

Bacterial quality of drinking water sources and antimicrobial resistance profile of Enterobacteriaceae in Bahir Dar city, Ethiopia

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ABSTRACT

A cross-sectional study was conducted to determine the bacterial quality and antimicrobial susceptibility profiles of Enterobacteriaceae from drinking water in Bahir Dar city. A total of 140 water samples were collected in the wet and dry periods from springs ($n = 4$), reservoirs ($n = 10$) and private tap water at households ($n = 126$). Bacteriological analysis of water was conducted using multiple tube method. Overall, 21.4%, 18.6% and 17.8% of drinking water samples had total coliforms (TC), faecal coliforms (FC) and *Escherichia coli*, respectively. All spring water samples and 29.2% of private tap water had the highest TC load (18 most probable number/100 mL, 95% CI: 100). For FC, 81.4% of the drinking water supplies tested complied with both World Health Organization and Ethiopian Standards. High levels of resistance (98–100%) were observed for ampicillin by Enterobacteriaceae and *Pseudomonas aeruginosa*. All *P. aeruginosa* isolates and 20 (66.7%) of *E. coli* revealed multiple drug resistance. Enterobacteriaceae and *P. aeruginosa* isolates exhibited high levels of antimicrobial resistance. The bacterial quality of drinking water in Bahir Dar city was poor. Microbial surveillance and monitoring with periodic assessment on physical integrity of the water pipelines need to be undertaken.

Key words | antibiotic resistance, *E. coli*, Ethiopia, thermotolerant coliforms, total coliforms, water

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INTRODUCTION

Water can be a vehicle for transmission of human pathogens. The World Health Organization (WHO) estimated that globally 3.4 million people die every year due to water-related diseases (WHO 2002). Moreover, in 2010, the annual incidence of diarrhea related to unsafe drinking water was 4.6 billion episodes and 2.2 million deaths (WHO 2010). Several infectious diseases are transmitted through water supplies contaminated with human and animal excreta particularly via faeces (WHO 1993). Outbreaks of waterborne diseases continue to occur throughout the world especially in developing countries (Jones *et al.* 2007; Reynolds *et al.* 2008). In Ethiopia, over 60% of the communicable diseases are due to poor environmental health conditions arising from unsafe and inadequate water supply linked with poor hygienic and sanitation practices (Abebe 1986).

Drinking water can be polluted with pathogenic organisms from the source, reservoirs and in the distribution network (Hein *et al.* 2007). Total coliforms (TC), thermotolerant (faecal) coliforms (FC) and *Escherichia coli* have been used to determine the microbial quality of drinking water worldwide (Ashbolt 2004). Therefore, bacteriological analyses of TC, FC and *E. coli* in water have been used worldwide to monitor and control the quality and safety of drinking water (Nichols *et al.* 2000). Studies indicate that water contamination with pathogens show seasonal variations (Islam *et al.* 2011).

Antimicrobial resistance (AMR) is a growing global problem. Antibiotic-resistant bacterial strains have been found in the human population, in foods, in livestock and in drinking water (Baquer *et al.* 2008; Samie *et al.* 2011). The entry of

antibiotics and resistant bacterial strains into the environment plays an important role in the spread of antibiotic resistance. However, in Ethiopia, water quality investigation has focused on the detection of TC and FC therefore there are no data on antimicrobial resistant bacteria isolated from drinking water sources.

In Bahir Dar city, there has been no study conducted on seasonal variations on the bacterial quality of drinking water and no data on antimicrobial susceptibility profile of Enterobacteriaceae species isolated from drinking water. The purpose of this study was therefore to assess the bacterial quality of drinking water during the wet and dry periods. Furthermore, this study aims to determine the AMR profile of Enterobacteriaceae isolates from drinking water sources in Bahir Dar city.

METHODS

Study design

A cross-sectional study was conducted in August 2012 (wet period) and in March 2013 (dry period) in Bahir Dar city.

Sample size

Sample size was calculated according to WHO (WHO 2004) guidelines based on the population size of Bahir Dar city. A total of 70 water sampling points were determined. The sampling distribution was based on the size of proportion of population in each clustered district of Bahir Dar city. Purposive sampling method was used to include springs and reservoirs used to store chlorinated water for distribution in the city. A simple random sampling technique using house numbers was used to select water sampling points from private tap water at households.

Water sampling points (source to point of consumption)

Water samples were gathered from 70 water sampling points during the wet period. Likewise, during the dry period, 70 water samples were collected from the same sampling points. Water samples were collected from the

main sources of two protected springs (namely, Areke and Lome), situated 10 kilometers away from Bahir Dar city. Water samples were also collected from the reservoirs and the distribution system in private tap water at households.

Collection of water samples

A total of 140 water samples were collected from springs ($n = 4$), reservoirs ($n = 10$) and private tap water at households ($n = 126$).

Four hundred millilitre samples were collected using sterile glass bottles in the morning (8 a.m. to 11 a.m.). The samples were quickly transported, using a cold chain box, to the Microbiology Laboratory at Bahir Dar Regional Health Research Laboratory Center.

Bacteriological analysis of water

Bacterial analyses of water were performed using the multiple-tube method. Within 6 hours of collection, all water samples were inoculated onto 11 tubes containing double strength MacConkey broth (Oxoid, UK) and single strength MacConkey broth (APHA 1998; WHO 2004). All inoculated tubes were incubated at 37 °C for 48 hours aerobically and examined for both acid and gas production.

Bottles and tubes that showed gas or acid production were considered as presumptive positive for TC. All presumptive positive samples were further inoculated into *E. coli* (EC) broth for *E. coli* and incubated for 48 hours at 37 °C and 48 hours at 44 °C for FC, respectively (APHA 1998). If there was no growth within 48 hours of incubation the tubes were deemed to be negative. The numbers of TC per 100 mL were enumerated from the number of presumptive positive tubes using most probable number (MPN) table (WHO 2004).

Isolation and identification of Enterobacteriaceae and *P. aeruginosa*

Isolation and identification of Enterobacteriaceae species and *P. aeruginosa* was done following standard operational procedures (WHO 2003). From presumptive positive tubes, a loop-full of culture was inoculated onto MacConkey agar

and blood agar and incubated at 37 °C for 24 hours. Phenotypic bacterial identification of Enterobacteriaceae species and *P. aeruginosa* was made manually in accordance with guidelines of the microbiology standards using the following biochemical media: Urea 40% broth, Simmons citrate agar, sulphide indole motility medium, Triple Sugar Iron Agar and Kligler Iron Agar and oxidase (Oxoid, UK) (WHO 2003). Reference strain *E. coli* ATCC 25922 with serial dilution was used to check whether MacConkey broth supports the growth of Enterobacteriaceae species isolated from drinking water or not.

Antibiotic susceptibility testing

Antibiotic susceptibility tests were performed using the Kirby-Bauer disc-diffusion method on Mueller Hinton agar (Oxoid, UK). Ciprofloxacin (5 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), tetracycline (30 µg), cotrimoxazole (25 µg), ampicillin (10 µg) and amoxicillin/clavulanic acid (30 µg) (Oxoid, UK) were used. Resistance and susceptibility data were interpreted according to CLSI (2010). Reference strain *E. coli* ATCC 25922 was used as control for interpretations of antibiotic susceptibility tests.

Data analysis

The data were entered into SPSS version 20 to compute χ^2 and determine the presence of association between wet and dry periods in terms of the presence and absence of TC, FT and *E. coli*. *p*-value >0.05 was considered non-significant.

RESULTS AND DISCUSSION

For bacteriological analysis, 140 drinking water samples were sampled from the springs, reservoirs and tap water at households (point of consumption) during wet and dry periods. During the wet period, TC, FC and *E. coli* were each detected from 17.1% of tap water samples. During the dry period, TC (17.1%), FC (14.3%) and *E. coli* (12.8%) were detected (Table 1). All water samples from protected springs had TC, FC and *E. coli* both in the dry and wet periods. The springs are located 10 km away from the city, thus, the water is collected via pipe and transported into the city and disinfected in a reservoir. Although water from the springs is not used as a source of drinking water directly, protection of the spring from animal contamination and flood is recommended.

During the wet period, two (40%) of the reservoirs used to store chlorinated water for distribution had TC but TC was not detected in the dry period. However, FC and *E. coli* were not detected in all reservoirs. The microbial quality of drinking water should be verified by surveillance and monitoring of indicator organisms. The presence of TC in drinking water samples indicates problems related to cleanliness and integrity of the distribution systems. In addition, FC and *E. coli* indicate faecal contamination of drinking water (WHO 2011).

In this study, the proportion of water samples positive for TC, FC and *E. coli* was high. These indicate the possibility of faecal pollutions of drinking water by enteric pathogens (WHO 2011). Therefore, disinfection was essential as per the guidelines of the WHO (WHO 2011). Overall, for FC, 81.4% of private piped drinking water supplies tested complied with WHO (2011) and Ethiopian Standards (ES) (ES 2001). However, in Ethiopia, a nationwide survey (Tadesse *et al.*

Table 1 | The proportion of indicator organisms detected from different water sources during wet and dry periods in Bahir Dar city, 2013

Water source	Indicator bacteria					
	Wet period			Dry period		
	TC	FC N (%)	<i>E. coli</i>	TC	FC	<i>E. coli</i>
Spring (<i>n</i> = 4)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
Tap water (<i>n</i> = 126)	12 (17.1)	12 (17.1)	12 (17.1)	12 (17.1)	10 (14.3)	9 (12.8)
Reservoirs (<i>n</i> = 10)	2 (40)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total (<i>n</i> = 140)	16 (22.8)	14 (20)	14 (20)	14 (20)	12 (17.1)	11 (15.7)

2010) reported that 72% of the piped drinking water was in compliance with WHO and ES. This difference may be due to the inclusion of piped water supplies from rural and semi-urban areas of the country in the previous nationwide study.

The proportion of TC (21.4%) and FC (18.6%) contamination from tap water at households was higher compared with TC (16%) and FC (7%) tap water contamination reported in Brazil (Nogueira *et al.* 2003). However, in Ethiopia, a study conducted in 2011 in the same area in Bahir Dar city reported 45.7% TC and 40% FC (Tabor *et al.* 2011). This difference may be attributable to sample size difference, methodology of bacterial analysis and seasonal differences.

The proportion of water contamination was higher at household level in private tap water. This indicated that the possible source of water contamination in Bahir Dar city might be from the water distribution pipelines because FC and *E. coli* were not detected in all reservoirs used to store and distribute water into the city (Table 1). However, further study on the physical integrity of the water pipelines needs to be investigated.

E. coli is the more precise indicator of recent faecal pollution. In any drinking water samples, *E. coli* must not be detected. The objective of zero *E. coli* per 100 mL of water is the goal for all water supplies (WHO 2004, 2011). However, it must be noted that *E. coli* as indicator organism for enteric viruses and protozoa has shortcomings. Since non-enveloped viruses and cysts of protozoa are more resistant to environmental conditions and chlorine disinfection, they may be present in the absence of *E. coli* in drinking water (WHO 2004, 2011). The microbial quality of drinking water is categorized based on the proportion of water samples negative for *E. coli*. Thus, for population size $\geq 100,000$, if *E. coli* is negative for 99%, 95%, 90% and 85% it is clustered as excellent, good, fair and poor, respectively (WHO 2004). In the present study, the proportion of water samples negative for *E. coli* is 82%; thus the microbial quality of the drinking water supplies tested in Bahir Dar city fell into the poor quality category.

Among 70 water sampling points, six (8.6%) had TC and FC both during wet and dry periods. There was no statistically significant difference in detection of TC, FC and *E. coli* ($p > 0.05$) during the wet and dry periods (Table 2). Overall, TC was detected in 23% and 18.5% of samples during the wet and dry periods, respectively. Furthermore, FC was detected in 20% and 17.1% of samples during the

Table 2 | TC, FC and *E. coli* detected from drinking water in wet and dry periods in Bahir Dar city, 2013

Seasons	Water samples tested N	Indicator bacteria		
		TC ^a N (%)	FC ^b N (%)	<i>E. coli</i> ^c
Wet	70	16 (22.8)	14 (20)	14 (20)
Dry	70	14 (20)	12 (17)	11 (15.7)
Total	140	30 (21.4)	26 (18.6)	25 (17.8)

^a p -value = 0.58; ^b p -value = 0.41; ^c p -value = 0.37.

wet and dry periods, respectively. In this study, 30 (21.4%) water samples were contaminated with TC. Overall, 17.8% of drinking water samples had *E. coli*.

The bacteriological analysis of drinking water samples did not reveal statistically significant differences during the wet and dry periods in terms of detection of TC, FC and *E. coli*. However, this study did not fully investigate the seasonal variation of bacterial quality of drinking water throughout the year as this study was carried out only in August and March which represents the situation during the peak wet and dry periods, respectively.

All water samples from springs and seven (29.2%) of tap water at households had the highest TC load (18 MPN/100 mL with 95% CI = 100). Furthermore, six (25%) water samples from tap water at household had 16 TC MPN/100 mL water (95% CI = 4–40) (Table 3). The presence of a high load of TC in drinking water indicates that the water has already been polluted by pathogens, thus it is of a high

Table 3 | MPN of TC/100 mL of water from different water sample locations in Bahir Dar city, 2013

TC (MPN/100 mL (95% CI))	Water sources		
	Reservoirs (n = 10)	Taps (n = 1260)	Spring (n = 4)
1 (1–4)	–	1	–
2 (1–6)	1	4	–
3 (1–9)	1	3	–
7 (2–17)	–	1	–
9 (2–21)	–	2	–
16 (4–40)	–	6	–
18 (100)	–	7	4
Total positive water samples	2	24	4

Table 4 | Antibiotic resistance profiles of Enterobacteriaceae and *P. aeruginosa* from drinking water sources in Bahir Dar city, Ethiopia, 2013

Antibiotics	Bacterial species				Total resistance
	<i>E. coli</i> (n = 35) N (%)	<i>P. aeruginosa</i> (n = 10)	<i>P. mirabilis</i> (n = 5)	<i>Citrobacter</i> spp. (n = 3)	
Amoxicillin ^a	35 (100)	10 (100)	4 (80)	3 (100)	51 (98)
Ampicillin	35 (100)	10 (100)	5 (100)	3 (100)	52 (100)
Ceftriaxone	7 (20)	5(50)	3 (60)	0	15 (28.8)
Ciprofloxacin	2 (5.7)	0	0	0	2 (3.8)
Chloramphenicol	13 (37.3)	10 (100)	3 (60)	2 (66.6)	28 (53.8)
Tetracycline	26 (74.3)	10 (100)	3 (60)	3 (100)	42 (80.8)
Gentamicin	10 (28.5)	4 (40)	0	2 (66.6)	16 (30.8)
Cotrimoxazole	16 (45.7)	10 (100)	3 (60)	2 (66.6)	31 (59.6)

^aAmoxicillin-clavulanic acid.

risk for human health (APHA 1998). The highest TC load was found from private tap water at households (Table 3). Therefore, 10.3% of the private tap water at households had 16–18 MPN/100 mL. This indicated poor bacterial quality of drinking water in Bahir Dar city (WHO 2004, 2011).

In the present study, AMR profiles of bacterial species of Enterobacteriaceae from drinking water sources were determined. The antibiotic resistance profiles of each bacterial species are depicted in Table 4. All of the Enterobacteriaceae isolates (*E. coli*, *P. mirabilis* and *Citrobacter* spp.) and *P. aeruginosa* showed resistance to ampicillin and amoxicillin with clavulanic acid. All *P. aeruginosa* isolates revealed resistance to chloramphenicol, tetracycline and cotrimoxazole. Low levels of resistance were seen for ciprofloxacin. All *P. aeruginosa* isolates and 66.7% of *E. coli* revealed multiple antibiotic resistances against three and more antibiotics (Table 5). Many studies

have shown the existence of antibiotic-resistant bacteria in drinking water (Xi et al. 2009; Coleman et al. 2012; Sahoo et al. 2012). This study is the first to document antibiotic resistance profiles of Enterobacteriaceae and *P. aeruginosa* isolates from drinking tap water in Bahir Dar city and in Ethiopia. Overall, the high levels of resistance by Enterobacteriaceae were observed for ampicillin and amoxicillin-clavulanic acid. In contrast, the lowest level of resistance (3.8%) was found to ciprofloxacin. In this study, 91.4% of *E. coli* from drinking tap water exhibited resistance to at least one of the eight tested antibiotics. Likewise, studies from India and Brazil reported 96% and 93% of *E. coli* resistant to at least one of the tested antibiotics, respectively (Coleman et al. 2012; Sahoo et al. 2012). In this study, all *P. aeruginosa* isolates from drinking water showed multiple antibiotic resistances to three and more antibiotics. However, all *P. aeruginosa* isolates were susceptible to ciprofloxacin (Table 4). High levels of AMR revealed by Enterobacteriaceae species indicated that antimicrobial resistant bacteria can spread to human beings through drinking water sources.

AMR profiles of *E. coli* from drinking water sources in Brazil, India, Nigeria and Egypt compared to the present study is shown in Table 6 (Lima-Brittencourt et al. 2007; Efuntoye & Apanpa 2010; Alzahrani & Gherbawy 2011; Coleman et al. 2012). *E. coli* revealed high levels of resistance against ampicillin and amoxicillin in almost all countries. However, *E. coli* showed different levels of resistance to various antibiotics in different countries. These differences may

Table 5 | Multiple drug resistance profiles of bacterial isolates from drinking water in Bahir Dar city, Ethiopia 2013

Bacteria spp.	Antibiogram							
	R0	R1	R2	R3	R4	R5	R6	MDR N (%)
<i>E. coli</i>	3	1	11	7	6	4	3	(66.7%)
<i>P. aeruginosa</i>	–	–	–	2	2	6	–	(100%)
<i>P. mirabilis</i>	1	1	–	–	1	1	–	(40%)
<i>Citrobacter</i> spp.	–	1	–	2	–	–	–	(75%)

R0 = susceptible to all eight tested antibiotics; R1 = resistance to only one antibiotic; R2–R8 = resistance to two and more antibiotics; MDR: resistance to three and more antibiotics.

Table 6 | Comparison of the percentage of antibiotic-resistant *E. coli* from drinking water sources in five countries

Antibiotics	Countries				
	Ethiopia	India ^a	Brazil ^a	Nigeria ^a	Egypt ^a
Ampicillin	100	47	100	82.9	76.9
Amoxicillin ^b	100	NA	100	100	NA
Ciprofloxacin	5.7	20–41	NA	2.9	34.6
Chloramphenicol	37.3	NA	100	17	53.8
Gentamicin	28.5	NA	0	11.4	NA
Cotrimoxazole	45.7	41–53	NA	100	38.5
Tetracycline	74.3	30–61	100	80	50

NA; not available.

^aReferences: India: Sahoo et al. 2012; Brazil: Coleman et al. 2012; Nigeria: Efuntoye & Apanpa 2010; Egypt: Alzahrani & Gherbawy 2011.

^b = Amoxicillin-clavulanic acid.

be related to the difference in antibiotic prescriptions across countries (Oancea & Stoia 2010).

CONCLUSION

Based on the proportion of *E. coli* positive water samples and population size, the microbial quality of drinking water in Bahir Dar city was poor, particularly at household levels in private tap water. A considerable proportion of water samples were not in compliance with WHO and ES. Thus, regular monitoring and surveillance on the microbial quality and safety of drinking water need to be conducted throughout the year. Further studies on physico-chemical parameters, physical integrity of pipelines and bacteria analysis on advanced indicator organisms need to be carried out. Enterobacteriaceae species and *P. aeruginosa* isolates from drinking water sources have shown high levels of antibiotic resistance to commonly prescribed antimicrobials. In addition to clinical isolates of bacteria, drug resistance surveillance from environmental isolates such as drinking water needs to be considered.

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