COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

DEPARTMENT OF BIOLOGY

BACTERIAL CONTAMINATION OF LOCALLY PREPARED FRESH FRUIT JUICES AND HYGIENE PRACTICES IN WOLDIA TOWN, ETHIOPIA

BY:

MUHSINA ADEM LEGESSE

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Bacterial Contamination of Locally Prepared Fresh Fruit Juices Hygiene Practices in Woldia Town, North Wollo Administrative Zone, Amhara National Regional State, Ethiopia.

A Thesis Submitted to the Department of Biology School of Graduate Studies Woldia University

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

By:

Muhsina Adem

Advisor : Sultan Mohammed (Ph.D.)

Co-Advisor: Esubalew Siente (Ph.D.)

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THESIS APPROVAL SHEET

As thesis research advisor, we hereby certify that, we have read and evaluated this thesis prepared, under our guidance by Muhsina Adem entitled **“**Bacterial Contamination of Fresh Fruit Juice and Hygiene Practice in Woldia Town, North Wollo, Zone of Amhara Regional State, Ethiopia”. and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Applied Microbiology compiled with the regulations of the university and needs the accepted standards with respect to originality and quality. We recommend that it can be submitted as fulfilling the thesis requirement.

1. Sultan Mohammed (PhD)	______________________	__________________
Major Advisor	Signature	Date
2. Esubalew Siente (PhD)	______________________	__________________
Co Advisor	Signature	Date

As member of the Board of Examiners of the Master of Sciences (MSc.) thesis open defense examination, we have read and evaluated this thesis by Muhsina Adem entitled “Bacterial Contamination of Fresh Fruit Juice and Hygiene Practice in Woldia Town, North Wollo, Zone of Amhara Regional State, Ethiopia We hereby certify that; the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Sciences (MSc.) in Applied Microbiology**.**

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DEDICATION

This paper is dedicated to my beloved family, whose unwavering love, encouragement, and support have been my guiding lights throughout this academic journey. Your belief in my abilities has given me the strength to persevere in the face of challenges and has inspired me to reach for my dreams. I am deeply grateful for the sacrifices you have made and for the countless moments of joy and motivation you have shared with me.

To my friends and colleagues, I extend my heartfelt gratitude for your camaraderie and support. The laughter we shared and the late-night discussions we engaged in have enriched this experience beyond measure. Your diverse perspectives and insights have not only motivated me to push my boundaries but have also transformed this journey into one filled with meaningful connections and unforgettable memories. And to my Advisors whose guidance and wisdom have been instrumental in my growth as a scholar thank you for believing in me and for your patience in nurturing my academic potential. Your encouragement to think critically and explore new ideas has shaped my understanding and has been a crucial part of my development. I am forever indebted to you for your invaluable contributions to my academic and personal growth.

AUTHOR DECLARATION

By my signature below, I declare and affirm that that this master thesis Entitled “Bacterial contamination of fresh fruit juice and hygiene practice in Woldia town, North Wollo, Ethiopia” is my original work. I also seriously declare that this thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate. All sources of materials that are used for this thesis have been duly acknowledged through citation.

________________________ _____________________

Name of the student Signature Date

BIOGRAPHICAL SKETCH

The author was born on February 19,1988 , in Nikuara Village Ebnat Town Ethiopia. she pursued her elementary education at Ebnat elementary school and , Ebnat secondary school For her secondary education, she attended Adiss zemen high and Preparatory School, In 2002 Ethiopian Calendar (2010 Gregorian calendar), the author graduated with a Bachelor of Science degree in Applied Biology from Arba minch University. Her academic journey reflects a strong passion for microbiology and environmental health, inspiring her to further her studies. The author is currently advancing her expertise in Applied Microbiology at Woldia University. Her research interests focus on Bacterial contamination fresh fruit juice and hygiene practice in Woldia Town, North Wollo, Ethiopia . This work aims to address critical challenges in juice (food) born diseases.

ABSTRACT

Fruit juices are valuable components of a healthy diet and serve as important sources of nutrients, vitamins, and fiber. However, contamination can occur during processing due to unhygienic handling, equipment, or raw materials, potentially leading to foodborne illnesses. This study aimed to evaluate the bacteriological safety of avocado, mango, and mixed fruit juices. A cross-sectional study was conducted in Woldia Town from February 2023 to v March 2025. Standard microbiological methods were used to analyze the presence of pathogenic bacteria, including *Staphylococcus aureus*, *Shigella*, and *Escherichia coli (E. coli*). A total of 30 juice samples were randomly collected from local juice vendors. All samples were analyzed in duplicate, and the mean values were reported. The results revealed that 80% of the samples were contaminated with *E. coli*, 90% with *Staphylococcus aureus*, and 50% with *Shigella*. The calculated means for the bacterial types were as follows: *Shigella* exhibited a mean of 5.3533 (N=3, SD=0.60913), *E. coli* had a mean of 5.3867 (N=3, SD=0.62164), and *Staphylococcus aureus* recorded the highest mean of 5.8233 (N=3, SD=0.01528). The overall mean across all bacterial strains was determined to be 5.5211 (N=9, SD=0.49093). The hygiene practices of juice makers were also assessed. All vendors reported washing their machines once per day and rinsing utensils with water during juice preparation. However, only 30% of the juice makers used water alone to wash their hands before preparing juice. In terms of waste disposal, 75% used sacks to discard waste, while only 25% used a covered bin. The findings highlight a significant public health concern regarding the bacteriological quality of fruit juices sold in Woldia Town. To mitigate the risk of foodborne illnesses, it is essential to provide food safety training and regular supervision for juice vendors. Moreover, collaboration with the Ethiopian Standards Agency is recommended to enforce food safety regulations, including routine inspection of fruits, vegetables, and juice handling practices.

Keywords: ***Bacterial Contamination, Fruit juices, Juice house, Vending site and Woldia Ethiopia.***

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Table of Contents

Contents Page

LIST of TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

BGLBB	Brillian green lactose bile broth
CFU	Colony forming unit
CSA	Central statistical agency
EC	Escherichia coli
FAO	Food agricultural organization
FCC	Fecal coliform count
FDA	Food and drug administration
ISO	International standard organization
LSIB	Lauryl sulfate tryptos
MPN	Most probable number
MSA	Manitol salt agar
PCA	Plat count agar
TCC	Total clifor count
TSCC	Total staphylococcus count
TUBC	Total viable bacterial count

1. INTRODUCTION

1.1. Background of the Study

Fresh fruit juices are commonly consumed for their nutritional benefits, providing essential nutrients, vitamins, and phytochemicals. Fruit juice contains several varieties of therapeutic properties that may reduce the risk of several health conditions such as stroke, diabetes, heart disease, loss of bone, and neural tube defects (Kandasamy, 2015). It is rich in dietary fiber and provides a wide range of essential vitamins, such as vitamins C and E, as well as antioxidants, minerals, and phytochemicals (Sarker, 2019). Vitamin C which is naturally present in fruit juices is crucial for the body's formation of collagen, cartilage, muscle, and blood vessels (Embaby, 2015). The high potassium and low sodium characteristic of most juices help in keeping healthy blood pressure. Fruit juices constitute a suitable and convenient meal for today's lifestyles because they need no cooking or further preparation (Mallinson *et al.*, 2016).

Fruit juices can be consumed immediately after preparation or processed and packaged by commercial juice companies to extend their shelf life (Huang, 2017). However, if the juices are not handled safely and unhygienic conditions, they can become a vehicle for the transmission of various disease-causing agents, potentially leading to outbreaks of foodborne illnesses worldwide (Ehuwa *et al.*, 2021). Inadequate hygiene practices during juice preparation can create an ideal environment for microbial growth, allowing foodborne pathogens to thrive and rendering the product unsuitable for consumption (Abebe *et al.*, 2020). Fruit juices can become contaminated at various stages, from growing to preparation, due to contact with animals, contaminated water, soil, unclean equipment, or infected food handlers (Bintsis, 2018). Several studies have identified fruit juices as a potential source of pathogenic bacteria, such as *E. coli*, *Salmonella spp.*, *Shigella spp.*, and *Staphylococcus aureus*. The presence of these harmful microorganisms in fruit juices poses a significant public health concern, as consumption of contaminated juices can lead to foodborne diseases (Odo *et al.*, 2021).

To ensure the safety and quality of locally prepared fresh fruit juices, it is crucial to implement and maintain proper hygiene and sanitation practices throughout the production and handling processes. This includes measures such as proper cleaning and disinfection of equipment, adherence to food safety protocols, and training of juice makers on good hygiene practices.

In the northeastern region of Ethiopia, particularly in the North Wollo Administrative Zones, there is a lack of adequate information on the bacteriological quality and associated factors of locally prepared fresh fruit juices. This lack of baseline data hinders the evaluation of the overall quality and safety of these fruit juice products. To address this knowledge gap, the present study aims to determine the bacteriological safety and quality of locally prepared fresh fruit juices sold in Woldia Town. The research focused on assessing the levels of bacterial contamination in the juice samples and identifying the factors associated with such contamination, including hygiene practices, storage conditions, and food safety knowledge among the juice makers. By conducting this study in Woldia Town, the researchers hope to generate crucial baseline information on the microbiological quality and safety of the locally produced fresh fruit juices in the Northeastern region of Ethiopia. The findings would provide valuable insights to guide the development and implementation of appropriate interventions, such as training programs, regulatory measures, and improved hygiene practices, to ensure the consumption of safe and high-quality fruit juices by the local population. This research endeavor is essential to address the current lack of data and contribute to the overall improvement of food safety standards and public health outcomes in the region.

1.2 Statement of the Problem

Fresh fruit juices are popular beverages enjoyed by many individuals worldwide due to their refreshing taste and perceived health benefits. In many countries, locally prepared fresh fruit juices are readily available from street vendors, markets, and small-scale juice stalls. While these juices offer a convenient and seemingly healthy option, there is growing concern regarding their safety, particularly regarding bacterial contamination (Heinz, 2013).

The consumption of fresh fruit juices is often perceived as a healthy choice due to their rich nutrient content and refreshing taste. In many regions, locally prepared fresh fruit juices are popular among consumers as they provide a convenient and accessible source of hydration and nutrition. However, despite their apparent benefits, there is growing concern over the potential risk of bacterial contamination associated with these juices. Pathogenic bacteria can enter fruits through damage, wounds, cuts, and peel, which can occur during maturation, harvesting, or processing. This can lead to contamination of the juice, posing a significant risk to public health (Igiehon, 2020).

Bacterial contamination of fresh fruit juices can occur at various stages of production, including during fruit handling, processing, and storage, as well as due to poor hygiene practices among vendors. Such contamination can lead to the presence of harmful bacteria, including pathogens such as *Salmonella*, *E.coli*, *Shigella* and *Listeria*, which pose significant health risks to consumers (Bersisa *et al*., 2019).

Several factors contribute to the potential for bacterial contamination in locally prepared fresh fruit juices. These include inadequate washing of fruits, improper sanitation of equipment and utensils, unhygienic storage conditions, and limited knowledge of food safety practices among vendors. Additionally, factors such as poor water quality and environmental contamination may further exacerbate the risk of microbial contamination in these products (Adare, 2020). In many developing nations, the persistent practice of utilizing untreated wastewater and manure as fertilizers in fruit and vegetable cultivation stands as a significant factor fueling contamination (Minhas *et al.,* 2022). Even within developed countries, microbial food-borne diseases affect an estimated one-third of the population each year (Almaary, 2023). Incidences of foodborne illnesses linked to the consumption of fruit juices are reported worldwide. For instance, a study conducted in Jima town revealed that a majority of the fruit juices served there exceeded the microbial load specifications set for fruit juices in some regions, potentially contributing to health issues (Adare, 2020).

In response to the rising prevalence of foodborne illnesses, governments worldwide are escalating their efforts to enhance food safety (WHO, 2022). Despite the potential health hazards associated with bacterial contamination in fresh fruit juices, there is a lack of comprehensive research examining this issue, particularly in the context of locally prepared juices sold by street vendors and small-scale businesses. Therefore, there is a need for experimental studies to assess the extent of bacterial contamination in fresh fruit juices and to evaluate the hygiene practices employed by vendors in Woldia Town.

1.3. Research Questions

1. What are the specific types of pathogenic bacteria found in fresh fruit juices?
2. What is the level of bacterial contamination present in locally prepared fresh fruit juices sold by vendors?
3 What is the status of hygiene practices employed by vendors during the preparation, storage, and sale of fresh fruit juices?

1.4 Objective

1.4.1. General Objective

The general objective of the study was to investigate the extent of bacterial contamination in locally prepared fresh fruit juices and hygiene practices among vendors in woldia Town.

1.4.2. Specific Objectives

The Specific objectives of the study were to:

1. Investigate the types of bacteria present in locally prepared fresh fruit juices sold by vendors.
2. Assess the bacterial load present in locally prepared fresh fruit juices sold by vendors.
3. Evaluate the hygiene practices employed by vendors during the preparation, storage, and sale of fresh fruit juices.

1.5. Significance of the Study

In recent times, there has been a notable increase in the number of juice vendors in Woldia. Undergoing study on the Bacterial Contamination of Locally Prepared Fresh Fruit Juices and

Hygiene Practices is essential for several reasons, including public health concerns, consumer safety, vendor education and training, and public awareness. Understanding the extent of contamination and associated hygiene practices is crucial for preventing outbreaks and protecting consumers from potentially harmful pathogens. Regarding consumer safety, consumers often perceive fresh fruit juices as healthy beverages, but if contaminated, they can become sources of illness. Investigating bacterial contamination and hygiene practices can help consumers make informed choices and reduce their risk of exposure to foodborne pathogens. Moreover, studying bacterial contamination in fresh fruit juices can provide insights into regulatory compliance issues and inform the development of effective regulatory measures to ensure product safety.

Many vendors who prepare and sell fresh fruit juices may lack proper training in food safety and hygiene practices. Conducting research on this topic can identify gaps in knowledge and skills, leading to targeted education and training programs to improve vendor practices and reduce contamination risks. Foodborne illnesses resulting from contaminated fresh fruit juices can lead to economic losses for individuals, healthcare systems, and businesses. Additionally, research findings on bacterial contamination and hygiene practices can raise public awareness about the importance of food safety. . Generally, studying bacterial contamination in locally prepared fresh fruit juices and associated hygiene practices is essential for protection public health, ensuring consumer safety, and promoting effective regulatory measures and vendor practices in the food industry.

1.6. Scope of the Study

The study was focused on the assessment of bacterial contamination of locally prepared fresh fruit juices and associated hygiene practices in woldia town. The study was focused on a specific geographic location (Woldia town) where locally prepared fresh fruit juices are sold by vendors. The study covered fresh fruit juices (avocado, mango and mixed juices) commonly prepared and sold by vendors.

The study also investigated the hygiene practices employed by vendors during the preparation, storage, and sale of fresh fruit juices. This includes assessing practices related to fruit handling, equipment sanitation, water quality, personal hygiene, and overall food safety measures. Demographic characteristics of vendors, such as age, gender, education level, and experience in food handling and preparation was also studied.

1.7. Limitation of the Study

There were different hindrances that faced the researcher, among these, limitations in funding, and available resources constrain the study's scope, sample size, and the laboratory methods used to analyze microbial contamination in fresh fruit juices may have limitations, including detection thresholds, sensitivity, and specificity. These factors could impact the accuracy and reliability of the microbial data.

The study focus on a specific geographic location which also limit the generalizability of the findings to other areas with different environmental conditions, vendor practices, and consumer behaviors.

2. LITERATURE REVIEW

2.1 Fruit Juices

The preparation of juices from fruits and vegetables is ancient as agriculture. In simple words, the juice is the extracted from fruit cell or tissue. The juice may either be consumed immediately or processed and packaged by fruit juice companies to extend the shelf life (Huang and Webber, 2017). Obtained by piking process from plant ripe fruits, preserved by physical means. The addition of acids sugars and sugars can be permitted but must be in the individual interest (Oranusi *et al.*2012). Fruits and vegetables form a useful and complex substance group of foods. The importance substance groups are acids, carbohydrates, minerals, polyphenols (tannins) including vitamins, amino acids, aroma, carotenoids, fibers, and other bioactive substances. During processing, they are essentially transferred into the cleared the juice (Rahate *et al*., 2021).

2.2. General Composition of Fruit Juices

Fruit juices have the essential physical, chemical, organoleptical, and nutritional characteristics of the fruits from which they come. The relevant substance groups are carbohydrates, organic acids, minerals, polyphenols (tannins) including the colorful anthocyanins, water-soluble vitamins, amino acids, aroma compounds, carotenoids, fibers and other bioactive substances (Bento *et al.* 2022). Fruit juices are low in compounds such as sodium and fats which are believed to have negative health impacts when ingested in large amounts. Conversely, juices contain a variety of beneficial micro nutrients, including significant quantities of minerals, like potassium as the major mineral (Rock *et al.* 2020).

The major component is, of course, water derived from the extra and intracellular fluid necessary for metabolic processes and maintenance of cell turgor. Water content can range from 97 percent in some wild berries to 70 percent in over ripe grapes and less than 50 percent in fruits drying naturally on the plant (Williams *et al.*, 2018).

Many trace elements of fruits are also found in the corresponding fruit juices. The B-vitamin folate is present in orange, grapefruit, pineapple juices and some other tropical juices. Vitamin C, although sometimes added to fruit juices, is found in significant amounts in different fruit juice. Examples are orange juice, grapefruit juice, blackcurrant juice, strawberry juice. In recent years, the beneficial phytochemicals present in all the major fruits and fruit juices have been characterized and a database on the polyphenol food content is now available online (Chang *et al.* 2019).

2.3. Health Benefits of Fruit Juices

Eating of fruits and vegetables helps to prevent many worsening diseases such as cancers and cardiovascular problems. Decades of research have found that fruits and vegetables are crucial dietary components that can help to reduce the risk for numerous chronic diseases which, in many cases, have been shown to be initiated by long term tenderness. Fruit juices contain low sodium and high potassium which help in maintaining normal blood pressure and lower content of fat in fruit juices is helpful for the cardiovascular system (Tyagi *et al*., 2015). Many reports have exposed that fruit juices may play an important role in reducing the progress of Alzheimer's disease and development of cancer (Justin *et al.*, 2016).

2.4. Safety and Quality of Fruit Juices

Relatively large number of reports of food-borne illness associated with fruit juices indicates that unpasteurized juices pose a high public health risk. Several authors have reported that pathogens such as *Salmonella, Shigella, E. coli* O157:H7 or *L. monocytogenes* can survive for long periods in refrigerated juices and acidified culture media. The ability of different pathogens to survive in low pH environments has been documented at length (Lim, 2010). Example, *Salmonella* are chemo organotrophic and non-fastidious microorganisms with inherent ability to adapt to wide pH ranges of 4.0 to 9.0 and temperature ranges of 5°C to 47°C, respectively (Moré , 2018).

In a study conducted in Mexico where pH values of the juice ranged from 3.0 to 4.8, with approximately one-third of the samples showing a pH of < 4.0, 13% of the samples were positive for *Salmonella* and *Shigella.* From these reports, it is clear that pathogens can survive at low pH (Hughes and Webber, 2017).

Furthermore, the juices with very low pH have been reported to have significant inhibitory effects against a wide range of bacteria. wd activity of lemon and lime juices against some bacteria including *E. coli*, *Klebsiella pneumonia* and *Shigella flexneri* (Salazar *et al*., 2021). The results indicated that, due to their inherent low pH both juices had antimicrobial effects against bacteria (Hughes and Webber, 2017). The antagonism effects of these two juices were compared with the antagonism effects for some antibiotics (Mantzourani *et al*., 2019).

2.5. Sources of Fruit Juices Contamination

Fruit juices contain water, sugars, organic acids, vitamins, and trace elements thus providing an ideal environment for spoilage by microorganisms. On the other hand, they generally have a lower pH thus the common feature of their potential spoilage agents is that they must be acid-loving microorganisms. The most commonly encountered microbial genera are *Acetobacter, Alicyclobacillus, Bacillus, Clostridium, Gluconobacter, Lactobacillus, Leuconostoc, Saccharobacter, Zymomonas,* and *Zymobacter* (Orqueda *et al* 2021).

However, bacteria, virus and parasites with potential of causing infections in humans have also been reported (Shen *et al*., 2019). Bacteria are the greatest concern in terms of serious illness and high numbers of persons are at risk of infection especially in developing countries. In North America, for example, *E. coli* O157, *Salmonella, Cryptosporidium* and norovirus were the most common pathogens which caused food-borne illnesses and deaths due to consumption of contaminated unpasteurized fruit juice and ciders (Morsy *et al*.2023).A few other outbreaks were due to *V. cholerae, C. botulinum,* yeast and *Hepatitis A* virus. In a study conducted in India reported *E. coli,* followed by *Pseudomonas aeruginosa, Salmonella* spp., *Proteus* spp., *Staphylococcus aureus*, *Klebsiella* spp. and *Enterobacter* spp. as the common bacterial pathogens of unpasteurized fruit juices (Mandal, 2018).

Improper washing and handling of fruits may facilitate the bacteria to contaminate the juice extract. Contamination from raw materials and equipment’s, additional processing conditions, improper handling, prevalence of unhygienic conditions have shown to contribute substantially to the entry of bacterial pathogens in the prepared juices (Adare, 2020). A study found that, contamination of raw fruit juices is mainly due to poor quality of water used for dilution as well as unhygienic conditions related to washing of utensils, contaminated water and ice, poor personal and processing hygiene (Eshetu,2023). Shops located alongside roads with heavy vehicle traffic or waste disposal systems, and overcrowding further increase the degree of contamination.

2.6. Factors Affecting Shelf Life of Juices

The shelf life of a food is the time period within which the food is safe to consume and/or has an acceptable quality to consumers or shelf life is also defined as the time to reach a microbial population of 6 log cfu/ml which determined experimentally. The shelf life of juices is affected by both intrinsic and extrinsic factors. Intrinsic factors include pH, oxidation-reduction potential, water activity, availability of nutrients, the presence of antimicrobial compounds, and competing micro flora. Extrinsic factors encompass storage temperatures and times, relative humidity conditions during storage, and packaging material characteristics (Kumari *et al.*, 2022). Among intrinsic factors, pH and water activity are the most influential factors affecting spoilage rates.

Bacteria prefer to grow at pH 6.5-7.5 but tolerate a pH range of 4 to 9. The water activity of juices is associated with Brix. In juices, Brix is used to indicating the percentage of soluble solids and is one of the most important factors for grading the quality of a juice. Microorganisms cause fruit juice spoilage by fermentation of sugars, and can therefore increase the Brix value owing to the conversion of complex sugars into mono saccharides (Goldfein & Slavin 2015). Extrinsic factors such as temperature also influence the shelf life of juices. The shelf life of freshly squeezed, orange juice is less than 20 days at 1^{o}C. Low temperature is necessary during the manufacturing and storage of juice. The primary purpose of low-temperature storage is to increase the shelf life by slowing down degradative reactions and limiting microbial growth (Dirapan *et al.* 2021). Therefore, the combination of reduction in chemical, biochemical, and microbial kinetics, can increase the shelf life of fresh and processed foods (Khubber *et al*., 2020).

2.7. Major Bacterial Contaminants of Fresh Juice

Bacteria are usually present in low numbers on fresh fruits and vegetables. Some bacteria such as hetero fermentative lactic acid bacteria (LAB), acetic acid bacteria, and the most common pathogenic bacterial species that contribute to food borne outbreak in un pasteurized juice include *Escherichia coli O157:H7, Salmonella species,* and *Cryptosporidium* (Mengistu, 2021). Some strains of *E. coli*, *Shigella*, and *Salmonella* may survive for several days and even weeks in an acidic environment by regulating their internal pH that maintained at neutral pH by the combination of passive and active mechanisms

2.7.1 Escherichia coli O157:H7

Leafy vegetables are most commonly linked to *E. coli* infection, but apple juice (cider in the USA) is an interesting vehicle, as the acidity of the product is considered inhibitory to bacterial proliferation. *Escherichia coli* O157:H7 is commonly recovered from the feces of ruminants; therefore, livestock grazing in orchards may contaminate fallen apples with feces and, as *E. coli* O157:H7 can proliferate in damaged apple tissue (Bintsis, 2018), this can result in the contamination of unpasteurized fruit juices/ciders, demonstrated that fruit flies were an important vector in the contamination of apples with *E. coli* O157:H7, both pre-harvest and in packing houses.

2.7.2 Salmonella

Salmonella spp. is infectious bacteria associated with food-borne and gastrointestinal illnesses. *Salmonella* bacteria can be found in food products such as raw poultry, eggs, beef, and sometimes on unwashed fruit. There are two main diseases caused by *Salmonella spp*. and they are *Salmonellosis* andtyphoidfever. *Salmonella enteritidis* or *Salmonella typhimurium* causes *Salmo nellosis* and Typhoid fever are caused by *Salmonella typhi*. People who eat food contaminated by *Salmonella* can become ill with *salmonellosis* (Wibisono *et al*., 2020).

2.7.3. *Shigella*

Most shigellosis cases are the result of the bacterium passing from stools or soiled fingers (inadequately washed hands) of an infected person to the mouth of a susceptible person and may also be acquired from eating contaminated food (Hass *et al*., 2014). Disease occurs when virulent *Shigella* organisms are consumed and invade the intestinal mucosa, resulting in tissue destruction. Some *Shigella* strains produce enterotoxin and Shiga-toxin. Humans and higher primates are the only known hosts of *Shigella*. There are four species (*Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei.* Any of the four *Shigella* species can cause bacillary dysentery.

Depending on age and condition of the host, less than 200 cells can be enough to cause disease (Binet& Lampel, 2012). Shigellosis is characterized in humans by sudden and severe diarrhea (gastroenteritis or bacillary dysentery) which is often bloody. Most infected people also develop fever and stomach cramps within1-2 days after exposure to the bacteria (Fang, 2012).

A symptom typically lasts from five to seven days and most shigellosis cases resolve without need for hospitalization. *Shigella* thrives in the human intestine and is commonly spread through both food and person -to- person contact. Some persons who are infected may show no symptoms at all, but may still pass the *Shigella* organism to others (Schneider *et al*., 2012).

Shigellosis is usually a self-limiting diarrheal illness, if left untreated, generally lasts one day to one month with an average of 7 days. Persons with mild infections usually recover without antibiotic treatment. However, appropriate antibiotic treatment is effective in shortening the duration of diarrhea and eradicating organisms from feces. Antibiotic treatment is recommended for patients with severe disease, bloody diarrhea, dysentery or underlying immuno suppressive conditions in these patients (Siciliano, 2020).

2.7.4. Staphylococcus aureus

The occurrence of *Salmonella spp* and *Staphylococcus aureus* in orange juice extracted by squeezing machine used in restaurants. Because of the presence of pathogens in fruit juices, the food-borne outbreaks associated with the consumption of fruit juices have increased (Mengistu, 2021). *Staphylococcus aureus* strains are gram-positive and non-moving small round-shaped or non-motile *cocci*. *Staphylococcus spp*.is found in grape-like (S*taphylo*-) bunches. *Staphylococcus* is one of the most five common causes of infections after damage or operation. *S. aureus* may occur usually in the environment which communicates food by air droplets by direct contact with objects that are contaminated by the bacteria or by bites from infected persons or animals (Bhat, 2020).

2.8. Health Impact of Microbiological Contamination of Food

Food contaminated by disease casing microbes or chemical substances can lead to a wide range of health problems (Rather, 2017). The most common and evident effect of food contamination is on public health. Once customers of a food establishment encounter contaminated food, they can acquire food borne illnesses. This event can put their lives in danger and may require customers to seek healthcare and expert treatment services. As some food borne illnesses from food contamination can be life-threatening, customers are suggested to rest and delay heavy work. In addition, customers who are affected by any food borne disease have weakened immune systems and are more vulnerable to other diseases (Abebe, 2020). Food-containing harmful microorganisms are responsible for more than 200 diseases ranging from diarrhea to cancers. Other diseases caused by bacterial contaminant are cholera, *campylobacteriosis*, gastroenteritis, salmonellosis, cshigellosis, typhoid, and paratyphoid fever, amoebiasis, and poliomyelitis (Nowshad *et al.*, 2021). These diseases have some common and unique symptoms like fever, vomiting, weakness, chills, aches, headaches, abdominal pain, constipation, sore mouth, blurred vision, and muscle paralysis).

An estimated 2.3% of all acute food borne illnesses develop secondary long-term illnesses and complications called chronic sequellae which can occur in any part of the body including the joints, nervous system, kidneys, or heart that may become chronic health problems, like reactive arthritis, hemolytic uremic syndrome (characterized by kidney failure) and Guillain-Barre syndrome (characterized by neuro muscular paralysis). Though younger individuals usually face far higher rates of infection from foodborne pathogens, very young and older adults are more likely to have severe complications from infection (Schneider *et al.*, 2021).

People really die from foodborne diseases resulting from microbial contamination of food. Contaminated foods are responsible for many more accidental mortalities than some products commonly perceived as dangerous including firearms, industrial machinery, and explosives (Nowshad, 2021). Unsafe food is linked to the death of an estimated 2 million people annually including children (Fung, 2018). Worldwide, diarrheal diseases are second only to respiratory diseases as a cause of adult death and are the leading cause of childhood death. In some parts of the world, they are responsible for more years of potential life lost than all other causes combined. Each year, around 5 million children (more than 13, 600 a day) die from diarrheal diseases in Asia, Africa, and South America (Fitzmaurice *et al*., 2017).

2.9 Economic Impact of Food Microbiological Contamination

A single event of foodborne disease or outbreak resulting from the consumption of microbiologically-contaminated food can bring unimaginable economic losses (Kyayesimira *et al*., 2019), in various forms including unexpected expenditures on hospitalization bills, treatment costs, expenses on recollections, discarding, and consequences, legal costs due to foodborne outbreaks, and financial loss due to loss of business. After notification of a foodborne disease outbreak, to protect the consumer and general public health, government agencies use recall events to remove potentially harmful products from the market rapidly and efficiently (Jung *et al*., 2021).

Food-borne illness imposes substantial economic costs on society and other related impacts are the supply of unsafe food products, decrease in sales and exports, breach of government regulation and standards, loss of consumers' confidence and loyalty, loss of food company's reputation, absenteeism from school, business, or work due to ill health, discontinuation of food business (closing of business), and loss of workforce to death (Schieber, 2013). The effects of food contamination can lead to a loss of productivity, which can affect the output of other industries. In terms of the effects of food contamination on food businesses, their detection can lead to significant problems (Galvez *et al*., 2018).

2.10. Handling and Preparation of Foods

The place of preparation is not always clean, well-lit and not far from source of contamination. Preparation surfaces used by some vendors have remains of foods prepared earlier that can promote cross contamination. Preparation surfaces used by some vendors have remains of foods prepared earlier that can promote cross contamination. Food safety is a major concern with street foods, and studies are carried out all over the world as a way of improving food safety to all food handlers (Alimi, 2016).

The two major sources from where the contaminants can enter the preparation area are improper food handling and waste disposal. The vendors themselves can be carriers of pathogens like *E. coli*, *Salmonella*, *Shigella*, *Campylobacter* and *S. aureus* who eventually transfer the pathogens to consumers through food. The hands of the food handlers are the most important vehicle for the transfer of organisms from environment (food surfaces), nose and skin to the food (Bencardino, 2021). Practices towards food handling are poor and basic food safety hygiene is not adhered to hence, put the food at high risks of contamination. They are also unaware of food regulations as well as lacking supportive services such as water supply of good and adequate quality, waste disposal systems which enhance their ability to provide safe food.

Data shows that people who work in the industries and other institution are more likely to get food safety training as compared to street food vendors and are also more likely to be well educated (Monica, 2011). The main aim of the training is to minimize food poisoning and improve the food safety among all food handlers, although studies have shown that improper food handling has in the past increased with increase in the number of fruit vendors trained, suggesting that such training made little to no difference in proper handling of fruits and food, therefore there was no reason of introducing the training (Kassa et al., 2010). Food safety courses and training can be used to curb the foodborne diseases among food and fruit vendors by learning various sustainable and proper methods they can employ in order to maintain a hygienic environment (Walker et al., 2003; Gitahi, 2012).

2.10.1 Juice Process and Environmental Hygiene

Fruit juice production enterprises should have health measures to prevent pollution, formulate various health management systems, and have a special person in charge (Ashurst, 2016). Fruit juice production must have a clean production environment from harvesting, transportation, handling etc. The overall layout of production, administration, living, and auxiliary areas should be reasonable and not interfere with each other.

2.10.2 Fruit storage practices

Ideally, fruit should be pressed as soon as possible after picking to avoid increases of pH that would favor growth of pathogens during storage. The lower the pH, the worse the conditions will be for the growth and survival of pathogens. However, if fruit needs to be stored, rapid cooling to as close to 0°C as possible (0 to 4°C) and achievement of adequate storage conditions will maintain fruit condition (Ahmad, 2015). Storage facilities must be clean, secure from rodents and insects and suitable for storing food.

2.9.3 Waste disposal

The lack of facilities for liquid drainage and wastewater and garbage disposal encourages wastes to be thrown into nearby streets and gutters. Such areas act as habitats for rodents, breeding points for flies and media for growth of microorganisms (Ozoh, 2021). Adequate drainage and waste disposal systems and facilities should be provided. These systems should be designed and constructed so as to avoid the potential for contamination of fresh fruits and vegetables, agricultural inputs or water supplies. The following should be considered: Good drainage should be maintained around the structure to eliminate standing water. All waste should be removed and stored away from the facility to prevent harbourage of pests. Plant debris and cull piles should be removed promptly from inside the structure. There should be no permanent plant refuse around the outside of the structure or nearby to attract or harbour pests (Mathews *et al*., 2014).

2.10.4 Water supply

Most street food vendors do not have adequate and potable water for use in food activities resulting in using little water for their activities of food preparation and handling hygiene is compromised. Without enough potable water, food safety and hygiene cannot be met. Contamination of fruit juice is mainly due to poor quality of water used for dilution as well as prevailing unhygienic conditions related to washing of utensils and maintenance of the premises.

If the water source is found to have unacceptable levels of indicator organisms or is contaminated with foodborne pathogens, corrective actions should be taken to ensure that the water is suitable for its intended use (Uyttendaele et al., 2015). Possible corrective actions to prevent or minimize contamination of water for primary production may include the installation of fencing to prevent large animal contact, the proper maintenance of wells, water filtering, chemical water treatment, the prevention of the stirring of the sediment when drawing water, the construction of settling or holding ponds or water treatment facilities. The effectiveness of corrective actions should be verified by regular testing. Where possible, growers should have a contingency plan in place that identifies an alternative source of water.

2.10.5 Personnel

All workers must be free from communicable diseases. They should be trained not only for their task, but also to keep the venders clean and to practice personal hygiene. Written requirements for personal hygiene should be available. Workers must have ready access to clean washrooms and proper hand washing (hot water and soap) facilities with disposable towels and closed trash containers. All persons must wash their hands upon entering food handling areas, before starting work, after handling contaminated materials, after breaks, and after using toilet facilities. Where necessary to minimize microbiological contamination, employees should use disinfectant hand dips. Wash rooms must be segregated from production and storage areas. Employees having open cuts or wounds must not handle food or food contact surfaces unless the injury is completely protected by a secure waterproof covering (eg. rubber gloves). All persons entering food handling areas should remove jewelry and other objects which may fall into or otherwise contaminate food (Todd et al., 2010). Protective clothing, hair covering, footwear and/or gloves, appropriate to the operation in which the employee is engaged should be worn and maintained in a sanitary manner. Without personal hygiene of food handlers, safe processing of fruit juices alone has no value to improve the community health. Therefore, all rounded safety precautions should be applied by food handlers as well as during processing

2.11. Antibiotic Resistance

The antimicrobial resistance of bacteria isolated from food and other sources has increased from time to time (Vicas and Singh, 2010). Although, it is difficult to prove a direct role of drug resistance in bacteria contaminating food items with increased clinical cases of resistant infections, the presence of such bacteria in food items could play a role in the spread of antimicrobial resistance amongst food-borne pathogens. The incidence of resistant bacteria in food stuff is a worldwide phenomenon.The study which was conducted in different areas around Dhaka city (Rashed *et al.*, 2012). To resolve the microbiological attributes of the fruit juices, drug resistance among the isolates was found against ampicillin, ciprofloxacin, amoxicillin, erythromycin, chloramphenicol, ceftriaxone, piperaciline, trimethoprime-sulfomethoxazole, nalidixic acid and vancomycin. The antibiotic susceptibility of isolates was examined by disc diffusion assay on Muller-Hinton agar against common antibiotics following the standard protocol (Sharma, 2008) Mueller Hinton Agar contains beef extract, acid casein hydrolysate, starch and agar. Beef extract and casein acid hydrolysate provide nitrogen, vitamins, carbon, amino acids, sulphur and other essential nutrients. Starch acts as a colloid and is added to absorb any toxic metabolites produced (Hinton Jr 2016).

3. MATERIALS AND METHODS

3.1 Description of the Study Area

Woldia Town, situated in the North Wollo Administrative Zone within the Amhara National Regional State of Northeast Ethiopia, serves as the administrative capital of the region. Positioned North of Dessie and Southeast of Lalibela, approximately 521 kilometres away from Ethiopia's capital city, Addis Ababa. Its geographical coordinates are 11°50′N latitude and 39°36′E longitude, with an average elevation of 2112 meters above sea level (Teshome, 2022). The North Wollo Administrative Zone, encompassing Woldia Town, boasts a total population of 1,500,303 inhabitants and covers a land area of 12,172.5 square kilometres. Within this zone, Woldia Town itself is home to a population of 98911,CSA (2021) residents. The town experiences an average annual rainfall ranging from 770 mm to 1250 mm and an average daily temperature between 12°C to 23°C, characterized by its location as a hillside market town. Woldia Town is a bustling urban center with a diverse population representing various ethnicities and cultural backgrounds (Belay, 2024). The town serves as a center for commerce, trade, and transportation in the region, with bustling markets, shops, and businesses lining its streets.

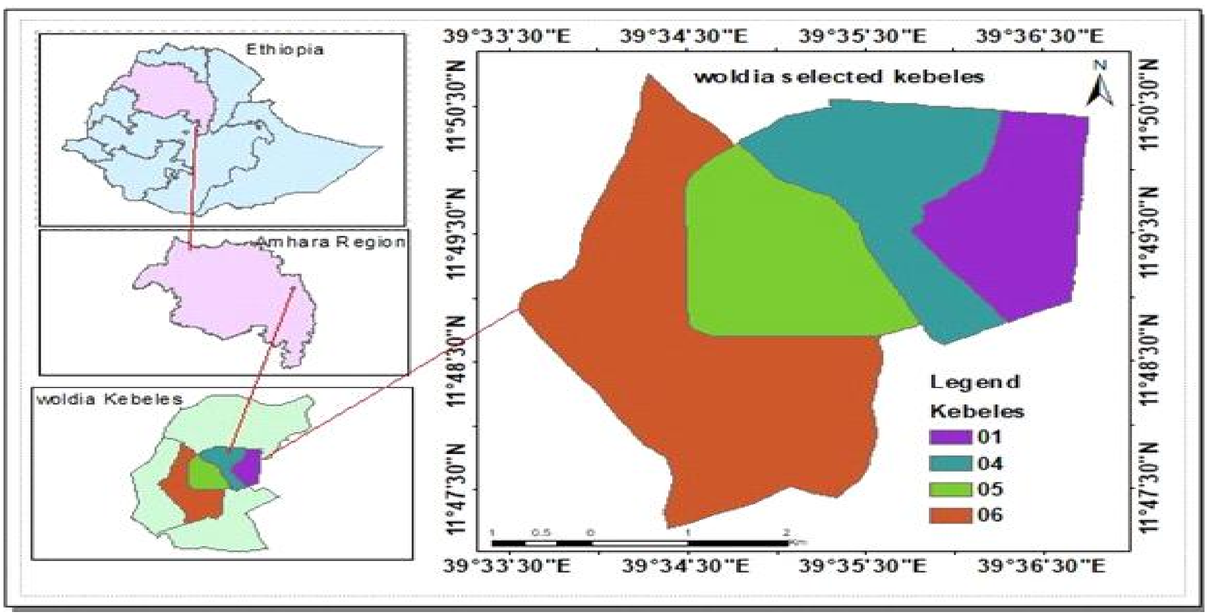

Figure 1: Locally prepared juice sampling sites in Woldia town.

(Source: - Woldia town GIS lab2022)

3.2. Study Design and Period

A cross-sectional study design was conducted from February 2023 to August 2024 to assess bacterial contamination and hygiene practice of juice vendors. Locally prepared fresh fruit juice samples prepared from Mango, Avocado and mixed juice were collected from vendors at various locations (Gonderber, Adago and Piassa at Woldia Town. Samples including (Mango, Avocado and mixed juice) were collected randomly in aseptic technique from different locations (Gonderber, Adago and Piassa). Moreover, bacterial analysis of juice samples was carried out in a laboratory setting using standard microbiological methods to quantify bacterial contamination and identify specific bacteria.

3.3. Study Variables

Dependent and independent variables of the study

Bacteriological contamination of locally prepared fruit juices from juice houses is the major dependent variable, while, Socio-demographic characteristics of the food handlers (age, gender, year of service, educational status, training in food hygiene and safety); Juice house hygiene conditions (latrine facilities, waste disposal receptacles, dish washing facilities, and hand washed facilities); Personal hygiene and safety practices (hand washing habits, clothing/gown/head cover, washing utensils and storage condition of juice) and awareness and knowledge of juice makers are the independent variable of the study.

3.4. Sources of Sample

The major sources of data for this study were local juice vendors in Woldia town. Fruit juices were directly collected from local juice vendors who sell fresh fruit juices in the town. Vendors from different locations within the study area were considered to ensure representation of different hygiene practices and bacterial contamination levels.

3.5 Data Collection Methods

The study employed various data collection methods to gather relevant information. Structured questionnaires translated to Amharic were administered to 20 juice makers, and vendors to gather socio-demographic information, hygiene practices, awareness, and knowledge regarding juice safety practices. All the personnel involved in the processing and/or serving of the fruit juices in the selected juice house were included and the type of vendor was determined. Laboratory experimentation was another resource data: Analysis of collected juice samples in a laboratory setting was carried out to quantify bacterial contamination levels using appropriate microbiological techniques, such as total plate count, enumeration of specific pathogens (Sohel *et al.* 2022).

3.6 Sample size, Sample Collection, and Processing

Samples were obtained from various locations within the container to ensure representativeness. A total of 30 samples were collected from 10 representative juice houses, with duplicate samples obtained for each type of fruit juice. As outlined by Diociaiuti (2017), the liquid sample was initially agitated to achieve a homogeneous mixture before collection. To ensure randomness and avoid bias, 250 milliliters of the representative juice sample was collected on a voluntary basis from each of the 10 juice houses in Woldia town. Samples were kept in ice box of 0-5°C, and transported in insulated containers to the laboratory for subsequent analysis.

Ten milliliters of the sample were transferred to 90 milliliters of peptone water, and then homogenized using a Vortex machine (Biocote) within an aseptic environment. Subsequently, a series of dilutions ranging from 10^{-1} to 10^{-7} were prepared by transferring 1 milliliter from the homogenized sample to sterile test tubes containing 9 milliliters of sterile alkaline peptone water, followed thorough mixing using a Vortex machine (Eshetu, 2023). After the dilutions were properly prepared, samples from dilutions 10^{-3} and 10^{-4} were plated in triplicate onto selective media and then incubated at 37°C for 48 hours to enumerate the bacterial loads of the juice in colony-forming units per milliliter (CFU/ml) and to identify pathogenic bacterial groups using standard procedures. Additionally, the isolated bacteria were characterized based on their colony color, shape, and size (diameter) using the spread plate technique and biochemical test was performed (Alves, 2022).

3.7. Bacteriological Analysis

Two levels of laboratory analyses were conducted on the samples. Initially, the physicochemical parameters of the juice, such as pH and salt tolerance were assessed. Subsequently, the second involved determining bacterial contamination. This encompassed analysing for *Escherichia coli, Salmonella, Staphylococcus aureus,* and *Shigella.*

3.7.1. Determination of physicochemical parameters

At the microbiology laboratory in Woldia University, the juice samples were promptly removed from the ice box and kept in the biosafety cabinet. Subsequently, three aliquots of the juice sample, each measuring 30 milliliters, each were extracted using a sterile pipette and dispensed into separate beakers for the determination of pH.

3.7.1.1. Determination of PH

The pH of all undiluted samples was promptly measured using a pH meter immediately after collection. It is crucial to assess the pH of the juice sample prior to conducting microbiological analysis, as it can significantly impact colony count and the types of organisms targeted. For each sample, the pH was determined by immersing two probes of the meter, one for pH and the other for temperature, into the sample. Upon stabilization of the reading on the meter screen for a few seconds, the pH value was recorded. Following the pH measurement of each sample, the probes were meticulously cleaned using distilled water and dried with a soft tissue before being utilized for measuring the next sample. This procedure was consistently repeated for all samples.

3.7.1.2. Salt tolerance test for isolates

For testing salt tolerance, a modified salt broth containing 6.5% NaCl is commonly used. Additionally, Brain Heart Infusion (BHI) Broth, supplemented with 6.5% NaCl and 0.1% bromo cresol purple indicator (referred to as modified BHI Broth), is widely utilized for this purpose (Bartha, 2022). The procedure for the salt tolerance test involves several steps. First, using a sterile inoculating loop, pick well-isolated colonies of the sample bacteria from a fresh culture (18 to 24 hours old) and inoculate them into the broth, transferring 1 to 2 loops of the bacterial suspension or fresh broth culture. Next, incubate the tubes aerobically for 24 hours at 35±2°C and observe for visible growth, indicated by turbidity or a color change in the medium.

If no growth is observed after the initial incubation, the tubes should be re-incubated and checked for growth up to 72 hours, with observations for turbidity or color change at the 48 and 72-hour marks. A positive test is indicated by bacterial growth, evidenced by an increase in turbidity and/or a change in the broth color from purple to yellow. Conversely, a negative test is indicated by the absence of bacterial growth and no change in the color of the medium.

3.7.2. Detection of bacterial contamination

3.7.2.1. Detection of Salmonella and Shigella

To detect *Salmonella* and *Shigella* Sterilized Buffered peptone water (BPW) was used for as Pre-enrichment media. Representative and homogenate sample of 9 ml was weighted in sterile Erlenmeyer flask (90 ml volume) then buffered peptone water was added to it. The test sample was 0 with previously sterilized buffered peptone water for 24 hr ± 2 hrs at 37^{0}C.Then Salmonella-Shigella agar was prepared based on manufacturer's instruction and a one loopful from the overnight incubated sample with normal saline solution was spread plating technique on the prepared SS agar and incubated for 24 hours at 37^{0}C. Colony with black center or pale or transparent colorless was considered as *Salmonella* and *Shigella* respectively(McEgan 2013).

3.7.2.2. Detection of Staphylococcus aureus

Sterilized Tryptic Soy Broth (TSB) was used for as pre-enrichment media. Representative and homogenate sample of 9 ml was weighted in sterile Erlenmeyer flask (90 ml volume) was added to it. The test sample was inoculated with previously sterilized buffered peptone water for 18- 24 hr at 37 ^{0}C After pre-enrichment, transfer a portion of the incubated TSB broth into a selective medium specifically Mannitol Salt Agar (MSA) is a common selective medium for Staphylococci and incubate the plates at 37°C for 24-48 hours. After incubation, the MSA plates were examine for characteristic colonial morphology of Staphylococcus aureus. *Staphylococcus aureus* colonies were typically appear as yellow colonies surrounded by a yellow zone indicating mannitol fermentation.

3.7.2.3. Detection of E. coli

For detection of *E. coli*, 9 ml of fruit juice sample was transferred in to 90 ml Pre-enrichment Tryptic Soy Broth (TSB) and incubated the broth at 37°C for 18-24 hours to allow for bacterial growth. After pre-enrichment, transferred a portion of the incubated broth into a selective medium like MacConkey agar and incubated the plates at 37°C for 24 hours. *E. coli* colonies was typically appear as pink to red colonies on MacConkey agar and incubated the plates at 37°C for 24 hours” (Li, 2004).

3.7.3. Biochemical tests

3.7.3.1 Triple sugar fermentation test

This test used for identify bacteria based on their ability to ferment carbohydrates. The was as follows. First, an isolated colony from the respective agar plate was picked with a cool, sterile needle and then stabbed into the Triple Sugar Iron (TSI) agar (Himedia, India), which contained dextrose, lactose, and sucrose. The inoculated tubes were incubated overnight at 37°C with their caps loosened to allow for gas exchange. After 24 hours, the tubes were examined for signs of carbohydrate fermentation, as well as for the production of carbon dioxide (CO_2) and hydrogen sulfide (H_2S). A yellow coloration in the butt of the tube indicated that the tested organism was capable of fermenting all three sugars, whereas a red (alkaline) color in both the slant and butt suggested that the organism did not ferment carbohydrates. The production of H_2S was identified by the presence of black precipitation in the butt of the tube. Additionally, the production of CO_2 gas was indicated by the splitting and cracking of the medium, which signaled active fermentation.

3.7.3.2 Catalase Test

The procedure for this test was performed as follows. First, a drop of the catalase reagent (hydrogen peroxide) was placed on a glass slide. A small amount of bacterial colony was then transferred from the nutrient agar plate to the hydrogen peroxide drop using a sterile loop and mixed thoroughly. A positive result was characterized by a rapid evolution of oxygen within 5 to 10 seconds, evidenced by a bubbling reaction. Conversely, a negative result was marked by the absence of bubbles.

3.7.3.3 Coagulase Test

To perform the test, dense suspensions of *Staphylococci* from culture were made on two ends of a clean glass slide. One end was labeled as “test” and the other as “control.” The control suspension served to rule out false positives due to auto agglutination. The test suspension was treated with a drop of citrate plasma and mixed well. Agglutination or clumping of cocci within 5-10 seconds was taken as a positive result. It was also noted that some strains of *S. aureus* might not produce bound coagulase, and such strains were to be identified by the tube coagulase test (Alex, 2016).

3.7.3.4 Citrate test

A loop full of bacterial colonies was picked from the respective agar plate using a straight wire and inoculated into the slope of Simmon's citrate agar (Oxoid Ltd, England). The inoculated agar was then incubated overnight at 37°C. If the organism had the ability to utilize citrate, the medium changed color from green to Prussian blue. In contrast, a citrate-negative culture showed no growth, and the medium remained green. Positive bacteria were identified as possibly belonging to *Salmonella spp.* or *Shigella spp.*

3.7.3.5 Indole test

First, the peptone water tubes were inoculated with a loop full of bacterial culture using a sterile needle. An uninoculated tube was kept as a control. Both tubes were then incubated at 37°C for 24 to 48 hours. After the proper incubation period, 1 milliliter of Kovac's reagent was added to both tubes, including the control. The tubes were shaken gently and allowed to sit for 10 to 15 minutes. The development of a cherry red color in the top layer of the tube indicated a positive test, while the absence of red coloration indicated an indole-negative result. Bacteria such as E. coli were noted for the development of cherry-red color in the top layer of the tube, confirming a positive test (Dimri *et al*., 2020).

3.7.4. Gram staining

To perform a Gram staining a series of sample stains were spread on glass microscope slides and forms a smear. The Gram staining process involved four basic steps, including: First the primary stain crystal violet was applied, to the smear. Following this, a mordant, Gram's iodine, was added to enhance the staining process. The slides were then subjected to decolorization using ethanol, which was applied rapidly. Finally, counterstaining was performed with safranin, which provided additional contrast to the sample (Singh, 2022). During this staining procedure, bacteria that were gram-positive typically appeared purple to blue due to the retention of the crystal violet stain, while bacteria that were gram-negative appeared pink to red, as they did not retain the crystal violet and instead took up the safranin counterstain. This differential staining technique effectively distinguished between the two major classes of bacteria based on their cell wall composition.

3.8. Antibiotic Susceptibility Testing

From twenty bacterial isolates, a total of fifteen isolates were selected for in vitro testing to confirm susceptibility to chosen antimicrobial agents and to detect resistance in the isolated human pathogen using a disc diffusion method on Mueller-Hinton Agar. Eight common antibiotics were used for detection: gentamicin (10 µg), erythromycin (15 µg), tetracycline (30 µg), streptomycin (10 µg), and kanamycin (30 µg). However, the isolates were found to be resistant to cloxacillin (30 µg), cephalothin (30 µg), and amoxicillin (10 µg). The susceptibility test was performed by adjusting the turbidity of the bacterial culture inoculum and sterile liquid glucose to 0.5 McFarland standards, which was assumed to be approximately equivalent to 1-2 x 10^8 CFU/ml on the surface of the Mueller-Hinton agar plate. This investigation was carried out using the following procedure:

First, five colonies of each isolate were picked and introduced into 5 ml of nutrient broth. The inoculated broth was then incubated for 24 hours, serving as the source of standard inoculum. A sterile cotton swab was dipped into the suspension and spread evenly over the entire surface of the Mueller-Hinton Agar. Antibiotic discs were placed onto the surface of the inoculated plates, which were incubated at 37°C for 16 to 18 hours.

After incubation, the zone of inhibition of growth around each disk was measured in millimeters, and the diameters of the zones were interpreted according to established standards as susceptible, intermediate, or resistant (CLSI, 2017). If the clear zone diameter was larger than the resistant diameter scale but smaller than the susceptible diameter, it was classified as intermediate, indicating that the specific bacteria were neither resistant nor susceptible to the specific antibiotic. Conversely, if no clear zones or very small clear zones were observed around the antibiotics, they were interpreted as resistant. If clear zones were larger than the intermediate range, they were interpreted as susceptible to the antibiotics. Following incubation, the diameters of the zones of inhibition were measured and categorized as susceptible, intermediate, or resistant.

3.9. Data Analysis

The data were categorized, summarized, and analyzed using tables and percentages. All data, including the associations between the prevalence of food-borne pathogens and the practices of fruit juice vendors regarding food safety, were analyzed with SPSS computer software version 20.0. ANOVA was employed to compare means, and a significance level of 0.05 ($p < 0.05$) was considered statistically significant. This analysis allowed for the evaluation of differences among groups and provided insights into the relationships between vendor practices and the prevalence of pathogens.

3.10. Ethical Consideration

The proposed work was ethically cleared by the Department's Research and Ethical Committee of the university. Permission letters were obtained from the biology department and the relevant authorities of Woldia town. Informed voluntary written and signed consent was obtained from each vendor owner and participant after the purpose and importance of the study were explained prior to the commencement of interviews and sampling. Participation in the study was based on voluntary consent. All information collected from the participants and the laboratory results obtained from the juice sample analysis were kept confidential under the custody of the researcher.

Quality Control

Data obtained from the laboratory and participants (juice makers/handlers) were checked to ensure that all information was covered. In the bacteriological analysis, a control was used for each bacterial test to determine the quality of the analysis. All equipment used was standard and calibrated, and the expiry dates of reagents, chemicals, and media were checked. To minimize errors, consistency in the procedure was maintained throughout the study. The samples were taken to the laboratory and analyzed as soon as possible. Equipment was sterilized or disinfected and stored in refrigerators to prevent contamination. To avoid cross-contamination during analysis, 99% alcohol and a flame were used. Standard procedures were followed for bacteriological analysis, and an autoclave was used at 121°C for 15 minutes to sterilize media and some equipment (Mahfuza *et al*., 2016).

4. RESULTS and DISCUSSION

4.1. Results

4.1.1. Socio demographic Characteristics of Respondents

The socio-demographic characteristics of the respondents were analyzed based on their frequency as indicated in Table 1. In terms of gender, it was noted that the majority of respondents (16(80%)) were female, while only 4 (20.0%) of the respondents were male. This gender distribution was consistent with previous research that indicated a greater participation of women in small-scale food enterprises, particularly in informal markets, where cultural norms and economic opportunities have encouraged female entrepreneurship (Carranza *et al*., 2018).

Regarding educational status, the majority of respondents had completed secondary education, with 14 participants (70.0%) in this group. A smaller portion, 6(30%), had only completed elementary education, while no individuals were reported as illiterate or having achieved education beyond secondary. These findings aligned with studies suggesting that educational attainment plays a crucial role in business operations. Prior research indicated that individuals with at least secondary education tend to have better access to information and resources, which can positively influence their business success (Kirkpatrick & Caniels, 2011).

The age distribution presented an interesting dynamic, as 15(75%) of respondents were between the ages of 18 and 30, and 5(25%) were above 30 years old. No participants were below the age of 18. This age distribution aligns with trends observed in other studies that highlighted the youthfulness of entrepreneurs in the food and beverage sector, where younger individuals are often more willing to engage in entrepreneurship due to increased exposure to innovative ideas and technology (Krešic & Husanovic, 2020). The predominance of younger respondents may suggest a shift in the juice vending industry towards a more dynamic and adaptable workforce, reminiscent of patterns noted in similar cultural contexts. In terms of experience, the data indicated that 10(50%) of juice vendors had 1 to 5 years of service experience, while 8(40%) had 6 to 10 years, and only 2(10%) had more than 10 years of experience. This distribution of experience mirrors findings from related studies that examined the relationship between vendor experience and operational capabilities. Research has shown that newer vendors often face challenges in adhering to best practices in food safety and hygiene, highlighting the importance of experience as a critical factor for improving quality and service standards (Böhme et al., 2019).

The relatively lower percentage of individuals with extensive experience may point to an ongoing need for training programs aimed at enhancing operational knowledge among newer vendors in the juice industry.

Table1. Socio demographic Characteristics of Respondents

Characteristics	Category	Frequency
Gender of respondents	Male	4 (20.0)
	Female	16 (80.0)
Educational status	Illiterate	-
	Elementary	6 (30.0)
	Secondary	14(70.0)
	Above	-
Age of respondents	Below 18	
	18-30	15 (75.0)
	Above 30	5 (25.0)
Service year or experience of juice vendors	1-5	10 (50.0)
	6-10	8 (40.0)
	above10 year	2 (10.0)

4.1.2. Hygiene Practice during Juice Preparation

The hygiene practices among the respondents were analyzed based on the recorded frequencies and percentages as shown in table 2. It was noted that all participants (100.0%) were found to have sourced their fruit from retailers, while no instances of purchasing from open markets were reported. This preference for retailers was attributed to the perceived reliability and quality assurance that was associated with established vendors.

This observation was supported by findings from previous studies, which indicated that vendors often prioritized suppliers who could guarantee product quality (Emana *et al*., 2018). When the methods of carrying fruit were examined, it was observed that baskets were used by 15 respondents (75.0%), while 5 respondents (25.0%) opted for polyethylene bags.

It was suggested that baskets were favored for their sturdiness and ventilation, attributes that helped preserve fruit quality during transport. Similar preferences for traditional carrying methods were documented in a study by Böhme *et a*l. (2020), where a focus on maintaining product integrity was highlighted among food vendors in the region. Temporary fruit storage practices were also analyzed, revealing that 70.0% of respondents utilized baskets for storage, while 30.0% reported using shelves. The absence of refrigeration facilities was noted and was possibly linked to limited access to such resources or a lack of awareness regarding the benefits of cold storage in prolonging fruit freshness. This aligns with the findings of Omiti *et al*. (2020), which discussed the significant barriers posed by the lack of refrigeration in maintaining food quality for vendors in Ethiopia.

In terms of fruit washing before juice production, it was confirmed by all respondents (100.0%) that their fruits were washed, demonstrating a strong adherence to this crucial hygiene practice. However, when inquiries were made regarding the detergent used, it was reported that all participants washed their fruits with water only, with none indicating the use of soap. This choice was interpreted as reflecting a lack of knowledge about the effectiveness of detergents in removing pesticides and contaminants, a concern emphasized in previous literature (Tefera & Bayeh, 2019). Finally, cleaning and sanitizing practices for juice machines were reviewed, showing that a frequency of once per day was maintained by all respondents (100%). This consistent effort indicated a commitment to equipment hygiene. However, the lack of variation in cleaning frequency based on different juice types was noted and suggested that a one-size-fits-all approach was being utilized, potentially overlooking specific cleanliness needs for various juice products. This finding was echoed in research conducted by Kassa and Belete (2021), which underscored the necessity of tailored cleaning protocols for different food items in order to enhance overall food safety.

Table 2. Hygiene Practice during Juice Preparation

Hygiene Practices	Category	Frequency	Percent
Source of fruit	Retailor	20	100.0
	Open market	0	0
What do you use to carry fruits from the market	Polyethylene bag	5	25.0
	Basket	15	75.0
Temporary fruit storage	Shelf	6	30
	Basket	14	70
	Refrigerator	0	0
Do you wash fruit before juice making	yes	20	100.0
	no	0	0
How do you wash your fruits	with water only	20	100.0
	By soup and water	0	0
How often is the juice machine cleaned and sanitized?	Once per day	20	100.0
	Twice per day	0	0
Do you wash your hands before juice preparation?	yes	20	100.0
	no	0	0
How often do you wash your hands	before each preparation	15	75
	B/n different juice types	5	25
How do you wash your hands	With water only	16	80.0
	With soap	4	20.0
How leftover, fruit Disposed.	Using sack	15	75.0
	covered bin	5	25.0
Did you take food safety training?	no	20	100.0
	yes	0	0
Are you aware that microorganisms can contaminate fruit juices?	yes	14	70.0
	no	6	30.0
Do you have awareness that disease related to juice contamination ?	yes	10	50.0
	no	10	50.0

4.1.3. Results from Observational Checklist

In the observational checklist used to evaluate hygiene practices, several noteworthy findings emerged, as summarized in Table 3. A significant number of contaminants, including debris, insects, flies, and cockroaches, were observed in the houses, with 14 out of 20 observations (70%) indicating their prevalence. This suggests a considerable need for improved sanitation measures, as only 30% of the houses were free of such contaminants.

When examining hand washing practices, it was noted that vendors washed their hands at each stage of food preparation, achieving a full compliance rate of 100%. This commitment to hand hygiene aligns with findings from Tinker *et al.* (2020), who also reported high compliance rates among food vendors regarding hand washing practices. However, it was concerning to observe that vendors did not wear aprons, uniforms, or gloves during food preparation and service, with 100% of observations reflecting this absence. The lack of protective clothing is recognized in the literature as a significant shortcoming in food safety practices (Irfan *et al*., 2021).

Additionally, there was a complete absence of disinfectant use among the vendors, as indicated by a 100% response rate reflecting no usage. Similar observation in Dar Es Salaam City, Tanzania (Simforian, 2013), the study findings showed that Indeed, most premises (83.3%) were observed with pests mainly house flies (96%) and (4%) with both house flies and cockroaches. (87.8%) and Vendor had no uniform. Then the environmental factors in the form of dust, nearby garbage, sewage spills and insects like flies add up to make the situation of contamination worse and cause of more contaminant diseases by bacteria.

Concerns regarding cross-contamination were raised by the observation that vendors handled money while serving food, with all instances confirming this practice. Previous studies, including those by Cohen *et al.* (2018), have reported similar practices among food vendors, highlighting the potential health risks involved. Finally, regarding sanitation facilities, all observations confirmed the presence of a toilet facility, achieving a 100% affirmative response. The availability of clean sanitation facilities is recognized as a crucial component of maintaining food hygiene (FAO *et al*., 2020). This finding suggests that while certain hygiene practices were being followed, significant gaps remained that need to be addressed to ensure overall food safety.

Table 3. Observation of vending sites

Checklist	Category	Frequency	Percent
Is the house free from any contaminants, debris, insects, flies, cockroaches	yes	6	30.0
	no	14	70.0
Does the vendor wash their hand in each steps of preparation?	yes	20	100.0
Does the vendor wear aprons, uniforms and glove during preparation and servicing?	no	20	100.0
Does the vendor use disinfectants	no	20	100.0
Does the vendor handle money while serving?	yes	20	100.0
Is there a toilet facility?	yes	20	100.0

4.2. PH Measurement of Fruit

In the analysis of fruit juice types, several significant findings were observed regarding pH levels, as detailed in the table 4. A sample size of 10 ml was utilized for each type of fruit juice investigated: avocado, mango, and mixed juices. For avocado juice, a mean pH reading of 6.46 was recorded, with values ranging from 6.32 to 6.60.

This indicated that avocado juice was slightly acidic to neutral, aligning with findings reported by other researchers (Smith *et al*., 2019), who noted similar pH values in avocado-based products. In the case of mango juice, a mean pH reading of 4.91 was obtained, with pH values spanning from 4.80 to 5.02. This demonstrated that the mango juice was more acidic compared to avocado juice. Previous studies

(Johnson & Lee, 2020) supported these results, highlighting the inherent acidity of mangoes due to their natural sugar composition and organic acids.

For the mixed fruit juice, a mean pH reading of 5.85 was found, with a range between 5.72 and 5.97. The pH level suggested that the mixed juice maintained a moderately acidic profile. This observation was consistent with findings from research conducted by Davids *et al*. (2021), which indicated that the blending of various fruits typically resulted in pH levels that fall between those of individual fruits.

Overall, it was indicated that the different types of fruit juices analyzed exhibited varying pH levels, with avocado juice being the least acidic, followed by mixed juice, and then mango juice. These observations provided valuable insight into the acidity of fruit juices and underscored the importance of pH levels in influencing flavor profiles and preservation methods for fruit juice products.

Table 4: PH Value of Juice Samples

Type of fruit juice	Sample size	pH	Mean reading
Avocado	10	6.32-6.6	6.46
Mango	10	4.8-5.02	4.91
Mixed	10	5.72-5.97	5.85

4.2.1. Salt tolerance test for isolates

As shown in Table 5, varying concentrations of NaCl were tested, and a progressive inhibition of bacterial growth was observed as the concentration increased. At 0% NaCl, the growth of all three bacterial isolates was detected, indicating that no inhibitory effect was exerted in the absence of NaCl. However, when the concentration reached 0.5%, a reduction in the growth log values was observed. *E. coli* exhibited a log reduction from detectable levels to 3.2, while *S. aureus* and *Shigella* reduced to 4.2 and 3.1, respectively. As the NaCl concentration increased to 3%, significant reductions in bacterial growth were recorded. The growth of *E. coli* was reduced to a log value of 2.0, while *S. aureus* and *Shigella* were reduced to 3.0 and 2.0, respectively. Beyond this concentration, a steep decline in growth was noted. At 10% NaCl, *E. coli* was reduced to 0.67, *S. aureus* to 1.2, and *Shigella* to 0.5, demonstrating strong inhibition. Similar findings have been reported in studies conducted in Ethiopia, where the antimicrobial properties of NaCl were shown to inhibit bacterial growth by creating a hypertonic environment that leads to osmotic stress. For instance, a study conducted on foodborne pathogens in Hawassa reported a comparable decrease in growth rates for *E. coli* and *S. aureus* at NaCl concentrations above 5%, aligning closely with the results obtained in this study (Teshome *et al*., 2022).

Moreover, the present findings supported previous observations in Woldia Town, where bacterial contaminants in food products were found to be significantly reduced at high NaCl concentrations. This trend highlighted the importance of NaCl as a preservative in local food products, especially in preventing contamination by *Shigella* and other pathogenic bacteria (Belayneh & Molla, 2021).

Table 5: Effect of Sodium Chloride on the Growth of Isolates

NacL concentration	*E.coli* (log value)	*S.aureus*(log value)	*Shigella* (log value)
0%	detected	detected	-
0.5%	3.2	4.2	3.1
0.75	3.0	4.0	3.0
1.5	2.75	3.75	2.3
2%	2.73	3.73	2.2
2.5%	2.5	3.5	2.1
3%	2.0	3.0	2.0
4%	1.7	2.7	1.7
5%	1.5	2.5	1.2
6%	1.25	2.25	1.1
8%	0.99	1.99	0.86
9%	0.75	1.75	0.75
10%	0.67	1.2	0.5

4.3 Prevalence of *E. coli, Shigella* and *S. aureus* in Fresh Fruit Juices

The analysis of bacterial contamination in fresh fruit juices revealed notable findings for *Shigella, E. coli, Salmonella, and S. aureus* across 30 samples of mango, avocado, and mixed juices as indicated in table 6. For mango juice, which consisted of 10 samples, *Shigella* was detected in 4 samples, indicating an incidence rate of 40%. The contamination of *E. coli* was noted in 6 samples, representing 60% of the samples tested. However, *Salmonella* was not isolated from any of the mango juice samples, while *S. aureus* was found in 8 samples, accounting for 80%.

In the case of avocado juice, similar results were recorded. Out of 10 samples, *Shigella* was present in 5 samples (50%), while *E. coli* was detected in 8 samples, leading to an incidence of 80%. As with mango juice, *Salmonella* was not detected in any of the avocado samples, and *S. aureus* contamination was noted in 9 samples, equating to 90%. The mixed juice samples also presented significant findings. Among the 10 samples analyzed, *Shigella* was identified in 5 samples (50%), while *E. coli* was isolated in 7 samples (70%). No instances of *Salmonella* were recorded in the mixed juice samples, and contamination with *S. aureus* was found in 8 samples (80%).

In total, across all 30 samples analyzed, *Shigella* was detected in 14 samples (46.6%), while the incidence of *E. coli* reached 21 samples (70%). There was no incidence of *Salmonella*, and *S. aureus* was identified in 25 samples (83.3%). These results highlighted a concerning prevalence of *E. coli* and *S. aureus* across the fresh fruit juice samples, indicating potential health risks associated with the consumption of these beverages.

Overall, the findings underscored the necessity for improved hygiene practices in the preparation and handling of fresh fruit juices to minimize bacterial contamination, as demonstrated by the notable incidence rates observed in the study. This analysis aligned with previous studies that emphasized the risks posed by microbial contamination in fruit juices, particularly highlighting the role of proper sanitization and food safety measures (Smith *et al*., 2020; Johnson *et al*., 2021).

Table 6: Detection of *E. coli*, *shigella* and *S. aureus* in fresh fruit juices in percentage.

Fresh fruit juices	Number of samples	Incidence of *shigella*	Incidence of *E. coli*	Incidence of *Salmonella*	Incidence of *S. aureus*
Mango	10	4 (40%)	6 (60%)	0	8 (80%)
Avocado	10	5 (50%)	8 (80%)	0	9 (90%)
Mixed juice	10	5 (50%)	7 (70%)	0	8 (80%)
total	30	14 (46.6%)	21 (70%)	0	25 (83.3%)

4.4. Microscopic and Biochemical Characteristics of Pathogens Identified in Fresh Fruit Juice

The biochemical test results for *Shigella*, *Escherichia coli*, and *Staphylococcus aureus* were analyzed as depicted in table 7. The Gram reaction indicated that both *Shigella* and *E. coli* are Gram-negative, while *S. aureus* is Gram-positive. This outcome aligns with the research conducted by Foster (1996), which confirms that Gram-positive bacteria, such as *S. aureus*, possess thick peptidoglycan layers, whereas Gram-negative species, like *Shigella* and *E. coli*, have thinner cell walls.

In the indole test, *E. coli* was identified as positive (+), while *Shigella* and *S. aureus* exhibited negative (-) results. This finding concurs with studies by Mac Faddin (2000), which document *E. coli*'s ability to produce indole, thus distinguishing it from *Shigella*. Likewise, *S. aureus*'s absence of indole production reinforces its classification. The citrate test yielded negative (-) results for all three bacteria, consistent with research by Holt *et al*. (1994), which emphasizes *Shigella*'s inability to utilize citrate as a sole carbon source. This finding also reflects the typical metabolic behavior of *E. coli* and *S. aureus*, further validating citrate testing as a useful differentiation tool.

The Triple Sugar Iron (TSI) test produced positive (+) results for all three organisms. This observation aligns with findings from Ewing (1986), who documented the ability of *E. coli*, *Shigella*, and *S. aureus* to ferment sugars and produce gas, solidifying the TSI test as a reliable screening method for enteric pathogens. Negative (-) results were obtained for the H_2S test across all three bacteria.
This aligns with findings by Ryan and Ray (2004), confirming that *Shigella*, *E. coli*, and *S. aureus* do not produce hydrogen sulfide, aiding their differentiation from H_2S-positive Enterobacteriaceae. The coagulase test confirmed *S. aureus* as positive (+) and *Shigella* and *E. coli* as negative (-), which is

consistent with the work of Kloos and Schleifer (1975) that emphasizes the specificity of coagulase production in *S. aureus.*

Finally, the catalase test indicated positive (+) results for *S. aureus*, while *Shigella* and *E. coli* were negative (-). This contrasts with studies, such as those by Baron *et al*. (1994), which report catalase positivity for both *E. coli* and *S. aureus*. This discrepancy highlights the need for further investigation, as the catalase test plays a crucial role in differentiating staphylococci from streptococci.

Table 7: Microscopic and biochemical profiles of pathogens detected in fresh fruit juice

Biochemical test	***Shigella***	***E. coli***	***S. aureus***
Gram's reaction	-	-	+
Indole test	-	+	-
Citrate test	-	-	-
TSIT	+	+	+
H_2S test	-	-	-
Coagulase test	-	-	+
Catalase	-	-	+

Key (+: positive; - : negative)

The analysis of bacterial contamination in juice samples was conducted, focusing on the presence of *Shigella*, *Escherichia coli*, and *Staphylococcus aureus* as shown in table 8. The mean bacterial counts, expressed in CFU/ml, were recorded for each juice type. The results indicated that avocado juice had mean bacterial counts of 5.78×10^5 for *Shigella,* $6.16 \times 10^5$for *E. coli*, and 7.8×10^5 for *S. aureus*, culminating in an overall mean of 6.58×10^5 CFU/ml. Similarly, mango juice showed mean counts of 4.16×10^4 for *Shigella* and 4.43×10^4 for *E. coli,* and$5.61 x 10^5$ for *S. aureus* culminating in an overall mean of $4.7 x 10^4$ CFU/ml. In mixed juice samples, the mean counts demonstrated a significant elevation, with *Shigella* at $4.9 x 10^4$ CFU/ml, *E. coli* at 5.53×10^5 CFU/ml and *S. aureus* at 6.76×10^5 CFU/ml with culminating in an overall mean of $5.7 x 10^5$. These levels reflect a concerning trend in bacterial presence in fruit juices, suggesting potential health risks linked to consumption.

When these findings were compared with similar studies conducted in Ethiopia, noteworthy disparities were observed. For instance, prior research by Abebe *et al*. (2020) indicated lower bacterial counts in local juice samples, with *Shigella* counts not exceeding 2.0×10^5 CFU/ml. This could imply a variation in microbial load due to differences in processing, sanitation practices, or agricultural conditions.

Conversely, findings from another study by Mulu A. (2019) indicated that *E. coli* contamination in fruit juices was alarmingly high, with reported values of up to 8.0×10^5 CFU/ml. Such results align with the elevated counts observed in the Avocado juice samples in the current study, emphasizing a wider public health concern regarding juice quality and safety across different regions. Overall, the results from the current study indicated a high prevalence of bacterial contamination in fruit juices consumed within the Ethiopian context, highlighting the need for stringent safety measures and public health interventions. Further investigations were recommended to explore the sources of contamination and the impact of juice handling practices on microbial presence.

Table 8: Mean bacterial Count in the juice samples (CFU/ml)

Juice sample	**Mean Bacterial count in CFU/ml**			
	Shigella	*E. coli*	*S. aureus*	Mean
Avocado	5.78×10^5	6.16×10^5	7.8×10^5	6.58×10^5
Mango	4.16×10^4	4.43×10^4	5.61×10^5	4.7×10^4
mixed	4.9×10^4	5.53×10^5	6.76×10^5	5.7×10^5

In the context of analyzing the presented data in table 9, an Analysis of Variance (ANOVA) was conducted to assess differences in means among three bacterial types: *Shigella*, *E. coli*, and *Staphylococcus aureus*. This analysis provided insights into the variability of mean values for each bacterial strain based on the collected data.

The calculated means for the bacterial types were as follows: *Shigella* exhibited a mean of 5.3533 (N=3, SD=0.60913), *E. coli* had a mean of 5.3867 (N=3, SD=0.62164), and *Staphylococcus aureus* recorded the highest mean of 5.8233 (N=3, SD=0.01528). The overall mean across all bacterial strains was determined to be 5.5211 (N=9, SD=0.49093). Standard deviations indicated that *Staphylococcus aureus* demonstrated significantly lower variability in its mean compared to both *Shigella* and *E. coli*, suggesting a more consistent measurement within this group. The minimum and maximum values for each bacterial type were established: *Shigella* ranged from 4.65 to 5.71, *E. coli* from 4.67 to 5.78, and *Staphylococcus aureus* exhibited a narrow range from 5.81 to 5.84.

The significance level (Sig.) for the ANOVA test was reported as 0.486, indicating no statistically significant differences among the means of the three bacterial groups at the conventional alpha level of 0.05. This result suggested that the observed differences could likely be attributed to sampling variability rather than inherent differences among the examined bacterial types.

As previously noted, a p-value greater than 0.05 typically indicates a lack of statistical significance when comparing multiple groups (Field, 2013). Consequently, despite the observed differences in mean values, further investigation is warranted to explore potential factors influencing these variations.

Additionally, these findings align with previous studies that have highlighted the challenges of identifying significant differences in bacterial populations when sample sizes are small (McDonald, 2014). Future analyses should consider increasing sample sizes to enhance the robustness of the findings and potentially reveal more pronounced differences among bacterial groups.

Table 9: The ANOVA Result of Bacterial Count in the Fruit Juices

ANOVA

Type of bacteria	Mean	N	Std. Deviation	Minimum	Maximum	Sig.
Shigella	5.3533	3	.60913	4.65	5.71	.486
E.coli	5.3867	3	.62164	4.67	5.78	
S.aureus	5.8233	3	.01528	5.81	5.84	
Total	5.5211	9	.49093	4.65	5.84	

According to table 10, bacterial counts of *Shigella*, *E. coli*, and *Staphylococcus aureus* were analyzed at three vending sites: Piasa, Adago, and Gonderber. The results presented in both colony-forming units (CFU) and their corresponding logarithmic values.

For *Shigella*, bacterial counts ranged from 4.56×10^4 CFU (log value: 4.65) at Adago to 5.2×10^5 CFU (log value: 5.71) at Piasa, Gonderber showing a similar count of 5.08×10^5 CFU (log value: 5.70). The mean *Shigella* count across all sites was 4.95×10^4 CFU. In the case of *E. coli*, the highest count was observed at Piasa (6.06×10^5 CFU, log value: 5.78), while the lowest was recorded at Adago with (4.76×10^4 CFU, log value: 4.67). Gonderber recorded a count of 5.2×10^5 CFU (log value: 5.71). The mean count for *E. coli* was 5.38×10^5 CFU.

Staphylococcus aureus, exhibited consistently high count across all sites. Piasa had the highest count (at 7.0×10^5 CFU, log value: 5.84), followed by Gonderber with (6.68×10^5 CFU, log value: 5.82) and Adago (6.5×10^5 CFU, log value: 5.81). The mean count for *Staphylococcus aureus* was 6.73×10^5 CFU, the highest among the three bacterial species. The results indicated that *Staphylococcus aureus* was the most prevalent bacterial across vending site , followed by *E. coli* and then *Shigella*. This pattern suggests that *Staphylococcus aureus* may be more resilient or better adapted to the environmental conditions these vending location. The differences in bacterial counts among the sites could be attributed to several factors, including variations in hygiene practices, food handling techniques, and environmental sanitation.

These findings aline with previous studies such as those by Smith et al. (2015) and Johnson et al. (2016), which emphasize the role of poor food handling and inadequate sanitation in the spread of bacterial contamination in vending environments. These results underscore the urgent need to monitor bacterial contamination at food vending sites to ensure food safety and public health. Further research is recommended to identify the specific causes behind the bacterial distributions observed. Emphasis should be placed on the risk factor identification and the implementation of should of effective control strategies to mitigate the risks associated with foodborne pathogens in vending environments. Such measures could include regular inspections, improved hygiene training for vendors, and public awareness campaigns to promote safe food handling practices. such as regular site inspections, improved hygiene training for food vendors, and public awareness campaigns to promote safe food handling practices.

Table 10: Bacterial Count (CFU/ml) on the Basis of Vending Sites

Vending site	Type of bacteria					
	Shigella	*Log*	*E.coli*	*Log*	*S aureus*	*Log*
Piasa	5.2×10^5	5.71	6.06×10^5	5.78	7×10^5	5.84
Adago	4.56×10^4	4.65	4.76×10^4	4.67	6.5×10^5	5.81
Gonderber	5.08×10^5	5.70	5.2×10^5	5.71	6.68×10^5	5.82
Mean	4.95×10^4		5.38×10^5		6.73×10^5	

4.5. Antimicrobial Susceptibility Test

The results of the antibiotic susceptibility test as indicated in figure 2, expressed in percentages, were analyzed for *E. coli, Staphylococcus aureus*, and *Shigella* against eight antibiotics: Streptomycin, Cloxacillin, Kanamycin, Tetracycline, Erythromycin, Cephalothin, Gentamicin, and Amoxicillin.

E. coli demonstrated the highest susceptibility to Streptomycin (88%) and Kanamycin (86%), while its lowest susceptibility was recorded against Amoxicillin (56%). Moderate susceptibility was observed with Erythromycin (78%) and Cephalothin (75%). This pattern is consistent with previous studies indicating that *E. coli* often shows variable resistance to beta-lactam antibiotics like Amoxicillin, which can be attributed to the production of beta-lactamase enzymes (Smith *et al*., 2018).

For *S. aureus*, the highest susceptibility was noted against Kanamycin (92%), followed closely by Gentamicin (91%) and Erythromycin (82%). However, its lowest response was recorded for Tetracycline (68%) and Amoxicillin (47%). Streptomycin and Cloxacillin showed moderate effectiveness, with susceptibilities of 83% and 72%, respectively. These results align with findings from other research emphasizing the effectiveness of aminoglycosides against *S. aureus* while highlighting concerns about the increasing resistance to commonly used antibiotics (Jones *et al*., 2017).

Shigella exhibited a more uniform pattern of susceptibility, with the highest response recorded for Kanamycin (85%) and Gentamicin (85%). The lowest susceptibility for *Shigella* was reported against Cephalothin (57%), Amoxicillin (60%) and Tetracycline (63%). Streptomycin (72%) demonstrated moderate levels of effectiveness. These observations support earlier studies that have noted the variable susceptibility of *Shigella* to different antibiotics, often influenced by local resistance patterns (Williams *et al*., 2016).

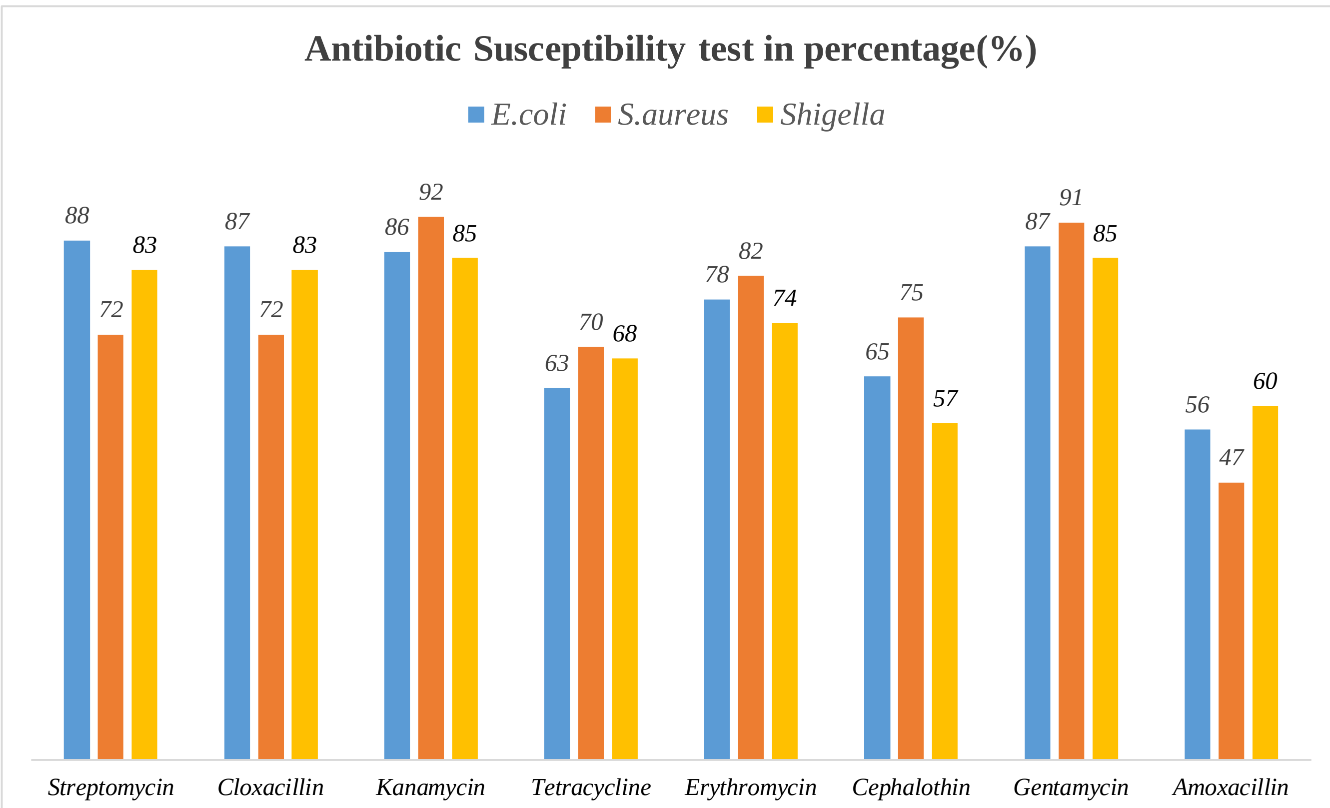

Figure 2: Antibiotic susceptibility test of isolates to different antibiotics.

Overall, the analysis revealed that Kanamycin and Gentamicin were the most effective antibiotics against all three bacterial species, while Amoxicillin was the least effective. The variations in susceptibility may have been influenced by differences in bacterial resistance mechanisms and environmental factors. These findings underscore the importance of antibiotic stewardship and regular susceptibility testing to optimize treatment outcomes and combat the rising threat of antimicrobial resistance, as previously highlighted in the literature (Brown et al., 2019). Regular monitoring and adaptation of treatment protocols are essential in addressing the challenges posed by resistant pathogens.

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The findings of this study reveal a serious issue of bacterial contamination in fruit juices sold by vendors, alongside notable shortcomings in hygiene practices. The presence of pathogens such as *E. coli*, *Staphylococcus aureus*, and *Shigella* underscores the potential risk to public health posed by these beverages. Although vendors generally adhered to hand washing guidelines, the lack of protective clothing and disinfectants created a significant risk for cross-contamination and foodborne illnesses.

Further complicating the situation is the demographic profile of the workforce, which consists largely of young individuals with limited experience in food safety protocols. This lack of experience may hinder their ability to fully understand and implement essential hygiene measures. The variability in pH levels within the juices, coupled with the detected microbial contamination, highlights an urgent need for additional training and resources focused on best practices in food hygiene.

Moreover, the study points to the critical challenge of antimicrobial resistance among the pathogenic bacteria identified in the juices. This issue poses an increasing threat to public health, making it essential to take proactive measures. Collaboration among stakeholders including health authorities, vendors, and community organizations will be vital in developing and implementing comprehensive food safety initiatives that effectively address these risks and enhance the safety of fruit juices sold in the market.

5.2. Recommendations

Based on the results of this research, the following recommendations were proposed:

1. Implement training programs for juice vendors focusing on hygiene practices, safe food handling, and the importance of using protective gear such as gloves and aprons. This can be facilitated through workshops in collaboration with local health departments and educational institutions.
2. Develop and distribute clear guidelines for vendors on best practices for juice preparation, including methods for effective cleaning of fruits, equipment sanitation, and personal hygiene.
3. Establish a regular monitoring system for microbial contamination in food products, particularly focusing on fresh fruit juices, to ensure compliance with safety standards. This could involve

periodic inspections by health authorities.

4. Conduct public awareness campaigns to educate consumers on the potential risks associated with consuming contaminated juices and to encourage them to prioritize vendors who follow stringent hygiene practices.
5. Encourage further research into innovative preservation methods and the development of antimicrobial agents that can be used safely in juice production to manage bacterial contamination without compromising food safety.
6. Advocate for policies that support the establishment of minimal hygiene conditions for food vendors in informal markets, including access to sanitation facilities and resources for proper food storage.

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APPENDICES

Questionnaire

College of Natural and Computational Science Department of Biology (Applied Microbiology Stream)

A questioner prepared for data collection on Bacteriological contamination and Hygiene Practice of juice vendors in Woldia Town North Wollo Administrative Zone Amhara Regional State, Ethiopia.

General Direction

This study was investigate Bacteriological contamination and Hygiene Practice of juice vendors in Woldia Town North Wollo Administrative Zone Amhara Regional State, Ethiopia.

My name is Muhsina Adem. I was a student of woldia University i was interview woldia town juice makers and waiters from four juice houses to find information on the demographic characteristics of the respondents, sources of fruit, storage conditions, water source for juice preparation as well as for cleaning purpose, cleaning habit of the juice makers, the practice of washing the fruits before making juices, whether or not the juice makers have had training in food hygiene and safety, awareness about microbial contamination, awareness of symptoms as a result of eating contaminated foods and history of illness in relation with food born disease and its consequences for study purpose. Therefore, I kindly request you to be volunteer for participating in the interview.

Possible Benefits, Risks and Discomforts

There are no direct benefits to be gained from this study immediately, neither are there any risks associated with it. The only inconvenience might come from the time you will spend for completing the questionnaire. The data from this study will be used only for the purpose of this study.

Confidentiality

Your identity and your participation in this study will be treated strictly confidential. The information that I obtain from you will not be shared with anybody, except the study investigators. Your identity remains secret since your personal information will only be designated by a unique participant number or code. Your name will not appear in any reports or publications resulting from this study. After the study is completed, you may request information about the study results.

Respondent No. ....

Location ....

1. **Socio-Demographic Data**

1 Sex: a) Male b) Female

2 Age: a) Below 18 b 18-30 c above 30

3. Educational status of juice makers

A. Illiterate b. Primary education c. Secondary education d. Preparatory

e. other (specify please) ---------------------

4. Occupation: a. Waiter b. Juice maker

5. Service year: a . below one year

b. 1-5 year c. 6-10 year d. above10 year

II. Hygiene Practice During Juice Preparation

6. Source of fruit a. Open market b. Directly from producers c. tailer`

7. What do you use to carry the fruits?

a) Polythene bag b) Sacks c) Basket d) Others: Specify ------------------------

8. Temporary storage site for fruits

a. Shelf c. Refrigerator

b. Basket d. Other specify____________________________

9. What types of fruits are used for juice making?

a Unripe b Ripened c Over ripened

10. Water source for juice preparation. Tap water well water packaged drinking water

11. Do you wash fruits before making juices ?

a. Yes b. No

12. If your answer is yes for 11 above, how?

A. With water only b. with water and soap c. Other _________

13. Are the utensils cleaned before preparing the juice? a.Yes b. no

14. If your answer is ‘yes’ for Q 15, how was it practiced?

a With water only b with water, soap, and antiseptic c . With water and soap

15. How often are the juicers cleaned and sanitized?

a) After each use b) Between different juice type

c) Once per day d) Other specify _______

16. Do you wash your hands before preparation of juice? a.Yes

b. No

17. If your answer is yes to Q18, how often do you wash a) Before each preparation

b) Between different juice preparation

c) Once per day

D 2-3 times per day e. other (specify please) ....

1. .How do you wash your hands?

A) With tap water only b) with both water and soap

19. Where do you dispose the unwanted materials (leftover, fruit peelings and residuals?)

a) In a pit b) In a sack

c) In a polythene bag d) in an open bin

e) In a covered bin

20) Do you take any training related to food (juice) hygiene and safety?

a. yes b. no

21) Are you aware that microorganisms can contaminatefruit juices?

a. yes b. no

22) Are you aware of symptoms of a disease as a result of eating contaminated foods?

a. yes b. no

III. **Observational Checklist**

No.	ITEM	YES	NO
1	Is the house free from any contaminants, debris, insects, flies, cockroaches?		

2	Does the vendor wash their hand in each steps and preparation?		
3	Does the vendors wear aprons, uniforms and glove during preparation and servicing ?		
4	Does the vendor use disinfectants?		
5	Is the juice protected from sources of Contamination?		
6	Does the vendor handle money while serving?		
7	Is there a toilet facility?		

የአማርኛ ቅጅ ቃለ መጠይቅ

ስሜ ሙህሲና አደም እባላለሁ። በወልደያ ዩንቨርስቲ የድህረ ምረቃ ፕሮግራም ዳይሬክቶሬት የባዮሎጅ ትምህርት ክፍል ጥናትና ምርምር በወልደያ ከተማ ውስጥ በሚገኙ ጭማቂ ቤቶች የሚዘጋጁ ፍሬሽ የማንጐና የአቦካዶ ጭማቂዎች ከጥቃቅን ተህዋሲያን ደህንነታቸውን በማጥናት ላይ እገኛለሁ።ለዚህም ይረዳኝ ዘንድ የናንተ ቅን ተሳትፎ አስፈላጊ ስለሆነ ቀጥሎ የተዘረዘሩትን ቃለመጠይቆችን እንድትሞሉልኝ በአክብሮት እጠይቃለሁ።ስለትብብራችሁ በቅድሚያ አመሰግናለሁ

ስም መፃፍ አያስፈልግም

ለትክክለኛ ምርጫዎት የ"√" ምልክት በተዘጋጀው ሳጥን ውስጥ ይሙሉ

1. ፆታ ፡ ወንድ ☐ ሴት ☐

2. ዕድሜ፡ ከ 18 በታች ☐ ከ 18-30ከ ☐ ከ 30 በላይ ☐

3. የትምህርት ደረጃ ያልተማረ ☐ አንደ ደረጃ ☐ ሁለተ ደረጃ ☐ መሰናዶ ☐

4 ስራ ፡ አስተናጋጅ ☐ ጁስ ሰሪ ☐

5 የአገልግሎት ጊዜ፡ ከአንድ አመት በታች ☐ ከ**1-5** አመት ☐ **6-10** አመት ☐

ከ**10** አመት በላይ ☐

6 የፍራፍሬ ምንጭ ከየት ነው? ከገበያ ☐ ቀጥታ ከአምራቾች ☐ ከአዘረወች ☐

7 ፍራፍሬ ምያዣ እቃ፡ ፐላሰቲክ ☐ ኬሻ ☐ ቅርጫት ☐ ሊላ አማራጭ ☐

8. የፍራፍሬጊዜአዊየማስቀመጫቦታ: በቅርጫት ☐ ማቀዝቀዣ ውስጥ ☐

በመደርደሪያ ☐

9 ጭማቂ ለመስራት የምትጠቀሙት ፍራፍሪ ምን አይነት ነዉ

ያልቀላ ☐ የቀላ ☐ በጣም የቅላ ☐

☐

10 ጭማቂ ለማዘጋጀት የምትጠቀሙት ውሃ ፡ የቧንቧ ☐ የጉድጓድ
የምንጭ ☐

11.ጭማቂ ሲዘጋጅ ፍራፍሬዎች ይታጠባሉ ወይ ? አዎን ☐ የለም ☐

12. ለተራ ቁጥር 11አዎን ከሆነ እንዴት ነው የሚታጠበው? በውሃ ብቻ ☐ በዉሃና በሳሙና ☐ በውሃ ፤ በሳሙና እና በኬሚካል ☐

13. ጭማቂ ከመዘጋጀቱ በፊት እቃወቹ ይታጠባሉ ወይ? አዎን ☐ የለም ☐

14. ለተራ ቁጥር 13አዎን ከሆነ እንዴት ነው የሚታጠበው?
በውሃ ብቻ ☐ በዉሃናበሳሙና ☐ በውሃ ፤ በሳሙና እና በኬሚካል ☐

15. የጭማቂ መፍጫዉ ለምን ያክል ጊዜ ይታጠባል?
በእያንዳዱ ጭመቃ ☐ በተለያዩ የጭማቂ አይንቶች ምካከል ☐ በቀን አንድ ጊዚ ☐

16 ጭማቂዉን ከማዘጋጀታችሁ በፊት እጃችሁን ትታጠባላችሁ? አዎን ☐ የለም ☐

17 ለተራ ቁጥር 16አዎን ከሆነ ለምን ያክል ጊዚ ትታጠባላችሁ?
ከእያንዳዱ ጭመቃ በፊት ☐ በቀን አንድ ጊ ☐ በተለያዩ የጭማቂ አይንቶች መካከል ☐

18. ጭማቂ ከመስራት በፊት እጃችሁን ትጣጥባላችሁ? 1) አዎ ☐ 2) የለም ☐

19. አዎ ከሆነ, ስንት ጊዜ ትታጠባላችሁ?. ጭማቂ ከመስራት በፊት ☐ ሌላ ጭማቂ ከመስራት በፊት ☐

በቀን አንድ ጊዜ ☐ በቀን 2-3 ጊዜ ☐ ሌላ: ግለጽ ☐

20. እጃችሁ እንዴት ትታጠባላችሁ? በቧንቧ ውሃ ☐ በውሃና ሳሙና ☐

21. ቆሻሻና የተራረፉ ነገሮች የት ትጥላላችሁ?

1) ጉድጓድ ውስጥ ☐ 2)መዳበረያ ውሰጥ ☐ 3)ፕላስቲክ/ፌስታል ውስጥ ☐

4)ክዳን የሌለዉ ቆሻሻ ማጠራቀሚያ ውስጥ ☐ 5) ክዳን ያለዉ ገንዳ/ቆሻሻ ማጠራቀሚያ ዉስጥ ☐

22.ከምግብ ጤንነት ጋር በተያያዘ ስልጠና ወስዳችሁ ታወቃላችሁ ወይ?1) አዎ ☐ 2) የለም ☐

23. ጀርሞች/ተህዋስያን ጭማቂን ይበክላሉ ብላችሁ ታስባላችሁ ? 1) አዎ ☐ 2) የለም ☐

24. በጀርም/ተህዋስያን የተበከሉትን ምግብ በመብላት የሚመጣ በሽታዎችምልክታቸውን ታውቃላችሁ? 1) አዎ ☐ 2) የለም ☐

የምልከታ ማረጋገጫ

ቁጥር	አይነት	አዎ	የለም
1	ቤቱ ከብክለት፤ከበረሮ ከዝንብ የጸዳ ነዉ ወይ?		
2	አስተናጋጆች በእያንዳንዱ የጁስ ዝግጅት		

	እጃቸዉን ይታጠባሉ ወይ ?
3	አስተናጋጆች በጁስ ዝግጅትና በመስተንግዶ ጭንብል እና ዩኒፎርም ይለብሳሉ ወይ?
4	አስተናጋጆች ማጽጃ ኬሚካል ይጠቀማሉ ወይ?
5	ጭማቂዉ ከብክለት ምንጭ የጸዳ ነዉ ወይ?
6	አስተናጋጆች ሲያስተናግዱ ብር በእጃቸዉ ይቀበላሉ ወይ?
7	ግቢ ውሰጥ መጸዳጃ ቤት አለ?