseudomonas, and *Staphylococcus aureus*. A higher number of bacterial isolates were obtained from storage container samples 41 (60.29%), and the lower bacterial isolates were from tap water 27 (39.7%). With regards to bacterial species, 23 (33.8%) were *E. coli*, followed by *Staphylococcus aureus* 17 (25%), *Salmonella* 12 (17.64%), *Klebsiella* 8 (11.76%), *Shigella* 7 (10.29%), and *Pseudomonas* 1 (1.4%).

Susceptibility of bacterial isolates to antibiotics. The antibiotic susceptibility of bacterial isolates was assessed, and the resulting mean resistance was depicted in Figure 2. The figure also highlights the variability among bacterial isolates in their resistance to commonly employed antibiotics, as illustrated by the standard errors from the mean. From all bacterial isolates tested the highest resistance was observed against ampicillin (96%) followed by amoxicillin (94%), cotrimoxazole (76.8%), chloramphenicol (36%), gentamycin 23%, ciprofloxacin (23%), and ceftriaxone (12%). The resistance observed against ampicillin was 100% for *Shigella, Pseudomonas, and Klebsiella*, 96% for *Staphylococcus*, 93% for *E. coli*, and 91% for *Salmonella. Salmonella* resistance to amoxicillin and ampicillin was found to be 100%, followed by cotrimoxazole (83%). In the same manner, *Shigella* were found highly resistant to ampicillin and amoxicillin (100%) followed by cotrimoxazole (86%) (Figure 2).

As shown in Figure 2, all isolated bacteria showed multiple drug resistance to 3 or more antibiotics. *E. coli* had higher level of resistance to many of the tested antibiotics like ampicillin (96%), amoxicillin (91%), cotrimoxazole (70%), and ciprofloxacin (35%). *Salmonella* was found the second most resistant bacterial isolate.

Discussion

Identification of drinking water contamination in food and drinking establishments was essential for the development and implementation of effective regulatory systems. The prevalence of total coliforms in tap water and storage containers water samples was found 80% and 100%, respectively. However, total coliforms must not be detected in any 100 ml of drinking water samples, according to WHO guidelines and Ethiopian standards[27,28] and the results of total coliforms obtained in this study exceeded the recommended values and were not safe for drinking. These findings were in line with the study conducted in Adama town, where all stored water was contaminated with total coliforms.[29] Likewise, a study done in Bangladesh on the water quality conditions of Sylhet city restaurants found that the drinking water of each restaurant was contaminated with total coliforms.[8]

The prevalence of total coliforms from storage container water samples in this study was found higher than the study conducted in Bule Hora (78%),[30] Bahir Dar (24.4%)[31] and South Gondar (50%). The higher prevalence of coliforms in water samples suggests the differences in storage and handling practices, which is a risk to the health of the consumers in food establishments. In addition to total coliforms, 20% of tap water and 26% of the storage container water samples were contaminated with *E. coli*. This finding was found lower compared to the study conducted in Cajamarca, Peru (37.3%),[32] Farta district, Northern Ethiopia (100%),[33] Adama (37%),[29] and higher than Addis Ababa[16] and Wageda, Northern Ethiopia (18%).[34] However, the prevalence of *E. coli* obtained in this study was found consistent with a study done in Bule Hora, Ethiopia (26.6%).[30]

The WHO guidelines recommend that drinking water should not contain any total coliforms or *E. coli*. The Ethiopian Standards Agency has also set a limit of 0 CFU/100 ml for total coliforms in drinking water.[27,28] The high levels of total coliforms and *E. coli* in the water samples indicate that the water is contaminated with fecal matter ([27]). This contamination could pose a serious health risk to people who drink the water. There are several possible reasons for the high levels of contamination in the drinking water in Shashemane town. One possibility is that the water is not being properly treated before it is distributed to the public. Another possibility is that the water is being contaminated after it is distributed, perhaps through leaks in the water pipes or through contamination of the water storage tanks. The high levels of contamination in

the drinking water in Shashemane town are a serious public health concern.

Staphylococcus aureus was found in 6% of tap water and 14% of the water samples from storage containers. This finding was lower than the prevalence reported in Sao Paulo, Brazil (25%)[35] and Jaju Sagar, India (100%).[36] The difference could be due to water sources and methods used for identification. The presence of the genus *Staphylococcus*, mostly *Staphylococcus aureus*, is considered an indicator of poor hygienic status employed in the field of production or distribution of drinking water.[37]

Klebsiella species were found in 4% of tap water and 8% of storage container water samples. Likewise, *Klebsiella* isolates from water containers were in line with the study in Cajamarca, Peru (8%),[32] and Andean, Peru (10.7%).[38] *Salmonella* species were also detected in 4 (8%) of the tap water and 7 (14%) of storage container water samples. In addition, *Shigella* was detected in 6% of tap and 8% of water samples from storage containers. The prevalence of the isolates of *Salmonella* in this study was lower than the study conducted in South Gondar (22.7%) and Bule Hora (24%).[30] *Shigella* species was found lower than the study done in Bule Hora (16%).[30] This might be due to the addition of residual chlorine to the pipeline, the efficiency of a water treatment plant, and the season of the year.

In this study highest antimicrobial resistance was observed against ampicillin (96%), followed by amoxicillin (94%), and cotrimoxazole (76.8%). This finding was consistent with a study in Bahir Dar, where 98 % to 100% resistance was observed against ampicillin.[31] Similarly study in Bule Hora reported that 100% resistance to ampicillin and amoxicillin.[30] Relatively lower resistance was observed against ciprofloxacin (23%) and ceftriaxone (12%). This finding agreed with findings from South Gondar, where higher resistance was observed to amoxicillin, and none of the bacterial isolates were detected as resistant to ciprofloxacin. Furthermore, in this study, the observed 100% resistance of *Salmonella, Shigella, Pseudomonas*, and *Klebsiella* against ampicillin was consistent with the study conducted in Bahir Dar (98%-100%), and Bule Hora (100%).[30,31] However, it was found lower than the findings of the study done in South Gondar, where 22.7% resistant *Salmonella* and 15% resistant *Shigella* were reported. In addition, *E. coli* resistance to amoxicillin (91%), and ampicillin (96%) observed was consistent with the study conducted in South Gondar, Ethiopia (94.4% and 94.4%) (23,22), and Bahir Dar, Ethiopia, where 100% resistance was reported for both amoxicillin and ampicillin.[31] But it was found higher than the findings from Camarja, where 3.4% resistance to amoxicillin and 28.2% resistance was detected against ampicillin.[32] The differences might be attributable to the used media and disk used in the investigation.

All bacterial isolates showed multiple drug resistance to 3 or more antibiotics. *E. coli* had higher level of resistance to many of the tested antibiotics like ampicillin (96%), amoxicillin (91%), cotrimoxazole (70%), and ciprofloxacin (35%). Likewise, isolates of *Pseudomonas* and *Klebsiella* showed 100% resistance to ampicillin and amoxicillin. A similar pattern of multidrug resistance was reported for Enterobacteriaceae.[30,31]

The isolates of *E. coli* had a higher level of resistance to many of the tested antibiotics like ampicillin (96%), amoxicillin (91%), cotrimoxazole (70%), and ciprofloxacin (35%). With regards to specific species, *Salmonella* species showed resistance to amoxicillin and ampicillin (100%), followed by cotrimoxazole (83%). Similarly, *Shigella* species isolates were highly resistant to ampicillin, amoxicillin (100%), and cotrimoxazole (86%). Regarding the *Staphylococcus* isolates, a high level of resistance was observed to ampicillin (89%), amoxicillin (82%), cotrimoxazole (76%), and lower resistance to gentamycin (12%), chloramphenicol (18%), ciprofloxacin (18%), and ceftriaxone (6%). Isolates of *Pseudomonas* and *Klebsiella* showed resistance to ampicillin and amoxicillin (100%) and were susceptible to ciprofloxacin, gentamycin, and ceftriaxone. The lowest antimicrobial resistance was detected against ciprofloxacin, gentamycin (23%), and ceftriaxone (12%), as depicted in Figure 2.

The drinking water contamination with these antimicrobial-resistant water-borne bacteria in the food and drinking establishments may result in outbreaks, resulting in treatment failure and prolonged morbidity, hospitalization, and increased mortality.[39,40]

Limitations of the study

The study was conducted for a few months, and the seasonal variation of the microorganisms present in the water was not addressed. In addition, pathogenic bacteria like *Vibrio cholerae*, viruses, fungi, and parasites (protozoa and helminths) were not investigated. Further, we did not assess factors that could be associated with bacterial contamination of the drinking water in food and drinking establishments.

Conclusions

In conclusion, this study showed that drinking water in food and drinking establishments was found vulnerable to microbiological contamination and it is a health risk to consumers. Water samples collected from storage containers had a higher level of contamination than tap water. The higher prevalence of fecal coliform in storage container water samples suggests hygienic storage and handling practices in food and drinking establishments. Antimicrobial resistant pathogenic bacteria such as *E. coli, Salmonella, Shigella, Klebsiella*, and *Staphylococcus aureus* was identified from both tap water and storage container samples. This indicates that food and drinking establishments were risk sites for the incidence of waterborne diseases and make treatment options limited due to the occurrence of antimicrobial resistant pathogens. Therefore, awareness should be given to food handlers and owners of the establishments on hygienic water handling practices by the regulatory bodies of Shashemane town and stakeholders.

Acknowledgements

We thank Hawassa University for the provision of laboratory facilities and the Shashemane town administration for their cooperation during data collection.

Author Contributions

Musa Bonso designed the study, conducted the laboratory analysis, and wrote manuscript. Dinaol Bedada and Simachew Dires supervised the study, edited, and approved the manuscript.

Data Availability Statement

All data are fully available without restriction.

ORCID iD

Dinaol Bedada https://orcid.org/0000-0003-1587-2242

REFERENCES

1. du Plessis A. Persistent degradation: global water quality challenges and required actions. *One Earth*. 2022;5:129-131.
2. Usman MA, Gerber N, von Braun J. *ZEF-Discussion Papers on Development Policy No. 221 The Impact of Drinking Water Quality and Sanitation on Child Health: Evidence from Rural Ethiopia*. 2016. Accessed July 10, 2023. http://ssrn.com/abstract=2809568Electroniccopyavailableat:https://ssrn.com/abstract=2809568
3. Yenew C, Kebede M, Mulat M. *Antimicrobial-Resistance Pathogen Load and Public Health Risk of Drinking Water in South Gondar, Ethiopia*. 2020. https://doi.org/10.21203/rs.3.rs-135494/v1
4. WHO. WHO guidelines for drinking-water quality (Recommendation). *Water Supply*. 2006;11(3–4).
5. Abubakar IR. Factors influencing household access to drinking water in Nigeria. *Utilities Policy*. 2019;58:40-51.
6. Mangin JM. Rural water supply in Southern Ethiopia: failures and alternatives. *Canadian Journal of Development Studies*. 2018;12:297-312.
7. Boro P, Soyam VC, Anand T, Kishore J. Physical Environment and hygiene status at food service establishments in a tertiary care medical college campus in Delhi: a cross-sectional study. *Asian J Med Sci*. 2014;6:74-79.
8. Tawfick MM, Elshamy AA, Mohamed KT, El Menofy NG. Gut commensal Escherichia coli, a high-risk reservoir of transferable plasmid-mediated antimicrobial resistance traits. *Infect Drug Resist*. 2022;15:1077-1091.
9. Santos GAC, Dropa M, Rocha SM, Peternella FAS, Razzolini MTP. Staphylococcus aureus and methicillin-resistant Staphylococcus aureus (MRSA) in drinking water fountains in urban parks. *J Water Health*. 2020;18:654-664.
10. Temesgen E, Hameed S. Assessment of physico-chemical and bacteriological quality of drinking water at sources and household in Adama town, Oromia Regional State, Ethiopia. *Afr J Environ Sci Technol*. 2015;9:413-419.
11. Cave R, Cole J, Mkrtchyan HV. Surveillance and prevalence of antimicrobial resistant bacteria from public settings within urban built environments: Challenges and opportunities for hygiene and infection control. *Environ Int*. 2021;157:Article 06836.
12. Gemeda BA, Assefa A, Jaleta MB, Amenu K, Wieland B. Antimicrobial resistance in Ethiopia: A systematic review and meta-analysis of prevalence in foods, food handlers, animals, and the environment. *One Health*. 2021;13:Article 100286. doi:10.1016/j.onehlt.2021.100286
13. Sitotaw B, Melkie E, Temesgen D. Bacteriological and physicochemical quality of drinking water in Wegeda town, Northwest Ethiopia. *J Environ Public Health*. 2021;2021:1-8.
14. Asefa YA, Alemu BM, Baraki N, Mekbib D, Mengistu DA. Bacteriological quality of drinking water from source and point of use and associated factors among households in eastern Ethiopia. *PLoS One*. 2021;16:Article 0258806.
15. Gizachew M, Admasie A, Wegi C, Assefa E. Bacteriological contamination of drinking water supply from protected water sources to point of use and water handling practices among beneficiary households of Boloso Sore woreda, Wolaita Zone, Ethiopia. *Int J Microbiol*. 2020;2020:1-10.
16. Girmay AM, Gari SR, Gebremariam AG, et al. 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. *J Water Sanit Hyg Dev*. 2020;10:969-978.
17. Girmay AM, Mengesha SD, Weldetinsae A, et al. Factors associated with food safety practice and drinking-water quality of food establishments in Bishoftu Town, Ethiopia. *Discov Food*. 2022;2:Article 35. doi:10.1007/s44187-022-00037-1
18. Edessa N, Geritu N, Mulugeta K. Microbiological assessment of drinking water with reference to diarrheagenic bacterial pathogens in Shashemane Rural District, Ethiopia. *Afr J Microbiol Res*. 2017;11:254-263.
19. Shine S, Muhamud S, Adanew S, Demelash A, Abate M. Prevalence and associated factors of diarrhea among under-five children in Debre Berhan town, Ethiopia 2018: A cross sectional study. *BMC Infect Dis*. 2020;20:174.
20. Centers for Disease Control and Prevention. *A Guide to Conducting Household Surveys for Water Safety Plans*. U. S. Department of Health and Human Services; 2008.
21. APHA. *Standard Methods for the Examination of Water and Wastewater*. 2005. https://doi.org/ISBNStandardMethods.
22. Ramírez-Castillo FY, Loera-Muro A, Jacques M, et al. Waterborne pathogens: Detection methods and challenges. *Pathogens*. 2015;4:307-334.
23. Hemraj V, Diksha S, Avneet G. A review on commonly used biochemical test for bacteria. *Innovare Journal of Life Science*. 2013;1:1-7.
24. Dires S, Birhanu T, Ambelu A, Sahilu G. Antibiotic resistant bacteria removal of subsurface flow constructed wetlands from hospital wastewater. *J Environ Chem Eng*. 2018;6:4265-4272.
25. Health protection agency, & England, P. H. Identification of Pseudomonas species and other Non-Glucose Fermenters. *UK Standards for Microbiology Investigations*. 2015:3.
26. Hudzicki J. *Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information*. American Society For Microbiology; 2012.
27. World Health Organization, & UNICEF. *Progress on drinking water, sanitation and hygiene: 2017 update and SDG baselines*; 2017.
28. Ethiopian Standards Agency. *Compulsory Ethiopian Standard- Water Quality Specifications: First Edition 2013*. ESA; 2013.
29. UNEP. Water quality monitoring and assessment of groundwater Technical Guidance Document Prepared by the UNEP GEMS/Water Capacity Development Centre for the United Nations Environment Programme. 2022. http://www.un.org/Depts/Cartographic/english/htmain.htm
30. Dhengesu D, Lemma H, Asefa L, Tilahun D. Antimicrobial resistance profile of Enterobacteriaceae and drinking water quality among households in Bule Hora Town, South Ethiopia. *Risk Manag Healthc Policy*. 2022;15:1569-1580.
31. Abera B, Kibret M, Goshu G, Yimer M. Bacterial quality of drinking water sources and antimicrobial resistance profile of Enterobacteriaceae in Bahir Dar city, Ethiopia. *J Water Sanit Hyg Dev*. 2014;4:384-390.
32. Larson A, Hartinger SM, Riveros M, et al. Antibiotic-resistant Escherichia coli in drinking water samples from rural andean households in Cajamarca, Peru. *Am J Trop Med Hyg*. 2019;100:1363-1368.
33. Genet GK, Desta HH. Bacteriological quality of drinking water from source to point of use among rural communities of Farta woreda in North West, Ethiopia. *Afr J Microbiol Res*. 2017;11:1069-1074.
34. Tambekar DH, Hirulkar NB, Banginwar YS, et al. Water hygiene behaviors in hotels and restaurants and their effects on its bacteriological quality. *Biotechnology*. 2006;5:475-477.
35. Shashemane town Administration. *Shashemane town administration 2021 Annual report(unpublished)*; 2022.
36. Chouhan S. Increased prevalence of coagulase-positive Staphylococcus aureus in municipal drinking water supplies. *Int J Plant Anim Environ Sci*. 2015;5:163-170.
37. Bencardino D, Amagliani G, Brandi G. Carriage of Staphylococcus aureus among food handlers: an ongoing challenge in public health. *Food Control*. 2021;130:Article 108362. doi:10.1016/j.foodcont.2021.108362
38. Hartinger SM, Medina-Pizzali ML, Salmon-Mulanovich G, et al. Antimicrobial resistance in humans, animals, water and household environs in rural andean peru: Exploring dissemination pathways through the one health lens. *Int J Environ Res Public Health*. 2021;18:Article 4604. doi:10.3390/ijerph18094604
39. Ashbolt NJ. Microbial contamination of drinking water and human health from Community Water Systems. *Curr Environ Health Rep*. 2015;2:95-106.
40. Mbanga J, Abia ALK, Amoako DG, Essack SY. Quantitative microbial risk assessment for waterborne pathogens in a wastewater treatment plant and its receiving surface water body. *BMC Microbiol*. 2020;20:Article 346. doi:10.1186/s12866-020-02036-7
41. Pascal B, Samie A, Makonto TE, et al. Microbial quality, diversity and antibioticsusceptibility profiles of bacterial isolates from borehole water microbial quality, diversity and antibiotic susceptibility profiles of bacterial isolates from borehole water used by schools in Greater Giyani Municipality, Mopani District, South Africa. *Afr J Microbiol Res*. 5