

## RESEARCH ARTICLE

# Enteric bacterial pathogens and their antibiotic-resistant patterns from the environmental sources in different regions of Ethiopia: A laboratory-based cross-sectional study

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## Abstract

**Background and Aim:** Antimicrobial resistance (AMR) resulting in the most significant public health and economic threat. Unfortunately, it is one of the missing topics on sustainable development goals (SDGs). Therefore, this laboratory-based study aimed at determining enteric bacterial pathogens and their antibiotic-resistant patterns from the environmental sources in different regions of Ethiopia.

**Methods:** A laboratory-based cross-sectional study was conducted by following the standard microbial culture and the Kirby-Bauer disc diffusion method for identification and AMR patterns of the enteric bacteria using a total of 180 environmental samples from January through June 2020. We employed descriptive statistics to examine the prevalence rate, comparability of results, and summary of AMR patterns of enteric bacteria and a 95% confidence Interval (CI) for considering the statistical significance and give conclusions by using Stata 14.1.

**Results:** The mean prevalence rates (SD) at 95% CI of AMR enteric bacterial pathogens were 53.13 (2.51)% (52.31, 53.95), 45 (1.85)% (44.40, 45.60), 32.5 (3.01)% (31.10, 33.00), and 31.12 (1.95)% (30.80, 31.45) in Wastewaters, leachate from solid waste dumping sites, waste receiving water bodies (Lake Tana at Bahir Dar and Boye Wetland at Jimma), and Soils sequentially. Specifically, *Escherichia coli*, *Shigella*, and *Salmonella* were 90 (3.10)% (89.00, 91.10), 67.5 (2.58)% (66.72, 68.41), and 45(1.58)% (44.48, 45.52), respectively, investigated in wastewater. In addition, solid waste dumping sites were contaminated with *E. coli* 80 (3.97)% (79.34, 80.66), *Shigella* 61 (2.87)% (59.06, 60.94), and *Salmonella* 42 (5.67)% (40.15, 43.85). This study implies that the waste discharges are the main source of contamination for AMR pathogens to the two aquatic water bodies.

**Conclusion:** The finding indicated that wastewater and solid waste dumping sites were important sources for AMR enteric pathogens. The finding might have indicated the tip of the iceberg about the environmental contamination with antimicrobial-resistant enteric pathogens.

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## KEYWORDS

antimicrobial-resistant, Bahir Dar, environmental sources, Ethiopia, Jimma, waste management

## 1 | INTRODUCTION

Globally, it is projected that antimicrobial-resistant (AMR) pathogens will pose the most significant public health and economic threat. By 2050, about 10 million people will die every year and the world's GDP will lose over 100 trillion USD. Hence, these figures are assumed to increase significantly if preventive measures could not be taken.<sup>1</sup> Unfortunately, it is one of the missing topics on sustainable development goals (SDGs).<sup>2</sup>

AMR has been occurring naturally over time but is accelerated by the inappropriate use of antimicrobial medicines in the health, animal, food, agriculture, and aquaculture sectors, and antimicrobial residues in soil, crops, sediments, and water.<sup>3,4</sup>

According to WHO, *Escherichia coli*, *Salmonella*, and *Shigella* species are classified priority tiers of pathogens for research, classified under critical, high, medium tier, respectively.<sup>5</sup> In response to this, global and local movements have been taking place for tracking the spread of that are difficult-to-treat infections.<sup>6</sup> AMR is also threatening SDGs on health, food security, environmental wellbeing, and socioeconomic developments and could affect the COVID-19 interventions. As AMR infections are becoming more common, modern medicine gets challenged much like the effects of COVID-19. This underscores that the high-potential AMR has to crush our healthcare structure and policies.<sup>7</sup>

The lessons we are learning from COVID-19 show that it is not the only threat that we are facing and indicates a dark future if we do not mobilize a growing response to the growing threat of AMR.<sup>8</sup>

In the study in Southern Ethiopia, 71.7% of AMR pathogens have been isolated from the wetland's outlets. The High public health concern is AMR pathogens have been isolated from the water samples of Lake Hawassa such as *E. coli*, *Shigella*, and *Salmonella*. Consequently, these may result in a serious threat to both public health and the environment.<sup>9</sup>

Treated wastewater used for irrigation has a greater role in the spread of AMR pathogens in urban agriculture in African cities.<sup>10</sup> Hence, the Ethiopian government has a greater tendency and effort to expand urban agriculture around the water bodies in different cities as mentioned by Ministries at different forums and meetings. With such and associated problems, the Ministry of Health of Ethiopia has considered AMR pathogens as a major threat and obstacle to ensure standard treatment and safeguard public health, where surveillance and knowledge generation is considered as the main strategy to tackle the problem.<sup>11</sup>

Almost all countries are taking significant measures for tracking AMR. However, serious gaps remain unattended due to a considerable lack of data and action in the environment sectors to prevent environmental contamination. Information on incidence, prevalence,

and trends of AMR must be collected to better understand and respond to the spread of AMR.<sup>12</sup>

Ethiopia begins to give more attention to and efforts on disseminating information about AMR to the community and establishing a national strategy and action plan to prevent and contain AMR. Holistic approaches needed to address AMR in Ethiopia are generating baseline data, identifying major gaps, suggesting and developing appropriate strategies and policy for action, suggesting and developing appropriate interventions, ongoing monitoring, and evaluation, and scaling up successful interventions in different areas.<sup>13</sup>

Determining the level of AMR, identification of contamination source, and the development of new recommendations and practices that continue for reducing the spread of AMR are pillars for global and local public health response, policy intervention, and reshaping of strategies. Therefore, this laboratory-based prevalence study is aimed to determine the prevalence rate and environmental source tracking of AMR enteric pathogens in Bahir Dar, Northwest Ethiopia, and Jimma in Southwest Ethiopia.

## 2 | METHODS

### 2.1 | Study design and period

A laboratory-based cross-sectional study was carried out from January through June 2020 at Bahir Dar, Northwest Ethiopia, and Jimma, Southwest, Ethiopia.

### 2.2 | Study setting selection and sample collection

Those standards more likely considered the situational condition of the countries. Samples were collected using the standard set by the United States Environmental Protection Agency sampling standards and District laboratory practices of tropical countries.<sup>14,15</sup> Following the above widely accepted laboratory standards, 120 samples from wastewater of Jimma and Felege Hiwot Comprehensive Specialized Hospitals, soil from the two hospitals compounds (using transect sampling method), and solid waste dumping sites were taken by considering the hospitals' given long-term care facilities and no any study concerned on AMR in the environmental media carried out as far as. Besides, 40 water and 40 sediment samples from waste receiving water bodies (Lake Tana and Boye wetlands) were collected. The assumption here is the waste discharges of the communities and hospitals of each city are discharging their waste to these water bodies. The samples were transported to the laboratory in a cold box with ice packs withholding a temperature of <4°C immediately after collection for processing and analysis by packed separately.

## 2.3 | Sample processing techniques of enteric bacterial isolation and AMR testing

### 2.3.1 | Sample preparation

The 25 mL or gram collected samples were homogenized with sterile 225 mL of 0.1% (w/v) bacteriological peptone in the flask for 5 min.<sup>16</sup>

### 2.3.2 | Enteric bacteria isolation and identification technique

A 0.1 mL of the prepared diluted sample directly inoculated on differential and selective agar media after being enriched with primary and secondary enrichment media and incubated at 37°C for 18–24 h. After incubation, The suspected isolates were transferred to Nutrient agar for further characterization, and morphological and biochemical tests including Gram reaction test, motility test, oxidase test, catalase test, triple sugar iron agar (TSI) test, lysine iron agar test (LIA), urease test, glucose, mannitol, and sucrose fermentation tests per the internationally accepted standard of ET ISO 707, 2012 for more authenticity (Figure 1).

### 2.3.3 | Multiple antibiotic-resistant profile testing

The slanted cultures were subcultured and purified. The pure colonies were inoculated into Nutrient Broth and incubated at 37°C for 18 to 24 h. After incubation, the turbidity of the culture was adjusted to 0.5 McFarland Standard to bring the cell density to approximately  $10^7$  to  $10^8$  cfu/mL. The 0.5 McFarland turbidity standard was prepared by mixing 0.05 mL  $\text{BaCl}_2$  (1%)

with 9.95 mL  $\text{H}_2\text{SO}_4$  (1%). Muller-Hinton (MH) (Oxoid) plates were prepared and warmed to ambient temperature for plating. A sterile cotton swab was dipped into 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° of each spreading. The plates were allowed to dry before applying antimicrobial discs. The following standard and Oxoid drug discs were used: Vancomycin (VA) disk of 30 µg oxide; Cotrimoxazole (COT) disk of 25 µg oxide; Ciproflaxicillin (CIP) disk of 5 µg oxide; Doxycycline (DC) of 30 µg, Amoxicillin (AMX) disk of 10 µg oxide, Erythromycin (ERYC) disk of 15 µg oxide, Ampicillin (AMP) disk of 10 µg oxide, Ceftriaxone (CRO) disk of 30 µg oxide, Chloramphenicol (CHL) disk of 30 µg oxide, Penicillin (PEN) of 10 µg oxide, Novobiocin (NB) disk of 30 µg oxide, Cloxacillin (CLOXA) disk of 1 µg oxide, Cefalotin (CET) disk of 30 µg oxide, and Gentamicin (GEN) disk of 10 µg oxide, which were commonly used and clinically important antibiotics in Ethiopian healthcare facilities. After incubation at 37°C for 18 to 24 h, inhibition zones were measured and scored as susceptible, intermediate, or resistant based on the guidelines developed from the Clinical and Laboratory Standards Institute of US (CLSI, 2017). The *E. coli* ATCC 25922, *Salmonella ser. Choleraesuis* ATCC 10708, and *Shigella flexneri* ATCC 12022 were used as reference strains for antibiotic disk control.<sup>17</sup>

## 2.4 | Data quality control

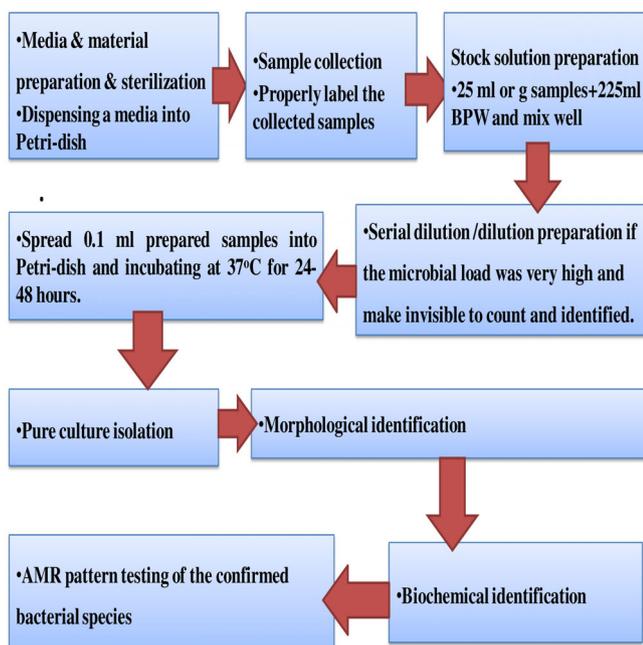
We assigned the qualified, competent, and proficient laboratory personals for the laboratory analysis and data collection, as well as the personnel that interpreted the results and those that were involved in the monitoring of AMR. Before the actual data collection, training, and discussion with 2 supervisors, 3 data collectors, and 2 laboratory technicians were undertaken for 2 d. 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 also used.

## 2.5 | Data management and analysis

The data were coded and entered using Epi info 7 (Centers for Disease Control and Prevention) and exported to Stata version 14.1. Stata 14.1 software (StataCorp) was used for data management and further analysis. Descriptive statistics were employed to examine the prevalence rate, comparability of results, and cumulative and summary of AMR patterns of different enteric bacteria. The 95% confidence interval (CI) was used for considering statistical significance.

## 2.6 | Ethical approval

Ethical clearance was obtained from the Institutional Review Board of the Jimma University and an official letter was submitted to the



**FIGURE 1** The logical framework shows the laboratory procedure followed during sample analysis of the study, 2020

relevant authorities. The relevant authorities were well informed to get the assurance of the study and confidentiality maintained at pertaining samples and the Institutional Review Board of the Jimma University approved it with Ethical approval of the Research protocol letter with its reference number IRB00010/2020. Finally, all waste materials generated during this research were well sterilized before disposing to the Environment.

### 3 | RESULTS

The present study analyzed the prevalence rate and AMR patterns of enteric bacteria against five commonly used antibiotics VA disk of 30 µg oxide; COT disk of 25 µg oxide; CIP disk of 5 µg oxide; DC disk of 30 µg, AMX disk of 10 µg oxide, ERYC disk of 15 µg oxide, AMP disk of 10 µg oxide, CRO disk of 30 µg oxide, CHL disk of 30 µg oxide, PEN of 10 µg oxide, NB disk of 30 µg oxide, CLOXA disk of 1 µg oxide, CET disk of 30 µg oxide, and GEN disk of 10 µg oxide, based on their mean inhibition zone (MIZ) among 180 samples from each environmental compartment (wastewater, soil, solid waste dumping site, and waste receiving water bodies) and identify the major sources among the different environmental media based on the point estimate (prevalence rate) that were generated from the laboratory data.

#### 3.1 | The Overall AMR patterns of enteric bacteria in the environment

The finding of this study has shown possible environmental sources related to AMR enteric pathogens (see Table 1).

#### 3.2 | The MDR-level enteric bacteria isolated from different environmental sources

In this study, multiple antibiotic-resistant (MDR) levels of *E. coli* was 83.63% (95% CI: 80.5%-88%), *Salmonella* was 85% (95% CI: 75%-92%), and *Shigella* was 76.25% (95% CI: 62%-85%) (Table 2).

*Escherichia*, *Salmonella*, and *Shigella* were isolated at varied rates from wastewater, solid waste dumping sites, soil, and waste receiving water bodies. The primary sources of enteric bacteria were determined based on their prevalence rate (Figure 2).

The main findings of the study and the prevalence of AMR enteric bacteria were a statistically significant difference between different environmental media. The prevalence of AMR enteric pathogens was 53.13% (95% CI: 51.23%-59.56%), 45% (95% CI: 41%-48%), 32.5% (95% CI: 29.23%-35.45%), and 31.12% (95% CI: 28.78%-34.23%) in the wastewater, solid waste dumping sites, waste receiving water bodies, and soil, respectively (Figure 2).

And also, most of the isolates were resistant to AMX, COT, VA, CLOXA, CRO, ERYC, CET, and CHL; none was resistant to CIP, AMP, PEN, GEN, and NB (Table 1).

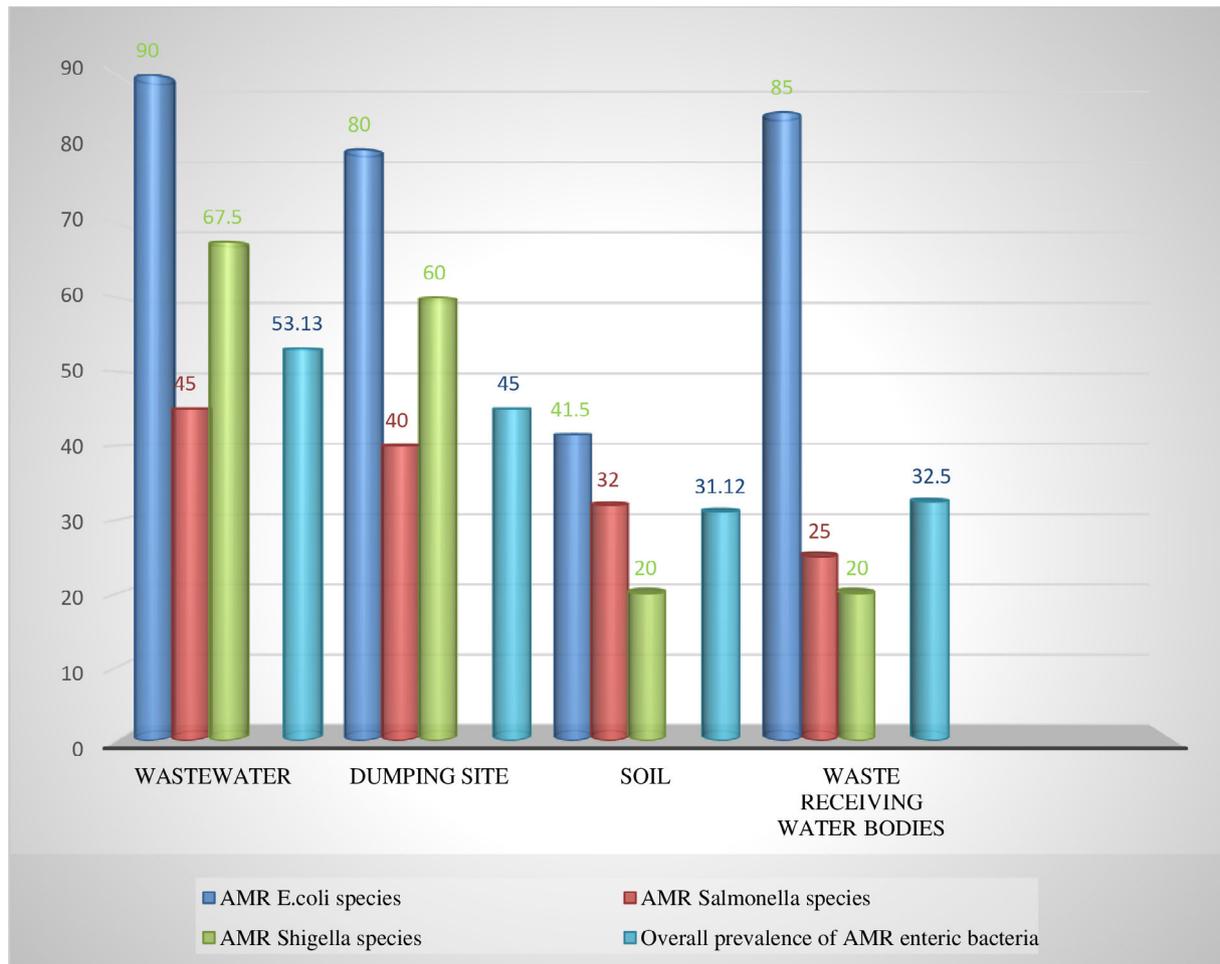
TABLE 1 Overall resistance level of enteric bacteria isolated from wastewater, soil, solid waste dumping site, and waste receiving water bodies

FBB species	Resistance		Sensitive		MAR index
	Antibiotics	MIZ	Antibiotics	MIZ	
<i>Escherichia coli</i>	AMX, COT, VA, CLOXA, CRO, ERYC, CET, and CHL	8.07 mm (95% CI: 6.63, 9.8)	CIP, AMP, PEN, GEN, NB, and DC	25.50 mm (95% CI: 2.2, 29.45)	0.57
<i>Salmonella</i>	AMX, COT, VA, CLOXA, CRO, ERYC, CET, and CHL	7.77 mm (95% CI: 5.90, 9.43)	CIP, AMP, PEN, GEN, NB, and DC	27.50 mm (95% CI: 23.25, 30.45)	0.57
<i>Shigella</i>	AMX, COT, VA, CLOXA, CRO, ERYC, CET, CHL, and DC	8.75 mm (95% CI: 6.98, 9.90)	CIP, AMP, PEN, GEN, and NB	19 mm (95% CI: 16.5, 23.2)	0.64

Abbreviations: AMP, ampicillin; AMX, amoxicillin; CET, cefalotin; CHL, chloramphenicol; CI, confidence interval; CIP, ciprofloxacin; CLOXA, cloxacillin; COT, cotrimoxazole; CRO, ceftriaxone; DC, doxycycline; ERYC, erythromycin; GEN, gentamicin; MIZ, mean inhibition zone; NB, novobiocin; PEN, penicillin; VA, vancomycin.

**TABLE 2** The MDR level of enteric bacteria isolated from different environmental media of the Bahir Dar and Jimma areas, Ethiopia, March 2020

Enteric bacteria	Wastewater	Solid waste dumping site	Waste receiving water bodies	Soil	MDR level (%)
<i>Escherichia coli</i>	n = 36 29 (80.5%)	n = 32 27 (84%)	n = 68 60 (88%)	n = 17 14 (82%)	83.63
<i>Salmonella</i>	n = 18 15 (83%)	n = 16 12 (75%)	n = 20 18 (90%)	n = 13 12 (92%)	85
<i>Shigella</i>	n = 27 23 (85%)	n = 24 20 (83%)	n = 16 10 (62.5%)	n = 8 6 (75%)	76.25

**FIGURE 2** The environmental sources of AMR enteric bacteria based on its prevalence rate, March 2020. AMR, antimicrobial resistance

## 4 | DISCUSSION

Identification and determination of the extent of AMR contamination and key sources in low-income countries are very crucial to combat the spread of AMR.<sup>18</sup>

In this study, the enteric bacterial species were detected with a high prevalence rate and MDR level (resistance to more than three antibiotics) in the hospital wastewater. It is supported by the study conducted in Hawassa reported a high prevalence of *Salmonella*,

*Shigella*, and *E. coli* with MDR ( $\geq 3$  antibiotics) detected from the effluents of hospitals.<sup>19</sup>

It is agreeing with the study conducted in South Africa indicated that about 90% of enteric bacteria were isolated from the hospital wastewater and were resistant to commonly prescribed antibiotics like SXT and VA, because high usage of antibiotics to treat infections in patients serves as a selective pressure for resistance development and transmitted via various routes such as hospital wastewater, discharged patients, and healthcare worker.<sup>20</sup>

In the subgroup laboratory analysis of this paper, the solid waste dumping site is the second environmental source of AMR enteric bacteria next to wastewater. This is supported by the study conducted in Tanzania where a high prevalence rate of AMR enteric bacteria with 56% of overall MDR on a solid waste dumpsite is reported.<sup>20</sup> Municipal dumpsite represents an end-point of biodegradable and unrecyclable garbage from various human activities demonstrating the microbial complexity and showing the role of such dumpsites as hot-spots for the emergence of new pathogens.<sup>21</sup>

In this laboratory-based prevalence study, the wastewater receiving bodies were almost with equal statistical significance contributing sources for AMR enteric pathogen with solid waste dumping site, but lower than the occurrence of AMR enteric pathogens in wastewater. The statistical significance value indicated that the dumping site was the main contributing factor for AMR enteric bacteria occurrence on waste receiving water bodies. A comparable survey conducted by a pan-European urban setting provided that wastewater and solid waste dumping sites were contributing to AMR enteric bacteria prevalence rate on the receiving water bodies.<sup>22</sup>

A similar study conducted in Egypt revealed that most isolated enteric bacteria were resistant to amoxicillin, SXT, and VA.<sup>23</sup> It is agreeing with the study done on Oslo University Hospital, Norway, *E. coli* in urban wastewater samples were highly prevalent and seemed to represent well the other pathogens in the town and it has a high degree of resistance to approximately all tested antimicrobials.<sup>24</sup>

The laboratory-based comparative statistics results of this study; wastewater is the primary source of AMR enteric with the prevalence rate ranging between 51.23% and 59.56%. So, it requires more priority and emphasis to reduce the spread of AMR enteric pathogens. While there are no statistically significant differences for the occurrence of AMR on dumping sites and waste receiving water bodies, it indicates that the dumping site may be the contributing factor for the spread of AMR in waste receiving water bodies. This is agreeing with the study conducted on Kakamega town, Kenya, 2018, the prevalence rate of AMR *E. coli* species was 100% in wastewater, about 93% of the sludge, and about 66% of the solid waste dumping site, and the prevalence rate of AMR enteric bacteria varied from one environmental source to the other.<sup>25</sup>

These data now allow research programs beyond surveillance activities. Ethiopia is considered a poor but stable country, according to “The World 2030” and moving toward transition and growth economies. Failure to control AMR and the spread of AMR will affect the progress towards the sustainable development goals (SDGs 1, 2, 3, and 8). Therefore, this paper will build onto the next 2021 Health Sector Transformation Plan (HSTP) and “Strategy for the Prevention and Containment of Antimicrobial Resistance (AMR) for Ethiopia” initiated in 2017.

#### 4.1 | Strength and limitation of the study

The strength of the study

- Assigned qualified, competent, and proficient laboratory personals and

- Used reference strains
- Followed an internationally accepted standard of ET ISO 707, 2012 for more authenticity
- Used appropriate sample size for the Laboratory analysis

The limitation of the study

- The study conducted using the Culture Method rather than the molecular technique because no availability of Primers and even PCR machines in the study area due to a lack of resources
- The study done during the dry seasons but, the contamination of AMR enteric bacteria may vary throughout the seasons
- A study done at a point in time due to resources and time constraints

## 5 | CONCLUSION

This laboratory-based prevalence study concluded that wastewater and solid waste dumping sites were important sources for AMR enteric pathogens. And also one of the major sources of AMR pathogen contamination of aquatic systems (waste receiving water bodies like Lake Tana) is the source of fish, recreation, vegetables, and the kidneys of the earth (Wetland like Boye Wetland).

The finding of this study has shown possible environmental sources related to AMR enteric pathogens. Identification of these sources would help different sectors including the Ministry of Health, Nongovernmental organizations, and other responsible bodies to pay attention to the major environmental sources of AMR and encourage decision makers to design and implement effective interventions at the sources. We recommend the improvement of waste treatment methods and the use of effective infection prevention measures to reduce the spread of AMR in the environment and analysis of AMR genes might indicate a true picture of the problem in developing countries. Besides, minimizing irrational drug use would help to reduce AMR in the environment.

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### AUTHOR CONTRIBUTIONS

Conceptualization, Data curation, Methodology, Writing—original draft, Writing—review and editing: Argaw Ambelu  
Funding Acquisition, and Writing—review and editing: Getnet Mitike

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All authors have read and approved the final version of the manuscript.

Chalachew Yenew had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

## TRANSPARENCY STATEMENT

Chalachew Yenew affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

## CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

## ARRIVE GUIDELINE

This study was carried out in compliance with the ARRIVE guideline recommendations for Human subject study.

## CONSENT TO PUBLISH

Not applicable.

## DATA AVAILABILITY STATEMENT

All data and materials are available from the corresponding author. So at a reasonable request, the corresponding author shared it via Email.

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