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DRINKING WATER ANTIMICROBIAL RESISTANCE ENTERIC BACTERIAL LOAD AND PUBLIC HEALTH RISK IN NORTHWEST, ETHIOPIA: A LABORATORY-BASED CROSS-SECTIONAL STUDY

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ABSTRACT

Background: The security of the drinking water supply has been sullied by antimicrobial-resistant bacteria at the source, within the dispersion framework, and amid families dealing with, which may cause intense or incessant wellbeing issues. Therefore, this study aimed at determining the antimicrobial-resistant bacterial contamination, health risk, and associated factors of drinking water in Northwest Ethiopia.

Methods: A laboratory-based cross-sectional study was employed by taking 60 water samples collected from the household tap and drinking water storage container by following the standard microbial analysis method. Besides, a sanitary survey was conducted for the municipal water supply system from March to May 2020. Descriptive statistics and multiple linear regression models were employed.

Results: The prevalence rate of multidrug resistance *Escherichia coli* species was 80% (95% CI: 76.9-81.2 %), Salmonella species was 40% (95% CI: 38.7-45%) and Shigella species was 60% (95% CI: 56.9-65%). The overall Health risk index (HRI) of drinking water showed that 45.83%, 41.67%, and 12.5% of them were categorized as low, intermediate, and high-risk classes, respectively. The load and health risk could be strongly correlated with the low residual chlorine of drinking water.

Conclusions: The contamination of drinking water with antimicrobial-resistant waterborne bacteria in the community could indicate an occurrence of treatment failure. Hence, proper drinking water treatment and strict supervision are needed to prevent the contamination of the water and related consequences.

Keywords: Antibiotic-resistant bacteria, Drinking water, Health risk, Ethiopia

BACKGROUND

The prevalence of antimicrobial-resistant pathogens, including waterborne antibiotic-resistant bacteria, is ever increasing. The widespread emergence of AMR bacteria has become one of the grimmest challenges in low-income countries including Ethiopia resulting from irrational antibiotic consumption, prescription without susceptibility test, self-medication, and prolonged hospitalization (1). Some experimental studies and surveillance in Ethiopia showed that *E. coli, Shigella*, and *Salmonella* species developed resistance to frequently recommended antibiotics (2).

In Ethiopia, 54% of households that use an improved source of drinking water. Nine households in every ten used non-treated drinking water. Emerging risks and challenges are those that are coming into existence because of changes in the environment. Assessment of the qualities of urban water source and tap water distribution systems in Arba-Minch town showed that the distribution lines were contaminated with Waterborne Bacteria (WBB) such as *Salmonella* and *Shigella* (3).

A study conducted in North Gondar, on the other hand, showed that 50% of water samples collected from water lines contaminated with indicator WBB *E.coli* (4). Assessment of the level of AMR contamination and source identification is highly relevant for policy intervention. Therefore, This study aimed at determining the antimicrobial-resistant contamination, health risk, and associated factors of drinking water in Northwest Ethiopia.

METHODS

Study design and period

A laboratory-based cross-sectional study was carried out for a month from March to May 2020 at Debre Tabor town, Debub Gondar Zone of the Amhara Region, about 100 kilometers southeast of Gondar and 50 kilometers east of Lake Tana. This historic town has a latitude and longitude of 11°51′N 38°1′E with an elevation of 2,706 meters above sea level.

Sample size determination and sampling technique

A total of 60 water samples each from the drinking water tap and storage container of the household was taken using random sampling techniques (5).

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Water sample collection

For bacteriological analysis, drinking water samples were collected using an autoclaved bottle containing two drops of sodium thiosulfate (10% $Na_2S_2O_3$) for complete neutralization of any residual chlorine present and preserving microbial contamination. Before taking a sample from the tap, the mouth of the tap was cleaned with a clean cloth to remove any dirt if present (6). Then, the drinking water of the tap was flushed for 5 min and then sterilization of the mouth of a tap was done with a spirit of flame followed by cooling it with water to run for 1–3 min at a medium flow (7). Then the sterilized bottle was opened, filled with water, leaving a small air space, and shaked before analysis. The collected water samples from each source were labeled and kept in a cold box containing ice freezer packs (<4ºC) and transported to Felege Hiwot Referal Hospital.

Sample processing techniques of WBB isolation and susceptibility testing

Sample preparation

Every 10 ml of sample was aseptically homogenized into 90 ml of sterile peptone water in a clean 250 ml sterile jar, shaken, and 1:10 dilutions were made using Poured techniques (8). The water samples of the tap and storage container were further diluted by mixing 1ml of each homogenized sample and 9ml of sterile 0.85% physiological saline solution (NaCl) to make 10^{-5} dilutions using a vortex mixer. For the water samples collected from the household tap and storage container, homogenized samples were plated.

WBB isolation and identification technique

0.1ml of the prepared diluted sample was directly inoculated on differential and selective agar media after primary and secondary enrichment, and incubated at 37 °C for 18–24 hours. After incubation, lactose and precipitated bile salts were added for presumptive identification of *E. coli* on the selective medium MacConkey agar. Thiosulfate and Ferric Citrate were used to observe hydrogen sulfide production for presumptive identification of *Salmonella* and *Shigella* on Selective medium SS agar.

MDR profile testing

We have performed the M D R testing for all the isolated WBB species. The slanted cultures were subcultured and purified. The pure colonies were inoculated into Nutrient Broth and incubated at $37^{O}C$ for 18-24 hours.

After incubation, the turbidity of the culture was adjusted to 0.5 McFarland Standard to bring the cell density to approximately 10^7-10^8cfu/ml. A sterile cotton swab dipped the standardized suspension. The culture was spread evenly over the entire surface of the Muller-Hinton agar plates by swabbing in three directions at 90^0 of each spreading. The plate allowed drying before applying antimicrobial discs. The following standard and Oxoid drug discs used: Vancomycin (VA) of 30µg; *Cotrimoxazole* (SXT) of 25µg; Ciproflaxicillin (Cip) of 5µg; *Doxycycline* (DC) of 30µg and Amoxicillin (AMX) of 2 µg, that were commonly used antibiotics in Ethiopian healthcare facilities based on the guidelines developed from Clinical and Laboratory Standards Institute of US (9).

Health risk analysis

To measure the sanitary condition and analyze the risk to health matrix,the World Health Organization (**WHO**) standards recommended to determine the degree of contamination were used(10). Besides, data on sanitary inspection of water sources were collected using the standard format described by WHO and UNICEF (11).

An observation checklist (sanitary inspection form) containing ten items and consisting of a set of questions with yes or no answers was used. A risk factor was assigned with a score of 1 for yes, while a risk factor with a score of 0 for no. A combination of the scores for each item was used to determine the sanitary risk scores, which were categorized into four categories: 0-2, 3-5, 6-8, and 9-10 for low, moderate, high, and very high risk of contamination, respectively.

Quality control

We assigned qualified, competent, and proficient laboratory personnel's for the laboratory analysis and data collection, as well as the personnel that interpreted the results and those that were involved in the monitoring of AMR. Before the actual data collection, training, and discussion with 02 supervisors, 03 data collectors, and 02 laboratory technicians were undertaken for 02 days. Triplicate and duplicate samples were collected. Information on each sampling site and identification of the sampling locations were done by *Global Positioning System* (*GPS*). To check the sterility of the prepared media, 5% of the prepared batch of media was incubated overnight and checked for microbial growth in the media, and reference strains were also used.

Data analysis

The data were coded and entered using Epi info-7 and exported to STATA version 16. then Mean prevalence of AMR bacteria, variability, and linear regression were executed by using STATA statistical software version 16. We conducted a multiple linear regression model to determine the relationship between AMR WBB in drinking water with associated factors.

Ethics Approval

Ethical clearance was obtained from the Institutional Review Board of the Jimma University and an official letter was submitted to the concerned bodies. The concerned bodies were informed to get the assurance of the study and confidentiality was maintained at all levels of the study. Informed consent was obtained from all participants and the Institutional Review Board of the Jimma University approved it with Ethical approval of Research protocol letter with its reference number IRB00010/2020.

RESULTS

We collected 60 drinking water samples each from the household tap and storage container.Households use both improved and unimproved water sources for their daily water consumption. Based on the present survey, about 112 (93.33%) and 8(6.67%) households used improved and unimproved sources of water; respectively. the Household hygienic practices for handling of drinking water, presence and concentration of *Salmonella*, *Shigella*, *TC,* and *E. coli*, MDR level and Health risks was determined. We also compared the microbiological quality results of drinking water samples from household taps and a storage container with national/WHO/EPA guidelines.

Household hygienic practices for handling and WBB analysis of drinking water

In this study, we assessed the hygiene of household taps and storage containers using sanitary inspection checklist. 63.9% of the respondents treated their drinking water, which was fetched from the tap before consumption. Out of the total respondents who treated their drinking water, 55.88% used ceramic water filters, while 23.53% added chorine and 20.58% boiled water. Among the drinking water assessed, 30.56% of the area around the tap was in an unhygienic environment. Besides, 36.11% of the drinking water storage containers had poor hygiene (Table 1).

Table 1: The hygienic status of household tap and storage containers found at Debre Tabor town, Ethiopia, 2020.

S.NO	Items	Alternative	Respondent (%)
1.	Do you use anything to make drinking water safer?	Yes	63.9
		No	36.1
2.	Methods used to make drinking water safer?	Boiling	20.58
		Adding chlorine	23.53
		Water filter	55.88
3.	Are there any flies, dust, and other contaminants around the area of the tap?	Yes	30.56
		No	69.44
4.	Are there any flies, dust, and other contaminants around the area/on the storage container?	Yes	36.11
		No	63.89

The results showed that 27 % of the drinking water samples collected from the household tap were contaminated with TC with an overall mean±SD (11.19±11.45) whereas 50% of the drinking water samples collected from a storage container were positive with TC value of 47.15±27.0. The adjusted mean±SD of *E.coli* counts for drinking water samples collected from the household tap and storage container were 3.45±1.70 and 6.32±7.61 sequentially. Besides, 23% of the drinking water samples of the Tap contaminated with *Shigella* at a rate of 3.45±1.72while 3% of drinking water samples of household containers had *Shigell*a at the rate of 2.32±1.6. Moreover, 33.33% of drinking water from the tap was also contaminated with *Salmonella* with a rate of and 23% of the drinking water from storage containers contaminated with *Salmonella with a rate of* 3.02 ±2.71 (Table 2).

The prevalence rate of MDR *E. coli* species was *80*% (95% CI: 76.9-81.2 %) with resistance to AMX, SXT, and VA with a MIZ of 8.5mm (95% CI: 6.5-8.9mm), *Salmonella* species was 40% (95% CI: 38.7-45%) with resistance to Van, SXT, and AMX with a MIZ of 7.83mm (95% CI: 6.2-9.4mm) and *Shigella* species was 60% (95% CI: 56.9-65%) with resistance to SXT, AMX, VA, & DC with a MIZ of 7.65mm (95% CI: 6-8mm) (Table 3).

Table 2: Summary of WBB counts (% or Mean±SD in log CFU/100ml) of water samples collected from the tap and household storage containers of Debre Tabor town, Ethiopia, 2020

WBB	N	Frequency	Adjusted Mean±SD in log CFU/100ml	WHO, 2004 standard
Drinking-water of the tap				
TC	30	8 (27%)	11.19±11.45	
Shigella	30	7 (23%)	3.45±1.72	0 cfu/100ml(A)
Salmonella	30	10(33%)	2.87±2.63	0 cfu/100ml
E.coli	30	14 (46%)	3.45±1.70	0 cfu/100ml (A)
Drinking-water of the household storage container				
TC	0	15 (50%)	47.15±27.0	
Shigella	0	2 (3%)	2.32±1.61	0 cfu/100ml (A)
Salmonella	0	7 (23%)	3.02 ±2.71	0 cfu/100ml (A)
E.coli	0	10 (30%)	6.32±7.61	0 cfu/100ml(A)

Table 3: MDR level of WBB isolated from Drinking water in case of Debre Tabor Town, 2020.

WBB species	Resistance		Sensitive		MDR Rate
	Antibiotics	MIZ	Antibiotics	MIZ	
E.coli	AMX, SXT & VA	8.5mm (95% CI: 6.5-8.9mm)	CIP& DC	25.50mm (95%CI: 22, 29.45)	*80*%(95% CI: 76.9-81.2 %)
Salmonella	SXT, VA&AMX	7.83mm(95% CI: 6.2-9.4mm)	CIP& DC	27.50mm (95%CI: 23.25,30.45)	40% (95% CI: 38.7-45%)
Shigella	SXT, AMX, VA, & DC	7.65mm(95% CI: 6-8mm).	CIP	19mm (95% CI: 16.5, 23.2)	60% (95% CI: 56.9-65%)

Health risks of drinking water consumption contaminated with AMR

The overall Health risk index (HRI) of drinking water showed that 45.83%, 41.67%, and 12.5% of them were categorized as low, intermediate, and high-risk classes, respectively (Table 4).

Associated factors with AMR WBB in drinking water

According to the correlation analysis of the WBB, their growth parameters, and the level of Sanitary Risk (SI), most of the bacteriological parameters showed a significant correlation with the SI level of risk and a significant correlation with residual

Table 1: The hygienic status of household tap and storage containers found at Debre Tabor town, Ethiopia, 2020.

Sample	**SI**	***Total coliform***					***Fecal coliform***			
		1-10	10-100	100-000	>1000	0	1-10	10-00	100-1000	>1000
Tap	1-2	4	2	0	0	19	1	1	0	0
	3-5	8	11	0	0	7	5	0	0	0
	6-8	0	3	0	0	0	3	0	0	0
	9-10	0	0	0	0	0	0	0	0	0
HHSC*	1-2	4	18	0	0	12	3	1	0	0
	3-5	0	10	3	0	6	3	10	0	0
	6-8	0	1	0	0	0	0	1	0	0
	9-10	0	0	0	0	0	0	0	0	0
HRI		Low		45.83%						
		Intermediate		41.67%						
		High		12.5%						

***Where: HHSC: Household Storage Container, SR: Sanitary Risk**

chlorine concentration. The bacteriological parameters assessed *by TC* in the drinking water storage container showed a relative strong correlation with SI (*Correlation coefficients* (r) = 0.856**) and with residual chlorine concentration (*Correlation coefficients* (r) = 0.622**) (Table 5).

DISCUSSION

WBB of drinking water determination is a good representative of public health risk since it can be a medium for the transmission of pathogenic disease, particularly from fecal contamination. A similar study conducted in Nekemte town has shown that 37% of the drinking water was contaminated

Table 5: Correlation analysis output of WBB growth parameters, sanitary inspection risk score, *and drug use characteristics (CHXS) with AMR WBB load for each water sample at Debre Tabor town, Ethiopia, 2020.*

Growth parameters	AMR WBB					
	On Tap water			On Household storage container water		
	E.coli	*Salmonella*	*TC*	*E.coli*	*Salmonella*	*TC*
pH	0.106	0.144	0.097	-0.091	0.046	0.023
Conductivity	0.289	0.209	0.157	-0.037	0.089	0.061
Turbidity	0.164	0.084	0.207	0.001	0.008	0.116
Residual chlorine	0.674**	0.713**	0.633**	0.787**	0.620**	0.622**
Drug use CHXS	0.004	0.084	0.007	0.001	0.008	0.016
Sanitary Risk (SI)	0.711**	0.601**	0.493**	0.741**	0.562**	0.856**

**. Correlation is significant at the 0.05 level (2-tailed).

with *FC*, which was more than that of the present study(12). However, *E.coli's* finding in this study was higher than those from the studies conducted in Addis Ababa City (2.4%) (13)and Dharan, Nepal town (21.1%) (6).

This difference might be due to the difference in the safety and quality control of water through the evaluation of water sources and managing contamination of water supply. Moreover, it may associate with frequent pipe breakage, leakage, and passing of pipelines through the ditches and drainage systems.

The *E.coli* load was higher than the study conducted in Kolla diba town of Ethiopia (32.5%) (14) And lower than Babati town. Tanzania (86%) of drinking water samples contaminated with *E.coli*(15). The difference might be the treatment of drinking water and variation in climatic conditions.
According to Temesgen & Hameed (2015), there was high contamination of drinking water with AMR WBB due to improper treatment and the existence of poor sanitation (17). Assessment of the qualities of urban water source and tap water distribution systems in Arba-Minch town revealed that the distribution lines were the most contaminated with AMR WBB, such as *Salmonella* and *Shigella* (3).

The relationship between sanitary inspection scores and the bacteriological risk category is used to identify the level of risk of contamination due to AMR WBB. A study conducted by Tsega*et et al.* (2013)showed that the total sanitary risk score had a significant relationship with the level of fecal contamination(18). Moreover, a similar study conducted in Bahir Dar town showed that 45.7% and 11.4% of drinking water samples had low and very high-risk scores, respectively(19). The reason for the difference in risk score between the present study and the study conducted by Milkiyas *et al.* (2011) and Tsega et al. (2013) might be the hygiene and sanitation condition of the water storage container, awareness of the community towards water storage container and the dose of residual chlorine. The limitation of all previously conducted studies and the present study was that they used the membrane filter and culture method, not molecular analysis techniques.

CONCLUSIONS

The finding of this study has shown possible health hazards related to the consumption of drinking water. Identification of these hazards would help health officials to pay attention to safety and quality issues regarding drinking water. Moreover, it would contribute to the awareness of consumers and water sector officials about safety and quality issues related to the consumption of drinking water. It can encourage water sector officials to follow proper water treatment procedures during distribution up to consumption. The finding suggested the importance of water quality training for humans working in water sectors, implementation of water treatment, and strict follow-up of the implementation of acceptable hygienic practices might improve water quality. Besides, minimizing irrational drug use could also help to reduce AMR in drinking water and the environment.

List of Abbreviations

WBB: Waterborne Bacteria; CIs: Confidence Intervals; WHO: World Health Organization; US: United State; EPA: Environmental Protection Agency; *E. coli: Escherichia coli*; ARR: Antibiotic Resistance Rate; MIZ: Mean Inhibition Zone: MDR: Multidrug Resistance: mg/l: milligram per liter, pH: negative logarithmic concentration of hydrogen, SD: Standard Deviation: SI: Sanitary Inspection, TC: Total Coliform; UNICEF: United Nations Children's Fund

Declarations

Consent to Publish

Not applicable because no person's data is in the manuscript.

Availability of Data and Materials

All data and materials are available from the corresponding author. Thereforeat a reasonable request, the corresponding author shared it via email.

Competing Interests

The authors declared that they had no any financial and nonfinancial competing interests.

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Authors' Contributions

MK has been actively involved during the conception of research issues, MM is involved in the development of research proposals, and CY has been involved in conception of research issues, development of research proposals and writing of various parts of the research report and prepares the final manuscript. All authors have read and approved the final version of the manuscript. Chalachew Yenew had full access to all data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

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