ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Bacteriological quality, antimicrobial resistance profile of *Escherichia coli* and associated factors of tap water, at Gulelle and Yeka sub cities of Addis Ababa, Ethiopia.

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A research thesis submitted to the Department of Medical Laboratory Science, College of Health Sciences, Addis Ababa University, for partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (diagnostic and public health microbiology specialty).

May 2025

Addis Ababa, Ethiopia

Addis Ababa University

College of Health Sciences

Department of Medical laboratory sciences

This is to certify that the thesis prepared by Desalegn Fente, entitled: **Bacteriological quality, antimicrobial resistance profile of *Escherichia coli* and associated factors of tap water at Gulelle and Yeka sub cities of Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (diagnostic and public health microbiology specialty) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Acknowledgements

I would like to express my profound gratitude to my advisors, Melese Hailu (PhD) and Dessie Abera (PhD candidate), for their invaluable guidance, insightful comments, and meticulous corrections throughout the entirety of my work. Their support has been instrumental in shaping my work.

I extend my sincere appreciation to Tesfaye Legese (PhD candidate) for their contributions as participants from the Public Health Microbiology laboratory in the study area. Their voluntary involvement and unwavering support, including insightful feedback and the provision of data, were crucial, and I am grateful for the time they dedicated to assisting me during the thesis writing process.

My deepest gratitude goes to my brother, Abraham Fente, and my family for their unwavering support. Their encouragement has been a crucial source of strength throughout my academic journey. I also wish to express my profound appreciation to my wife, Tinsae Hailu. Her kindness, support, and continuous motivation have been invaluable, inspiring me to approach my education with enthusiasm and dedication. In conclusion, I recognize the collective contributions of all those mentioned, whose support has been instrumental in my academic achievements. Their encouragement and assistance have left a lasting impact on my educational experience.

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List of Abbreviations

AAWSA	Addis Ababa Water Sanitation Authority
AMB	Aerobic Mesospheric Bacteria
AMR	Antimicrobial Resistance
BGLB	Brilliant Green Lactose Bile Broth
CDC	Center of Disease Control
EC Broth	Escherichia coli broth
EPHI	Ethiopian Public Health Institution
FC	Faecal Coliforms
MF	Membrane Filtration
MTEC	Membrane Thermo Tolerant *Escherichia Coli*
SPSS	Statistical Package for Social Sciences
QC	Quality Control
TC	Total Coliform
ESBL	Extended spectrum beta lactamase
TT	Thermotolerant coliform
TSA	Tryptone soy agar
VRB	Violet red bile agar
WBI	Water borne illness
WHO	World Health Organization

Abstract

Background: Providing safe water, sanitation, and hygiene (WASH) services is a challenge in low-income countries.

Objective: This study aimed to assess the bacteriological quality of tap water, antimicrobial resistance profile of *Escherichia coli* and associated factors of tap water in Gulelle and Yeka sub-cities of Addis Ababa, Ethiopia.

Methods: A community-based cross-sectional study was conducted on 341 tap water samples collected from May 13 to July 26 2024. Sample culturing for the isolation of coliforms was carried out through the application of membrane filtration technique and aerobic plate count (APC) carried out by the pouring plate method. The coliforms were enumerated and presented in CFU/100 ml of the water sample and APC presented in CFU/ 100ml. The antimicrobial susceptibility testing was done using the Kirby–Bauer method on Mueller–Hinton Agar. The data were entered and analyzed using SPSS version 27, both bi-variable and multivariable logistic regression analyses were employed. A p-value of less than 0.05 was considered statistically significant.

Results: From a total 341 of tap water samples, 64 (18.8%), 50 (14.7%), 30 (8.8%), and 278 (81.5%) of them contaminated with total coliforms, thermotolerant, *E. coli*, and APC, respectively was indicate a contamination level. The isolated *E. coli* exhibited a significant level of antimicrobial resistance, with complete resistance observed to amoxicillin (100%), ampicillin (80%), and nitrofurantoin (70%). In contrast, all 30 *E. coli* were (100%) susceptible to ciprofloxacin and gentamicin, while 25 isolates (83.3%) demonstrated susceptibility to Tazobactam and chloramphenicol. Multi drug-resistances, amoxicillin (100%), ampicillin (80%), and nitrofurantoin (70%), were observed in *Escherichia coli***.** The contamination of tap water with *E. coli* was significantly linked to some factors, including the existence of damaged or leaking pipes (AOR: 9.9, 95% CI: 4.09-24.15), the close proximity of latrines to water sources (AOR: 7.19, 95% CI: 2.93-17.64), and inadequate waste management practices (AOR: 2.5, 95% CI: 1.02-6.3).

Conclusion and recommendation: In this research, the analysis of tap water samples revealed a significant level of contamination, indicating that the tap water was polluted at a rate of 64.7

CFU per 100 milliliters. This situation necessitates the implementation of water quality and antimicrobial resistance surveillance and monitoring.

Keywords: Antimicrobial resistance; bacteriological quality; *E. coli; tap* water; coliform.

1. Introduction

1.1. Background

Water is a natural resource and is essential to sustain life. Accessibility and availability of fresh clean water does not only play a crucial role in economic development and social welfare, but also it is an essential element in health, food production and poverty reduction [1]. safe drinking water is fundamental for the preservation of life and the enhancement of public health [2]. Contaminated water significantly contributes to the burden of disease, especially in developing countries, where waterborne pathogens are responsible for nearly 80% of health-related problems [3, 4]. The assurance of water quality necessitates comprehensive assessment and management, taking into account chemical, physical, and biological factors as outlined by the World Health Organization (WHO) and national regulatory bodies [5]. Despite international initiatives, such as the 2030 Agenda for Sustainable Development, the goal of providing universal access to safe drinking water continues to pose a considerable challenge, with more than 663 million individuals globally still deprived of this essential resource [6].

Drinking water standards are implemented to prevent harmful microbial and chemical contamination while enhancing its sensory and aesthetic qualities [7]. The microbiological quality of drinking water is determined by the presence of bacteria, viruses, and protozoa, many of which are responsible for diseases such as cholera, gastroenteritis, and diarrhea [8]. Among these, coliform bacteria, particularly *Escherichia coli* serve as primary indicators of fecal contamination. The detection of fecal *Streptococci* further substantiates pollution sources [7]. Microbiological assessments of drinking water help evaluate its hygienic conditions, ensuring compliance with safety standards. The primary sources of waterborne diseases include drinking water contaminated with human or animal waste. Historically, typhoid and cholera were among the earliest recognized waterborne illnesses and continue to pose significant public health threats worldwide. Enteric pathogens such as coliforms, *Salmonella* spp, *Vibrio* spp., and dysentery-causing agents contaminate water sources, often due to the discharge of untreated sewage into lakes and rivers [9, 10]. Regular monitoring of microbial contamination is crucial for maintaining safe drinking water supplies.

Water quality is influenced by multiple factors, including pollution, human activities, and natural phenomena [11]. In healthcare settings, access to clean water is vital for infection control, sanitation, and medical applications such as sterilization, intravenous fluid preparation, and food handling. Patients with compromised immune systems face heightened risks of nosocomial infections due to poor water quality [12]. Chlorination is the most widely used water disinfection method in many developing nations due to its affordability, effectiveness, and ease of monitoring [13]. Unlike some other disinfectants, chlorine provides residual protection, preventing microbial regrowth during storage and distribution. However, microbial resistance to disinfectants and antibiotics is an emerging concern [14].

Escherichia coli, a facultative anaerobe within the Enterobacteriaceae family, is commonly found in the intestines of warm-blooded mammals. While most strains are harmless, pathogenic variants such as *E. coli* O157:H7 pose serious health risks [15]. The ubiquitous presence of *E. coli* in sewage and its role as a fecal coliform highlight its importance as a water quality indicator [16]. The fecal coliforms serve as proxies for the potential presence of other fecal-origin pathogens, given their ease of cultivation and identification [17]. Antimicrobial resistance (AMR) has become a critical public health concern worldwide [18]. It not only threatens human health but also places a significant burden on healthcare systems and economic [19]. The isolation and antimicrobial susceptibility testing of fecal indicator bacteria from drinking water samples are increasingly vital [20]. Opportunistic *E. coli* strains and other indicator organisms can act as reservoirs for resistance genes, facilitating the transfer of resistance traits to pathogenic bacteria [21].

AMR significantly contributes to the increasing incidence of disease. In 2016, antimicrobial-resistant infections accounted for 40% of deaths among immune-compromised children under five. By 2017, this age group had experienced 5.4 million deaths, with 50% occurring in sub-Saharan Africa and 30% in Southern Asia[19]. In the United States alone, antibiotic-resistant infections affect more than 2.8 million individuals annually, resulting in approximately 35,000 deaths [22].

1.2. Statement of the Problem

Numerous individuals face challenges in obtaining safe drinking water. In contrast to the norm in Europe and North America, where households typically have access to clean and treated water, many developing nations do not share this privilege. The availability of both potable water and adequate sanitation facilities is severely restricted, resulting in a high incidence of waterborne diseases. At present, approximately 2.5 billion people do not have access to enhanced sanitation, and annually, over 1.5 million children succumb to illnesses related to diarrhea in Portugal [23]. The World Health Organization indicates that inadequate water quality, poor sanitation, and insufficient hygiene practices are responsible for 9.1% of the global disease burden and contribute to 6.3% of total deaths across the globe [24].

Diarrheal diseases are the most prevalent waterborne health issues, with an estimated 4.6 billion cases reported each year, resulting in around 2.2 million deaths. The overall health impacts of contaminated water, lack of proper sanitation, and inadequate hygiene are analyzed by examining a range of health outcomes, with a particular focus on diarrheal infections [24]. The risk factor is defined by faecal-oral transmission, which arises from a confluence of elements, including the consumption of contaminated tap water, inadequate water supply stemming from substandard hygiene practices, deficiencies in personal and domestic cleanliness, agricultural practices, contact with polluted water, and the lack of effective development and management of water resources or systems [25, 26].

The rise in disease prevalence is significantly influenced by the presence of antimicrobial-resistant bacteria and polluted water. *E. coli* in water bodies represents a major reservoir AMR, with a notably high rate of extended-spectrum beta-lactamase (ESBL) production [27]. Antimicrobial resistance in *Escherichia coli* represents a critical global challenge that affects both human and veterinary medicine. This bacterium is particularly adept at accumulating resistance genes, largely through horizontal gene transfer. The primary resistance mechanisms observed in *E. coli* include the acquisition of genes that encode ESBL, which render it resistant to broad-spectrum cephalosporins; carbapenemases, which provide resistance to carbapenems; 16S rRNA methylases, resulting in pan-resistance to aminoglycosides; and plasmid-mediated quinolone resistance (PMQR) genes, which confer resistance to fluoroquinolones [27].

The considerable discharge of fecal matter from infected individuals poses a serious threat to the contamination of wastewater systems as well as drinking water sources [28]. *Escherichia coli* was selected as the indicator bacterial species due to its prevalent application in assessing bacterial contamination in water and in examining trends related to antibiotic resistance [29]. While tap water is the principal source of drinking water for the community, there is a significant lack of detailed understanding concerning the degree of microbial contamination and the emergence of resistance [30].

Antimicrobial resistance (AMR) has now emerged as a chronic public health problem globally, with the forecast of 10 million deaths per year globally by 2050. AMR occurs when viruses, bacteria, fungi and parasites do not respond to antimicrobial treatments in humans and animals, thus allowing the survival of the microorganism within the host [31].

This research was conducted to evaluate Bacteriological quality, antimicrobial resistance profile of *Escherichia coli* and associated factors of tap water at Gulelle and Yeka sub cities of Addis Ababa, Ethiopia. Furthermore, the in light of the city's rapid population growth, infrastructure development, and the introduction of new water sources such as dams, springs, and wells, there is a notable absence of recent data regarding the quality and safety of drinking water. To fill this knowledge gap, the study analyzed water samples from two water sources, including household taps, across representative areas of selected sub-districts in Addis Ababa. The results offer essential insights into the microbial safety of the drinking water supply and the prevalence of antimicrobial-resistant *E. coli*, thereby contributing to public health protection.

1.3. Significance of the study

This study's findings contribute to a broader understanding of water handling practices within the local context. Analyzing microbial contamination levels in drinking water gives practical insights for public health authorities, quality assurance specialists, and policymakers. Evaluating the presence of these indicators microorganism in tap water is vital for recognizing the potential health threats associated with water consumption. Indicators such as *Escherichia coli* (*E. coli*) and thermotolerant can result in severe gastrointestinal illnesses in humans.

Moreover, this research empowers by providing a deeper comprehension of the microbial quality of the water. And it serves as a vital tool for raising awareness about preventing disease transmission through the consumption of tap water. Furthermore, this study contributes valuable global data that researchers can utilize to explore bacteriological quality, AMR and associated factors related to drinking tap water.

2. Literature Review

A 2017 study by *Bergeron et al*. in the United States investigated the quality of drinking water as well as the antimicrobial resistance of *Escherichia coli* and Enterobacteriaceae. *E. coli* isolates were widely susceptible to all the antibiotics used, but 35% of the isolates were intermediately resistant to neomycin, 24% of the isolates are totally or intermediately resistant to streptomycin, 2 isolates, 12%, are intermediately resistant to kanamycin, and 1 isolate, 6%, was intermediately resistant to tetracycline. There are 6 isolates, 40%, that were intermediately resistant to ampicillin, but 3 isolates, 19%, are intermediately resistant to ampicillin coupled with the β-lactamase inhibitor. Enterobacter cloacae isolates showed high amounts of total and intermediate resistance, with 10 isolates, 91%, showing resistance to ampicillin [32].

A 2020 study in the Eastern Indian Himalayas investigated the occurrence of antimicrobial-resistant Enterobacteriaceae pathogens in spring water used for drinking. The findings revealed that isolates of *Escherichia fergusonii* exhibits a resistance rate of 73.30% to ceftazidime. Additionally, *Escherichia coli* isolates demonstrated resistance to both ampicillin and amoxicillin, each at a rate of 66.66%, while *Klebsiella oxytoca* isolates shows a resistance of 66.66% to cefoxitin. In contrast, *Shigella flexneri* exhibits the lowest resistance rate of 5.55% to nofloxacin. The hierarchy of antibiotic resistance is observed in the following descending order: ampicillin, amoxicillin, cefoxitin, ceftazidime, streptomycin, amikacin, tetracycline, ciprofloxacin, norfloxacin, imipenem, and ofloxacin. Notably, bacterial isolates from East Sikkim displays resistance to the greatest number of antibiotics, and several multidrug-resistant (MDR) strains are identified [33].

A study conducted in 2017 investigated the occurrence of antibiotic-resistant *Escherichia coli* in drinking water sources within Hangzhou city, China, analyzing a total of 200 *E. coli* isolates. The findings reveal that 99 of these isolates (49.50%) demonstrated resistance to at least one of the 18 antibiotics assessed, resulting in an antibiotic resistance index of 0.13. Tetracycline (TE) is identified as the most commonly resisted antibiotic, with a resistance rate of 42.0%, followed by ampicillin (AM) at 29.0%, piperacillin (PIP) at 27%, trimethoprim/sulfamethoxazole (SXT) at 25.50%, and chloramphenicol (C) at 19.0%. Conversely, the isolates exhibit higher susceptibility to aztreonam (ATM) (94%), amoxicillin/clavulanate (AMC) (96.0%), ceftazidime (CAZ) (98.0%), and amikacin (AN) (99.0%). Furthermore, 48 isolates (24.0%) display multiple

resistances, with six isolates resistant to all antibiotic classes. A significant finding is that 75.51% of the multi-drug resistant *E. coli* (37 out of 49) are concurrently resistant to both tetracycline and β-lactams [34].

A cross-sectional investigation conducted in *Coleman B et al* 2012 in Canada reveals that a total of 129 individuals omit exposed to water contaminated with *E. coli* strains exhibiting resistance to one or more antibiotics. Among these, 112 individuals (87%) demonstrate resistance to tetracycline, 69 to sulfonamide, 44 to β-lactam, 44 to aminoglycoside, 13 to chloramphenicol, and five to a fluoroquinolone antibiotic. Notably, 60% (78 out of 129) of the participants consumed water contaminated with *E. coli* strains resistant to two or more antibiotic classes. Furthermore, of the rectal swabs analyzed, 357 out of 878 (41%) *E. coli* isolates are found to be resistant to at least one of the antibiotics under study. The highest resistance rates are observed for β-lactam, tetracycline, sulfonamide, and aminoglycoside antibiotics, while fluoroquinolones and chloramphenicol exhibit lower resistance rates [35].

A study conducted in 2019 in Urban Salvador, Brazil, reveal significant findings regarding the presence of fecal bacteria of human origin in water samples, with an average concentration of 2.1 × 10^5 copies of DNA per 100 mL, ranging from 0 to 7.6 × 10^5. Additionally, the research identified 40 isolates of Enterobacteriaceae that exhibit resistance to antibiotics such as ciprofloxacin, cefotaxime, and meropenem. Resistance is also noted against fluoroquinolones, aminoglycosides, trimethoprim/sulfamethoxazole, and extended-spectrum cephalosporins [36].

A study carried out in Zimbabwe in 2021 demonstrate that *E. coli* exhibit resistance to several antibiotics, including amoxicillin, ampicillin, cephalothin, oxytetracycline, nalidixic acid, and norfloxacin. However, the research indicates that all isolates are sensitive to amikacin and chloramphenicol. Additionally, *E. coli* demonstrates intermediate resistance to gentamicin, whereas the remaining two isolates showed susceptibility [37].

A cross-sectional study conducted in 2022 in Bule Hora Town, South Ethiopia, indicate that all isolates of Enterobacteriaceae exhibited complete resistance to amoxicillin and ampicillin. Resistance rates for *E. coli* are observed as follows: gentamicin at 25% (5 isolates), cotrimoxazole at 50% (10 isolates), chloramphenicol at 40% (8 isolates), ciprofloxacin at 35% (7 isolates), and tetracycline at 55% (11 isolates). Furthermore, analysis of drinking water samples from 60 homes indicate that 72.3% display resistance to five different antimicrobial agents,

while all samples demonstrate resistance to three specific agents: amoxicillin, ampicillin, and tetracycline [38].

In a cross-sectional study carried out in 2014 in Baher Dar, Ethiopia, it was found that 66.7% of *E. coli* strains are resistant to three or more antibiotics from a total of 140 water samples collected. Among the antibiotics tested, amoxicillin, ampicillin, ceftriaxone, and ciprofloxacin are highlighted [39].The mean resistance rates for *E. coli* isolates against ampicillin, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim are notably elevated, falling within the range of 15% to 30%. Resistance patterns are detected in all water samples, either singularly or in combination with other antibiotics [40, 41].

A study in Addis Ababa city, Ethiopia, shows a contamination rate of 10%. Consequently, total coliforms are detected in 204 samples, accounting for 7% of the total. The analysis shows that public faucets and reservoirs had the lowest total bacterial counts, whereas private wells had the highest. During the wet season, municipal tap water was found to be contaminated with both faecal and total coliforms, with a minor increase in overall coliforms observed during the rainy season [42].

3. Objectives

3.1. General objective

To assess the bacteriological quality, antimicrobial resistance profile of *Escherichia coli* and associated factors of tap water at selected sub cities of Addis Ababa, Ethiopia

3.2. Specific objective

- To assess the bacteriological quality of tap water by determining APC, TC, FC and *E. coli* enumeration in selected sub cities in Addis Ababa, Ethiopia.
- To determine the antimicrobial resistance pattern of *E. coli* isolated from tap water in selected sub cities in Addis Ababa, Ethiopia.
- To assess risk factors associated with tap water contamination in Gulelle and Yeka sub cities in Addis Ababa, Ethiopia.

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4. Materials and methods

4.1. Study area

Location

As the capital city of Ethiopia, Addis Ababa also holds the distinction of being Africa's diplomatic capital. Positioned centrally within the country, it is enveloped by mountains. The total area of the city is approximately 540 square kilometers, of which 18.2 square kilometers are classified as rural. The city is located at the base of the Entoto Mountains, which reach a height of 3,000 meters. Addis Ababa experiences a mild, Afro-Alpine climate and is situated at the coordinates of 9ºN and 38º45'E. Furthermore, it serves as the operational center for the African Union and various international organizations. The water supply for Gullelle, which borders Arada and Addis Ketema, utilizes a surface water source from Gufersa, there by serving as an appropriate representative for several sub-cities. Likewise, Yeka, situated near Bole, depends on the Legedadi deep well.

Population

As of 2025, the metro population of Addis Ababa is approximately 5.96 million, showing a 4.44% increase from 2024. The city is divided into 11 sub-cities and about 120 districts. Gulelle, located in the north, had a population of around 410,792 in 2016, making it the fourth most populous sub-city with 10 weredas and about 100,193 households. Yeka sub-city had an estimated 370,900 people and 90,463 households across 12 weredas, according to the 2016 Central Statistical Agency report [43].

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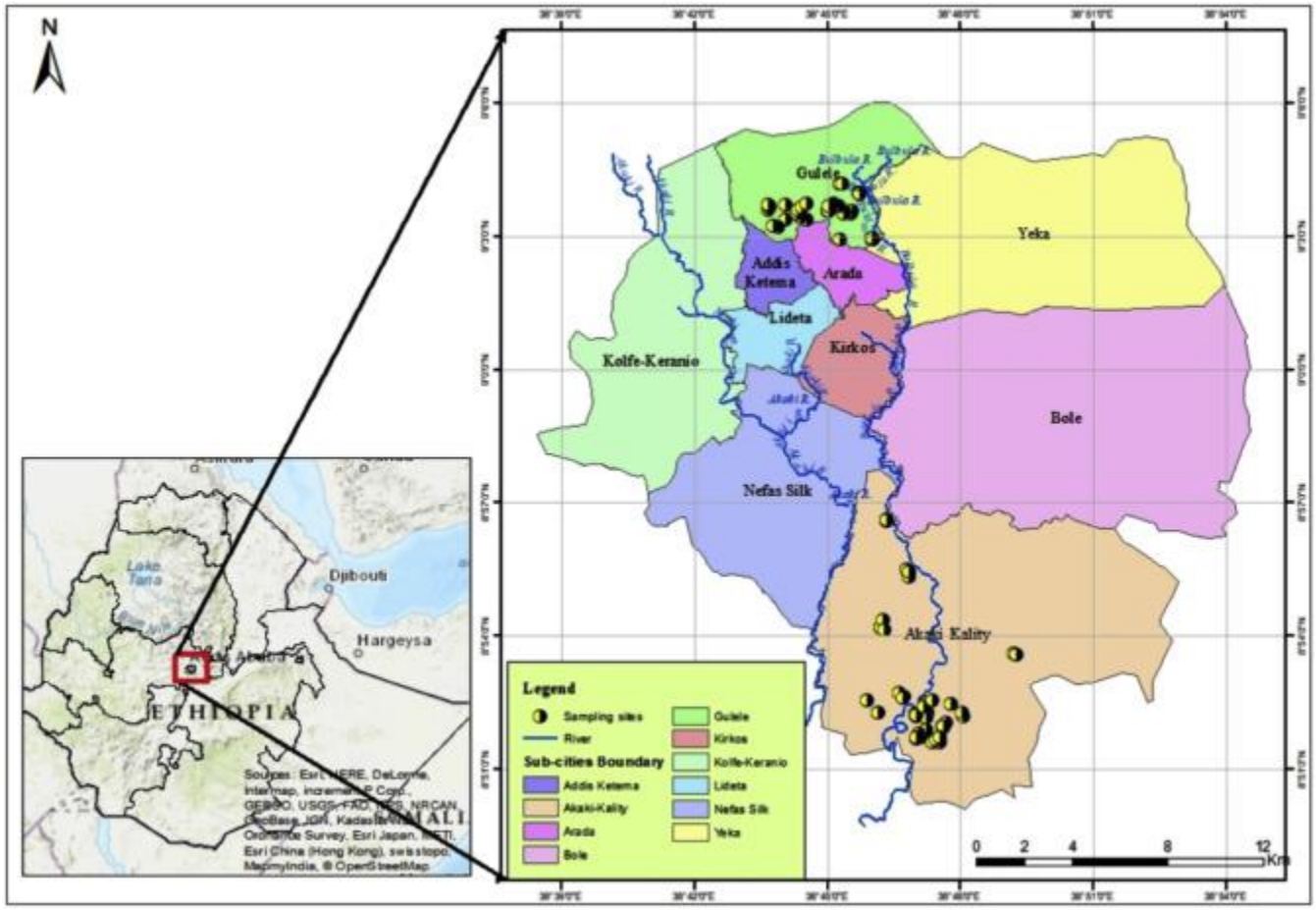

Figure 1. The map of study area [44].

4.2. Study design and period

A community-based cross-sectional study was conducted in two selected sub-cities, namely Gulelle and Yeka, from May to July 2024.

4.3. Population

4.3.1. Source population

All drinking tap water supplied for those selected sub- cities in Addis Ababa

4.3.2. Study Population

The study population was those who utilize tap water source and who fulfill the inclusion critrea in the selected sub city of Addis Ababa.

4.4. Eligibility criteria

4.4.1. Inclusion criteria

Each of the households in the study area utilized tap water exclusively was include in the study.

4.4.2. Exclusion criteria

Any type of drinking water source other than tap water such as packed water, reservoir, spring water.

4.5. Study variables

4.5.1. Dependent variables

- Bacteriological quality of tap water (determining Total coliform, thermotolerant and *E. coli*).
- Antimicrobial resistance pattern of *E. coli*

4.5.2. Independent variables

- Socio- demographic characteristics like, age, educational status of the household and children
- Tube or plastic lines quality of the materials
- Waste management practice
- Latrine distance from the pipe line
- Information on water handling practice

4.6. Sample size calculation and sampling techniques

4.6.1. Sample size calculation

It is determined by applying the following formula. The sample size (n) was determined using the formula. ($n = \frac{z^2 pq}{d^2}$) [45]. Due to a previous study, p= (0.33), q=1-p (0.67), and a Z-score with a 95% confidence interval of 1.96 was used. [46].

$$n = \frac{z^2 pq}{d^2}$$

$$n= (1.96)^2(0.333)\ (0.66.67)/\ (0.05)^2$$

$$n=341$$

4.6.2 Sampling techniques

The choice of Gulelle and Yeka sub-cities was selected based on lottery method. The calculated sample size was allocated for two sub-cities equally (171 samples from the Gulelle sub-city and 170 samples from the Yeka sub-city). The allocated sample size was distributed equally across 10 woredas in Gulelle and 12 in Yeka, resulting in the survey of 171 households in Gulelle (17 households per woreda) and 170 households in Yeka (14 households per woreda). Water sampling points were determined through a convenience sampling approach, which relied on house numbers. Data collection faced several challenges, including the unavailability of household representatives and inconsistent tap water supply; some households received water only once a week and stored it for periods extending up to a month. To mitigate these challenges, a second round of assessments was implemented, which facilitated a more accurate and thorough data collection process.

4.7. Data collection and Laboratory Method

4.7.1. Data collection procedure

In accordance with the guidelines established by the World Health Organization for the assessment of drinking water quality, water samples were collected aseptically in sterile containers for analysis. Tap opening was sterilized with a lighter or alcohol flame before

obtaining a sample for microbiological testing. During the collection procedure, the interior of the container and its lid were kept free from contact with fingers, clothing, or any unsterilized objects.

About 500 ml of water sample was obtained in Sterilized glass bottles. These samples were then transported to the Ethiopian Public Health Institute (EPHI) in a cool box within a 4-hour period. Any samples with residual chlorine were neutralized by adding 0.2 mL of sodium thiosulphate for every 100 mL of water. To minimize variations in microbial counts, the samples were preserved at temperatures between 2°C and 8°C in a dark setting until analysis, which was conducted within 6 hours of collection [47].

WHO sanitary observation checklist for drinking water sources was used to assess the contamination risks of water sources [48].

Sample flow chart procedure

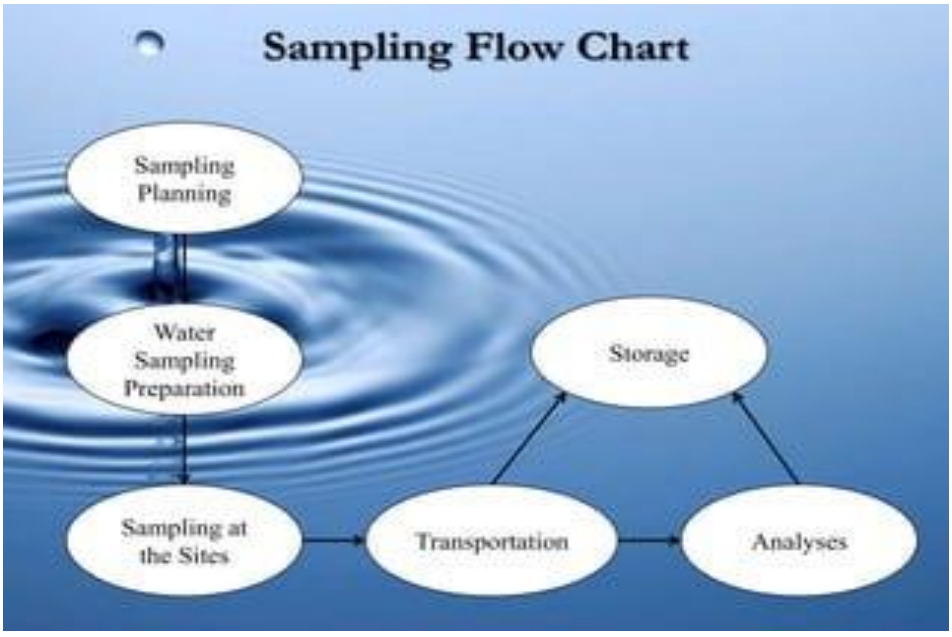

Figure 2. Sampling Flow Chart

4.7.2. Laboratory analysis

The Manual of Methods of Analysis, Microbiological Examination of Food and Water was used to perform microbiological examination of tap water [49].

Enumeration of Aerobic Colony Count (ACC)

To initiate the procedure, the sample bottle was gently inverted to achieve a uniform mixture. A volume of 1 ml from the sample was then dispensed into a sterile culture plate. Next, 20 ml of plate count agar (PCA) was introduced into the plate. The sample and PCA were mixed thoroughly and left at room temperature until the mixture solidified. The plates were incubated for 48 hours at 37 degrees Celsius, after which total viable counts were assessed using a digital colony counter [50].

Enumeration of Total Coliform (TC)

A water sample underwent filtration using a nitrocellulose membrane filter, which was specifically designed for the identification of total coliform and fecal coliform bacteria in water samples. This filter had a diameter of 47 mm and a pore size of 0.45 µm. The small pore size effectively captured bacterial cells present in the water during the filtration process. Following filtration, the retained microorganisms on the membrane is transferred to tryptosoya agar (TSA), which was placed in appropriately labeled petri dishes and pre-incubated for 1-2 hours at a temperature of 20-25°C (room temperature). After this initial incubation, the membranes were transferred to Violet Red Bile Lactose Agar plates, where they are incubated for 24 to 48 hours at 37°C for total coliform detection and at 44°C for thermotolerant detection. Organisms that fermented lactose rapidly produced pink colonies accompanied by a reddish-purple halo, while non-fermenters and late fermenters result in pale colonies. The pink colonies were subsequently counted using a colony counter machine [51].

The selected colonies were confirmed by conducting gas production tests in Brilliant Green Lactose Bile broth. A single colony of inoculum from each presumptive-positive violet and red bile agar petri dish were inoculated into tubes with 5 ml of Brilliant Green Lactose Bile broth, which contained inverted Durham tubes, and incubated at 37°C for 24 hours. The presence of gas in the Durham tubes was then evaluated. All broth tubes that exhibited gas formation were classified as positive for coliforms [51].

Enumeration of Thermotolerant (TT)

In the process of confirming fecal coliforms, five colonies were extracted from each presumptive-positive violet red bile agar petri dish. These colonies were inoculated into tubes

with 5 ml of EC broth, which contained inverted Durham tubes, and incubated at 44.5℃ for 24 to 48 hours. After 24 hours, the test tubes were examined for turbidity and gas production. Those with turbidity and gas production were taken as positive for thermotolerant [52].

Enumeration of *E. coli*

The methodology was continued from each positive E.C broth used in the identification of *Escherichia coli*. A single colony of inoculum was streaked in to nutrient broth, which was incubated at 44.5 ℃ for 24 hours. To confirm the presence of *E. coli*, a drop of Kovacs reagent was added to each tube. The tubes were examined for the emergence of a red ring, indicating a positive indole test and confirming the presence of *E. coli* [52].

Antimicrobial Susceptibility Patterns

Antibiotic susceptibility testing was performed on bacterial isolates utilizing the agar disc diffusion technique, as outlined by the Clinical and Laboratory Standards Institute (CLSI) 2023. A sterile wire loop was employed to select 3-5 pure colonies from tryptosoya agar (TSA), which were then emulsified in nutrient broth and gently mixed to achieve a uniform suspension. The turbidity of the suspension was subsequently adjusted to match the optical density of McFarland 0.5 standards to ensure a consistent inoculum size. A sterile cotton swab was then immersed in the suspension and used to evenly spread the bacterial suspension across the entire surface of Mueller-Hinton agar [53].

The antimicrobials utilized for disc diffusion testing were sourced from the antimicrobials recommended by the Clinical and Laboratory Standards Institute (CLSI) in 2023, with the following concentrations: Augmentin (30 µg), ampicillin (10 µg), amoxicillin (30 µg), chloramphenicol (30 µg), Tazobactam (30/10 µg), Cefepime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), gentamicin (10 µg), nitrofurantoin (300 µg), Meropenem (10 µg), ciprofloxacin (5 µg), tobramycin (10 µg), amikacin (30 µg), and tetracycline (30 µg).

Antibiotic discs were placed on the inoculated plates with the aid of sterile forceps and incubated at 36℃ for a time frame of 18 to 24 hours. After incubation, the diameters of the inhibition zones around the discs were measured to the nearest millimeter using a metal caliper, and the isolates were classified as sensitive, intermediate, or resistant following the standard protocol [53].

4.8. Data Quality Assurance

Standard Operating Procedures (SOPs) were adhered to in all procedures, like checking expiration dates and quality control parameters of media specified by CLSI. Systematic visual inspections were conducted to identify any cracks in the media or plastic petri dishes, variations in fill levels, hemolysis, and signs of freezing, bubble formation, and contamination.

The evaluation of the medium's quality using quality control (QC) procedures, with *E. coli* serving as a positive control. Moreover, site assessments and preliminary testing were carried out before data collection began. Data collection took place by trained personnel after the public health microbiology laboratory provided on-site training and orientation. Prior to data entry, the collected data were examined for completeness and representativeness.

4.9. Data interpretation and analysis

Data was entered into SPSS version 27 software for analysis. The analysis of factors associated with *E. coli* contamination of tap water was conducted using both bi-variable and multivariable logistic regression techniques. Variables that exhibited a p-value < 0.25 in the bivariate tests were deemed suitable candidates for inclusion in the multivariable analysis to account for potential confounding effects. Once the relevant variables were identified, the multivariable analysis was conducted, incorporating all selected variables. The Hosmer and Lemeshow goodness-of-fit test was employed to determine whether the assumptions necessary for multiple logistic regressions were satisfied, if p value > 0.05.

The adjusted odds ratios (AOR) and their respective 95% confidence intervals were calculated to assess the strength of the associations. All statistical tests were two-tailed, with a significance level set at $p < 0.05$. The findings are subsequently presented through narrative descriptions and descriptive statistics, including tables and figures.

4.10. Ethical considerations

Following an ethical review and subsequent approval by the Departmental Research and Ethics Evaluation Committee of the Addis Ababa University Department of Medical Laboratory Sciences, the Ethiopian Public Health Institution's institutional ethical review board issued an official approval letter to the Public Health Microbiology Laboratory department. Prior to the

collection of water samples, informed consent was secured. Throughout the duration of the study, all selected homeowners were evaluated, consulted, and made aware of the study's objectives. Their consent was sought for participation in the research.

4.11. Dissemination of the result

Upon completion of the research, the findings will be presented to the Department of Medical Laboratory Sciences at the College of Health Sciences, Addis Ababa University, as well as to St. Paul Specialized Hospital and other relevant organizations. Additionally, the results of the thesis will be submitted for publication in a national or international peer-reviewed journal.

4.12. Operational definitions

Tap water is water supplied through a tap, a water dispenser valve.

Bacteriological safety water: Water that has no detectable coliform (TC or EC) bacteria in 100mL of water sample.

Multi-drug resistance (MDR) refers to *Escherichia coli*, specifically *E. coli* strains that exhibit resistance to a minimum of one antimicrobial agent across three or more distinct categories [54].

Contamination level: Contamination level is defined based on bacterial counts in 100 mL of water as per WHO guidelines: [25].

Latrine distance- near latrine distance in which close to tap water (less than 5 meter) and far distance mean those latrine have a distance of > 5 meter [55].

Waste management – poor water management mean these have dirty around tap water and good water management mean those have clean area around tap water [56].

5. Results

5.1. Socio-demographic characteristics of respondents

The study examined a total of 341 households. The age distribution among the heads of these households was evenly distributed across all categories, the mean of age for the study participants were 37.3± 40.16. The substantial 271 (79.5 **%)** of female respondents may reflect the societal expectation that women are primarily responsible for water collection. A considerable proportion of household heads had achieved primary and higher education. Water collection was mainly performed by adult women. (Table1).

Table 1. Socio-demographic characteristics of respondents at selected sub-city of Addis Ababa, Ethiopia, 2024 (n = 341).

Characteristic	**Categories**	**Frequency (n)**	**Percentage (%)**
Age of household-head (years)	18-24	93	27.3**(%)**
	25-34	102	29.9**(%)**
	35-44	63	18.5**(%)**
	>45	80	23.5**(%)**
Sex of household-head	Female	271	79.5**(%)**
	Male	70	20.5**(%)**
Education level of household-head	No formal education	37	10.9**(%)**
	Primary school	112	32.8**(%)**
	Secondary school	69	20.2**(%)**
	Higher education	122	35.8**(%)**
No of family in the household	Less than 3	101	29.6**(%)**
	4-6	240	70.4**(%)**
Persons fetching water	Children's	59	17.3**(%)**
	Adult male	51	15.5**(%)**
	Adult women	231	67.8**(%)**
Do you have children	Yes	137	40.2**(%)**

	No	204	59.8**(%)**
The water line from the house	In-hose	294	86.2**(%)**
	Out-side house	47	13.8**(%)**

5.2. Bacteriological quality of tap water

The mean concentrations of total APC, TC, TT, and *E. coli* in tap water samples collected from the Gulelle and Yeka sub-cities of Addis Ababa were found to be 341.3 ± 392.6, 23.6 ± 71.8, 8.9 ± 31, and 4.9 ± 20.9, respectively. These findings indicate that the tap water is contaminated, as evidenced by the recorded levels of TC, APC, *E. coli*, and TT.

In relation to the occurrences of total coliforms (TC), analysis of 341 tap water samples revealed the presence of TC in 64 samples, representing 18.8% of the total. Among these, 2 samples (0.6%) were categorized as low risk, 32 samples (9.4%) as intermediate risk, and 30 samples (8.8%) as high risk. Furthermore, coliforms were identified in 50 samples, accounting for 14.7% of the total, with 43 samples (12.6%) classified as intermediate risk and 7 samples (2.1%) as high risk.

Additionally, our research indicated that *E. coli* was detected in 30 samples, which corresponds to 8.8% of the total. Within this group, 1 sample (0.3%) was classified as low risk, 24 samples (7%) as intermediate risk, and 5 samples (1.5%) as high risk. Moreover, aerobic plate count was found in 278 samples, representing 81.5% of the total tap water samples analyzed (Table 2).

Table 2: Level of bacterial contamination of tap water samples collected from Gulelle and Yeka sub city, Addis Ababa, Ethiopia, 2024 (n = 341).

Conformity with Standards* (CFU/100 ml)	**Categories**	**TT, F (%)**	***E. coli*, F (%)**	**TC, F (%)**	**APC**	
	0 (safe)	291 (85.3)	311 (91.2)	277 (81.2)	**Categories**	**F (%)**
	Bacteriological and Parasitological Quality	<1.0	1 (0.3)	2 (0.6)	≤ 100	63 (18.5)
	and Safety Assessment of Public Municipal	43 (12.6)	24 (7)	32 (9.4)		
	101-1000 (High risk)	7 (2.1)	5 (1.5)	30 (8.8)	>100	278 (81.5)
	>1000 (Very high)	<1.0	<1.0	<1.0		
Mean ±SD		8.9 ± 3.1	4.9 ± 20.9	23.6 ± 71.8	341.3 ± 392.6	
%CV		348.3	426.5	304.2	115	

Abbreviation: APC-aerobic plate count, TC-total coliform, TT- Thermotolerant and *E. Coli*-*Escherichia coli* classification Source: WHO (2011) WHO (2011): According to WHO (2011) Zero (0) CFU/100ml=Safe; 1 to 10 CFU/100ml = Reasonable Quality; 11-100CFU/100ml = Polluted; and >100CFU/100ml=Dangerous; N=Number of water samples.

Sub analysis of bacteriological quality of tap water

The examination of tap water samples collected from Gulelle sub-city revealed average concentrations of APC, TC, TT, and *E. coli* at 317.4 ± 406.1, 64.2 ± 37.1, 36.9 ± 11.6, and 27.3 ± 7.2, respectively. In addition, the tap water samples from Yeka sub-city exhibited mean concentrations of APC, TC, TT, and *E. coli* at 365.3 ± 378.3, 10.1 ± 32.8, 6.2 ± 23.5, and 2.6 ± 11.1, respectively. There was statically significant variation in the mean of total coliform and *E. coli* between two sub-cities (Table 3).

Table 3. Sub-analysis on the mean of APC, TC, TT, and *E. coli* (c.f.u/mL) of tap water samples collected from Gulelle and Yeka sub-city, Addis Ababa, Ethiopia, 2024 (n = 341).

Sub city	APC		TC		TT		*E. coli*	
	mean ±SD	**%CV**	**mean ±SD**	**%CV**	**mean ±SD**	**%CV**	**mean ±SD**	**%CV**
Gulelle	317.4± 406.1	128	37.1 ±94.2	254	11.6 ±36.9	318.1	7.2±27.3	379.1
Yeka	365.3± 378.3	103.5	10.1 ±32.8	324.7	6.2 ±23.5	391.6	2.6±11.1	427
P value	0.26		**<0.001**		0.11		**0.04**	

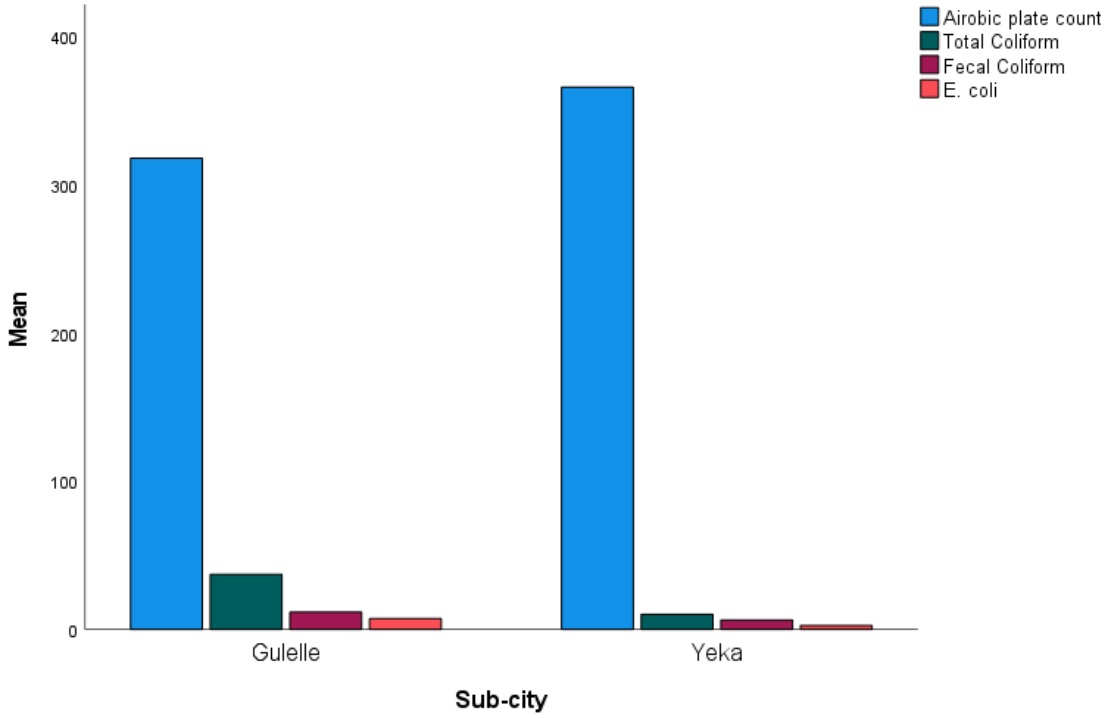

Figure 3. Sub-analysis of the concentration of APC, TC, TT, and *E. coli* (c.f.u/mL) of tap water samples collected from Gulelle and Yeka sub-city, Addis Ababa, Ethiopia, 2024 (n = 341).

5.3. Antimicrobial susceptibility pattern of *E. coli* isolates

The antimicrobial susceptibility testing results for the *E. coli* isolates were, involved 15 selected antimicrobial agents, as detailed in Table 5. Among the 30 *E. coli* isolates tested, all (100%) exhibited resistance to amoxicillin, while 24 isolates (80%) were resistant to ampicillin and 21

isolates (70%) to nitrofurantoin. Conversely, the isolate demonstrated all 30 isolates (100%) being susceptible to ciprofloxacin and gentamicin and 25 isolates (83.3%) showing susceptibility to tazobactam and chloramphenicol. Additionally, 26 isolates (86.7%) were found to be susceptible to meropenem (Table 4).

Table 4: Antibiotic resistance profiles of *E. coli* from tap water sources in selected sub-city in Addis Ababa, Ethiopia, 2024 (n = 30).

Antibiotics	***E. coli*** **Resistance** **N (F)**	***E. coli*** **Intermediate** **N (F)**	***E. coli*** **Sensitive** **N (F)**	**Zone diameter breaking point, mm**		
				S	**I**	**R**
Augmentin (30 µg)	17 (56.7%)	10(33.3%)	3(10%)	≥18	14-17	≤13
Amoxicillin (20/10 µg)	30 (100%)	0	0	≥18	14-17	≤13
Amikacin (30 µg)	0	10(33.3%)	20(66.7%)	≥17	15-16	≤14
Chloramphenicol (30 µg)	2 (6.7%)	3(10.0%)	25(83.3%)	≥18	13-17	≤12
Ceftriaxone (30 µg)	6 (20%)	6(20%)	23(60%)	≥23	20-22	≤19
Cefepime (30 µg)	2 (6.7%	6(20%)	22(73%)	≥25	19-24	≤18
Ceftazidime (30 µg)	3 (10%)	12(40%)	15(50%)	≥21	18-20	≤17
Nitrofurantoin (300 µg)	21 (70%)	0	9(30%)	≥17	15-16	≤14
Tobramycin (10 µg)	1 (3.3%)	6(20%)	23(76.7%)	≥15	13-14	≤12
Meropenem (10 µg)	1 (3.3%)	3(10%)	26(86.7%)	≥19	16-18	≤15
Tazobactam (30/10 µg)	0	5(16.7%)	25(83.3%)	≥21	17-20	≤16
Ciprofloxacin (5 µg)	0	0	30(100%)	≥26	22-25	≤21
Gentamicin (10 µg)	0	0	30(100%)	≥15	13-14	≤12
Tetracycline (30 µg)	9 (30%)	0	21(70%)	≥15	13-14	≤11
Ampicillin (10 µg)	24 (80%)	6(20%)	0(0%)	≥17	14-16	≤13

The *E. coli* strains isolated from tap water samples displayed multidrug resistance (MDR), showing complete resistance (100%) to amoxicillin, significant resistance (80%) to ampicillin, and moderate resistance (70%) to nitrofurantoin. Conversely, all isolates were entirely susceptible (100%) to ciprofloxacin and gentamicin, while 83.3% remained susceptible to tazobactam and chloramphenicol. These results underscore the existence of MDR *E. coli*, indicating resistance to at least three classes of antibiotics, which include β-lactams (amoxicillin, ampicillin) and nitrofurans (nitrofurantoin).

5.4. Factors associated with the presence of *E. coli* in the Tap water sources

Table 5 demonstrates that both bivariate and multivariable logistic regression analyses were utilized to identify the independent predictors of *E. coli* contamination in tap water. Findings from the multivariable logistic regression analysis demonstrated that factors such as damaged or leaking pipes, the distance of latrines from tap water sources, and the waste management system were statistically significant in relation to *E. coli* contamination of tap water, indicated by a p-value of less than 0.05 and a 95% confidence interval.

A study revealed that households with leaks or damaged pipes are 9.9 times more likely to have *E. coli* contaminated tap water compared to their counterparts (AOR: 9.9, 95% CI: 4.09-24.15). Furthermore, households with latrines positioned within 10 meters are 7.19 times more likely to have *E. coli* contaminated tap water compared to those with latrines located more than 10 meters away (AOR: 7.19, 95% CI: 2.93-17.64). In addition, households that practice poor waste management are 2.5 times more likely to have *E. coli* contaminated tap water than those that maintain good waste management practices (AOR: 2.5, 95% CI: 1.02-6.3).

Table 5. The associated factors related to *E. coli* contamination of tap water sources in selected sub-city in Addis Ababa, Ethiopia, 2024 (n = 30).

Parameters	**Category**	***E. coli*, N(F)**		**COR (95%CI)**	**P value**	**AOR (95%CI)**	**P value**
		Positive	**Negative**				
Leak or damage pipe	Yes	16 (53.3%)	35 (11.3%)	9.01 (4.05-20)	< 0.001	9.9 (4.09 – 24.15)	< 0.001
	No	14 (46.7%)	276 (88.7%)	1		1	
Cleaning of pipe before fetching water	Yes	6 (20%)	98 (31.5%)	1	0.197	1	0.209
	No	24 (80%)	213 (68.5%)	1.84 (0.73-4.65)		1.94 (0.69-5.43)	
Latrine distance from tap water	Near	20 (66.7%)	80 (25.7%)	5.78 (2.6-12.86)	< 0.001	7.19 (2.93-17.64)	< 0.001
	Far	10 (33.3%)	231 (74.3%)	1		1	
Waste management system	Poor	21 (70%)	157 (50.5%)	2.3 (1.02-5.16)	0.046	2.5 (1.02-6.3)	0.046
	Good	9 (30%)	154 (49.5%)	1		1	

Abbreviations: AOR, adjusted odds ratio; COR, crude odds ratio and 1 = reference groups.

6. Discussions

Access to clean drinking water is a fundamental human right and plays a vital role in health and well-being [57]. In our study, the average prevalence of bacterial indicator organisms in the tap water samples was found to be around 18.8% for total coliform, 14.7% for thermotolerant, and 8.8% for *E. coli*. In this study, the levels of total coliform, thermotolerant coliform, and *E. coli* in tap water samples were surpass the recommended limits set by the World Health Organization and national standards, which specify <1.0 CFU/100 mL of sample [58, 59].

The total contamination rate of TC in household tap water was found to be 18.8%, with 0.6% classified as low risk, 9.4% as intermediate risk, and 8.8% as high risk. Additionally, FC contamination in tap water was observed at a rate of 14.7%, with 12.6% categorized as intermediate risk and 2.1% as high risk. These findings align with studies conducted in Brazil and Baher Dar, Ethiopia, where the prevalence of total coliform in tap water was reported to be 16% and 24.4%, respectively [39, 60].

In contrast to our findings, a study carried out in Bahir Dar city reported a total coliform prevalence of 45.7% and a thermotolerant prevalence of 40% [61]. A national study in South Gondar also reported a total coliform prevalence of 50% [62]. In addition, a higher prevalence of faecal coliform was reported from studies done in the Gedeo Zone, Southern Ethiopia (50.2%), and South Darfur, Sudan [63, 64]. Furthermore, a study in Cajamarca, Peru, reported a total coliform prevalence of about 55.4% [65]. Such differences may stem from variations in sample sizes, the bacterial analysis methodologies employed, and seasonal factors.

The presence of *E. coli* is a more precise indicator of recent faecal pollution. It is essential that drinking water samples show no detection of *E. coli*. The objective of zero *E. coli* per 100 mL of water is a benchmark for all water supplies [66]. The current study, however, found that the overall prevalence of *E. coli* in tap water was 8.8%, with 0.3% falling into the low-risk category, 7% into the intermediate risk category, and 1.5% into the high-risk category. The higher prevalence of *E. coli* was seen at Gulelle sub city than Yeka sub city. The results of our study are comparable to those from Egypt, which reported an overall prevalence of *E. coli* at 5.3% [67].

Findings from studies conducted in Peru, India, Pakistan, as well as in Bule Hora, Bahir Dar, Arba Minch town, Shashemane, and Ghana, have demonstrated higher prevalence rates of *E.*

coli. The observed rates were 37.3%, 36.2%, 35%, 26.7%, 17.8%, 81.1%, 68%, and 79.6%, respectively [38, 39, 65, 68-72] The observed variation in the prevalence of *E. coli* in water samples might be explained by the differences in both the sample sizes and the water source types across the studies conducted.

This study investigated the antibiotic resistance (AMR) profiles of *E. coli* isolated from tap water sources. The results indicated that all *E. coli* isolates exhibited resistance to amoxicillin, with higher resistance levels also observed for ampicillin (80%) and nitrofurantoin (70%). On the other hand, the isolates demonstrated full susceptibility to ciprofloxacin and gentamicin. Additionally, a high rate of susceptibility was recorded for meropenem (86.7%), Tazobactam, and chloramphenicol (83.3%); cefepime (73%); and tetracycline (70%), suggesting a lower level of resistance to these antibiotics. Previous investigations have identified the occurrence of antibiotic-resistant bacteria in tap water sources [35, 73].

Our findings align with a study conducted in Baher Dar, which reported the isolate of *E. coli* exhibited higher resistance to ampicillin and amoxicillin, while lower resistance was noted for ciprofloxacin [39]. Additionally, a Brazilian study indicated that isolated strains of *E. coli* exhibited higher antimicrobial resistance to ampicillin and amoxicillin and lower resistance levels to gentamicin [35]. Moreover, a study in Nigeria also revealed higher resistance of *E. coli* to ampicillin and amoxicillin, alongside lower resistance against ciprofloxacin, chloramphenicol, and gentamicin [74].

A study conducted in Egypt reported that isolated *E. coli* strains from tap water exhibited a higher resistance to ampicillin while demonstrating lower resistance levels to ciprofloxacin, chloramphenicol, and tetracycline [75]. Further study carried out in Ghana has shown that *E. coli* isolates from tap water exhibited a higher resistance to nitrofurantoin, with a resistance rate of 65%. In comparison, the resistance to cefepime, meropenem, and piperacillin-tazobactam was considerably lower, at 12%, 3%, and 5%, respectively, thereby supporting our findings [76].

A study done in Peru revealed a low resistance level to gentamicin and tetracycline. In contrast to our findings, this study noted a low resistance rate to amoxicillin and ampicillin, recorded at 2.6% and 32%, respectively [65]. Additionally, the resistance of *E. coli* to ciprofloxacin in current study was lower than in a study from Bangladesh, which reported 100% resistance [77].

This study found that 80% of *E. coli* strains obtained from tap water exhibited resistance to at least three antibiotics tested. Similarly, investigations in India, Brazil, and Debre Tabor demonstrated that the rates of multi MDR *E. coli* strains found in tap water were 96%, 93%, and 80%, respectively [35, 68, 78]. On the other hand, current results were higher than those reported in studies from Ghana, China, and Peru, which indicated MDR rates of 48.2%, 24.5%, and 25%, respectively [34, 72, 79].

The differences in these results may stem from varying antimicrobial usage patterns, healthcare practices, surveillance systems, and sanitation conditions across different countries, which can significantly influence *E. coli* resistance levels. Moreover, the characteristics of the samples analyzed and their sources of water sample may also contribute to the observed differences [80, 81]. The excessive use of these antibiotics, along with improper disposal practices, may facilitate the emergence of resistance [82].

The multivariable logistic regression analysis revealed that *E. coli* contamination of tap water was associated with the presence of damaged or leaking pipes. The findings of this study align with a study conducted in Shashemane, Ethiopia and US, which identified leaks in water pipes as a significant contributor to higher levels of tap water contamination with *E. coli* [71, 83].

Current investigation revealed an association of proximity of latrines to water sources and poor waste management practices with contamination of tap water with *E. coli*. The same findings were reported from studies done in Nepal and Ethiopia, which found significant *E. coli* contamination in water sources located within 10 meters of latrines, particularly in areas with shallow water tables and poor waste management [84, 85]. Studies carried out in Indonesia and Zambia further illustrated that inadequate waste management and the presence of substandard latrines significantly contributed to *E. coli* contamination in tap water [86, 87].

To ensure the quality of tap water and promote community health, it is important to engage in regular monitoring and public health education focused on raising awareness about the prevention of bacterial contamination in tap water.

7. Strength and limitation of the study

This investigation sought to deliver an extensive evaluation of the bacteriological quality of tap water, however that successfully integrating the antimicrobial resistance patterns of *E. coli*.

The study primarily concentrated on the antimicrobial resistance (AMR) profile *of Escherichia coli*, limiting its scope to this specific microorganism. Consequently, the research did not assess the antimicrobial resistance patterns of other species.

8. Conclusion and recommendation

8.1. Conclusion

In conclusion, a substantial proportion of tap water samples failed to meet the World Health Organization's standards, which stipulate a coliform count of zero per 100 ml. This finding underscores the critical necessity for continuous monitoring and assessment of the microbial quality and safety of tap water throughout the year. The detection of *E. coli* in tap water was significantly associated with various factors, such as damaged or leaking pipes, the proximity of latrines to water sources, and inadequate waste management practices. The study revealed a high degree of contamination in tap water samples, with notable levels of *E. coli* and other coliforms surpassing WHO safety thresholds. Identified risk factors for contamination included compromised piping systems, the closeness of latrines to water sources, and ineffective waste management. Furthermore, the antimicrobial resistance (AMR) profile indicated multidrug resistance among *E. coli* isolates, highlighting the urgent need for regular water quality monitoring, enhanced sanitation practices, and regulated antibiotic usage to reduce public health risks.

8.2. Recommendation

- Our study recommends that, further water source-based studies to identify more the microbiological quality of the tap water.
- Leak or damage pipe should be inspected by the Addis Ababa Water & Sewerage Authority and users for immediate action as it contributes to tap water contamination.
- To prevent tap water contamination, a significant distance between the latrine and tap water source should be ensured.
- Effective waste management system should be in place to prevent bacterial contamination of tap water.
- It is recommended that additional efforts be made to identify antimicrobial-resistant bacteria species other than *E. coli*, employing more effective detection methods to tackle the potential human health threats posed by environmental exposure to these resistant bacteria.

- Continuous monitoring and surveillance of the microbial quality and safety of tap water is strongly advised.
- It is essential to provide health education and raise awareness regarding the appropriate use of tap water within the community to enhance public health and reduce the incidence of waterborne diseases.

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ANNEXES

Annex 1: English Version of Participant Information Sheet

Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences

Title: Assessment of the bacteriological quality of tap water, the antimicrobial resistance profile of *Escherichia coli* and associated factors at selected sub city of Addis Ababa, Ethiopia.

Introduction: This information sheet is prepared by the principal investigator to clarify the study that you are asked to take part in. If there is any uncertainty before you decide to participate or not.

Purpose: This study will primarily benefit drinking tap water users by providing a better understanding and awareness of water handling practices in today's world, as well as assessing the bacteriological quality of tap water and the antimicrobial resistance of *E. coli* and its correlation with contamination of tap water, with the ultimate goal of benefiting consumers by preventing disease spread through consumption in Addis Ababa, Ethiopia.

Confidentiality: Any information that we are going to collect about you during this research will be kept confidential. Your name and identity on the request paper will be changed to **Confidentiality code** for the purpose of this study. Samples and information given by the participants will serve only for this research not for any other purpose.

Benefit: There will be no direct benefit to you. However, the findings from this study will be utilized to improve to our society by managers, administrators, and policy makers. So, you are indirectly benefiting yourself, other patients and the society at large by involving into this quality related laboratory research. The finding will have benefits in ensuring a sustainable AAWSA Ethiopia.

Person to contact: Please direct any questions or problems you may encounter during this study to the principal

Investigator: Desalegn Fente, Department of Medical Laboratory

Tel: +251910535604, Email: fentedesalegn88@gmail.com

Annex II: Amharic Version of Participant Information Sheet

አዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይንስ ኮሌጅ፣ የሕክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል

ርዕስ፡- በኢትዮጵያ አዲስ አበባ ከተማ የቧንቧ ውሃ ባክቴሪያዊ ጥራት ግምገማ, *Escherichia coli* የፀረ ፀረ ባክቴሪያ መድኃኒት የመቋቋም ፕሮፌይል ና ተያያዥ ምክንያቶች ግምገማ ።

መግቢያ፡ ይህ የመረጃ ወረቀት የተዘጋጀው እርስዎ እንዲሳተፉበት የተጠየቁትን ጥናት ለማብራራት በዋና መርማሪው ነው። ለመሳተፍ ወይም ላለመሳተፍ ከመወሰንዎ በፊት ግልጽ ያልሆነ ነገር ካለ ይጠይቁ።

ዓላማው፡- ይህ ጥናት በዋነኛነት የመጠጥ ውሃ ተጠቃሚዎችን የውሃ አያያዝ የተሻለ ግንዛቤ እንዲኖራቸው እንዲሁም የቧንቧ ውሃ የባክቴሪያሎጂ ጥራት እና የኢ.ኮሊ ፀረ ተህዋሲያን መቋቋም እና ከቧንቧ ውሃ መበከል ጋር ያለውን ትስስር ለመገምገም የሚረዳ ይሆናል።

ምስጢራዊነት፡ በዚህ ጥናት ወቅት ስለእርስዎ የምንሰበስበው ማንኛውም መረጃ በሚስጥር ይጠበቃል። ለዚህ ጥናት ዓላማ በጥያቄ ወረቀቱ ላይ ያለው ስምዎ እና ማንነትዎ ወደ ሚስጥራዊነት ኮድ ይቀየራል። በተሳታፊዎች የተሰጡ ናሙናዎች እና መረጃዎች ለዚህ ምርምር ብቻ የሚያገለግሉት ለሌላ ዓላማ አይደለም።

ጥቅማ ጥቅሞች: ለእርስዎ ቀጥተኛ ጥቅም አይኖርም. ሆኖም፣ ከዚህ ጥናት የተገኙት ግኝቶች በአስተዳዳሪዎች፣ እና ፖሊሲ አውጪዎች ህብረተሰባችንን ለማሻሻል ይጠቅማሉ። ስለዚህ በዚህ ከጥራት ጋር የተያያዘ የላብራቶሪ ጥናት ውስጥ በመሳተፍ እራስዎን፣ ሌሎች ተገልጋዮችን እና ህብረተሰቡን በተዘዋዋሪ መንገድ የሚጠቀም ነው። ግኝቱ ቀጣይነት ያለው በአዲስ አበባ ውሃ እና ፍሳሽ ባለስልጣን (AAWSA) ኢትዮጵያን በማረጋገጥ ረገድ ፋይዳ ይኖረዋል።

ስለዚህ ጥናት ምንም አይነት ጥያቄ ካሎት በማንኛውም ጊዜ] ለመጠየቅ ነፃነት ሊሰማዎት ይገባል::

ተመራማሪ፡ ደሳለኝ ፈንቴ፣ የሕክምና ላቦራቶሪ ባለሙያ

ስልክ፡ +251910535604 ኢሜል፡ fentedesalegn88@gmail.com

Annex III: English Version of Informed Consent Form

I, the undersigned, confirm that, as I give consent to participate in the study, **I** have read the information sheet above and clearly understood the purpose and anticipated benefit of the research. I hereby assure with my signature below that I, without any coercion or forceful act by the researcher, have decided to voluntarily participate in the study to contribute my part by providing information on the assessment quality of water and antimicrobial resistance of *E. coli* and its correlation with contamination of tap water, Addis Ababa, Ethiopia**.** The aim of the study has been explained to me in the language I understand.

Unique ID No _____Signature _____ Date_____

Date of data collection _____Start time _____End Time_____ participant's Name_____ Signature_____

I thank you for your cooperation

Please direct any questions or problems you may encounter during this study to:

Desalegn Fente Kassa

Cell phone number +251910535604

E-mail. fentedesalegn88@gmail.com

Annex IV: Amharic Version of Informed Consent Form

እኔ፣ በስሩ የፈረምኩት፣ በጥናቱ ለመሳተፍ ፍቃድ ስሰጥ፣ ከላይ ያለውን የመረጃ ወረቀት እንዳነበብኩ እና የምርምሩ አላማ እና የተጠቀሰውን ጥቅም በግልፅ እንደተረዳሁት አረጋግጣለሁ። ከዚህ በታች ባለው በፊርማዬ አረጋግጣለሁ፣ በተመራማሪው በኩል ምንም አይነት አስገዳጅነት እና ሃይለኛ እርምጃ ሳይወስድ በጥናቱ ላይ በፈቃደኝነት ለመሳተፍ የውሃ ጥራት ግምገማ እና የኢ.ኮላይ ፀረ ተህዋሲያን መቋቋም እና ከቧንቧ ውሃ ብክለት ጋር ያለው ግንኙነት፣ አዲስ አበባ፣ ኢትዮጵያ። የጥናቱ አላማ በምረዳው ቋንቋ ተብራርቶልኛል።

ልዩ መታወቂያ ቁጥር _____ ፊርማ _____ ቀን_____

መረጃ የሚሰበሰብበት ቀን _____የመጀመሪያ ሰዓት ____ የማጠናቀቂያ ሰአት__የተሳታፊው ስም _____ ፊርማ_____

ስለ ትብብርዎ አመሰግናለሁ።

እባክዎን በዚህ ጥናት ወቅት ሊያጋጥሙዎት የሚችሉትን ማንኛውንም ጥያቄዎች ወይም ችግሮች ወደዚህ ያቅርቡ፡-

ደሳለኝ ፈንቴ ካሳ

የሞባይል ስልክ ቁጥር +251910535604

ኢ-ሜይል fentedesalegn88@gmail.com

Annex V: English Version of Questionnaires

Part I: Demographic characteristics of the respondent

Address:

City _____

Kebele _____

Village (Location) _____

1. Gender

☐ Male ☐ Female

2. Age: _____

3. Education situation

☐ No formal education

☐ Primary school

☐ Secondary school

☐ Higher education

4. Number of people in the family

A. Male _____
B. Female _____
C. Total_____

5. Do you have children?

☐ Yes ☐ No

Part two: Question related to tap water

6. Source of water supply near the house

☐ Tap water (In-hose)

☐ Bono's (Out-side house)

7. Who usually fetches water for your household?

☐ Children's

☐ Adult male

☐ Adult female

☐ If there is another

8. The cleanliness around the source of drinking tap water is

☐ Clean

☐ It's not clean at all.

9. The presence of damaged, broken or leakage in water pipes

☐ Yes ☐ No

10. The distance of the pipeline from the toilet is close?

☐ Yes ☐ No

11. Is there a practice of disposing of waste near tap water?

☐ Yes ☐ No

12. Do you have a habit of cleaning of pipe before fetching water?

☐ Yes ☐ NO

Thank you for your participation!!

Annex VI: Amharic Version of Questionnaires

ክፍል አንድ፡-የተጠያቂው የ ስነ -ህዘበ አወቃቀር ባህርያት በተመለከተ

አድራሻ:

ከፍለ ከተማ _____

ቀበሌ _____

መንደር (መገኛ ቦታ) _____

1. ጾታ

☐ ወንድ

☐ ሴት

2. ዕድሜ: ----------

3. የትምህርት ሁኔታ

☐ መደበኛ ትምህርት የለም

☐ የመጀመሪያ ደረጃ ትምህርት

☐ ሁለተኛ ደረጃ ትምህርት

☐ ከፍተኛ ትምህርት

4. በቤተሰብ ውሰጥ ያለ ሰው ብዛት:

ሀ) ወንድ _____

ለ) ሴት_____

ሐ) ድምር_____

5. ልጆች አሉዎት?

☐ አዎ

☐ አይ

ክፍል ሁለት፥ ከቧንቧ ውሃ ጋር የተያያዘ ጥያቄ

6. በቤት ዉሰጥ የትኛው አይነት የውሃ አቅርቦት አለ ?

ሀ) የቧንቧ ውሃ

ለ) የቦኖ

7. ብዙ ጊዘ ጊዜ ውሃውን ማን ነው የሚቀዳው ?

A. ልጆች

B. ውጣት ወንድ
C. ወጣት ሴት
D. ሌላ ካለ

8. በመጠጥ ውሃ ዙሪያ ያለው ንፅህና
A. ንጹህ
B. ጨርሶ ንጹህ አይደለም.

9. የውሃ ቱቦዉ የተበላሸ, የተሰበረ ወይም የማንጠባጠብ ባህሪ አለው?

☐ አዎ
☐ አይ

10. ከመጸዳጃ ቤት ያለው የቧንቧ መስመር ርቀት ቅርብ ነው?

☐ አዎ
☐ አይ

11. ከቧንቧ ውሃ አጠገብ ቆሻሻን የማስወገድ ልምድ አለ?

☐ አዎ
☐ አይ

12. ውሃ ከመቅዳትዎ በፊት ቧንቧን የማጽዳት ልምድ አሎት?

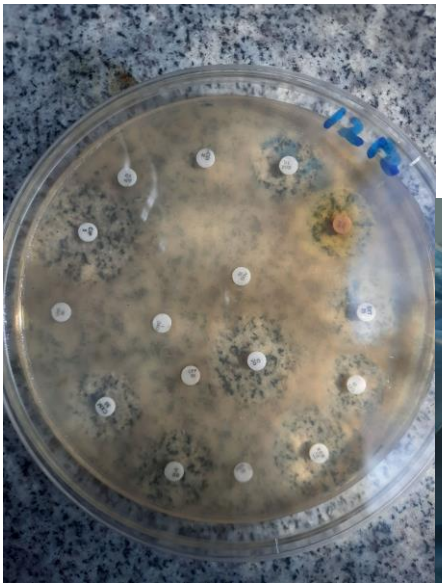
☐ አዎ
☐ አይ

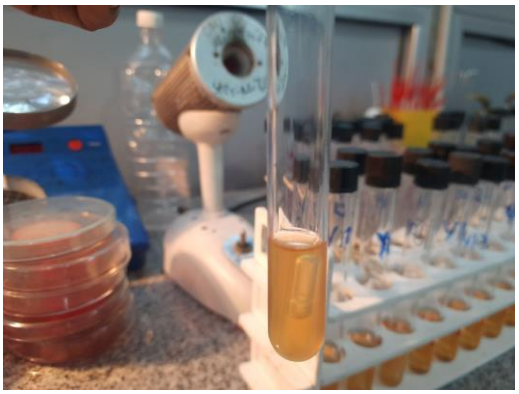
ስለ ተሳትፍዎ እናመሰግናለን !!

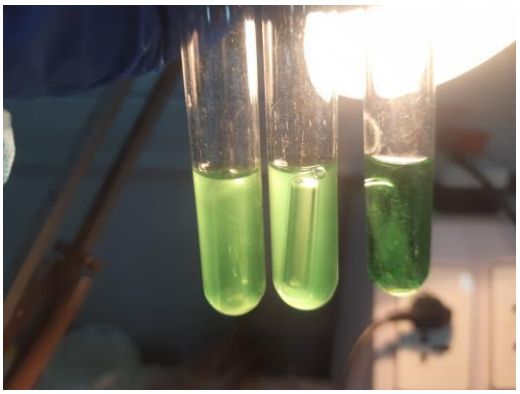
Annex VII: Laboratory work

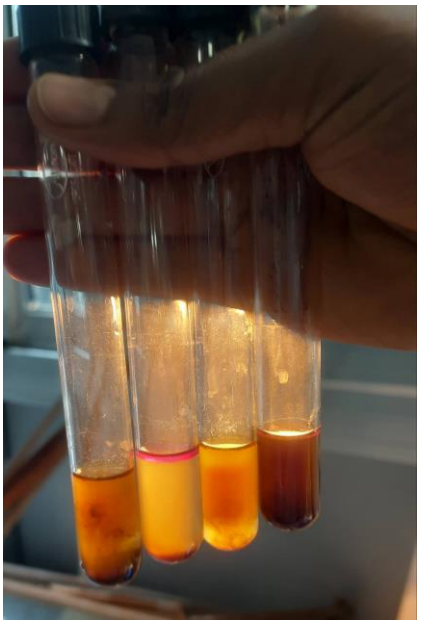
Part I. Sample collection


Part II. Microbiological examination








D3?

DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Desalegn Fente

Signature: _____

Date: _____/25_____

This thesis has been submitted with our approval as advisors.

Advisor: Dr. Melese Hailu (PHD)

Signature: _____

Date: _____/25_____

Place: Addis Ababa, Ethiopia.

Advisor: Mr. Dessie Abera (PHD fellow)

Signature: _____

Date: _____/25_____

Place: Addis Ababa, Ethiopia.