2023-06

Bacteriological and Physicochemical Quality and Antibiotic Resistance Profile of Bacteria Isolates from Drinking Water in Bahir Dar Zuriya Rural Kebeles, West Gojjam, Ethiopia

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A THESIS SUBMITTED TO THE DEPARTMENT OF BIOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF SCIENCE DEGREE IN BIOLOGY (APPLIEDE MICRO BIOLOGY)

BY

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JUNE, 2023

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APPROVAL SHEET

As a thesis research adviser, I hereby certify that I have read and evaluated this thesis prepared under my guidance, by Zinash Wessen entitled "**Bacteriological and physicochemical quality and antibiotic resistance profile of bacteria isolates from drinking water in Bahir Dar Zuriya Rural Kebele, west Gojjam, Ethiopia.** I recommend the paper to be submitted as fulfilling the requirement for the Degree of Master of Science in Biology (Applied Microbiology).

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As members of the board of examiners for the MSc thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Zinash Wesen and examined the candidate. We recommend the thesis to be accepted as fulfilling the requirements for the Degree of MSc in Biology.

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DECLARATION

I the undersigned, MSc. student declare that this thesis is my original work in partial fulfillment for the requirements for the degree of Master of Science in Applied Microbiology. All the sources of the materials used for this thesis and all people and institutions who gave support for thesis work are fully acknowledged.

Zinash Wessen	________________________	_________________
Student Name	Signature	Date

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ACKNOWLEDGEMENTS

First of all, I would like to thanks Bahir Dar University institution and Biology department to give me this chance. Next I would like to express my heartfelt thanks to my advisor Dr. Baye Sitotaw for his intellectual guidance; advice and follow- up from the beginning to completion of my thesis and direct/guide me in each step of my work by providing every information and material. I would like also to thank Mr Misganaw Liyew for their technical and material support during laboratory work. I would like to thank Bahir Dar Zuriya Rural community for give me appropriate information. I would like to thank my friends and colleagues who helped me to sample collection and the development of the thesis. Finally, my grateful thanks also all my family members for their financial, moral and ideal supporting and initiating to start this program.

LIST OF ACRONYMS AND ABBREVIATIONS

FC	Fecal Coliforms
FDRE	Federal Democratic Republic of Ethiopia
ISO	International Standard Organization
MOH	Ministry Of Health
MPN	Most Probability Number
SDG	Sustainable Development goal
SPSS	Statistical Package for Social Science
TC	Total Coliforms
TDS	Total Dissolves Solids
UNICEF	United Nations Children’s Emergency Fund
WHO	World Health Organization
WWAP	World Water Assessment Program

***ABSTRAC*T**

Drinking water must be free from pathogenic micro-organisms, chemical, physical contaminants and other pollutants that constitute a danger to a person's health. The objective of this study is to assess the bacteriological and physicochemical quality of drinking water in Bahir Dar Zuryia Rural District. A cross-sectional study was conducted from source of water and household container in Bahir Dar Zuryia Rural Kebele community from April 2022 to august 2022. Sixty water samples were collected from hand dug wells, river, hand pump and house hold containers from Bahir Dar Zuryia Rural Kebele and analyzed for total coliforms and fecal coliforms using the MPN method and the isolation of pathogens using selective medium for each pathogen. Antibacterial susceptibility profiling of the isolated bacteria was conducted on Mueller Hinton Agar, using a common Kirby-Bauer disk diffusion technique. The physicochemical parameters namely Temperature was measured by thermometer on site and pH was measured using a portable digital pH meter, Conductivity and Total Dissolved Solid (TDS) were measured by portable conductivity and TDS meter and Turbidity of the samples was determined using a turbidity meter in the laboratory. From a total of 60 samples, 91.6 % (55/60) and 40 % (24/60) of the drinking water samples were tested positive for Total Coliforms (TC) and Fecal Coliform respectively. From total pathogens, 16 (37.2%), 15 (34.8%) and 12 (27.9%) were positive for Pseudomonas spp. Salmonella spp. and Shigella spp. were found respectively. Most of isolated pathogen bacteria 97.6%% were resists at least one antibiotic and 62.8 % (27/43) bacteria were resist two and more than two antibiotics. The mean value of temperature, pH, conductivity, total dissolved solid and turbidity in all water samples were recorded 19.6^{0}c, 6.76, 327µs/cm, 198.5 mg/l and 8.81 NTU, respectively. Among physicochemical parameters, pH, Conductivity and total dissolved solid mostly in line with WHO standards but temperature and turbidity water sample were not fulfilling WHO and National Standard Guide line. In this study, there is high level of total and faecal contamination of drinking water in Bahir Dar Zuriya Rural Kebele. High risk scores found in the drinking water suggest poor source and household container protection, poor sanitation conditions and practices.

Key Words: antibiotics resistance, Bacteriological parameter, Drinking water quality physicochemical parameters

1. INTRODUCTION

1.1. Background of information

Water is a precious resource to humans and other living creatures and is required for the survival of life on Earth and evidenced by its many uses (drinking, cooking, washing, irrigation, and farming) (Rukeh *et al*., 2007). Access to safe drinking water and adequate sanitation is a fundamental human right and a basic component of public health. However, lack of access to these amenities is one of the key issues confronting developing countries in the twenty-first century (Adil *et al*., 2021). Drinking water must be free from pathogenic microorganisms, free from chemical, physical contaminants and other household uses that constitute a danger to a person's health. For a country to maintain optimal health and development there has to be a continuous supply of safe drinking water for its population (Miner *et al*., 2016).
Worldwide, 159 million people gathered drinking water from surface water sources directly, with 58% of those individuals living in sub-Saharan Africa (WHO, 2017). Delivering tap water to every rural household in many low-income nations, notably those in Africa, may be challenging. The Ethiopian government has been paying attention to the benefits of choosing improved water supplies for the community, including access to water closer to their homes, which improves their health status, saves them time and energy, and increases their productivity in their jobs and educational pursuits. The current generation of improved water sources may not always produce water of high quality. Chemicals and pathogenic microorganisms may contaminate it (Zinabu Alemu *et al*., 2015).

Drinking water is contaminated with chemical and biological pollutants at any point from the source all the way to the household container. Some chemicals and pathogens may contaminate the water at the source, during transportation, distribution, or handling of the water in households or at point of use. Consequently, millions of people are suffering from diseases related to water, sanitation, hygiene, such as diarrhea, skin diseases, and trachoma (Muhammed Yasin *et al*., 2015. Rich and poor, young and old, and residents of industrialized and developing nations are all susceptible to diarrheal disease; nevertheless, poverty and unhygienic living conditions are strongly correlated (Girmay Mekonen *et al*., 2020). The World Health Organization estimates that diarrheal disease causes four billion instances of diarrhea and 1.8 million deaths each year contributing up 4.1% of the total daily global disease burden (WHO, 2014). According to the aforementioned estimation, the use of contaminated drinking water, inadequate sanitation, and poor hygiene practices are responsible for 88% of the burden (Haseena *et al*., 2017). An estimated 50% of children in Ethiopia are undernourished, while 60 to 80% of communicable diseases are attributed to poor sanitation and hygiene facilities and restricted access to safe water. Children in the nation suffer from communicable diseases most frequently as a result of contaminated water and poor sanitation. Wastes from faulty sanitation, agricultural practices, and other activities that reach the water distribution networks are the main sources of water pollutants (Milkiyas Tabor *et al*., 2011). Regular assessment and monitoring of drinking water quality should be conducted in the chain from the source to the end user (household) storage. In order to prevent water-related diseases, it is necessary to preserve the water supply from contamination and to regularly monitor water sources. Continuous examination of water quality analysis based on enumeration of indicator organisms is among the methods of assessing the hygienic condition of water. Other crucial water quality metrics for drinking water are generally acknowledged to include physicochemical factors including turbidity, pH, temperature, nitrate, and others. According to Mengesha Admassu *et al*. (2004), these variables either have a direct impact on microbiological quality or have an impact on disinfection effectiveness and human health.

Studies in the different localities of Ethiopia have reported a very high level of coliform contamination in drinking water systems. For example, 100% in south Gonder of Wegeda town (Baye Sitotaw *et al*., 2021), again 100 % in North Wolo Zone of Kobo town (Baye Sitotaw and Molla Nigus, 2021), 89 % in Awi zone of Addis Kidam Town (Baye Sitotaw and Mulu Geremew, 2021), and 92.31% in West Gojam of Mecha district (Mekuanint Lewoyehu, 2021). As a result, acute diarrhea has been affecting under five children (Natnael *et al*., 2021; Mernie *et al*., 2022). Antibiotics are chemicals that either kill or inhibit the growth of bacteria by inhibiting the synthesis of proteins, nucleic acids, cell walls, and folic acid (Walsh, 2003). The persistence of antibiotic-resistant bacteria (ARB) in the aquatic environment due to the overuse and misuse of human and veterinary antibiotics is an issue of global concern. Abiotic factors such as disinfectants, chemical co-pollutants (e.g. metals and biocides), and physicochemical conditions and biotic factors such as bacterial adaptation and stress response induction can favor the spread of ARBs in drinking water (Sanganyado and Gwenzi, 2019). The current study focus on the bacteriological and physicochemical quality of drinking water from the water source and the household container, isolation of antibiotic resistance bacteria and hygiene and sanitation practices of the consumers in Bahir Dar Zuriya Rural Kebele.

1.2. Statement of the problem

Due to contamination from anthropogenic and natural phenomenon, it has been difficult to ensure the quality and safety of drinking water for the majority in poor setting nations. The major problem of the study of rural area is shortage of potable drinking water access and supply coverage, environmental management, uncontrolled liquid and solid waste disposal, agricultural and other activities that reach the networks that provide water (Belaynew Muche, 2016).
Water collected from sources with good microbial quality may become contaminated during storage in households (Dagnaw Tadesse *et al*., 2010; Metadel Adane *et al*., 2017) and when water is handled during storage in households, it may be subjected to further contamination (WHO, 2011). In rural community, majority of population use hand pump and other source of water. There is a shortage of water and people store water for a long time for drinking purpose. Sometime peoples find drinking water from one place to other place and water are contaminated by the pathogens during transporting and put long time.

Several factors can affect the physicochemical quality of drinking water. The major pollution sources are industrial wastes, improper sanitation, and agricultural and other activities (Milkiyas Tabor *et al*., 2011). The linkages between agriculture activity and wash are also crucial in rural areas (Usman *et al*., 2018). Increasing agricultural activity and agro-chemicals for example, herbicides, pesticides, inorganic fertilizers, etc. can be helped by irrigation and rainfall that flow into ground water sources. In Bahir Dar zuriya rural kebele, Groundwater is the primary source of water used for drinking purpose. Many studies have been conducted on assessment of bacteriological and physicochemical quality of drinking water, sanitation and hygiene (Mengesha Admassu *et al*., 2004; Milkiyas Tabor *et al*., 2011; Molla Gedefaw *et al*., 2015). However, the previous study does not show the presence of pathogenic bacteria and antibiotic resistance bacteria in Bahir Dar zuriya rural kebele. Therefore, this study was filled the information gap about bacteriological, physicochemical quality and antibiotics resistance bacteria of drinking water from its source and customers household container in Bahir Dar Zuriya Rural Kebele.

1.3. Objectives

1.3.1. General objective

- To assess bacteriological and physicochemical quality and antibiotic resistance profile of bacterial isolates from drinking water in Bahir Dar Zuriya Rural Kebele, West gojjam, Ethiopia.

1.3.2. Specific objectives

- ✓ To enumerate total and feacal coliforms in drinking water in rural community
- ✓ To isolate and identify pathogenic bacteria in drinking water
- ✓ To determine the antibiotic resistance profile of bacterial isolates
- ✓ To assess sanitary survey of drinking water in rural community
- ✓ To assess physicochemical quality of drinking water in rural community

1.4. Significant of the study

The current study was able to indicate the bacteriological and physicochemical quality and antibiotic resistance profile of bacterial isolates from drinking water in the study areas. It is useful in order to estimate the disease burden attributable to water, sanitation and hygiene. It shows the water quality status of the rural area drinking water supply from the WHO standards. It gives a hint or information to carry out water quality assessment (monitoring), planning, and water quality management for the respective body. The outcomes of this finding will be helpful to researchers by serving as a stand point and to go for further study.

2. LITERATURE RIVIEW

2.1. Drinking water

Water is an essential element of the environment, however due to both natural and human-caused activities, the quality of surface and groundwater has been declining for a long time. Hydrological, atmospheric, climatic, topographical, and lithological elements are examples of natural variables that affect the quality of water. Examples of anthropogenic activities that have a negative impact on water quality include mining, livestock raising, waste production and disposal (industrial, municipal, and agricultural), increased sediment run-off or soil erosion as a result of land-use change and heavy metal pollution (Uddin *et al*., 2021).

All people, regardless of nationality, religion, race, wealth, or creed, are entitled to basic human rights, including access to clean drinking water. Diseases including cholera, diarrhea, dysentery, and polio can spread because of contaminated drinking water and insufficient sanitation. Poor drinking water quality has an adverse effect on the health of consumers. At least 2 billion people worldwide were said to consume water that was tainted with feces (WHO, 2018). The situation has slightly improved as a result of many developing nations' recent commitment to reducing. However, the situation is far from ideal, especially in rural regions, and this somewhat improved condition can potentially be harmed by the growing demand for water and the decreased availability of water due to population expansion and economic development (Li and Qian, 2018). In order to achieve harmony between people, resources, and the environment, there is still a long way to go (Li *et al*., 2017). Thankfully, a lot of academics are working hard in the field of drinking water studies, and the situation has marginally improved.

In developing countries, like Ethiopia, have suffered from a lack of access to safe drinking water from improved sources and to adequate sanitation services (WHO, 2006). According to national and international guidelines, all individuals should have access to 50-100 liters of water per person per day, or a minimum of 20 liters (UNDP, 2006). Water is obtained from rivers, streams, shallow wells, springs, lakes, ponds, and rainfall in Ethiopia's rural areas and communities for human consumption, drinking, washing (bathing, laundry), and food preparation, among other uses. Water may be harmful to health and spread infections if it isn't made safe or treated for human consumption (Desalegn Amenu *et al*., 2013). Due to open field defecation practices, animal waste, and sewage system effluent, human excreta are the predominant source of pollutants in these water sources. As a result, the majority of rural villages rely on tainted or questionable water sources, putting residents at risk of contracting the water must be safe for drinking and other household uses. Drinking water must be free from pathogenic (disease-causing) micro-organisms (tiny living organisms that you can see only with a microscope), and free from chemical and physical contaminants that constitute a danger to a person's Health. Water must be within safe physical reach, in or near the home, school, or healthcare facility. It must also be free from colour and odour. According to the World Health Organization (WHO), the water source has to be within 1000 m of the home, and collection time should not exceed 30 minutes (UNDESA, 2014).

2.1.1. Source of drinking water contamination

Water pollution occurs when unwanted materials enter in to water, changes the quality of water and harmful to environment and human health (Briggs, 2003). Discharge of domestic and industrial effluent wastes, leakage from water tanks, marine dumping, radioactive waste and atmospheric deposition are major causes of water pollution. Heavy metals that disposed off and industrial waste can accumulate in lakes and river, proving harmful to humans and animals. Toxins in industrial waste are the major cause of immune suppression, reproductive failure and acute poisoning. Infectious diseases, like cholera, typhoid fever and other diseases gastroenteritis, diarrhea, vomiting, skin and kidney problem are spreading through polluted water (Khan and Ghouri, 2011. Human health is affected by the direct damage of plants and animal nutrition. Water pollutants are killing sea weeds, mollusks, marine birds, fishes, crustaceans and other sea organisms that serve as food for human. Insecticides like DDT concentration is increasing along the food chain. These insecticides are harmful for humans (Owa, 2013).

Major sources of water pollution

i. Domestic sewage

ii. Industrialization

iii. Population growth

iv. Pesticides and fertilizers

v. Plastics and polythene bags

vi. Urbanization

vii. Weak management system

It is reported that 75 to 80% water pollution is caused by the domestic sewage. Waste from the industries like, sugar, textile, electroplating, pesticides, pulp and paper are polluting the water (Kamble, 2014). Polluted river have intolerable smell and contains less flora and fauna. 80% of the world's population is facing threats to water security (Owa, 2013). Large amount of domestic sewage is drained in to river and most of the sewage is untreated. Domestic sewage contains toxicants, solid waste, plastic litters and bacterial contaminants and these toxic materials causes water pollution. Different industrial effluent that is drained in to river without treatment is the major cause of water pollution (Kamble, 2014). Hazardous material discharged from the industries is responsible for surface water and ground water contamination. Contaminant depends upon the nature of industries. Toxic metals enter in to water and reduced the quality of water. 25% pollution is caused by the industries and is more harmful (Desai and Vanitaben, 2014). Increasing population is creating many issues but it also plays negative role in polluting the water (Guo *et al*., 2012).

Increasing population leads to increase in solid waste generation. Solid and liquid waste is discharged in to rivers. Water is also contaminated by human excreta. In contaminated water, a large number of bacteria are also found which is harmful for human health. Government is incapable to supply essential needs to citizens because of increasing number of population. Sanitation facilities are more in urban areas than rural areas. Polythene bag and plastic waste is a major source of pollution. Waste is thrown away by putting it in to plastic bags. It is estimated that three core people of urban areas defecate in open. 77% people are using flush latrines and 8% are using pit latrines. Pesticides are used to kill bacteria, pest and different germs (Desai and Vanitaben, 2014).

Chemical containing pesticides are directly polluting the water and affect the quality of water. If pesticides are excess in amount or poorly managed then it would be hazardous for agriculture ecosystem. Only 60% fertilizers are used in the soil other chemicals leached in to soils polluting the water, cyanobacteria are rich in polluted water and excess phosphate run off leads to eutrophication. Residues of chemicals mix with river water due to flooding, heavy rainfall, excess irrigation and enter in the food chain. These chemicals are lethal for living organisms and many vegetables and fruits are contaminated with these chemicals (Kamble, 2014). Trace amounts of pharmaceutical in water also causes water pollution and it is dangerous to human health (Haseena *et al*., 2017)

2.1.2. Impact of Water Born Disease

There is a greater association between pollution and health problem. Disease causing microorganisms are known as pathogens and these pathogens are spreading disease directly among humans. Some pathogens are worldwide some are found in well-defined area. Many water borne diseases are spreading man to man (Halder and Islam, 2015). Heavy rainfall and floods are related to extreme weather and creating different diseases for developed and developing countries. 10% of the population depends on food and vegetables that are grown in contaminated water. Many waterborne infectious diseases are linked with fecal pollution of water sources and results in fecal-oral route of infection. Health risk associated with polluted water includes different diseases such as respiratory disease, cancer, diarrheal disease, neurological disorder and cardiovascular disease (Nel and Markotter, 2009).

Nitrogenous chemicals are responsible for cancer and blue baby syndrome. Mortality rate due to cancer is higher in rural areas than urban areas because urban inhabitants use treated water for drinking while rural people don't have facility of treated water and use unprocessed water. Poor people are at greater risk of disease due to improper sanitation, hygiene and water supply. Contaminated water has large negative effects in those women who are exposed to chemicals during pregnancy; it leads to the increased rate of low birth weight as a result fetal health is affected. Poor quality water destroys the crop production and infects our food which is hazardous for aquatic life and human life. Pollutants disturb the food chain and heavy metals, especially iron affects the respiratory system of fishes (Halder and Islam, 2015). An iron clog in to fish gills and it is lethal to fishes, when these fishes are eaten by human leads to the major health issue. Metal contaminated water leads to hair loss, liver cirrhosis, renal failure and neural disorder (Chowdhury *et al*., 2016).

2.2. Bacteriological quality of drinking water

Bacteriological water quality is defined in terms of the absence or presence of indicator organisms. Drinking water does not cause an infectious disease if it is free from indicator organisms (WHO, 2011).Access to safe drinking water is one of the basic human rights and is extremely important for health. For a country to maintain optimal health and development there has to be a continuous supply of safe drinking water to its population (Miner *et al*., 2016). However, most of the world's population lacks access to adequate and safe water (Dagnew Tadesse *et al*., 2010). According to WHO estimation, about 1.1 billion people globally drink unsafe water and the vast majority(88 %) of diarrheal disease reported across the globe is attributable to unsafe water, sanitation and hygiene (WHO, 2002).Furthermore, around 250 million infections each year, which results in 10–20 million deaths world-wide, occur due to water-borne diseases (Zamxaka *et al*.,2004). The wide spread of a number of diseases such as cholera, dysentery and *salmonellosis* are mainly due to the lack of safe drinking water and adequate sanitation that ends up in death of millions of people in developing countries every year. Diarrhea is the major cause for the death of more than 2 million people per year world-wide, majority of which are children aged less than 5 years (WHO, 2002).

Majority of Ethiopia population does not have access to safe and reliable sanitation facilities besides insufficient hygienic practices related to food, water and personal hygiene. Accordingly, more than 75 % of the health problems in Ethiopia were due to infectious diseases attributed to unsafe and inadequate water supply, and unhygienic waste management, with human excreta being the major problem (WWAP, 2004). The World Health Organization (WHO) of 2017 guidelines for drinking water and quality drinking water standards for Ethiopia recommend that coliform bacteria must not be detectable in any 100 ml sample of all water directly intended for drinking. Faecal contamination of drinking water is a major problem in both urban and rural communities of Ethiopia, where surface water sources like rivers, wells and lakes are used for drinking (Gobena *et al*., 2017; Moe and Rheingan, 2006).

2.3. Indicator microorganisms

2.3.1. Total coliform bacteria

Total coliform bacteria are a diverse group of Gram-negative, non-spore forming, aerobic, and facultative anaerobic bacteria that can thrive in the presence of moderately high bile salt concentrations and ferment lactose to produce acid or aldehyde within 24 hours at 35–37 °C (WHO, 2006). *Escherichia coli* and thermo tolerant coliforms are a subset of the total coliform group that can ferment lactose at higher temperatures. Total coliforms produce the enzyme b-galactosidase as part of the lactose fermentation process. *Escherichia, Citrobacter*, *Klebsiella*, and *Enterobacter* were once thought to be the only genera that coliform bacteria belonged to; however, the category is now more diverse and includes genera like Serratia and Hafnia. Both faeces and environmental species are included in the total coliform group (Ashbolt *et al.*, 2001).

Total coliforms are generally measured in 100-ml samples of water. Based on the creation of acid from lactose or the manufacture of the enzyme b-galactosidase, a number of reasonably straightforward techniques are available. The procedures include membrane filtration followed by incubation of the membranes on selective media at 35–37 °C and counting of colonies after 24 h. Alternative techniques include P/A testing, most probable number procedures, and microliter plate or tube procedures, Field test kits are available (WHO, 2006).

According to WHO microbiological criteria (WHO, 2004), Coliform bacteria must not be found in 100 ml samples of water for the water to be certified safe; their presence in water indicates harmful bacterial contamination (Chalchisa *et al*., 2017). Several studies on the bacteriological quality of drinking water in Ethiopia's various regions have been conducted and get high amount of total and feacal coliform. According to Dessalegn Amenu *et al*. (2013), the average counts of TC were 1.5-133.05 CFU/100ml, and the average counts of FC were 0.34- 54 CFU/100ml. The TC and FC were both above WHO recommended limits for drinking water quality (1-10CFU/100ml for TC, 0CFU/100ml for FC) in all samples. This is due to a lack of effective water treatment, poor water handling methods, and inadequate water source protection. As a result, water source protection combined with sanitation and hygiene promotion programs can improve the quality of rural water supplies. According to Milkiyas Tabor *et al*. (2011), 77% of the drinking water samples in Bahir Dar City (northwest Ethiopia) had high-risk scores and tested positive for total coliform levels.

2.3.2. *Escherichia coli* and thermotolerant coliform bacteria

Escherichia coli are the predominant member of the facultative anaerobic portion of the human colonic normal flora The bacterium's only known natural habitat is the large intestine of warm-blooded animals, and since *E. coli*, with a few notable exceptions, typically does not survive well outside of the intestinal tract, its presence in environmental samples, food, or water typically indicates recent faecal contamination or subpar sanitation practices in food-processing facilities. The population of *E. coli* in these samples is influenced by the extent of faecal pollution, lack of hygienic practices, and storage conditions (Krieg *et al*., 1984). Even if the simple presence of *E. coli* in food or water does not explicitly mean that pathogenic germs are present, it does suggest that there is a higher probability of their presence of other faecal-borne bacteria and viruses, many of which, such as *Salmonella* spp. or hepatitis A virus, are pathogenic (Brüssow *et al*., 1993). For this reason, *E. coli* is widely used as an indicator organism to identify food and water samples that may contain unacceptable levels of fecal contamination (Atlas *et al*., 1993).

Escherichia coli is considered a more specific indicator of fecal contamination than fecal coliforms since the more general test for fecal coliforms also detects thermotolerant non-fecal coliform bacteria (Francy *et al*., 2013). The *E. coli* test advised by the US Environmental Protection Agency (EPA) checks for the absence of an enzyme that is specific for the *E. coli* organism to confirm presumptive faecal coliforms. This test separates *E. coli* from non-fecal thermotolerant coliforms. *E. coli* analysis is a contemporary approach that has been used as an indicator organism because it provides conclusive evidence of recent faecal contamination more specific, and used to estimate disease risk. World health organization recommends zero *E. coli* per 100 ml of drinking water (Genet Gedamu and Desta Haftu, 2017; WHO, 2012).

2.4. Bacterial pathogens

According to Dzwairo *et al*. (2006), waterborne pathogens worldwide infect 250 million people annually with diseases that cause 10 to 20 million fatalities (Dzwairo *et al*., 2006). These pathogens continue to occur as outbreaks and contribute to 80% of health problems in developing countries (Jyana *et al*., 2009). Ethiopia is one of the developing countries where only 57 and 28% of its population have access to safe water and sanitation coverage respectively. A total of 60–80% of the population suffers from waterborne and water-related diseases. This places a significant financial and social burden on the country with such a large number of people suffering from these devastating diseases (WHO, 2015).

The majority of bacterial infections that could spread through water affects the digestive system and is expelled in the faeces of infected people and other animals. However, there are also some waterborne bacterial pathogens, such as *Legionella*, *Burkholderiapseudomallei* and atypical mycobacteria that can grow in water and soil. The routes of transmission of these bacteria include inhalation and contact (bathing), with infections occurring in the respiratory tract, in skin lesions or in the brain (WHO, 2008). In Arba-Minch town, evaluation of the tap water supply and distribution systems revealed that the distribution lines were infected with waterborne Bacteria (WBB) like *Salmonella* and *Shigella* (Ameya *et al*., 2018).

2.4.1. *Salmonella* spp.

Salmonella is a natural inhabitant in the gastrointestinal tract of many animals, including birds, reptiles, livestock, and humans (Whiley *et al*., 2017). Salmonellosis caused by non typhoidal *Salmonella* ranks among the highest in all gastroenteritis cases linked to food consumption, affecting the health of approximately one million people annually in the United States alone, resulting in medical costs of $3.7 billion (Brandl *et al*., 2013). It is estimated that Salmonella species causes 93.8 million cases of gastroenteritis worldwide annually with 155,000 deaths (Majowic *et al.*, 2010). The causative source for salmonellosis has traditionally been attributed to animal origin (Sivapalasingam *et al*., 2004; Hintz *et al*., 2010).

2.4.2. *Shigella* spp.

A number of large waterborne outbreaks of shigellosis have been recorded. As the organisms are not particularly stable in water environments, their presence in drinking-water indicates recent human faecal pollution. Available data on prevalence in water supplies may be an underestimate, because detection techniques generally used can have a relatively low sensitivity and reliability. The control of *Shigella* spp. in drinking-water supplies is of special public health importance in view of the severity of the disease caused. *Shigella* spp. is relatively sensitive to disinfection. Within a WSP, control measures that can be applied to manage potential risk include protection of raw water supplies from human waste, adequate treatment and protection of water during distribution. *Escherichia coli* (or, alternatively, thermo tolerant coliforms) is a generally reliable index for *Shigella* spp. in drinking-water supplies (Alamanos *et al*., 2000; Pegram *et al*., 1998).

2.5. Antibiotics resistance

The prevalence of antimicrobial-resistant pathogens, including water borne antibiotic-resistant bacteria, is ever increasing. The widespread emergence of antibiotics resistance bacteria has become one of the grimmest challenges in low-income countries including Ethiopia resulting from irrational antibiotic consumption, prescription without susceptibility test, self-medication, and prolonged hospitalization (Tamiru *et al*., 2017). Some experimental research and monitoring program in Ethiopia revealed that *Salmonella*, *Shigella*, and *E. coli* species become resistant to regularly prescribed antibiotics (Feleke Moges *et al*., 2014). A better source of drinking water is used by 54% of households in Ethiopia. The term "emerging risks" refers to dangers and issues that are developing as a result of environmental changes.

Aquatic habitats are known to be a reservoir for antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB), which are serious public health issues, around the world (Baquero *et al*., 2008). Antibiotics from sewage and agricultural runoff, which are a result of the extensive and growing use of antibiotics, are selected for and enriched for by naturally existing ARB and ARGs in the aquatic environment (Baquero *et al*., 2008; Zhang *et al*., 2009). The level of amoxicillin, rifampin, and chloramphenicol resistance increased dramatically even though the bacterial concentration was really reduced after the water was treated. Chlorine, a disinfectant that is frequently employed, is found to favour ARB, according to several researches. The proportion of multidrug-resistant (MAR) bacteria significantly increased after flash mixing with chlorine, according to Siedlecka *et al*. (2020) research. Adefisoye and Olaniran (2022) showed that chlorination of sewage significantly increased the proportion of bacteria resistant to ampicillin and cephalothin (cefalotin), and they also observed a significantly increased proportion of MAR strains during chlorination in laboratory experiments. Other investigations showed a correlation between the susceptibility of ARB to a disinfectant and the susceptibility of bacteria that are susceptible to antibiotics (Fraise, 2002), proving that disinfection does not favor ARB but rather promotes the emergence of antibiotic resistance. Untreated water obtained from springs, hand dug wells, rivers and boreholes poses a substantial risk of human exposures to ARB and ARGs in developing nations study in the US isolated Enterococcus spp. resistant to erythromycin, clindamycin, tetracycline and vancomycin in groundwater samples downstream and upstream of swine concentrated feeding operations (Sapkota *et al*., 2007).

2.6. Sanitary survey status of drinking water

Access to water is a requirement for both health and subsistence, which was why The Millennium Development Goal aim is defined in terms of sustainable access to an affordable supply of drinking water. It is commonly acknowledged that the advancement of social, economic, and human rights depends on the provision of facilities for better and high-quality water supply and sanitation. For the purpose of promoting health and preventing disease, environmental sanitation is crucial. It is described in terms of personal hygiene, restrooms, and the surrounding area. Sanitation and hygiene practices have little impact on the availability of water (Water Aid, 2009). Effective water and sanitation practices can help people be more resistant to the potential risks of water-borne diseases. These actions include safe water storage and piping, instruction on hygienic behavior, and sanitary sewage disposal (WHO, 2017). Pit latrines, when utilized by adults and for the disposal of newborn faeces, have been shown to reduce diarrhoea by 36% or more, cholera by 66%, and worm infestations by between 12 and 86%, according to the FDRE MOH (2005). Diarrheal illness can be decreased by 35% or more by washing hands with soap (or a replacement) and water after coming into contact with stools. Increased body and face cleansing can help prevent eye and skin infections. The prevalence of diarrhoea often declines by 15% when the water supply is improved.

2.7. Physicochemical quality of drinking water

Water for human consumption must be free from living and non-living organisms, toxic elements and chemical substances in concentration large enough to affect health. The addition of various kinds of pollutants through sewage, industrial effluents, agricultural runoff, etc., into the water main stream brings about a series of changes in the physicochemical characteristics of the water, which have been the subject of several investigations (Bernard; Ayeni, 2012). Likewise, human activities are a major factor that determines the quality of surface waters directly and indirectly by atmospheric pollution, effluent discharges and agricultural practice (Sillanpää *et al.*, 2004).Hence, water, which infiltrates through the soil and accumulates in underground aquifers and this water have had lengthy exposure to calcium carbonate and sulfate are typically hard and alkaline (Gunten, 2003).

Many chemicals found in drinking water sources may be the cause of adverse human health effects, affect the acceptability of water and lower the effectiveness of water treatment. The health impacts related to chemicals in drinking water are mainly those that cause adverse effects after long term exposure. The severity of this health effect depends upon the chemical; and its concentration, as well as the length of exposure. There are only a few chemicals that can lead to health problems after a single exposure, except through massive accidental contamination of drinking water supply (WHO, 2006). The main problems associated with chemical components of drinking water arise primarily from their ability to cause adverse health effects after prolonged periods of exposure, especially in the developing countries can be traced to lack of safe and wholesome water supply (WHO, 2004). Then, the health impact associated with chemical elements of drinking water differs from microbial contamination, which arises from prolonged exposure to chemicals.

On the other hand, some chemicals in drinking water could be beneficial or detrimental health effects depending on its concentration and total amount ingested. And yet, there is some evidence that magnesium can have protective effects against heart diseases or inverse relation with cardiovascular diseases in general. The important effects of magnesium on humans among the numerous study variables involved in the water story are that Mg appears preeminent. Also, its importance is both quantitative and qualitative intakes of water; magnesium may palliate an "absolute" Mg deficit and its multiple consequences, particularly on the nephron-cardio vascular apparatus (Durlach *et al*., 1989). However, if the concentration of sodium exceeds from the recommended amount, it may cause to increase blood pressure (FDEP, 2014). Though recent findings suggest that high sodium intake could result in high blood pressure (hypertension) that causes cardiovascular disease, stroke, and coronary heart disease, and mortality. Reducing salt intake lowers blood pressure and also reduces the incidence of cardiovascular diseases (Geleijnse *et al*., 2003; Bochud *et al*., 1989; WHO, 2012).

2.7.1. Temperature

Temperature is the main factor which affects almost all chemical and biological reactions (Delpa *et al*., 2009). It can influence the pH, dissolved oxygen, redox potential and microbial activity (Park *et al*., 2010). Higher temperature can favor the growth of microorganisms and encourage the biofilm formation in the distribution and storages containers which could lead to environmental reservoir for pathogenic microorganism (Wingender and Flemming, 2011).

Cool water is generally more palatable than warm water and temperature will impact on the acceptability of a number of other inorganic constituents and chemical contaminants that may affect taste. High water temperature enhances the growth of microorganisms and may increase taste, odour, colour and corrosion problems (WHO, 2006).

The elevated temperatures generally result from the enhanced microbial activities, which feed upon a high load of solid waste, mostly organic ones (Khapekar *et al*., 2008; Singh and Dey, 2014). The temperature affects microbial growth and other vital water attributes (Sakyi and Asare, 2012). The studies have revealed that any increase in temperature directly affects the rate of chemical reactions (Akhigbe *et al*., 2018), and every degree (◦C) rise in temperature significantly affects the quality of biochemical reactions (Nartey *et al*., 2012). The high temperature of water enhances the pace of chemical processes and reduces the solubility of CO_2, O_2 and NH3 (Akhigbe *et al*., 2018). The high temperatures also intensify respiration rates, thus augmenting O_2 utilization and putrefaction of organic matter (Peirce *et al*., 1998). Various anthropogenic activities such as the dumping-off wastes generated from the commercial, household and industrial units into our aquatic ecosystems add high organic matter content (Guerrero *et al*., 2013) together with other associated pollutants. Bacterial and phytoplankton growth double in warm environs instantly in a short period of time, as water temperature also influences the physicochemical and biological characteristics of freshwaters. The temperature of water at the same time regulates the metabolic and reproductive behavior of aquatic organisms. Any rise in temperature boosts metabolic activities in aquatic organisms (Crawford *et al*., 2005; Ho and Frenzel, 2012).

2.7.2. Total dissolved solids (TDS)

Several inorganic and some organic minerals or salts, including potassium, calcium, sodium, bicarbonates, chlorides, magnesium, sulfates, and others, can be dissolved by water. These minerals gave the water an undesirable flavor and muted color. This is a crucial variable while using water. Water with a high TDS rating is one that has a high mineral content. The recommended TDS level for drinking purposes is 500 mg/l, with a maximum limit of 1000 mg/l. In Wondo Genet campus Meride and Ayenew reported that 118.19 mg/l (Meride and Ayenew, 2016).

2.7.3. Electrical Conductivity (EC)

The electric conductivity is the ability of a substance to conduct electricity. The conductivity of water is a more-or-less linear function of the concentration of dissolved ions. Conductivity itself is not a human or aquatic health concern, but because it is easily measured, it can serve as an indicator of other water quality problems. It is used to give an indication of the amount of inorganic materials in the water including, calcium, bicarbonate, nitrogen, phosphorus, iron, sulfur and others (WHO, 2003). If the conductivity of a stream suddenly increases, it indicates that there is a source of dissolved ions in the vicinity. The sources of EC may be an abundance of dissolved salts due to poor irrigation management, minerals from rain water runoff, or other discharge. EC is also a measure of the water quality parameter TDS or salinity (Atekwana *et al.*, 2004). Therefore, conductivity measurements can be used as a quick way to locate potential water quality problems. Conductivity is measured in terms of conductivity per unit length, and meters or micro siemens/cm. Storm water runoff, sewage effluent, catchment geology and agricultural effluent running into streams have a significant influence on the conductivity of stream water.

2.7.4. Turbidity

The physical quality of drinking water can be measured by its turbidity level; high turbidity can result in increased microbiological and chemical contamination (Mann *et al*. 2007). Turbidity in drinking-water is caused by particulate matter that may be present from source water as a consequence of inadequate filtration or from re suspension of sediment in the distribution system. It may also be due to the presence of inorganic particulate matter in some ground waters or sloughing of biofilm within the distribution system. The appearance of water with a turbidity of less than 5 NTU is usually acceptable to consumers, although this may vary with local circumstances. Particulates can protect microorganisms from the effects of disinfection and can stimulate bacterial growth. In all cases where water is disinfected, the turbidity must below so th at disinfection can be effective. Turbidity is also an important operational parameter in process c ontrol and can indicate problems with treatment processes, particularly coagulation, sedimentatio n and filtration. No health-based guideline value for turbidity has been proposed; ideally, however, median turbidity should be below 0.1 NTU for effective disinfection, and changes in turbidity are an important process control parameter (WHO, 2006).

Turbidity less than 1 NTU are necessary for effective disinfection, either chemical (chlorine or ozone) or physical (UV or irradiation) disinfection methods, and turbidity levels greater than 5NTU are a clear indication of the presence of solids (potentially harmful) in the water (WHO, 2011). Six of the 26 sites, five of the eleven samples in Simada, and none of the samples for Quarit had turbidity levels exceeding 5 NTU. In wells during the height of the dry season, turbidity may increase due to low water yield; however, turbidity also indicates the presence of contaminants (Tilahun *et al.*, 2012).

2.7.5. Hydrogen Ion Concentration (pH)

pH is the negative logarithm of hydrogen ion activity and its value expresses the intensity of the activity or alkalinity condition of water under normal condition temperature (T°C) and pressure. Most reactions in gas water rock systems involve or are controlled by the pH of the system, it related to taste, and odor problems. pH-value in natural water is affected by the concentration of bicarbonate and carbonate ions. The pH value for all water samples is in the optimum range (6.5-8.5). Some water samples are described as alkaline water, and the others are close to neutral. The water in a pure state has a neutral (pH=7), while the rain has a natural acidic pH of about 5.6 because it contains CO2 and SO2. It measured by pH Electrode meter, or Acidity Index paper (WHO, 2006). Nigatu Tsega *et al* (2013) reported as The mean pH of tap water, protected dug well, protected spring, open dug well and open spring were 7.5 ± 0.4, 7.3 ± 0.4, 7.3 ± 0.4, 6.8 ± 0.3and 6.7 ± 0.4, respectively. All tap and protected dug wells met national and WHO guidelines. However, 3 (27.3%) and 5 (45.5%) of open dug wells and open springs had pH values below the recommended national and WHO limits.

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in Bahir Dar Zuriya rural kebele around Bahir Dar city in Amara Regional state, located in the north western Ethiopia (Figure 1). It is found in West Gojjam Zone. This district has 36 rural kebele and total population size is 228821 from this 117520 are male and 111302 female. Its area is 151,119 hectares, and it is located at an altitude of 1700 to 2300 meters above sea level. Its extension is between 11°20′N 11°55′N latitude and 37°04′E 37°50′E longitude. The climate is tropical with four seasons (dry period, Small rains, rainy and dry spell between the long and small rains) and 50% of the rain falls are in July and August and 18% falls during October to February. The maximum rain fall (499.6mm) was in July and minimum was in January (1.8mm). The maximum temperature usually occurs in March to May. The mean monthly maximum was 27.7^0c and minimum was 13^0c (Goshu and Akoma, 2011). In Bahir Dar zuriya rural kebele the main water sources are unprotected springs, hand pump, hand dug wells and river for all domestic uses.

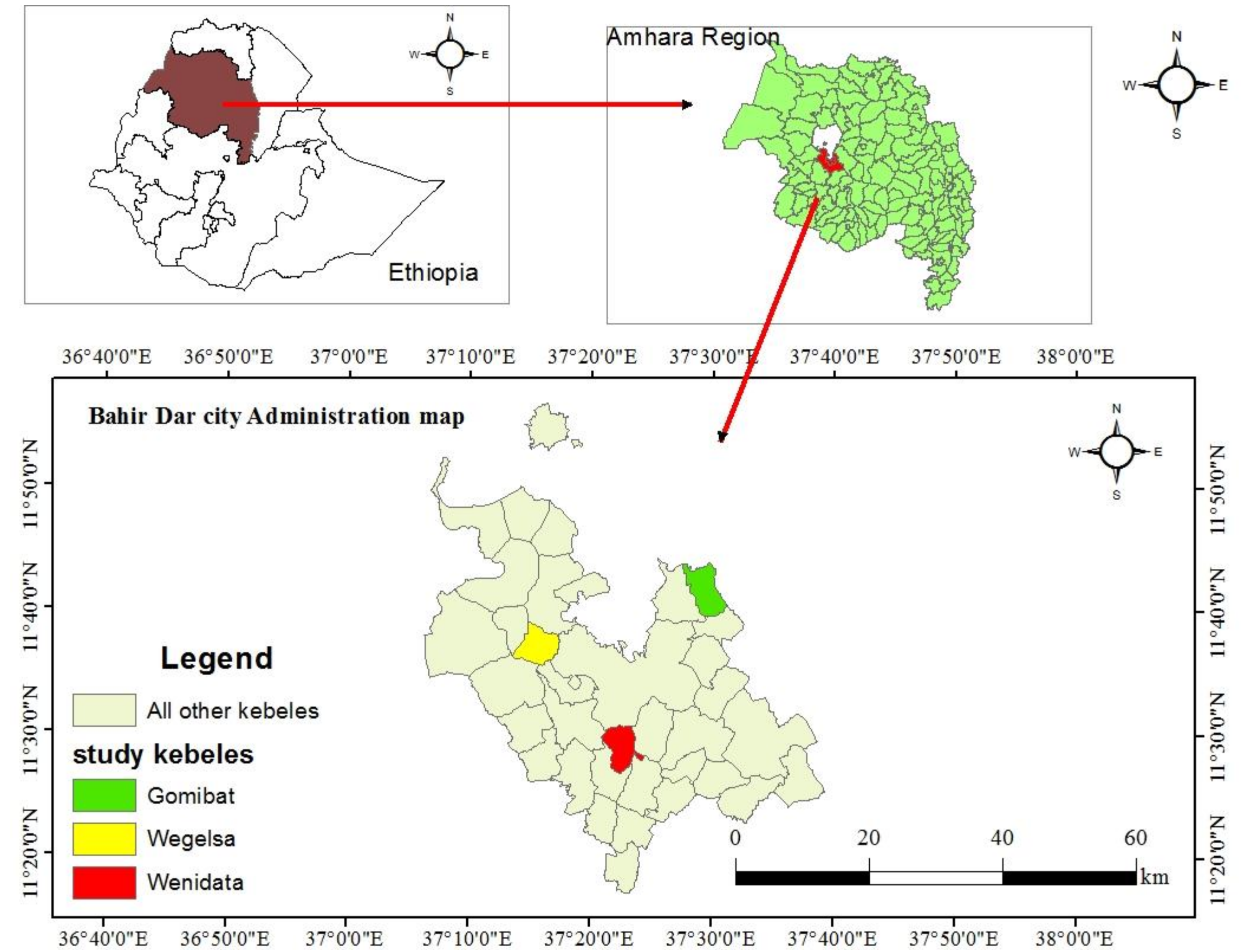


Figure 1: Map of Ethiopia, Amhara region, Bahir Dar zuriya woreda, and Bahir Dar City Administration.

3.2. Sample size

A total of 60 water sampling points were determined from 24 water sample from Gombat, 20 from Wegelsa and 16 from Wendata kebele. The number of households from each selected kebele was calculated based on the population size proportion. A simple random sampling technique was used to select water sampling points from source and household. Water samples were collected from four sampling points' 8 water samples from Hand Dug Well, 17 from hand pumped Well water, 5 from surface water (river) and 30 from house hold container). Water sample was collected from water source with house hold container randomly in three Kebele.

3.3. Study Design

A cross-sectional study was conducted to assess the bacteriological and physicochemical quality of drinking water from source Hand Dug Well, pumped Well water, surface water and household container from Bahir Dar Zuriya Rural Kebele community from April 2022 to august 2022.

3.4. **Sampling Procedures**

250ml Water sample was collected in sterilized glass bottles. Water samples were transported in an icebox and transported to the microbiology laboratory within 6 hours of collection. The laboratory tests were begun immediately after the sample had arrived at the microbiology laboratory, Bahir Dar University, Ethiopia.

3.5. Bacteriological Quality Analysis of Water Samples

Enumeration was done using the Most Probable Number technique (MPN). For presumptive test fifteen test tubes were used per sample where five tubes contain sterile 10 ml double strength and ten tubes contain 10 ml single strength Makconky broth, all tubes with inverted Durham tubes. With a sterile pipette, 10 ml of the water sample was aseptically dispensed into each of the first five culture tubes containing the double strength Makconky broth. Into the rest of the ten tubes containing sterile single strength Makconky broth, 1 ml of the sample was inoculated into each of the five culture tubes, while 0.1 ml sample was inoculated into the remaining five tubes all with inverted Durham tubes. The tubes were gently shaken to distribute the sample uniformly throughout the medium and incubated at 37°C and 44^0c for 24 hours for Total coliform and Fecal coliform respectively. After 24 hours of incubation, the cultures were observed for color change (acid production) and gas formation. For the confirmatory test, a loop full of culture from test tubes that showed gas production was transferred to the brilliant-green lactose bile (BGLB) broth tube and incubated for 24 to 48 hours at 35°C for Total coliform and 44.5^0c for Fecal coliform. The bacteria present in the water reproduce and produce acid with or without gas. From the number of tubes inoculated and the number with a positive reaction, the most probable number (MPN) of bacteria present in the original water sample can be determined statistically (APHA *et al.*, 1992). The numbers of coliforms of positive tubes were estimated from most probable number (MPN/100ml) tables and finally results reported as MPN/100ml. The presence of *E. coli* was from FC positive tubes streaked on Eosin–Methylene Blue plate and incubated for 24 hours at 37°C. The presence of golden greenish shiny color was taken as evidence for the presence of *E. coli*. The risk level (based on coliform counts) of the drinking water was calculation based on WHO (2012). Based on this, fecal coliform counts of (MPN/100 ml) <1 low risk, 1-10 intermediate risk, 11-100 high risk, and > 100 very high risk were considered.

3.6. Isolation and Identification of Bacteria

To test for the presence *Salmonella*, *Shigella and Pseudomonas* species of pathogens was used to selective medium for each pathogen. Loop full of each Fecal positive sample was aseptically streaked into each selective medium agar in petridish and incubated at 37 °C for 24 h. *Salmonella* spp. and *Shigella* spp. were isolated on SS agar plates and *Pseudomonas* isolation agar, which is a selective and differential medium, for the isolation of *Pseudomonas* spp. were used. These isolates were identified to the genus level using colony characteristics, cell morphology, Gram test and a serious of other biochemical tests. Suspected non-lactose fermenting bacterial colonies were further characterized having inoculated into the following biochemical test: Triple Sugar Iron (TSI) agar, Simmon's Citrate agar, Sulfur Indole motility (SIM) medium, Lysine Iron agar, Urea agar, and fermentation tubes of glucose, sucrose and Mannitol. Finally, the proportions of each positive pathogen samples were determined based on the above biochemical results and by colony morphology.

3.7. Antibiotics resistance testing

All isolates were tested for antibiotic susceptibility to commonly used antibiotics. The standard Kirby-Bauer`s disc diffusion method was performed to determine their antimicrobial susceptibility profiles following standard procedures (Bauer, 1966, Biemer, 1973). Bacterial inoculum was prepared by suspending 4-5 morphologically identical colonies from each isolate in 5 ml nutrient broth (HiMedia, India) and incubated for 4 hours at 37°C. The bacterial suspension was compared with 0.5 McFarland turbidity standards to achieve about $1.5x10^8$ CFU/mL. After adjusting the turbidity, the surface of the prepared Mueller Hinton Agar (MHA) medium (Accumix, India) was evenly inoculated three times using a sterile cotton swab while rotating the plate with the culture. The plates were left at room temperature for 15-20 minutes to let dry. The antibiotic discs included gentamicin (GN, 10 μg), tetracycline (TE, 30 μg), ciprofloxacin (CIP, 5 μg), nalidixic acid (NA, 30 μg), erythromycin (E, 15 μg and Cefoxitin (CE, 30 μg) (Becton, Dickinson, and Company, Sparks, Maryland, USA). The discs were aseptically laid on the surface of the inoculated agar plates with proper spacing using sterile forceps and incubated at 37°C for 18-24 hours. The diameter of the inhibition zone around the discs was measured to the nearest millimeter and interpreted as sensitive (S), intermediary resistant (I), or resistant (R) according to the defined breakpoints in Clinical and Laboratory Standards Institute (CLSI, 2020).

3.8. Physicochemical Analysis of Parameter

Analysis of physicochemical parameters of water was determined after collection and arrival to the laboratory by standard methods of water and waste examination (APHA, 1998). Water samples collected were analyzed by standard methods (APHA, 1998). Physicochemical parameter was done using standard analytical techniques and instruments. Temperature (^{0}C) were measured by thermometer on site and pH were measured using a portable digital pH meter, conductivity and total dissolved solid were measured by portable conductivity and TDS meter (Bante 901p) and Turbidity of the samples was determined using a turbidity meter(AL250T-IR) in the laboratory.

3.9. Sanitation survey and hygienic practices

Structured questionnaire is used to obtain information on the sanitary condition at the household level that may affect drinking water quality. The questionnaires was first developed in English and translated into Amharic (a local language) and then the responses were translated back into English. 30 households were interviewed using structured questionnaire. The questionnaires were also used to obtain information on sanitary integrity and the potential hazards in the environment that may affect drinking water quality. All the drinking water sources and household drinking water handling practices were evaluated based on standard checklists recommended by WHO (2012) and the risk level of each sample was determined as described in WHO (2012).

3.10. Data Analysis

Data were analyzed using SPSS statistical software (version 21). The results were presented in a descriptive statistics such as mean, range and frequencies. Significant differences in the mean values of measured parameters among the source and household container drinking water samples were tested using independent t-test. The values of mean bacterial counts and physicochemical parameters were compared with WHO guidelines, which is more or less similar to the Ethiopian standards for drinking water quality. In all cases, statistical significance was considered at a 95% confidence interval and a p value ≤ 0.05

3.11. Ethical clearance

Ethical clearance was obtained from the Ethical clearance committee of Bahir Dar University. Data at the households were collected after informed consent was assured from the households. The study objectives were clearly explained to the households and each household was assured that the information provided would be kept confidential.

4. RESULTS AND DISCUSSION

4.1. Bacteriological Quality of Drinking Water in Bahir Dar Zuriya Rural Kebele

In this study, a total of 60 water samples were collected from water source and household water containers in three kebele. Out of this, 91.6 %(55/60) and 40 %(24/60) of the drinking water samples were tested positive for total coliforms (TC) and fecal coliform respectively. Majority water samples was above the WHO guidelines (0 faecal coliform counts per 100 ml) for drinking water (table 1). Out of the total 96.6% (29/30) household container water sample were positive TC and 86.6% (26/30) water sample from source positive TC. Likewise, 43.3% (13/30) of water samples from households' containers and 36.66% (11/30) of the water samples from the source were tested positive for FC. The result was above WHO and national standard of drinking water. The variation of the mean counts of total coliforms among the source and households' containers water samples was not significant (p=0.994) and also faecal coliform counts between source and households' containers was not significant (p=0.551). The highest number recorded (1600 MPN/100 ml) and the lowest number recorded (0 MPN/ml) TC and FC counts were recorded in the water samples taken from households' containers and Source. The mean count of TC and FC in households' containers and source was 723.6 ± 127.6, 725 ± 132.1, 65.9 ± 53.7 and 120.5 ± 74.1 respectively.

Table 1: Coliform counts (MPN/ml) in the drinking water samples from the source and households' containers in Bahir Dar Zuriya Rural Kebele (n= 60).

Indicator	Sample	Mean ± SD	WHO standard	National standard	p-value
TC	HHC	723.6 ± 127.6	0	10	
	Source	725 ± 132.1			0.994
FC	HHC	65.9 ± 53.7	0	0	
	Source	120.5 ± 74.1			0.551

HHC; House Hold container

In terms of risk to human health, all river source 5 (100%) had high number of total coliform (>100 MPN/100ml) and 1(20%), 1 (20%), 1(20%) and 2 (40%) had low risk, risk, high risk and very high risk to FC respectively (Table 2). Two (25%) and 6 (75%) Hand dug Well had high risk and very high risk TC respectively and 4 (50%), 1(12.5%), 2 (25%) and 1 (12.5%) had low risk, risk, high risk and very high risk to FC respectively. 4 (23.5%), 2 (11.8%), 3 (17.6%), 8 (47%) 0f hand pump had low risk, intermediate risk, high risk and very high risk to TC respectively and 14 (82.4%), 2 (11.8%), 1 (5.9%) and 0 had low risk, risk, high risk and very high risk to FC respectively. All river and hand dug well, 64.7% of hand pump and 96.7% of household container water samples had total coliform counts above the national and WHO recommended limits. Similarly 80% of river, 50% of hand dug well, 17.6% of hand pump and 43.3% of house hold container of water samples had feacal coliform counts above the national and WHO recommended limits.

Table 2: Overall risk-to-health Classification of drinking water samples from the source and households' containers in Bahir Dar Zuriya Rural Kebele

	Total coliform(MPN/100ml)				Feacal coliform(MPN/100ml			
Risk level	<2	2-10	11-100	>100	<2	2-10	11-100	>100
River (n=5)	0	0	0	5 (100%)	1 (20%)	1 (20%)	1 (20%)	2 (40%)
Hand dug Well (n= 8)	0	0	2 (25%)	6 (75%)	4 (50%)	1 (12.5%)	2 (25%)	1 (12.5%)
Hand pump (n=17)	4 (23.5%)	2 (11.8%)	3 (17.6%)	8 (47%)	14 (82.4%)	2 (11.8%)	1 (5.9%)	0
HHC	1 (3.3%)	0	5 (16.7%)	24 (80%)	17 (56.75)	7 (23.3%)	4 (13.3%)	2 (6.7%)

Risk level, <2 MPN/100ml= low risk; 2-10=intermediate risk; 11-100= high risk; >100= very high risk

4.2. Frequency of pathogenic bacteria in drinking water sample in Bahir Dar Zuriya Rural Kebele

In this study the pathogenic species that were most frequently isolated from feacal contaminated water samples 16 (37.2%), 15 (34.8%) and 12 (27.9%) were positive for *Pseudomonas* spp. *Salmonella* spp. and *Shigella* spp. were found respectively (Figure 2). Among the total FC positive water samples, 8 (53.3%) of *Salmonella* spp. were found in household container and 7 (46.7%) in water sources, 6 (50%) of *Shigella* spp. were found in water source and 6 (50%) in household container, *Pseudomonas* spp. were found 7 (44%) in household container and 9 (56%) in water source.

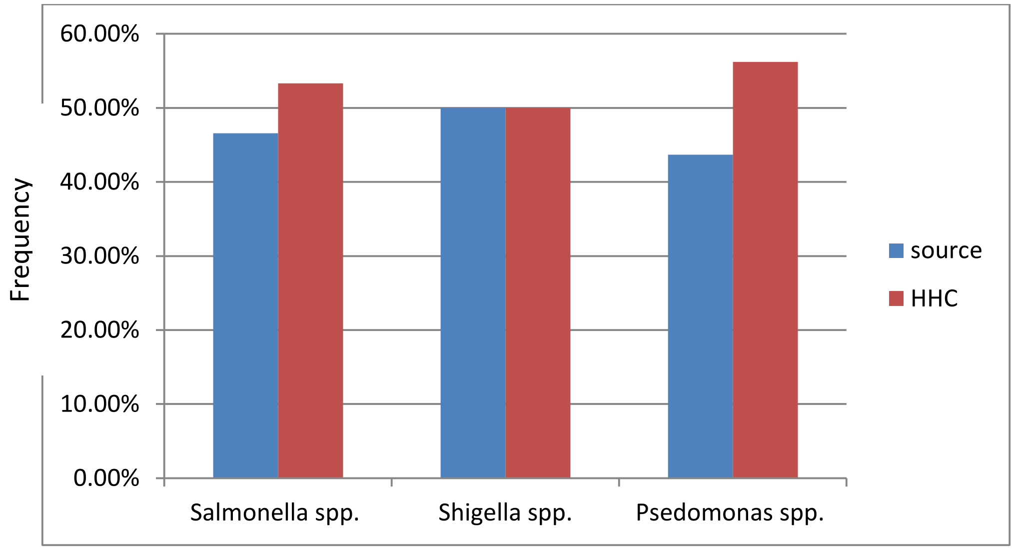


Figure 2**:** frequency of isolated bacteria from water source and Household container from positive water samples in Bahir Dar Zuriya Rural Kebele (n= 43).

4.3. Antibiotic resistance susceptibility pattern of bacterial isolates from Household container and Water source of drinking water

Most of isolated pathogen bacteria 97.6%% were resists at least one antibiotic. The highest number of bacteria resistance shows from cefoxtin 95.3 % (41/43), erythromycin 58.1 % (25/43), gentamycin 4.6% (2/43), ciprofloxacin 32.5% (14/43), and tetracycline were 23.2% (10/43) and 0% nalidix acid resists respectively (Table 3). The most frequency of susceptible pathogen were recorded in nalidix acid (98.7%) and 96% of pathogen were susceptible by gentamycin antibiotics**.**

Table 3: Antibiotic resistance profiles of bacterial isolates from Household container and Water source from Bahir Dar Zuriya Rural Kebele

type of Pathogen (N)		Gentamycin (%)	Tetracycline (%)	Ciprofloxine (%)	Nalidix Acid (%)	Erythromycin (%)	Cefoxtin (%)
	R	1 (6.6%)	5 (33.3%)	5 (33.3%)	0	11 (73.4%)	15 (100%)
Salmonella(15)	I	0	0	2(13.4%)	0	2(13.3%)	0
	S	14 (93.4%)	10 (66.7%)	8 (53.3%)	15 (100%)	2 (13.3%)	0
	R	1 (8.3%)	1 (8.3%)	4 (33.3%)	0	6 (50%)	10 (83.3%)
Shigella(12)	I	0	0	1(8.3%)	0	2 (16.7%)	0
	S	11 (91.7%)	11 (91.7%)	7 (58.4%)	12(100%)	4 (33.3%)	2 (16.7%)
	R	0	4 (25%)	5 (31.3%)	0	8 (50%)	16 (100%)
Pseudomonas(16)	I	0	0	1 (6.2%)	0	1 (6.3%)	0
	S	16(100%)	12(75%)	10 (62.5%)	16 (100%)	7 (43.7%)	0
Total	R	2 (4.6%)	10 (23.2%)	14 (32.5%)	O	25 (58.1%)	41 (95.3%)
	I	0	0	4 (9.3%)	0	5 (11.6%)	0
	S	41 (95.3%)	33 (76.7%)	25 (58.1%)	43 (100%)	13 (30.2%)	2 (4.6%)

R-resistance, I- intermediate, S- Susceptible

In multi-drug resistance test out of the total 62.8 % (27/43) bacteria were resist two and more than two antibiotics (Table 4). According to each pathogen, 31. 3%, 46.7%, and 16.7% 0f *Pseudomonas*, *Salmonella*, *Shigella* species were multiple antibiotic resistances against three and more than three antibiotics respectively.

Table 4: Multi-drug resistance profiles of bacterial isolates from Household container and Water source from Bahir Dar Zuriya Rural Kebele

Types of pathogen	Total Isolation	R2 (%)	R3 (%)	R4 (%)	R5 (%)	R6 (%)	MDR (%)
Pseudomonas spp.	16	4 (25)	2 (12.5)	3 (18.8)	0	0	31.3
Salmonella spp.	15	4 (26.7)	4 (26.7)	2 (13.3)	1(6.6)	0	46.7
Shigella spp.	12	5 (41.7)	1(8.3)	0	1(8.3)	0	16.7
Total	43	13 (30.2)	7(16.2)	5 (11.6)	2 (4.6)	0	62.8

R2: Resistant to two antibiotics, R3: Resistant to three antibiotics, R4: Resistant to four antibiotics, R5: Resistant to five antibiotics, R6: Resistant to six antibiotics and MDR: Multi drug resistance.

4.4. Sanitary survey status of drinking water system at the source and household level in Bahir Dar Zuriya Rural Kebele

The household use different types of water sources as their primary source. Households survey in the study area use mainly three different types of primary sources to meet their needs. Out of 30 water source, 17(56.7%) were improved hand pump and others were unimproved. 76.7% households were collected from one source and other 23.3% household collected from multiple source.63.3% water source were cracked and unclean (Table 5). all household (100%) were not chlorinated and treat at home level. 10% water source were present animal and human faeces near to water source and 83.3% had farming activities – herbicide and pesticides water source. about 26.7% of population in the study area use private hand dug well water and other 73.3% of population gain drinking water from public water source.73.3 % of population has not gain enough water.

Table 5: Sanitary Survey result of Drinking water source and household in Bahir Dar Zuriya Rural Kebele (n=30).

Questions		Frequency	Percent (%)
Source of drinking water			
	Hand dug well	8	26.7
	Hand pump	17	56.7
	River	5	16.7
Do you use drinking water from multiple sources?			
	Yes	7	23.3
	No	23	76.7
Does your household store drinking water in small container?			
	Yes	30	100
	No	0	0
Do you cover water container			
	Yes	30	100
	No	0	0
Do the drinking water container used for other purposes?			
	Yes	4	13.3
	No	26	86.7
Do you treat water at household level?			
	Yes	0	0
	No	30	100
Ways of fetching water from distant sources			
	Jerrican	30	100
Do you know that water can be a vehicle to transmit diseases waterborne diseases?			
	Yes	30	100
	No	0	0

Which of the following diseases can be transmitted through polluted drinking water?			
	Amebae	4	13.3
	Amebae and giardia	14	46.7
	Others	12	40
Is there hand washing facility at the household level?			
	Yes	1	3.3
	No	29	96.7
If yes for question 12, what type of hand washing facility does the household have?			
	Small container	1	3.3
	NA	29	96.7
If not for question 12, why?	Economic reason	1	3.3
	Lack of awareness	28	93.3
	NA	1	3.3
Is there soap or detergent at the place for hand washing?			
	Yes	0	0
	No	30	100
Ways of deposing children stool			
	In the environment	20	66.7
	In toilet	10	33.3
Was there an incidence of waterborne diseases acute diarrhea in the family that last less than one week?			
	Yes	13	43.3
	No	17	56.7

Do you worry about waterborne diseases that may infect you family from the drinking water you frequently used?

Yes	19	63.3
No	11	36.7

Which problem is most serious in your family?

Quality	12	40
Quantity	4	13.3
Both	12	40
No	2	6.7

Does the family use multiple source of drinking water?

Yes	9	30
No	21	70

Does your household have a large storage tank?

Yes	2	93.3
No	28	6.7

Do you use privet tap well	Yes	8	26.7
	No	22	73.3

If No. for question 5, how long does it take to go there, get water, and come back? (Put in minute or hours)

5-10	10	33.3
11-20	9	30.1
>20	11	36.6

What type of water do you use for other purpose other than drinking (washing, cooking)

The one used for drinking	24	80
Other	6	20

Who is responsible to collecting water?

Children	5	16.7
Mother	21	70
Mother and children	3	10
Mother and father	1	3.3

Where do you dispose waste water?

	In garden	30	100
Do you (the family) have privet toilet?			
	Yes	12	40
	No	18	60
If yes for question 11, what kind of toilet facility do members of your household usually use?			
	Unimproved	12	40
	NA	18	60
Do the family have shared toilet?	Yes	2	6.7
	No	28	93.3
Do you family member wash hands after toilet use?			
	Yes	29	96.7
	No	1	3.3
Does water collector wash her hands before collecting drinking water?			
	Yes	30	100
	No	1	0
Is there a shortage of drinking water?			
	Yes	13	43.3
	No	17	56.7
How much drinking water does the family get per day? (Mention in liter?			
	1 to 2 jerican	12	40
	3 t0 4 jerican	13	43.3
	>4 jerican	5	16.7
Do you think that the quantity of drinking water supplied is sufficient?			
	Yes	12	40
	No	18	60

Is there evidence of cracks in the water line? (Observation of turbid water?)

	Yes	7	23.3
	No	23	76.7
Is there aesthetic discomfort on the drinking water?			
	Comfort	13	43.3
	Taste	7	23.3
	Temperature	1	3.3
	Temperature, smell, taste	7	23.3
	Temperature, taste	2	6.7
Is the water supplied from your main source usually acceptable?			
	Yes	11	36.7
	No	19	63.3
Is live stoke (cattle, poultry and others) present?			
	Yes	29	96.7
	No	1	3.3
Are animal faces present in the house?			
	Yes	14	46.7
	No	16	53.3

sanitary risk score was computed as qualitative risk category (low, medium, high, and very high risks) for each water source by putting the number of positive factors as a range (8-13, 13–18, 18-23 and 23–28) of the total number of factors being assessed. According to the result obtained from the sanitary inspection of the assessed water sources (Table 6). The percentage of the water sources clustered into Low, Intermediate, High and very high contamination risk category were 36.7, 60, 3.3% and 0 respectively (Table 6).

Table 6: Survey check lists contamination risk level category at the Drinking water source and household in Bahir Dar Zuriya Rural Kebele

Sanitation score	Frequency	Percent	Risk category
8-13	11	36.7	Low risk
13-18	18	60	Medium risk
18-23	1	3.3	High risk
23-28	0	0	Very high risk
Total	30	100	

4.5. Physicochemical Drinking Water Quality in Bahir Dar Zuriya Rural Kebele

As shown table 7, the mean Temperature of river, hand dug well and hand pump water were 19.8± 0.2, 20.25±0.59 and 20.4± 0.19^{0}c respectively. The minimum temperature were recorded in river source19.8± 0.2^{0}c. All water samples were above the recommended national and WHO limits. The mean pH of river hand dug well and hand pump were 6.9±0.26, 6.9± 0.1 and 6.6± 0.085 respectively. Majority of source water pH met national and WHO guidelines. However, 9 (27.3%) of source had pH values below the recommended national and WHO limits. The mean value of conductivity values of river, hand dug well, hand pump and household container were 130± 9.6, 312.3± 73.66, 345.9± 76 µs/cm and total dissolve solid records 126.5± 1.98, 184.1± 28.2, 213.1± 31.6 mg/l in river hand dug well and hand pump respectively.

Table 7: Mean physicochemical values of drinking water samples from the water source in Bahir Dar Zuriya Rural Kebele

Source	Temperature(T^{0}c) Mean ± SD	P^H Mean ± SD	Conductivity (µs/cm) Mean ± SD	total dissolve solid(mg/l) Mean ± SD	Turbidity(NTU) Mean ± SD
River (n=5)	19.8± 0.2	6.9±0.26	130± 9.6	126.5± 1.98	7.7± 2.5
HDW (n=8)	20.25±0.59	6.9± 0.1	312.3± 73.66	184.1± 28.2	8.53± 4.1
HP(n=17)	20.4± 0.19	6.6± 0.085	345.9± 76	213.1± 31.6	7.4± 2.3

HDW=Hand dug well, HP= hand pump

The mean value of temperature, pH, conductivity, TDS and turbidity in all water sources were recorded 20.23±0.19^{0}c, 6.69±0.07, 319.3±48.64 µs/cm, 199.3±19.96mg/l and 8.87±1.96 NTU respectively. The mean value of temperature, pH, conductivity, TDS and turbidity in all households 'containers were recorded 18.97±0.27^{0}c, 335.45±47.77 µs/cm, 198.5 ±20.34 mg/l and 8.75±1.56 NTU respectively. Majority of water sample were met the recommended national and WHO physicochemical limits. Majority 17 (56.6%) of water source and 16(53%) of household container samples had turbidity values above the recommended WHO limits (Table 8).

Table 8: comparing the mean (n=30) physicochemical values of drinking water samples from the source and households' containers in Bahir Dar Zuriya Rural Kebele

		Mean SD	Range	p- value	WHO
Temperature(T^{0}c)	Source	20.23±0.19	18-23.67	0.00	<15
	HHC	18.97±0.27	16-22		
pH	Source	6.69±0.07	5.69-7.49	0.225	6.5-8.5
	HHC	6.83±0.08	5.61-7.71		
Conductivity(µs/cm)	Source	319.3±48.64	105- 1079	0.797	<1000
	HHC	335.45±47.77	101- 1070		
Total dissolve solid(mg/l)	Source	199.3±19.96	113- 536	0.979	
	HHC	198.5 ±20.34	110-538		<500
Turbidity(NTU)	Source	8.87±1.96	0.4-36.1	0.341	
	HHC	8.75±1.56	0.88-32.7		<5

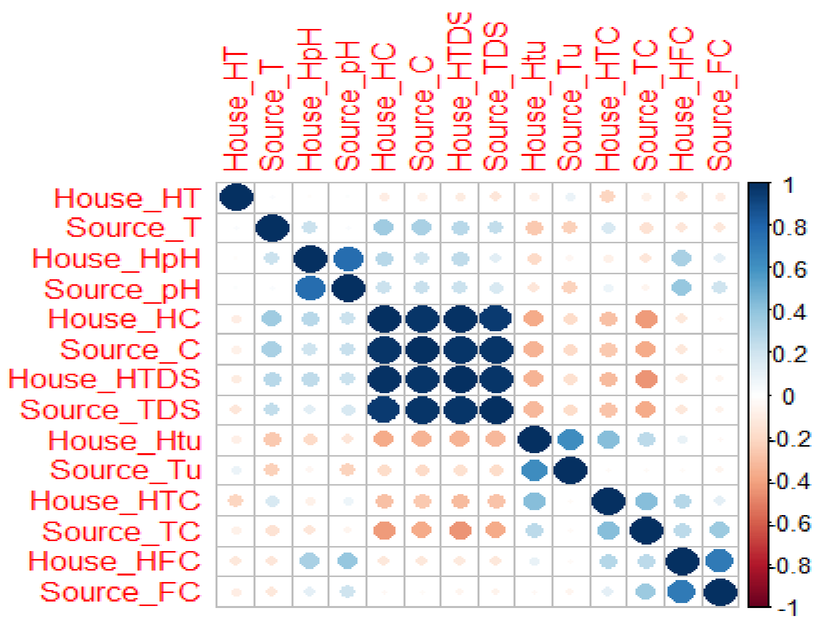
4.6. Correlation among Bacteriological and Physicochemical parameters

The data were analyzed using Pearson's correlation to see the correlation of bacterial indicator counts with temperature, pH, conductivity, TDS and turbidity of source and household water container (Table 9). In the correlation analysis the bacteriological and physicochemical parameters were found to be significantly correlated with each other. Household temperature was negatively correlated to all bacteriological parameters or HHC temperature with HHC TC, HHC temperature with source TC, HHC temperature with HHC FC and HHC temperature with source FC.

Table 9: Correlation among bacteriological and physicochemical parameters

Physicochemical parameter	Bacteriological parameter			
	HHC TC	Source TC	HHC FC	Source FC
HHC Temperature	-0.21	-0.08	-0.12	-0.10
Source Temperature	0.16	-0.15	-0.13	-0.12
HHC pH	-0.08	-0.13	0.32	0.11
Source pH	-0.08	-0.05	0.38	0.20
HHC Conductivity	-0.29	-0.42	-0.12	-0.04
Source Conductivity	-0.26	-0.37	-0.12	-0.02
HHC TDS	-0.31	-0.44	-0.11	-0.05
Source TDS	-0.28	-0.37	-0.12	-0.06
HHC Turbidity	0.42	0.26	0.09	-0.03
Source Turbidity	-0.02	-0.02	-0.02	-0.05

HHC= Household container; TC= Total Coliform; FC= Feacal Coliform



T= temperatures; C= Conductivity; TU= turbidity

Figure 3: Correlation among bacteriological and physicochemical parameters

4.7. Discussion

4.7.1. Bacteriological Quality of Drinking Water in Bahir Dar Zuriya Rural Kebele

The World Health Organization guidelines for drinking water and quality drinking water standards recommend that coliform bacteria must not be detectable in any 100 ml sample of all water directly intended for drinking (WHO, 2017). However, the current study showed that majority 91.6 % (55/60) and 40 % (24/60) of the drinking water samples were tested positive for total coliforms (TC) and fecal coliform respectively. It was above the WHO acceptable range for drinking water. Except few water source all water source were contaminated by coliform bacteria. Based on observation there is lack of treatment, lack of sanitation, lack of management of water source and environmental sanitation. The same study in Ethiopia such as in Adis Kidame Town reported that 89% for total coliforms and 77% of the samples for faecal coliforms, respectively (Baye Sitotaw and Mulu Geremew, 2021), in North gonder reported that 77% of positive faecal coliform is found in the households (Atalay Getachew *et al*., 2021), in Wogeda Town 94.16% of total coliform and 82.5% of fecal coliform were tested positive (Baye Sitotaw *et al*., 2021), in shashemane rural district Edessa Negera *et al*.(2017) also found that 92.6% of hand dug well water samples are contaminated with fecal coliforms.

The results of this study also indicated a poor drinking water handling practices at the household level. This was shown by the observation that most of the drinking water samples from the household containers (96.6%) were found to be contaminated with coliforms, indicating poor drinking water handling at household level. Of course, most of the drinking water samples from the sources 80% (24/30) water sample were positive TC, showing that the causes for drinking water contamination are likely both poor drinking water handling practices and lack of treatment, poor sanitation, poor management of water source and environmental sanitation in the sources. For instances, a study by Luvhimbi *et al.* (2022) in Limpopo Province of South Africa, Baye Sitotaw *et al*. (2021) in Wogeda town (Northern Ethiopia), Baye Sitotaw and Molla Nigus (2021) in Kobo town (North west Ethiopia), Sebsibe *et al*. (2021) in Fiche (Central Ethiopia) indicated significantly higher levels of contamination in water samples from households' container than from the sources. Out of the total 96.6% (29/30) household container water sample were positive TC and not satisfy WHO and national standard of drinking water. Similar study done in Adama town, from household water container, 29 (55.8%) samples had FC concentrations within the recommended level of WHO and National standard and 23 (44.2%) above the standard limits (Temesgen Eliku and Hameed Sulaiman, 2015) The major factors for drinking water contamination at household levels are related to hygiene and sanitation practices (Ondieki *et al*., 2022) which have to be addressed through education, support and monitoring.

The majority of water source in Ethiopia had bacteriological counts that were highly extremely for drinking, particularly for TC and FC. Forty three percent (43%) of Ethiopia's rural population obtains their drinking water from unprotected water sources (UNDP, 2018). In this study, most of water samples taken from river, hand dug well and hand pump had very high pollution levels categorized under dangerous. All river and hand dug well source (100%) and 76.5% of hand pump were contaminated by total coliform and also 80% river source, 50% hand dug well and 17.7% hand pump were contaminated by feacal coliform. Similar study in shashemane rural district,100% and 91.6% water samples from rivers and hand dug wells water, respectively (Edessa Negera *et al*., 2017), study in North Gondar done from protected spring and protected well water samples, 71.43% and 28.6% had levels of total coliform (TC) and faecal coliform /thermotolerant (TTC/FC) count, respectively (Mengesha Admassu *et al*., 2004).Study done by Dessalegn Amenu *et al*. (2013) in the surrounding area of dire dawa town and Nigatu Tsega *et al*. (2013) in Bahir Dar. Lack of protective infrastructure, poor administration by the local government, and residents with low socioeconomic position could all be contributing factors to unprotected water sources. However some research indicates that even protected water sources can become contaminated with human waste (Bain *et al*., 2014). According to risk classification, all river source (100%), 6(75%) of hand dug well and 8(47%) of hand pump were at very high risk category for total coliform (>100 MPN/100ml). regarding fecal coliform, 2 (40%) water sample of river, 12.5 % of hand dug well and 5.9% of hand pump were very high risk category of fecal coliform. Similar study done in Bahir Dar had high risk categories (Nigatu Tsega *et al*., 2013).

4.7.2. Frequency of isolated pathogenic bacteria

A number of potentially pathogenic bacteria were retrieved from drinking water samples further confirming the level of bacterial contamination in the system. Particularly, the detection of isolates related to *Salmonella*, *Shigella* and *Pseudomonas* species could show a serious concern as most members of these genera are pathogenic. In the present study, *Salmonella* (25 %) was the predominant pathogen isolated from the drinking water sample, with the second most isolated being *Shigella* and *Pseudomonas.* Majority number of pathogenic bacteria (52%) gain in household container water sample compared to water sample from source. Similar study done in Tigrai, Ethiopia, *Escherichia coli* (20.3%) was the predominant pathogen (Aderajew Gebrewahd *et al*., 2020). A study in rural areas of Ethiopia found that about 74% and 58% of the water samples from water sources and household storage were positive for *E. coli* (Dessalegn Amenu *et al*., 2013). In this study high number of pathogen were isolated from various water sources especially from rivers. The high prevalence of bacterial pathogens in river water sources might be due to contamination of human and animal feces, introduction of microorganisms by birds and wild animals and behavioral practices associated with use of the river water sources for bathing, washing clothes, and dumping waste into the river.

4.7.3. Antibiotic resistance profile of the isolates bacteria

Majority of the pathogen bacteria in this study (97.6%) were resists at list one antibiotic and commonly used antibiotics that also imply complicated public health concern as a result of drinking water contamination. High level of antibiotics resistance was seen in Cefoxtin (94.5%) and low level of resistance was seen in Nalidix acid antibiotics. Another study done in Bahir Dar by Bayeh Abera *et al*. (2014) in lowest level of resistance was found to ciprofloxacin. In this study high level of antibiotics resistance by *Salmonella* (41%) was observed. In multi-drug resistance test out of the total 62.8 %(27/43) bacteria isolates were resist two and more than two antibiotics. Comparing each pathogen to pathogens by melti drug resistance, 31.3%, 46.7% and 16.7%, of *Pseudomonas, Salmonella* and *Shigella* species revealed multiple antibiotic resistances against three and more than three antibiotics respectively.

Antibiotic resistance emerges in four important genetic reactors. The first reactor serve as Human and animal feces. The secondary reactor implicates any environment where susceptible people are packed and exposed to bacterial exchange, including hospitals, nursing homes, farms, and other such establishments. The effluent and any biological leftovers created in the secondary reactor are transferred to the tertiary reactor, which includes. The soil, surface or groundwater mediums operate as the fourth reactor, combining and competing with ecological species as the microbes created in the other three reactors do. Water is a crucial component in each of the four genetic reactors (Ghemaout and Elboughdiri, 2020). In this study according to sanitary survey, the hand dug wells, rivers and hand pump are surrounded by different agricultural activities, had poor water drainage systems, had no protections, it leads to the well contacted by animals and humane feces also found near to the wells which increased the risk for contamination of well water.

4.7.4. Sanitary survey status of drinking water system at the source and household level in Bahir Dar Zuriya Rural Kebele

In additions to the bacteriological and physicochemical parameters, visual assessment of water source and environment surrounding water sources, taking account the condition and practices in the water sources that pose on actual or potential danger to drinking water and health and well-being of the consumer was under taken by using logical questions. Unhygienic practices play important role in the transmission of diseases caused by pathogenic microorganism in drinking water. Improper sanitation behavior such as a frequency of latrine, animal contact, protection, cracking waste disposal, collect water in the apron area agricultural and human activities can affect the quality of drinking water. In this study majority of population was not gain enough water and lower than the national and WHO minimum water consumption level of 20 l/p/d and accessible water supply is within safe physical reach from the home or institution, usually within 1 km or a 30-minute round trip (WHO, 2008). In this study 56.7% of male and 66.7% of female are illiterate household members. There is no any form of drinking water treatment at household or at the source. There is no hand washing facility at household level and majority of the study population have not aware hand washing facility.

4.7.5. Physicochemical Drinking Water Quality in Bahir Dar Zuriya Rural Kebele

Temperature measurements are very useful in understanding the trend of physical, chemical and biological activity which is enhanced by the variation of temperature. In water resources, a high temperature can encourage the growth of organisms (Nigatu Tsega *et al*., 2013). In the present study, the maximum temperature recorded from source water sample 20.23±0.19^{0}c and minimum temperature recorded in the households 'containers18.97±0.27^{0}c. This is beyond the WHO standards of <15^{0}c. So, higher amount of bacterial contamination present in the water source comparing from house hold container water. Similar study done in Bahir Dar, Nigatu Tsega *et al*.(2013), the average temperature recorded was 22.71±1.01^{0}c and ranged between 19.97-25.67ºC, in Nekemt town Gonfa Duressa *et al*. (2019) range from 20.5 to 20.8 ^{0}C. Similar minimum temperature recorded in Wogeda town (Northern Ethiopia) from households' containers fetched from tap water 16.1°C (Baye Sitotaw *et al*., 2021).

The significance of water's hydrogen ion concentration (pH) is demonstrated by how it influences biological and chemical processes that only take place within a limited range (Kolawole *et al*., 2013). In the present study, some water sample pH concentration trend was found to be slightly acidic, and the other sample pH range fell within the permissible range of 6.5 to 8.5 for drinking waters (WHO, 2011). In the present study the pH were recorded between 5.61-7.71.This finding is in agreement with Mekuanint Lewoyehu (2021) who reported a similar range for pH of water used for drinking in Mecha, Amara Region. For human consumption, pH values lower than 6.5 have been considered to be overly acidic and can result in diseases such acidosis. Low pH values are also dangerous for the environment and have synergistic effects on heavy metal toxicity in water bodies, and this study disagrees with Baye Sitotaw and Mulu Geremew (2021) in Adis kidame town, Baye Sitotaw *et al* (2021) in Wogeda town (Northern Ethiopia).

The ability of a solution to convey an electrical current is measured by the electrical conductivity of the solution, which includes water. Its capability is dependent on the ion's existence, total concentration, mobility, and measuring temperature. Because it provides a good indication of the amount of dissolved elements in water, conductivity is a crucial component in determining the quality of the water (Muhammad, 2004). In this study, the electric conductivity was the range between 101-1079 µs/cm and the conductivity of household water was slightly greater than source water. Majority of water sample met WHO standards <1000 µs/cm. these value is agreement from Baye Sitotaw *et al* (2021) in Wogeda town (Northern Ethiopia) but greater than Mekuanint Lewoyehu(2021) who reported ranged between 34 and 304 µs/cm, Baye Sitotaw and Mulu Geremew (2021) in Adis kidame town.

Total dissolved solids in drinking water came from sewage, urban runoff, industrial wastewater, and natural sources (WHO, 2004). According to Talling (2009), runoff from residential, agricultural areas, as well as contaminated soil, are the main sources of TDS. In this study except few samples, all household container water and source water sample lied within desirable range for drinking purposes. This study is agreed with Mekuanint Lewoyehu (2021).

One measure of the plant's treatment effectiveness is the ability to manage turbidity. As there is more suspended load present, there is lighter scattering. Physicochemical parameters closely linked to the microbiological safety of drinking water (Murphy, 2007).Therefore, turbidity has to be correlated with bacterial contamination, and the probable existence of pathogens that are of human health concern (Olson, 2004). The high acceptable level of turbidity for drinking water is 0.00 NTU, while the high acceptable level is 5 NTU, according to WHO regulations (WHO, 2011).In this study, 50% of water sample were above recommended value and the range between 0.4-36.1 NTU. Similar study done by Mekuanint Lewoyehu (2021) reported the turbidity values in the range 0.7–46 NTU.

4.7.6. Correlation among Bacteriological and Physicochemical parameters

The selected water quality parameters were examined to determine if one parameter could be used as a proxy indicator for the other. Some of the physico-chemical parameters were found to be significantly correlated with each other. For instance household temperature was negatively correlated to all bacteriological parameters or HHC temperature with HHC TC (r= -0.20), HHC temperature with source TC (r= -0.08), HHC temperature with HHC FC (r= -0.12) and HHC temperature with source FC (r= -0.10). Moreover a correlation for turbidity and bacterial indicator had strong positive correlation both household container and source water. According to (WHO, 2011), high levels of turbidity can protect microorganisms form the effect of disinfection. Similarly the study conducted at Metropolis, Ghana by Karikari and Ampoto (2013) showed that there was a significant positive correlation between pH and turbidity (r = 0.79) and also pH and turbidity were positively correlated with total coliform.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

Bacteriological quality of most drinking water samples analyzed in the current study did not meet the standards set for drinking water by the WHO guideline value 0 CFU/100 ml. there is high level of total and faecal contamination of drinking water in Bahir Dar zuriya rural kebele. High risk scores found in the drinking water suggest poor source and house hold container protection, poor sanitation conditions and practices. The most important risk factors for drinking water quality in Bahir Dar zuriya rural kebele include unhygienic or unclean water source, agricultural activities that use pesticide and herbicide, and unhygienic water handling practices at the household level, shortage of water, solid waste disposal technique and Other socio demographic factors, Occupational status, income; are factors for presence of total and faecal coliform contamination in drinking water. Majority of improved hand pump water source are cracked, unclean and no treatment system like chlorine. Due to this reason, there is high number of bacteria pathogen in the study area. These pathogen bacteria from drinking water have shown high levels of antibiotic resistance to commonly prescribed antibiotics and high number of melti drug resistance bacteria is present. Majority of Physicochemical parameters of water sample in both source and house hold container drinking waters are not acceptable for drinking purpose. Especially temperature and turbidity in all samples are not met national and WHO standard.

5.2. Recommendations

Based on the results and conclusions of this study, the following recommendations are formulated:

Indicator bacterial counts in the majority sampled water from source and household have above the guidelines set for human use. Wastes from both livestock and human were found to be causes of the problem, so there is clearly and crucial need to develop safe water supplies and basic sanitation in the area. Community must keep the water sources safe by properly constructed fences, regular maintenance's and supervisions of water sources and proper disposal of human and animal wastes. Minimizing fecal contamination of water with livestock and human wastes had a dramatic impact on reducing water sources pollution. It should be given to create awareness in the community to improve hygiene, such as to develop a habit of using latrines, develop water treatment system at house hold and water source and separate drinking water from irrigation purpose. The water sector as a service provider is expected to achieve safe and adequate water provision to consumers and always would be assure both physicochemical and bacteriological drinking water quality standards and give education for community how to use and proper cleaning of water storage container at household container and also give awareness and inform the people who uses river and hand dug well source to be the necessary of water treatment of this water before they can be used for drinking purposes. In those study area except a few, there is no improved and clean water source. So Government and community should be given attention of construct improved water source for rural community. Future studies are needed to determine the seasonal variations in the contamination level of the water sources, to quantify pathogen loads and antibiotics resistance profile in both the water sources and household container.

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7. APPENDICES

Appendix 1: Structured questionnaires to collect information about the status of drinking water quality

Village name………………code……………… and location …………………………

Dear respondents, I am a student in Bahir Dar University; I am working my research in rural in Bahir Dar town and around Bahir Dar city. I am interested in learning more about your sanitary and hygienic practices in relation to microbial and physicochemical contamination of drinking water. I hope you will help me by answering this question none of your answer will be available to anyone. Do not give you your name. All the information you give me will be kept private. We really need your honest response to better understand on sanitary and hygienic practices towards microbiological quality of water. The result of the study will help fully serves an important input to intervention programs that aim at improving drinking water quality of the communities. I thank you in advanced for taking your time to answer the question.

Part one: Drinking water handling practices, knowledge and attitudes

1. Source of drinking water
    A. Tap
    B. Hand dug well
    C. Spring
    D. River
    E. Other ………………
2. Do you use drinking water from multiple sources?
    A. Yes
    B. No
3. Does your household store drinking water in small containers? (Can you show me?)
    A. Yes
    B. No
4. If yes for question 3, what type of water container do you use?
    A. clay pot,
    B. Jerry can,

C. bucket,

D. Other………………

5. Do you cover water container?

A. yes

B. No

6. Do the drinking water container used for other purpose(s)?

A. Yes

B. No

7. Do you treat water at household level?

A. Yes

B. No

8. If yes for question 7, what types of treatment do you use?

A. filtration,

B. chlorine based,

C. boiling,

D. Other………………

9. Ways of fetching water from distant sources

A. Jerry can

B. Clay pot

C. Bucket

D. Other……

10. Do you know that water can be a vehicle to transmit diseases /waterborne diseases/)

A. Yes

B. No

11. Which of the following diseases can be transmitted through polluted drinking water?

A. Amebae

B. Giardia

C. Cholera

D. HIV

E. Diabetes

12. Is there hand washing facility at the household level?

A. Yes

B. No

13. If yes for question 12, what type of hand washing facility does the household have?
    A. Tap
    B. From storage container
    C. Other…………..
14. If not for question 12, why?
    A. Economic reasons
    B. No need
15. Is there soap or detergent at the place for hand washing?
    A. Yes
    B. No
16. Ways of deposing children stool
    A. Dumped in to the toilet
    B. Through in to the environment
    C. Through in to garbage container
17. Was there an incidence of waterborne diseases/acute diarrhea/ in the family that last less than one week?
    A. Yes
    B. No
18. Do you worry about waterborne diseases that may infect you/family from the drinking water you frequently used?

    A. Yes

    B. No

19. Which problem is most serious in your family?
    A. drinking water quality
    B. Drinking water quantity
    C. Both
    D. Other……….
    E.

Part two: Source of water and Environmentalsanitation(multiple responces are possible at some questions)

1. Source of drinking water:
    A. Tap improved(treated)
    B. unimproved(untreated)
    C. Borehole/treated
    D. Open shallow well/ hand pulled
    E. surface water/lake, pond, river/
    F. Other…………….
2. Do the family use multiple source of drinking water
    A. Yes, mention……………………….
    B. No
3. Does your household have a large storage tank?
    A. Yes B. No
4. Have there been any time in the last week/month/ when you have not been able to store sufficient water to meet your needs?
    A. Yes B. No
5. Do you use privet tap/ well/
    A. Yes B. No
6. If No. for question 5, how long does it take to go there, get water, and come back?(put in minute or hours)……………………….
7. What type of water do you use for other purpose other than drinking(washing, cooking)
    A. The one used for drinking
    B. Other mention………………
8. Who is responsible to collecting water?
    A. Mother C. Children
    B. Father D. Other
9. Where do you dispose waste water?
    A. Dispose in to sewage system C. Dispose in to garden
    B. Dispose in to pond D. Others
10. How do the households dispose solid waste?

A. Compost C. removed by other

B. Burning

11. Is live stoke (cattle, poultry and others) present?

A. Yes B. No

12. Are animal faces visible in the house?

A. Yes B. No

13. Evidence of Open defecation

A. Yes B. No

14. Do you (the family) have privet toilet?

A. Yes B. No

15. If yes for question 11, what kind of toilet facility do members of your household usually use?

A. improved

B. unimproved

16. Do the family have shared toilet?

A. Yes B. No

17. If yes for question 13, how many households in total use this toilet facility, including your own household?

18. Do you/ family member wash hands after toilet use?

A. Yes, always

B. Yes, some times

C. Not at all

D.

19. Do water collector wash her/his hands before collecting drinking water?

A. Yes B. No

20. Is there a shortage of drinking water?

A. Yes B. No

21. If yes for question 17, how frequent was the discontinuity? ………………………….

22. How much drinking water does the family get per day? (Mention in liter?

23. Do you think that the quantity of drinking water supplied is sufficient?

A. Yes B. No

24. Is there evidence of cracks in the water line? (Observation of turbid water?)

A. Yes B. No

25. Is there aesthetic discomfort on the drinking water?

A. Temperature C. taste

B. smell, D. other

26. Is the water supplied from your main source usually acceptable?

A. Yes B. No

Appendix 2 : MPN table

Table 10.5 MPN index and 95 per cent confidence limits for various combinations of positive results when five tubes are used per dilution (10 ml, 1.0 ml, 0.1 ml portions of sample)

Combination of positives	MPN index per 100 ml	95 % confidence limits		Combination of positives	MPN index per 100 ml	95 % confidence limits	
		Upper	Lower			Upper	Lower
0-0-0	<2	-	-	4-2-0	22	9.0	56
0-0-1	2	1.0	10	4-2-1	26	12	65
0-1-0	2	1.0	10	4-3-0	27	12	67
0-2-0	4	1.0	13	4-3-1 4-4-0	33 34	15 16	77 80
1-0-0	2	1.0	11	5-0-0	23	9.0	86
1-0-1	4	1.0	15	5-0-1	30	10	110
1-1-0	4	1.0	15	5-0-2	40	20	140
1-1-1	6	2.0	18	5-1-0	30	10	120
1-2-0	6	2.0	18	5-1-1 5-1-2	50 60	20 30	150 180
2-0-0	4	1.0	17	5-2-0	50	20	170
2-0-1	7	2.0	20	5-2-1	70	30	210
2-1-0	7	2.0	21	5-2-2	90	40	250
2-1-1	9	3.0	24	5-3-0	80	30	250
2-2-0	9	3.0	25	5-3-1	110	40	300
2-3-0	12	5.0	29	5-3-2	140	60	360
3-0-0	8	3.0	24	5-3-3	170	80	410
3-0-1	11	4.0	29	5-4-0	130	50	390
3-1-0	11	4.0	29	5-4-1	170	70	480
3-1-1	14	6.0	35	5-4-2	220	100	580
3-2-0	14	6.0	35	5-4-3	280	120	690
3-2-1	17	7.0	40	5-4-4	350	160	820
4-0-0	13	5.0	38	5-5-0	240	100	940
4-0-1	17	7.0	45	5-5-1	300	100	1,300
4-1-0	17	7.0	46	5-5-2	500	200	2,000
4-1-1	21	9.0	55	5-5-3	900	300	2,900
4-1-2	26	12.0	63	5-5-4 5-5-5	1,600 >1,600	600 -	5,300 -

Source: After APHA, 1992

Appendix 3 : Photo shows sanitary inspection of hand dug well and hand pump

Appendix 4: Photo shows laboratory work and results

Appendix 5 : Antibiotics resistance test