



WOLLEGA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE
DEPARTMENT OF BIOLOGY

**MICROBIAL SAFETY AND QUALITY OF FOODBORNE BACTERIAL
PATHOGENS FROM COMMON FRESH FRUITS AND VEGETABLES
IN LOCAL MARKET OF NEKEMTE TOWN ,EAST WOLLEGA
ZONE,OROMIA,ETHIOPIA**

MSc THESIS
BY
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NEKEMTE, ETHIOPIA



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PATHOGENS FROM COMMON FRESH FRUITS AND VEGETABLES IN LOCAL
MARKET OF NEKEMTE TOWN, EAST WALLAGA ZONE

A thesis is submitted to the School of Graduate Studies, department of Biology in partial fulfilment of the requirement for the award of degree of masters in Microbiology and microbial biotechnology.

By

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THESIS SUBMISSION APPROVAL SHEET

As members of the Board of Examining of the Final MSc thesis open defense, we certify that we have read and evaluated the thesis prepared by **Girma Melaku** under the title “**Microbial safety and quality of food borne bacterial pathogens from common fresh fruits and vegetables in local market of Nekemte town East wollega zone**” and I recommend that the thesis be accepted as fulfilling the thesis requirement for the Degree of Masters in microbiology.

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Certification of the Final Thesis

I hereby certify that all the correction and recommendation suggested by the board of examiners are incorporated into the final thesis entitled “**microbial safety and quality of foodborne bacterial pathogens from common fresh fruits and vegetables in local market of Nekemte town of East Wollega Zone**” by Girma Melaku.

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DEDICATION

This certifies that Girma Melaku's master's thesis, "microbial safety and quality of foodborne bacterial pathogens from common fresh fruits and vegetables in local market of Nekemte town of East Wollega Zone," which was approved as a partial fulfilment of the requirements for the award of the master's degree in Biology by Wollega University's School of Graduate Studies through the College of Natural and Computational Science, is authentic work completed by him under my supervision.

Major Advisor: Desalegn Amenu (PhD) Signature_____ Date_____

STATEMENT OF THE AUTHOR

I declare that this thesis is entitled “**microbial safety and quality of foodborne bacterial pathogens from common fresh fruits and vegetables in local market of Nekemte town of East Wollega Zone**” is my work and that all sources of materials used for this thesis have been used appropriately acknowledged. This thesis is submitted in partial fulfilment of the requirements for MSc. degree in Biology at Wollega University. I seriously declare that this thesis is not conducted to any other institution and anywhere for the award of any degree. The thesis deposited at the university Library to make available to burrowers under rules of the Wollega University Library. Brief Quotations from this thesis are allowable without special permission if accurate acknowledgement of the source is made. However, requests for permission extended quotation from reproduction in part of this manuscript may be granted by Wollega University. The school of graduates Studies in all other instances, Permission must be obtained from the author.

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LISTS OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CDC	Center for disease Control
CFU	Colony forming unit
CLSI	Clinical and Laboratory Standard Institute
FAO	Food and Agricultural Organization
FCC	Fecal coliform count
FDA	Food and Drug Administration
FMHE	Federal Ministry of Health Ethiopia
GAPs	Good Agricultural practices
GHPs	Good Handling practices
LAB	Lactic acid bacteria
MRSA	Methicillin resistant staphylococcus aureus
SPBC	Staphylococcus Bacterial Count
SPP	Species
SPSS	Statistical package for social science
TCC	Total coliform count
TSI	Triple sugar iron
VRSA	Vancomycin resistant staphylococcus aureus
XLD	Xylose lysine deoxycholate agar

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ABSTRACT

Fresh fruits and vegetable products are nutritious and healthy for human consumption, they are potential carriers of foodborne pathogens when contaminated during production, harvesting, transportation, and marketing of the produce. Therefore, this study sought to assess foodborne bacterial pathogens from fresh fruits and vegetable products sold in local markets of Nekemte town. The study involved a total of 180 samples comprising 30 samples each of mangoes, papaya, avocado, tomatoes, cabbage, and carrot. The samples were collected from three different market stations: Bake Jama, Darge, and Bordi in Nekemte town. Microbial analysis of the samples was carried out using the spread plate method on selective and differential media like manitol salt agar, violet red bile agar, eosin methylene blue and macconkey agar for total counts of Staphylococcus, fecal coliform, total coliform, and Escherichia coli. The results revealed that the maximum count of Staphylococcus was found in mango (6.98 log CFU/g), followed by cabbage (6.54 log CFU/g), while the minimum count of Staphylococcus was found in avocado (5.43 log CFU/g). For fecal coliforms, the maximum count was found in cabbage (6.52 log CFU/g), followed by papaya (6.41 log CFU/g), and avocado (6.01 log CFU/g), while the minimum count of fecal coliforms was found in carrot (4.42 log CFU/g). For total coliforms, the maximum count was found in mango (6.23 log CFU/g), followed by papaya (6.15 log CFU/g). The minimum total coliform counts were found in carrot (5.42 log CFU/g) and tomato (5.51 log CFU/g). From the total bacterial isolates, the dominant bacterial species isolated were Staphylococcus spp. (24.44%), followed by Bacillus spp. (20.83%), Enterobacter spp. (16.67%), and Micrococcus spp. (14.17%). Out of 180 samples, pathogenic bacteria were detected as follows: Staphylococcus aureus in 17.22% of samples, Salmonella in 9.44%, Shigella in 8.33%, and Escherichia coli in 6.67% of the samples. The bacterial isolates were also subjected to the study of antimicrobial susceptibility pattern by the disc diffusion method on Mueller Hinton agar medium. The bacterial isolates were found to be sensitive to ampicillin, ciprofloxacin, gentamicin, and trimethoprim/sulfamethoxazole (SXT). From the overall findings, it is evident that contamination could take place during harvesting, post-harvest handling, transportation, and display at market stalls and vendors' practices. The presence of fecal coliform bacteria and antibiotic-resistant bacteria suggests health risks. It is recommended that microbiological surveys, improvement of hygiene practices, and training of vendors on handling practices should be implemented for better safety and quality of fresh fruits and vegetables.

Keywords: avocado, count, fruits, isolate, mango, papaya, vegetable

1. INTRODUCTION

1.1 Background of the study

Fresh fruits and vegetables are defined as unprocessed or minimally processed plant-derived foods whose natural form, nutritional value, and moisture content are preserved without undergoing any kind of heat treatment, canning, and/or chemical preservation (FAO, 2019). Fresh fruits and vegetables include leafy vegetables, roots, tubers, fruits, and salad crops, which are usually eaten in the raw state or after minimal processing. Due to their high moisture and nutrient content, these foods provide an environment where microbes can thrive, including those pathogenic microorganisms that are foodborne (WHO, 2015).

Fresh fruits and vegetables are known for their nutritional and health benefits, thus playing an essential role as food sources of vitamins, minerals, and dietary fibers (Senjuti et al., 2014). Foodborne diseases resulting from contaminated foods are increasingly becoming a worldwide concern for public health. Fresh produce contaminated with disease-causing organisms is known to cause serious illness and death worldwide (Hannan et al., 2014; Hanson et al., 2012). The disease-causing organisms of foodborne illness are microbial pathogens, chemicals, and toxins produced by these microorganisms. The severity of foodborne illness depends on several factors such as the type of disease-causing organisms, dose of exposure, and host susceptibility and can lead to acute and chronic illness, death, and economic losses (WHO, 2004; Assefa et al., 2015).

Fresh produce of fruits and vegetables is often contaminated with disease-causing organisms at different stages of growth and development from the pre-harvest stage through the entire food chain up to the consumer. Pre-harvest contamination of fresh produce occurs through contact with soil, dust, contaminated water, and use of raw sewage and manure as fertilizers in agricultural fields (Jung et al., 2014; Razzaq et al., 2014; Senjuti et al., 2014; Hannan et al., 2014). Additional risk of foodborne illness occurs during harvesting, transportation, storage, and display of produce at markets because of poor hygienic practices and handling of produce (Isa et al., 2014).

Different foodborne pathogens have been identified as causative agents in the contamination of fruits and vegetables, and outbreaks of gastroenteritis due to contaminated fruits and vegetables have been reported worldwide (Jeddi et al., 2014). The most common bacterial pathogens responsible for the contamination of fruits and vegetables include *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, *Aeromonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Vibrio* spp., and *Pseudomonas* spp. (Nwachukwu and Chukwu, 2013; Senjuti et al., 2014).

In developing countries, foodborne diseases associated with contaminated fruits and vegetables are common, but many cases remain unreported because of limited diagnostic facilities and inadequate surveillance systems. Foodborne microbial pathogens are among the leading causes of illness worldwide. According to the World Health Organization (WHO), unsafe food causes approximately 866 million cases of foodborne illness and more than 1.5 million deaths globally each year. Even in developed countries, foodborne diseases continue to pose a significant public health challenge, affecting millions of people annually (WHO, 2026). Consequently, food safety has become a major global concern, prompting governments and international organizations to strengthen food safety systems and implement measures to protect public health and economic interests (WHO, 2026).

Nekemte town is known to be a major site for the purchase and distribution of various fruits and vegetables, which are considered an attractive site for residents and vendors. Markets in this town frequently sell fresh fruits such as avocado, mango, papaya and vegetables like tomato, cabbage and carrot. These fresh fruits and vegetables, however, are acquired from different farmlands without proper handling practice. Contamination is a result of unclean marketing environments, hazardous containers, and insufficient hygiene. This study was undertaken to microbial safety and quality of foodborne bacterial pathogens from common fresh fruits and vegetables in local markets of Nekemte town.

1.2. Statement of the problem

Globally, foodborne diseases remain a major public health concern, and fresh fruits and vegetables have increasingly been implicated in outbreaks of microbial infections. Recent reviews have identified *Salmonella*, *E. coli*, and *Listeria monocytogenes* as major pathogens associated with fresh produce contamination. In addition, contamination from irrigation water, animal manure, poor sanitation, and unhygienic market conditions contributes significantly to microbial hazards. In developing countries, particularly in sub-Saharan Africa, inadequate food safety systems, limited access to clean water, poor waste management, and weak regulatory enforcement increase the risk of contamination of fresh produce. Ethiopia faces similar challenges due to rapid urbanization, expanding fresh produce markets, and the common practice of consuming raw vegetables and fruits.

Several scholars have investigated the microbiological quality and safety of fruits and vegetables in different regions. Abakari et al. (2018) reported contamination of fresh produce with pathogenic bacteria in Ghana. Aijuka et al. (2015) documented microbial contamination and antimicrobial resistance among foodborne pathogens in East Africa. Asfaw et al. (2023) found considerable microbiological and parasitic contamination of vegetables marketed in Debre Berhan, Ethiopia. Gazu et al. (2023) highlighted that foodborne diseases remain an important but underreported public health burden in Ethiopia. Due to the rapid increase of street fruits and vegetables purchased in nekemte town, the farmers are attracted toward cultivating different kinds of fruits and vegetables as well (Abadias *et al*, 2008). So, the economical contribution of fruit and vegetables production by the rural area of study site is great enormous. In Ethiopia no continuous assessment of food safety has been prepared in fruits and vegetables where fresh fruit and vegetables are sold and consumed. Therefore, this study was tried to isolate and characterize food borne pathogens from fresh fruit and vegetables in nekemte town. Fresh fruits and vegetables in Nekemte town are widely consumed and economically important, but they are vulnerable to microbial contamination at multiple points from farm to market. This contamination can include pathogenic and antibiotic-resistant bacteria, posing serious public health risks. Despite this, there is no local study on the prevalence, characterization, and antimicrobial resistance of foodborne pathogens in fresh produce, creating a critical knowledge gap for food safety interventions.

1.3 Significance of the study

This study will help consumers become aware of the potential health risks associated with contaminated fresh produce and encourage proper washing and hygienic handling practices. It also provide valuable information to fruit and vegetable vendors, farmers, and food handlers about the importance of maintaining good agricultural, transportation, storage, and market hygiene practices to reduce contamination.

In addition, the study will assist local health authorities, food safety regulators, and policymakers in designing evidence-based strategies and interventions to improve the microbial safety of fresh fruits and vegetables in Nekemte town. The data generated can serve as baseline information for monitoring foodborne pathogens and strengthening food safety surveillance programs. Furthermore, by determining the prevalence of major foodborne bacterial pathogens and their antimicrobial susceptibility patterns, the study contributes to the understanding of antimicrobial resistance among foodborne bacteria, which is an emerging global public health challenge.

1.4 Objectives

1.4.1 General objective

The main objective of this study was to assess food borne bacterial pathogens from common fresh fruits and vegetables, in Nekemte town, East Wollega Zone.

1.4.2 Specific objectives

The specific objectives were:

- ❖ To evaluate the microbiological quality of fresh fruits and vegetables in reference to indicator bacteria (Total coliform, fecal coliform, *E.coli*, *Staphylococcus spp*)
- ❖ To determine the microbial safety analysis of fresh fruits and vegetables in reference to food borne pathogens (*E. coli*, *Salmonella*, *Shigella*, *S.aureus*).
- ❖ To identify and characterize the dominant fresh fruits and vegetables dominant micro flora highly dominates the fruits and vegetables.
- ❖ To evaluate the antimicrobial susceptibility patterns of food borne pathogens isolated from fresh fruits and vegetables isolated in this study.

2. REVIEW LITERATURE

2.1. Food borne pathogens

Foodborne pathogens are harmful microorganisms, including bacteria, viruses, parasites, and fungi, that contaminate food and cause foodborne diseases in humans. Common bacterial foodborne pathogens include *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Campylobacter spp.*. These microorganisms can contaminate food at any stage of the food chain, from production and harvesting to processing, transportation, storage, and preparation. Consumption of contaminated food may result in symptoms such as diarrhea, vomiting, abdominal pain, fever, and, in severe cases, kidney failure, septicemia, or death. According to the World Health Organization (WHO), foodborne diseases remain a major global public health concern, affecting millions of people every year. (WHO, 2025). Fresh fruits and vegetables are increasingly recognized as important vehicles for the transmission of foodborne pathogens because they are often consumed raw or with minimal processing.

2.2 The safety and microbial quality of fresh fruits and vegetables

Fresh fruits and vegetables are widely consumed and undergo little processing, their safety and microbiological quality are major public health concerns. Since fresh produce is frequently eaten raw, any microorganisms that were present at harvest, such as pathogens or spoilage organisms, may reach consumers without being removed by heat treatment. Bacteria including *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* can survive on product surfaces due to microbial contamination, which can originate from soil, irrigation water, manure, animals, or handling techniques. Produce's microbiological quality is impacted by conditions that encourage the growth of microorganisms during post-harvest handling in addition to contamination levels. Temperature abuse, incorrect washing, and cross-contamination during storage or transportation can greatly increase microbial loads. Cut fruits and vegetables, for instance, offer nutrient-rich conditions that promote quick bacterial growth, hastening spoiling and raising concerns about food safety. According to studies, utilizing adequately sanitized wash water and keeping produce at low temperatures are crucial stages in preventing microbial growth (Gil et al., 2015).

Testing for indicator organisms such total coliforms, *E. coli*, aerobic plate counts, yeasts, and molds is commonly used to evaluate the microbiological quality. These markers aid in determining potential fecal contamination and evaluating hygienic settings.

For example, the presence of *E. coli* indicates the presence of pathogens and is a strong indicator of recent fecal contamination. These indicators are used by regulatory bodies and international food safety standards to assess adherence to Good Agricultural Practices (GAPs) and Good Handling Practices (GHPs), guaranteeing that produce satisfies acceptable safety thresholds prior to reaching consumers (Feng et al., 2022).

2.3 Sources of contamination of fresh fruits and vegetables.

2.3.1 Pre-harvest Sources of Contamination

Soil, excrement, irrigation water, water used to apply pesticides and fungicides, dust, insects, poorly composted manure, domestic and wild animals, and human handling are all potential pre-harvest sources of contamination. Since irrigation water may contain enteric infections including *Salmonella*, *E. Coli* O157:H7, and *Cryptosporidium* from adjacent livestock operations or sewage overflow, it is regarded as one of the most important risk factors. Inadequately handled soil amendments can also lead to contamination, allowing pathogens to survive for long periods of time (Taban and Halkman, 2011).

2.3.2 Post-harvest Sources of Contamination

Faeces, human handling, harvesting tools, shipping containers, domestic and wild animals, insects, dust, ice, etc. are examples of post-harvest causes of contamination. Another significant factor is wash water, which may quickly transmit infections from a single infected item to an entire lot if it is not adequately chlorinated or changed on a regular basis. Reusing containers and packaging materials without properly cleaning them can also be dangerous. In general, there are several chances for cross-contamination during the post-harvest stage, especially in large-scale commercial operations (Jeddi *et al.*, 2014).

2.3.3 Personal Hygiene–Related Contamination

In order to avoid microbial contamination of fresh fruit, workers' personal cleanliness is crucial. Food handlers can spread diseases including *Shigella*, *Staphylococcus aureus*, and Norovirus to fruits and vegetables by not adequately washing their hands after using the restroom, handling animals, or touching contaminated surfaces.

Contamination hazards are greatly increased when workers handle product when sick, don't wear protective gloves properly, or employ unhygienic methods when harvesting, sorting, and packing. Therefore, maintaining stringent sanitation regulations and providing training in good hygiene are essential elements of food safety in the produce sector (Lambrechts et al., 2014).

2.3.4 Storage-Related Sources of Contamination

The introduction and survival of microorganisms on fresh fruits and vegetables are significantly influenced by storage conditions. Pathogens and spoilage organisms can proliferate quickly due to inadequate refrigeration or temperature abuse, particularly on chopped or broken food where nutrients are more readily available. When fresh produce is kept next to raw meats, dirty crates, or infected containers, cross-contamination may also happen. While extended storage raises the possibility of pathogen development, humidity levels and condensation can produce wet conditions conducive to microbial growth. Key tactics to reduce contamination during storage include efficient temperature management, food type separation, and storage facility sanitation (James & James, 2010).

2.4 Mechanism to control contaminations

Applying Good Agricultural Practices (GAPs) is crucial to reducing microbiological risks during the pre-harvest stage, which is where contamination control of fresh fruits and vegetables starts. Using clean and safe irrigation water, properly composting manure, limiting domestic and wild animals' access to producing fields, and maintaining worker cleanliness through training and hand washing facilities are all important strategies (FAO/WHO, 2008). The risk of introducing infections like *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* is further decreased by field sanitation techniques such cleaning tools, harvest equipment, and containers. Contamination is further avoided at the farm level by maintaining soil health, keeping an eye on water quality, and putting efficient waste management in place (Beuchat, 2002). Preventing cross-contamination and lowering microbial burdens during processing, storage, and distribution are the main goals of post-harvest control methods. Freshness is preserved and microbial growth is slowed via efficient temperature control, such as quick cooling and preserving the cold chain. Other developments that have demonstrated efficacy in lowering pathogen survival on produce include antimicrobial packaging, ultraviolet (UV) treatment, and changed atmosphere packaging (Siroli et al., 2017). Together,

these integrated mechanisms help ensure the microbial safety of fresh fruits and vegetables from farm to consumer.

2.5 Nutritional values and health benefits of fresh fruit and vegetables

Vitamins, minerals, dietary fiber, and a variety of phytochemicals that promote healthy bodily processes are all abundant in fresh fruits and vegetables. They supply essential micronutrients like potassium, folate, vitamin C, and vitamin A, which are necessary for cellular functions, the immune system, and vision. Important antioxidants found in fruits and vegetables, such as flavonoids, phenolic compounds, and carotenoids, help shield the body from oxidative stress. They are perfect for hydration and controlling weight because of their high water content and low energy density (Slavin & Lloyd, 2012). Fresh fruit and vegetable consumption is closely linked to a lower risk of chronic illnesses, such as type 2 diabetes, cardiovascular disease, and several types of cancer. Plant-based dietary fiber promotes healthy digestion, reduces cholesterol, and aids in blood glucose regulation, all of which help prevent metabolic diseases. Colourful fruits and vegetables include bioactive chemicals and antioxidants that have anti-inflammatory and anti-carcinogenic qualities. Higher daily consumption of fruits and vegetables is consistently associated with lower all-cause mortality and better long-term health outcomes, according to large epidemiological studies (Aune et al., 2017).

Fruits and vegetables enhance immune resilience, maintain a healthy body weight, and improve gut microbiome variety, all of which contribute to general health and wellbeing in addition to preventing disease. While green vegetables include folate and vitamin K for healthy blood and bones, many fruits, including citrus and berries, offer vitamin C and polyphenols that boost immune system activity. Compared to separate supplements, the combination of nutrients and phytochemicals in plant-based diets optimizes body functioning more successfully. Therefore, for the best nutrition and illness prevention, international health organizations advise eating a range of fruits and vegetables every day (WHO, 2020).

2.6 Economic Importance of Fresh Fruits and Vegetables

Because of their high demand, nutritional value, and contribution to food security, fresh fruits and vegetables are essential to both national and international economies. They are important parts of the agriculture industry because they supply vital vitamins, minerals, and dietary fiber that promote public health and lower healthcare expenses related to non-communicable

diseases (Slavin & Lloyd, 2012). As consumer awareness of healthy diets increases, the market demand for fresh produce continues to rise, strengthening their economic value.

Millions of small-holder farmers, particularly in developing nations, rely heavily on the production of fruits and vegetables. Compared to staple crops, these crops frequently yield better yields per unit area, allowing farmers to increase household income, diversify their sources of income, and lessen poverty (Weinberger & Lumpkin, 2007). Fruit and vegetable growing is labor-intensive in many rural areas, giving women and young people the chance to work in planting, harvesting, sorting, packaging, and marketing.

Fresh fruits and vegetables make up a sizeable portion of the world's agricultural exports and play a major role in international trade. Exporting seasonal products to markets in Europe, the Middle East, and North America boosts the economies of nations with favorable climates, like those in Africa, Asia, and Latin America (FAO, 2020). This trade enhances foreign exchange earnings, supports agricultural modernization, and strengthens global market integration.

2.7 Pathogens in fruits and vegetables as a cause of disease

Fresh fruit and vegetable pathogens are now a significant cause of foodborne diseases globally. Pathogens that contaminate the surface or internal tissues can quickly spread to consumers because the majority of product is eaten raw or with little processing. *Salmonella* species, *Escherichia coli* O157:H7, *Shigella* species, and *Listeria monocytogenes* are common bacterial infections linked to produce-related outbreaks. These organisms are hard to get rid of by washing alone since they can withstand challenging climatic conditions and stick firmly to surfaces. Therefore, even if contaminated fruits and vegetables seem clean and fresh, they actually present serious hazards (Beuchat, 2002).

These pathogens can cause everything from minor gastrointestinal disorders to serious, sometimes fatal illnesses. For instance, *Listeria monocytogenes* can cause meningitis and septicaemia, especially in pregnant women and immune compromised people, whereas *E. coli* O157:H7 can cause haemorrhagic colitis and haemolytic uremic syndrome, especially in youngsters and the elderly. It is becoming better acknowledged that fresh produce can carry these infections (Herman et al., 2015). Globalized supply chains, rising raw food consumption, and intricate distribution networks that enable widespread exposure when contamination occurs are all contributing factors to the rising number of produce-related outbreaks. To lessen the burden of diseases linked to contaminated fruits and vegetables, it is

crucial to establish excellent farming and handling practices, strengthen food safety systems, and raise consumer knowledge (Carstens et al., 2019).

2.8 Transmission of foodborne disease

When pathogenic bacteria or their toxins contaminate food at any stage of production or consumption, foodborne illnesses are mainly spread. Poor hygiene practices, such as insufficient hand washing, incorrect food handling, or cross-contamination between raw and prepared meals, are frequently the cause of contamination. Through contaminated surfaces, utensils, and food contact situations, bacteria like *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes* can spread with ease.

When proper sanitation is not maintained, these bacteria can live on fruit and vegetable surfaces and equipment for extended periods of time, increasing the risk of transmission (WHO, 2023; CDC, 2022). Inadequate food preparation and storage in homes, marketplaces, and food service facilities can also result in transmission. Foods that are left at room temperature for extended periods of time or that are not cooked to temperatures high enough to destroy pathogens foster the growth of microorganisms. Furthermore, bacteria can be transferred to food through direct contact by sick food handlers, especially those with gastrointestinal diseases. Foodborne infections like *Shigella* and norovirus continue to spread largely due to poor personal hygiene, such as not washing hands after using the restroom (CDC, 2022; Todd et al., 2008).

2.9 Incidence of pathogens on fresh fruits and vegetables

Bacterial pathogens, such as *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*, have been found on a variety of food products, including leafy greens, tomatoes, melons, and berries, according to numerous studies. These infections might come from polluted soil, irrigation water, animals, or human contact. Fresh produce is currently one of the main categories linked to foodborne outbreaks, according to surveillance data collected globally (Beuchat, 2002).

Because of their vast surface area and frequent ingestion without cooking, leafy greens in particular exhibit a high prevalence of contamination. Products like lettuce, spinach, and cabbage frequently include fecal indicator bacteria and pathogenic strains connected to significant outbreaks, according to investigations conducted by public health organizations. For instance, *E. Coli* O157:H7 outbreaks linked to spinach in the US and Europe demonstrate

how farm-level contamination can have significant effects on public health. Detecting and eliminating infections during washing is made more difficult by their capacity to integrate into plant tissue (Herman et al., 2015).

Global monitoring statistics show that handling conditions along the supply chain, local water quality, and farming methods all have an impact on the prevalence of infections on fresh fruit. Research from both industrialized and developing nations has demonstrated that inadequate sanitation infrastructure, inconsistent water treatment, or a lack of hygiene education increase the risk of microbiological contamination. Due to centralized processing and extensive distribution networks, the risk of cross-contamination increases along with urbanization and industrial farming. Stricter regulations, better farming methods, and ongoing surveillance are still essential for lowering the prevalence of pathogens on fresh produce (Carstens et al., 2019).

2.10 Indicator organisms

Microorganisms used to evaluate the microbiological safety and quality of food, water, and environmental samples are known as indicator organisms. Coliforms, *Escherichia coli*, Enterococci, and total aerobic mesophiles are common indicator organisms that are used to assess the efficacy of sanitation and identify fecal contamination. Indicator organisms aid in locating sources of contamination and evaluating compliance with sanitary procedures during production and handling of fresh fruits and vegetables. Since *E. coli* usually comes from warm-blooded animals and does not survive well in outdoor situations, it is largely acknowledged as the most accurate sign of recent fecal contamination. Elevated levels of aerobic plate counts or total coliforms on produce surfaces could be a sign of unhygienic handling, insufficient washing, or poor agricultural water quality. Therefore, these markers are crucial instruments for detecting contamination hazards prior to pathogen growth or disease (Beuchat, 2002).

Additionally, indicator organisms are essential to quality control initiatives and regulatory standards. Thresholds of indicator organisms are used by several food safety regulations, including Hazard Analysis and Critical Control Point (HACCP) systems and Good Agricultural Practices (GAP), to gauge compliance. Because of this, indicator organisms are essential to contemporary food microbiology and public health monitoring (Feng et al., 2022).

2.11 Antibiotic Resistance of pathogens

One of the biggest risks to global public health is antibiotic resistance in pathogenic bacteria. When bacteria develop the capacity to withstand exposure to antibiotics that would typically inhibit or kill them, this is known as resistance. Genetic mutations or the horizontal transfer of resistance genes via plasmids, transposons, and bacteriophages are frequently the cause of this. *Escherichia coli*, *Salmonella species*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are among the pathogens that have become resistant to several antibiotic classes, making treatment more difficult and worsening the illness. Antibiotic resistance has increased due to the extensive use of antibiotics in agriculture, animal care, and human medicine (Ventola, 2015).

There has been a growing focus on the existence of antibiotic-resistant bacteria in the food chain, which includes fresh fruits and vegetables. Manure fertilizers, polluted soil, irrigation water, and improper handling techniques can all contaminate produce with resistant bacteria. Research has demonstrated that resistant variants of *Salmonella*, *E. Enterococcus* and *coli* can live on produce surfaces and spread to customers, increasing the risk of diseases that are challenging to treat. Resistance genes like bla, tet, and sul may be present in these organisms and can spread to other bacteria in the human gut (Martínez, 2009).

Increased morbidity, mortality, and financial burden are frequently linked to antibiotic-resistant diseases. Longer hospital stays, more costly treatments, and alternate antibiotic therapies which may be less successful or more toxic are all necessary for infections brought on by resistant strains. For instance, serious gastrointestinal disorders and bloodstream infections that are difficult to treat are associated with multidrug-resistant *Salmonella* and *E. coli*. The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) further highlights the critical need for international effort to limit resistance (CDC, 2019).

3. MATERIALS AND METHODS

3.1. Description of the study Area

The study was conducted in Nekemte town. The town is located in the East Wallaga zone of the Oromia, Regional State, 328 km from Addis Ababa. The town has a latitude and longitude of 9°5'N and 36°33'E, respectively, and an elevation of 2,088 m above sea level. The average annual rainfall 1854.9mm and the average temperature ranges from 14⁰c to 26⁰c.agriculture is dominant livelihood of the population in the study area. The major crops grown in the study area of Nekemte include cereals like maize, sorghum, teff, wheat, barley and horticultural crops like fruits avocado, mango, papaya, vegetables such as cabbage, and root crops like carrot. For this study three Gulit (small markets area) namely Bord, Bake Jama, and Darge sites were selected.

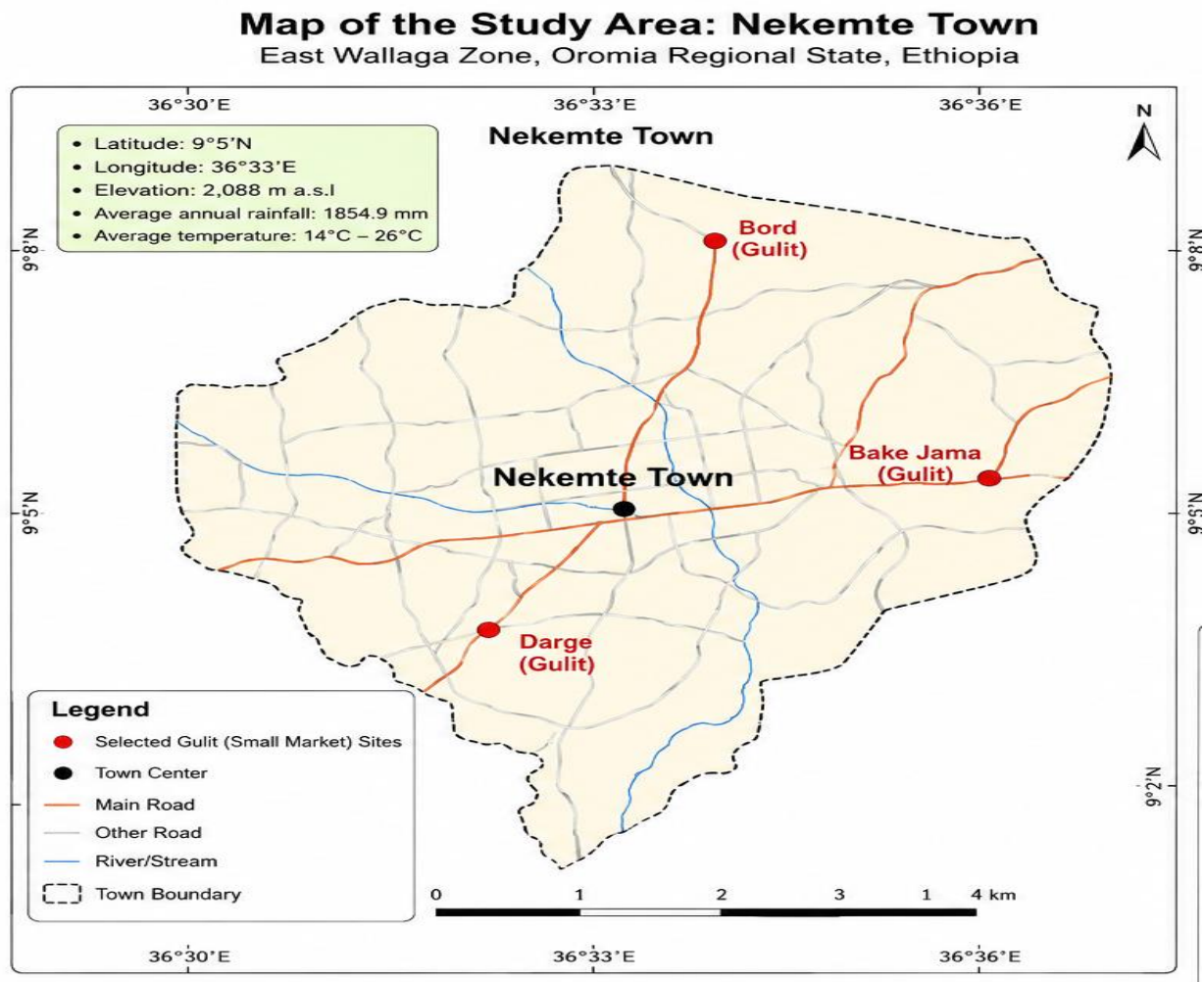


Figure 1. Map of study area

3.2 Study design

This study used an experimental design to assess the microbiological quality and safety of fresh fruits and vegetables in Nekemte town.

3.3 Sample Size

A total of 180 fresh fruit and vegetable samples were used in this study, and these samples were collected from three major markets in Nekemte town, namely Bordi, Bake Jama, and Darge. The sample size included 30 samples for each of the fruits and vegetables such as avocado, papaya, mango, carrot, cabbage, and tomato. The sample size of 30 is commonly used in microbiological studies to have adequate statistical power and reliable analysis(FAO,2019,ISO6887-1,2017;Cochran1977).

The Cochran formula for calculating sample size is:

$$n_0 = Z^2 P (1-P) / e^2$$

Where:

n_0 = required sample size

Z = Z-value corresponding to the confidence level (1.96 for 95% confidence)

p = estimated proportion (if unknown, 0.5 is commonly used)

e = margin of error (0.073) Or (0.73%)

3.4 Data collection methods

Data for this study were obtained through laboratory-based experiments, focusing on the microbiological quality and safety of fresh fruits and vegetables.

3.4.1 Laboratory experiment

Collected samples were processed for microbial analysis. Microorganisms were isolated and characterized, and the identified pathogens were tested for antibiotic susceptibility using standard laboratory techniques.

3.4.2 Sample collection and sampling techniques

A total of 180 samples of six different kinds of fresh fruits and vegetables, 30 for each of avocado, mango, papaya, tomato, carrot, and cabbage, were collected from three different local markets located in Nekemte town, which include Bordi, Bake Jama, and Darge.

Random samples of the fruits and vegetables were collected from the market vendors using the lottery system, and each type of fruit and vegetable was collected three times from each market. The fruits and vegetables were then put into a sterile and labeled plastic bag and immediately taken to the Department of Biology, Microbiology Laboratory at Wollega University for analysis (Figure 1). The samples were stored in refrigerator until the microbial analysis were conducted.



Figure 2: fresh fruit and vegetable samples collected from study area

3.4.3 Sample preparation

After collection, the samples were transferred into the lab and rinsed with distilled water or tap water. Thereafter, ten grams of each of the food samples were measured and put into a multifunctional food blender, and 90ml of distilled water was gradually added and blended for 2 or 3 minutes. Then, 1ml of each of the sample from the blended food was added to 9ml of sterile distilled water in test tubes and diluted up to 10^{-5} . 0.1ml of each of the diluted food samples from 10^{-1} to 10^{-5} was spread on pre-solidified media. After this, the plates were inverted and incubated for 24hours at 37°C . The colonies were enumerated from the plate containing microbial colonies between 30 and 300. The colonies were uncountable if they were more than three hundred colonies per sample. The colonies were counted and then converted into log colony forming units (Log CFU).

3.5 Microbial enumeration

Microbes were enumerated using the spread plate technique. The homogenate from sample preparation in distilled water was used for the procedure to count *staphylococcus*, *Enterobacteriaceae* like total coliform, fecal coliform and *Escherichia coli* (ISO 4833-1:2013).

3.5.1 Staphylococcus spp, count

For enumeration of *Staphylococcus*, 0.1 ml of the homogenate sample was transferred and spread plated on Mannitol salt agar (MSA). Then, the plate was inverted and incubated at 37°C for 24 hours. Colonies that were typical golden yellow counted as staphylococcus species (Acco *et al.*, 2003).

3.5.2 Escherichia coli count

For enumeration of *E. coli*, 0.1 ml of the homogenate was transferred and spread plated on pre-solidified surface MacConkey agar. Then, the plates were incubated for 24 hours at 37°C. After that, all pink colonies were enumerated as *Escherichia coli*.

3.5.3 Total Coliform count (TCC)

From appropriate dilutions, 0.1 ml of the aliquot was spread plated on pre-solidified surfaces of Violet Red Bile Agar (VRBA) (Oxoid) plates. Then the plates were incubated at 37°C for 24 hrs. Purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms (Weil *et al.*, 2006).

3.5.4 Faecal coliform count

To count faecal coliform, 0.1 ml of the homogenate sample was transferred and spread on eosin methylene blue agar using a cotton swab. Then, it was incubated for 24 hours at 37°C. After incubation, the colonies were counted.

3.6 Detection of foodborne Pathogens

3.6.1 Detection of Salmonella and Shigella

Ten grams of fresh fruit and vegetable samples were combined with ninety millilitres (90ml) of buffered peptone water and incubated at 37°C for 24 hours in order to detect *Salmonella* and *Shigella* species. Next, 10 ml of nutrient broth (Oxoid) was combined with 1 ml of pre-enrichment broth culture, and the mixture was once more cultured for 24 hours at 37°C. After then, Xylose Lysine Deoxycholate Agar (XLD) was streaked with a loopful of suspension

from a tube. The plates containing typical *Shigella* colonies (red colonies on XLD agar) and typical *Salmonella* colonies (red colonies with black cores on XLD agar) were analysed. The following common biochemical tests were used to examine these cultures.

3.6.1.1 Citrate Utilization Test

To measure citrate utilization, the slant was streaked and incubated for 24 hours at 37 °C. Citrate is the source of carbon. Growth and a shift from green to blue in color were regarded as *Salmonella* presumptive. *Shigella* lacks this black color because it is citrate negative.

3.6.1.2 Triple Sugar Iron Agar

After streaking the slant, it was incubated for 24 hours at 37°C to identify the production of H₂S and the fermentation of lactose, glucose, and sucrose. The red slant and acidic (yellow) butt were present, and H₂S produced was regarded as presumptive for *Salmonella* species. An acidic and an alkaline (red) slant (yellow) butt with no gas production was a presumptive for *Shigella* spp.

3.6.1.3 Methyl red test

The Methyl Red test was performed to determine the ability of bacterial isolates to produce stable acid end products from glucose fermentation. A loopful of the test organism was inoculated into sterile MR-VP broth containing glucose and incubated at 37°C for 24–48 hours. After incubation, 3–5 drops of methyl red indicator were added to the broth culture. The development of a red color indicated a positive result, showing that the organism produces stable mixed acids and maintains a low pH (below 4.4). A yellow or orange color indicated a negative result, suggesting that the organism does not produce stable acids and instead follows alternative metabolic pathways.

3.6.2 Detection of *Staphylococcus aureus*

Golden yellow colonies on the Mannitol Salt Agar plates were aseptically selected after the *Staphylococci* were counted. They were then placed in six millilitres (6ml) of nutrient broth and cultured for 24 hours at 37°C for additional purification. After that, a loopful of the nutrient broth culture was spread out on nutrient agar and cultured for 24 hours at 37°C. Procedure for these tests:

3.6.2.1 Catalase test

Two to three drops of 3% hydrogen peroxide is introduced to a small amount of a bacterial colony that has been put onto a clean glass slide for the test; instantaneous bubbling signifies a catalase-positive reaction. Like other *Staphylococcus* species, *Staphylococcus aureus* is catalase-positive, which helps set it apart from taxa like *Streptococcus* and *Enterococcus* that are catalase-negative.

3.7 Microbial analysis

Microbial analysis, refers to a systematic study of microorganisms that includes morphological analysis, Gram staining, microscopic identification, and biochemical characterization of microorganisms.

Morphological Analysis: The characteristics of colonies formed on culture medium, such as , shape, size, elevation, color, and margin, are studied to obtain preliminary information about microbial groupings (Pelczar et al., 2006).

Gram Staining: Gram staining was performed according to the method originally developed by **Gram (1884)**. This differential staining technique distinguishes bacteria into Gram-positive and Gram-negative groups based on differences in their cell wall structure.

Microscopic Identification: Microscopic examination was used to observe the morphology and arrangement of bacterial cells, including cell shape, cellular arrangement, spore formation, and motility, which are important characteristics for the preliminary identification of microorganisms (Koch, 1877; Bergey, 1923).

Biochemical Characterization: Biochemical characterization was carried out using standard biochemical tests, including catalase, oxidase, urease, citrate utilization, and carbohydrate fermentation tests. These tests determine the metabolic and enzymatic characteristics of bacterial isolates and aid in their identification (MacConkey, 1905; Simmons, 1926; Christensen, 1946; Kovács, 1956).

3.7.1 Identification of micro-flora

Following the determination of the samples' bacterial load, a loopful of four distinct colonies, ranging in size and number from one to four, were chosen at random from countable plates and purified by repeated streaking. Several morphological and biochemical tests were used to identify the isolates of dominating aerobic viable bacteria to the species level APHA (2015).

3.7.1.1 Microscopic examination

3.7.1.2 Gram staining

Prepare clean glass slide and add a drop of water on clean glass slide, air dried, and heat fixed by placing the slide gently over the flame of Bunsen burner. The pure culture isolates were stained according to Gram's techniques. The smear was stained with crystal violet for 1 minute, and then gently rinsed with tap water. Then, the slide was flooded with iodine solution for 1 minute and rinsed under tap water for 3 seconds. After rinsing, the smear was decolorized with 95% of ethanol for 20 seconds and rinsed slides gently under tap water for 3 seconds. Thereafter, the smear was counter stained by safranin and dried by absorbent paper. The stained cells were then examined with the oil immersion objective lens of the light microscope. At the end of the Gram Staining, the gram positive bacteria was characterized by a purple color while a gram negative bacteria was stained pink color.

3.7.1.3 Identification of Cell morphology

Identification of cell morphology includes cell shape, organization, endospore presence or absence and movement. These were carried out by gram staining techniques and observing under microscope.

3.7.2 Biochemical tests

3.7.2.1 Citrate Utilization Test

The ability of microbes, especially those belonging to the Enterobacteriaceae family, to use citrate as their only carbon source and ammonium ions as their only nitrogen source is assessed biochemically using the citrate utilization test. The enzyme citrate permease, which carries citrate into the cell and is processed there to produce alkaline end products, is expressed by bacteria that are able to use citrate. The bromothymol blue indicator in Simmons citrate agar changes from green to blue as a result in positive indicator (Cappuccino & Sherman, 2014). The test isolates were inoculated into test tubes containing Simmon's Citrate Agar by stabbing to the bottom of the tubes and streaked, then incubated at 37°C for 24hrs. After incubation, the colour changed from green to blue was observed.

3.7.2.2 Catalase Test

The catalase test was used to determine which species generate the catalase enzyme, which breaks down hydrogen peroxide (H_2O_2) into water and oxygen. A smear of a loop full of bacteria was made. One drop of 3% H_2O_2 was added onto a clean grease-free slide.

Formation of bubbles was observed for each bacterial isolate. The formation of this bubble indicates that the presence of catalase enzyme.

3.7.2.3 Urease Test

The urease test was performed to determine the ability of the isolated bacteria to produce the enzyme urease. A pure colony of the test organism was aseptically inoculated onto the surface of the medium and incubated at 37°C for 24–48 hours. After incubation, the tubes were examined for color change. A shift in color from yellow/orange to pink indicated a positive result due to the production of ammonia from the hydrolysis of urea, which increases the pH of the medium. No color change indicated a negative result.

3.7.2.4 Oxidase test

The oxidase test was used to identify bacteria that produce cytochrome c oxidase that catalyze the transport of electrons between electron donors in the bacteria and reagent 1% tetramethyl-p-phenylene diamine. First filter paper was soaked with the substrate tetramethyl-p-phenylene diamine dihydro chloride. The paper was moistened with sterile distilled water, and the colony to be tested was picked with sterile loop and smeared in the filter paper. The inoculated area of paper was observed for color change to intense deep blue or purple within 10-30 seconds, as a positive test.

3.7.2.5 Indole test

The indole test is a useful biochemical method to differentiate enteric bacteria based on their ability to produce indole from the amino acid tryptophan. The test is performed by inoculating the organism into tryptone broth and incubating it at 37°C for 24 hours. Thereafter Kovac's reagent is added. The formation of a red ring on the surface indicates a positive result (indole production), while no color change or a yellow ring indicates a negative result.

3.7.2.6 Triple Sugar Iron Agar

In order to identify the fermentation of glucose, sucrose, and lactose as well as the generation of H₂S, the butt was punctured, the slant was streaked, and it was incubated at 37°C for 24 hours. The test organism was found to have an acidic (yellow) butt and an alkaline (red) slant, along with the generation of H₂S. Once more, the isolated organism showed an acid (yellow) butt and an alkaline (red) slant with no gas generation.

3.8 Antimicrobial Susceptibility Test

The isolated organisms underwent antibiotic sensitivity profiling using Kirby Bauer disc diffusion method. The antibiotic panels that were examined consisted of ampicillin, gentamicin, tetracycline, oxacillin, erythromycin, vancomycin, sulphamethaxazol and ciprofloxacin. Culture of pure bacterial isolates were inoculated into nutrient broth and incubated at 37°C for 24 hours. The bacterial suspension was adjusted to 0.5MC Farland turbidity standards and transferred to Muller-Hinton agar plate using sterile cotton swab against all over agar surface. This turbidity standard prepared by mixing 1% of barium chloride with 1% of sulphuric acid according to its procedure. To ensure contact with the agar surface, antibiotic-impregnated discs were placed on it and gently tapped. After that, the plates were incubated for 24 hours at 37°C. Finally using a transparent ruler, the diameters of the zones of inhibition were measured to the nearest whole millimetre and classified as intermediate, susceptible, or resistant in accordance with CLSI guidelines (2017).

3.9 Data analysis

Data analysis was performed by using statistical package for social science (SPSS) software using version 25. The bacterial count was expressed as mean± standard deviation. The mean values of fresh fruits and vegetables compared by one way ANOVA. The mean difference was considered significant at p-value less than zero point zero five ($p < 0.05$).

4. RESULT

4.1 Evaluation of the Microbiological Quality (Load) of Fresh Fruits and Vegetables

Fresh fruits and vegetables exhibited different microbial loads depending on the type of produce and sampling location. Table 1 shows the total counts of *Staphylococcus*, total coliforms, faecal coliforms, and *Escherichia coli* (log CFU/g) detected in the samples. The mean microbial counts were 6.09 log CFU/g for *Staphylococcus* 5.90 log CFU/g for total coliforms, 5.60 log CFU/g for faecal coliforms, and 5.51 log CFU/g for *E. coli* (table1).

Among the tested samples, avocado had the minimum mean count of *Staphylococcus* (5.43 log CFU/g), whereas mango had the maximum (6.98 log CFU/g). Similarly, mango showed the maximum total coliform count (6.23 log CFU/g), while carrot had the minimum (5.41 log CFU/g). The results also indicated that total coliform and *E. coli* counts were comparable in tomato samples, whereas total coliform and faecal coliform counts were similar in avocado samples. Among all fresh fruits and vegetables examined, papaya had the maximum mean faecal coliform count, indicating a relatively higher level of faecal contamination compared with the other produce types analyzed (table 1).

Table 1. Microbiological Quality of Fresh Fruits and Vegetables (Indicator Bacteria)

Total Coliforms, Fecal Coliforms, *E. coli*, *Staphylococcus aureus*

Sample Type	N	Total Coliforms (Mean $\pm$ SD)	Fecal Coliforms (Mean $\pm$ SD)	<i>E. coli</i> (Mean $\pm$ SD)	<i>Staphylococcus</i> (Mean $\pm$ SD)
Mango	30	6.23 $\pm$ 0.05 ^a	4.61 $\pm$ 0.05 ^c	5.83 $\pm$ 0.05 ^b	6.98 $\pm$ 0.05 ^a
Papaya	30	6.15 $\pm$ 0.05 ^a	6.61 $\pm$ 0.05 ^a	5.94 $\pm$ 0.05 ^a	5.44 $\pm$ 0.05 ^c
Avocado	30	6.01 $\pm$ 0.05 ^b	6.01 $\pm$ 0.05 ^b	5.99 $\pm$ 0.05 ^a	5.43 $\pm$ 0.05 ^c
Tomato	30	5.51 $\pm$ 0.05 ^c	4.43 $\pm$ 0.05 ^c	5.51 $\pm$ 0.05 ^c	5.89 $\pm$ 0.05 ^b
Carrot	30	5.42 $\pm$ 0.05 ^c	4.42 $\pm$ 0.05 ^c	4.21 $\pm$ 0.05 ^b	6.23 $\pm$ 0.05 ^b
Cabbage	30	5.78 $\pm$ 0.05 ^b	6.52 $\pm$ 0.05 ^a	5.58 $\pm$ 0.05 ^c	6.54 $\pm$ 0.05 ^a
Total	180	5.90 $\pm$ 0.05	5.60 $\pm$ 0.05	5.51 $\pm$ 0.05	6.09 $\pm$ 0.05

Data represent mean $\pm$ standard deviation, a, b, c = mean within column with the same letter for same count are not significantly different (P<0.05).

4.2 Prevalence of Foodborne Pathogens Isolated from Fresh Fruits and Vegetables

The prevalence of foodborne pathogens isolated from fresh fruits and vegetables is presented in Table 2. Out of the 180 samples examined, 17 (9.44%) were positive for *Salmonella* spp. Avocado had the highest prevalence, with 6 (20%) positive samples, followed by carrot with 4 (13.33%) and papaya with 3 (10%). No *Salmonella* spp, were detected in tomato samples. A total of 31 (17.22%) samples were positive for *Staphylococcus aureus*. Avocado showed the highest prevalence with 9 (30%) positive samples, whereas mango and tomato had the lowest prevalence, each with 3 (10%) positive samples. *Shigella* spp. were isolated from 15 (8.33%) samples. Papaya and avocado had the highest prevalence, each with 4 (13.33%) positive samples, while tomato and cabbage had the lowest prevalence, each with 2 (6.67%) positive samples. No *Shigella* spp, was detected in mango samples (table 2).

Escherichia coli was detected in 12 (6.67%) of the samples. Tomato had the highest prevalence with 4 (13.33%) positive samples, followed by cabbage with 3 (10%). Mango had

the lowest prevalence with 1 (3.33%) positive sample; whereas no *E. coli* was isolated from avocado samples (table 2).

Tables 2. Detection of some Foodborne Pathogens Isolated from fresh Fruits and Vegetables in nekemte town.

Sample Type	Sample Size	<i>E. coli</i> (%)	<i>S. aureus</i> (%)	<i>Shigella spp</i> (%)	<i>Salmonella spp</i> (%)
Mango	30	1 (3.33)	3 (10)	0 (0)	2 (6.67)
Papaya	30	2 (6.67)	5 (16.67)	4 (13.33)	3 (10)
Avocado	30	0 (0)	9 (30)	4 (13.33)	6 (20)
Tomato	30	4 (13.33)	3 (10)	2 (6.67)	0 (0)
Carrot	30	2 (6.67)	7 (23.33)	3 (10)	4 (13.33)
Cabbage	30	3 (10)	4 (13.33)	2 (6.67)	2 (6.67)
Total	180	12 (6.67)	31 (17.22)	15 (8.33)	17 (9.44)

4.3 Identification of Dominant Microflora Isolated from Fresh Fruits and Vegetables

A total of 360 bacterial isolates were recovered from fresh fruit and vegetable samples. Six bacterial genera were identified: *Enterobacter spp.*, *Pseudomonas spp.*, *Bacillus spp.*, *Lactic acid bacteria (LAB)*, *Staphylococcus spp.*, and *Micrococcus spp.*(table 3).

Among all isolates, *Staphylococcus spp.* was the predominant bacterial genus, accounting for 24.44% of the total isolates, followed by *Bacillus spp.* (20.83%). The highest occurrence of *Staphylococcus spp.* was observed in cabbage (29.47%), papaya (28.39%), and avocado (22.58%). *Bacillus spp.* was most frequently isolated from mango (26.09%), avocado (20.97%), papaya (20.27%), and cabbage (20.00%). Carrot samples were mainly dominated by *Enterobacter spp.* (27.27%), followed by *Micrococcus spp.* (18.18%) and *Bacillus spp.* (15.91%). In cabbage, *Staphylococcus spp.* (29.47%) was the dominant isolate, followed by *Bacillus spp.* (20.00%) and *Enterobacter spp.* (14.74%)(table3).

LAB was commonly isolated from avocado (16.13%), tomato (15.38%), and cabbage (12.63%). *Pseudomonas spp.* showed the highest frequency in mango (15.22%) and avocado (12.90%). *Micrococcus spp.* was the least frequently isolated bacterial genus from both fruit and vegetable samples. The distribution of the dominant bacterial genera isolated from the different fresh fruit and vegetable samples are presented in Table 3.

Table 3: frequency distribution (%) of dominant micro-flora isolated from fresh fruit and vegetables in Nekemte town.

Sample Types	No. of Isolates	Enterobacter spp (%)	Pseudomonas spp (%)	Bacillus spp (%)	Lactic Acid Bacteria (%)	Staphylococcus spp (%)	Micrococcus spp (%)
Mango	46	10(21.74)	7(15.22)	12(26.09)	5(10.87)	9(19.57)	3(6.52)
Papaya	74	9(12.16)	6(8.12)	15(20.27)	9(12.16)	21(28.39)	14(18.92)
Avocado	62	11(17.74)	8(12.9)	13(20.97)	10(16.13)	14(22.58)	6(9.68)
Tomato	39	4(10.26)	5(12.82)	9(23.08)	6(15.38)	8(20.51)	7(17.95)
Carrot	44	12(27.27)	5(11.36)	7(15.91)	4(9.09)	8(18.18)	8(18.18)
Cabbage	95	14(14.74)	9(9.47)	19(20)	12(12.63)	28(29.47)	13(13.68)
Total	360	60(16.67)	40(11.11)	75(20.83)	46(12.78)	88(24.44)	51(14.17)

4.4 Antimicrobial Susceptibility Patterns of Foodborne Pathogens Isolated

A total of 17 bacterial isolates were subjected to antimicrobial susceptibility testing. Among the *E. coli* isolates (N = 5), the highest resistance was observed against ampicillin (60%) and tetracycline (60%), while the highest susceptibility was recorded for ciprofloxacin (60%).

Salmonella isolates (N = 4) exhibited 100% resistance to ampicillin but showed 100% susceptibility to gentamicin and ciprofloxacin. The *Shigella* isolates (N = 2) demonstrated 100% susceptibility to ciprofloxacin, 50% susceptibility to trimethoprim-sulfamethoxazole (SXT), and 50% resistance to ampicillin. Among the *S. aureus* isolates (N = 6), 66.67% were sensitive to oxacillin, whereas 100% were resistant to vancomycin and 66.67% were resistant to erythromycin. The antimicrobial susceptibility profiles of the isolated foodborne pathogens are presented in Table 5.

Table 4: Antimicrobial susceptibility patterns of pathogenic bacterial isolates from fresh fruits and vegetables

Pathogens	Antibiotics	Sensitive (S) %	Intermediate (I) %	Resistant (R) %
<i>E. coli</i> (N=5)	AMP (30 µg)	40%	0%	60%
	CIP (5 µg)	60%	40%	0%
	TE (30 µg)	20%	20%	60%
<i>Salmonella spp</i> (N=4)	AMP (30 µg)	0%	0%	100%
	GEN (10 µg)	100%	0%	0%
	CIP (5 µg)	100%	0%	0%
<i>Shigella spp</i> (N=2)	SXT(5 µg)	50%	50%	0%
	CIP (5 µg)	100%	0%	0%
	AMP (30 µg)	50%	0%	50%
<i>S. aureus</i> (N=6)	OX (1 µg)	66.67%	33.33%	0%
	VAN (30 µg)	0%	0%	100%
	ERY (15 µg)	0%	33.33%	66.67%

N= number of isolate

AMP =Ampicillin, CIP=Ciprofloxacin, TE=Tetracycline, GEN= Gentamycin, OX=Oxacillin, ERY=Erythromycin, VAN=Vancomycin, SXT= sulphamethaxazol/ Trimethoprim.

5. DISCUSSION

The variation in microbial loads observed among the different fruits and vegetables may be associated with differences in their surface characteristics, moisture content, sugar composition, and handling practices. The maximum *Staphylococcus* count observed in mango agrees with the findings of Nguyen et al. (2014), who reported that fruits with smooth and moist surfaces are more susceptible to contamination through human handling. Likewise, Dümen et al. (2012) suggested that fruits with high sugar content support greater microbial survival than oily fruits such as avocado.

The maximum total coliform count detected in mango is consistent with the findings of Castro-Rosas et al. (2012), who reported elevated coliform contamination in tropical fruits because of their high moisture content and exposure to contamination during harvesting, transportation, and marketing. In contrast, the minimum coliform count observed in carrot may be related to its firm surface and reduced surface moisture, which can limit bacterial attachment, as reported by Beuchat (1995) and Alam et al. (2013).

The comparable levels of total coliforms and *E. coli* observed in tomato, as well as the similarity between total and faecal coliform counts in avocado, agree with the findings of Feng et al. (2002) and Alegbeleye et al. (2018). These studies indicated that contamination from soil, irrigation water, manure, and poor handling practices can result in similar distributions of these microbial groups. The maximum faecal coliform count recorded in papaya is in agreement with Castro-Rosas et al. (2012), who attributed the increased contamination of tropical fruits to their soft surface and susceptibility to post-harvest contamination. The presence of faecal coliforms in fresh produce may indicate inadequate hygiene during cultivation, irrigation, harvesting, transportation, and marketing.

The present study found that 17 (9.44%) of the samples were contaminated with *Salmonella* spp., with avocado showing the highest prevalence (20%), while tomato showed no contamination. This finding agrees with Abakari et al. (2018), who reported that *Salmonella* contamination varies among fruits and vegetables depending on their surface characteristics, cultivation environment, and handling practices. The absence of *Salmonella* in tomato samples is also consistent with Nguyen et al. (2014), who suggested that the acidic nature and smooth surface of tomatoes reduce bacterial attachment and survival.

The overall prevalence of *S. aureus* was 31 (17.22%), with avocado exhibiting the highest contamination level (30%) and both mango and tomato showing the lowest prevalence (10%). Similar findings were reported by Abakari et al. (2018), who observed that fruits with rough or irregular surfaces are more likely to harbor *S. aureus* because they facilitate bacterial attachment. Likewise, Dümen et al. (2012) reported that produce with smooth or waxy surfaces, such as tomatoes and mangoes, generally has lower *S. aureus* contamination due to reduced microbial adhesion. *Shigella spp.* were detected in 15 (8.33%) of the samples, with papaya and avocado having the highest prevalence (13.33%), while tomato and cabbage had the lowest prevalence (6.67%). No *Shigella spp.* were isolated from mango samples. These results are comparable with those of Castro-Rosas et al. (2012), who reported that *Shigella* contamination in fresh produce is influenced by handling practices and exposure to contaminated water. The low prevalence observed in tomato and the absence of *Shigella* in mango are also in agreement with the findings of Gómez-Aldapa et al. (2019) and Nguyen et al. (2014).

In the current study, *E. coli* was isolated from 12 (6.67%) of the samples. Tomato had the highest prevalence (13.33%), followed by cabbage (10%), whereas mango had the lowest prevalence (3.33%), and no *E. coli* was detected in avocado. The relatively high contamination in tomato is similar to the findings of Ramos et al. (2014), who associated higher *E. coli* prevalence with contaminated irrigation water and inadequate washing practices. The contamination observed in cabbage is also supported by Abadias et al. (2008) and Beuchat (2002), who reported that leafy vegetables are particularly susceptible because of their large surface area and contact with soil. The low prevalence in mango and the absence of *E. coli* in avocado agree with the findings of Barak et al. (2011) and Little et al. (2003), who suggested that protective fruit skins, antimicrobial compounds, and peeling before consumption reduce the risk of contamination.

The present study showed that *Staphylococcus spp.* (24.44%) was the predominant bacterial genus, followed by *Bacillus spp.* (20.83%). This finding agrees with Viswanath et al. (2019), who reported *Staphylococcus spp.* as the most common contaminant in ready-to-eat foods due to frequent human handling and its ability to survive on environmental surfaces. Similarly, Barbosa et al. (2017) identified *Bacillus spp.* as one of the dominant microorganisms associated with fresh produce because of its spore-forming capacity.

In this study, the highest proportions of *Staphylococcus spp*, were found in cabbage (29.47%), papaya (28.39%), and avocado (22.58%). These values are lower than those reported by Eni et al. (2010) and Little et al. (2003), who observed higher *Staphylococcus* contamination levels in commonly handled fruits and vegetables. The differences may be related to variations in post-harvest handling practices, sanitation measures, market conditions, and environmental factors.

Bacillus spp, occurred at relatively high frequencies in mango (26.09%), avocado (20.97%), papaya (20.27%), and cabbage (20.00%). Similar observations were reported by Ray and Bhunia (2013) and Montville and Matthews (2008), who documented the widespread occurrence of *Bacillus* species on fresh produce due to their persistence in soil and resistance to environmental stress through spore formation. The predominance of *Enterobacter spp*, (27.27%) in carrot samples may be associated with soil contamination, since root vegetables are in direct contact with soil during growth and harvesting. Comparable findings have been reported in studies of fresh vegetables where Enterobacteriaceae were commonly isolated from root crops. LAB were frequently detected in avocado (16.13%), tomato (15.38%), and cabbage (12.63%), supporting the findings of Di Cagno et al. (2013) and Fleet (2011), who reported that lactic acid bacteria naturally colonize fruits and vegetables and may increase during storage or early fermentation processes.

The highest frequencies of *Pseudomonas spp*, were observed in mango (15.22%) and avocado (12.90%). This is consistent with the reports of Nwachukwu et al. (2014) and Barth et al. (2009), who found *Pseudomonas species* to be common spoilage organisms of fresh produce because of their ability to grow under refrigerated and moist conditions.

Micrococcus spp, was the least abundant bacterial genus isolated in the present study. This agrees with Ray and Bhunia (2014), who reported that although *Micrococcus species* are common environmental microorganisms, they usually occur at lower frequencies than *Staphylococcus*, *Bacillus*, and *Enterobacteriaceae* because they are less competitive in nutrient-rich plant environments. Overall, the bacterial profile observed in this study is generally consistent with previous reports, although differences in the percentages of individual bacterial groups may reflect variations in agricultural practices, environmental conditions, handling procedures, storage methods, and market hygiene.

The present study revealed that *E. coli* isolates showed high resistance to ampicillin (60%) and tetracycline (60%), which is comparable with the findings of Aijuka et al. (2015) and

Van et al. (2007), who reported ampicillin resistance ranging from 55–80% and tetracycline resistance of 45–70% among foodborne *E. coli* isolates. The 60% susceptibility to ciprofloxacin observed in this study agrees with Rasheed et al. (2014), who reported that fluoroquinolones remain relatively effective against *E. coli*, although resistance is gradually increasing.

The 100% resistance of *Salmonella* isolates to ampicillin is consistent with studies by Tadesse et al. (2012) and Thung et al. (2016), which documented widespread ampicillin resistance associated with the dissemination of β -lactamase-producing strains. In contrast, the 100% susceptibility to gentamicin and ciprofloxacin agrees with reports by Akinyemi et al. (2010) and Antunes et al. (2016), indicating that these antibiotics remain effective against many non-typhoidal *Salmonella* isolates.

The *Shigella* isolates showed 100% susceptibility to ciprofloxacin, 50% susceptibility to SXT, and 50% resistance to ampicillin. Similar patterns have been reported by Rahman et al. (2011), who found 60–90% resistance to ampicillin among *Shigella* isolates from food and water sources. The moderate susceptibility to SXT is also comparable to the findings of Khan et al. (2013), who reported increasing resistance to trimethoprim-sulfamethoxazole due to its extensive use in developing countries.

In the present study, *S. aureus* isolates exhibited 66.67% sensitivity to oxacillin and 66.67% resistance to erythromycin. These findings are similar to those of Normanno et al. (2007) and Hennekinne et al. (2012), who reported substantial erythromycin resistance among foodborne *S. aureus* strains. The observed oxacillin sensitivity suggests a relatively low occurrence of methicillin-resistant *S. aureus* (MRSA), which agrees with the findings of Pu et al. (2009).

However, the 100% resistance to vancomycin observed in this study differs markedly from most previous reports, where vancomycin-resistant *S. aureus* (VRSA) remains uncommon. Loomba et al. (2010) reported reduced susceptibility to vancomycin in some isolates, but complete resistance was rare. The discrepancy may be associated with local antimicrobial use patterns, methodological differences, or the limited number of isolates tested, and therefore warrants further molecular investigation. Overall, the antimicrobial susceptibility patterns observed in this study indicate that resistance to commonly used antibiotics, particularly ampicillin, is widespread among foodborne pathogens, whereas ciprofloxacin and gentamicin generally remain effective therapeutic options.

6. CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

This findings demonstrate that fresh fruits and vegetables sold in the study area can serve as potential vehicles for the transmission of foodborne pathogens and may pose a public health risk when consumed raw or without adequate washing and handling.

The microbiological quality evaluation revealed the presence of indicator bacteria, including total coliforms, fecal coliforms, *Escherichia coli*, and *Staphylococcus* spp., in a considerable proportion of the analyzed samples. The detection of these indicator microorganisms suggests possible fecal contamination and inadequate hygienic practices during production, harvesting, transportation, storage, and marketing. Maximum counts of indicator bacteria also indicate poor sanitary conditions and reflect the potential presence of other pathogenic microorganisms.

The microbial safety analysis confirmed the occurrence of important foodborne pathogens, including *E. coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus*, in fresh fruits and vegetables. Among the tested produce, some commodities showed higher contamination levels than others, indicating that the type of produce, handling practices, and environmental exposure may influence microbial contamination.

The study also identified and characterized the dominant microflora associated with fresh fruits and vegetables. The predominance of bacterial groups commonly associated with environmental sources, human handling, and fecal contamination suggests that contamination can occur throughout the food supply chain.

The antimicrobial susceptibility testing of the isolated foodborne pathogens demonstrated varying resistance and sensitivity patterns against the tested antibiotics. Some isolates showed resistance to commonly used antimicrobial agents, while others remained susceptible to more effective drugs. Out of isolated foodborne bacterial pathogens *E.coli* and *staphylococcus aureus* more resistant the tested antibiotics while *Shigella* spp less resistant the tested antibiotics.

6.2 RECOMMENDATION

Based on the current study, the following recommendations were made

- ✚ Farmers should adopt Good Agricultural Practices (GAPs) to minimize microbial contamination during cultivation and harvesting.
- ✚ Vendors should receive training on safe food handling practices and the prevention of cross-contamination.
- ✚ Consumers should thoroughly wash fresh fruits and vegetables with clean potable water before consumption.
- ✚ Public awareness programs should be conducted to educate consumers about foodborne pathogens and safe food handling practices.
- ✚ Local health offices and food safety regulatory agencies should establish routine microbiological monitoring and inspection programs for fresh fruits and vegetables sold in markets.
- ✚ Public health institutions should organize continuous training programs for farmers, vendors, and food handlers regarding food hygiene and sanitation.
- ✚ Further studies should include a larger sample size and cover additional fruits and vegetables from different seasons and locations.

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APPENDICES
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APPENDEX I



Figure 3: gram staining and microbial count

APPENDIX- II

Table 5: Biochemical characterization of bacterial isolates from fresh fruits and vegetables

Bacterial isolates	No. of isolates	Isolate codes	Suspected source	Gram reaction	Catalase	Citrate Utilization	Oxidase	Urease	Indole	TSI agar
<i>Staphylococcus aureus</i>	9, 21, 28, 8, 14, 8	MA-1, PA-2, CB-3, CR-4, AV-5, TO-6	Mango, Papaya, Cabbage, Carrot, Avocado, Tomato	+	+	-	-	-	-	-
<i>Bacillus spp</i>	12, 9, 7, 15, 13, 19	MA-1, TO-2, CR-3, PA-4, AV-5, CB-6	Mango, Tomato, Carrot, Papaya, Avocado, Cabbage	+	+	+	+	+	-	+
<i>Pseudomonas spp</i>	9, 8, 6, 7, 5, 5	CB-1, AV-2, PA-3, MA-4, CR-5, TO-6	Cabbage, Avocado, Papaya, Mango, Carrot, Tomato	-	+	+	+	-	-	-
<i>Lactic acid bacteria</i>	6, 4, 12, 10, 5, 9	TO-1, CR-2, CB-3, AV-4, MA-5, PA-6	Tomato, Carrot, Cabbage, Avocado, Mango, Papaya	+	-	-	-	-	-	+
<i>Enterobacter spp</i>	9, 14, 12, 11, 10, 4	PA-1, CB-2, CR-3, AV-4, MA-5, TO-6	Papaya, Cabbage, Carrot, Avocado, Mango, Tomato	-	+	+	-	-	-	+
<i>Micrococcus spp</i>	6, 8, 13, 3, 14, 7	AV-1, CR-2, CB-3, MA-4, PA-5, TO-6	Avocado, Carrot, Cabbage, Mango, Papaya, Tomato	+	+	-	+	-	-	-
Total	360									

Keys :(-)=Negative, (+) = Positive, MA-Mango, PA-Papaya, AV-Avocado, CB-cabbage, CR-Carrot, TO-Tomato, TSI =Triple sugar iron

APPENDIX-III

Guideline levels used to determine the microbiological quality of fruits and vegetables

Table -6 Guideline Levels for Microbiological Quality of Fruits and Vegetables

Microorganism	Satisfactory Level	Acceptable Level	Unsatisfactory Level	Potentially Hazardous Level
Total Coliforms (TCC)	< 10 ³ CFU/g	10 ³ – 10 ⁵ CFU/g	> 10 ⁵ CFU/g	—
Fecal Coliforms (FCC)	< 10 ² CFU/g	10 ² – 10 ⁴ CFU/g	> 10 ⁴ CFU/g	—
Escherichia coli	< 10 ² CFU/g	10 ² – 10 ³ CFU/g	> 10 ³ CFU/g	≥ 10 ⁴ CFU/g
Staphylococcus aureus	< 10 ² CFU/g	10 ² – 10 ³ CFU/g	> 10 ³ CFU/g	≥ 10 ⁴ CFU/g
Salmonella spp.	Not detected in 25 g	—	Detected in 25 g	Always hazardous
Shigella spp.	Not detected in 25 g	—	Detected in 25 g	Always hazardous

Interpretation

Satisfactory: Safe for consumption; good hygienic quality

Acceptable: Marginal quality; indicates some contamination

Unsatisfactory: Poor hygiene; corrective action needed

Potentially Hazardous: Unsafe; poses health risk.