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ASSESSMENT OF BACTERIOLOGICAL LOAD FROM SHELLE RIVER AT WOLDIA TOWN, NORTH WOLLO, ETHIOPIA

BY:

YOHANNES AYALEW

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ASSESSMENT OF BACTERIOLOGICAL LOAD FROM SHELLE RIVER AT WOLDIA TOWN, NORTH WOLLO, ETHIOPIA

A thesis Submitted to Department of Biology, School of Graduate Studies Woldia University

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Applied Microbiology

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THESIS APPROVAL SHEET

As thesis research advisor, we hereby certify that, we have read and evaluated this thesis prepared, under our guidance by Yohannes Ayalew Zewdie, Entailed with "Assessment of Bacteriological Load from Shelle River Waste Water at Woldia Town, North Wollo, Ethiopia" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Applied Microbiology compiled with the regulations of the university and needs the accepted standards with respect to originality and quality. We recommend that it can be submitted as fulfilling the thesis requirement.

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As members of the Examining Board of the Final M.Sc. Open Defense, We certify that we have read and evaluated the thesis prepared by Yohannes Ayalew Zewdie, Entailed with "Assessment of Bacteriological Load from Shelle River Waste Water at Woldia Town, North Wollo, Ethiopia". and we recommend that it be accepted as fulfilling the thesis requirement for the degree of master of Science in Applied Microbiology.

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External Examiner	Signature	Date

DEDICATION

I dedicate this work to my family for their unwavering support, love, and encouragement throughout this journey. Their belief in me has been my greatest source of motivation. To my advisors thank you for your invaluable guidance and wisdom. This paper is a reflection of all the knowledge and insight you have imparted to me. I also dedicate this work to my friends for their constant encouragement and companionship, which made this academic journey more enjoyable and meaningful.

AUTHOR DECLARATION

I declare that, this master thesis entitled "Assessment of Bacteriological Load from Shelle River Waste Water at Woldia Town, North Wollo, Ethiopia**"** is my original work. I also seriously declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Yohannes Ayalew Zewdie

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Date _____

BIOGRAPHICAL SKETCHE

The author was born on December 25, 1987, in Addis Amba village, Kobo Town Ethiopia. He pursued his elementary education at Kobo Ewuket Chora (06) and Kobo Catholic Kidus Joseph School, For his secondary education, he attended Kobo High School, and Woldia General Preparatory School, In 2003Ethiopian Calendar (2011 Gregorian calendar), the author graduated with a Bachelor of Science degree in Applied Biology from Samara University. His academic journey reflects a strong passion for microbiology and environmental health, inspiring him to further his studies. The author is currently advancing his expertise in Applied Microbiology at Woldia University. His research interests focus on bacteriological water quality, its implications for public health, and sustainable environmental practices. This work aims to address critical challenges in waterborne diseases and the management of wastewater.

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ACRONYMS AND ABBREVIATION

ARGs	Antibiotic Resistant Genes
BGLB	Brilliant Green Lactose Bile
CFU	Colony Forming Units
EAEC	*Entero Aggregative E.coli*
EHEC	*Entero Hemorrhagic E.coli*
EIEC	*Entero Invasive E.coli*
EPA	Environmental Protection Agency
EPEC	*Entero Pathogenic E.coli*
ETEC	*Entero Toxigenic E.coli*
FCC	Fecal Coliform Count
FIB	Fecal Indicator Bacteria
HE	Hekton Enteric
LB	Lactose Broth
MHA	Mueller Hinton Agar
MPLs	Maximum Permissible Limits
MPN	Most Probable Number
MSA	Mannitol Salt Agar
PCA	Plate Count Agar
PW	Peptone Water
SC	Selenite Cysteine
TCC	Total Coliform Count
XLDX	Xylose Lysine Deoxy Chocolate

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ABSTRACT

The contamination of river water is a pressing environmental and public health issue, particularly in developing countries lacking adequate treatment facilities. This study evaluates the bacteriological load from the Shelle River in Woldia Town, North Wollo, Ethiopia. A laboratory-based cross-sectional study design was employed to collect 25 samples river water samples from five sites along the river from February to May 2024, Seven bacterial isolates namely: Escherichia coli, Proteus sp., Salmonella sp., Staphylococcus sp., Shigella sp., Klebsiella sp., and Enterobacter sp., each exhibiting distinct biochemical traits were identified. Notably, E. coli and Staphylococcus aureus were prevalent at 20%, indicating significant fecal contamination risks. Mean colony counts revealed alarming contamination levels, especially at the Hospital site, where E. coli reached 4.14 Log10 CFU/ml. Fecal coliform counts averaged 1.8 x 10^3 CFU/ml, highlighting critical water quality issues. The environmental competency of the identified pathogens was assessed, revealing E. coli and S. aureus showed remarkable tolerance to pH levels of 5, 7, and 9, with resilience to NaCl concentrations of 1% and 3%. Both pathogens demonstrated optimal temperature tolerance between 25°C and 37°C and exhibited high heavy metal resistance to $CdCl_2$ and $Pb(NO_3)_2$. In contrast, other pathogens showed varying levels of tolerance, with moderate resistance to extreme pH and salinity. Antibiotic susceptibility tests indicated varied resistance patterns, with E. coli showing 90% susceptibility to streptomycin, while other isolates exhibited concerning resistance trends. These results underscore the heavy bacterial contamination of the Shelle River, emphasizing the urgent need for effective wastewater treatment strategies to mitigate risks to public health and the environment.

Key Words: ***Antibiotic susceptibility, Bacterial Isolates, Fecal Contamination, Shelle River, Woldia Town***

1. INTRODUCTION

1.1Background of the Study

Water is essential for all forms of life, covering approximately 75% of the Earth's surface. Pure water is a colorless, odorless, and tasteless liquid with unique physical and chemical properties. However, many people lack access to clean and healthy drinking water, which can lead to waterborne infections and deaths (WHO & UNICEF, 2021). Microorganism-mediated water pollution is a significant global concern. Effluents from sources such as fecal matter, hospitals, industries, and cattle farms increase the bacterial load in water bodies (Pandey & Kass, 2014; Haile *et al.,* 2020).

Wastewater refers to water that has been altered by the introduction of substances, rendering it unsafe for certain uses, such as drinking (Tchobanoglous *et al*., 2003). Activities heavily rely on water, often resulting in the discharge of "waste" into water sources. Wastewaters can contain large quantities of pathogenic bacteria, posing serious risks to human health (Goel, 2006). In many parts of the world, health problems and diseases have resulted from the discharge of untreated or inadequately treated wastewater (Prüss-Üstün *et al.,* 2008). The use of wastewater for irrigation is a common practice, particularly in developing countries facing water shortages (Haj Jami *et al*., 2013).

Water bodies can be contaminated from various point and non-point sources, leading to the presence of pathogenic organisms and other toxic substances that exceed health protection standards (Mcheik *et al.,* 2018; Okereke *et al.,* 2016). Freshwater resources contaminated by microbes remain a significant global issue. However, wastewater irrigation can play an important role in enhancing urban food security and nutrition (Raschid-Sally & Jayakody, 2008). Studies show that wastewater agriculture accounts for over 50% of urban vegetable supply in parts of Asia and Africa (Thebo *et al*., 2017; Drechsel & Keraita, 2014). However, the microbial quality of irrigation water is critical, as contamination from animal or human waste can introduce pathogens into crops during pre- and post-harvest stages (Perulli *et al.,* 2019; Amoah *et al.,* 2007). It is estimated that one-tenth or more of the world's population currently eats food produced using wastewater irrigation (Win penny *et al*., 2010).

Polluted surface waters and sediments can contain a variety of pathogenic microbes, including bacteria, viruses, and protozoa.

In many developing countries, untreated wastewater is the primary source of irrigation water for producing edible fruits and vegetables (Toze *et al*., 2006). Sediments can contain 100-1000 times higher bacterial counts than the overlying water, making them a stable indicator of long-term water quality risks. Fecal indicator bacteria like *E. coli* and *Enterococcus spp*. are commonly used to monitor the microbial quality of water sources. Bacteria are among the most common microbial pathogens found in wastewater.

The practice of unintentional indirect reuse of waste water in developing countries is largely responsible for an estimated 4 billion cases of diarrhea per year, causing 2.2 million deaths, mainly in children under five (Kumar *et al.,* 2022). *Salmonellosis* and dysentery-like infections caused by pathogenic *E. coli* strains are common examples. Bacterial contamination can result from a combination of factors, including non-point sources, landscape use, transport in streams, and environmental conditions. The growth and reproduction of bacteria in River sediments can also be a source of outbreaks. *Pseudomonas spp*. is extensively present in the environment and known for their antibiotic resistance, posing the risk of spreading antibiotic-resistant genes. Effluents from various sources in developing countries can introduce emerging contaminants, including bacteria, into water bodies without prior treatment (Almakki *et al*., 2019; Laffite *et al*., 2016).

Vegetables are a staple in global diets due to their nutritional value, yet the unmonitored practice of irrigating them with wastewater poses significant risks to both human health and the environment. Contamination of vegetables and fruits can arise during irrigation, growth, harvesting, and transportation, ultimately resulting in the transmission of pathogens through consumption (Iwu, Okoh *et al.* 2019; Singh *et al*., 2008; Flimar *et al.,* 2010). Consumption of raw, wastewater irrigated vegetables and crops is a major pathway for pathogen transmission, causing diseases like gastroenteritis, cholera, and chemical toxicity (Mcheik *et al.,* 2018). In addition to pathogenic microorganisms, vegetables grown using wastewater irrigation can accumulate excessive heavy metals, posing short- and long-term health risks (Mcheik *et al*., 2018).

Water pollution is a significant global concern, as it is linked to the onset of numerous fatal diseases, responsible for the deaths of over 14,000 people every day (World Health Organization, 2023). Contaminated water sources can lead to various health issues, including cholera, diarrhea, dysentery, hepatitis A, typhoid, and polio, which disproportionately affect vulnerable populations, particularly children under five years old. The impact of water pollution extends beyond immediate health risks; it also affects economic productivity and social well-being. Access to clean water is essential for maintaining public health, and the lack of it can lead to increased healthcare costs and reduced quality of life for affected individuals and communities. Efforts to combat water pollution must focus on improving water management practices, enhancing sanitation infrastructure, and raising awareness about the importance of clean water for health and well-being. Addressing these issues is crucial for reducing the burden of waterborne diseases and ensuring a healthier future for all.

This is a growing concern in Ethiopia, where the consumption of vegetables irrigated with wastewater is increasing (Gashaye and Agriculture, 2020). The practice of unintentional indirect reuse of wastewater, which often contains harmful pathogens, poses significant health risks, particularly in regions with limited access to clean water. Consequently, the researcher aims to assess the bacteriological load of Shelle River in Woldia Town, located in the North Wollo Administrative Zone of the Amhara National Regional State, Ethiopia. Understanding the microbial content of this wastewater is crucial for addressing the public health implications associated with its use in agriculture.

1.2 Statement of the Problem

Water pollution is the contamination of water bodies (e.g. lakes, rivers, oceans, ground water). This may be defined in terms of the undesirable changes in the microbial, chemical and physical properties of water which are not favorable to all those living things utilizing water for their lives. The two basic forms of water pollution; are changing the types and amounts of materials carried by water, and altering the physical characteristics of a body of water (Gupta *et al*., 2009). Water pollution occurs in many forms, and from a wide range of sources. Agriculture may contribute to water pollution from feedlots, pastures, and croplands. Mining, petroleum drilling, and landfills may also be major sources of water pollution. Other water pollution sources, related to humans, are sanitary sewers, storm sewers, industry, and construction (Asubiojo,2016). Nutrients (Nitrogen and Phosphorus) wastewater high in nutrients entering a water body can cause harmful algal blooms that impact water quality and kills aquatic organisms. Water with too high or too low pH also presents a risk to the environment (biota) and human health when there is close human contact (Okereafor *et al*., 2020). The problem in developing countries is more alarming than that of industrialized nations. In addition to pesticides, natural phenomena such as volcanoes, algae blooms, storms, and earthquakes also cause major changes in water quality and the ecological status of water. Water pollution has many causes and characteristics. If the quality of water is changed by the presence of toxins, it becomes potentially harmful to these life forms, instead of sustaining them (Agrawal *et al*, .2010).

Water pollution is a significant global issue, with microbial contamination of freshwater resources being a major problem, particularly in developing countries facing water scarcity. Untreated wastewater is often used for irrigation, leading to the introduction of pathogens into crops and posing risks to human health and the environment. Despite the known risks, there is a noticeable dearth of research on the bacteriological content of polluted river water in developing countries. This study aims to fill this gap by examining the bacteriological load of Shelle River located in Woldia Town. This Rivers receives various sources of pollution, including industries, car washes, household waste, human and animal excreta, and waste from cafes and restaurants. Therefor the study aims to assess the bacteriological quality of the Shelle river water and provide insights into the extent of microbial contamination in the study area.

1.3 Research Questions

The study was intend to answer the following research questions.

1. What are the most prevalent pathogenic bacteria found in the river?

2. What is the status of bacterial load of Shelle riverat different points of the river?

3. What is the status of antibiotic susceptibility profile of the identified pathogenic bacteria to some common antibiotics?

1.4 Objectives of the Study

1.4.1 General objectiveassess the bacteriological quality of water in Shelle river of Woldia Town, North Wollo Zone, Amhara National Regional State, Ethiopia.

1.4.2 Specific objectives

- Todentify the most prevalent pathogenic bacteria present in the Shelle river water.
- Assess the bacterial load of Shelle River at different sampling points of the river.
- Determine the antibiotic susceptibility profile of the identified pathogenic bacteria to selected commonly used antibiotics.

1.5 Significance of the Study

The results of this study would help researchers and relevant stakeholders to develop their knowledge and raise awareness among the local community regarding the bacteriological quality of polluted water used for irrigation. This research would provide insights into the common risk factors associated with the bacterial load in polluted water, which is crucial for consumers of vegetables and fruits irrigated with this water. Furthermore, the findings might serve as baseline data for researchers interested in this area. The information might help for policy makers and the community to improve waste water management as well as promote safe irrigation practices in the study area.

Overall, this study aims to contribute to a better understanding of the bacteriological contamination of wastewater resources in Woldia Town, which is essential for protecting public health hazard and the environment.

1.6. Scope of the Study

This study focuses on the bacteriological assessment of wastewater in the Shelle River within Woldia Town. The research was conducted from February to May 2024 aiming to evaluate the bacterial contamination of the water from the Shelle River.

This includes the isolation, enumeration, and identification of the most prevalent pathogenic bacteria present in the river water. By concentrating on the bacteriological characteristics of the Shelle River water, this study aims to provide comprehensive understanding of the bacteriological quality of this water source within the specified time frame and geographical location.

2. LITERATURE REVIEW

2.1. Waste Water

Pure water is colorless, odorless, tasteless liquid with a unique set of physical and chemical properties. However, water containing various materials dissolved or suspended in it is said to be polluted and unsafe for human recreation or consumption. Water may become contaminated by two forms of pollutants: toxic chemicals or pathogenic microorganisms. Probably the largest single source of potentially pathogenic microbes is animal feces (including humans), which contains billions of bacteria per gram. Although most intestinal microbes are non-pathogenic, some cause enteric disease, such as the organisms which cause typhoid fever (*Salmonella typhi*), cholera (*Vibrio cholera*e), and bacterial dysentery (*Shigella Flexner*). Furthermore, waste water contaminated with feces of different sources are carriers of viral and protozoan pathogens that could pose human health problems ((Peacock, Chur-Hansen *et al.* 2012).

2.2 Source of Waste Water

The major source of waste water including residential, commercial districts and institutional facilities discharged from sanitary facilities (Palamuleni, 2002). Water pollutants may originate from point sources or from dispersed sources. A point-source pollutant is one that reaches water from a single pipeline or channel, such as a sewage discharge or outfall pipe. Dispersed sources are broad, unconfined areas from which pollutants enter a body of water. Surface runoff from farms, for example a dispersed source of pollution, carrying animal wastes, fertilizers, pesticides, and silt into nearby streams (Zhang *et al.*, 2003). Urban storm water drainage, which may carry sand and other gritty materials, petroleum residues from automobiles, and road deicing chemicals, is also considered a dispersed source because of the many locations at which it enters local streams or lakes. Point-source pollutants are easier to control than dispersed-source pollutants, since they flow to a single location where treatment processes can remove them from the water. Such control is not usually possible over pollutants from dispersed sources, which cause a large part of the overall water pollution problem. Dispersed-source water pollution is best reduced by enforcing proper land-use plans and development standards (Fan, 2004).

2.3. The Use of Waste Water for Crop Irrigation

In many countries, especially in developing countries, the use of wastewater for crop irrigation has gained considerable attention due to the shortage of fresh water. Agriculture is the most common area in which untreated wastewater is reused. The use of waste water for crop irrigation has further increased in recent years because it has certain advantages such as providing the essential nutrients and organic matter, saving water and nutrients, and reducing water contamination (Zhang and Sheen, 2017, Murtaza *et al*., 2010).

Wastewater also contains quite sufficient quantities of macronutrients (N, P, and K) are added to soil and highly needed for plant growth (Murtaza, Ghafoor *et al.* 2010). Therefore, it is a great temptation for poor farmers to irrigate crops with wastewater as it can reduce the crop production cost (Scott *et al.*, 2010).The global wastewater discharge reaches 400 billion m^3/year, contaminating about 5500 billion m^3 of water per year (Zhang and Chen, 2017). The nature and the quality of the wastewater used vary within and between the countries. It has been stated that 15 million m^3/day of reclaimed water is being used by approximately 44 countries for irrigation purposes (Win penny *et al.*, 2010).

The use of untreated wastewater for urban and peri-urban agriculture accounts for about 11% of all the irrigated croplands worldwide and it is estimated that about 10% of the global population consumes wastewater irrigated foods (Gashaye and Agriculture 2020). The practice of crop irrigation with wastewater is quite different in various countries. For instance, in low income countries, the practice is not well-regulated, and the environmental and economic issues are poorly understood (Murtaza *et al*., 2010). Moreover, some less developed and low-income countries such as Asia, Latin America, and Africa, use raw sewage for irrigation purpose without any treatment, while, middle- and high-income countries used wastewater for irrigation after treatment (Alobaidy, Al-Sameraiy *et al*. 2010).

There is also a considerable difference in the collection, treatment, and reuse of wastewater for crop irrigation between low and high-income countries (Murtaza *et al*., 2010).This variation among the two groups can be due to several factors such as the availability of fresh water for crop irrigation, the availability of resources to treat wastewater, the awareness among the farming community about environmental and human health issues related to wastewater crop irrigation, and the implementation of laws of wastewater use in the agriculture sector (Khalid *et al.*, 2017, Murtaza *et al.*, 2010).

Moreover, there are social, economic, and corporate issues that are also affecting the wastewater treatment and reuse for crop irrigation between low and high-income countries. Poverty is also considered an important factor behind the use of wastewater for crop irrigation, especially in less-developed countries. Farmers with small agricultural land holdings prefer to use wastewater for irrigation despite the availability of fresh water due to the low-cost of crop production by adopting this irrigation practice (Qadir, Wichelns *et al.* 2010). In some developing countries, the limited public awareness, the less commercial development of wastewater recycle/reuse, and the social setup are also some of the other key challenges faced in the area of wastewater use for the agricultural sector which clearly showed the presence of an alarming gap among the developed and developing countries on the environmentally-friendly practices and environmentally sustainable approaches regarding water treatment. Hence, there is a need at the global level to do more and shrink down this gap (Khalid *et al*., 2018).

2.4. Irrigation Water and Potential Pathogens

At the turn of the century only five organisms directly linked to food borne infections had been identified (Wadhwa, Glynn *et al.* 2002). At present more than 40 such organisms are commonly associated with illnesses contracted from food or water, with symptoms ranging from a slight fever to lethal septicemia or even the Guillain-Barre Syndrome. Despite major advances made in the preventative health arena over the last century, food borne diseases still remain a significant problem one that is grossly underestimated as many third world countries have no food safety systems in place where these cases have to be reported ((Temkin, Anderson *et al.* 2007).

The main sources of contamination of vegetables are from soil, water and air (Wadhwa *et al*., 2002), although the recent increase in the use of reclaimed water for irrigation has seen the rise of a new and definitely significant threat to the industry as well as consumer health. Over the years researchers have seen that all microorganisms can be categorized into three groups depending on the relationship in which they associate with humans (Wadhwa *et al.*, 2002). Some organisms can live inside the human body in complete harmony and are known as the “symbionts”.

These microbes will not cause infection or harm their host in any way, and in some cases may even be beneficial through the production of secondary metabolites such as Vitamin K. The second group, the "opportunists", will have no detrimental effects on their host under normal conditions, but they possess enough virulence to cause illness if the individual has a stressed immune system.

The last groups are the "pathogens". These organisms are virulent enough to infect even a healthy individual under normal physiological conditions (Wadhwa *et al*., 2002; Gourabathini *et al*., 2008). The more virulent the organism, the smaller the number of organisms needed to cause infection. As a vast number of potential pathogens can be found in just about any place on earth, it is nearly impossible to detect and quantify each type of these organisms (Dewedar & Bahgat, 1995; Savichtcheva & Okabe, 2006) and therefore "indicator" organisms are used to simplify things. An indicator is defined as an organism which shares the same habitat as the pathogens under observation. In the case of a fecal indicator, the organism should always be found in faces, it would not be able to multiply outside of the intestinal tract, it would be more or less as resistant to environmental factors and disinfectants as the pathogens themselves, a strong correlation should exist between the presence of the pathogens and the indicator, and the organism should be relatively easy and safe to cultivate and enumerate under laboratory conditions ((Savichtcheva and Okabe 2006). Total coliforms, fecal coliforms and Enterococci species are widely used as indicators of fecal contamination in water bodies (Savichtcheva & Okabe, 2006) although several intrinsic properties make them less than ideal for this purpose.

These include their rapid decay rate, their ability to proliferate outside the intestinal tract, their inability to withstand disinfectants, their shortcomings in identifying the source of fecal pollution and the fact that they can be of non-fecal origin, problems with cultivation under laboratory conditions and a low association with the presence of enteric pathogens. It can be concluded that, as yet, a single organism that can be used as an indicator of fecal pollution, has not been identified (Savichtcheva & Okabe, 2006) as the survival characteristics and decay-rates of different pathogens vary under identical environmental condition. . At this stage researchers agree that, although not ideal, fecal coliforms and *E. coli* are the best indicators of fecal pollution available.

2.5. Impacts of Wastewater Irrigation

The supply of wastewater expands with population growth in large cities, towns and villages throughout the developing world. In many communities, the volume of wastewater has increased faster than the ability to build and operate treatment facilities, and as a result more wastewater is released into open ditches or discharged into agricultural drains and the use of such wastewater for crop irrigation in the agricultural sector has the potential for both negative and positive effects on the soil productivity, crop production, and human health as it carries various viruses, bacteria, nematodes, and protozoa, which can cause different diseases (Qadir *et al.*, 2010). Wastewater may contain unwanted chemical constituents that pose health and environmental risks. The negative effects of crop irrigation with wastewater are primarily due to the presence of high total suspended and dissolved solids, high nutrient contents, and potentially toxic elements ((Li, Wang *et al*. 2017). Plants can accumulate trace elements especially heavy metals in or on their tissues due to their great ability to adapt to variable chemical properties of environment. Thus, plant intermediate reservoirs from soil and partly from water and air, move to man and animals and pose human health problems through food chain contamination as continues irrigation of crops with polluted water is responsible for the raise of heavy metals content in agricultural soil and in food crops (Li *et al.*, 2017).

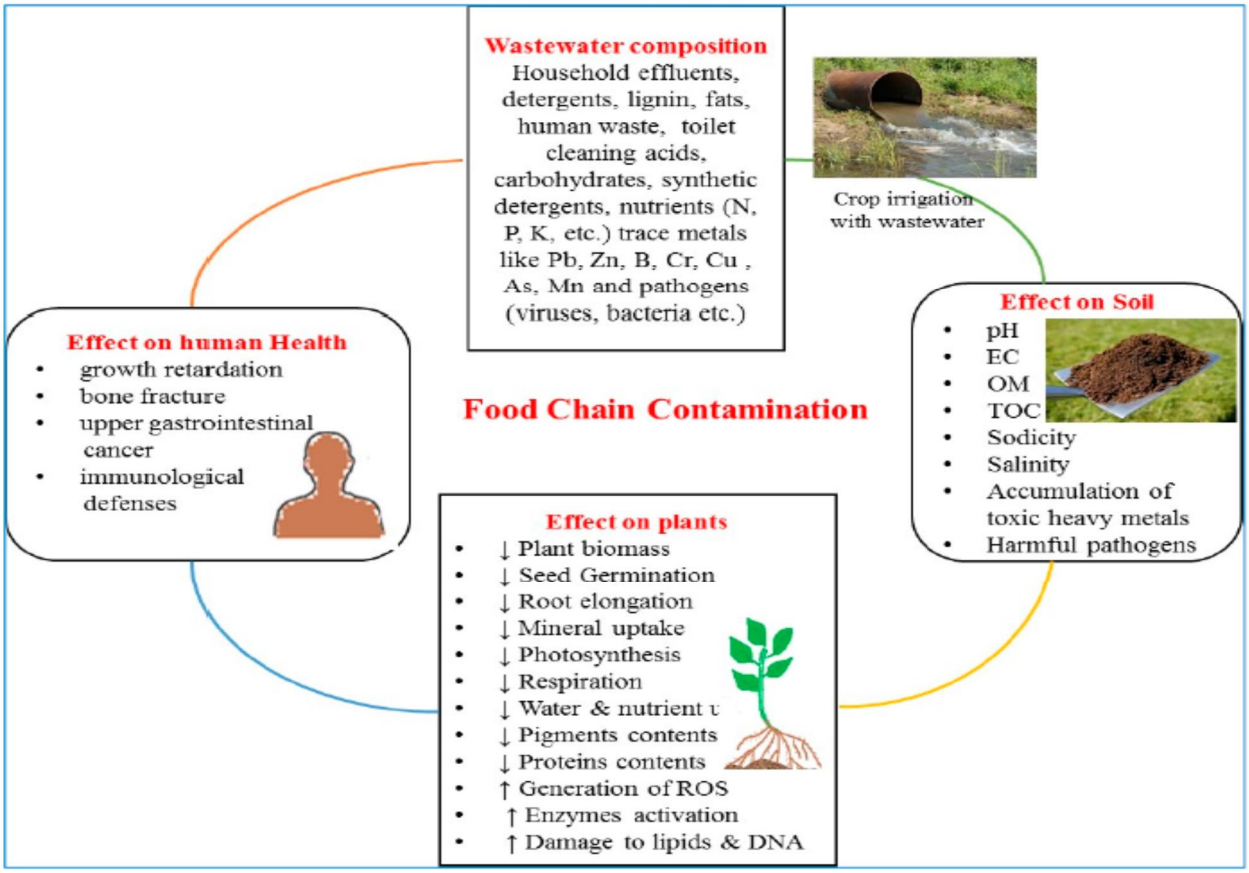

Figure1. The possible food chain contamination by wastewater irrigation (Adopted from Khalid *et al*., 2018).

2.6. The Effect of Waste water on Potentially Toxic Element Accumulation in Plants

The soil is the direct pathway for the contamination of plants by potentially toxic elements (heavy metals) via root uptake. Vegetables and crops irrigated by wastewater take up high concentrations of toxic heavy metals which may cause health risks to the consumers. Several studies have demonstrated that wastewater irrigated plants may absorb and accumulate these elements in concentrations greater than the maximum permissible limits (MPLs) with serious public health implications (Khalid, Shahid *et al.* 2018). High concentrations of Cr, Cd, Co, Pb, Cu, Zn, and Ni were reported in spinach, cabbage, radish, and forage grasses grown on sewage sludge-amended soils than the allowable level (Singh, Agrawal *et al.* 2010).

The soil-plant transfer of toxic elements after irrigation with wastewater depends on several factors relating to the soil, plant, and wastewater. Heavy metals may exist in soil in different forms such as either as free metal ion or complexed with various organic, inorganic components in the soil (Saifullah *et al*., 2015, Shahid *et al*., 2013).

The transfer of toxic elements from the soil to the plant mainly depends on their chemical speciation (Shahid *et al*., 2012). Anthropogenic application of the heavy metals via wastewater contributes their accumulation in higher level mainly in the topsoil and are usually have higher mobility and bio availability compared to those deposited from their parent material (Shahid *et al*., 2012). The uptake and accumulation of heavy metals in different parts of the plant play an important role in their health Effects. Potentially toxic elements may accumulate at high levels in plants after wastewater irrigation. The excessive concentration of toxic elements in plant tissues is capable of inducing various physiologically, morphologically, and biochemically toxic effects by disrupting the nutrient and water uptake and transport, altering the nitrogen metabolism, disrupting the activity of ATPase, reducing photosynthesis, interfering with plant growth, dysfunctioning the plant photosynthetic machinery in chloroplasts, and causing stomatal shutting (Shahid *et al*., 2014). Potentially toxic elements are also capable of inducing toxic effects to other living organisms, including human beings, at very low levels due to the absence of proper defense mechanisms to mitigate the toxic effects of these metals and to remove them from the body. Therefore, much attention is given worldwide to food safety and risk assessment (Khalid *et al*., 2018).

2.7. The Effects of Waste water Irrigation on Human Health

The irrigation of crops with wastewater poses significant risks to human health due to the presence of hazardous microorganisms found in animal and human fecal waste. Contaminated irrigation water can infiltrate potable water sources and soil, leading to the growth of unsafe plants. Understanding the source and quality of irrigation water is crucial, as poorly managed or contaminated water can expose consumers to harmful pathogens. When the quality of irrigation water is inadequate or its contamination is unmanageable, it becomes essential to implement controlled measures to mitigate the risk of crop contamination (WHO, 2012).

In many developing countries, untreated human waste is often utilized in agriculture, contributing significantly to food production and income generation in rapidly urbanizing areas. However, when these wastes are applied without proper treatment or health protection measures, there is a heightened risk of pathogen "recycling" among urban and peri-urban populations, leading to increased incidence of diseases (Ntajal, 2022). The challenge is exacerbated by the rapid increase in wastewater volume, which often outpaces the capacity to construct and maintain effective treatment facilities. Consequently, much wastewater is discharged into open ditches or agricultural drains, further heightening health risks. The use of polluted irrigation water, along with fresh animal manure, has been linked to elevated levels of total coliform contamination in vegetable samples, highlighting the potential for foodborne illnesses (Alegbeleye and Sant'Ana, 2023).

To protect human health, it is imperative that wastewater is properly treated before being used for crops intended for raw consumption. This safety measure should be coupled with good agricultural practices during the cultivation of fresh vegetables. Additionally, thorough washing of vegetables and the use of food-grade antibacterial chemicals for adequate durations can significantly reduce microbial loads and eliminate pathogens, safeguarding public health (Desta and Diriba, 2016).

2.8. Water Quality Indicator Organisms

Indicator organisms may be employed to reflect the microbiological quality of waters relative to their safety from water borne pathogens. In general, indicator organisms are most often used to assess water safety /sanitation or environmental condition (Savelli, Bradshaw *et al.* 2019). Indicator organisms are typically used to demonstrate the potential presence or absence of different groups of pathogenic microorganisms. The use of indicators is attractive because it reduces the complexity and cost of analyzing sludge or environmental media (soil, water, air) for individual pathogens (Carroll 2016). Indicator microbes are generally selected because they are abundant in the matrix, a relatively rapid, accurate and cost-effective analytical method for enumerating them is available and a reasonably strong correlation exists between the presence/absence of the indicator and a particular pathogen or group of pathogens (McKee and Cruz 2021).

The strength of the correlation will determine the effectiveness and accuracy of the indicator organisms as a measure of pathogen occurrence (New Hampshire department of environmental services, 2003). There are different groups of indicator organisms. However, aerobic Mesophiles (25-40 ^{0}C), total coliform (35-37 ^{0}C) and fecal coliforms (44 or 44.5 ^{0} C) are the well-known indicator microbes (Carroll 2016).

3. MATERIALS AND METHODS

3.1. Description of Study Site

Woldia Town is located in the North Wollo Zone of the Amhara National Regional State, Ethiopia. The town is situated at an absolute location of 11°50' N latitude and 39°36' E longitude. Woldia Town is approximately 360 km from Bahir Dar, the capital city of the Amhara National Regional State, and 520 km from Addis Ababa, the capital city of Ethiopia. The population of Woldia Town is estimated to be around 100,000 people (as of 2020 ESA data). The town is characterized by a rugged topography, with an elevation of 2112 meters above sea level and surrounded by Guba Lafto woreda. Woldia experiences an average annual rainfall of 850 mm and an average daily temperature of 22°C (Fisha Semaw 2018).

The major economic activities of the people in Woldia Town include agriculture, livestock rearing, trade, and small-scale industries. The town serves as a regional hub for commercial and administrative activities in the North Wollo Zone. The Shelle River, which is the focus of this study, is an important water resource for the local community, used for various purposes, including irrigation of agricultural lands.

3.2 Study Design and Duration of the Study

The study design for this research was a experimental-based cross-sectional study. This approach was used to evaluate the bacteriological contamination of the Shelle River over the study period from February 2024 to May 2024. In a cross-sectional study design, data were collected at a specific point in time, allowing for the assessment of the current situation regarding the bacteriological quality of the Shelle River. The laboratory-based aspect of the study involves the collection of water samples from the river, followed by the analysis of these samples in a controlled laboratory setting. This study design was chosen to provide a snapshot of the bacteriological load and profiles present in the Shelle River water within the given time frame.

The results generated from this cross-sectional assessment were to identify the prevalence of pathogenic bacteria and their antibiotic susceptibility patterns, which is crucial for understanding the potential public health risks associated with the use of this water source.

3.3. Sample Collection

Wastewater samples were collected from the septic tank before being released to the open field according to guidelines of wastewater sampling technique (APHA, 2005). For this study, a total of 25 wastewater samples were collected from the Shelle River. The samples were taken from five different sampling points along the River, with five samples collected from each point. The wastewater samples were collected following the procedures recommended by the American Public Health Association (Carroll, 2016). The samples are taken from the approximate half-depth of the Rivers to avoid collecting debris and sediments. The wastewater was collected in sterile, wide-mouth, screw-capped bottles with a capacity of 250 ml. All samples kept in ice box and transported to the Microbiology laboratory for bacterial load analysis

3.4. Isolation and Enumeration of Bacteria

For the bacterial isolation and enumeration, the following protocols were followed: From each wastewater sample, appropriate dilutions ranging from 10^{-1} to 10^{-8}were prepared. Then, using the spread plate technique, 0.1 ml of the diluted samples was streaked onto growth solid media and the inoculated plates then be incubated at 37°C for 24-48 hours.

After incubation, the bacterial colonies on the plates were counted by using colony counter (Model J2) and recorded as colony-forming units per milliliter (CFU/ml) of the wastewater sample, as per the recommendations in Carroll (2016). The plates containing 30 to 300 colonies at two consecutive dilutions (10^{-4} and 10^{-5}) were used to calculate the final results.

The number (N) of CFU/ml of the test was calculated using the following formula:

$$N = \sum C / (v \times (n1 + 0.1 \times n2) \times d)$$

Where:

$\sum C$ is the sum of colonies on all plates counted

V is the volume applied to each plate

n1 is the number of plates counted at the first dilution

n2 is the number of plates counted at the second dilution

D is the dilution factor from which the first count was obtained

The final result was rounded to two significant figures and expressed as a number between 1.0 and 9.9 multiplied by 10^x, where x is the appropriate power of 10, as recommended by Maturin and Peeler (2001) and (Wore, Stevens *et al*. 2003).

3.4.1. Mesophilic bacteria counts

From each collected wastewater sample, 100 ml was homogenized with 225 ml of 0.1% peptone water (PW) to prepare the initial homogenized sample. Serial dilutions of the homogenized sample were prepared up to the 10^{-6} dilution. From the appropriate dilutions (10^{-4} and 10^{-5}), 0.1 ml of the aliquot was spread-plated in triplicates on Plate Count Agar (PCA) plates using a sterile glass rod. The inoculated PCA plates were then incubated in an inverted position at 37°C for 24-48 hours under aerobic conditions. After the incubation period, the bacterial colonies on the plates were counted using a colony counter. The data were recorded as colony-forming units per milliliter (CFU/ml) of the wastewater sample, as recommended by Vasavada and Critzer (2015). This comprehensive procedure, involving sample homogenization, serial dilutions, and spread-plating on PCA media, ensures the accurate enumeration of the bacterial load present in the Shelle river samples. The triplicate plating and standardized incubation conditions further enhance the reliability and reproducibility of the results.

3.4.2. Staphylococcus aureus count

From each collected wastewater sample, 100 ml was homogenized with 225 ml of 0.1% peptone water (PW) to prepare the initial homogenized sample. Serial dilutions of the homogenized sample were then prepared up to the 10^{-8} dilution. Two appropriate dilutions (10^{-4} and 10^{-5}) were selected for further analysis. Using the spread plate technique, 0.1 ml of each selected dilution was inoculated onto the surface of solid Mannitol Salt Agar (MSA) plates. The inoculated MSA plates were then incubated in an inverted position at 30 ± 2°C for 24 to 48 hours. After the incubation period, the plates containing 30 to 300 yellow colonies were counted and identified as *Staphylococcus aureus*. The bacterial count was expressed as colony-forming units per milliliter (CFU/ml) of the wastewater sample, as per the recommendations by (Bennett *et al*. 2015).

3.4.3. E. coli count

From each collected water sample, 100 ml was homogenized with 225 ml of 0.1% peptone water (PW) to prepare the initial homogenized sample. The homogenized sample was then diluted up to $10^{-6.}$ From each selected dilution, 1 ml of the aliquot was inoculated into multiple test tubes (using the three-tube method) containing 10 ml of lactose broth with Durham tubes.

The inoculated lactose broth tubes were incubated at 35°C for 24 hours. After incubation, the test tubes were examined visually for gas production, which indicates the fermentation of lactose. The gas-negative tubes were further incubated for an additional 24 hours and re-examined at 48 hours. For the gas-positive tubes, a loop full of the suspension was transferred into tubes containing EC broth for confirmatory tests after 24 hours of incubation, a loopful of the suspension from the EC broth tubes was streaked onto Levine's Eosin Methylene Blue (L-EMB) agar plates. The L-EMB agar plates were incubated at 35°C for 18 to 24 hours. Colonies that appeared dark-centered and flat, with or without a metallic sheen, were considered to be *E. coli* colonies. The *E. coli* colonies on the L-EMB agar plates were counted, and the results were expressed as colony-forming units per milliliter (CFU/ml) of the water sample, as recommended by(Hunter, Erskine *et al.* 2015).

3.4.4. Salmonella-Shigella Count From each wastewater sample, 100 ml was homogenized with 225 ml of Buffered Peptone Water (BPW). The BPW-diluted samples were then incubated at 37°C for 24 hours. This pre-enrichment step allows for the metabolic recovery and proliferation of *Salmonella* cells in the wastewater samples, increasing the number of target organisms to a detectable level, as recommended by (Andrews *et al*, .2018). After the pre-enrichment in BPW, 1 ml of the sample was transferred into a tube containing 10 ml of Selenite Cysteine (SC) broth. The SC broth culture was incubated at 37°C for 24 hours. This selective enrichment step helps to inhibit the growth of non-target microorganisms, such as Gram-positive bacteria and coliforms, while allowing for the rapid multiplication of *Salmonella* species. After the selective enrichment, a loopful of the SC broth culture was streaked onto the following selective agar plates: *Salmonella-Shigella* (SS) agar, Xylose Lysine Deoxycholate (XLD) agar and Hektoen Enteric (HE) agar the inoculated agar plates were incubated at 37°C for 24 ± 3 hours. After the incubation, colonies with a black dot at the center (due to hydrogen sulfide production) were presumptively identified as *Salmonella* colonies, as per the recommendations by (Andrews *et al*.., 2018).

3.4.5. Total coliform count (TCC)

For total coliform most probable number (MPN) analysis, three consecutive dilutions of the water sample (10^{-6}, 10^{-7}, and 10^{-8}) were prepared. From each of these three dilutions, 1 ml was inoculated into 5 separate tubes containing Lactose Broth (LB) with inverted Durham's tubes. The inoculated LB tubes were incubated at 35°C for 48 hours, as recommended by Kornacki *et al.* (2015). After incubation, the LB tubes were examined for turbidity and gas production, which indicate a positive result for coliforms.

For the LB tubes showing a positive result (turbidity and gas production) a confirmatory test was carried out by taking a loop full of the suspension to tubes containing 2% Brilliant Green Lactose Bile (BGLB) broth. The BGLB broth tubes were then incubated at 35°C for 48 hours. After incubation, the BGLB broth tubes were examined for growth and gas production, which confirms the presence of coliforms. The number of positive BGLB broth tubes for each dilution was recorded. Using the MPN table provided in the Bacteriological Analytical Manual (FDA, 2010), the total coliform count was calculated and expressed as Most Probable Number per milliliter (MPN/ml), as recommended by Kornacki *et al*. (2015).

3.4. 6. Fecal coliform count (FCC)

Three consecutive dilutions of the water sample (10^{-6},$10^{-7}$and 10^{-8}) were prepared. From each of these three dilutions, 1 ml was inoculated into 5 separate tubes containing Lactose Broth (LB) with inverted Durham's tubes. The inoculated LB tubes were incubated at 35°C for 24 hours, as recommended by Kornacki *et al.* (2015). After incubation, the LB tubes showing presumptive positive results (turbidity and gas production) were gently mixed. A loop full of the culture from each positive LB tube was transferred to tubes containing Brilliant Green Lactose Bile (BGLB) broth using a sterile inoculating loop. The BGLB broth tubes were then incubated at 45.5°C for 24 hours. After incubation, the BGLB broth tubes that showed gas production were considered positive for fecal coliforms. The number of positive BGLB broth tubes for each dilution was recorded. Using the MPN table provided in the Bacteriological Analytical Manual, the fecal coliform count was calculated and expressed as Most Probable Number per milliliter (MPN/ml), as recommended by Kornacki *et al*. (2015).

3.5. Biochemical Characteristics of the Isolates

3.5.1. Gram reaction test

For this activity, a thin smear of the bacterial isolate was prepared on a clean glass slide using sterile distilled water. The smear was allowed to air-dry and then heat-fixed. The fixed smear was flooded with crystal violet stain and allowed to stand for 1 minute. The slide was then washed with tap water to remove the excess crystal violet. Gram's iodine solution was applied to the slide and allowed to stand for 1 minute, acting as a mordant. The slide was washed with 95% ethanol to decolorize the sample. The slide was immediately washed with tap water to stop the decolonization process. Finally, Safranine counterstain was added to the slide and allowed to stand for 1 minute. The slide was washed with tap water, air-dried, and viewed under a microscope using the 100x oil immersion objective. Gram-positive bacteria were appear purple or blue in color, while Gram-negative bacteria appeared pink or red in color. This detailed protocol, based on the description provided by Dimir *et al*. (2020), outlines the step-by-step procedure for performing the Gram staining technique to determine the Gram reaction type of the bacterial isolates.

3.5.2. Triple sugar fermentation test

The test medium was prepared containing 0.1% glucose, 1% lactose, and 1% sucrose. A colony of the bacterial isolate was picked using a sterile inoculating needle and inoculated by stabbing into the TSI agar slant. The inoculated TSI tube was incubated at 37°C overnight with the cap loosened. After 24 hours of incubation, the TSI tube was examined for the following: Carbohydrate fermentation: A yellow color in the butt (bottom) of the tube indicated that the organism could ferment all three sugars (glucose, lactose, and sucrose). H_2S production: A black precipitate in the butt of the tube indicated the production of hydrogen sulfide (H_2S) by the organism. CO_2 production: Cracking or splitting of the medium indicated the production of carbon dioxide (CO_2) by the organism. If the slant (upper part) and butt of the TSI tube appeared red, it indicated that the organism was a non-fermenter of the tested carbohydrates.

3.5.3. Catalase test

A clean glass slide was taken and a drop of 3% hydrogen peroxide (H_2O_2) solution was placed on the slide. Using a sterile inoculating loop, a small amount of the bacterial colony from the nutrient agar plate was transferred and mixed into the hydrogen peroxide drop. The slide was observed for the formation of bubbles within 5-10 seconds. A positive catalase test was indicated by the rapid evolution of oxygen bubbles within 5-10 seconds. This showed that the bacterial isolate produced the catalase enzyme, which decomposes hydrogen peroxide and a negative catalase test resulted in no bubble formation, indicating the absence of the catalase enzyme in the bacterial isolate.

3.5.4. Coagulase test

A dense suspension of the bacterial isolate was made on a clean glass slide. A drop of citrate plasma was added to the bacterial suspension and mixed well, then, the slide was observed for agglutination or clumping of the cocci within 5-10 seconds. A positive result indicated the presence of bound coagulase, typical of *S. aureus*. A control suspension without plasma was used to rule out any auto-agglutination.

Tube coagulase test detects the presence of free coagulase. The bacterial isolate was inoculated into a tube containing plasma. The tube was incubated and observed for the formation of a solid gel clot, indicating a positive result for *S. aureus.* While most *S. aureus* strains produce both free and bound coagulase, some strains may only produce bound coagulase. Free coagulase is heat-labile, while bound coagulase is heat-stable. The slide coagulase test is used for screening, while the tube coagulase test is used for confirmation of *S. aureus* identification. As described by (Kannan, Rajasekaran *et al*. 2023) the coagulase test is a key biochemical test for the identification of *Staphylococcus aureus.*

3.5.5. Citrate test

Citrate Agar Composition (Ammonium hydrogen phosphate: 1.0 g, Di-potassium hydrogen phosphate: 1.0 g, Sodium chloride: 5.0 g, Sodium citrate: 2.0 g, Magnesium sulfate: 0.2 g, Bromothymol blue: 0.08 g, Agar: 13.0 g, and Distilled water: 1000 mL) was used for citrate test. Using a straight wire, a loop full of the bacterial colonies was inoculated into a Simmons Citrate Agar slant. The inoculated slant was incubated overnight at 37°C. A positive citrate utilization test was indicated by a change in the medium color from green to Prussian blue. A negative citrate utilization test showed no growth and the medium remained green.

3.5.6. In dole Test.

For the purpose, inoculated tube of peptone water broth with a loopful of the bacterial culture using a sterile inoculating needle was used. An inoculated peptone water broth tube was used as a control and both tubes were incubated at 37°C for 24-48 hours. After incubation, 1 mL of Kovac's reagent was added to both the inoculated and control tubes and the tubes were shaked and allowed to stand for 10-15 minutes. A positive iodole test is indicated by the development of a cherry-red color in the top layer of the inoculated tube, while a negative iodole test showed no color change, and the top layer of the inoculated tube remains colorless. As described by Ban Mahmood and Aziz (2021), the indole test is a useful biochemical test for the identification of microorganisms that can produce the tryptophanase enzyme.

3.5.7. Methyl Red Test

Pure bacterial cultures under investigation were inoculated into tubes containing MR-VP (Methyl Red-Voges-Proskauer) and then, Incubated at 35°C for 24-48 hours. After incubation, approximately 5 drops of methyl red indicator solution was added to the tubes. Interpretation: If the color of the medium changes to a stable red within a few minutes, the test was considered positive for the Methyl Red test. If the indicator turns yellow, the test was considered to negative, indicating the organism did not produce and maintain a low pH from glucose fermentation.

3.5.8. Voges - Proskauer test

Tubes containing MR-VP (Methyl Red-Voges-Proskauer) broth with the pure bacterial culture under investigation were inoculated, and Incubated at 35°C for up to 24 hours. After incubation, approximately 5 drops of Barrett’s reagent (a mixture of 5% α-naphthol and 40% potassium hydroxide solutions) was added to the tubes and the tubes were shacked gently to mix the reagent.

A positive VP test is indicated by the formation of a pink-red complex in the medium, while a negative VP test shows no color change, and the medium remains colorless.

3.5.9. Urease Test

As described by Tindal *et al*., (2007), the urease test is an important biochemical assay for identifying microorganisms that possess the ability to hydrolyze urea.

A fresh 18-24 hour old bacterial culture was inoculated onto the surface of urea agar slants, and then incubated at 35°C with the caps loosely closed.

The color change of the medium was observed at 8 hours, 12 hours, and 24 hours of incubation. A positive urease test is indicated by the development of a deep pink or red color in the medium, signifying an increase in pH due to urease production. A negative urease test shows no color change, and the medium remains yellow, indicating the lack of urease activity.

3.6. Eco physiological test for Isolates

To evaluate the pH tolerance of *Escherichia coli, Staphylococcus aureus, Shigella* spp., *Salmonella* spp., Klebsiella spp., Proteus spp., and Enterobacter spp., culture media with varying pH levels (3, 5, 7, 9, and 11) were prepared using appropriate buffers. Each medium was inoculated with the bacterial strain and incubated under optimal growth conditions, with growth monitored using spectrophotometry or colony-forming unit (CFU) counts (MOUSUM, 2021). For salt tolerance, media containing increasing concentrations of sodium chloride (1%, 3%, 5%, 7%, and 10%) were utilized. Similarly, inoculation was performed, and growth was monitored over time (Sharma, Kulkarni et al. 2016).

Temperature tolerance was assessed by incubating bacterial cultures at a range of temperatures (10°C, 25°C, 37°C, 42°C, and 50°C) in nutrient-rich media. Growth rates were measured using optical density (OD600) or CFU enumeration (Eze, El Zowalaty et al. 2019).

Heavy metal tolerance was determined by adding specific concentrations of heavy metal salts (100 µg/mL cadmium chloride, 500 µg/mL lead nitrate and 50 µg/mL mercury chloride) to the media. A series of increasing concentrations was prepared, inoculated with the test organisms, and growth inhibition or tolerance was assessed. Controls without the stress factor were included in all tests for comparison, and experiments were conducted in triplicate for statistical reliability (Lima e Silva, Carvalho et al. 2012).

3.7. Antibiotic Susceptibility Test of the Isolates

As mentioned by Nur (2020) and Hinton Jr (2016), the disk diffusion method provides a standardized and reliable way to assess the antibiotic susceptibility profile of bacterial isolates. The Kirby-Bauer disk diffusion method was used to determine the antibiotic susceptibility of bacterial isolates. By prepare a bacterial suspension in 0.5% saline, adjusting the turbidity to match the 0.5 McFarland standard was prepared and standardized bacterial suspension with OD600 = 1.0 generally corresponds to ~8 x 10^8 cells/mL for *E. coli*, but this varies by species and instrument and was inoculated onto the surface of (Muller Hilton Agar) using a sterile cotton swab, creating a lawn.

According to CLSI (2019)The breaking point/standard to determine the breaking point of the inhibition zone diameter so as to categorize the isolates in to resistant(less thanfifteen , susceptible (greater than 21) and intermediate between (16-20mm).

Antibiotics (gentamycin (10µg) erythromycin (15µg), tetracycline (30µg), ciprofloxacin (5µg), amoxicillin (10µg, streptomycin (30µg), and cephalothin (30µg)) impregnated on paper disks were placed on an inoculated agar plate, and the zone of inhibition around each disk was measured to infer susceptibility..Then, aseptically place the antibiotic disks (10-30 µg concentrations) were aseptically placed onto the MHA surface using sterile forceps, gently pressing them down and incubated at 35-37°C for 18-20 hours. After incubation, measure the diameter of the clear zones of inhibition around each antibiotic disk. The size of the inhibition zone corresponds to the susceptibility of the isolate to that particular antibiotic. A larger zone indicates susceptibility, whiles a smaller or absent zone indicates resistance.

3.8. Data Analysis

The results from the various experiments were systematically organized into tables and figures. This facilitated the clear presentation and visualization of the data. The data was analyzed using SPSS software version 22. Descriptive statistics were employed to summarize the data. For experiments conducted in triplicates, the average values were considered for the analysis. The countable dilutions were used to calculate the average number of colonies in terms of colony forming units per milliliter (CFU/mL) and a p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

4. RESULTS AND DISCUSSION

4.1 Identification and Biochemical Test of Isolates

The data presented in Table 1 were analyzed to gain insights into the characteristics of seven bacterial isolates identified through various biochemical tests. Each isolate displayed distinct phenotypic and metabolic traits, contributing to our understanding of their potential pathogenicity and environmental role. Isolate WUSR1 was identified as *Escherichia coli*, categorized as a rod-shaped, Gram-negative bacterium. Observations indicated that gas and acid production occurred during fermentation on Triple Sugar Agar (TSA), reflecting significant metabolic activity. While the iodole test was performed, the results were not consistently documented; however, positive results were obtained for both the citrate and catalase tests. Notably, the methyl red and Voges-Proskauer tests were not conducted. Overall, these findings align with previously documented characteristics of *E. coli*, confirming its metabolic profile. The identification of WUSRS1 as *Escherichia coli*, a rod-shaped and Gram-negative bacterium, is consistent with findings reported in various studies. Significant metabolic activity indicated by gas and acid production on Triple Sugar Agar (TSA) is a well-documented characteristic of *E. coli* (Berkley *et al*., 2005; Kaper *et al.*, 2004). The positive citrate and catalase tests further corroborate its established metabolic profile, reflecting classic biochemical behavior (Madigan *et al.*, 2015).

Isolate WUSR2 was identified as *Proteus* sp., recognized for its circular and Gram-negative morphology. This isolate exhibited positive results for H_2S production, gas production, and acid production on TSA, confirming its metabolic capabilities. The indole and citrate tests also yielded positive outcomes, while the methyl red and Voges-Proskauer tests were not performed. The results were consistent with existing literature on *Proteus* species, particularly regarding their known fermentation patterns and metabolic activity. This aligns with known properties of *Proteus mirabilis* and other *Proteus* species, which are well-established for their ability to produce H_2S in culture media and demonstrate various fermentation patterns (Friedman *et al.*, 2008; Watanabe *et al.*, 2009). The results of the indole and citrate tests further reflect the metabolic versatility associated with *Proteus* species (Gurung *et al.*, 2015).

Isolate WUSR3 was categorized as *Salmonella* sp., a rod-shaped, Gram-negative organism. It demonstrated positive results for H2S production, gas production, and acid production, suggesting its pathogenic potential. Positive reactions were also observed for the indole and citrate tests, whereas the Voges-Proskauer test was not conducted. These findings corroborate previous studies highlighting the prevalence of *Salmonella* in clinical and environmental samples, further emphasizing its public health significance. The biochemical characteristics of WUSR3 as *Salmonella* sp. match substantial literature that emphasizes its pathogenic potential, especially regarding H_2S, gas, and acid production on TSA. Previous studies have reported similar findings, indicating the prevalence of *Salmonella* in both clinical and environmental niches, particularly in Ethiopia, where it is a significant concern in food safety and public health (Tameru *et al.*, 2013; Thakur *et al.*, 2018).

Isolate WUSR4 was classified as *Staphylococcus* sp., characterized by an irregular shape and Gram-positive nature. This isolate exhibited positive results across all biochemical tests, including the coagulase, methyl red, and Voges-Proskauer tests. Such comprehensive positivity suggests a strong likelihood of pathogenicity, aligning with existing knowledge of *Staphylococcus* strains being associated with various human infections. WUSR4's classification as *Staphylococcus* sp. showcases consistent positive results across biochemical tests, implicating its potential pathogenicity. *Staphylococcus* strains, particularly *Staphylococcus aureus*, are known for their ability to cause various infections due to their coagulase-positive nature (Weller & Lentz, 2019; DeLeo *et al*., 2010). The comprehensive positivity in biochemical tests supports the characterization of pathogenic staphylococci, reinforcing their clinical relevance.

Isolate WUSR5 was identified as *Shigella* sp., a circular, Gram-negative organism. Negative results for H_2S production were recorded, while gas and acid production were positive. The methyl red test also yielded positive results, indicative of mixed acid fermentation. The coagulase test was not performed for this isolate. Existing literature acknowledges *Shigella* as a significant public health concern, supporting the findings from this analysis and underlining the importance of monitoring this pathogenic bacterium. The identification of WUSR5 as *Shigella* sp. aligns with findings that highlight this genus as a significant public health concern in Ethiopia and elsewhere.

The negative H_2S production coupled with positive gas and acid production is characteristic of *Shigella*, which typically undergoes mixed acid fermentation (Liu *et al*., 2015). Existing literature imparts a pressing need for continuous surveillance of *Shigella* species, given their association with dysentery outbreaks (Tammara *et al*., 2020).

Isolate WUSR6 was recognized as *Klebsiella* sp., described as circular and Gram-negative. This isolate recorded negative results for H_2S production but showed positive outcomes for gas production, acid production, and the methyl red tests. The coagulase test was not conducted, which leaves some uncertainty regarding its pathogenic capacity. The metabolic pathways described for this isolate appeared to contrast with previous studies, suggesting further investigation into the diversity of *Klebsiella* in the region is warranted. The recognition of WUSR6 as *Klebsiella* sp. adds an intriguing aspect to the research, especially given the negative H_2S production. This finding contrasts with studies indicating diverse metabolic pathways in various *Klebsiella* strains (Patil *et al.*, 2017). The differing results highlight the need for further investigation into local *Klebsiella* diversity in Ethiopia to better understand their pathogenic and environmental roles (Nash *et al*., 2020).

Lastly, isolate WUSR7 was classified as *Enterobacter* sp., characterized as a rod-shaped, Gram-negative bacterium. This isolate exhibited positive results for H_2S production, gas production, acid production, and the methyl red test. The coagulase test was not performed, which limits the understanding of its pathogenic potential. These findings are consistent with literature that recognizes *Enterobacter* as a common inhabitant of various environments, highlighting its potential role in both ecological and clinical settings. WUSR7's classification as *Enterobacter* sp. aligns with existing literature that recognizes this genus as common in various environments, capable of causing opportunistic infections (Jo *et al*., 2016). The positive results in biochemical tests, particularly for H_2S production and acid production, support its versatile metabolic capabilities, emphasizing the need for awareness regarding its potential pathogenicity (Nazari *et al.*, 2020). In conclusion, the analysis of these seven bacterial isolates provided substantial insights into their biochemical characteristics and potential pathogenic behaviors. The results reinforce the importance of continued surveillance and study of these microorganisms in both clinical and environmental contexts, as understanding their traits can aid in public health measures and inform treatment protocols.

Table1. Biochemical Characteristics of Isolates From Shelle River of Woldia Town in 2024

			TSA Fermentation									
Isolates	Shape	Grams test	H_2S Production	Gas Production	Acid production	Indole test	Citrate test	Catalase test	Methyl red test	Vogues proskauer test	Coagulase Test	Probable bacteria
WUSR1	Rod	Gm^{-ve}	_	+	+	+	_	_	+	_	_	*E.coli.*
WUSR2	Circular	Gm^{-ve}	+	+	+	+	_	_	+	_	_	*Proteus sp.*
WUSR3	Rod	Gm^{-ve}	+	+	+	+	+	_	+	_	_	*Salmonella sp.*
WUSR4	Irregular	Gm^{+ve}	+	+	+	+	_	+	+	+	+	*Staphylococcus sp.*
WUSR5	Circular	Gm^{-ve}	-	+	+	+	+	-	+	_	+	*Shigella ps.*
WUSR6	Circular	Gm^{-ve}	-	+	+	+	+	-	+	_	-	*Klebsiella sp.*
WUSR7	Rod	Gm^{-ve}	+	+	+	+	-	-			-	*Enterobacter sp*

Key: (-) negative result, (+) for positive, ($Gm^{+ve)}$ for gram positive (Gm^{-ve}), for gram negative, (TSA) Triple Sugar iron Agar.

4.2. Prevalence of Isolates from the Shelle River

In Table 3, the frequency and incidence of isolates from the Shelle River were presented. The isolates were recorded along with their corresponding frequency and occurrence percentages. It was shown that *E. coli* and *Staphylococcus aureus* each accounted for 5 occurrences, reflecting a frequency of 20%.

Additionally, *Klebsiella* was noted with 4 occurrences, resulting in a frequency of 16%. *Shigella*, *Salmonella*, and *Proteus* were each observed 3 times, equating to a frequency of 12% for each. Finally, *Enterobacter* was recorded 2 times, which represented a frequency of 8%. Thus, the data illustrated the diversity and prevalence of the bacterial isolates present in the Shelle River.

The prevalence of *Escherichia coli* and *Staphylococcus aureus* in the Shelle River, each recorded at a frequency of 20% with five occurrences, underscores a troubling trend that has been observed in various studies throughout Ethiopia. Specifically, research conducted by Egeda and Taye (2018) highlighted *E. coli* as a predominant indicator of fecal contamination in rivers within Addis Ababa. This finding underscores the critical importance of monitoring *E. coli* levels in assessing water quality. The pollution stemming from both human and animal sources significantly impacts the integrity of water bodies, posing serious health risks to communities that rely on these sources for their daily needs.

In addition to *E. coli*, the presence of *Klebsiella* in the Shelle River at a frequency of 16% is consistent with findings from other studies in the region. For instance, (Beyene *et al.*2019) reported the isolation of *Klebsiella pneumonia* in untreated water supplies, demonstrating its ability to survive and proliferate in contaminated environments. This prevalence points to critical water sanitation challenges faced in various Ethiopian regions, particularly in areas where the local population depends on surface water for drinking and domestic purposes. The persistence of such pathogens highlights the urgent need for effective water treatment and sanitation measures to safeguard public health. Furthermore, the occurrence of *Shigella* and *Salmonella*, both noted at a frequency of 12%, validates other research suggesting that these pathogens are commonly found in contaminated water sources across Ethiopia.

A study by Belayneh and Wondimu (2019) reported a high incidence of *Shigella* and *Salmonella* in drinking water, signaling significant public health hazards linked to inadequate water treatment practices. Given the implications for waterborne diseases, this situation emphasizes the necessity for improved surveillance and management strategies aimed at controlling the spread of these enteric pathogens and protecting community health. Lastly, the detection of *Proteus* and *Enterobacter*, noted at frequencies of 12% and 8% respectively, echoes findings from Kebede and Alemayehu (2017), who isolated a variety of Gram-negative bacteria from local river systems.

The presence of these microorganisms is often indicative of fecal contamination, further suggesting that the water quality in the Shelle River may be significantly compromised due to insufficient sanitation infrastructure. This situation presents a compelling need for comprehensive sanitation improvements and public health initiatives to enhance drinking water safety in Ethiopia.

Table 2.The Frequency and the Incidence Isolates from Shelle Stream

Isolates	Frequency	Occurrence in percentage
E.coli	5	20
S.aureus	5	20
Shigella	3	12
Salmonella	3	12
Klebsella	4	16
Proteus	3	12
Enterobacter	2	8

4.3. Mean Count of Bacterial Isolates from Water Samples

In Table 4, the mean colony count of bacterial isolates from different sampling points of the Shelle River was presented. The data demonstrated varying levels of contamination across several locations, which were categorized by contamination levels expressed in Log10 values. The isolates of *E. coli* were counted, revealing values that ranged from 1.8 at the Teaching College to 4.14 at the Hospital site.

This level of fecal coliform presence is concerning, as *E. coli* serves as an indicator of fecal contamination and potential pathogenic bacteria in water (Apun *et al.*, 2008). The high levels observed at the Hospital could suggest localized contamination sources, such as waste discharge. According to the World Health Organization (WHO), the acceptable limit for *E. coli* in drinking water is zero, highlighting the significance of these findings (WHO, 2017). Similarly, the mean counts for *S. aureus* indicated a maximum of 4.33 at the Hospital and a minimum of 2.57 at the Teaching College. *S. aureus* is notorious for causing foodborne illnesses and is often associated with poor hygiene conditions (Fertner *et al.*, 2019). The high counts in the Hospital may indicate environmental reservoirs or inadequate hygiene practices that require further investigation.

The *Shigella* counts showed significant contamination, with a peak value of 5.2 at the Hospital and a low of 2.01 at the Teaching College. The presence of *Shigella* in environmental water has been linked to outbreaks of shigellosis, primarily spread through contaminated water (Bottone, 2010). The detection of 2.01 at the Teaching College still reflects a concerning level, as even low counts can pose public health risks, especially in susceptible populations.

The presence of *Salmonella* was recorded with a maximum count of 3.7 at the Hospital and a low of 1.67 at the Teaching College. The presence of *Salmonella*, associated with gastrointestinal illness, is significant and can be indicative of broader issues of environmental contamination and public health (Bennett *et al.*, 2019). Additionally, *Klebsiella* was isolated with the highest level found at the Hospital (4.21) and the lowest at the Teaching College (2.75). *Klebsiella* species are known to be opportunistic pathogens that can cause severe infections (Patel et al., 2019). The decreased count at the Teaching College (2.75) still reflects a potential risk, particularly in immune compromised individuals. The count for *Proteus* varied significantly, reaching 5.1 at the Hospital and decreasing to 2.54 at the Teaching College. Lastly, *Enterobacter* was detected with counts ranging from 1.87 at the Teaching College to 3.57 at the Hospital. *Proteus* species are often found in polluted waters and can indicate fecal contamination (Sokouti *et al.*, 2016). Lastly, the presence of *Enterobacter* is noteworthy as it can be part of a spectrum of pathogens, sometimes associated with nosocomial infections (Nicolle *et al*., 2020).

Overall, the results were analyzed, and it was concluded that substantial microbial contamination was identified in various locations. This analysis underscored the importance of monitoring water quality and highlighted potential public health risks associated with increased bacterial counts in water sourced from the Shelle River.

Table3. Mean Colony Count of Bacterial Isolates from Different Sampling Points of Shelle River

Isolates	Sampling site and contamination level in Log1o value				
	Hospital	Menehariya	Save the children	Health center	Teaching college
E.coli	4.14	3.4	2.6	2.3	1.8
S.aureus	4.33	4.05	3.02	2.88	2.57
Shigella	5.2	4.81	3.75	2.42	2.01
Salmonella	3.7	3.21	2.85	2.45	1.67
Klebsella	4.21	4.01	3.87	3.5	2.75
Proteus	5.1	3.9	3.55	2.97	2.54
Enterobacter	3.57	3.42	2.95	2.43	1.87

4.4 Fecal Coliform Count

In Table 5, the total fecal coliform counts from various sampling sites along the Shelle River were compiled, and mean values were calculated. The data were characterized in terms of colony-forming units per milliliter (CFU/ml). The fecal coliform count at the Hospital was noted to have a range from 1.9×10^2 to 2.8×10^3, with a mean value calculated at 2.1×10^3 CFU/ml. The high counts observed at the Hospital site (2.1×10^3 CFU/ml) suggest a critical area for intervention. Hospitals often generate significant waste, including medical waste and human waste, which can contaminate nearby water bodies if not managed properly. Research indicates that improper waste disposal practices can lead to serious health risks, with contaminated water sources being a common pathway for diseases such as cholera and dysentery (Corvine *et al*., 2020).

In the context of Ethiopia, where waterborne diseases remain a leading cause of morbidity and mortality, addressing sources of contamination from healthcare facilities is vital (Ethiopian Public Health Institute, 2019).

At the Menehariya site, the mean fecal coliform count ranged from 1.3 x 10^2 to 2.6 x 10^3, resulting in a mean of 2.0 x 10^3 CFU/ml. This reinforces findings from studies in similar Ethiopian settings, where rural and peri-urban areas have been shown to struggle with water quality issues due to a lack of infrastructure and inadequate sanitation practices (Hassan *et al.*, 2018).

In the Save the Children sampling site, the total fecal coliform count ranged from 2.0 x 10^2 to 3.0 x 10^3, leading to a calculated mean value of 2.2 x 10^3 CFU/ml. additionally, the Save the Children sampling site recorded the highest count, indicating potential contamination from nearby human activities or sanitation-related issues. Research has shown that areas with high human density and poor sanitation facilities are particularly vulnerable to fecal contamination in water sources (Fadnes *et al.*, 2015).

For the Health Center, the counts ranged from 1.3 x 10^2 to 3.0 x 10^3, with a mean value established at 1.9 x 10^3 CFU/ml. Finally, the Teaching College recorded counts that varied from 0.9 x 10^2 to 2.2 x 10^3, resulting in a mean value of 1.6 x 10^3 CFU/ml. Health Centers, while intended to provide healthcare, can inadvertently contribute to water pollution if waste management systems are not effectively implemented (Moges *et al.*, 2019).

Educational institutions, often frequented by large numbers of students, can also play a role in spreading contamination if sanitary practices are inadequate. The overall means of means across the sampling sites were assessed, leading to a mean value of 1.8 x 10^3 CFU/ml. The findings indicated notable levels of fecal coliform contamination across all sampling locations, highlighting potential public health risks associated with water quality in the Shelle River.

Table 4: Total Fecal Coliform Count Obtained from Selected Shelle River Sample Sites

Sample site	Minimum mean value (CFU/ml)	Maximum mean value (CFU/ml)	Mean Value(CFU/ml)
Hospital	$1.9x10^2$	$2.8x10^3$	$2.1x10^3$
Menehariya	$1.3x10^2$	$2.6x10^3$	$2.0x10^3$
Save the Children	$2.0x10^2$	$3.0x10^3$	$2.2x10^3$
Health Center	$1.3x10^2$	$3.0x10^3$	$1.9x10^3$
Teaching College	$0.9x10^2$	$2.2x10^3$	$1.6x10^3$

4.5. Eco-Physiological Characteristics of Identified Pathogens

The environmental competency of the identified pathogens was analyzed based on their tolerance to diverse conditions (Table 5). Each pathogen exhibited unique characteristics influencing its ability to survive in challenging environments. *E. coli* demonstrated remarkable environmental adaptability, showing significant tolerance to pH levels of 5, 7, and 9, with resilience to NaCl concentrations of 1% and 3%. However, its tolerance decreased at higher salt concentrations. Temperature tolerance was optimal between 25°C and 37°C, but a notable decline was observed at 42°C and 50°C. Additionally, *E. coli* exhibited high tolerance to heavy metals such as $CdCl_2$ and $Pb(NO_3)_2$, signifying its capability to persist in contaminated environments. These observations are consistent with prior studies, which highlight the adaptive mechanisms of *E. coli* in various settings, especially in polluted water sources (Ibekwe et al., 2017; Yang et al., 2021). Similarly, *S. aureus* exhibited high environmental resilience. It tolerated pH levels of 5, 7, and 9 effectively but showed low tolerance at extreme pH values (3 and 11). Its resilience extended to NaCl concentrations of 1% and 3%, with optimal growth temperatures between 25°C and 37°C. Heavy metal tolerance for $CdCl_2$ and $Pb(NO_3)_2$ was also significant, reflecting its ability to withstand environmental

stressors. Previous research supports these findings, noting *S. aureus's* persistence in high-salinity and metal-contaminated environments (da Silva Fernandes et al., 2021; Otto, 2022).

Table5.pH, NaCl, Temperature and Heavy Metals Resistance of the Identified Pathogens

Identified pathogens	pH					NaCl (%)					Temperature (^{0}C)					Heavy Metals (µg/ml)		
	3	5	7	9	11	1	3	5	7	10	10	25	37	42	50	$CdCl_2$ (100)	$Pb(NO_3)_2$ (500)	$HgCl_2$ (50)
E. coli	-	++	+++	++	+	+++	++	+	-	-	-	++	+++	+	+	+++	++	+
S. aureus	-	++	+++	++	+	+++	+++	+	-	-	-	++	+++	+	+	+++	+++	+
Shigella	-	+	+++	+	-	++	+	-	-	-	-	++	+++	-	-	+	-	_
Salmonella	-	++	+++	++	+	+++	++	+	-	-	-	++	+++	-	-	+++	++	_
Klebsiella	-	+	++++	-	-	++	++	-	-	-	+	++	+++	+	-	+	+	_
Proteus	-	+	+++	-	-	++	+	-	-	-	-	+	+++	+	+	+	+	+
Enterobacter	-	+	+++	-	-	++	+	-	-	-	-	+	+++	+	-	+	+	+

"-"= No growth, "+" = slight growth, "++" = Moderate growth, "+++" = high growth

Salmonella demonstrated considerable environmental competency, tolerating pH levels of 5, 7, and 9 but displaying sensitivity to extreme pH levels (3 and 11). Its tolerance to NaCl concentrations was moderate, and its optimal temperature for survival was up to 37°C. The pathogen also exhibited high resilience to heavy metals, including $CdCl_2$ and $Pb(NO_3)_2$. Studies have confirmed *Salmonella's* ability to adapt to various environmental niches, facilitating its survival and transmission (Wang et al., 2018; Fong et al., 2020).

Klebsiella displayed notable tolerance to pH 7 and moderate tolerance at pH 5, with limited resilience to high NaCl concentrations. It thrived across a range of temperatures but exhibited sensitivity at 50°C. Moderate tolerance to heavy metals was observed, especially for $CdCl_2$ and $Pb(NO_3)_2$, indicating its potential for survival in contaminated environments. These findings align with research highlighting *Klebsiella's* adaptability, particularly its resistance to heavy metals and environmental stressors (Podschun & Ullmann, 1998; Hoque et al., 2020). In contrast, *Shigella*, *Proteus*, and *Enterobacter* demonstrated moderate environmental

competency. *Shigella* showed high tolerance at pH 7, moderate resilience to NaCl, and low heavy metal tolerance.

Proteus exhibited moderate tolerance across various conditions but lacked the adaptability of other pathogens. *Enterobacter* displayed high tolerance at pH levels of 5, 7, and 9, with moderate resilience to NaCl and heavy metals. These behaviors highlight their varying levels of environmental adaptability and persistence in adverse conditions, as documented in earlier studies (Mukherjee et al., 2016; Dsouza et al., 2020). The environmental competency of these pathogens underscores their ability to survive and thrive under challenging conditions, emphasizing the importance of understanding their adaptability for public health and environmental management. Their persistence in diverse environments has significant implications for transmission and contamination in human and animal populations.

4.6. Antibiotic Susceptibility Test of the Isolates

The results of the antibiotic sensitivity test were analyzed and interpreted based on the susceptibility patterns of the tested bacterial isolates. The findings provided insight into the effectiveness of various antibiotics among isolates. The isolates were subjected to antibiotic sensitivity testing against gentamycin (10 µg), erythromycin (15 µg), tetracycline (30 µg), ciprofloxacin (5 µg), streptomycin (10 µg), and kanamycin (30 µg). Susceptibility was observed for these antibiotics in isolates such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.*, and *Enterobacter spp.*, as indicated by large clear zones of inhibition. Conversely, resistance was observed against cloxacillin (30 µg), cephalothin (30 µg), and amoxicillin (10 µg), as shown in Figure 2.

The susceptibility profile of *E. coli* revealed high sensitivity to streptomycin, with a 90% susceptibility rate, indicating its potential effectiveness against this isolate. However, lower susceptibility was recorded for tetracycline (60%) and erythromycin (40%), suggesting partial resistance. Moderate susceptibility to cloxacillin (78%) and kanamycin (70%) was also noted, reflecting varying degrees of resistance to these antibiotics.

Staphylococcus aureus exhibited high susceptibility to streptomycin (80%), cloxacillin (78%), and kanamycin (70%). However, the susceptibility to tetracycline (60%) and erythromycin (50%) was reduced, signaling emerging resistance trends that could complicate therapeutic options. These findings are consistent with reports that *S. aureus* can develop resistance to certain antibiotics over time (CLSI, 2019).

Salmonella isolates were highly susceptible to streptomycin (90%) and kanamycin (80%), with moderate susceptibility to cloxacillin (70%) and tetracycline (60%). Susceptibility to erythromycin (50%) and cephalothin (40%) was notably lower, which may necessitate the consideration of alternative treatment strategies, especially for multidrug-resistant strains (Wang et al., 2018). A similar pattern was observed in *Shigella*, where high susceptibility to streptomycin (90%) and moderate susceptibility to cloxacillin (70%) and kanamycin (60%) were recorded. However, reduced susceptibility to erythromycin (50%) highlighted potential limitations in treatment options for *Shigella*-related infections.

Proteus spp. showed high susceptibility to streptomycin (80%) and moderate susceptibility to kanamycin (70%) and tetracycline (60%). Lower effectiveness was noted for erythromycin (50%) and cephalothin (40%), consistent with previous findings that indicate variability in susceptibility patterns within this genus (Mukherjee et al., 2016). For *Enterobacter spp.*, moderate susceptibility was observed across all tested antibiotics, with the highest rates for streptomycin and kanamycin (60%). The lowest susceptibility was reported for erythromycin (40%), reflecting limited treatment options for infections caused by this pathogen. *Klebsiella spp.* demonstrated moderate susceptibility to streptomycin (60%) and kanamycin (60%), with reduced effectiveness against tetracycline (50%) and erythromycin (40%). This trend aligns with earlier studies that highlight increasing resistance in *Klebsiella* isolates (Podschun & Ullmann, 1998).

Overall, the results of the antibiotic sensitivity test revealed substantial variability in the susceptibility of different bacterial isolates. The observed resistance patterns underscore the critical need for continued monitoring of antibiotic efficacy to inform treatment protocols and public health interventions. These findings also emphasize the importance of implementing antimicrobial stewardship programs to address the growing challenge of antibiotic resistance globally.

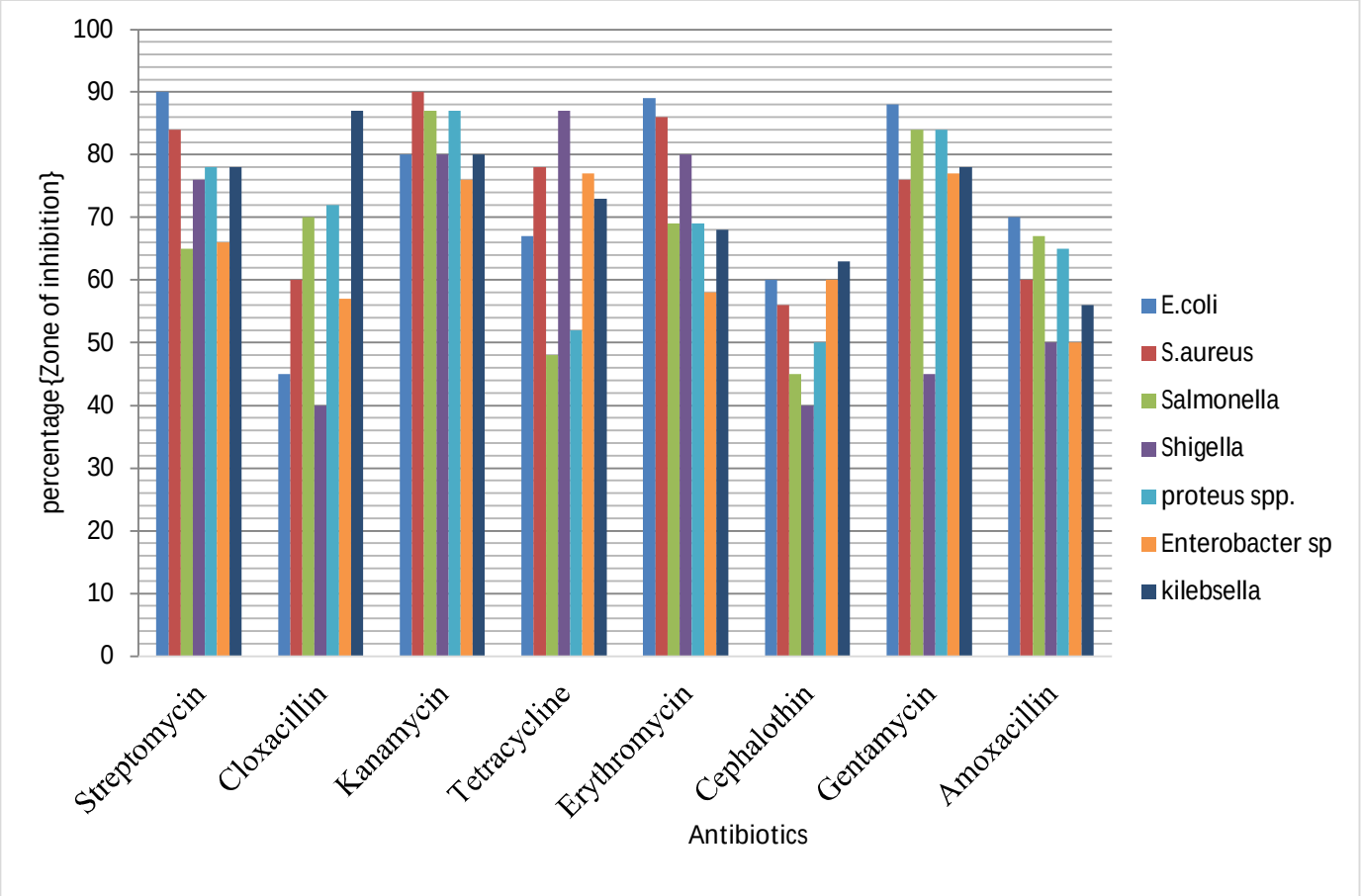

Figure2. Antibiotic susceptibility isolates to different antibiotics

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The investigation of bacterial isolates from the Shelle River offered critical insights into their biochemical profiles, prevalence, environmental adaptability, and antibiotic resistance. Each isolate displayed unique characteristics that relate to their potential pathogenicity and ecological function. The detection of species such as *Escherichia coli*, *Proteus* spp., *Salmonella* spp., *Staphylococcus* spp., *Shigella* spp., *Klebsiella* spp., and *Enterobacter* spp. confirmed the presence of contamination in the water, raising significant public health concerns. The frequent occurrence of *E. coli* and *Staphylococcus aureus* aligns with earlier Ethiopian studies, reflecting widespread fecal contamination and underscoring the need for continuous water quality surveillance. The presence of *Klebsiella*, *Shigella*, and *Salmonella* also points to persistent issues with poor sanitation and inadequate water treatment.

Bacterial count analysis revealed considerable contamination, especially at the hospital discharge point, which recorded the highest levels of *E. coli*, *S. aureus*, and other pathogens. This indicates localized pollution sources and highlights the necessity for improved waste disposal systems in healthcare facilities to reduce waterborne disease risks. The environmental tolerance of the identified bacteria varied, with *E. coli* and *S. aureus* exhibiting strong adaptability is a key factor in their persistence in both environmental and clinical settings. Meanwhile, the moderate resilience of *Shigella*, *Proteus*, and *Enterobacter* suggests their potential to endure under adverse conditions, meriting further research into their public health implications.

Antibiotic susceptibility tests showed notable differences in resistance across the isolates. Although many strains remained responsive to antibiotics like streptomycin and kanamycin, there were worrying levels of resistance to cloxacillin and erythromycin. These findings stress the urgent need for robust antimicrobial stewardship initiatives to address the growing threat of antibiotic resistance, which compromises effective treatment options for bacterial infections. In conclusion, this study underscores the critical importance of routine monitoring of microbial pathogens in water sources, particularly in regions with inadequate sanitation. Strengthened surveillance, improved hygiene, and comprehensive public health measures are vital to protect communities and ensure the availability of clean and safe water.

5.1. Recommendations

Based on the findings from the study of bacterial isolates from the Shelle River, the following recommendations are proposed:

- Implement regular monitoring of water quality in the Shelle River to assess levels of contamination, particularly focusing on fecal coliforms and pathogenic bacteria such as *E. coli*, *Salmonella*, and *Shigella.* This will help identify contamination sources and enable timely interventions.
- Promote and enforce better waste management and sanitation practices, especially in healthcare facilities, to minimize the discharge of untreated waste into water bodies. Establishing proper waste disposal systems is crucial to prevent further contamination.
- Conduct community awareness programs regarding the risks associated with contaminated water sources. Emphasize the importance of boiling or treating water before consumption, especially in areas with known contamination.
- Establish a surveillance system to monitor antibiotic resistance patterns among the bacterial isolates. This data is essential for informing treatment protocols and ensuring effective management of infections caused by these pathogens.
- Work closely with local health authorities to develop strategies for controlling the spread of waterborne diseases. This may include improving access to clean water and sanitation facilities, especially in rural and peri-urban areas.
- Encourage further research on the diversity and pathogenicity of bacterial isolates in the region. Understanding local strains' behavior can inform public health strategies and improve treatment options.
- Invest in water treatment infrastructure to ensure safe drinking water for communities relying on the Shelle River. This includes both physical treatments (filtration, chlorination) and biological treatments (using natural purification techniques).
- Provide training for healthcare workers on the identification and management of infections caused by these pathogenic bacteria, emphasizing antibiotic stewardship to mitigate resistance.

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