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Microbial Quality and Safety Assessment of Fresh Lettuce in Addis Ababa, Ethiopia

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DECLARATION

I, the undersigned, declare that this thesis entitled **Microbial Quality and Safety Assessment of Fresh Lettuce in Addis Ababa, ETHIOPIA** is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

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Acronyms/Abbreviation

VRBA	Violet-red bile agar
PCA	Plate count agar
MSA	Mannitol salt agar
FDA	Drug Administration
WHO	World Health Organization
AD	Anno Domini
STEC	Shiga toxigenic *Escherichia coli*
E. coli	*Escherichia coli*
AMC	Aerobic Mesophilic Count
YM Agar	Yeast and Mold agar
MR-VP	Methyl Red -Voges Proskauer Test
PAR	Photosynthetically active radiation
TCA	Tricarboxylic acid
MAP	Modified atmospheric packaging
TiO2	Titanium dioxide membrane
UV	Ultraviolet
N/G	No growth
AMB	Total Aerobic Mesophilic Bacteria
W/v	Weight by volume
GAPs	Good Agricultural Practices
GMPs	Good Manufacturing Practices
Aw	Water activity
Ct	Cut Off

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Abstract

Lettuce is high in water content (94-95%) and low in calories. It's also high in vitamins, minerals, and bioactive substances including polyphenols, carotenoids, and chlorophyll, all of which have health benefits. Food-borne illness outbreaks linked to the consumption of ready-to-eat vegetables are on the rise. but the recent information about the microbial loads and safety aspects of fresh lettuce are lacking. In this study, the microbial quality and safety assessment of fresh lettuce in Addis Ababa, Ethiopia were investigated. The general objective was to assess the microbial quality and safety and identify the major food-borne pathogens from fresh lettuce samples collected from selected marketplaces in Addis Ababa. A stratified random sampling was used to select 110 vegetable sellers in Addis Ababa, Ethiopia. Detection of hygiene indicators microorganisms: total coliforms, aerobic mesophilic bacteria (AMB), yeast, mold and *Enterococci*, *E. coli*, and other food-borne pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, were analyzed from 110 fresh lettuce samples. The study reveals that the fresh lettuce samples collected from selected marketplaces in Addis Ababa were contaminated with various microorganisms, including total coliform, *Staphylococc*i, *Fecal streptococci* and *E. coli* this indicates the poor hygienic conditions of the study area. As a confirmatory test, 33 positive representative isolates after the biochemical test were taken and detected by polymerase chain reaction (PCR). Among the analyzed samples, only 2 samples Addis Ketema and Lideta sub cities were positive for *E. coli* and *P. aeruginosa*, respectively. This contamination poses a potential biological health hazard to general consumers, as they are at risk of contracting food-borne infections. The findings underscore the urgent need for improved hygiene and safety measures in the production and handling of lettuce to safeguard consumers from potential biological health risks in Addis Ababa.

Keyword: *Vegetable's, Addis Ababa, quality, hygiene, biological health hazards, safety, lettuce*

1. INTRODUCTION

Lettuce (*Lactuca sativa L.*) is an Asteraceae family member that originated in the Mediterranean. It is a thriving and diversified plant that is found throughout the world [22]. The earliest grown lettuce appeared as a therapeutic vegetable in numerous prehistoric manuscripts as early as 2680 BC. The Asteraceae are family with a huge number of species, ranging from 23,000 to 30,000 [22]. The lettuce consists of seven varieties: Cos (also known as Romaine), Butterhead, Leaf (also known as Cutting), Stalk (or Asparagus), Crisphead (also known as Iceberg), Latin, and Oilseed. The most prevalent goods on the market are full heads of lettuce and fresh-cut lettuce. Colors, forms, and nutrition all influence customer purchasing decisions [22].

Lettuce is high in water content (94-95%) and low in calories. It's also high in vitamins, minerals, and bioactive substances including polyphenols, carotenoids, and chlorophyll, all of which have health benefits [58]. Furthermore, phytochemical compositions and contents change across varieties. Some researchers have found that red lettuce contains greater phenolic contents than green lettuce. As a result, red lettuce is an excellent source of antioxidants in the daily diet [22,58]. The contaminated lettuce frequently causes human bacterial, viral, and parasite epidemics, including *E. coli* and *Salmonella*.

Food-borne illness outbreaks linked to the consumption of ready-to-eat vegetables are on the rise [59]. Outbreaks of illnesses caused by bacteria, viruses, and parasites have been epidemiologically linked to the consumption of a wide variety of vegetables and, to a lesser extent, fruits [59]. Furthermore, vegetable surveillance has revealed that these foods can be contaminated with a variety of bacterial pathogens such as *Salmonella spp*., *Shiga toxigenic E. coli (STEC) Shigella spp*., *Listeria monocytogenes,* and *Campylobacter spp*. [22].

According to Ijabadeniyi [4,22], the Food and Drug Administration (FDA) recalled prepackaged fresh spinach owing to an *E. coli* outbreak in California, USA. The author also mentioned that fresh tomatoes consumed in restaurants in the United States were blamed for the *S. typhimurium* outbreak. In addition, an *E. coli* O157: H7 outbreak in the northern United States was linked to lettuce from Taco Bell restaurants [22].

The rise in outbreaks of foodborne illnesses caused by fresh produce is the result of dietary changes, including increased per capita consumption of fresh or minimally processed fruits and vegetables as well as increased use of salad bars and meals eaten away from home [22]. Changes in production and processing methods, distribution patterns, and practices are factors that have contributed to an increase in foodborne diseases caused by raw vegetables [9,21].

According to Ijabadeniyi [2], The World Health Organization (WHO) has identified several reasons for the increase in foodborne infection and poisoning outbreaks [2]. These include microbial adaptation, increased international trade, and skepticism [2,10]. Furthermore, changes in lifestyle oriented towards convenience, consumer demand for healthy food free of chemical preservatives and with a longer shelf life, as well as changes in human demographics and behavior have all contributed to an increase in foodborne infections [2,10,21].

According to Suslow *et al*. [11,21], the microbial quality of irrigation water is critical, because water contaminated with animal or human waste can introduce pathogens into vegetable products during preharvest and postharvest activities via direct or indirect contamination. Therefore, the microbiological quality of irrigation water is critical for the safety of fresh and minimally processed vegetables [12,21]. Furthermore, Ibenyassine *et al*. [13,21] reported that contaminated irrigation water and surface runoff water could be the primary sources of pathogenic microorganisms contaminating fruits and vegetables in a field-susceptible population and an increase in worldwide travel.

Because of contamination with microorganisms in human and animal intestinal habitats, such as *Salmonella* and *Listeria spp*. [14,15,21], water from rivers that receive both human and animal waste poses a health risk. Therefore, the microbiological quality and safety of fresh vegetables are major concerns for all stakeholders worldwide. Food-borne illness outbreaks linked to lettuce and ready-to-eat vegetables are a major public health concern [14,15,21].

According to the Centers for Disease Control and Prevention (CDC) [8] leafy greens, mainly lettuce, have been linked to 78 foodborne disease outbreaks in the United States between 2014 and 2021[8]. In addition, consumer reports states that between 2006 and 2019, romaine and other leafy greens, such as spinach and bags of spring mix, have been involved in at least 46 multistate *E. coli* outbreaks [8]. Investigators have linked five multistate outbreaks (three STEC, one *Listeria*, and one *Salmonella* outbreak) and 82 illnesses to consumption of packaged leafy greens, including spinach, romaine, a "power greens" blend, and other packaged salads in 2021 [8]

A study conducted in Addis Ababa, Ethiopia, evaluated the microbiological quality of lettuce irrigated with wastewater from the highly polluted Akaki River [52]. The study found that the mean fecal coliform levels of irrigation water ranged from 4.29-5.61 log 10 MPN 100 ml $^{-1}$, while on lettuce, the concentrations ranged from 3.46-5.03 log 10 MPN 100 g $^{-1}$. Compared with the WHO recommendations and international standards, the fecal coliform in irrigation water and lettuce samples exceeded the recommended levels [10]. A study conducted in Jimma town, Western Ethiopia [21], reported that the microflora of vegetables and irrigation river samples was dominated by *Bacillus species* (32.7%) followed by *Enterobacteriaceae* (25%) and *Micrococcus* (16%). *Staphylococcus aureus* and *Salmonella spp*. were detected in 24.0% and 20.7% of the samples, respectively [21].

While these studies provide valuable information about the microbial load of fresh vegetables in Ethiopia, but the recent information about the microbial loads and safety aspects of fresh lettuce are lacking. Since then, there may be changes in agricultural practices, water sources, and other factors that could affect the microbial quality of fresh vegetables. Therefore, it would be beneficial to evaluate the current microbial quality of fresh vegetables in Ethiopia. This study could help to identify potential risks and provide recommendations for improving the safety of fresh vegetables.

One of the main reasons why new research is needed on lettuce contamination is the changing nature of the produce industry. Modern agricultural practices have shifted towards large-scale farming and centralized production, leading to increased risk of contamination. Lettuce is often grown near animal farms, posing a high risk of contamination from animal waste. Additionally, lettuce is usually consumed raw, making consumers vulnerable to any pathogens present on the surface of the leaves.

Another critical factor is the evolving nature of these pathogens. As bacteria adapt and develop resistance to existing control measures, it becomes essential to continually research and update these measures to effectively reduce the risk of contamination. This is especially important given the severity of the illnesses caused by these bacteria, which can lead to severe complications and even death in vulnerable populations such as children, older adults, and those with compromised immune systems.

Furthermore, increased globalization and trade have made the source of contamination more challenging to identify. In the case of the recent *E. coli* outbreak, the source of contamination was initially thought to be romaine lettuce from Arizona, but it was later discovered to be from a different growing region in California [8]. Without continuous research and advanced detection methods, it is difficult to trace the origin of contamination and prevent further outbreaks.

Moreover, recent studies have also highlighted the possibility of contamination through irrigation water. With the use of contaminated water in the irrigation of crops, lettuce can easily become contaminated and carry pathogens onto consumers' plates. This highlights the need for research not just on the lettuce itself but also on potential sources of contamination in the production process.

In general, the recent outbreaks of contamination have shown the need for new research on lettuce. With changing agricultural practices, evolving pathogens, and a mix of potential sources of contamination, it is crucial to continuously update and improve control measures. The current study can help to identify and address the root causes of contamination, develop more effective prevention methods and ultimately ensure the safety of the leafy green vegetable that is beloved by many consumers.

2. OBJECTIVES OF THE STUDY

2.1 General Objective

The general objective of this study is to assess the microbial quality and safety of fresh lettuce samples collected from selected marketplaces in Addis Ababa and identify the major food-borne pathogens in lettuce.

2.2 Specific Objectives

The specific objective is: -

- To assess the microbial loads, quality, and safety of fresh lettuce.
- To identify the major food-borne pathogens using conventional methods and PCR detection technique.

3. LITERATURE REVIEW

3.1. History of lettuce

Lettuce, also known by its scientific name Lactuca sativa, is a delightful leafy green vegetable that finds its place in a wide array of salads and sandwiches. Its roots trace back to the distant realms of ancient times, captivating our imagination with tales from days long gone. The Mediterranean region holds the honor of being the birthplace of lettuce, where it first sprouted and captured the attention of both Greeks and Romans alike. As centuries passed, lettuce embarked on a journey across oceans and continents, leaving its indelible mark on countless cuisines. In this intriguing exploration, we shall uncover the rich tapestry of lettuce's history, unraveling its cultivation practices and unearthing its cultural significance [22]

Lettuce, the leafy green vegetable that we enjoy today, has its roots in the ancient civilizations that once thrived. Evidence of people consuming lettuce can be found in the burial sites of Egyptian pharaohs from a time long ago. Pliny the Elder, an esteemed writer from ancient Rome, tells us that lettuce was first grown and cultivated in Egypt around the 6th century BC [22]. The ancient Egyptians held the belief that lettuce symbolized fertility and incorporated it into their religious ceremonies. Additionally, they attributed medicinal qualities to this leafy green, often employing the juice of wild lettuce to promote restful slumber.

In the ancient times, the Greeks were known to grow lettuce and they held the belief that it possessed magical abilities to cure different ailments. Hippocrates, who is revered as the founding figure of medicine, used to recommend lettuce as a remedy for blood-related issues and individuals suffering from poor vision. Additionally, the Greeks also connected lettuce with Hypnos, the god of slumber, and firmly believed that consumption of this leafy green would bring about tranquil sleep [22].

During the expansion of the Roman Empire, lettuce experienced a surge in cultivation and appreciation. The Romans, being adventurous and dominant, disseminated their fondness for lettuce to various regions of Europe, where it swiftly captivated the elite. This verdant vegetable made its presence felt in numerous delectable dishes, as referenced by Apicius, a distinguished ancient Roman writer, in his celebrated culinary work, The Roman Cookery Book. In the era of the middle Ages, lettuce persisted in its cultivation and consumption across Europe. In the olden days, lettuce was predominantly cultivated in monasteries, not so much for consumption but rather for its healing qualities. It was said to possess aphrodisiac properties and monks were strictly prohibited from indulging in it [22].

During the century when European voyages of exploration were taking place lettuce was introduced to the Americas. Hernando de Soto, a conquistador brought lettuce seeds to the world, where it quickly became a popular ingredient, in local cuisine and spread to other regions through trade [22]

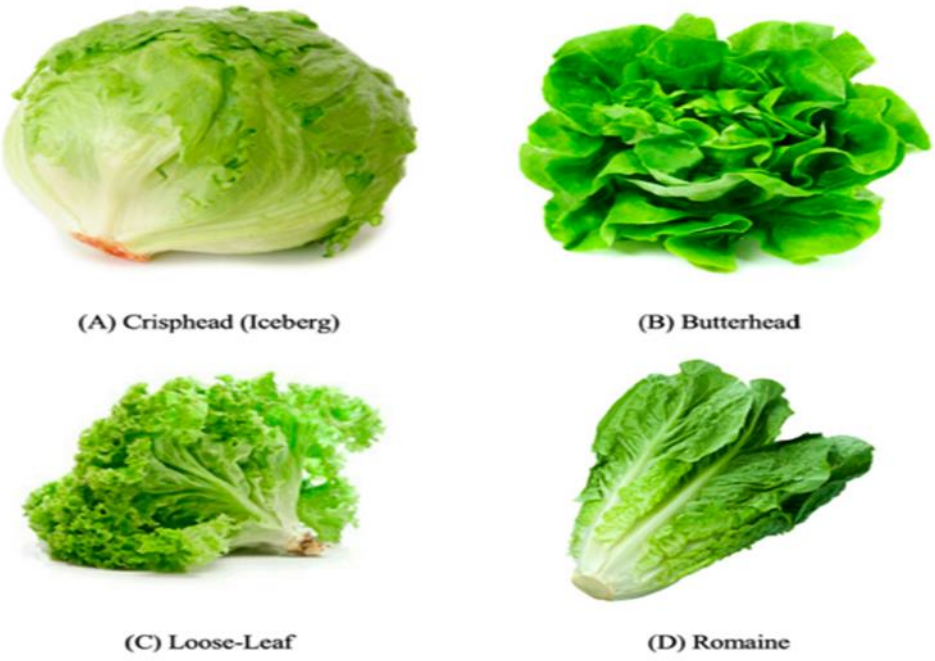

Figure 1 frequently grown lettuce types (Source: Min shi et al. [22])

Nowadays lettuce is enjoyed everywhere around the globe. There are four types of lettuce – head lettuce, romaine lettuce butterhead lettuce and looseleaf lettuce – each with its unique taste and appearance. This vegetable is not low in calories. Also packed with essential vitamins and minerals making it an important component of a healthy diet. Leafy green vegetables like baby leaf spinach Swiss chard rocket (arugula) and various types of lettuces play a role in nutrition due to their abundance of necessary nutrients. However there has been an increase in reported cases of illnesses associated with the consumption of these vegetables, in times [22].

The primary bacteria that cause these outbreaks are *Shigatoxin producing Escherichia coli (STEC) Salmonella spp. Yersinia spp. Listeria monocytogenes*. Apart, from pathogens there is also concern about antibiotic bacteria in ready to eat salads especially those containing leafy green vegetables. The overall safety and quality of leafy greens are often compromised, underscoring the need, for advancements in controlling harmful microorganisms across the entire agricultural value chain. This highlights the importance of implementing measures to suppress the growth and spread of these pathogens [8].

One of the challenges in ensuring the microbiological safety of fresh leafy green vegetables is the fact that they are typically consumed raw, without undergoing any heat treatment that could eliminate viruses. To address this issue, various methods have been explored, including the use of aqueous ozone treatment to minimize bacterial contamination. Traditional disinfectant washes, such as chlorine solutions, may not be sufficient in ensuring food safety, as they may not effectively reach internalized germs. Moreover, existing commercial cleaning and distribution techniques may not adequately control human infections caused by pathogens that can adhere to leaf surfaces and infiltrate the interior tissues of the vegetables [8].

3.2. History of lettuce contamination

For millennia, people have included lettuce in their meals as a vital and adaptable leafy green vegetable. It is an excellent supplement to any diet since it is a rich source of vitamins, minerals, and dietary fiber. But lettuce's history is also entwined with the health hazards associated with contamination [8,10].

Any stage of the manufacturing process, from planting to processing and packing, can result in contamination of lettuce. This essay seeks to present a thorough analysis of the literature on the history of lettuce contamination, covering its causes, consequences, and current preventative strategies. Knowing the background of contaminated lettuce is essential to creating tactics that can safeguard customers and raise the security of this popular vegetable [8,10].

In 60 B.C., Emperor Claudius Caesar of ancient Rome died after consuming a meal of lettuce tainted with ergot fungus, marking the first documented occurrence of foodborne sickness linked to lettuce intake. Over the ensuing centuries, there were numerous other documented cases of illnesses linked to lettuce, such as the notorious 'Dancing Plague' of the 16th century, in which people in Strasbourg, France experienced hallucinations, convulsions, and other symptoms following ingestion of tainted lettuce [8,10].

Typhoid fever epidemics in the 19th and early 20th centuries were frequently linked to lettuce. According to Butts, the contamination was thought to have resulted from the usage of human excrement as fertilizer in lettuce fields. With the advent of hygienic regulations, this practice was eventually outlawed with the introduction of sanitary standards for food production [8,10,59].

When outbreaks of the fatal *E. Coli O157:H7* strain of bacteria were connected to lettuce intake in the US and Canada in the 1990s, lettuce contamination grabbed headlines once more [8]. It was determined that the animal excrement applied to the lettuce fields as fertilizer was the cause of the contamination. Stricter guidelines and standards for the growing, processing, and packaging of lettuce were brought about by this occurrence.

3.3. Contributing factors to contamination

From farm to fork, there are a number of ways that harmful microbes can contaminate lettuce. The main sources of pollution include soil, water, and fertilizer made from animal dung. Inadequate sanitation in processing and packaging facilities, as well as careless handling and storage procedures, are other contributing causes [8].

Water: A vital component of growing lettuce, water quality plays a crucial role in avoiding infection. Produce affected by irrigation or washing with tainted water may have germs that render lettuce unfit for human eating [8]. Sometimes the poisoning of lettuce fields occurs from tainted runoff water from neighboring farms or livestock operations.

Because lettuce is often cultivated in open areas, it is susceptible to soil pollution. If appropriate precautions are not followed, pathogenic bacteria can live in the soil for long periods of time and infect lettuce during harvesting and packing [18-21]. Animal dung: Growing lettuce is a typical approach that involves using untreated animal excrement as fertilizer. On the other hand, germs like Salmonella and E. coli can infect the manure and spread to the lettuce, rendering it unfit for human consumption. Furthermore, there may be a higher chance of contamination if lettuce fields are next to animal farms [8] [18-21].

Inadequate handling and storage procedures: Lettuce may be exposed to temperature abuse during transit and storage, which fosters the development of harmful germs. Contaminated lettuce can spread to other fresh products and cause extensive epidemics if improperly handled and stored [21].

3.4. Causes of Contamination

Lettuce contamination can arise from several sources, such as inappropriate farming methods, inappropriate handling techniques, and external circumstances. Because animal manure can harbor dangerous pathogens like *Salmonella* and *E. coli*, it is one of the primary sources of contaminated lettuce [8,21]. Workers' inadequate personal hygiene, cross-infection during harvesting and packaging, and tainted water used for irrigation and washing lettuce are other causes of contamination.

Furthermore, feral animals nearby lettuce fields may potentially be a source of infection. It is possible for dangerous germs, including *E. coli*, to enter lettuce fields from wild animals by their excrement. Although thorough cleaning and disinfection can lessen contamination, it might be challenging to manage wildlife's presence in agriculture [18-21].

3.5. Effects of Contamination

Lettuce contamination may have serious health effects on the general public, ranging from moderate food poisoning to serious infections and even death. The most typical signs of food poisoning linked to lettuce include nausea, vomiting, diarrhea, and cramping in the stomach. In extreme situations, these symptoms can result in dehydration and hospitalization [10].

Outbreaks of contaminated lettuce can have a serious negative effect on public health, leading to serious infections, hospital stays, and even fatalities. Frequent symptoms linked to contaminated lettuce are fever, vomiting, diarrhea, and stomach discomfort. But occasionally, infections might result in more serious side effects such hemolytic uremic syndrome (HUS), which can be fatal, particularly for elderly and young individuals [10].

Furthermore, contamination can have a negative financial impact on farmers, producers, and merchants as a result of recalls, legal actions, and reputational harm [8,10]. The 2006 *E. coli* epidemic in the US caused the lettuce sector to lose an estimated $500 million [8].

3.6. List of recorded outbreaks

A significant green vegetable that has been consumed by people for generations is lettuce. But despite its widespread use and health advantages, lettuce has a lengthy history of being linked to a number of illness outbreaks. There have been several documented instances of lettuce being connected to foodborne disease outbreaks from antiquity to the present. This article will examine some of the most notable lettuce outbreaks from various historical periods and their impact on human health [8,10].

3.6.1 Ancient Greece and Rome (400 BC - 400 AD)

In 401 BC, there was the first known epidemic of lettuce in ancient Greece. Soldiers who ate wild lettuce during Xenophon's Ten Thousand army's withdrawal had excruciating stomachaches. It is believed that this episode is the first incidence of lettuce poisoning ever recorded. Lettuce was regarded as a basic item and was often eaten in ancient Rome. But when the plague struck the city in AD 65, the main cause was said to be eating tainted lettuce. Due to this incident, people started to think of lettuce as a "poisonous" vegetable, which decreased its appeal [8,10,22].

3.6.2. 17th to 19th Century

Lettuce remained connected to a number of outbreaks between the 17th and 19th centuries, particularly in Europe. A typhoid epidemic in Germany in 1682 was traced back to the eating of tainted lettuce. Similar events involving lettuce intake and typhoid epidemics were documented in England in 1745. Manure fertilizer for lettuce fields was connected to epidemics of milk-borne illnesses including typhoid and diphtheria in the late 1800s. These outbreaks were mostly caused by unhygienic circumstances and a lack of hygienic handling and production procedures for lettuce [8,10,22].

3.6.3. 20th Century

Lettuce consumption increased in the 20th century in terms of both quantity and diversity. On the other hand, epidemics became more common as a result. A botulism outbreak that was linked to canned lettuce was reported in the United States in 1925. Salmonellosis infections in the 1950s were associated with lettuce; the illness was caused by tainted manure. In 1996, radish sprouts tainted with *E. coli* caused over 400 individuals in Japan to become ill, making it one of the worst outbreaks in recent memory. Water containing lettuce seeds which were thought to be the cause of the *E. coli* contamination was used to irrigate the sprouts [8,10,22].

3.6.4. 21st Century

In the twenty-first century, epidemics related to lettuce persist despite enhanced restrictions and safety precautions. The cause of an *E. coli* epidemic in the US in 2006 was identified as pre-packaged lettuce. 2011 saw the connection between imported Egyptian fenugreek seeds used for sprouting and an *E. coli* epidemic in Germany. Over 200 Americans contracted *E. coli* in 2018 as a result of eating tainted romaine lettuce, marking the most recent and extensive incident involving lettuce. There were five fatalities and several hospital admissions as a result of the outbreak, which was connected to tainted irrigation water [8,10,22,59].

These incidents highlight the ongoing risk of consuming lettuce contaminated with harmful bacteria. Farmers and producers are constantly working towards implementing stricter sanitary measures and traceability systems to prevent such outbreaks. These events demonstrate the continuous danger of eating lettuce tainted with dangerous microorganisms. To avert such outbreaks, farmers and producers are always trying to put in place more stringent sanitary regulations and traceability programs [8,10,22,59].

In conclusion, the history of documented lettuce outbreaks should serve as a reminder to the food business of the significance of safe and sanitary handling procedures. It also emphasizes the necessity of more stringent laws and oversight to guarantee the security of our food supply. We may hope to lessen the possibility of future outbreaks on lettuce and other commodities with ongoing efforts to improve food safety.

3.7. Food Preservation

The process of managing and preserving food to extend its shelf life, reduce spoilage, and preserve its nutritional content is known as food preservation. The need for food preservation methods is rising as a result of population growth and the fast urbanization of the world. As a result, several food preservation techniques have been developed to satisfy the expanding demands of the populace. Foods have been preserved by a variety of techniques since ancient times, including pickling, smoking, salting, and drying. Technological developments in recent years have allowed for the development of contemporary preservation techniques including canning, freezing, and radiation [61].

Reducing food waste and preserving food safety depend on proper food preservation. Food may be kept in storage for extended periods of time without going bad thanks to it, which is particularly useful when food is scarce. This paper's goal is to present a thorough analysis of the literature on food preservation, including its procedure and various techniques [61].

3.7.1. The process of Food Preservation

Inhibiting or eliminating the natural compounds, enzymes, and microbes that cause food to deteriorate is the process of food preservation. Foodborne infections and food deterioration are both caused by microorganisms found in food. Therefore, stopping or reducing the proliferation of these enzymes and microbes is the main goal of food preservation [61].

Food may be preserved using a variety of techniques, some of which are more appropriate for particular kinds of food than others. One of the earliest and most popular techniques for preserving food is drying. This entails lowering the food's moisture level, which stops bacteria from growing. There are three methods for drying: oven, freeze, and sun. According to studies, the most popular technique for preserving food in poor nations is sun-drying (Gustavsson et al., 2011). It is a well-liked option for small-scale food preservation as it is affordable and energy-efficient [61].

Another age-old technique for preserving food, especially meat and fish, is salting. By removing moisture from the meal, salt stops microbes from growing. According to studies, salted goods have been kept for millennia with no changes made to technology (Tapia et al., 2019). However, excessive consumption of salt can have adverse health effects, making this method less popular in modern times [61].

Canning is a more modern method of food preservation, introduced in the early 19th century. This method involves heating food to a high temperature, sealing it in airtight containers, and then cooling it. The high temperature kills microorganisms, and the airtight seal prevents oxygen from entering, preventing spoilage. Canned foods have a longer shelf life and do not require refrigeration, making them convenient for storage and transportation. However, concerns have been raised about the health risks associated with food stored in metal containers and the potential for bacterial contamination during the canning process [61].

Freezing is another popular method of food preservation, which has gained popularity in recent years. This method involves storing food at low temperatures, usually below 0°C, which slows down or halts the growth of microorganisms. Freezing helps preserve the texture, flavor, and nutritional value of the food, making it a preferred method for preserving fruits and vegetables. However, freezing can be an expensive method of preservation as it requires specialized equipment and energy, making it less accessible in developing countries [61].

Irradiation is a modern technology used for food preservation by exposing food to ionizing radiation. This technique kills microorganisms, insect pests, and parasites, and can also delay the ripening process, therefore extending the shelf life of the food. Studies have shown that irradiation does not affect the taste, nutritional value, or safety of food . However, there have been concerns about the potential health risks associated with consuming irradiated food, leading to limited consumer acceptance [61].

3.8. Prevention and Control Measures

In recent years, significant efforts have been made to prevent and control contamination in lettuce production. These include stricter regulations and guidelines for food safety, improved agricultural practices, and increased surveillance and testing for harmful bacteria [61]. To effectively control and prevent microbial contamination in lettuce, individuals and the food industry can adopt the following measures [61]

- Good Agricultural Practices (GAPs): These are a set of guidelines that help farmers to produce safe and healthy food by minimizing the risks of microbial contamination. GAPs include proper irrigation, composting, and pest control practices, as well as regular monitoring and testing of soil, water, and crops [61].

- Good Manufacturing Practices (GMPs): These are a set of guidelines that help food manufacturers to produce safe and healthy food by minimizing the risks of microbial contamination. GMPs include proper sanitation, hygiene, and pest control practices, as well as regular monitoring and testing of equipment, surfaces, and products [61]

- Hurdle Technology: This is a combination of different control strategies that work together to prevent microbial contamination in food production environments. Hurdle technology includes thermal, non-thermal, biocontrol, natural, and chemical methods [62].

- Natural Antimicrobials: These are compounds that are naturally present in some foods and have antimicrobial properties. Natural antimicrobials can be used as an alternative to synthetic chemicals for controlling microbial contamination in fresh produce

- Sanitary design and maintenance of production facilities: Proper design and maintenance of production facilities, including equipment and packaging materials, can prevent cross-contamination and the growth of pathogens on the lettuce [61].

- Food safety training and education: Educating farmers, handlers, and consumers on proper handling, storage, and cooking practices can significantly reduce the risk of contamination and foodborne illnesses associated with lettuce [61].

- Hydroponic production: Hydroponic systems, which grow plants without using soil, have shown promising results in reducing the risk of microbial contamination in lettuce. It eliminates the use of soil and animal manure, two of the primary sources of contamination, and can also be grown in a controlled environment, reducing the risk of contamination from water and other external factors [63].

In conclusion lettuce microbial contamination has been a longstanding health concern, resulting in numerous outbreaks and illnesses. The primary sources of contamination include contaminated water, soil, animal manure, and poor handling and storage practices. However, with the implementation of proper preventive measures and future strategies, it is possible to ensure a safe supply of lettuce for consumers. Continuous monitoring, education, and research in this area are essential to prevent and control outbreaks and protect public health.

3.8.1. Good Agricultural Practices (GAPs)

Farmers must adhere to Good Agricultural Practices (GAPs). These are practical guidelines that provide a framework for safe and sustainable agricultural practices. One important aspect of GAPs is water quality management, as irrigation water is a common source of contamination in lettuce production. Farmers must ensure that their water sources are tested regularly and meet national and international standards for agricultural use [61,62].

Another crucial aspect of GAPs is soil management. Proper soil management practices, such as crop rotation and composting, can help to reduce the presence of harmful bacteria and fungi in the soil. This can not only prevent contamination of lettuce during production but also improve the overall health and quality of the soil for future crops. Additionally, GAPs recommend the use of clean and sanitized equipment, tools, and containers to prevent cross-contamination during harvesting and processing of lettuce [61,62].

Proper hygiene and sanitary practices are also emphasized in GAPs. This includes personal hygiene for farmers, workers, and visitors, as well as the maintenance of clean and sanitary facilities, such as restrooms and handwashing stations. This is crucial in preventing the spread of pathogens from human sources to lettuce crops.GAPs also advocate for the use of safe and approved pesticides, fertilizers, and other chemicals in lettuce production. These should be used in accordance with recommended rates and application methods to prevent excessive residue and adverse effects on human health and the environment. Farmers should also keep accurate records of all chemical use, as well as any potential sources of contamination, such as neighboring farms or livestock operations [61,62].

Proper harvesting and post-harvest handling practices are also essential components of GAPs. This includes harvesting only when lettuce is at its peak maturity and using clean and sanitized containers for transportation. Temperature control is also crucial to prevent bacteria growth during transportation and storage. Farmers must have appropriate storage facilities and employ proper cooling methods to maintain the freshness and quality of lettuce. Finally, GAPs emphasize the importance of training and education for farmers, workers, and other involved parties in the production and handling of lettuce. This includes educating them on the potential risks and methods to prevent contamination, as well as proper sanitation and hygiene practices [61,62].

In conclusion, adherence to GAPs is essential for safe and sustainable lettuce production. By following these guidelines, farmers can not only prevent and control bacterial contamination but also ensure the quality and freshness of their produce for consumers. Implementing GAPs requires dedication and ongoing efforts, but the benefits in terms of food safety and sustainability make it a worthwhile investment for all involved in the lettuce production process.

3.8.2. Good Manufacturing Practices (GMPs)

Good Manufacturing Practices (GMPs) play a crucial role in ensuring the safety and quality of lettuce production. Lettuce, being a leafy green vegetable, is highly susceptible to microbial contamination from various sources including soil, water, and human handling. This poses a significant risk to consumers who may be exposed to harmful pathogens such as E. coli, Salmonella, and Listeria, which can cause serious foodborne illnesses. Therefore, it is essential for lettuce producers to implement GMPs to effectively control and prevent microbial contamination [61,62].

The first step in implementing GMPs for lettuce production is to establish a clean and sanitary production environment. This includes designing facilities and equipment in a way that minimizes the risk of contamination, such as using non-porous surfaces that are easy to clean and disinfect. Regular cleaning and maintenance procedures must also be followed to ensure that all equipment and surfaces are free from any potential sources of contamination. Another critical aspect of GMPs is maintaining strict personal hygiene among workers. This includes proper hand washing, wearing appropriate protective clothing, and regular health screenings to prevent the spread of any illnesses. Employees should also be adequately trained on proper handling techniques to minimize the risk of cross-contamination between different areas and products [61,62].

Furthermore, GMPs also involve monitoring and controlling the quality of water used in lettuce production. Water is not only used for irrigation but also in the washing and rinsing of lettuce. Therefore, it is essential to regularly test and treat the water to ensure it meets the necessary quality standards. Additionally, proper storage and handling of water must be practiced to prevent any potential contamination. Proper harvesting, storage, and transportation practices are also crucial in preventing microbial contamination in lettuce production. Harvesting equipment must be regularly cleaned and disinfected, and proper temperature controls must be in place during storage and transportation to prevent the growth of harmful bacteria. Furthermore, vehicles used to transport lettuce should be properly sanitized to avoid the risk of contamination during transit [61,62].

In addition to these measures, GMPs also involve implementing a robust pest control program to prevent pests and insects from contaminating lettuce crops. This includes maintaining a clean and well-maintained production area, using pest-resistant packaging, and regularly inspecting and treating the crops for any signs of infestations [61,62].

In conclusion, GMPs play a pivotal role in ensuring the safety and quality of lettuce production. By establishing a clean and sanitary production environment, maintaining strict personal hygiene, monitoring water quality, and implementing proper harvesting, storage, and transportation practices, producers can effectively control and prevent microbial contamination in lettuce production. By following these GMPs, consumers can have confidence in the safety and quality of the lettuce they consume, and the risk of foodborne illnesses can be greatly reduced.

3.8.3. Hurdle technology

Hurdle technology is a preservation method that combines multiple hurdles or barriers to prevent the growth of microorganisms, thereby extending the shelf life of food products. These hurdles often include factors such as pH, temperature, water activity, and antimicrobial substances. The aim of using hurdle technology is to prevent or delay the growth of spoilage and pathogenic microorganisms, while maintaining the sensory and nutritional attributes of the food product. [62].

3.8.3.1. Biotic Hurdles

Biotic hurdles in hurdle technology refer to the use of living organisms to prevent the growth of other microorganisms. One of the most commonly used biotic hurdles is the use of bacteria, specifically lactic acid bacteria (LAB). LAB are desirable bacteria that are naturally found in many fermented foods. These bacteria produce lactic acid, which lowers the pH of the food product, making it difficult for other microorganisms to grow. In addition to lowering the pH, LAB also produce antimicrobial compounds such as bacteriocins, which inhibit the growth of spoilage and pathogenic bacteria [61,62].

Studies have shown that LAB can effectively reduce the growth of pathogens such as Listeria monocytogenes and Escherichia coli in food products, making them a valuable biotic hurdle in hurdle technology[62]. However, the effectiveness of LAB as a biotic hurdle may differ depending on the type of food product and the specific strains of LAB used. Another biotic hurdle used in hurdle technology is the use of bacteriophages. Bacteriophages are viruses that infect and kill specific bacterial strains. They have been found to be effective in reducing the growth of foodborne pathogens such as *Salmonella, Listeria*, and Staphylococcus in various food products [62]. Bacteriophages are considered a more targeted approach compared to other biotic hurdles like LAB, as they only target specific bacterial strains. However, there are concerns about the potential transfer of antibiotic resistance genes by bacteriophages, which may limit their use in hurdle technology [62].

3.8.3.2. Abiotic Hurdles

Abiotic hurdles refer to the use of non-living barriers to prevent the growth of microorganisms in food products. These hurdles include pH, temperature, water activity, and antimicrobial substances. pH is one of the most widely used abiotic hurdles in hurdle technology. The pH of food products plays a crucial role in controlling the growth of microorganisms, as many bacteria are unable to grow in acidic conditions. Studies have shown that a pH lower than 4.5 can effectively inhibit the growth of most bacteria, making it an important hurdle in preserving food products [62].

Temperature is another important abiotic hurdle in hurdle technology. Lowering the temperature of a food product inhibits the growth of microorganisms by slowing down their metabolic activities. For example, temperatures below 5°C can effectively inhibit the growth of most spoilage bacteria, while temperatures above 60°C can destroy the cells of pathogenic bacteria. However, temperature alone may not be enough to prevent the growth of bacteria, making it essential to combine it with other hurdles [62].

Water activity (Aw) is a measure of the free water available in a food product. It is often used as an abiotic hurdle in hurdle technology, as most bacteria require high levels of water to grow [62]. By reducing the Aw of a food product, its shelf life can be extended, as water is essential for cell growth and reproduction. Many preservation techniques like drying and salting reduce the Aw of food products, making them an effective abiotic hurdle in hurdle technology [62].

Antimicrobial substances are also used as abiotic hurdles in hurdle technology. These include natural substances like salt, sugar, and spices, as well as chemical preservatives like nitrates and sulfites. These substances inhibit the growth of microorganisms by altering their cell membranes or metabolic processes [62]. However, concerns have been raised about the potential adverse effects of chemical preservatives on human health, making their use in hurdle technology a subject of debate [62].

In Conclusion Hurdle technology is an effective preservation method that has gained popularity in the food industry. The use of biotic hurdles like LAB and bacteriophages, as well as abiotic hurdles like pH, temperature, Aw, and antimicrobial substances, has been found to effectively prevent the growth of microorganisms in food products. However, the effectiveness of these hurdles may vary depending on the food product and the specific strains and concentrations used. Therefore, it is crucial to carefully select and combine these hurdles to achieve optimal results. Further research is needed to explore and optimize the use of biotic and abiotic hurdles in hurdle technology to ensure the production of safe and high-quality food products.

3.8.4. Natural Antimicrobials

The use of natural antimicrobials has gained much attention in recent years. Natural antimicrobials are compounds derived from plants, animals, and microorganisms that have the ability to inhibit the growth and survival of pathogenic microorganisms. These antimicrobial compounds can be found in various forms such as essential oils, organic acids, bacteriocins, and plant extracts. Essential oils, which are extracted from plants through distillation or cold pressing, have been extensively studied for their antimicrobial properties due to their broad-spectrum activity against bacteria, fungi, and viruses. Similarly, organic acids such as citric acid, lactic acid, and acetic acid have shown strong antimicrobial activity against foodborne pathogens [61,62].

Another type of natural antimicrobial, bacteriocins, are protein-based compounds produced by certain bacteria that inhibit the growth of closely related species. These compounds have gained attention as potential alternatives to synthetic preservatives due to their effectiveness at low concentrations and lack of adverse effects on human health. Plant extracts, including garlic, onion, and oregano, have been reported to possess antimicrobial activity due to their high content of phenolic compounds and essential oils [61,62].

Additionally, these natural antimicrobials have antioxidant properties that can help extend the shelf life of fresh produce by reducing the formation of reactive oxygen species. Moreover, the use of natural antimicrobials can also improve the flavor and nutritional value of fresh produce, making it a favorable option for both producers and consumers. Overall, the use of natural antimicrobials is a promising approach to ensure the safety and quality of fresh produce, while also providing a more natural and sustainable solution compared to synthetic preservatives [61,62].

3.8.5. Sanitary design

Sanitary design plays a crucial role in ensuring the safety and quality of lettuce and other food products. Contamination control is of utmost importance in the lettuce industry, as lettuce is often consumed raw and any contamination can pose serious health risks to consumers. Sanitary design refers to the design and construction of food processing facilities and equipment in a way that minimizes the risk of contamination and allows for effective cleaning and sanitation. This article aims to explore the significance of sanitary design in lettuce contamination control and its impact on food safety [61,62].

Sanitary design principles are essential in preventing lettuce contamination throughout the production process. Starting from the farm, proper sanitation practices should be implemented to minimize the risk of microbial contamination. This includes maintaining clean and well-maintained facilities, ensuring proper waste management, and implementing effective pest control measures. Additionally, the design of harvesting and handling equipment should prioritize hygiene, with smooth surfaces that are easy to clean and sanitize [61,62].

In processing facilities, the design of lettuce washing and packaging equipment should adhere to sanitary design principles. Equipment should be constructed with materials that are resistant to corrosion, easy to clean, and capable of withstanding frequent sanitization processes. The layout of the facility should also be designed to minimize the risk of cross-contamination, with clear separation between raw and processed lettuce, and proper ventilation systems to prevent the buildup of airborne contaminants [61,62].

3.8.6. Food Safety Training

Food safety training and education play a crucial role in ensuring the control of lettuce contamination. With the increasing concerns over foodborne illnesses and outbreaks, it is imperative to have proper measures in place to prevent contamination and protect public health. Lettuce, being a widely consumed leafy green vegetable, is particularly susceptible to contamination by pathogens such as *E. coli* and *Salmonella*. Therefore, comprehensive training programs and educational initiatives are essential to equip individuals involved in the food industry with the knowledge and skills necessary to mitigate the risks associated with lettuce contamination [61,62].

Introduction to food safety training and education is vital in addressing the challenges posed by lettuce contamination. These programs aim to educate food handlers, including farmers, processors, distributors, and food service personnel, about the potential hazards associated with lettuce and the best practices to prevent contamination. By understanding the sources and routes of contamination, individuals can implement effective control measures at each stage of the food supply chain. This includes proper handling, storage, and preparation techniques, as well as maintaining hygienic conditions in production and processing facilities [61,62].

3.8.7. Hydroponic Production

Hydroponic production of lettuce has gained significant attention in recent years due to its potential for high yield and efficient use of resources. This soilless cultivation method involves growing plants in nutrient-rich water solutions, providing an ideal environment for plant growth. However, like any agricultural system, hydroponics is not immune to contamination risks. Contamination can occur through various sources such as water, nutrient solutions, equipment, and even human handling. Therefore, it is crucial to implement effective contamination control measures to ensure the safety and quality of hydroponically grown lettuce [61,63].

Controlling contamination in hydroponic lettuce production begins with proper hygiene practices and sanitation protocols. Regular cleaning and disinfection of equipment, tools, and growing surfaces are essential to minimize the risk of microbial contamination. Additionally, strict personal hygiene measures should be followed by workers, including handwashing and the use of protective clothing. Water quality is another critical factor in contamination control. Regular testing and monitoring of water sources and nutrient solutions are necessary to detect any potential contaminants and take appropriate corrective actions [61,63].

To further enhance contamination control in hydroponic lettuce production, it is recommended to implement a comprehensive integrated pest management (IPM) program. This involves the use of biological controls, such as beneficial insects, to manage pests and reduce the reliance on chemical pesticides. Regular scouting and monitoring of plants for signs of pests or diseases can help identify potential issues early on and prevent their spread. Additionally, maintaining a clean and well-maintained greenhouse environment can help minimize the risk of pest infestations. By implementing these contamination control measures, hydroponic lettuce producers can ensure the production of safe and high-quality lettuce for consumers [61,63].

4. Materials and Methods

4.1. Description of the Study Area.

The study was conducted at Addis Ababa, the capital and largest city of Ethiopia [24]. Addis Ababa grew from a settlement of an estimated 15,000 people in 1888 to more than 3.6 million in 2020 [25] According to the Central Statistical Agency, the population is estimated to surpass 5 million in 2036. However, the city's growth rate has declined in the last couple of decades from 6.9% annually over the 1961-62 period to 2% from 2007 to 2013 Addis Ababa's population is almost ten times larger than Ethiopia's second-largest city, Gondar [26] Most of Ethiopia's administrative, diplomatic, and commercial activities are concentrated in the city. Addis Ababa exhibits a robust economic interconnection and enjoys a well-established road infrastructure network that facilitates strong connectivity with other prominent urban hubs within Ethiopia. It is also connected with Djibouti's port, through a railway, and to Nairobi, with a recently upgraded highway. The city serves as a node for the flow of goods and services to other parts of the country [24].

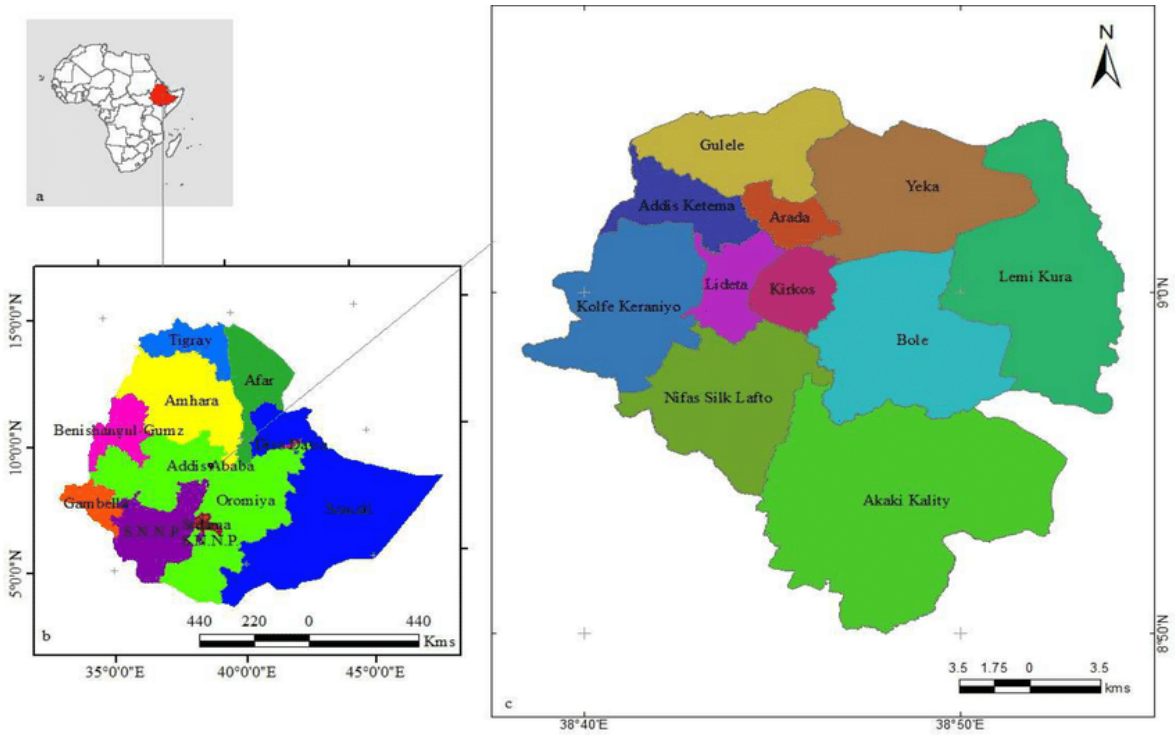

Figure 2 Map of Addis Ababa, with Sub-Cities. Source: Ethio GIS (2022)

4.2. Study Design and Sample Collection

A cross-sectional study design was used to select 110 vegetables sellers in Addis Ababa, Ethiopia. These various locations were chosen by stratified random sampling based on food joint proximity as well as sampling convenience. The research area was divided into 11 sub-city 's from sub-city 1- sub-city 11. Sub-city 1, Addis Ketema, Sub-city 2, Akaki Kaliti, Sub-city 3, Arada, Sub-city 4, Bole, Sub-city 5, Gullele, Sub-city 6, Kirkos, Sub-city 7, Kolfe Keranio, Sub-city 8, Lemi kura, Sub-city 9, Lideta, Sub-city 10, Nifas Silk-Lafto, Sub-city 11, Yeka. A total of 110 fresh vegetable samples (10 samples from each sub-city) were aseptically collected using sterile polythene zip lock bags, kept in an ice chest at 0-4 °C, transported to the BGI Ethiopia Microbiology Laboratory, Addis Ababa. The microbial analysis was performed within 2-4 hours. The study period run from November 2022 to May 2023.

4.3. Sample Preparation

Packaging bags was aseptically opened with a sterile stainless-steel knife, and 25 g portions was weighted and shaken for three minutes in 225 ml of sterile 0.1 percent (w/v) buffered peptone water to homogenize the samples [21]. Detection of hygiene indicators, *E. coli*, total coliforms, aerobic mesophilic bacteria (AMB), yeast, mold and *Enterococci*, *E. coli O157:H7*, and other food-borne pathogens such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, were analyzed from the sample homogenate.

4.4. Bacterial Isolation and Counting

4.4.1. Total Aerobic Mesophilic Bacteria (AMB)

A 0.1 ml of homogenized vegetable samples from appropriate dilution was pipetted and spread plated on a standard pre-solidified Plate Count Agar (PCA) medium and incubated at 32°C for 72 hours. Plates with colonies ranging from 30 to 300 was counted after incubation [21].

4.4.2. Total Coliform

A 0.1 ml of homogenized vegetable samples from appropriate dilution was pipetted and spread on BD Endo Agar. After incubating plates at 35°C for 18-24 hours, total coliforms from all vegetable samples were counted on Endo Agar. Coliforms are defined as red to pink colonies surrounded by precipitated bile [27].

4.4.3. Staphylococci

A 0.1 ml of homogenized vegetable samples from appropriate dilution was pipetted and spread on Mannitol Salt Agar (MSA) was incubated for 36 hours at 32°C. The golden yellow colonies were picked and purified aseptically [27]. Then, the colonies were subjected to tests like gram staining, KOH test, motility test, catalase test, motility, citrate utilization, indole test, sulfide production test and coagulase.

4.4.4. Yeast and Mold Counts

A 0.1 ml of homogenized vegetable samples from appropriate dilution was pipetted and spread on Yeast and Mold agar (YM Agar) supplemented with 250 mg Chloramphenicol. The plates were incubated for 3-5 days at 25-28°C. Yeasts and Molds was counted separately after incubation. Smooth (non-hair) colonies without a periphery (margin) extension were considered and counted as yeasts. Molds was counted as hairy colonies with periphery extension [27].

4.4.5. *E. coli*

A 0.1 ml of homogenized vegetable samples from appropriate dilution was pipetted and spread on CHROMagarTM *E. coli* and incubated in aerobic conditions at 37 °C for 24 hours. All blue colonies were classified as *E. coli* [28].

4.4.6. *Pseudomonas Species*

A 0.1 ml of homogenized vegetable samples from appropriate dilution was pipetted and spread on BD Pseudomonas Agar and incubated in aerobic conditions at 35 ± 2°C for 18-24 hours. All samples were seen under UV light. A positive result is indicated by a Blue-green colonies, greenish pigment diffusing into the surrounding medium, fluorescence under UV light [29].

4.4.7. *Fecal Streptococci*

A 0.1 ml of homogenized vegetable samples from appropriate dilution was pipetted and spread on Enterococcosel agar. The plates were incubated for 24 hours at 35 ± 2°C and colonies with beige color and strong black halos were considered as *fecal Streptococci*.

4.5. Purification

After enumeration colonies with distinct morphological differences such as color, size, and shape were picked from plates and aseptically transferred into a tube containing 5 mL tryptic soya broth and incubated at 37∘C for 24–48hours. The cultures were purified by repeated plating and pure cultures were identified using standard biochemical tests. The tests include, gram staining test, indole test, MR-VP test, citrate utilization test, motility test and catalase test and cell morphology under microscope were done [21].

4.6. Morphological and Biochemical Characterization

4.6.1. Cell morphology

4.6.1.1. Microscopic Examination

Cultures were grown overnight and wet mounted on microscopic slides. Cell shape (regular rods and cocci), cell arrangement (single, pairs, chains, cluster, tetrads) were examined under light microscope using oil immersion objectives (100x).

4.6.1.2. Gram staining

A smear of pure isolates was prepared on a clean slide and allowed to air-dry and heat-fix. The heat-fixed smear was flooded with crystal violet dye for 1 minute and rinsed under tap water for 3 seconds. Then, the slide was flooded with iodine solution for 1 minute and rinsed under gently flow tap water for 3 seconds. After rinsing, the smear was decolorized with 96% of ethanol for 20 seconds and washed slide gently under tap water for 3 seconds. Thereafter, the smear was counterstained with safranin and dried using absorbent paper. Finally, the air-dried smear was observed under the oil immersion objective. After the gram staining, gram-negative bacteria are stained pink/red and gram-positive bacteria are stained blue/purple [60].

4.6.2. Biochemical Tests

4.6.2.1. KOH-test

Gram characteristics of the isolates were indirectly done according to Gregersen (1978). A colony was aseptically picked from the surface of MRS Agar plate and it was then mixed with one or two drops of 3% KOH solution using an inoculating loop on a clean microscopic slide for 10 seconds to 2 minutes. Colonies were considered Gram-negative when the KOH solution became viscous and threads of 0.5-2.0 cm followed the loop upon raising from the suspension. Colonies that failed to show the characteristic of thread-like structure were considered as Gram- positive [37].

4.6.2.2. Catalase Test

A catalase test was carried out by flooding young colonies with a 3% solution of H_2O_2. The formation of bubbles indicated the presence of catalase and no bubbles for the absence of catalase enzyme [36].

4.6.2.3. Citrate utilization test

The slant was streaked and the tube was incubated at 37°C for 24 hours in Citrate agar to determine citrate utilization as a sole source of carbon. The presence of growth and color change from green to blue was considered as presumptive for *Salmonella spp*. [35].

4.6.2.4. Motility, Sulphide and Indole production test

The SIM medium was stabbed to the bottom and incubated at 37°C for 24 hours for the determination of H_2S production, indole production, and motility [34]. If the bacteria are motile, they will move away from the point of inoculation. The SIM medium contain ferrous sulfate. If the bacteria produce hydrogen sulfide, the ferrous sulfate will react with it to form a black precipitate, no black precipitate is considered as negative for the production of hydrogen sulfide After incubation, Kovac’s reagent is added to the medium. If the bacteria produce indole, the reagent will react with it to form a red color, no formation of red color was considered negative [34].

4.6.2.5. Coagulase test

Coagulase test was done using slide test. Briefly, a colony of the pure isolates was emulsified in a drop of distilled water on two ends of a clean glass slide to make thick suspensions. One was labeled as test and the other was as control. A loopful of human blood plasma was added to one of the suspensions and mixed gently. Clumping within 10 seconds was observed for coagulase-positive organisms [33].

4.6.2.6. Methyl Red Voges-Proskauer (MR-VP) test

This medium was stabbed to the bottom and incubated at 37°C for 24 hours for the determination of acid production for methyl red and acetoin production in Voges – Proskauer. Methyl red was added to the MR tube. A red color indicates a positive result (glucose can be converted into acidic end products such as lactate, acetate, and formate). A yellow color indicates a negative result; glucose is converted into neutral end products. For Voges - Proskauer first alpha-naphthol and then potassium hydroxide was added to the VP tube. The culture was allowed to sit for about 15 minutes for color development to occur. If acetoin was produced, then the culture turns a red color (positive result); if acetoin was not produced then the culture appears yellowish to copper in color (a negative result). [32].

4.7 PCR Detection

The GeneDisc Plate for Specified Microorganisms is a real-time PCR assay kit that enables the detection of specific microorganisms enables the detection of *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans,* and *Burkholderia cepacia complex*.[64]

4.7.1 Analysis of DNA Samples

4.7.1.1 DNA Extraction Protocol

The process began by using a sterile pipette to transfer a pure colony from a petri dish into a lysis tube. This tube was then centrifuged for 5 minutes at 10,000 g ± 250 g. The supernatant was discarded into a microbiological waste bin, and 500 µL of dilution buffer was added to the lysis tubes. The lysis tube was then vortexed vigorously to resuspend the pellet. The samples were placed in an Ultra-Lyser sonication bath for 8 minutes at 80% power, followed by heating the lysis tubes for 10 minutes at 102 °C ± 2 °C. Another 500 µL of dilution buffer was added to the lysis tube to dilute the sample at a 1:2 ratio. Finally, the DNA sample was briefly centrifuged before being added to the GeneDisc Plate. [64]

4.7.1.2 PCR Run

Gene Disc Plate and the corresponding master mix was removed from the refrigerator 30 minute before start of PCR so that it defrost then the barcode located on the Gene Disc Plate and on the ID card contained in the Master Mix bag was Scanned then the sample names was Entered and we start machine by clicking ok the Gene Disc Cycler will then automatically heat the thermal blocks to the temperatures required for this assay [64]

4.7.1.3 Gene Disc Plate Loading

The master mix vial was vortexed for 2 seconds at low speed, then briefly centrifuge for 2-5 seconds then 36µL of the Master Mix was added to each microcentrifuge tube and the caps was closed the DNA samples was centrifuged in a bench centrifuge for 15 seconds then 36µL from each of the DNA extracts was pipetted and transferred to the corresponding microcentrifuge tubes containing Master Mix. In addition, the tube was closed to prevent cross-contamination.

The filling cap was placed on the top of the Gene Disc Plate, was gently pressed the cap to ensure that there is no leakage and click on the PLAY button to start the vacuum when the vacuum progress bar reaches 10% complete, the Gene Disc Plate was gently tapped on the bench to remove any residual bubbles before the vacuum stops. Waited for the vacuum to be released before removing the cap. [64]

Four drops of mineral oil were loaded into each Gene Disc Plate sector to prevent evaporation the filling cap was placed onto the Gene Disc Plate and was started the vacuum cycle by clicking on the PLAY button on Gene Disc Cycler. Plate tapping is not needed for this vacuum cycle the cap was removed at the end of the vacuum cycle and cleaned by wiping with alcohol. The wells were checked if they are filled correctly. Since partially or unevenly filled wells may cause the assay to be aborted. [64]

The Gene Disc Plate was carefully inserted into the Gene Disc Cycler and the lid of the Gene Disc Cycler was closed. After 55 minutes, at the end of the PCR run, the Gene Disc Plate was removed and discarded into a suitable waste bin even if all the sectors were not used. [64]

4.7.1.4. Interpretation of Results

Internal software processes the results of the Gene Disc for specified microorganism's analysis. Results are presented in the "Samples" tabs. Amplification curves are displayed in the "Curves" tab. Amplification curves may be used to evaluate data in cases when e.g., amplification curves look atypical. [64]

4.8. Data Analysis

Bacterial enumerations were calculated as colony forming units per gram (CFU g−1) and colony forming units per milliliter (CFU mL−1) and converted into log10 values. The one-way analysis of variance (ANOVA) and Tukey Multiple Range Test was conducted to figure out the differences among the groups' means at a significance level of $P < 0.05$. All statistical analyses were carried out using SPSS version 27.

5. RESULT

5.1. Microbial Analysis

5.1.1. Aerobic Mesophilic Count

Aerobic Mesophilic Count were tested on plate count agar. The mean value of aerobic mesophilic bacteria among the eleven sub cities was 10.59±0.11 log CFU/g. The maximum value was 10.79±0.22 log CFU/g from Lemi Kura sub-city (Site-A) and the minimum value was 10.04±0.22 log CFU/g from Lemi Kura sub-city (Site-B) (Table 9). From Table 12 the lowest mean value of aerobic mesophilic bacteria was recorded from Addis Ketema sub-city with 10.47±0.04 log CFU/g (Table 1) and the largest mean value was 10.66±0.05 log CFU/g which was from Kirkos sub-cities (Table 6). There are statistically significant differences in aerobic mesophilic counts among the sub-cities.

Table 1 Microbiological assessment of fresh lettuce samples collected from Addis Ketema sub-city, Addis Ababa

Site	**Microbial count (log cfu/g, mean ± standard deviation)**						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	10.36±0.05	N/G	N/G	4.46±0.03	N/G	10.51±0.04	10.55±0.01
B	N/G	N/G	N/G	N/G	N/G	10.49±0.04	N/G
C	10.36±0.05	N/G	9.96±0.06	4.39±0.03	N/G	10.52±0.04	N/G
D	10.39±0.05	N/G	N/G	4.41±0.03	N/G	10.50±0.04	N/G
E	10.36±0.05	N/G	N/G	4.42±0.03	N/G	10.47±0.04	10.54±0.01
F	10.39±0.05	N/G	9.87±0.06	4.47±0.03	N/G	10.53±0.04	N/G
G	10.35±0.05	N/G	N/G	4.45±0.03	N/G	10.45±0.04	N/G
H	10.31±0.05	N/G	N/G	4.44±0.03	N/G	10.45±0.04	N/G
I	10.50±0.05	N/G	N/G	4.42±0.03	N/G	10.47±0.04	N/G
J	10.32±0.05	N/G	N/G	4.38±0.03	N/G	10.38±0.04	N/G
P-value	0.00	N/G	0.17	0.00	N/G	0.00	0.17

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; *Staph: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E. coli*;

5.1.2. *Total Coliform*

Total coliforms were tested on ENDO Medium. The mean value of total coliform bacteria among the eleven sub cities was 7.10±0.15 log CFU/g. From Table 12 the lowest mean value of total coliform was recorded from Kirkos sub-city with 5.27±0.09 log CFU/g (Table 6)and the largest mean value was 9.67±0.21 log CFU/g which was from Akaki Kality sub-cities (Table 2). Among the eleven sub cities, there is no statistically significant variation in *total coliform* counts with p-value of 0.39.

Table 2 Microbiological assessment of fresh lettuce samples collected from Akaki Kaliti sub-city, Addis Ababa

Site	**Microbial count (log cfu/g, mean ± standard deviation)**						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	10.35±0.21	N/G	N/G	4.50±0.08	N/G	10.61±0.11	N/G
B	10.47±0.21	N/G	N/G	4.65±0.08	N/G	10.63±0.11	N/G
C	10.72±0.21	N/G	9.92±0.08	4.53±0.08	N/G	10.53±0.11	N/G
D	10.70±0.21	N/G	N/G	4.60±0.08	N/G	10.61±0.11	N/G
E	10.81±0.21	N/G	10.02±0.08	4.45±0.08	N/G	10.45±0.11	N/G
F	10.84±0.21	N/G	N/G	4.60±0.08	N/G	10.67±0.11	N/G
G	10.85±0.21	N/G	10.07±0.08	4.60±0.08	N/G	10.72±0.11	N/G
H	10.93±0.21	N/G	N/G	4.50±0.08	4.68±0.00	10.61±0.11	N/G
I	N/G	N/G	N/G	N/G	N/G	10.72±0.11	N/G
J	10.97±0.21	N/G	N/G	4.41±0.08	N/G	10.41±0.11	10.68±0.00
P-value	0.00	N/G	0.08	0.00	0.34	0.00	0.34

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; *Staph: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E.coli*; N/G: no growth

Table 3 Microbiological assessment of fresh lettuce samples collected from Arada sub-city, Addis Ababa

Site	**Microbial count (log cfu/g, mean ± standard deviation)**						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	N/G	N/G	N/G	N/G	N/G	10.58±0.08	N/G
B	N/G	N/G	N/G	N/G	N/G	10.45±0.08	N/G
C	10.60±0.13	N/G	10.01±0.02	4.61±0.09	N/G	10.67±0.08	N/G
D	10.76±0.13	N/G	10.03±0.02	4.76±0.09	N/G	10.68±0.08	N/G
E	10.82±0.13	N/G	N/G	4.55±0.09	N/G	10.56±0.08	N/G
F	10.76±0.13	N/G	9.99±0.02	4.45±0.09	N/G	10.46±0.08	N/G
G	10.90±0.13	N/G	N/G	4.49±0.09	N/G	10.66±0.08	N/G
H	10.96±0.13	N/G	N/G	4.54±0.09	N/G	10.63±0.08	N/G
I	10.98±0.13	N/G	N/G	4.61±0.09	N/G	10.57±0.08	N/G
J	10.96±0.13	N/G	N/G	4.66±0.09	N/G	10.64±0.08	N/G
P-value	0.00	N/G	0.08	0.00	N/G	0.00	N/G

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; Staph*: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E. coli*; N/G: no growth

5.1.3. *Staphylococci*

Staphylococci were tested on Mannitol salt agar. From the positive samples of *S. aureus* among the eleven sub cities the highest percentage was recorded from two sub-city's Addis Ketema sub-city (site-a,e)(Table-1) and Lemi Kura sub-city (site-b,f) (Table-9) with 20% and the lowest positive samples were recorded from Akaki Kality sub-city (site-j),Bole sub-city (site-f), Gullele sub-city (site-j), Kirkos sub-city (site-c), Kolfe Keranio sub-city (site-h), Nifas Silk Lafto sub-city (site-i) and Yeka sub-city (site-f) (Table 2,4-7,10,11) with only 10% being positive for the target microorganism. and Arada and Lideta sub-cities have no growth at all (Table-12).

Table 4 Microbiological assessment of fresh lettuce samples collected from Bole sub-city, Addis Ababa

Site	**Microbial count (log cfu/g, mean ± standard deviation)**						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	10.47±0.15	N/G	N/G	4.62±0.07	N/G	10.76±0.08	N/G
B	10.59±0.15	N/G	N/G	4.63±0.07	4.67±0.00	10.71±0.08	N/G
C	10.73±0.15	N/G	10.05±0.05	4.65±0.07	N/G	10.71±0.08	N/G
D	10.72±0.15	N/G	N/G	4.70±0.07	N/G	10.64±0.08	N/G
E	N/G	N/G	N/G	N/G	N/G	10.63±0.08	N/G
F	10.77±0.15	N/G	9.95±0.05	4.63±0.07	N/G	10.70±0.08	10.64±0.00
G	N/G	N/G	N/G	N/G	N/G	10.60±0.08	N/G
H	10.91±0.15	N/G	9.97±0.05	4.58±0.07	N/G	10.52±0.08	N/G
I	N/G	N/G	N/G	4.53±0.07	N/G	10.53±0.08	N/G
J	N/G	N/G	N/G	4.64±0.07	N/G	10.70±0.08	N/G
P-value	0.01	N/G	0.08	0.00	0.34	0.00	0.34

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; Staph: Staphylococci; Fs: Fecal *streptococci*; Ps: *Pseudomonas*; Ec: *E. coli*; N/G: no growth

Coagulase positive *S. aureus* were observed in Addis Ketema sub-city (site-a,),Akaki Kality sub-city (site-j),Bole sub-city (site-f),Gullele sub-city (site-j), Kirkos sub-city (site-c), Kolfe Keranio sub-city (site-h), Nifas Silk Lafto sub-city (site-i) and Yeka sub-city (site-f) (Table-1,2,4-7,10,11) with 10% being positive but from Lemi Kura sub-city (site-b,f) 20% were found to be positive. Coagulase positive *S. aureus* from the different sub-cities were unevenly distributed from one block to another. The mean value of Coagulase positive *S. aureus* among the eleven sub cities was 1.06±0.03 log CFU/g. From Table 12 the maximum value is 2.11±0.01 log CFU/g from Addis Ketema sub-city and the lowest mean value of *Staphylococci* is from Yeka sub-city with 1.05±0.00 log CFU/g. There is no statically significant difference in *Staphylococci* counts among the sub-cities.

Table 5 Microbiological assessment of fresh lettuce samples collected from Gulele sub-city, Addis Ababa

Site	Microbial count (log cfu/g, mean ± standard deviation)						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	N/G	N/G	N/G	N/G	N/G	10.75±0.11	N/G
B	10.6±0.16	N/G	9.94±0.04	4.63±0.09	N/G	10.67±0.11	N/G
C	N/G	N/G	N/G	N/G	N/G	10.70±0.11	N/G
D	10.63±0.16	N/G	N/G	4.67±0.09	N/G	10.46±0.11	N/G
E	10.71±0.16	N/G	9.97±0.04	4.73±0.09	N/G	10.66±0.11	N/G
F	10.77±0.16	N/G	10.02±0.04	4.51±0.09	N/G	10.48±0.11	N/G
G	N/G	N/G	N/G	N/G	N/G	10.68±0.11	N/G
H	N/G	N/G	N/G	N/G	N/G	10.47±0.11	N/G
I	10.97±0.16	N/G	N/G	4.74±0.09	N/G	10.55±0.11	N/G
J	10.97±0.16	N/G	N/G	4.52±0.09	N/G	10.48±0.11	10.62±0.00
P-value	0.01	N/G	0.08	0.00	N/G	0.00	0.34

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; *Staph: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E. coli*; N/G: no growth

5.1.4. Yeast and Mold

Yeast and Mold were tested on Yeast and Mold agar (YM Agar) . . From the eleven sub cities, only 9.1% of all samples tested positive for yeast and the rest of the samples, 82.7% tested negative and 8.2% of samples had no growth at all. Mold was not detected in all of the samples tested. From all sub cities the highest percentage of positive samples recorded from Kolfe Keranio sub-city with 30% of the samples collected and the lowest positive samples recorded from 3 sub-cities Lemi Kura, Akaky Kaliti and Bole with only 10% being positive for the target microorganism and the rest of the sub-cities got similar negative result.

Table 6 Microbiological assessment of fresh lettuce samples collected from Kirkos sub-city, Addis Ababa

Site	Microbial count (log cfu/g, mean ± standard deviation)						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	10.45±5.56	N/G	9.53±4.54	4.57±0.36	N/G	10.63±0.05	N/G
B	N/G	N/G	N/G	N/G	N/G	10.67±0.05	N/G
C	10.67±5.56	N/G	9.11±4.54	4.08±0.36	N/G	10.60±0.05	10.7±0.00
D	N/G	N/G	N/G	N/G	N/G	10.63±0.05	N/G
E	N/G	N/G	N/G	N/G	N/G	10.75±0.05	N/G
F	10.56±5.56	N/G	9.52±4.54	3.89±0.36	N/G	10.59±0.05	N/G
G	N/G	N/G	N/G	N/G	N/G	10.68±0.05	N/G
H	10.45±5.56	N/G	N/G	N/G	N/G	10.73±0.05	N/G
I	N/G	N/G	N/G	N/G	N/G	10.65±0.05	N/G
J	10.59±5.56	N/G	N/G	4.54±0.36	N/G	10.65±0.05	N/G
P-value	0.01	N/G	0.08	0.00	N/G	0.00	0.34

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; *Staph: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E. coli*; N/G: no growth

Table 7 Microbiological assessment of fresh lettuce samples collected from Kolfe Keranio sub-city, Addis Ababa

Site	Microbial count (log cfu/g, mean ± standard deviation)						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	10.35±0.25	N/G	10.01±0.04	4.38±0.13	N/G	10.71±0.08	N/G
B	N/G	N/G	N/G	N/G	4.55±0.02	10.56±0.08	N/G
C	10.63±0.25	N/G	10.04±0.04	4.71±0.13	N/G	10.51±0.08	N/G
D	N/G	N/G	N/G	N/G	4.59±0.02	10.61±0.08	N/G
E	10.78±0.25	N/G	N/G	4.58±0.13	N/G	10.70±0.08	N/G
F	N/G	N/G	N/G	N/G	N/G	10.61±0.08	N/G
G	N/G	N/G	N/G	N/G	4.55±0.02	10.57±0.08	N/G
H	10.88±0.25	N/G	9.96±0.04	4.62±0.13	N/G	10.57±0.08	10.66±0.00
I	N/G	N/G	N/G	N/G	N/G	10.62±0.08	N/G
J	10.97±0.25	N/G	N/G	4.72±0.13	N/G	10.44±0.08	N/G
P-value	0.01	N/G	0.08	0.00	0.08	0.00	0.34

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold*; Staph: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E. coli*; N/G: no growth

The mean value of yeast and mold among the eleven sub cities was 0.42±1.32 log CFU/g. From Table 12 the lowest mean value of yeast and mold was recorded from Lemi Kura sub-city with 0.44±1.40 log CFU/g and the largest mean value was 1.37±2.20 log CFU/g which was recorded from Kolfe Keranio sub-city. There were no statistically significant differences in yeast and mold counts among the sub-cities.

Table 8 Microbiological assessment of fresh lettuce samples collected from Lideta sub-city, Addis Ababa

Site	Microbial count (log cfu/g, mean ± standard deviation)						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	N/G	N/G	N/G	N/G	N/G	10.60±0.05	N/G
B	N/G	N/G	N/G	N/G	4.72±0.19	10.65±0.05	N/G
C	10.58±0.15	N/G	9.98±0.05	4.63±0.08	N/G	10.55±0.05	N/G
D	10.72±0.15	N/G	9.96±0.05	4.58±0.08	N/G	10.56±0.05	N/G
E	10.72±0.15	N/G	10.05±0.05	4.60±0.08	N/G	10.53±0.05	N/G
F	N/G	N/G	N/G	N/G	N/G	10.67±0.05	N/G
G	10.85±0.15	N/G	N/G	4.58±0.08	N/G	10.63±0.05	N/G
H	N/G	N/G	N/G	N/G	4.45±0.19	10.66±0.05	N/G
I	N/G	N/G	N/G	N/G	N/G	10.57±0.05	N/G
J	10.97±0.15	N/G	N/G	4.70±0.08	N/G	10.65±0.05	N/G
P-value	0.01	N/G	0.08	0.00	0.17	0.00	N/G

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; *Staph: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E.coli*; N/G: no growth

Table 9 Microbiological assessment of fresh lettuce samples collected from Lemi Kura sub-city, Addis Ababa

Site	Microbial count (log cfu/g, mean ± standard deviation)						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	N/G	N/G	N/G	N/G	N/G	10.79±0.22	N/G
B	10.64±0.18	N/G	9.89±0.33	3.61±0.67	N/G	10.04±0.22	10.30±0.34
C	N/G	N/G	N/G	N/G	N/G	10.52±0.22	N/G
D	10.43±0.18	N/G	9.34±0.33	3.60±0.67	N/G	10.61±0.22	N/G
E	10.80±0.18	N/G	N/G	N/G	N/G	10.77±0.22	N/G
F	10.77±0.18	N/G	9.30±0.33	4.47±0.67	4.41±0.00	10.65±0.22	10.78±0.34
G	N/G	N/G	N/G	N/G	N/G	10.61±0.22	N/G
H	N/G	N/G	N/G	N/G	N/G	10.71±0.22	N/G
I	10.35±0.18	N/G	N/G	4.35±0.67	N/G	10.68±0.22	N/G
J	10.70±0.18	N/G	N/G	4.43±0.67	N/G	10.77±0.22	N/G
P-value	0.01	N/G	0.08	0.00	0.34	0.00	0.17

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; *Staph: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E. coli*; N/G: no growth

5.1.5. *E. coli*

E. coli was tested on CHROMagar ™ medium. The mean value of *E. coli* bacteria among the eleven sub cities was 2.88±0.09 log CFU/g. The maximum value was 3.00±0.02 log CFU/g from Arada sub-city and the minimum value was 1.98±0.06 log CFU/g Addis Ketema sub-city (Table 12). The lowest percentage of positive *E. coli* samples were recorded from Addis Ketema sub-city with only 20% (Table-1) being positive for the target microorganism and the rest of the sub-cities had similar positives result of 30% (Table-1-11). The maximum and the minimum values was from Kirkos sub-city site-g and site-d respectively (Table-6). There was no statically significant variation in *E.coli* counts among the sub-cities.

Table 10 Microbiological assessment of fresh lettuce samples collected from Nifas Silk Lafto sub-city, Addis Ababa

Site	Microbial count (log cfu/g, mean ± standard deviation)						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	N/G	N/G	N/G	N/G	N/G	10.45±0.10	N/G
B	N/G	N/G	N/G	N/G	N/G	10.70±0.10	N/G
C	10.60±0.11	N/G	9.99±0.02	4.48±0.15	N/G	10.49±0.10	N/G
D	10.78±0.11	N/G	9.97±0.02	4.32±0.15	N/G	10.53±0.10	N/G
E	10.82±0.11	N/G	10.00±0.02	4.72±0.15	N/G	10.59±0.10	N/G
F	10.81±0.11	N/G	N/G	4.61±0.15	N/G	10.60±0.10	N/G
G	10.90±0.11	N/G	N/G	4.71±0.15	N/G	10.62±0.10	N/G
H	10.88±0.11	N/G	N/G	4.33±0.15	N/G	10.42±0.10	N/G
I	10.96±0.11	N/G	N/G	4.50±0.15	N/G	10.69±0.10	10.58±0.00
J	10.93±0.11	N/G	N/G	4.68±0.15	N/G	10.66±0.10	N/G
P-value	0.00	N/G	0.08	0.00	N/G	0.00	0.34

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; *Staph: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E. coli*; N/G: no growth

5.1.6. *Pseudomonas Aeruginosa*

Pseudomonas Aeruginosa were tested on Pseudomonas medium. Hundred percent (100%) of all samples tested negative for the targeted microorganism.

5.1.7. *Fecal streptococci*

Fecal streptococci were tested on Enterococcosel Medium. The mean value of *Fecal streptococci* bacteria among the eleven sub cities was 2.91±0.52 log CFU/g. The maximum value was 4.76±0.10 log CFU/g from Arada sub-city (Site-d) (Table-3) and the minimum value was 3.60±0.67log CFU/g from Lemi Kura sub-city (Site-d) (Table-9). From Table 12 the lowest mean value of *Fecal streptococci* was from Lemi Kura sub-city with 1.71±0.36 log CFU/g and the highest mean value was 4.08±0.08 log CFU/g which was from Akaki Kality sub-city. There is statistically significant difference in *Fecal streptococci* counts among the sub-cities.

Table 11. Microbiological assessment of fresh lettuce samples collected from Yeka sub-city, Addis Ababa

Site	Microbial count (log cfu/g, mean ± standard deviation)						
	TC	Ps	Ec	Fs	YM	AMB	Staph
A	10.53±0.19	N/G	10.05±0.05	4.60±0.13	N/G	10.56±0.11	N/G
B	N/G	N/G	N/G	N/G	N/G	10.41±0.11	N/G
C	10.55±0.19	N/G	9.95±0.05	4.30±0.13	N/G	10.42±0.11	N/G
D	N/G	N/G	N/G	N/G	N/G	10.65±0.11	N/G
E	10.72±0.19	N/G	N/G	4.37±0.13	N/G	10.76±0.11	10.52±0.00
F	N/G	N/G	N/G	N/G	4.58±0.03	10.63±0.11	N/G
G	10.90±0.19	N/G	N/G	4.68±0.13	N/G	10.48±0.11	N/G
H	10.88±0.19	N/G	10.03±0.05	4.56±0.13	N/G	10.62±0.11	N/G
I	10.99±0.19	N/G	N/G	4.52±0.13	4.54±0.03	10.55±0.11	N/G
J	N/G	N/G	N/G	N/G	N/G	10.59±0.11	N/G
P-value	0.01	N/G	0.08	0.00	0.17	0.00	0.34

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; *Staph: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E. coli*; N/G: no growth

Table 12 Summary of the microbiological assessment of fresh lettuce samples among the eleven sub-cities in Addis Ababa

Site	Microbial count (log cfu/g, mean ± standard deviation)						
	TC	Ps	Ec	Fs	YM	AMB	Staph
Addis Ketema	9.33±0.05	N/G	1.98±0.06	3.98±0.03	N/G	10.47±0.04	2.11±0.01
Akaki Kality	9.67±0.21	N/G	3.00±0.08	4 .08±0.08	0.47±0.00	10.60±0.11	1.07±0.00
Arada	8.67±0.13	N/G	3.00±0.02	3.67±0.10	N/G	10.59±0.08	N/G
Bole	6.42±0.15	N/G	3.00±0.05	3.70±0.05	0.47±0.00	10.65±0.08	1.06±0.00
Gullele	6.46±0.16	N/G	2.99±0.04	2.70±0.10	N/G	10.59±0.11	1.06±0.00
Kirkos	5.27±0.09	N/G	2.82±0.24	1.71±0.35	N/G	10.66±0.05	1.07±3.38
Kolfe Keranio	5.36±0.25	N/G	3.00±0.04	2.30±0.14	1.37±0.02	10.59±0.08	1.07±0.00
Lideta	5.38±0.15	N/G	3.00±0.05	2.31±0.05	0.92±0.19	10.61±0.05	N/G
Lemi Kura	6.37±0.18	N/G	2.85±0.33	2.05±0.10	0.44±0.00	10.62±0.22	2.11±0.34
Nifas Silk Lafto	8.67±0.11	N/G	3.00±0.02	3.64±0.16	N/G	10.57±0.10	1.06±0.00
Yeka	6.45±0.19	N/G	3.00±0.05	2.70 ±0.14	0.91±0.03	10.57±0.11	1.05±0.00
Average	7.10±0.15	N/G	2.88±0.09	2.91±0.52	0.42±1.32	10.59±0.11	1.06±0.03
P-value	0.39	N/G	1.00	0.00	0.22	0.03	0.94

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold*; Staph: Staphylococci; Fs: Fecal streptococci; Ps: Pseudomonas; Ec: E. coli*; N/G: no growth

5.2. PCR Analysis Result

Thirty-three positive representative samples from the biochemical test were taken and PCR analysis were conducted and only 2 samples from Addis Ketema and Lideta sub cities were positive for *E. coli* and *P. aeruginosa*, respectively. Table-13 show Addis Ketema sub-city PCR result and it was positive for *E. coli* it had a positive curve at 37.95. Table-14 Lideta sub-city PCR result and it was positive for *P. aeruginosa* it had a positive curve at 35.88.

Table 13 Addis Ketema sub-city PCR Result

Addis Ketema Sub-city

Target	Ct	Results
B. cepacia complex	0	Not detected
C. albicans	0	Not detected
E. coli	37.95	Presence
P. aeruginosa	0	Not detected
S. aureus	0	Not detected
Salmonella spp.	0	Not detected

Ct=cut off, E. coli=Escherichia coli, P.aeruginosa=Pseudomonas aeruginosa, S.aureus=Staphylococcus aureus, C. albicans=Candida albicans and B. cepacia complex=Burkholderia cepacia complex

Table 14 Lideta sub-city PCR Result

Lideta Sub-city

Target	Ct	Results
B. cepacia complex	0	Not detected
C. albicans	0	Not detected
E. coli	0	Not detected
P. aeruginosa	35.88	Presence
S. aureus	0	Not detected
Salmonella spp.	0	Not detected

Ct=cut off, E. coli=Escherichia coli, P.aeruginosa=Pseudomonas aeruginosa, S.aureus=Staphylococcus aureus, C. albicans=Candida albicans and B. cepacia complex=Burkholderia cepacia complex

5.3 Correlation matrix

The correlation analysis of bacteria isolates, using Kendall's tau_b, reveals that the correlation coefficients between Total Coliform (TC) and *E.coli*, fecal streptococci, yeast and mold, aerobic mesophilic count, and *Staphylococci* were 0.020, 0.101, -0.079, -0.070, and 0.022, respectively with no significant correlations at the 0.05 level. For *E.coli*, the correlation coefficients with fecal streptococci, yeast and mold, aerobic mesophilic count, and *Staphylococci* were -0.053, -0.024, -0.119, and -0.190, respectively with the correlation with *Staphylococci* being significant at the 0.05 level.

The correlation coefficients for fecal streptococci with yeast and mold, aerobic mesophilic count, and *Staphylococci* were 0.040, 0.140, and -0.057, respectively with the correlation with aerobic mesophilic count being significant at the 0.05 level. The correlation coefficients for yeast and mold with aerobic mesophilic count and *Staphylococci* were 0.062 and 0.094, respectively both not significant at the 0.05 level. Lastly, the correlation coefficient between aerobic mesophilic count and *Staphylococci* was -0.031, not significant at the 0.05 level. The sample size for all correlations was 110. These results provide a comprehensive understanding of the relationships between different types of bacteria, and further research may be needed to understand the implications of these correlations.

Table 15 Correlation Analysis on Bacteria Isolates among the eleven sub-cities in Addis Ababa

Variable	TC	Ec	Fs	YM	AMB	Staph
TC	1.000					
Ec	0.020	1.000				
Fs	0.101	-0.053	1.000			
YM	-0.079	-0.024	0.040	1.000		
AMB	-0.070	-0.119	.140*	0.062	1.000	
Staph	0.022	-.190*	-0.057	0.094	-0.031	1.000

TC: Total coliform; AMB: Aerobic mesophilic bacteria; YM: Yeast and Mold; *Staph: Staphylococci; Fs: Fecal streptococci; Ec: E.coli;*

5.4. Prevalence of pathogenic microorganism in fresh lettuce

The results of the analysis revealed that 74 out of 110 samples tested positive for total coliform, representing 67.3% prevalence. *Staphylococci* were found in 18 samples, indicating a prevalence of 16.4%. *E. coli* was detected in 34 samples, accounting for 30.9% prevalence. *Fecal Streptococci* were present in 105 samples, with a prevalence of 95.5%. Interestingly, none of the samples showed any *Pseudomonas* growth, however, PCR detection shows the presence of *E. coli* and *P. aeruginosa*.

6. DISCUSSION

This study investigated the presence and levels of various microorganisms of lettuce in different sub-cities of Addis Ababa. Microbiological analyses revealed contamination of lettuce at the study location. The lettuces were contaminated with different flora, including fecal streptococci, total coliforms, *E. coli*, aerobic mesophilic count, staphylococci, yeast and mold.

Aerobic mesophilic counts were positive in all sub-cities with mean log cfu/g values ranging from 10.47 to 10.66. greater than earlier Moroccan findings, Ibenyassine *et al*. [38], while other research found a lower count ranging from 2 to 8 $logCFUg^{-1}$, including one from Awetu River in Jimma Town, Southwestern Ethiopia by D.Weldezgina *et al*. with an average count of 6.94 to 8.06 log CFU g^{-1}.

According to the London Health Protection Agency. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods, the article said “If sampled at the point of production AMCs are likely to categorize foods as “satisfactory”, whereas if sampled at the end of shelf-life an AMCs can normally be expected to approach the upper “borderline” limit and type of packaging, and the capturability of injured organisms will also contribute further to the variation of reproducibility between microbiological results.” [65]

In general, no definition exists for the allowable level of microorganisms for lettuce provided in Ethiopia [21]. However, the Technical Guidelines for Hazard Analysis and Critical Control Points-Total Quality Management (HACCPTQM) specify the microbiological quality of raw foods harboring aerobic mesophilic bacteria can be classified as good (4 log10 CFU/g), average (4.0-6.7 log10 CFU/g), bad (6.7-7.7 log10 CFU/g), and spoiled (>7.7 log10 CFU/g) [31]. So, the result detected on this research consistent with hazardous conditions since aerobic organisms reveal the sample's contamination and the presence of favorable circumstances for microbe proliferation [21] that might be due to poor handling and the use of contaminated water used on the irrigation of the lettuce [21]

Staphylococci were found in nine sub-cities as well, with mean log cfu/g values ranging from 1.05 to 2.11. Lower than the findings of Degaga *et al.* [27] with count of 4.0 and 6.0 log CFU/g. it was also Lower than result conducted in an urban area located in Daloa, Centre-West Côte d'Ivoire by Kouassi *et al*. [49] with result of $6x10^5$ to $9.2x10^5$ CFU/g this might be due to the use of contaminated water for lettuce cultivation.

Similar to this research Coagulase positive *Staphylococcus aureus* has been reported in an urban area located in Daloa, Centre-West Côte d'Ivoire by Kouassi *et al*. [49] .The positive samples have a mean value of 1.06±0.03 log CFU/g which is low According to the London Health Protection Agency. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods [65].

Eni *et al.* [47] demonstrated that *S. aureus* is a common source of contamination in vegetables. It has been documented that the production of enterotoxin takes place when the counts of *Staphylococcus aureus* reach 6 log CFU/g. [47]. *Staphylococci* are common bacteria that can be found on human skin and their presence suggests on the surface of vegetables may be attributed to contact with unwashed hands during the selection process prior to purchase [48].

Yeast and mold were detected in six sub-cities, with mean log cfu/g values ranging from 0.44 to 1.37. Lower than the findings of Degaga *et al* with ≤2.9 log CFU/g [27]. And also lower than the finding of Clément *et al.* [30] with CFU/g load for yeasts and molds ranged from $3.4x10^5$to $9.3x10^5$. According to Mritunjay and Kumar [44], yeast and mold counts vary from 0.3 to 5.5 log cfu/g. Some molds can create mycotoxins and allergens, and huge populations of conidia can cause health problems [27].

All sub-cities also tested positive for total coliforms, with mean log cfu/g values ranging from 5.27 to 9.67. The mean counts of total coliforms in the vegetable samples analyzed in this study are comparatively lower than the findings reported by Nipa *et al*. [39], who recorded counts exceeding 1100 MPN/100mL from salad vegetables.

Similarly, our results are lower than those reported by Ashenafi [41] for raw consumed food like tomatoes in Ethiopia, where total coliform counts were 1.5 × 10^3 MPN/10g and fecal coliform counts were 3.7 × 10^2 MPN/10g. The variation in counts observed can be attributed to factors such as initial contamination levels, storage conditions, and the hygiene practices of utensils and vegetable handlers [21].

E. coli was also detected in all sub-cities. With mean log cfu/g values ranged from 1.98 to 3.00. this study has lower result than reported by Woldetsadik *et al* [51] which reported mean fecal coliform concentrations in lettuce were 3.46-5.03 log10 MPN 100 g^{-1} from lettuce (*Lactuca sativa*) irrigated with wastewater in Addis Ababa, Ethiopia. This study was also lower than the findings of Girmaye *et al*. [52] who reported that the fecal coliform counts of lettuce samples grown in wastewater ranged from 2.3×10^5 to 3.1×10^5 CFU/g from two farms around Adama Town (Ethiopia).

This research had higher result than Maïwore *et al*.[54] which studied the influence of some bacteriological sources of contamination on the quality of lettuce consumed at Maroua (Cameroon) were the fecal coliform counts of lettuce samples were 1.80 Log UFC/g. This means the data in this research shows the lettuce have been grown using wastewater and consumption of this lettuce raw without washing can lead to adverse health risks to consumers.

According to the London Health Protection Agency. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods, the acceptable amount of *E.coli* amount should be <20 CFU/g and result between <20 and 10^4 show moderate contamination any and any result more than $>10^4$ $_{CFU/g}$ is considered high and is "UNSATISFACTORY: Potentially injurious to health and/or unfit for human consumption" and the amount which were found show unsatisfactory result and because *E.coli* Originates from the intestinal tract of man and animals the result shows the lettuce may have been irrigated with fecally contaminated water. [65]

In terms of *fecal streptococci*, all sub-cities tested positive for its presence, with mean log cfu/g values ranging from 1.71 to 4.08, which were lower than the findings of Ait-Mouheb *et al.*[53] which studid the effect of untreated or reclaimed wastewater drip-irrigation for lettuces and leeks on yield, soil and fecal indicators, who recorded *fecal streptococci* counts in one sample of lettuce leaves at a low concentration level of < 100 MPN g^{-1} . This research also have lower fecal streptococci count than Atidégla *et al* which studied vegetable contamination by the fecal bacteria of poultry manure: case study of gardening sites in Southern Benin, were fecal streptococci count on lettuce were 5 to 8 log cfu/g.

According to the London Health Protection Agency. Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods, the acceptable amount of *fecal streptococci* amount should be <20 CFU/g and result between <20 and 10^4 show moderate contamination any and any result more than $>10^4$ CFU/g is considered high and is "UNSATISFACTORY: Potentially injurious to health and/or unfit for human consumption" and the amount which were found show 2.05 to 4.62 log cfu/g and this result shows acceptable amount of *fecal streptococci* contamination. [65]

Pseudomonas aeruginosa was not found in any of the sub-cities, which is unusual. The dominance of coliforms over *Pseudomonas aeruginosa* among the bacterial taxa isolated from lettuce is unexpected. This finding contradicts with Lund's [58] finding that the majority of bacteria discovered on plant surfaces are gram-negative and belong to the *Pseudomonas* or *Enterobacteriaceae* families.
However, no growth is not unique to this study because Zhang *et al*. [57] found no bacterial pathogens such as *Shigella* and *E. coli* in any of the green vegetable and fruit samples collected in Canada. As a result, contamination of leafy vegetables by bacterial pathogens was discovered to be infrequent and sporadic occurrence [56].

Out of the 33 PCR analysis only Addis Ketema and Lideta sub cities had a positive PCR result. Table 13 show Addis Ketema sub-city PCR result and it was positive for *E.coli* it had a positive curve at 37.95. Table 14 show Lideta sub-city PCR result and it was positive for *P. aeruginosa* it had a positive curve at 35.88.

The PCR analysis was only conducted on 33 representative samples and do not show the full scale of contamination and the PCR analysis also detect not only live cells but also dead cells it also analyzes only pre-loaded 6 microorganisms and cannot be taken as the full representation of the microorganisms present on the lettuce samples. Because the PCR analysis can detect not only live cells but also dead cells as well. The Lideta sub-city PCR result was positive for *P. aeruginosa* doesn't mean the microorganism was alive on the lettuce sample.

Even though this study tried to assess the microbial quality and safety of freshly prepared lettuce samples. This study is limited by only analyzing one but not all; ready to eat vegetables. On the other hand, this study only shows the microbial load of lettuce but don't examine how they are contaminated. Even though this study tried to take a representative sample it may not show the depth of the problem of the entire city, Addis Ababa.

7. CONCLUSION

This analysis uncovered a high amount of microorganisms, a sign of poor handling, process and temperature control. Notably, hazardous bacterial strains such *Escherichia coli*, coagulase-positive *Staphylococcus aureus*, were consistently found in the samples from the 11 examined sub cities, along with fecal coliforms, enteric bacteria, mesophilic aerobic bacteria, yeasts, and molds.

This study shows the inadequate hygienic conditions of lettuce in the study area and consumption of these lettuce may be dangerous to consumers. Because consumers are at risk of contracting food-borne infections. This analysis underscores the urgent need for improved hygiene and safety measures in the production and handling of lettuce to safeguard consumers from potential biological health hazards.

8. RECOMMENDATION

- The PCR analysis was only conducted on 33 representative isolates and do not show the full scale of contamination and the PCR analysis also detect not only live cells but also dead cells it also analyzes only pre-loaded 6 microorganisms so a better PCR analysis method must be used to show the full scale of the contaminant microorganisms found on lettuce.
- All lettuce samples were contaminated with different microorganisms so a proper washing method must be employed to decrease the damage caused by the microorganisms.
- This research only showed the level of contamination of lettuce found in the market and don't show how they were contaminated so more research must be conducted to find the source of the contamination in order to control the contamination at the source.
- This research only focuses on lettuce and don't cover other ready to eat vegetable's so more research must be conducted on other ready to eat vegetables in order to see the full scale of the danger.

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10. List of Appendix

Appendix 1 Results of one-way ANOVA

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
TOTAL COLIFORM	Between Groups	276.215	10	27.621	1.079	0.386
	Within Groups	2534.582	99	25.602		
	Total	2810.797	109			
E.COLI	Between Groups	9.252	10	0.925	0.041	1.000
	Within Groups	2211.643	99	22.340		
	Total	2220.895	109			
fecal streptococci	Between Groups	27.658	10	2.766	4.682	0.000
	Within Groups	58.482	99	0.591		
	Total	86.140	109			
yeast and mold	Between Groups	22.753	10	2.275	1.345	0.218
	Within Groups	167.500	99	1.692		
	Total	190.253	109			
aerobic mesophilic count	Between Groups	0.234	10	0.023	2.137	0.028
	Within Groups	1.086	99	0.011		
	Total	1.321	109			
Staphylococci	Between Groups	44.412	10	4.441	0.412	0.938
	Within Groups	1067.224	99	10.780		
	Total	1111.636	109			

ANOVA Effect Sizes[a,b]				
		Point Estimate	95% Confidence Interval	
			Lower	Upper
TOTAL COLIFORM	Eta-squared	0.098	0.000	0.131
	Epsilon-squared	0.007	-0.101	0.043
	Omega-squared Fixed-effect	0.007	-0.100	0.042
	Omega-squared Random-effect	0.001	-0.009	0.004
E.COLI	Eta-squared	0.004	0.000	0.000
	Epsilon-squared	-0.096	-0.101	-0.101
	Omega-squared Fixed-effect	-0.095	-0.100	-0.100
	Omega-squared Random-effect	-0.009	-0.009	-0.009
fecal streptococci	Eta-squared	0.321	0.113	0.392
	Epsilon-squared	0.253	0.023	0.331
	Omega-squared Fixed-effect	0.251	0.023	0.329
	Omega-squared Random-effect	0.032	0.002	0.047
yeast and mold	Eta-squared	0.120	0.000	0.161
	Epsilon-squared	0.031	-0.101	0.076
	Omega-squared Fixed-effect	0.030	-0.100	0.076
	Omega-squared Random-effect	0.003	-0.009	0.008
aerobic mesophilic count	Eta-squared	0.178	0.000	0.235
	Epsilon-squared	0.094	-0.101	0.158
	Omega-squared Fixed-effect	0.094	-0.100	0.157
	Omega-squared Random-effect	0.010	-0.009	0.018
Staphylococci	Eta-squared	0.040	0.000	0.024
	Epsilon-squared	-0.057	-0.101	-0.074
	Omega-squared Fixed-effect	-0.056	-0.100	-0.074
	Omega-squared Random-effect	-0.005	-0.009	-0.007
a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.				
b. Negative but less biased estimates are retained, not rounded to zero.				

Homogeneous Subsets

TOTAL COLIFORM

sub-city s		N	Subset for alpha = 0.05 1
Tukey HSD[a]	KIRKOS	10.00	5.27
	KOLFE KERANIO	10.00	5.36
	LEMI KURA	10.00	5.38
	LIDETA	10.00	6.37
	BOLE	10.00	6.42
	YEKA	10.00	6.45
	GULELE	10.00	6.46
	NIFAS SILK LFTO	10.00	8.67
	ARADA	10.00	8.67
	ADDIS KETEMA	10.00	9.33
	AKAKI KALITY	10.00	9.67
	Sig.		0.69
Tukey B[a]	KIRKOS	10.00	5.27
	KOLFE KERANIO	10.00	5.36
	LEMI KURA	10.00	5.38
	LIDETA	10.00	6.37
	BOLE	10.00	6.42
	YEKA	10.00	6.45
	GULELE	10.00	6.46
	NIFAS SILK LFTO	10.00	8.67
	ARADA	10.00	8.67
	ADDIS KETEMA	10.00	9.33
	AKAKI KALITY	10.00	9.67

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

E.COLI

sub-city s		N	Subset for alpha = 0.05 1
Tukey HSD[a]	ADDIS KETEMA	10	1.98

	KIRKOS	10	2.82
	LIDETA	10	2.85
	GULELE	10	2.99
	NIFAS SILK LFTO	10	3.00
	BOLE	10	3.00
	LEMI KURA	10	3.00
	AKAKI KALITY	10	3.00
	KOLFE KERANIO	10	3.00
	YEKA	10	3.00
	ARADA	10	3.00
	Sig.		1.00
Tukey B[a]	ADDIS KETEMA	10	1.98
	KIRKOS	10	2.82
	LIDETA	10	2.85
	GULELE	10	2.99
	NIFAS SILK LFTO	10	3.00
	BOLE	10	3.00
	LEMI KURA	10	3.00
	AKAKI KALITY	10	3.00
	KOLFE KERANIO	10	3.00
	YEKA	10	3.00
	ARADA	10	3.00

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

sub-city s		N	1
Tukey HSD[a]	LIDETA	10	3.18
	KIRKOS	10	3.37
	ADDIS KETEMA	10	
	AKAKI KALITY	10	
	KOLFE KERANIO	10	
	YEKA	10	
	NIFAS SILK LFTO	10	
	ARADA	10	
	GULELE	10	
	BOLE	10	
	LEMI KURA	10	
	Sig.		1.00
Tukey B[a]	LIDETA	10	3.18
	KIRKOS	10	3.37

	ADDIS KETEMA	10	
	AKAKI KALITY	10	
	KOLFE KERANIO	10	
	YEKA	10	
	NIFAS SILK LFTO	10	
	ARADA	10	
	GULELE	10	
	BOLE	10	
	LEMI KURA	10	

yeast and mold

sub-city s		N	Subset for alpha = 0.05 1
Tukey HSD[a]	ADDIS KETEMA	10	0.00
	ARADA	10	0.00
	GULELE	10	0.00
	KIRKOS	10	0.00
	NIFAS SILK LFTO	10	0.00
	LIDETA	10	0.44
	BOLE	10	0.47
	AKAKI KALITY	10	0.47
	YEKA	10	0.91
	LEMI KURA	10	0.92
	KOLFE KERANIO	10	1.37
	Sig.		0.41
Tukey B[a]	ADDIS KETEMA	10	0.00
	ARADA	10	0.00
	GULELE	10	0.00
	KIRKOS	10	0.00
	NIFAS SILK LFTO	10	0.00
	LIDETA	10	0.44
	BOLE	10	0.47
	AKAKI KALITY	10	0.47
	YEKA	10	0.91
	LEMI KURA	10	0.92
	KOLFE KERANIO	10	1.37

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

sub-city s		N	1
Tukey HSD[a]	ADDIS KETEMA	10	10.47
	YEKA	10	10.57
	NIFAS SILK LFTO	10	10.57
	ARADA	10	10.59
	GULELE	10	10.59
	KOLFE KERANIO	10	10.59
	AKAKI KALITY	10	10.60
	LEMI KURA	10	10.61
	LIDETA	10	10.62
	BOLE	10	
	KIRKOS	10	
	Sig.		0.10
Tukey B[a]	ADDIS KETEMA	10	10.47
	YEKA	10	10.57
	NIFAS SILK LFTO	10	10.57
	ARADA	10	10.59
	GULELE	10	10.59
	KOLFE KERANIO	10	10.59
	AKAKI KALITY	10	10.60
	LEMI KURA	10	10.61
	LIDETA	10	10.62
	BOLE	10	
	KIRKOS	10	

Staphylococci

sub-city s		N	Subset for alpha = 0.05 1
Tukey HSD[a]	ARADA	10	0.00
	LEMI KURA	10	0.00
	YEKA	10	1.05
	NIFAS SILK LFTO	10	1.06
	GULELE	10	1.06
	BOLE	10	1.06
	KOLFE KERANIO	10	1.07

	AKAKI KALITY	10	1.07
	KIRKOS	10	1.07
	LIDETA	10	2.11
	ADDIS KETEMA	10	2.11
	Sig.		0.94
Tukey B[a]	ARADA	10	0.00
	LEMI KURA	10	0.00
	YEKA	10	1.05
	NIFAS SILK LFTO	10	1.06
	GULELE	10	1.06
	BOLE	10	1.06
	KOLFE KERANIO	10	1.07
	AKAKI KALITY	10	1.07
	KIRKOS	10	1.07
	LIDETA	10	2.11
	ADDIS KETEMA	10	2.11

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.000.

Appendix 2 Prevalence of pathogenic microorganism

Microorganism	*Prevalence Among Tested Samples*	*Positive Results*	*No Growth*
Total Coliform	74 (67.3%)	Yes	-
Staphylococci	18 (16.4%)	Yes	-
E. coli	34 (30.9%)	Yes	15 samples
Fecal Streptococci	105 (95.5%)	Yes	4 samples
Pseudomonas	All 110 samples (100.0%)	No	-

Appendix 3 Correlations

Correlations

			TOTAL COLIFORM	E.COLI	fecal streptococci	yeast and mold	aerobic mesophilic count	Staphylococci
Kendall's tau_b	*TOTAL COLIFORM*	Correlation Coefficient	1.000	0.020	0.101	-0.079	-0.070	0.022
		Sig. (2-tailed)		0.798	0.133	0.325	0.298	0.784
		N	110	110	110	110	110	110
	E.COLI	Correlation Coefficient	0.020	1.000	-0.053	-0.024	-0.119	-.190*
		Sig. (2-tailed)	0.798		0.472	0.781	0.105	0.029
		N	110	110	110	110	110	110
	fecal streptococci	Correlation Coefficient	0.101	-0.053	1.000	0.040	.140*	-0.057
		Sig. (2-tailed)	0.133	0.472		0.600	0.031	0.459
		N	110	110	110	110	110	110
	yeast and mold	Correlation Coefficient	-0.079	-0.024	0.040	1.000	0.062	0.094
		Sig. (2-tailed)	0.325	0.781	0.600		0.424	0.304
		N	110	110	110	110	110	110
	aerobic mesophilic count	Correlation Coefficient	-0.070	-0.119	.140*	0.062	1.000	-0.031
		Sig. (2-tailed)	0.298	0.105	0.031	0.424		0.684
		N	110	110	110	110	110	110
	Staphylococci	Correlation	0.022	-.190*	-0.057	0.094	-0.031	1.000

	Coefficient						
	Sig. (2-tailed)	0.784	0.029	0.459	0.304	0.684	
	N	110	110	110	110	110	110

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix 4 BIOCHEMICAL TEST

NO	MEDIA	TARGET MOB	COLOUR ON MEDIA	SHAPE OF MOB	SUB-CITY	SITE	CATALASE	GRAM STAIN	KOH	MOTILITY	SULFUR	INDOL	CITRATE	Methyl Red	Voges-Proskauer	coagulase
1	MSA	Staphylococci	YELLOW	COCCI	LEMI KURA	B	+	-	+	+	-	+	+	-	+	+
2	MSA	Staphylococci	YELLOW	COCCI	LEMI KURA	F	+	-	+	+	-	+	+	-	+	+
3	MSA	Staphylococci	YELLOW	COCCI	YEKA	F	+	-	+	+	-	+	-	-	+	+
4	MSA	Staphylococci	YELLOW	COCCI	BOLE	F	+	+	+	+	-	+	-	-	+	+
5	MSA	Staphylococci	YELLOW	COCCI	KOLFE KERANIO	H	+	-	+	+	-	+	-	-	+	+
6	MSA	Staphylococci	YELLOW	COCCI	KIRKOS	C	+	-	+	+	-	+	+	-	+	+
7	MSA	Staphylococci	YELLOW	COCCI	NIFAS SILK LAFTO	I	+	-	+	+	-	+	-	-	+	+
8	MSA	Staphylococci	YELLOW	COCCI	AKAKI KALITY	J	+	-	+	+	-	+	+	-	+	+
9	MSA	Staphylococci	YELLOW	COCCI	ADDIS KETEMA	E	+	-	+	+	-	+	+	-	+	-
50	MSA	Staphylococci	YELLOW	COCCI	GULELE	J	+	-	+	+	-	+	+	-	+	+
101	MSA	Staphylococci	YELLOW	COCCI	ADDIS KETEMA	A	+	-	+	+	-	+	-	-	+	+

NO	MEDIA	TARGET MOB	COLOUR ON MEDIA	SHAPE OF MOB	SUB-CITY	SITE	CATALASE	GRAM STAIN	KOH	MOTILITY	SULFUR	INDOL	CITRATE	Methyl Red	Voges-Proskauer
1	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LEMI KURA	A	+	-	+	+	-	-	+		+
2	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	LEMI KURA	B	+	-	+	+	-	-	+	-	+
3	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LEMI KURA	C	+	-	+	+	-	-	+	+	-
4	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	LEMI KURA	D	+	-	+	+	-	-	-	-	+
5	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LEMI KURA	E	-	-	+	+	-	-	+	-	+
6	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	LEMI KURA	F	+	-	+	+	-	-	+	-	+
7	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LEMI KURA	G	+	-	+	+	-	-	-	-	+
8	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LEMI KURA	H	+	+	-	+	-	-	-	-	+
9	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	LEMI KURA	I	+	+	-	+	-	-	+	-	+
10	ENDO MEDIA	TOTAL COLIFORM	PINK (METALIC SHINE)	ROD	LEMI KURA	J	-	-	-	+	-	-	+	-	+
11	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	YEKA	A	+	-	-	+	-	-	-	-	+
12	ENDO MEDIA	TOTAL COLIFORM	WHITE	COCCI	YEKA	B	+	-	-	+	-	-	+	-	+
13	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	YEKA	C	+	-	-	+	-	-	-	-	+
14	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	YEKA	D	+	-	-	+	-	-	-	-	+
15	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	YEKA	E	+	-	-	+	-	-	-	-	+
16	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	YEKA	F	+	-	-	+	-	-	-	-	+
17	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	YEKA	G	+	-	-	+	-	-	-	-	+
18	ENDO MEDIA	TOTAL COLIFORM	PINK	COCCI	YEKA	H	+	-	-	+	-	-	-	-	+
19	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	YEKA	I	+	-	-	+	-	-	-	-	+
20	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	YEKA	J	-	-	-	+	-	-	-	-	+
21	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	BOLE	A	+	-	-	+	-	-	-	-	+

22	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	BOLE	B	-	+	-	+	-	-	-	-	+
23	ENDO MEDIA	TOTAL COLIFORM	PINK (METALIC SHINE)	ROD	BOLE	C	-	-	+	-	-	-	+	-	+
24	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	BOLE	D	+	-	+	+	-	-	-	-	+
25	ENDO MEDIA	TOTAL COLIFORM	WHITE	COCCI	BOLE	E	+	-	+	+	-	-	+	-	+
26	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	BOLE	F	+	-	+	+	-	-	+	-	+
27	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	BOLE	G	+	+	-	+	-	-	-	-	+
28	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	BOLE	H	-	-	+	-	-	-	-	-	+
29	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	BOLE	I	+	-	+	+	-	-	+	-	+
30	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	BOLE	J	+	-	+	+	-	-	+	-	+
31	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	KOLFE KERANIO	A	+	-	+	+	-	-	-	-	+
32	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	KOLFE KERANIO	B	+	-	+	+	-	-	+	-	+
33	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	KOLFE KERANIO	C	-	-	+	+	-	-	-	-	+
34	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	KOLFE KERANIO	D	+	-	+	+	-	-	-	-	+
35	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	KOLFE KERANIO	E	+	-	+	+	-	-	-	-	+
36	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	KOLFE KERANIO	F	+	-	+	+	-	-	-	-	+
37	ENDO MEDIA	TOTAL COLIFORM	WHITE	COCCI	KOLFE KERANIO	G	+	-	+	+	-	-	-	-	+
38	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	KOLFE KERANIO	H	+	-	+	+	-	-	-	-	+
39	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	KOLFE KERANIO	I	+	-	+	+	-	-	-	-	+
40	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	KOLFE KERANIO	J	+	-	+	+	-	-	-	-	+
41	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	GULELE	A	+	-	+	+	-	-	-	-	+
42	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	GULELE	B	+	-	+	-	-	-	-	-	+
43	ENDO MEDIA	TOTAL COLIFORM	WHITE	COCCI	GULELE	C	-	-	+	+	-	-	+	-	+
44	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	GULELE	D	+	-	+	+	-	-	-	-	+
45	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	GULELE	E	-	-	+	+	-	-	+	-	+
46	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	GULELE	F	-	-	+	+	-	-	+	-	+

47	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	GULELE	G	+	-	+	+	-	-	-	-	+
48	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	GULELE	H	+	-	+	+	-	-	-	-	+
49	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	GULELE	I	+	-	+	+	-	-	+	-	+
50	ENDO MEDIA	TOTAL COLIFORM	PINK (METALIC SHINE)	ROD	GULELE	J	+	-	+	+	-	-	+	-	+
51	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LIDETA	A	-	-	+	+	-	-	-	-	+
52	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LIDETA	B	+	-	+	+	-	-	+	-	+
53	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	LIDETA	C	+	-	+	+	-	-	-	-	+
54	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LIDETA	D	+	-	+	+	-	-	-	-	+
55	ENDO MEDIA	TOTAL COLIFORM	PINK	COCCI	LIDETA	E	+	-	+	+	-	-	-	-	+
56	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LIDETA	F	-	-	+	+	-	-	-	-	+
57	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	LIDETA	G	+	-	+	+	-	-	-	-	+
58	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LIDETA	H	+	-	+	+	-	-	-	-	+
59	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	LIDETA	I	+	-	+	+	-	-	-	-	+
60	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	LIDETA	J	+	-	+	+	-	-	-	-	+
61	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	KIRKOS	A	+	-	+	+	-	-	-	-	+
62	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	KIRKOS	B	+	-	+	+	-	-	-	-	+
63	ENDO MEDIA	TOTAL COLIFORM	PINK	COCCI	KIRKOS	C	+	-	+	+	-	-	+	-	+
64	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	KIRKOS	D	+	-	+	+	-	-	-	-	+
65	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	KIRKOS	E	+	-	+	-	-	-	+	-	+
66	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	KIRKOS	F	-	-	+	+	-	+	+	-	+
67	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	KIRKOS	G	+	-	+	+	-	-	-	-	+
68	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	KIRKOS	H	-	-	+	+	-	-	-	-	+
69	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	KIRKOS	I	-	-	+	+	-	-	+	-	+
70	ENDO MEDIA	TOTAL COLIFORM	PINK	COCCI	KIRKOS	J	+	+	-	+	-	-	+	-	+
71	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	NIFAS SILK LAFTO	A	+	-	+	-	-	-	-	-	+

	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	NIFAS SILK LAFTO	B	+	-	+	+	-	-	+	-	+
73	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	NIFAS SILK LAFTO	C	+	-	+	+	-	-	-	-	+
74	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	NIFAS SILK LAFTO	D	-	-	+	+	-	+	-	-	+
75	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	NIFAS SILK LAFTO	E	+	-	+	+	-	-	-	-	+
76	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	NIFAS SILK LAFTO	F	+	-	+	+	-	-	-	-	+
77	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	NIFAS SILK LAFTO	G	+	-	+	+	-	-	-	-	+
78	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	NIFAS SILK LAFTO	H	+	-	+	+	-	-	-	-	+
79	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	NIFAS SILK LAFTO	I	-	-	+	+	-	-	-	-	+
80	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	NIFAS SILK LAFTO	J	+	-	+	+	-	-	-	-	+
81	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	AKAKI KALITY	A	+	-	+	+	-	-	-	-	+
82	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	AKAKI KALITY	B	+	-	+	+	-	-	-	-	+
83	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	AKAKI KALITY	C	+	+	-	+	-	-	+	-	+
84	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	AKAKI KALITY	D	+	-	+	+	-	-	-	-	+
85	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	AKAKI KALITY	E	+	-	+	+	-	-	+	-	+
86	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	AKAKI KALITY	F	+	-	+	+	-	-	+	-	+
87	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	AKAKI KALITY	G	+	-	+	+	-	-	-	-	+
88	ENDO MEDIA	TOTAL COLIFORM	PINK (METALIC SHINE)	ROD	AKAKI KALITY	H	+	-	+	-	-	-	-	-	+
89	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	AKAKI KALITY	I	-	-	+	+	-	-	+	-	+
90	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	AKAKI KALITY	J	+	-	+	+	-	-	+	-	+
91	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	ARADA	A	-	-	+	+	-	+	-	-	+
92	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	ARADA	B	-	-	+	+	-	-	+	-	+
93	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	ARADA	C	+	-	+	+	-	-	-	-	+
94	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	ARADA	D	+	-	+	+	-	-	-	-	+
95	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	ARADA	E	+	-	+	-	-	-	-	-	+
96	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	ARADA	F	+	-	+	+	-	-	-	-	+

97	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	ARADA	G	-	+	-	+	-	-	-	-	+
98	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	ARADA	H	+	-	+	+	-	-	-	-	+
99	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	ARADA	I	+	-	+	+	-	-	-	+	-
100	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	ARADA	J	+	-	+	+	-	-	-	-	+
101	ENDO MEDIA	TOTAL COLIFORM	PINK (METALIC SHINE)	ROD	ADDIS KETEMA	A	+	-	+	+	-	-	-	-	+
102	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	ADDIS KETEMA	B	-	-	+	+	-	-	-	-	+
103	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	ADDIS KETEMA	C	+	-	+	-	-	-	+	-	+
104	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	ADDIS KETEMA	D	+	+	-	+	-	-	-	-	+
105	ENDO MEDIA	TOTAL COLIFORM	WHITE	COCCI	ADDIS KETEMA	E	+	-	+	+	-	-	+	-	-
106	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	ADDIS KETEMA	F	+	-	+	+	-	-	+	-	+
107	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	ADDIS KETEMA	G	+	-	+	-	-	-	-	+	-
108	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	ADDIS KETEMA	H	+	-	+	-	-	-	-	+	-
109	ENDO MEDIA	TOTAL COLIFORM	PINK	ROD	ADDIS KETEMA	I	+	-	+	+	-	-	+	-	+
110	ENDO MEDIA	TOTAL COLIFORM	WHITE	ROD	ADDIS KETEMA	J	+	-	+	-	-	+	+	-	+

NO	MEDIA	TARGET MOB	COLOUR ON MEDIA	SHAPE OF MOB	SUB-CITY	SITE	CATALASE	GRAM STAIN	KOH	MOTILITY	SULFUR	INDOL	CITRATE	Methyl Red	Voges-Proskauer
1	CHROMO	*E.coli*	BLUE	ROD	LEMIKURA	A	+	-	+	+	-	-	+	-	+
2	CHROMO	*E.coli*	PINK	ROD	LEMIKURA	B	+	-	+	+	-	-	+	-	+
3	CHROMO	*E.coli*	BLUE	ROD	LEMIKURA	C	+	-	+	+	-	-	+	+	-
4	CHROMO	*E.coli*	PINK	ROD	LEMIKURA	D	+	-	+	+	-	-	-	-	+
5	CHROMO	*E.coli*	BLUE	ROD	LEMIKURA	E	-	-	+	+	-	-	+	-	+
6	CHROMO	*E.coli*	BLUE	ROD	LEMIKURA	F	+	-	+	+	-	-	+	-	+
7	CHROMO	*E.coli*	PINK	ROD	LEMIKURA	G	+	-	+	+	-	-	-	-	+
8	CHROMO	*E.coli*	BLUE	ROD	LEMIKURA	H	+	+	-	+	-	-	-	-	+
9	CHROMO	*E.coli*	BLUE	ROD	LEMIKURA	I	+	+	-	+	-	-	+	-	+
10	CHROMO	*E.coli*	BLUE	ROD	LEMIKURA	J	-	-	-	+	-	-	+	-	+
11	CHROMO	*E.coli*	PINK	ROD	YEKA	A	+	-	-	+	-	-	-	-	+
12	CHROMO	*E.coli*	BLUE	COCCI	YEKA	B	+	-	-	+	-	-	+	-	+
13	CHROMO	*E.coli*	PINK	ROD	YEKA	C	+	-	-	+	-	-	-	-	+
14	CHROMO	*E.coli*	BLUE	ROD	YEKA	D	+	-	-	+	-	-	-	-	+
15	CHROMO	*E.coli*	BLUE	ROD	YEKA	E	+	-	-	+	-	-	-	-	+
16	CHROMO	*E.coli*	BLUE	ROD	YEKA	F	+	-	-	+	-	-	-	-	+
17	CHROMO	*E.coli*	BLUE	ROD	YEKA	G	+	-	-	+	-	-	-	-	+
18	CHROMO	*E.coli*	PINK	ROD	YEKA	H	+	-	-	+	-	-	-	-	+
19	CHROMO	*E.coli*	BLUE	ROD	YEKA	I	+	-	-	+	-	-	-	-	+
20	CHROMO	*E.coli*	BLUE	ROD	YEKA	J	-	-	-	+	-	-	-	-	+
21	CHROMO	*E.coli*	BLUE	ROD	BOLE	A	+	-	-	+	-	-	-	-	+
22	CHROMO	*E.coli*	BLUE	ROD	BOLE	B	-	+	-	+	-	-	-	-	+
23	CHROMO	*E.coli*	PINK	ROD	BOLE	C	-	-	+	-	-	-	+	-	+

24	CHROMO	*E.coli*	BLUE	ROD	BOLE	D	+	-	+	+	-	-	-	-	+
25	CHROMO	*E.coli*	BLUE	COCCI	BOLE	E	+	-	+	+	-	-	+	-	+
26	CHROMO	*E.coli*	PINK	ROD	BOLE	F	+	-	+	+	-	-	+	-	+
27	CHROMO	*E.coli*	BLUE	ROD	BOLE	G	+	+	-	+	-	-	-	-	+
28	CHROMO	*E.coli*	PINK	ROD	BOLE	H	-	-	+	-	-	-	-	-	+
29	CHROMO	*E.coli*	BLUE	ROD	BOLE	I	+	-	+	+	-	-	+	-	+
30	CHROMO	*E.coli*	BLUE	ROD	BOLE	J	+	-	+	+	-	-	+	-	+
31	CHROMO	*E.coli*	PINK	ROD	KOLFE KERANIO	A	+	-	+	+	-	-	-	-	+
32	CHROMO	*E.coli*	PINK	ROD	KOLFE KERANIO	B	+	-	+	+	-	-	+	-	+
33	CHROMO	*E.coli*	BLUE	ROD	KOLFE KERANIO	C	-	-	+	+	-	-	-	-	+
34	CHROMO	*E.coli*	BLUE	ROD	KOLFE KERANIO	D	+	-	+	+	-	-	-	-	+
35	CHROMO	*E.coli*	BLUE	ROD	KOLFE KERANIO	E	+	-	+	+	-	-	-	-	+
36	CHROMO	*E.coli*	BLUE	ROD	KOLFE KERANIO	F	+	-	+	+	-	-	-	-	+
37	CHROMO	*E.coli*	BLUE	COCCI	KOLFE KERANIO	G	+	-	+	+	-	-	-	-	+
38	CHROMO	*E.coli*	PINK	ROD	KOLFE KERANIO	H	+	-	+	+	-	-	-	-	+
39	CHROMO	*E.coli*	BLUE	ROD	KOLFE KERANIO	I	+	-	+	+	-	-	-	-	+
40	CHROMO	*E.coli*	BLUE	ROD	KOLFE KERANIO	J	+	-	+	+	-	-	-	-	+
41	CHROMO	*E.coli*	PINK	ROD	GULELE	A	+	-	+	+	-	-	-	-	+
42	CHROMO	*E.coli*	BLUE	ROD	GULELE	B	+	-	+	-	-	-	-	-	+
43	CHROMO	*E.coli*	BLUE	COCCI	GULELE	C	-	-	+	+	-	-	+	-	+

44	CHROMO	*E.coli*	BLUE	ROD	GULELE	D	+	-	+	+	-	-	-	-	+
45	CHROMO	*E.coli*	PINK	ROD	GULELE	E	-	-	+	+	-	-	+	-	+
46	CHROMO	*E.coli*	PINK	ROD	GULELE	F	-	-	+	+	-	-	+	-	+
47	CHROMO	*E.coli*	BLUE	ROD	GULELE	G	+	-	+	+	-	-	-	-	+
48	CHROMO	*E.coli*	BLUE	ROD	GULELE	H	+	-	+	+	-	-	-	-	+
49	CHROMO	*E.coli*	BLUE	ROD	GULELE	I	+	-	+	+	-	-	+	-	+
50	CHROMO	*E.coli*	BLUE	ROD	GULELE	J	+	-	+	+	-	-	+	-	+
51	CHROMO	*E.coli*	BLUE	ROD	LIDETA	A	-	-	+	+	-	-	-	-	+
52	CHROMO	*E.coli*	PINK	ROD	LIDETA	B	+	-	+	+	-	-	+	-	+
53	CHROMO	*E.coli*	PINK	ROD	LIDETA	C	+	-	+	+	-	-	-	-	+
54	CHROMO	*E.coli*	PINK	ROD	LIDETA	D	+	-	+	+	-	-	-	-	+
55	CHROMO	*E.coli*	BLUE	COCCI	LIDETA	E	+	-	+	+	-	-	-	-	+
56	CHROMO	*E.coli*	BLUE	ROD	LIDETA	F	-	-	+	+	-	-	-	-	+
57	CHROMO	*E.coli*	BLUE	ROD	LIDETA	G	+	-	+	+	-	-	-	-	+
58	CHROMO	*E.coli*	BLUE	ROD	LIDETA	H	+	-	+	+	-	-	-	-	+
59	CHROMO	*E.coli*	BLUE	ROD	LIDETA	I	+	-	+	+	-	-	-	-	+
60	CHROMO	*E.coli*	BLUE	ROD	LIDETA	J	+	-	+	+	-	-	-	-	+
61	CHROMO	*E.coli*	PINK	ROD	KIRKOS	A	+	-	+	+	-	-	-	-	+
62	CHROMO	*E.coli*	BLUE	ROD	KIRKOS	B	+	-	+	+	-	-	-	-	+
63	CHROMO	*E.coli*	BLUE	COCCI	KIRKOS	C	+	-	+	+	-	-	+	-	+
64	CHROMO	*E.coli*	PINK	ROD	KIRKOS	D	+	-	+	+	-	-	-	-	+
65	CHROMO	*E.coli*	BLUE	ROD	KIRKOS	E	+	-	+	-	-	-	+	-	+
66	CHROMO	*E.coli*	BLUE	ROD	KIRKOS	F	-	-	+	+	-	+	+	-	+
67	CHROMO	*E.coli*	PINK	ROD	KIRKOS	G	+	-	+	+	-	-	-	-	+
68	CHROMO	*E.coli*	BLUE	ROD	KIRKOS	H	-	-	+	+	-	-	-	-	+

69	CHROMO	*E.coli*	BLUE	ROD	KIRKOS	I	-	-	+	+	-	-	+	-	+
70	CHROMO	*E.coli*	BLUE	COCCI	KIRKOS	J	+	+	-	+	-	-	+	-	+
71	CHROMO	*E.coli*	PINK	ROD	NIFAS SILK LAFTO	A	+	-	+	-	-	-	-	-	+
72	CHROMO	*E.coli*	BLUE	ROD	NIFAS SILK LAFTO	B	+	-	+	+	-	-	+	-	+
73	CHROMO	*E.coli*	PINK	ROD	NIFAS SILK LAFTO	C	+	-	+	+	-	-	-	-	+
74	CHROMO	*E.coli*	PINK	ROD	NIFAS SILK LAFTO	D	-	-	+	+	-	+	-	-	+
75	CHROMO	*E.coli*	BLUE	ROD	NIFAS SILK LAFTO	E	+	-	+	+	-	-	-	-	+
76	CHROMO	*E.coli*	BLUE	ROD	NIFAS SILK LAFTO	F	+	-	+	+	-	-	-	-	+
77	CHROMO	*E.coli*	BLUE	ROD	NIFAS SILK LAFTO	G	+	-	+	+	-	-	-	-	+
78	CHROMO	*E.coli*	BLUE	ROD	NIFAS SILK LAFTO	H	+	-	+	+	-	-	-	-	+
79	CHROMO	*E.coli*	BLUE	ROD	NIFAS SILK LAFTO	I	-	-	+	+	-	-	-	-	+
80	CHROMO	*E.coli*	BLUE	ROD	NIFAS SILK LAFTO	J	+	-	+	+	-	-	-	-	+
81	CHROMO	*E.coli*	BLUE	ROD	AKAKI KALITY	A	+	-	+	+	-	-	-	-	+
82	CHROMO	*E.coli*	BLUE	ROD	AKAKI KALITY	B	+	-	+	+	-	-	-	-	+
83	CHROMO	*E.coli*	PINK	ROD	AKAKI KALITY	C	+	+	-	+	-	-	+	-	+
84	CHROMO	*E.coli*	BLUE	ROD	AKAKI KALITY	D	+	-	+	+	-	-	-	-	+
85	CHROMO	*E.coli*	PINK	ROD	AKAKI KALITY	E	+	-	+	+	-	-	+	-	+
86	CHROMO	*E.coli*	BLUE	ROD	AKAKI KALITY	F	+	-	+	+	-	-	+	-	+
87	CHROMO	*E.coli*	PINK	ROD	AKAKI KALITY	G	+	-	+	+	-	-	-	-	+
88	CHROMO	*E.coli*	BLUE	ROD	AKAKI KALITY	H	+	-	+	-	-	-	-	-	+

89	CHROMO	*E.coli*	BLUE	ROD	AKAKIKALITY	I	-	-	+	+	-	-	+	-	+
90	CHROMO	*E.coli*	BLUE	ROD	AKAKIKALITY	J	+	-	+	+	-	-	+	-	+
91	CHROMO	*E.coli*	PINK	ROD	ARADA	A	-	-	+	+	-	+	-	-	+
92	CHROMO	*E.coli*	PINK	ROD	ARADA	B	-	-	+	+	-	-	+	-	+
93	CHROMO	*E.coli*	BLUE	ROD	ARADA	C	+	-	+	+	-	-	-	-	+
94	CHROMO	*E.coli*	BLUE	ROD	ARADA	D	+	-	+	+	-	-	-	-	+
95	CHROMO	*E.coli*	BLUE	ROD	ARADA	E	+	-	+	-	-	-	-	-	+
96	CHROMO	*E.coli*	PINK	ROD	ARADA	F	+	-	+	+	-	-	-	-	+
97	CHROMO	*E.coli*	BLUE	ROD	ARADA	G	-	+	-	+	-	-	-	-	+
98	CHROMO	*E.coli*	BLUE	ROD	ARADA	H	+	-	+	+	-	-	-	-	+
99	CHROMO	*E.coli*	BLUE	ROD	ARADA	I	+	-	+	+	-	-	-	+	-
100	CHROMO	*E.coli*	BLUE	ROD	ARADA	J	+	-	+	+	-	-	-	-	+
101	CHROMO	*E.coli*	BLUE	ROD	ADDISKETEMA	A	+	-	+	+	-	-	-	-	+
102	CHROMO	*E.coli*	BLUE	ROD	ADDISKETEMA	B	-	-	+	+	-	-	-	-	+
103	CHROMO	*E.coli*	PINK	ROD	ADDISKETEMA	C	+	-	+	-	-	-	+	-	+
104	CHROMO	*E.coli*	BLUE	ROD	ADDISKETEMA	D	+	+	-	+	-	-	-	-	+
105	CHROMO	*E.coli*	BLUE	COCCI	ADDISKETEMA	E	+	-	+	+	-	-	+	-	-
106	CHROMO	*E.coli*	PINK	ROD	ADDISKETEMA	F	+	-	+	+	-	-	+	-	+
107	CHROMO	*E.coli*	BLUE	ROD	ADDISKETEMA	G	+	-	+	-	-	-	-	+	-
108	CHROMO	*E.coli*	BLUE	ROD	ADDISKETEMA	H	+	-	+	-	-	-	-	+	-
109	CHROMO	*E.coli*	BLUE	ROD	ADDISKETEM	I	+	-	+	+	-	-	+	-	+

110	CHROMO	*E.coli*	BLUE	ROD	A ADDISKETEMA	J	+	-	+	-	-	+	+	-	+

NO	MEDIA	TARGET MOB	SHAPE OF MOB	SUB-CITY	SITE	COLURE	CATALASE	GRAM STAIN	KOH	MOTILITY	SULFUR	INDOL
1	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	LEMI KURA	A	BEJE	-	+	-	+	-	-
2	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	LEMI KURA	B	BEJE	-	+	-	-	-	-
3	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	LEMI KURA	F	BEJE	-	+	-	+	-	-
4	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	LEMI KURA	H	BEJE	-	+	-	-	-	-
5	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	LEMI KURA	I	BEJE	-	+	-	-	-	-
6	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	LEMI KURA	J	BEJE	-	+	-	-	-	-
7	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	LEMI KURA	A	WHITE	-	+	-	+	-	-
11	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	YEKA	A	BEJE	-	+	-	-	-	-
12	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	YEKA	B	BEJE	-	+	-	-	-	-
13	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	YEKA	C	BEJE	-	+	-	-	-	-
14	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	YEKA	D	BEJE	-	+	-	-	-	-
15	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	YEKA	E	BEJE	-	+	-	-	-	-
16	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	YEKA	F	BEJE	-	+	-	-	-	-
17	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	YEKA	G	BEJE	-	+	-	-	-	-
18	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	YEKA	H	BEJE	-	+	-	-	-	-
19	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOC	YEKA	I	BEJE	-	+	-	-	-	-

		i	OCCUS										
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	YEKA	J	BEJE	-	+	-	-	-	-
20													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	BOLE	A	BEJE	-	+	-	-	-	-
21													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	BOLE	B	BEJE	-	+	-	-	-	-
22													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	BOLE	C	BEJE	-	+	-	-	-	-
23													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	BOLE	D	BEJE	-	+	-	-	-	-
24													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	BOLE	E	BEJE	-	+	-	-	-	-
25													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	BOLE	F	BEJE	-	+	-	-	-	-
26													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	BOLE	G	BEJE	-	+	-	-	-	-
27													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	BOLE	H	BEJE	-	+	-	-	-	-
28													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	BOLE	I	BEJE	-	+	-	-	-	-
29													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	BOLE	J	BEJE	-	+	-	-	-	-
30													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KOLFE KERANIO	A	BEJE	-	+	-	-	-	-
31													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KOLFE KERANIO	B	BEJE	-	+	-	-	-	-
32													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KOLFE KERANIO	C	BEJE	-	+	-	-	-	-
33													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KOLFE KERANIO	D	BEJE	-	+	-	-	-	-
34													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KOLFE KERANIO	E	BEJE	-	+	-	-	-	-
35													

	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	KOLFE KERANIO	F	BEJE	-	+	-	-	-	-
36												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	KOLFE KERANIO	G	BEJE	-	+	-	-	-	-
37												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	KOLFE KERANIO	H	BEJE	-	+	-	-	-	-
38												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	KOLFE KERANIO	I	BEJE	-	+	-	-	-	-
39												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	KOLFE KERANIO	J	BEJE	-	+	-	-	-	-
40												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	GULELE	A	BEJE	-	+	-	-	-	-
41												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	GULELE	B	BEJE	-	+	-	-	-	-
42												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	GULELE	C	BEJE	-	+	-	-	-	-
43												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	GULELE	D	BEJE	-	+	-	-	-	-
44												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	GULELE	E	BEJE	-	+	-	-	-	-
45												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	GULELE	F	BEJE	-	+	-	-	-	-
46												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	GULELE	G	BEJE	-	+	-	-	-	-
47												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	GULELE	H	BEJE	-	+	-	-	-	-
48												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	GULELE	I	BEJE	-	+	-	-	-	-
49												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	GULELE	J	BEJE	-	+	-	-	-	-
50												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC OCCUS	LIDETA	A	BEJE	-	+	-	-	-	-
51												
	ENTEROC OCCIOSEL	Fecal Streptococci	STRIPTOC	LIDETA	B	BEJE	-	+	-	-	-	-
52												

		i	OCCUS										
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	LIDETA	C	BEJE	-	+	-	-	-	-
53													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	LIDETA	D	BEJE	-	+	-	-	-	-
54													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	LIDETA	E	BEJE	-	+	-	-	-	-
55													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	LIDETA	F	BEJE	-	+	-	-	-	-
56													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	LIDETA	G	BEJE	-	+	-	-	-	-
57													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	LIDETA	H	BEJE	-	+	-	-	-	-
58													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	LIDETA	I	BEJE	-	+	-	-	-	-
59													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	LIDETA	J	BEJE	-	+	-	-	-	-
60													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KIRKOS	A	BEJE	-	+	-	-	-	-
61													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KIRKOS	B	BEJE	-	+	-	-	-	-
62													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KIRKOS	C	BEJE	-	+	-	-	-	-
63													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KIRKOS	D	BEJE	-	+	-	-	-	-
64													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KIRKOS	E	BEJE	-	+	-	-	-	-
65													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KIRKOS	F	BEJE	-	+	-	-	-	-
66													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KIRKOS	G	BEJE	-	+	-	-	-	-
67													
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	KIRKOS	J	BEJE	-	+	-	-	-	-
68													

	ENTEROC	Fecal		NIFAS	A	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	SILK								
69		i	OCCUS	LAFTO								
	ENTEROC	Fecal		NIFAS	B	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	SILK								
70		i	OCCUS	LAFTO								
	ENTEROC	Fecal		NIFAS	C	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	SILK								
71		i	OCCUS	LAFTO								
	ENTEROC	Fecal		NIFAS	D	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	SILK								
72		i	OCCUS	LAFTO								
	ENTEROC	Fecal		NIFAS	E	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	SILK								
73		i	OCCUS	LAFTO								
	ENTEROC	Fecal		NIFAS	F	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	SILK								
74		i	OCCUS	LAFTO								
	ENTEROC	Fecal		NIFAS	G	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	SILK								
75		i	OCCUS	LAFTO								
	ENTEROC	Fecal		NIFAS	H	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	SILK								
76		i	OCCUS	LAFTO								
	ENTEROC	Fecal		NIFAS	I	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	SILK								
77		i	OCCUS	LAFTO								
	ENTEROC	Fecal		NIFAS	J	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	SILK								
78		i	OCCUS	LAFTO								
	ENTEROC	Fecal		AKAKI	A	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	KALITY								
79		i	OCCUS									
	ENTEROC	Fecal		AKAKI	B	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	KALITY								
80		i	OCCUS									
	ENTEROC	Fecal		AKAKI	C	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	KALITY								
81		i	OCCUS									
	ENTEROC	Fecal		AKAKI	D	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	KALITY								
82		i	OCCUS									
	ENTEROC	Fecal		AKAKI	E	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	KALITY								
83		i	OCCUS									
	ENTEROC	Fecal		AKAKI	F	BEJE	-	+	-	-	-	-
	OCCIOSEL	Streptococc	STRIPTOC	KALITY								
84		i	OCCUS									
	ENTEROC	Fecal		AKAKI	G	BEJE	-	+	-	-	-	-
85	OCCIOSEL	Streptococc	STRIPTOC	KALITY								

		i	OCCUS									
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	AKAKI KALITY	H	BEJE	-	+	-	-	-	-
86												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	AKAKI KALITY	I	BEJE	-	+	-	-	-	-
87												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	AKAKI KALITY	J	BEJE	-	+	-	-	-	-
88												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ARADA	A	BEJE	-	+	-	-	-	-
89												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ARADA	B	BEJE	-	+	-	-	-	-
90												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ARADA	C	BEJE	-	+	-	-	-	-
91												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ARADA	D	BEJE	-	+	-	-	-	-
92												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ARADA	E	BEJE	-	+	-	-	-	-
93												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ARADA	F	BEJE	-	+	-	-	-	-
94												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ARADA	G	BEJE	-	+	-	-	-	-
95												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ARADA	H	BEJE	-	+	-	-	-	-
96												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ARADA	I	BEJE	-	+	-	-	-	-
97												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ARADA	J	BEJE	-	+	-	-	-	-
98												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ADDIS KETEMA	A	BEJE	-	+	-	-	-	-
99												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ADDIS KETEMA	B	BEJE	-	+	-	-	-	-
100												
	ENTEROC OCCIOSEL	Fecal Streptococc i	STRIPTOC OCCUS	ADDIS KETEMA	C	BEJE	-	+	-	-	-	-
101												

	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	ADDIS KETEMA	D	BEJE	-	+	-	-	-	-
102												
	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	ADDIS KETEMA	E	BEJE	-	+	-	-	-	-
103												
	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	ADDIS KETEMA	F	BEJE	-	+	-	-	-	-
104												
	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	ADDIS KETEMA	G	BEJE	-	+	-	-	-	-
105												
	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	ADDIS KETEMA	H	BEJE	-	+	-	-	-	-
106												
	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	ADDIS KETEMA	I	BEJE	-	+	-	-	-	-
107												
	ENTEROCOCCIOSEL	Fecal Streptococci	STRIPTOCOCCUS	ADDIS KETEMA	J	BEJE	-	+	-	-	-	-
108												

Appendix 5 Some Pictures of the stud

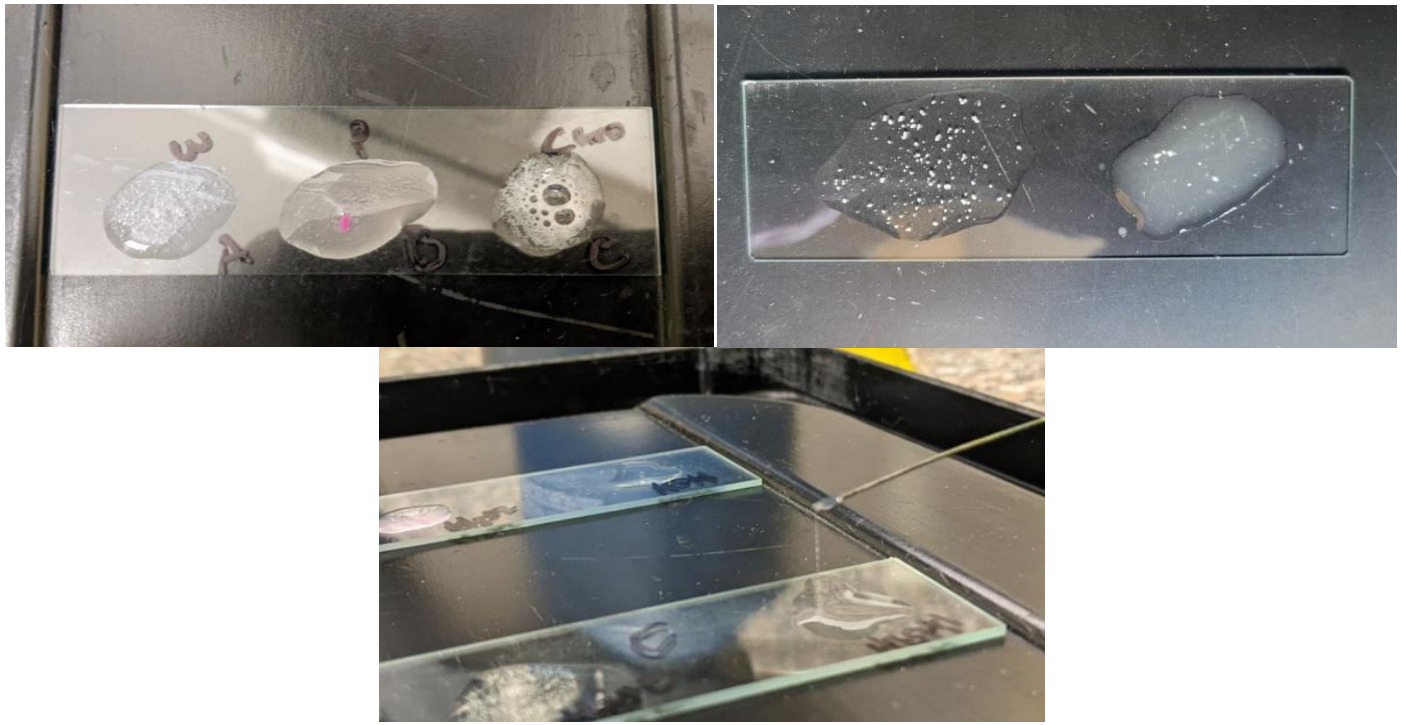

Figure: Some chemical tests for pathogens

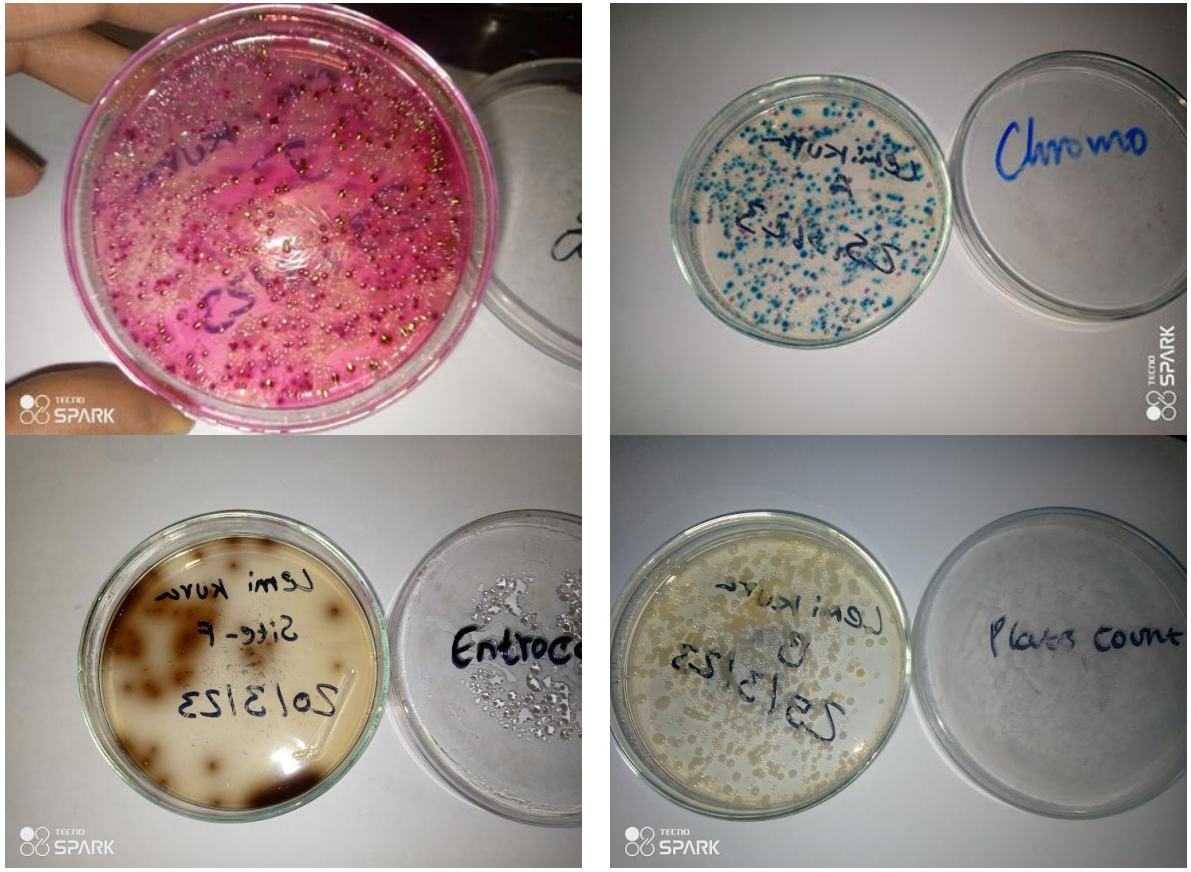

Figure: Some microbial isolates on Agar

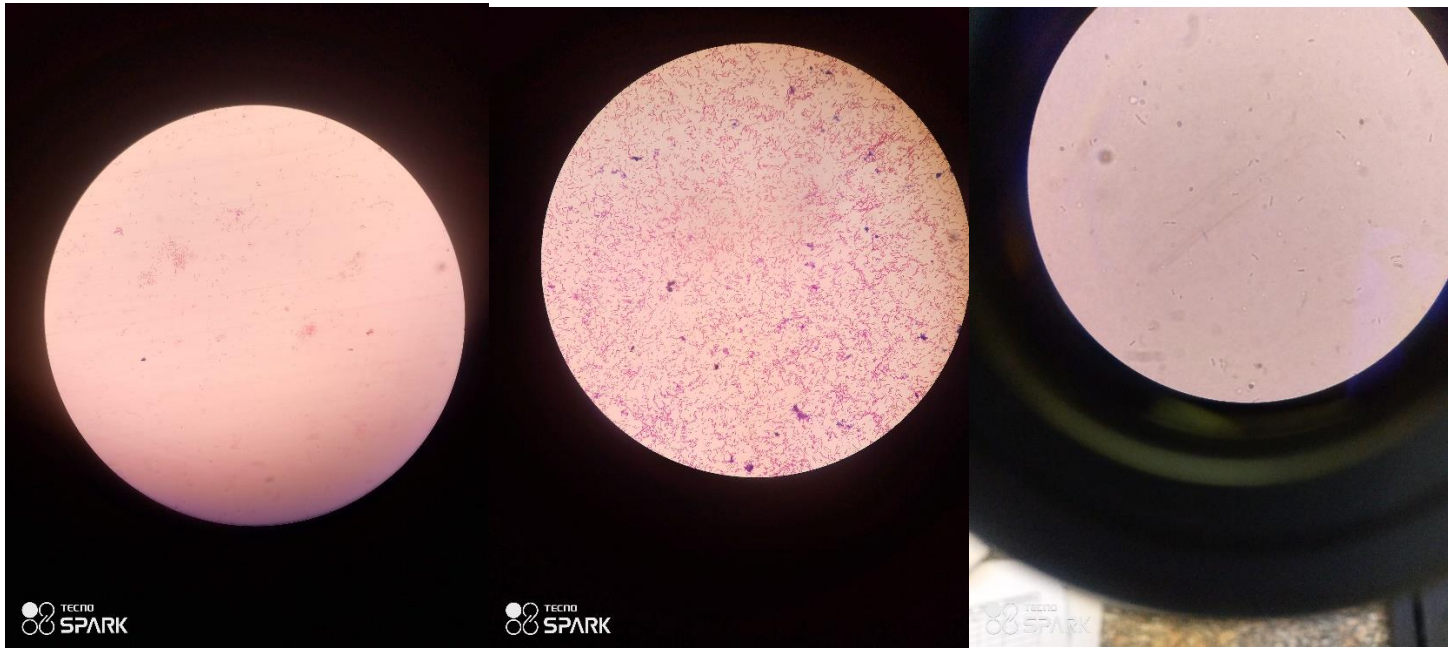

Figure: Microscopic View of Some microbial Isolates

Figure: Some biochemical tests for pathogens

Figure: workflow

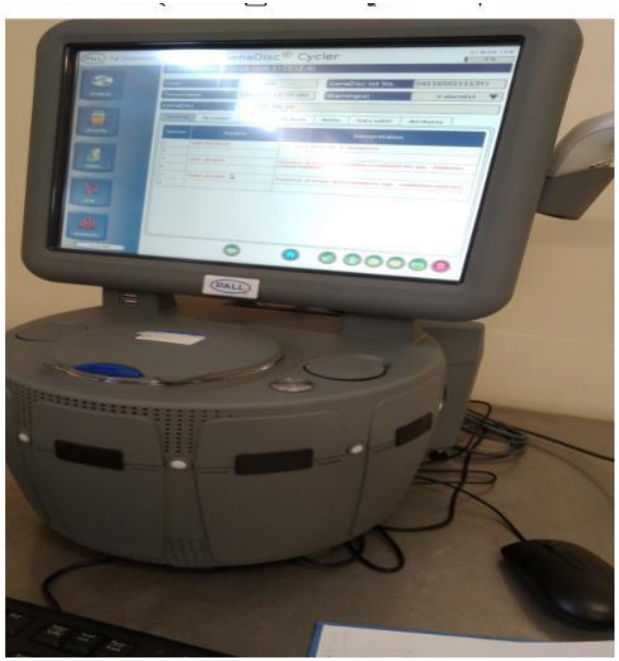

Figure: PCR machine in BGI ETHIOPIA A.A brewery