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Bacteriological Quality and Associated Factors of Ready-to-consume Juices in Yeka Sub-City, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Hana Mekonnen, entitled: **"Bacteriological Quality and Associated Factors of Ready-to-Consume Juices in Yeka Sub-City, Addis Ababa, Ethiopia"** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards for originality and quality.

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Abbreviations

AAU	Addis Ababa university
AMB	Aerobic Mesophilic bacteria
AMR	Antimicrobial resistance
AST	Antibiotic Susceptibility Test
ATCC	American Type Culture Collection
BGLBB	Brilliant Green Lactose Bile Broth
CDC	Center of disease control
CDT	Combination disk test
CFU	Colony forming unit
CLSI	Clinical and Laboratory Standards Institute
CSA	Central Statistical Agency
DRERC	Department of Research and Ethical Review Committee
EPHI	Ethiopian public health institute
ESBL	Extended spectrum beta-lactamase
FBI	Food born illness
FDA	Food and drug administration
MDR	Multidrug resistance
MPN	Most Probable Number
MRSA	Methicillin-resistant *S. aureus*
PCA	Plate count agar

SDR	Single drug resistance
SOP	Standard operating procedure
SPSS	Statistical Package for the Social Sciences
TSA	Tripton soy agar
VRBA	Violate red bile agar
WHO	World health organization
XLD	Xylose Lysine Deoxycholate

Abstract

Background: Ready-to-consume fruit juices are highly valued for their nutritional benefits, however, pose significant public health risks due to microbial contamination caused by poor hygiene, improper handling, and inadequate sanitation practices, particularly in low-income countries like Ethiopia.

Objective: To assess the bacteriological quality and associated factors of ready-to-consume fruit juices in Yeka sub-city, Addis Ababa, Ethiopia.

Method: A cross-sectional study was conducted from January to June 2024, involving 189 juice samples collected from 63 local vendors. The plate count method was used to determine aerobic plate counts, total coliform, and thermotolerant coliform counts, while the spread plate method was used for Staphylococcus count on Mannitol Salt Agar. *Escherichia coli* detection involved transferring positive thermotolerant coliform samples to Nutrient broth, followed by confirmation using Kovacs reagent. For *Salmonella* and *Shigella*, Buffered Peptone Water and Rappaport Vassiliadis enrichment broths were used, with Xylose Lysine Deoxycholate Agar serving as the selective medium; pinkish colonies with black centers were confirmed through biochemical tests. Antimicrobial susceptibility testing was performed on Muller Hinton Agar for all bacterial isolates. Vendor hygiene practices were evaluated using structured questionnaires and checklists, and data were analyzed using SPSS version 27. The Kruskal-Wallis H test was used to compare medians among different juice types. Bivariate logistic regression and multiple logistic regression were applied to assess relationships between dependent and independent variables, with a p-value < 0.05 considered statistically significant.

Results: The analysis revealed that the median for aerobic colony count, total coliform, thermotolerant coliform, and staphylococcal count across all samples were 7.14×10^6 CFU/ml, 2.8×10^6 CFU/ml, 6.4×10^4 CFU/ml, and 2.2×10^3 CFU/ml, respectively. From a total of 189 samples, the pathogens identified included *E. coli*, which was found in 29.1% of cases, *S. aureus* in 45.5%, and *Salmonella* in 6.9%. The presence of hand washing facilities, frequency of hand washing, and the cleaning agents used were significant contributing factors for the presence of *Salmonella*, with AOR of 5.34 (95% CI: 1.06–26.81, $p = 0.002$), 0.07 (95% CI: 0.01–0.15, $p < 0.001$), and 0.127 (95% CI: 0.028–0.57, $p < 0.001$), respectively. Moreover, hair cover usage was

also significantly associated with *S. aureus* detection with (AOR = 2.9, 95% CI: 1.2-7.1, p = 0.016).

E. coli exhibited moderate resistance to ampicillin and tetracycline, with 10.9% multidrug-resistant (MDR). *Salmonella* demonstrated 100% resistance to ampicillin and tetracycline, with a 15.38% MDR level. Moreover, 41.9% of *S. aureus* identified were MDR with resistant to tetracycline (51.2% n=44/86), penicillin (90.7% n=78/86), and oxacillin (66.3% n=57/86). Methicillin-resistant *S. aureus* (MRSA) was identified in 58.1% of *S. aureus* isolates, while extended-spectrum beta-lactamase (ESBL)-producing *E. coli* was detected in 21.8% of *E. coli* isolates.

Conclusion: The findings from this study clearly indicate the poor hygienic conditions of these juices and the consumers are at risk of getting food-borne infections and underscore the urgent need for improved hygiene practices, regular monitoring, and targeted public health interventions to reduce microbial risks in ready-to-consume fruit juices in Addis Ababa, Ethiopia.

Key words: *risk factors, fecal Coliform, fruit juices, total coliform, antimicrobial resistance.*

1. Introduction

1.1 Background

Fruit juice is the squeezed form of fruits or vegetables typically extracted from one or more types [1]. Globally, the consumption of fruit juice is high, offered by various establishments such as roadside kiosks, cafes, and restaurants, often blended with ice. Communities worldwide attribute substantial nutritional and health benefits to fruit and vegetable juice. Essential nutrients present in fruits include minerals, dietary fibers, vitamins, and important phytochemicals [2-4]. Public health authorities globally advocate for the consumption of fruit juice owing to its high nutritional value, positioning its increased intake as a critical public health objective [5].

Despite the nutritional advantages of fruit juice, improper handling, sanitation, and preparation practices can result in exposure to food-borne illnesses, given its susceptibility to various pathogens. The nutrient-rich nature of fruit juices provides a conducive environment for microbial growth [6]. Contamination Sources include the use of unclean water for juice preparation and serving, as well as poor hygienic practices and contaminated utensils [7]. Microbial pollutants, such as total coliforms, fecal coliforms, and fecal streptococci, are often found in water used for juice preparation. Additionally, harmful bacteria, including *Vibrio*, *Escherichia coli*, *Shigella*, and *Salmonella* spp., can proliferate under unsanitary conditions [8]. The microbial composition of fruits and vegetables can therefore serve as an indicator of the hygiene standards maintained during harvesting, transportation, storage, and processing [9].

Food born illness poses a significant global public health concern, with far-reaching health and economic implications. As such, ensuring the safety and quality of fruit and fruit products is critical in addressing these challenges [10]. In developing countries, the lack of adequate systems for routine diagnosis and reporting of food borne pathogens often leads to an underestimation of outbreaks caused by contaminated fruits and vegetables before, during, and after harvest [11]. For instance, in Ethiopia, facilities involved in the preparation and sale of fresh fruit juice frequently lack ongoing food safety and quality inspections, resulting in higher microbiological counts in most fruit juices provided to customers, which are believed to cause health complications [12].

This study aimed to assess the bacteriological quality of ready-to-drink juices by analyzing essential microbial indicators, including total viable count, coliforms, *E. coli*, and staphylococcal count. Additionally, it seeks to identify the presence of pathogens such as *Salmonella, Shigella*, and *S. aureus*, while also evaluating their susceptibility to antimicrobial agents. Furthermore, the study examined the associated risk factors with bacterial contamination of ready-to-drink juices and the occurrence of methicillin-resistant Staphylococcus aureus (MRSA), as well as extended-spectrum beta-lactamase and carbapenemase-producing enterobacteriaceae.

1.2 Statement of the Problem

Currently, the consumption of freshly squeezed fruit juices is significantly high among a large portion of the global population due to their ease of preparation and the fact that they do not require additional cooking. Moreover, many nutrition and health professionals recommend their consumption because fruits are believed to help prevent and manage various non-communicable diseases. Conversely, numerous outbreaks of human illnesses have been associated with contaminated juices [12]. Recently, there has been a notable increase in cases of diseases linked to the intake of raw fruits, vegetables, and unpasteurized fruit juices [13]. The health effects of consuming contaminated juice depend on several factors, such as the specific type of pathogenic bacteria present, the quantity of toxins produced, and the consumer's immune system strength.

Globally, an estimated 1.9 million people die each year, and approximately 33 million disability-adjusted life years are attributed to foodborne or waterborne microbial infections, which are among the leading causes of illness in developing countries [14, 15]. Microbiological contaminants, including *Salmonella spp., Escherichia coli, Enterobacter spp., Staphylococcus aureus,* and *Serratia spp*., are commonly found in fruit juices and vegetables. These pathogenic bacteria can lead to various health issues, ranging from mild to severe, in both immunocompromised and immunocompetent individuals. For example, *Escherichia coli* can cause conditions such as renal failure, pneumonia, skin infections, respiratory illnesses, and diarrhea [10].

In Africa, the use of unsafe water for food processing and cleaning poses a significant public health challenge. Each year, approximately 92 million people suffer from illnesses, and 137,000 deaths are attributed to the consumption of contaminated food [16]. The absence of reliable data from food borne illness surveillance systems in low- and middle-income countries impedes the implementation of effective risk-based food safety management strategies [17].

In Ethiopia, the growing demand for fruit juice has driven the rapid expansion of fruit juice businesses. However, food safety monitoring in these establishments remains insufficient, particularly in addressing microbial contamination linked to poor vendor hygiene. This gap increases the risk of foodborne illness outbreaks [18].

Although some studies have examined the bacteriological quality of juices in Ethiopia, they have not sufficiently explored the risk factors for microbial contamination or the presence and drug resistance patterns of pathogenic bacteria. This lack of comprehensive data has contributed to recurring food borne disease outbreaks in the country, with annual incidence rates ranging from 3.4% to 9.3% [19]. In Addis Ababa, as in other towns across Ethiopia, foodborne illness outbreaks are increasing both periodically and seasonally. These outbreaks are often associated with pathogenic bacteria in vegetables and other raw consumables, as well as elevated levels of non-harmful bacteria that exceed acceptable limits [20].

Hence this study aims to assess the bacteriological quality, the factors associated with the contamination of ready-to-consume juices, and the drug susceptibility patterns of isolates in Yeka sub-city, Addis Ababa, Ethiopia.

1.3 Significance of the study

This study provides information about the bacteriological qualities of juice ready to drink, which contributes to the broader understanding of food safety practices within the local context. It benefits the public health authorities, quality assurance professionals, and policymakers by investigating the microbial contamination levels in commercially available fruit juices with implications for the hygienic practices of vendors, which promotes safer consumption of fruit and vegetable juices in Ethiopia. Moreover, this study helps vendors by providing a deeper comprehension of the microbial quality and safety of their prepared juices. Furthermore, this study contributes valuable global data that researchers can utilize to explore bacteriological safety and associated factors related to fresh fruit juices.

1.4 Research questions

1. To what extent are fresh fruit juices provided by roadside sales facilities contaminated by bacteria?
2. What associated factors will contribute to the bacteriological contamination of fresh juice?
3. What will be the contamination prevalence of fresh juice by pathogenic bacteria such as *E. coli*, *Shigella* spp., *Salmonella* spp., and *Staphylococcus aureus*?
4. Will *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* isolates develop first-line drug resistance?
5. Will Methicillin-Resistant Staphylococcus aureus (MRSA), Extended-Spectrum Beta-Lactamase (ESBL) and carbapenemases-producing Enterobacteriaceae detected from fresh juice samples?

2. Literature review

2.1 Bacteriological quality of fruit juices

Fruit juices are known to enhance consumer health by helping to prevent a range of non-communicable diseases [21]. However, they are susceptible to contamination at various stages of their production. Such contamination can lead to serious health risks and amplify the potential for public health crises. A variety of studies have examined the contamination levels in fresh juices.

A cross-sectional study was carried out in New York in the United States of America in 2004, revealing the occurrence of 213 diseases associated with the consumption of fruit juice. *E. coli* O111:H, which produces Shiga toxin, and *Cryptosporidium parvum* were isolated and analyzed from environmental and clinical materials [22, 23]. Additionally, in 2005, authorities from 23 states reported 152 cases of *Salmonella enterica* serovar *Typhimurium* infections linked to commercially sold, unpasteurized orange juice [24]. According to the CDC, the most frequent foodborne pathogens in North America that caused illnesses and deaths from unpasteurized fruit juice and cider were *Salmonella spp., E. coli O157* and *O111, Cryptosporidium,* and norovirus. Other causes of epidemics included yeast, *C. botulinum, V. cholerae,* and hepatitis A virus [25].

A cross-sectional study was conducted in Hyderabad city, India, in 2015 on 150 samples of fruit juices for microbiological analysis. Results: 77.3% of the juices were contaminated with fecal coliforms, 73.3% with *S. aureus*, 48.6% with *Shigella spp*., and 42.6% with *E. coli*. Specific hygiene practices and food safety knowledge were associated with the prevalence of food-borne pathogens (p-value of 0.05) [26]. Another cross-sectional study was conducted in Vidarbha, India, in 2013 on 115 fruit juice samples collected from various locations. The finding showed that 105 juice samples were tested positive for *Salmonella spp.*, while 10 samples remained free from pathogens [27].

A similar cross-sectional study was conducted in Port Harcourt Metropolis, Nigeria, in 2013. Fifteen samples of packaged fruit juices, which include pineapple, orange, and apple juice, were analyzed for their microbial content. The study isolates *Saccharomyces spp., Lactobacillus spp., Flavobacterium spp., Bacillus spp.,* and *Micrococcus spp.* from those juice samples [28].

A cross-sectional study in Western Uganda in 2021 on unpackaged fruit juice showed consistent levels of bacterial contamination (5.2 to 5.4 log CFU/ml) and total coliform counts (4.9 to 5.3 log CFU/ml), attributed to the use of unclean water for juice preparation [29].

A cross-sectional study in Jimma town in 2006 analyzed a total of 90 samples (30 samples each for avocado, papaya, and pineapple) from six randomly selected cafés and/or restaurants. The study found the mean aerobic mesophilic bacteria counts (CFU/ml) for avocados, papayas, and pineapples to be 8.0 x 10^6, 3.1 x 10^7, and 7.9 x 10^6, respectively, with higher yeast counts in avocado (4.5 x 10^5 CFU/ml) and pineapple (5.0 x 10^6 CFU/ml) compared to papaya (6.2 x 10^3 CFU/ml) [12].

A cross-sectional study in Shewarobit town, Ethiopia, in 2018 analyzed 16 fruit juice samples (avocado and papaya) from five cafeterias and three restaurants, finding total viable counts between 1.3 × 10^5 and 2.9 × 10^5 CFU/ml, total coliform counts between 0.1 × 10^5 and 2.4 × 10^5 CFU/ml, and Staphylococci counts between 0.2 × 10^5 and 1.7 × 10^5 CFU/ml [30]. Another cross-sectional study was conducted in Bahir Dar, Northwest Ethiopia, in 2012, including ninety samples (30 each of mango, pineapple, and water) from six purposively selected juice houses. Results showed that about 96.7% of the samples had a total viable count above 4 log CFU/ml, exceeding the Gulf Standard 2000 for fruit juices [31].

2.2 Hygienic related risk factors for microbial quality of juices

Fruit juices sold on the street are popular beverages in many cities. However, these drinks frequently represent major microbiological dangers due to poor hygienic procedures during preparation, storage, and distribution. According to the study conducted in Dares Salaam city, Tanzania, in 2013, 78.9% of juice preparation and vending locations were unsanitary, allowing juice contamination. It has been concluded that the overall handling, preparation techniques, and bacterial quality of unpasteurized fruit juices require significant improvement to ensure safety for consumers. Implementing strict hygiene protocols and regular inspections could help mitigate these risks and promote healthier practices among vendors. The gender of the juice vendors was

a risk factor for microbiological contamination of fruit juices (P = 0.05), with female vendors having a considerably higher *E. coli* contamination rate than male vendors [32].

A cross-sectional study was conducted in Eastern Ethiopia in 2020 on 78 fruit juice samples. found that the bacteriological quality of locally prepared fresh fruit juice sold in juice houses was significantly related to educational status ($\chi^2 = 31.663$), training in food hygiene and safety ($\chi^2 = 23.04$), method of fruit preservation ($\chi^2 = 17.98$), place to keep the juice ($\chi^2 = 13.7$), action done with the juice gone bad ($\chi^2 = 12.78$), frequency of cleaning materials used to keep the juice ($\chi^2 = 12.78$), type of dishwashing ($\chi^2 = 19.75$), availability of handwashing equipment ($\chi^2 = 12.78$), and types of waste receptacles ($\chi^2 = 26.25$) (*P*-value <.05) [33].

Another cross-sectional study was also conducted in Bahir Dar Town from December 2014 to April 2015 among 80 (40 avocado and 40 guava) fresh fruit samples and found that there were statistically significant differences between *Salmonella spp.* detection and fruit safety/management training (x^2=4.977 and P-value=0.03). The study also discovered statistically significant differences in bacteria detection and washing of fruits before juice preparation (χ^2 = 5.714 and P-value=0.02), fruit handling practices (χ^2 =6.502 and P-value=0.01), and hand washing before and after fruit handling (χ^2 =4.286 and P-value=0.04) [34].

2.3 Antimicrobial resistance of bacterial isolates

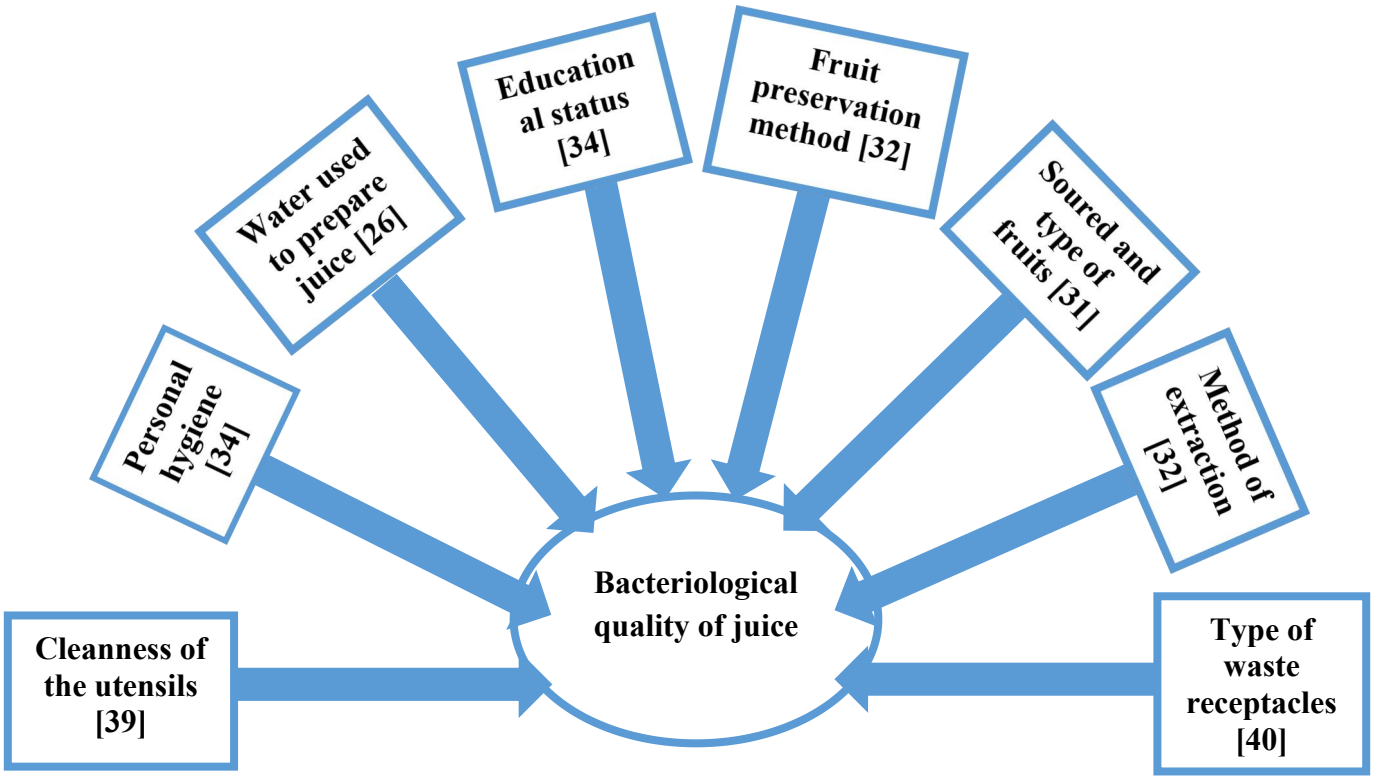
Bacterial drug resistance is becoming an increasing public health concern, as it can result in treatment failures, with some bacteria exhibiting multi-drug resistance patterns. Neglecting recommended hygiene practices by food producers led to microbial contamination and facilitated the spread of antibiotic resistance [35].

A study conducted in Pakistan in 2018 among 125 fresh fruit juice samples showed that all Gram-negative rods (GNR) were resistant to ampicillin. However, most *E. coli* isolates were resistant to ceftazidime (72.4%) and ceftriaxone and cefepime (68.9%), while most *K. pneumoniae* isolates were resistant to cefepime (72%) and ceftriaxone (64%). All *S. aureus* isolates were resistant to penicillin, while most (64%) were resistant to piperacillin. The most effective drugs against bacteria were vancomycin and imipenem. Another significant finding was

that the prevalence of multidrug-resistant strains was notably high, raising concerns about the effectiveness of standard treatments [36].

Another cross-sectional study was conducted to assess the antibiotic resistance of 30 isolates (10 each for *E. coli, Listeria,* and *Salmonella)* from retail meat in the Ibadan municipal abattoir, Nigeria, in 2011. The antibiotic sensitivity profile showed that all the isolates were resistant to three or more antibiotics, including tetracycline. The incidence of antibiotic resistance in virulent strains, *E. coli* O157:H7 (60%) and *Salmonella typhi* (60%), was higher than in the non-virulent strains, *E. coli* (40%) and *Salmonella spp.* (50%), respectively. The overall incidence of antibiotic resistance in *Listeria* strains was relatively lower (37.5%) than in the other pathogens [38].

A study done in Egypt in 2012, analyzing a total of 1000 food samples obtained from different food products, and 80 isolates were identified as *E. coli*. The results showed that 85% and 95% of the *E. coli* isolates from food and clinical origins, respectively, were resistant to ampicillin. When combinations of conventional beta-lactams with beta-lactamase suicidal inhibitors were tested, the recorded resistance was much lower. Only 15-20% of the isolated *E. coli* strains were resistant to cephalosporins and the new generation of beta-lactams [37].



References: [26, 31, 32, 34, 39, 40].

Figure 1: Conceptual frame work.

3. Materials and Methods

3.1 Study area

The study was carried out in Yeka Sub-City, Addis Ababa. Addis Ababa is the capital city of Ethiopia and is administratively divided into 11 sub-cities. According to the Ethiopian Demographic Health Survey 2022 by the Central Statistical Agency, the population of Addis Ababa was estimated to be 5,228,000. Among the 11 sub-cities of Addis Ababa, Yeka Sub-City is located in the northeastern region of the city, and the population was projected to be 424,217 in 2016 [41]. It was the third most populous sub-city in Addis Ababa and shared borders with the districts of Gullele, Arada, Kirkos, and Bole. The sub-city had many licensed juice vendor cafeterias and roadside juice houses. According to the Yeka Sub-City Food and Drug Administration Office, around 447 facilities were registered as vegetable and fruit-selling houses in the 12 woredas of the sub-city.

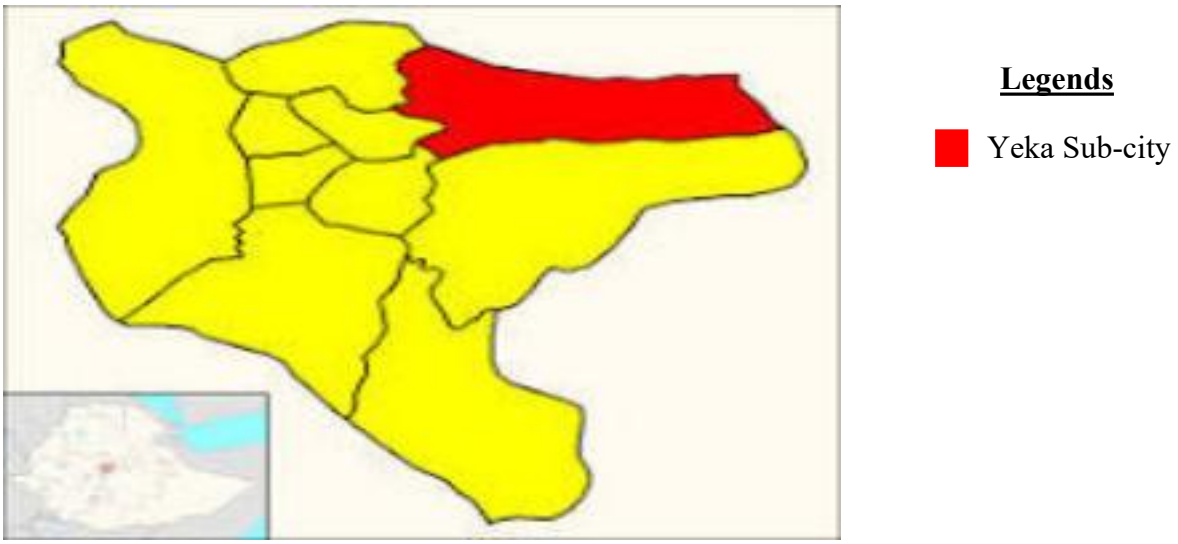


Figure 2: Map of Yeka Sub-City, Addis Ababa (Wikimedia Commons)

3.2 Study Design and Period

A cross-sectional study was conducted from January 2024 to June 2024.

3.3 Source population

The source of the population was roadside fresh juice houses that prepared and sold fruit juices and juice makers working in the juice houses at Yeka Sub-City.

3.4 Study Population

Roadside fresh juice houses that existed in woredas 1, 2, 5, and 9 of Yeka Sub-City and fulfilled the eligibility criteria were considered as the study population.

3.5 Eligibility criteria

3.5.1 Inclusion criteria

- All voluntary roadside fresh juice houses that existed in selected woredas (1, 2, 5, and 9) and were licensed by the sub-city were included.

3.5.2 Exclusion criteria

- All roadside fresh juice houses that were not opened during the study period were excluded.

3.6 Study variables

3.6.1 Dependent Variable

- Aerobic colony count, total coliform count, thermotolerant coliform count, and Staphylococcus count
- Prevalence of *Salmonella* species, *Shigella* species and *S. aureus* from ready to consume juices
- Antimicrobial susceptibility patterns of *E. coli, Salmonella* species, *Shigella* species and *S. aureus*.
- Prevalence of Methicillin Resistance Staphylococcus aureus (MRSA)
- Prevalence of extended spectrum beta-lactamase and carbapenemase producing enterobacteriaceae.

3.6.2 Independent Variable

- Method of juice preservation
- Storage of prepared juice

- The water used to prepare and wash the fruits
- Personal hygiene of makers
- Cleanness of utensils
- Clothing of juice makers
- Waste Receptacles

3.7 Sample size determination

The required sample size was determined by single population formula $n = Z^2\alpha/2\ P\ (1- P)/\ d^2$

Where: n: Sample population

Z = Z score for 95 % confidence interval = 1.96,

p =11% (The prevalence of *E. coli* in avocado juice samples which was previously done in Ethiopia) [42].

d = tolerable error =5 %.

1-p=Q= negative proportion

There by $n = ((1.96)^2 \times 0.11*(1-0.11))/ (0.05)^2 = 151$. To reduce errors arising from complaint non-response rate, 10% of sample size added giving a sample of **168.** However, our study used larger sample size (189) than minimum calculated sample size.

3.8 Sampling technique

By using the lottery method, 4 woredas (woreda 1, 2, 5, and 9) were selected from the 12 woredas of Yeka Sub-City. The calculated sample size was distributed to each selected woreda conveniently, by considering the number of licensed juice house existed in each woredas and by their willingness. Then, three types of each commonly consumed fruit juice (avocado, papaya, and mixed/spice) from each juice house were collected.

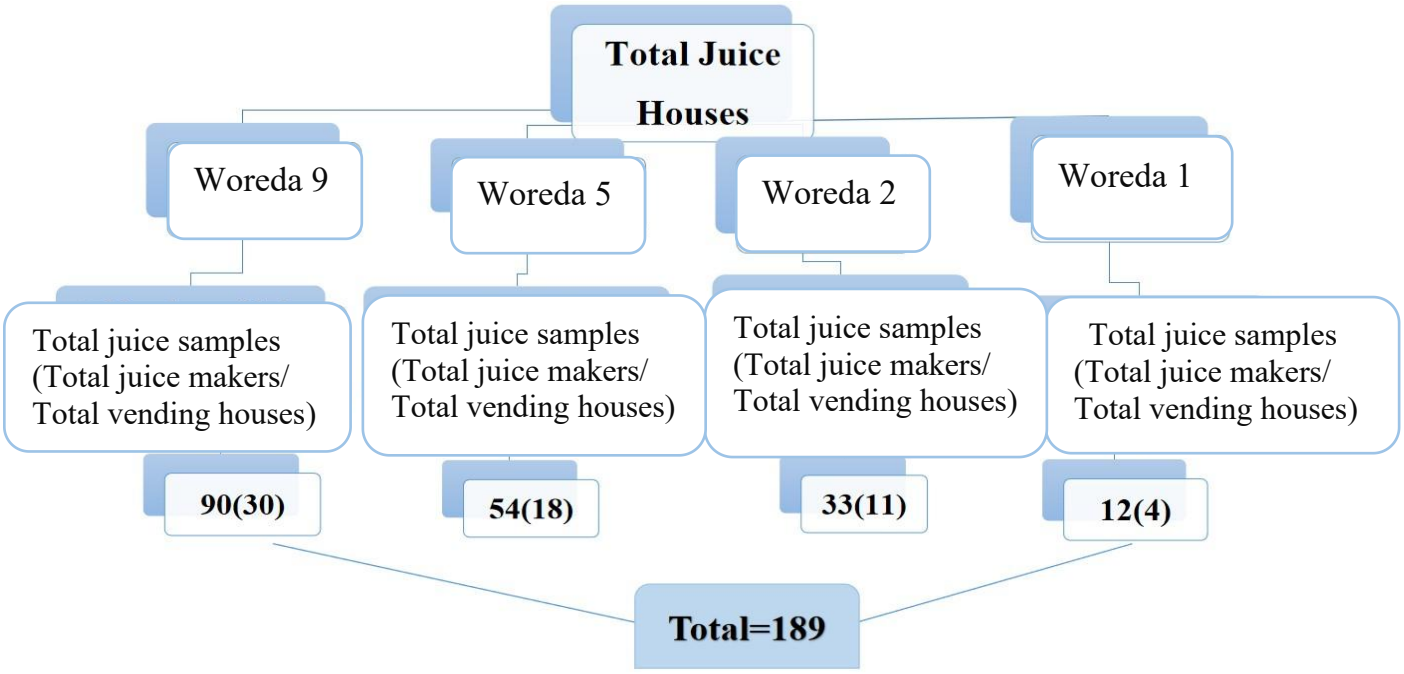


Figure 3: Sampling technique of food handlers from study site.

3.9 Sample collection and transportation

A total of 189 samples of different varieties (avocado, papaya and mixed) of locally made fresh fruit juices were collected from Yeka Sub-City's selected woredas between January 2024 and June 2024. A total volume of 250 ml of samples from each house was collected on a voluntary basis from roadside juice houses in wide-mouth sterile containers aseptically, labeled, and immediately transported to the EPHI Food Microbiology National Reference Laboratory in an ice-box. Associated risk factors were also collected using a pre-structured questionnaire and additional hygienic practices were evaluated by using an observational checklist.

3.10 Laboratory analysis

3.10.1 Microbiological Analysis of juice sample

Aerobic colony count: The sample bottle was mixed by gentle inversion. Subsequently, 1 ml of the sample was poured into a sterile culture plate. A total of 20 ml of plate count agar (PCA) (HiMedia Laboratories plc, Mumbai, India) was added to the plate. The sample and PCA were thoroughly mixed and allowed to stay at room temperature until the mixture solidified.

Following this, the plates were incubated for 48 hours at 37 degrees Celsius. Finally, the total viable counts were determined using a digital colony counter [43].

Coliform Count: Nine milliliters of sterile saline solution were used as the diluent. Additionally, one milliliter of juice sample was transferred to prepare 10^{-2} to 10^{-3} ten-fold serial dilutions. Subsequently, five milliliters of tryptone soya agar (Hi Media Laboratories plc, Mumbai,India), previously cooled to 45.0±1.0°C were added to appropriately labeled petri dishes. These petri dishes were pre-incubated for 1-2 hours at 20-25°C. Following this, they were covered with 10-15 ml of violet red bile agar (Hi Media Laboratories plc, Mumbai,India) at a temperature of 45.0±1.0°C. One-milliliter aliquots from each dilution ((10^{-2},10^{-3}) were aseptically transferred to each petri dish. The petri dishes were then incubated at 37°C for 24 hours in an inverted position.

During this incubation period, typical colonies were counted, and selected colonies were confirmed by testing for gas production in Brilliant Green Lactose Bile broth (Oxoid LTD, Basingstoke, Hampshire, England). A loopful of inoculum from all presumptive-positive violet red bile agar petri dishes was inoculated into tubes containing 5 ml of Brilliant Green Lactose Bile broth with inverted Durham tubes. These tubes were incubated at 37°C for 24 hours. Observations were made for gas formation in the Durham tubes. Any positive Brilliant Green Lactose Bile broth tubes were considered positive for coliform confirmation [44].

Thermotolerant Coliform Count: Approximately 5 ml of tryptone soya agar previously cooled to 45.0 ± 1.0 °C was added to appropriately labeled petri dishes and Pre-incubated for 1-2 hours at 20 – 25 °C (room temperature) then covered with 10 - 15 ml of violet red bile agar at a temperature of 45.0 ± 1.0 °C. One ml aliquots from each dilution (10^{-2},10^{-3}, 10^{-4} , 10^{-5}) was aseptically transferred to each petri dish and incubated at 44.5°C for 24 hours. The colonies were counted using a colony counter. Subsequently, a confirmatory test for fecal coliforms was done. Five colonies were taken from each presumptive-positive violet red bile agar petri dish and inoculated into tubes containing 5 ml of EC broth with inverted Durham tubes. These tubes were incubated at 44.5°C for 24 hours. Confirmation was obtained by gas production and turbidity [44].

The number (N) of CFU/ml of test sample was calculated as follows:

$$N(CFU/ml) = \sum \frac{C}{V\ (n1\ +\ 0.1n2)\quad d}$$

Where: C: sum of colonies on all plates count

V: The volume applied to each plate

n1: The number of plates counted at the first dilution

n2: The number of plates counted at the second dilution

d: The dilution from which the first count obtained

Finally, the results obtained from analysis was rounded off to two significant figures and expressed as a number between 1.0 and 9.9 multiplied by 10x, where x is the appropriate power of 10 [45].

E. coli: Samples from positive test tubes for fecal coliform were inoculated onto nutrient broth (Oxoid ltd, Basingstoke, England) and incubated at 44.4 degrees Celsius for 24 hours. After 24 hours, a drop of Kovacs reagent (HiMedia Laboratories Pvt. Ltd, Mumbai, India) was added. The formation of a red ring was used as proof for Indole positivity and hence for the presence of *E. coli* [44].

S. aureus: An appropriate dilution of a 0.1 mL aliquot was spread-plated in duplicate on pre-solidified plates of Mannitol Salt Agar (HiMedia Laboratories Pvt. Ltd, Mumbai, India) and incubated at 37°C for 24 hours. Yellow colonies on Mannitol Salt Agar plates were counted, calculated, and picked aseptically, then transferred to 5 mL nutrient broth for further purification and incubated at 37°C for 24–36 hours. To obtain distinct colonies, a loop of culture from the nutrient broth was streaked on the pre-solidified surface of nutrient agar and incubated at 37°C for 24–36 hours. Finally, the distinct colonies were characterized using the gram staining microbiological method. Gram-positive cocci with a clustered arrangement under the microscope were subjected to catalase, coagulase, and hemolysis tests for confirmation [46].

***Salmonella and Shigella* Spp:** Pre-enrichment (buffered peptone water) (HiMedia Laboratories Pvt. Ltd, Mumbai, India) was used and incubated at 37°C for 24 hours to diminish the risk of obtaining false-negative results. From each pre-enriched sample, one ml was transferred into tubes containing 10 ml of Rappaport Vassiliadis broth (HiMedia Laboratories Pvt. Ltd, Mumbai, India) and thoroughly *mixed* for two minutes. Following mixing, the tubes were incubated at 41.5°C for 48 hours. A loopful of culture from the Rappaport Vassiliadis broth was streaked onto Xylose Lysine Deoxycholate (XLD) (Oxoid LTD, Basingstoke, Hampshire, England) agar and incubated at 37°C for 24 hours for *Salmonella* spp and Shigella spp.

Typical colonies which had a slightly transparent zone of reddish color and a black center, and a pink-red zone surrounding the colonies, were considered as presumptive *Salmonella* spp, and red colonies were considered as presumptive *Shigella*. Presumptive colonies from XLD agar were picked aseptically, streaked onto nutrient agar for purification, and incubated at 37°C for 24 hours. Pure colonies were transferred aseptically into TSB slants as stock cultures and stored at 4-5°C in the refrigerator. The pure cultures were then subjected to biochemical tests like the Citrate utilization test, Motility test, Lactose fermentation, H2S gas production test, Lysine Iron agar test, MR-VP test, and Urea hydrolysis test [47].

3.10.2 Antimicrobial Susceptibility Testing for *E. coli*, *Salmonella* Spp, *shigella.* and *S. aureus*

Antimicrobial susceptibility testing for *E. coli*, *Salmonella* spp., *shigella* and *S. aureus* was performed on Mueller Hinton Agar (MHA) (Oxoid LTD, Basingstoke, Hampshire, England) plates following the standardized disk diffusion techniques.

To determine the drug susceptibility pattern of these isolates, the following 14 commonly prescribed drugs were used: Ampicillin (10 g), Amoxicillin clavulate (20/10µg), gentamicin (10 g), chloramphenicol (30 g), tetracycline (30 g), Ceftazidime (30 µg), erythromycin (15g), sulphamethoxazole-trimethoprim (25 g), Norfloxacin, Nitrofurantoin, ciprofloxacin (5g), oxacillin (10 g), Meropenem (10µg) and penicillin (10 g). *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used as reference strains for quality control of the antibiotics used [48]. The zone of inhibition was measured to the nearest millimeter and isolates

were classified as sensitive, intermediate and resistant according to the standardized table supplied by CLSI [49].

3.10.3 Extended-spectrum beta-lactamase e*nterobacteriaceae* detection

Extended-spectrum beta-lactamase (ESBL) production was analyzed according to CLSI guidelines for those *E. coli* isolates that showed reduced susceptibility or an inhibition zone of ≤ 22 millimeters for Ceftazidime (30µg), and it was considered as potential beta-lactamase producers and confirmed by the combination disc method according to CLSI [49].

Combined disk (double disk potentiate) Test (CDT)

Ceftazidime(30µg) was placed with ceftazidime with clavulanic acid (30 µg/10 µg) on an MHA plate inoculated with bacterial suspension of 0.5 McFarland standard, then incubated at 37^0c for 18 hrs. An increase in inhibition zone of diameter of > 5 mm or 50% increment for a combination disc against ceftazidime disc alone was confirmed as extended spectrum beta-lactamase producer.

3.10.4 Methicillin Resistance *Staphylococcus aureus* (MRSA)

Disk diffusion test method was performed for all isolates of *S. aureus* using 30 µg cefoxitin disk on bacterial suspension (0.5Mcfraland standard) inoculated into MHA plat and incubated at 37^oc for 18 hrs. A zone of inhibition < 21 mm diameter was interpreted as methicillin-resistant (MRSA) according to the CLSI guideline [50].

3.11 Data Quality Assurance

All requisite procedures and protocols were adhered to in accordance with the manufacturer's guidelines. Overall, comprehensive safety measures encompassing pre-analytical, analytical, and post-analytical phases were implemented to ensure the accuracy and precision of the test outcomes

3.11.1 Pre- analytical phases

The juice samples that were collected were inspected to ensure they were labeled correctly. Standard operating procedures were routinely applied during the collection, transportation, preparation, and storage of each sample.

3.11.2 Analytical phases

The methods were assessed with known negative and positive control materials. Then the test was performed based on SOP.

3.11.3 Post analytical phases

The data were documented on a registration format sheet, which included the identification number of each participant, and the results were subjected to a verification process prior to their release for further analysis.

3.12 Data Entry and Analysis

The data were analyzed using SPSS version 27, which was then cleaned and verified. The Kruskal-Wallis H test was used to compare the medians among types of juice. Moreover, bivariate and multivariate logistic regression analyses were utilized to ascertain the risk factors linked to the contamination of ready-made juices. The model's goodness of fit was evaluated using the Hosmer and Lemeshow test. The variables that had a p-value below 0.25 in the bivariate logistic regression analysis were subjected to a multivariate logistic regression analysis.

The Adjusted Odds Ratio (AOR) was computed at a 95% confidence level to show the strength of the associations identified. A significance level of 95% CI, with a P-value below 0.05, indicates statistical significance. The output of the data was organized and presented using texts, tables, and figures.

3.13 Operational Definitions

Bacteriologically safe juice: refers to juice that is free from harmful bacteria and safe for consumption.

Gulf standards: for bacterial levels, above which the presence of bacteria results in health hazards, causing spoilage of fruit juices and leading to forborne illnesses.

Satisfactory: Results within or below the expected microbiological thresholds (total viable bacterial count ≤ 5 x 10^3 CFU/ml, total coliforms ≤ 10 CFU/ml, absence of fecal coliforms and *E. coli*, Staphylococcus ≤ 100 CFU/ml, and absence of *Salmonella/Shigella* in 25 ml of sample) pose no food safety concern and indicate good microbiological quality [51].

Unsatisfactory: The results exceed the expected microbiological limits (total viable bacterial count > 1.0 x 10^4 CFU/ml, total coliforms > 100 CFU/ml, presence of fecal coliforms and *E. coli,* Staphylococcus > 1.0 x 10^3 CFU/ml, and presence of *Salmonella/Shigella* in 25 ml of sample), indicating poor food handling practices. Therefore, corrective actions are necessary to restore proper food hygiene or safety [52].

Indicator organisms: non-pathogenic but are used as indicators to assess the presence of pathogenic microorganisms.

3.14 Ethical considerations

Before conducting the research, ethical clearance was obtained from the Department of Research and Ethical Review Committee (DRERC) of the Department of Medical Laboratory Science, AAU, Protocol number: DRERC/749/24/MLS/. A support letter was also obtained from the Food and Drug Administration (FDA) of Yeka sub-city. The purpose of the research was then clearly explained by the principal investigator to the roadside juice vendors in Yeka Sub-City, Addis Ababa. Written consent was obtained from each individual participant prior to conducting the study. Participants were informed that their results were confidential and not exposed to others.

3.15 Dissemination of the result

The results of the study will be conveyed to Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences. Additionally, the findings will be shared with Food and Drug Administration (FDA) of Yeka sub-city, Addis Ababa, Ethiopia. Furthermore, the study results will be submitted for publication in a national or international peer-reviewed journal.

4 Results

4.1 Socio demographic characteristics of study participants

A total of 63 juice vending facilities from the Yeka sub-city of Woreda 1, 2, 5, and 9 were recruited for the study. Among 63 respondents, 49 (76.2%) were high school and above in their educational status, 56 (88.9%) of them being within the age range of < 35 years. All of the 63 participants were females (Table 1).

Table 1: Sociodemographic characteristics of study participants in Yeka sub-city, Addis Ababa, Ethiopia. 2024 (n=63).

Parameter	Category	Frequency (%)
Sex of respondents	Male	0
	Female	63(100%)
Age of respondents	< 35	56 (88.9%)
	≥ 35	7(11.1%)
Educational status of respondents	No formal education	0
	Primary school	15(23.8%)
	High school and above	49(76.2%)

4.2 Hygiene and handling practice of the study participants

Among the 63 vendors, shelves were employed as temporary storage for fruits, while refrigerators served as short-term storage for the squeezed fruit juice. All respondents indicated that they used tap water for washing utensils and diluting juice, without any form of water treatment. Notably, 60 (95.2%) of the food handlers prepared fruit juice with bare hands, and only 3 (4.8%) utilized kitchen gloves to protect against contamination. In terms of personal protective equipment, 49 (77.8%) of the juice makers wore aprons, while 14 (22.2%) did not. Additionally, 18 (28.6%) of the makers did not cover their hair (Table 2).

In terms of hand hygiene practices, 47/63 (74.6%) respondents used only water for cleaning their hands, while 16/63 (25.4%) of them utilized both water and soap. The frequency of hand washing was noted, with 53/63 (84.1%) respondents washing their hands either before or after food preparation, and 10/63 (15.9%) respondents doing so both before and after. Furthermore, 28/63 (44.4%) respondents indicated that they regularly washed utensils either before or after use.

Regarding the protection of vending houses from sunlight and dust, 44/63 (69.8%) respondents reported adequate protection, whereas 19/63 (30.2%) respondents did not. Additionally, 17 juice houses (27%) were classified as congested, while 46 (73%) were not. Notably, 54 vending houses (85.7%) had handwashing facilities located near or within the kitchen, in contrast to 9 houses (14.3%) that lacked such facilities (Table 2).

Table 2: Hygiene and handling practice of fruit juice houses and juice makers in Yeka sub-city, Addis Ababa, Ethiopia. 2024 (n=63).

Parameter	Category	Frequency (%)
Work experience	<=1 year	46(73%)
	2-4 years	16(25.4%)
	>4 years	1(1.6%)
Frequency of washing utensils	Once	28(44%)
	Pre and post preparation	7(11.1%)
	Either pre or post preparation	28(44%)
Hand washing facilities near or inside the kitchen	Presence	9(14.3%)
	Absence	54(85.7%)
Frequency of washing hand	Either before or after each preparation	53(84.1%)
	Both before and after each preparation	10(15.9%)
Use of kitchen gloves	Yes	3(4.8%)
	No	60(95.2)
Cleaning agent used in hand After toilet	Water only	16(25.4%)
	water with Soap	47(74.6%)
Protections of vending sell from sun, dust and wind	yes	44(69.8%)
	No	19(30.2%)
Clothing of juice makers	Wear apron	49(77.8%)
	Didn't wear apron	14(22.2%)
Waste disposal	Plastic bag	21(33.3%)
	Bun with cover	42(66.7%)
Type of juice house	Congested	17(27%)

	Non congested	46(73%)
Hair cover	covered	45(71.4%)
	Uncovered	18(28.6%)
Finger nail size	Short and appropriate	63(100%)
	Long	0
Place to store prepared juice	Refrigerator	63(100%)
	Squeezing machine	0

4.3 Factors associated with the contamination of pathogenic bacteria in juice samples

Table 3 demonstrates the bivariate and multivariate logistic regression analyses used to identify the independent predictors of pathogenic bacteria contamination in juice samples. The study highlights several key hygiene-related factors associated with bacterial contamination of the juice samples.

The absence of hand-washing facilities near or inside the kitchen, with 5.34 times higher odds of *Salmonella* detection compared to their presence (AOR = 5.34, 95% CI: 1.06–26.81, p = 0.002). Adequate handwashing practices, where washing hands both before and after food preparation, significantly reduce the odds of *Salmonella* contamination, with an AOR indicating a 93% reduction in risk compared to washing hands only before or after (AOR = 0.07, 95% CI: 0.01–0.15, p < 0.001). The use of soap after toilet use also reduces the odds of *Salmonella* detection by 87% compared to using water alone (AOR = 0.127, 95% CI: 0.028–0.57, p < 0.001).

Moreover, the study finds a significant association with *S. aureus* detection; individuals who didn't use hair cover are about 2.9 times more likely to experience food contamination compared to those who use hair cover (AOR = 2.9, 95% CI: 1.2-7.1, p = 0.016) (Table 3).

Table 3: Multivariate logistic regression showing factors associated with the contamination of juice samples in Yeka sub-city, Addis Ababa, Ethiopia. 2024 (n=189).

Parameters	Category	*S. aureus* N (%)		AOR (95% CI) p-value	*E. coli* N (%)		AOR (95% CI) p-value	*Salmonella* N (%)		AOR (95% CI) p-value
		Pos	Neg		Pos	Neg		Pos	Neg	
Hand washing facilities near or inside the kitchen	Presence	70 (81.4%)	90 (87.4%)	1	11 (20%)	18 (13.4%)	1	6 (46.2%)	154 (87.5%)	1
	Absence	16 (18.6%)	13 (12.6%)	1.4 (0.5-3.6) 0.44	44 (80%)	116 (86.6%)	1.7 (0.66-4.4) 0.26	7 (53.8%)	22(12.5 %)	**5.34 (1.06-26.81) 0.002***
Frequency of washing hand at the time of preparations	Either before or after	73 (84.9%)	86 (83.5%)	1.5(0.6-3.8) 0.39	46 (83.6%)	113 (84.3%)	0.9(0.35-2.7) 0.9	10 (76.9%)	20 (11.4%)	1
	Both before and after	13 (15.1%)	17 (16.5%)	1	9 (16.4%)	21 (15.7%)	1	3 (23.1%)	156 (88.6%)	**0.07(0.01-0.15) <0.001***
Cleaning agent used to wash hand after toilet	Water only	66 (76.7%)	75 (72.8%)	0.91(0.43-1.9) 0.81	46 (83.6%)	95 (70.9%)	0.5(0.21-1-1.26) 0.15	9 (69.2%)	39 (22.2%)	1

	water with Soap	20 (23.3%)	28 (27.2%)	1	9 (16.4%)	39 (29.1%)	1	4 (30.8%)	137 (77.8%)	**0.127(0.028-0.57) <0.001***
Protections of vending sell from sun, dust and wind	yes	59 (68.6%)	70 (70.9%)	0.98(0.22-4.01) 0.9	39 (70.9%)	93 (69.4%)	0.9(0.4-2.02) 0.8	3 (23.1%)	129 (73.3%)	2.6(0.35-20.2) 0.33
	No	27 (31.4%)	30 (29.1%)	1	16 (29.1%)	41 (30.6%)	1	10 (79.9%)	47(26.7%%)	1
Clothing of juice makers	Wear apron	69 (80.2%)	78 (75.7%)	0.46 (0.17-1.2) 0.12	42 (76.4%)	105 (78.4%)	0.8(0.3-2.3) 0,74	9 (69.2%)	138 (78.4%)	0.9 (0.08-12.2) 0.99
	Didn't wear apron	17 (19.8%)	28 (24.3%)	1	13 (23.6%)	29 (21.6%)	1	4 (30.8%)	38 (21.6%)	1
Waste disposal	Plastic bag	59 (68.6%)	67 (65%)	0.97(0.5-1.9) 0.94	41 (74.5%)	85 (63.4%)	0.6(0.3-1.4) 0.31	4 (30.8%)	122 (69.3%)	1.7(0.29-9.9) 0.5
	Bun with cover	27 (31.4%)	36 (35%)	1	14 (25.5%)	49 (36.6%)	1	9 (69.2%)	54 (30.7%)	1
Type of juice house	Congested	30 (34.9%)	21 (20.4%)	0.49(0.24-1.01) 0.06	13 (23.6%)	38 (28.4%)	1.5(0.68-3.3) 0.3	3 (23.1%)	48 (27.3%)	4.7(0.48-4.5) 0.18

	Non-congested	56 (65.1%)	82 (79.6%)	1	42 (76.4%)	96 (71.6%)	1	10 (79.2%)	128 (72.7%)	1
Hair cover	Uncovered	29 (33.7%)	25 (24.3%)	**2.9(1.2-7.1) 0.016***	19 (34.5%)	35 (26.1)	1	4 (30.8%)	50 (28.4%)	1
	covered	57 (66.3%)	78 (75.5%)	1	36 (65.5%)	99 (73.9%)	1.6(0.68-4.1) 0.25	9 (69.2%)	126 (71.6%)	0.3(0.27-3.4) 0.34

Abbreviations: AOR=Adjusted odds ratio; **CI**=Confidence Interval; **Pos** = Positive; **Neg** = Negative and **1**= reference groups. Statistically significant level (p-value < 0.05, 2-tailed).

4.4 Aerobic colony count (ACC), Coliform Count (CC), Thermotolerant Coliform Count (TCC) and staphylococcal count (SC) of Fruit Juice Samples

From a total of one hundred eighty-nine (189) samples of locally prepared fresh fruit juices, the median aerobic colony count (ACC) was found to be highest in avocado juice, at 9.74×10^6 CFU/ml. The media values of ACC for avocado, papaya, and mixed (spris) juices were 9.74×10^6 CFU/ml, 4.98×10^6 CFU/ml, and 6.7×10^6 CFU/ml, respectively. The differences in the median of ACC among the various fruit juice types were not statistically significant (P =0.171) (Table 4).

The median total coliform counts for avocado, papaya, and mixed (spris) juice were determined to be 4.28×10^6 CFU/ml, 2.76×10^6 CFU/ml, and 1.36×10^6 CFU/ml, respectively. The analysis showed that there were no statistically significant differences in the median total coliform counts across the different fruit juice types (P = 0.106). The median thermotolerant coliform (TCC) counts for avocado, papaya, and mixed (spris) juices were 3.5×10^4 CFU/ml, 4.17×10^4 CFU/ml, and 1.16×10^5 CFU/ml, respectively. Each juice type exhibited a minimum count of less than 1 CFU/ml. Additionally, the median TCC did not present significant differences among the fruit juices (P = 0.31).

Furthermore, the overall median *staphylococcal* count for all samples was determined to be 2.2×10^3 CFU/ml. In particular, the median *staphylococcal* counts for avocado, papaya, and mixed (spris) juice were recorded at 3.08×10^3 CFU/ml, 2.01×10^3 CFU/ml, and 1.73×10^3 CFU/ml, respectively. The lowest counts were noted in all sample types, which were less than 1 CFU/ml. The analysis revealed that there were no statistically significant differences in median *Staphylococcal* counts among the different types of fruit juices (P = 0.11), as shown in Table 4.

The sub-analysis also indicated the overall maximum, minimum, and median values for aerobic colony count, coliform count, thermotolerant coliform count, and *staphylococcal* count of avocado, papaya, and mixed (spris) juices within each woreda of the study site, as illustrated in the table below (Table 5).

Table 4: Overall microbial distribution (maximum, minimum, and median) of locally prepared fresh fruit juice samples in Yeka sub-city, Addis Ababa, Ethiopia. 2024 (n=189)

Bacterial count	Status	Bacteriological loads of fruit juice samples			
		Avocado	Papaya	Mixed (spris)	All samples
ACC (CFU/ml)	Minimum	4.2×10^4	4.2×10^3	6.4×10^2	6.4×10^2
	Maximum	1.80×10^8	1.5×10^8	2.6×10^8	2.6×10^8
	Median	9.74×10^6	4.98×10^6	6.7×10^6	7.14×10^6
	P-value	0.171			
CC (CFU/ml)	Minimum	4.4×10^2	2.13×10^2	3×10^2	2.13×10^2
	Maximum	1.03×10^8	1.1×10^8	3.4×10^7	1.1×10^8
	Median	4.28×10^6	2.76×10^6	1.36×10^6	2.8×10^6
	P-value	0.106			
TCC (CFU/ml)	Minimum	< 1	< 1	< 1	< 1
	Maximum	6.1×10^5	5.1×10^5	5.2×10^6	5.2×10^6
	Median	3.5×10^4	4.17×10^4	1.16×10^5	6.4×10^4
	P-value	0.31			
SC (CFU/ml)	Minimum	< 1	< 1	< 1	< 1
	Maximum	3.3×10^4	9.9×10^3	8.7×10^3	3.3×10^4
	Median	3.08×10^3	2.01×10^3	1.73×10^3	2.2×10^3
	P-value	0.11			

Abbreviations: ACC= Aerobic colony count; **CC=**Coliform Count; **TCC**=Thermotolerant coliform count; **SC** = Staphylococcal count and **CFU**=Colony forming Unit. **Kruskal-Wallis H test** was used to compare the medians and statistically significant at p-value <0.05.

Table 5: Microbial distribution (maximum, minimum, and median) of locally prepared fresh fruit juice samples by study sites and types of fruit juices in Yeka sub-city, Addis Ababa, Ethiopia. 2024 (n=189)

Bacterial count	Status	Bacteriological loads of fruit juice samples											
		Woreda 1			Woreda 2			Woreda 5			Woreda 9		
		Avo	pa	Mix	Avo	pa	Mix	Avo	Pa	Mix	Avo	Pa	Mix
ACC (CFU/ml)	Mini	1.68 $\times10^5$	2.04 $\times10^5$	6.4 $\times10^2$	2.5 $\times10^5$	1.34 $\times10^5$	8 $\times10^3$	1.16 $\times10^5$	8 $\times10^4$	1.68 $\times10^4$	4.2 $\times10^4$	4.2 $\times10^3$	2.4 $\times10^4$
	Max	6.1 $\times10^5$	8 $\times10^5$	4.04 $\times10^5$	3.8 $\times10^6$	9.68 $\times10^5$	1.5 $\times10^7$	1.8 $\times10^8$	1.5 $\times10^8$	2.6 $\times10^7$	2.1 $\times10^6$	4.8 $\times10^6$	3.2 $\times10^6$
	Median	3.52 $\times10^5$	4.05 $\times10^5$	2.32 $\times10^5$	9.74 $\times10^5$	5.94 $\times10^5$	1.8 $\times10^6$	3.2 $\times10^7$	1.55 $\times10^7$	2.25 $\times10^7$	5.14 $\times10^5$	8.82 $\times10^5$	5.1 $\times10^5$
CC (CFU/ml)	Min	4.4 $\times10^2$	2.5 $\times10^4$	3 $\times10^2$	8.2 $\times10^4$	3.6 $\times10^4$	1.9 $\times10^3$	1.04 $\times10^5$	1.14 $\times10^4$	1.04 $\times10^4$	8.2 $\times10^3$	2.13 $\times10^2$	6.860 $\times10^3$
	Max	1.08 $\times10^5$	3.32 $\times10^5$	3.8 $\times10^4$	6.48 $\times10^5$	7.71 $\times10^5$	1.05 $\times10^7$	1.03 $\times10^8$	1.1 $\times10^8$	3.4 $\times10^7$	6.6 $\times10^5$	2.82 $\times10^6$	5.3 $\times10^5$

	Median	3.35 $\times10^4$	1.09 $\times10^5$	2.3 $\times10^4$	3.36 $\times10^6$	3.6 $\times10^5$	1.13 $\times10^6$	1.44 $\times10^7$	8.92 $\times10^6$	4.0 $\times10^6$	2.08 $\times10^5$	2.66 $\times10^5$	1.75 $\times10^5$
TCC (CFU/ml)	Mini	< 1	< 1	1.50 $\times10^2$	< 1	< 1	< 1	<1	< 1	< 1	<1	< 1	< 1
	Max	1.26 $\times10^5$	3.2 $\times10^5$	2.2 $\times10^5$	6.1 $\times10^5$	3.04 $\times10^5$	5.2 $\times10^6$	2.01 $\times10^5$	5.1 $\times10^5$	1.6 $\times10^5$	2.1 $\times10^5$	5.6 $\times10^4$	1.2 $\times10^5$
	Median	3.16 $\times10^4$	1.34 $\times10^5$	6.35 $\times10^4$	1.09 $\times10^5$	7.22 $\times10^4$	5.34 $\times10^5$	2.84 $\times10^4$	6.22 $\times10^4$	4.3 $\times10^4$	1.19 $\times10^4$	5.86 $\times10^3$	1.46 $\times10^4$

Abbreviations: **ACC**=Aerobic colony Count; **CFU**=Colony forming: **CC**=Coliform Count; **TCC**=Thermotolerant colony count; **Min**=Minimum; **Max**=Maximum; **Avo**=Avocado; **Pa**=Papaya: **Mix**=Mixed.

4.5 Bacteriological status of locally prepared fresh fruit juices

The findings indicate that 186 fruit juice samples, or 98.4%, had ACC that exceeded $1.0x10^4$ CFU/ml, which is considered unsatisfactory. Additionally, 178 samples, representing 94.2%, had CC higher than $1.0x10^2$ CFU/ml, also deemed unsatisfactory. Furthermore, 79 samples, accounting for 41.8%, showed TCC above 0 CFU/ml, while 102 samples, or 54%, recorded SC exceeding $1.0x10^3$ CFU/ml, both classified as unsatisfactory (see figure 4).

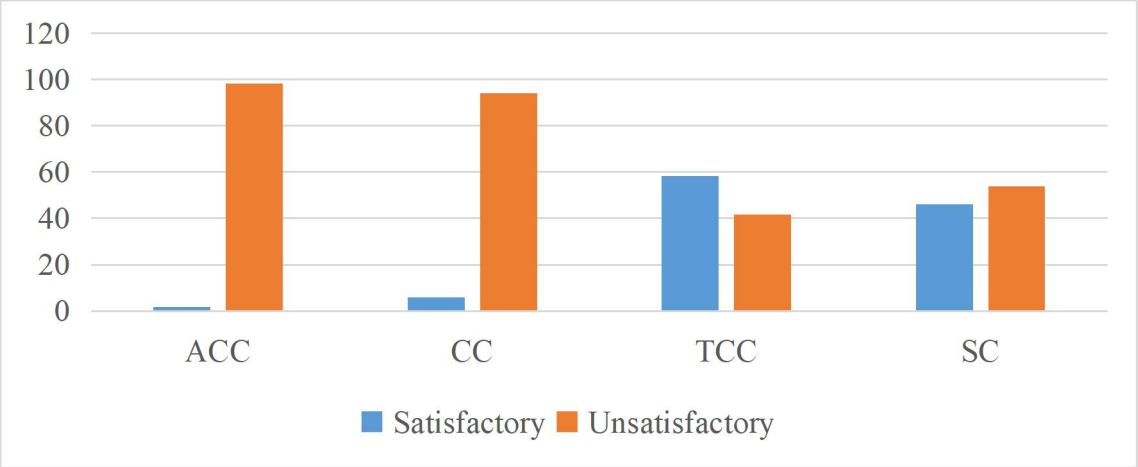


Figure 4: Bacteriological quality status (ACC, CC, TCC, and SC) of locally prepared fresh fruit juice samples in Yeka sub-city, Addis Ababa, Ethiopia. 2024. (Gulf reference standard used).

4.6 Occurrence of *E. coli*, *S. aureus, Salmonella* and *Shigella* in juice samples

Out of 189 juice samples, the highest percentage of *E. coli* was found from mixed (spris) samples, which was 34.9% (n=22/63), and also the lowest *E. coli* was isolated from papaya samples, which was 22.2% (n=14/63).

The investigation into the presence of *Salmonella* in the juice samples yielded positive results of 6.3% (n=4/63) for avocados, 7.9% (n=5/63) for papayas, and 6.3% (n=4/63) for mixed samples. In terms of *S. aureus*, the highest isolation rate was found in avocado samples at 49.2% (n=31/63), while papaya samples exhibited the lowest rate at 39.7% (n=25/63). In our study, no juice samples were positive for *Shigella* spp. In summary, the overall prevalence of *E. coli, S. aureus*, and *Salmonella* across 189 fruit juice samples was determined to be 29.1% (n=55/189), 45.5% (n=86/189), and 6.9% (n=13/189), respectively, as detailed in Table 6.

Table 6: Detection of *E. coli*, *S. aureus,* and *Salmonella* in juice samples in Yeka sub-city, Addis Ababa, Ethiopia. 2024 (n=189).

Sample Type	No of sample	Positive *E. coli*	Positive *S. aureus*	Positive *Salmonella*	Total positive *E. coli*	Total positive *S. aureus*	Total positive *Salmonella*
Avocado	63	19(30.2%)	31(49.2%)	4(6.3%)			
Papaya	63	14(22.2%)	25(39.7%)	5(7.9%)	55 (29.1%)	86(45.5%)	13(6.9%)
Mixed (Spris)	63	22(34.9%)	30(47.7%)	4(6.3%)			

4.7 Antibiotics susceptibility patterns of *S. aureus, E. coli* and *Salmonella* isolates

This analysis illustrated that bacteria were detected in 154 juice samples, which included 55 samples of *E. coli*, 86 samples of S. *aureus*, and 13 samples of *Salmonella,* out of 189 juice samples.

The isolated *S. aureus* were moderately resistant to tetracycline 51.2% (n=44/86), penicillin 90.7% (n=78/86), and oxacillin 66.3% (n=57/86). However, they were moderately susceptible to erythromycin 91.9% (n=79/86) and gentamycin 96.5% (n=83/86). Moreover, completely susceptible (100%, n=86/86) to ciprofloxacin, chloramphenicol, sulfamethoxazole-trimethoprim, nitrofurantoin, and norfloxacin.

The *E. coli* isolates demonstrated complete susceptibility (100%, n=55/55) to gentamicin, sulfamethoxazole-trimethoprim, norfloxacin, and nitrofurantoin. However, the isolates showed moderate resistance to ampicillin 58.2% (n=32/55), tetracycline 58.2% (n=32/55), ceftazidime 23.6% (n=13/55), chloramphenicol 9.1% (n=5/55), and ciprofloxacin 7.3% (n=4/55).

All *Salmonella* isolates tested were found to be fully resistant (100%, n=13/13) to ampicillin and tetracycline. However, they exhibited complete susceptibility (100%, n=13/13) to ciprofloxacin, chloramphenicol, sulfamethoxazole-trimethoprim, ceftazidime, and meropenem. Notably, norfloxacin and nitrofurantoin demonstrated moderate susceptibility, with both showing a rate of 84.6% (n=11/13) (Table 7).

Table 7: Antibiotic susceptibility pattern of *S. aureus, E. coli,* and *Salmonella* isolates from fruit juice samples in Yeka sub-city, Addis Ababa, Ethiopia. 2024 (n=154).

Antibiotics	***S. aureus* (N=86)**			***E. coli* (N=55)**			***Salmonella* (N=13)**		
	S (%)	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**
AMP (10µg)	NA	NA	NA	0 (0%)	23 (41.8%)	32(58.2 %)	0 (0%)	0 (0%)	13(100%)
PEN (10µg)	8 (9.3%)	0 (0%)	78 (90.7%)	NA	NA	NA	NA	NA	NA
AMC (20/10µg)	NA	NA	NA	33 (60%)	9 (16.4%)	13(23.6 %)	13(100 %)	0 (0%)	0 (0%)
T (30µg)	26(30.2%)	16(18.6%)	44(51.2%)	8(14.5 %)	15(27.3 %)	32(58.2 %)	0 (0%)	0 (0%)	13(100%)
GN (10µg)	83(96.5%)	0 (0%)	3 (3.5%)	55 (100%)	0 (0%)	0 (0%)	13(100 %)	0 (0%)	0 (0%)
CIP (5µg)	86 (100%)	0 (0%)	0 (0%)	50(90.9 %)	1(1.8%)	4(7.3%)	13(100 %)	0 (0%)	0 (0%)
SXT (25µg)	86 (100%)	0 (0%)	0 (0%)	55 (100%)	0 (0%)	0 (0%)	13(100 %)	0 (0%)	0 (0%)
NOR (10µg)	86 (100%)	0 (0%)	0 (0%)	55 (100%)	0 (0%)	0 (0%)	11(84.6%)	2(15.3 %)	0 (0%)
C30 (30µg)	86 (100%)	0 (0%)	0 (0%)	39(70.9 %)	11(20%)	5 (9.1%)	13(100 %)	0 (0%)	0 (0%)
NIT (50µg)	86 (100%)	0 (0%)	0 (0%)	55 (100%)	0 (0%)	0 (0%)	11(84.6%)	2(15.3 %)	0 (0%)
ERY (15µg)	79 (91.9%)	0 (0%)	7 (8.1%)	NA	NA	NA	NA	NA	NA
CAZ (30µg)	NA	NA	NA	38(68.9 %)	4 (7.5%)	13 (23.6%)	13(100 %)	0 (0%)	0 (0%)

MRP (10 µg)	NA	NA	NA	52(94.5 %)	3 (5.5%)	0 (0%)	13(100 %)	0 (0%)	0 (0%)
OX (10µg)	29 (33.7%)	0 (0%)	57(66.3%)	NA	NA	NA	NA	NA	NA

Abbreviations: AMP = Ampicillin, **AMC**=Amoxicillin clavulate, **PEN** = Penicillin, **CIP** = Ciprofloxacin, **T**=Tetracycline, **CAZ**=Ceftazidime, **C30**=Chloramphenicol, **NOR**=Norfloxacin, **GN**=Gentamycin, **NIT**=Nitrofurantoin, **SXT** = Sulphamethoxazole-trimethoprim, **E**=Erythromycin, **MRP**=Meropenem, **OX**=Oxicillin**, N**=Number of isolates, **R** = Resistant, **I** = Intermediate, **S** = Sensitive, **NA** = Not Applicable

4.7.1 The prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA)

The methicillin-resistant *Staphylococcus aureus* (MRSA) test was conducted by using the cefoxitin (30 µg) drug; from a total of 86 tested *S. aureus* isolates, 58.1% (n=50/86) of them were resistant to cefoxitin (30 µg), which is interpreted as MRSA by the CLIS standard of 2024.

4.7.2 The prevalence of Extended Beta Lactamase (ESBL) and Carbapenem's producing isolates

Extended Beta Lactamase (ESBL) tests were performed for 68 (55 *E. coli* and 13 *Salmonella*) isolates by the combination disc (CDT) method. In this study, 19.1% (n=13/68) of isolates were suspected of ESBL and tested and confirmed by using the phenotypic confirmatory combination disk method according to CLSI. The confirmation test shows, 21.82%. of *E. coli* (n=12/55) isolates were ESBL producers (Figure 5), and no *Salmonella* isolates showed ESBL production. In this study none of the isolates exhibit resistance to meropenem (10 µg), but three isolates were intermediate.

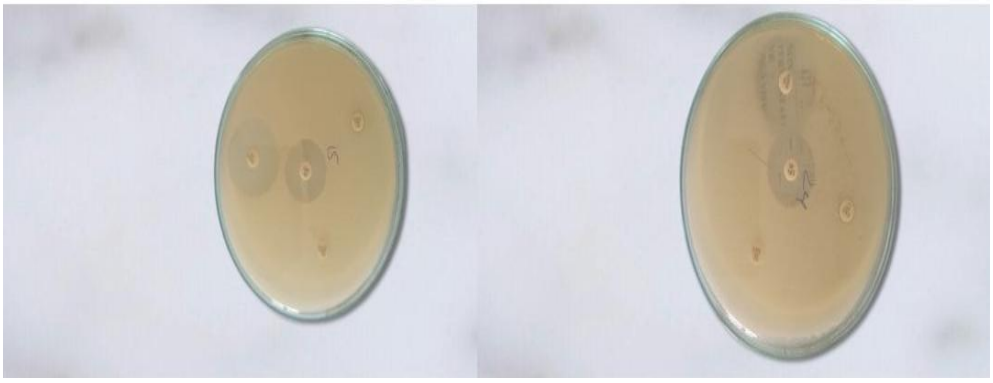


Figure 5: ESBL positive *E. coli* using combination disk method isolated from fruit juices in Yeka sub-city, Addis Ababa, Ethiopia. 2024.

4.8 Prevalence of multi-drug resistance (MDR) isolates

Among the total isolates (n=154), multidrug resistance was recorded in 28.57% (n=44/154). The isolated *S. aureus, E. coli*, and *Salmonella* isolates showed 41.9% (n = 36/86), 10.9% (n = 6/55), and 15.38% (n = 2/13) multi-drug resistance, respectively (Table 8).

Table 8: Distribution of muti-drug resistant isolates from fruit juices in Yeka sub-city, Addis Ababa, Ethiopia. 2024 (n=154).

Bacteria strain (N)	**R0**	**R1**	**R2**	**R3**	**R4**	**R5**	**MDR Status**
S. aureus (86)	1 (1.16%)	30 (34.88%)	19 (22.09%)	26 (30.23%)	10 (11.63%)	0	R4
E. coli (55)	0	23 (41.82%)	26 (47.27%)	3 (5.45%)	2 (3.6%)	1 (1.82%)	R5
Salmonella (13)	0	0	11 (84.62%)	2 (15.38%)	0	0	R3
Total	1 (1.12%)	53 (59.55%)	56 (62.9%)	31 (34.83%)	12 (13.48%)	1 (1.12%)	

Note: **R0** = Sensitive, **R1**=Resistance for one antibiotic, **R2**=Resistance for two antibiotics **R3**=Resistance for three antibiotics, **R4**=Resistance for four antibiotics, **R5**=Resistance for five.

For *S. aureus,* although the isolates resistance patterns varied, resistance to penicillin (10 µg), oxacillin (10 µg), and tetracycline (30 µg) was commonly observed. In the case of Gram-negative bacteria such as *E. coli* and *Salmonella,* resistance to tetracycline (30 µg) and ampicillin (10 µg) was frequently observed (Figure 6 and 7).

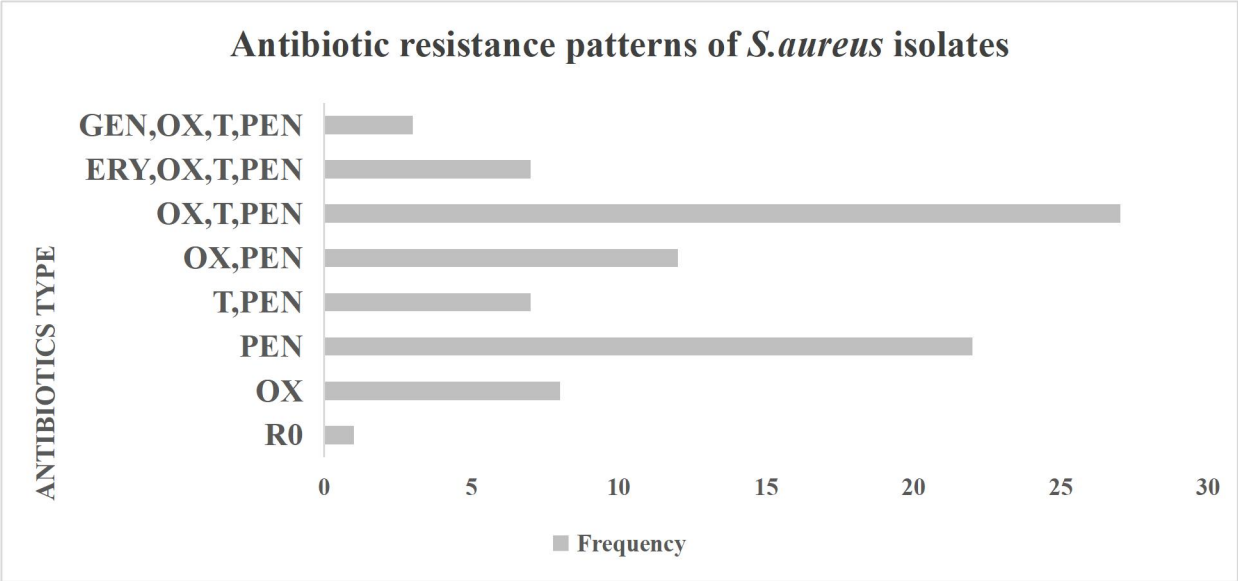


Figure 6: Distribution of MDR *S. aureus* by drug types isolated from fruit juices in Yeka sub-city, Addis Ababa, Ethiopia. 2024. **Abbreviations:PEN** = Penicillin**, T**=Tetracycline, **GN**=Gentamycin**, E**=Erythromycin, **OX**=Oxicillin.

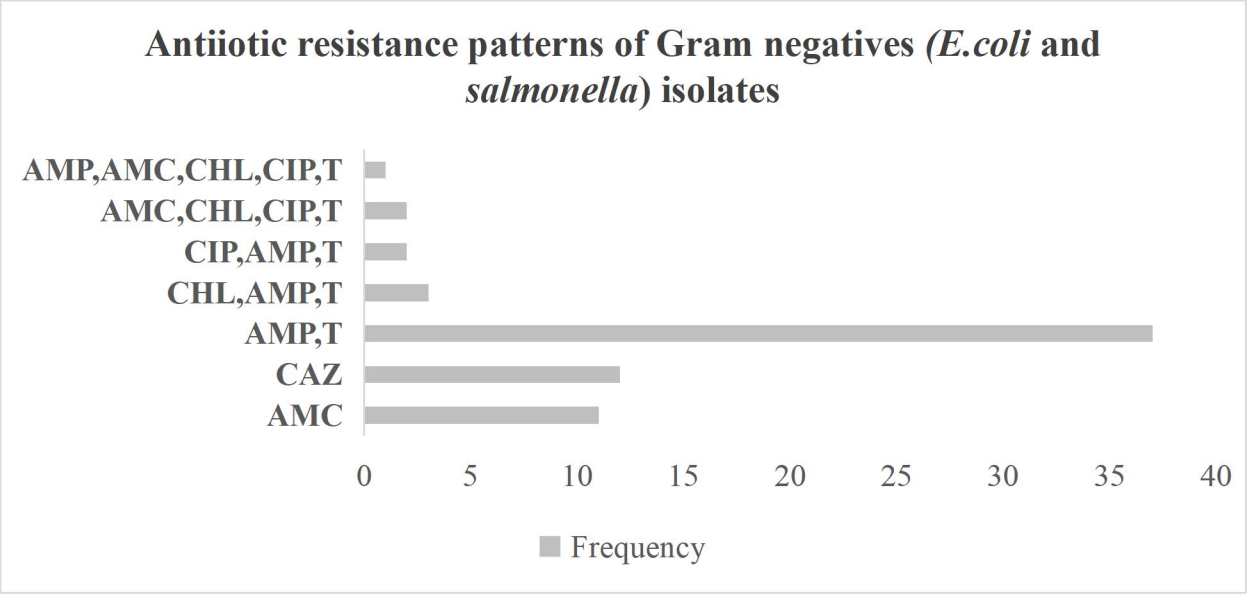


Figure 7: Distribution of MDR Gram negative isolated from fruit juices in Yeka sub-city, Addis Ababa, Ethiopia. 2024. **Abbreviations: AMP** = Ampicillin, **AMC**=Amoxicillin clavulate, **CIP =** Ciprofloxacin, **T**=Tetracycline, **CAZ**=Ceftazidime, **CHL**=Chloramphenicol**.**

5 Discussion

Fruit juices are highly nutritious and convenient, requiring no cooking, making them ideal for modern lifestyles. However, they can become vehicles for harmful pathogens if safety and hygiene are not maintained, potentially causing foodborne outbreaks globally [31]. The rise in antimicrobial resistance (AMR) among foodborne pathogens underscores the need for improved knowledge, attitudes, and practices (KAP) among manufacturers to ensure juice safety [12].

In our study, all of the study participants were female, which aligns with findings from similar studies conducted in Jimma, Shewarobit, and Mekelle, Ethiopia [12, 30, 52]. These results reflect traditional and cultural roles of women in food and beverage preparation. The majority of respondents were under 35 years old, more likely due to migration patterns, where adults move to urban areas like Addis Ababa in search of better job opportunities. Notably, 76.2% of the respondents had completed high school or higher education, while the percentage of individuals with no formal education was comparatively lower than findings reported in similar studies in Ethiopia [53].

All of the vendors were seen to use refrigerators and shelves to temporarily store squeezed juice and fruits, respectively. Juice quality can be preserved by refrigeration, but incorrect handling and inconsistent hygiene standards can negate these advantages by introducing impurities during preparation and storage. The sub-city does not have a continuous water supply of tap water. Due to this, stored water is commonly used to wash and prepare juices. Using untreated water to wash utensils and dilute juice was one of the main problems found. A major cause of food borne illnesses, microbial contamination, is a real risk when water treatment measures are not implemented. This finding aligns with studies indicating that untreated water is a major source of contamination in food preparation environments, particularly in low-resource settings [33].

The use of personal protective equipment (PPE) among vendors was minimal, with only 4.8% wearing gloves, although 77.8% reported wearing aprons. This is concerning, as bare-hand contact with food has been strongly associated with the transmission of food borne pathogens, such as *S. aureus*, *Escherichia coli*, and *salmonella* [54]. Hair covering was also inadequate, as

28.6% of vendors did not use hair covering, which raises the possibility of physical contamination [55, 56].

The investigation revealed that the use of soap after toilet use significantly reduces the odds of *Salmonella* detection by 87% relative to the use of water alone (AOR = 0.127, 95% CI: 0.028–0.57, $p < 0.001$). This result is consistent with studies carried out in Bahir Dar Town and eastern Ethiopia [31, 33]. In addition, washing hands both before and after food preparation significantly lowers the likelihood of *Salmonella* detection (AOR = 0.07, 95% CI: 0.01–0.15, $p < 0.001$) compared to washing hands only before or after food preparation. These findings emphasize the importance of enhanced training about keeping sanitation habits.

Infrastructure-related issues have further heightened the risks of contamination. The absence of hand-washing stations near kitchens is associated with a 5.34-fold increase in the likelihood of *Salmonella* contamination (AOR = 5.34, 95% CI: 1.06–26.81, $p = 0.002$). Additionally, while 81% of vending houses were adequately protected from environmental factors such as sun and dust, 19% were not. This observation diverges from results reported in other studies conducted in Addis Ababa and Mekelle, Ethiopia [52, 53].

In our study, the total bacterial load in juice samples was indicated by aerobic colony count (ACC), with a median of 7.14×10^6 CFU/ml. However, in Eastern Ethiopia, a study found lower microbial loads, with an average of 2.4×10^5 CFU/ml, which is relatively lower than our finding [33]. It was found that 98.4% of juice samples had bacterial counts that were above the Gulf standard of 1.0×10^4 CFU/ml. This finding aligns with the results observed in Bahir Dar Town, where 96.7% of samples exceeded the standard [31]. In addition to this, our study is comparable with 100% prevalence recorded in Hossaena [57].

The results of this study indicated that total coliform counts ranged from 2.13×10^2 CFU/ml to 3.4×10^7 CFU/ml, with a median value of 2.8×10^6 CFU/ml, which is higher than the results of a similar investigation in Jessore, Bangladesh [58]. A striking 94.2% of the juice samples exceeded the Gulf Standard limit of 1.0×10^2 CFU/ml, in contrast to the 64.1% observed in Eastern Ethiopia [33]. This variation may stem from poor hygiene practices, which can lead to the contamination of fruits and juices by harmful microorganisms, thus threatening consumer health.

The Gulf Standard of 2000 states that food containing faecal coliforms is dangerous and should not be consumed. However, this study found that 41.8% of fruit juice samples (79 samples) were contaminated with faecal coliform with a median value of 6.4×10^4 CFU/ml. This is lower than findings from Dar es Salaam, Tanzania [59]. The observed variations may be due to poor hygiene, quality of raw materials, or inadequate storage conditions. No significant difference in FC count between sample types was observed ($P > 0.05$), contrary to a similar study in Eastern Ethiopia [33].

The study revealed a median *Staphylococcus* count of 2.2×10^3 CFU/ml, which is lower than the finding reported in Mekelle (7.2×10^7 CFU/ml) and Debre Markos ($0.14 \pm 0.03 \times 10^5$ CFU/ml) [60, 61]. Moreover, 54% of the samples were found to exceed the Gulf standard ($>1.0 \times 10^3$ CFU/ml), which is lower than 93.4% reported in Bahir Dar Town [31]. This indicates that there may be issues with handling practices, possibly during the processes of harvesting, transportation, or preparation.

Staphylococcus aureus is commonly found in the nasal passages, throat, and on human skin. Though often harmless, it can produce enterotoxins that cause food poisoning, especially in temperatures ranging from 10°C to 45°C, with an optimal range of 35°C to 40°C [60]. In our study, 45.5% (86) of juice samples were contaminated by *S. aureus*. The highest contamination was observed in avocado samples (49.2%), while papaya samples had comparatively the lowest contamination (39.7%). This aligns with a similar study in Ethiopia reporting a 37.21% prevalence of *S. aureus* [61].

Escherichia coli, a gram-negative bacterium, serves as a significant marker for fecal contamination in food products [62, 63]. In this study, 29.1% (55) of juice samples tested positive for *E. coli*. This finding is comparable to an Indian study showing a 27.7% prevalence [64] but lower than studies in Arba Minch, Ethiopia (50%) and Tamale, Ghana (88%) [65, 66]. Conversely, it was higher than a study in Greece, which found a 3.34% prevalence [67]. The highest contamination was observed in mixed (spris) juices (34.9%), consistent with findings in Addis Ababa, where 33.3% of mixed samples were contaminated [53]. The survival of *E. coli* is often unaffected by the acidic nature of some juices.

The presence of *Salmonella* or *Shigella* in food items poses serious health risks. Gulf standards specify that these pathogens should not be detectable in 25 ml of juice samples [68]. In this study, *Salmonella* was found in 6.9% of the samples, while Shigella was absent. This prevalence is comparable to a study conducted in Axum town, Ethiopia, which reported a 5% prevalence of Salmonella [69]. However, it is lower than the prevalence observed in Eastern Ethiopia (24.4%) and Northwestern Ethiopia (39.1%) [33, 70]. An Indian study also indicated a higher Salmonella contamination rate of 38.8% [64]. Overall, this study found contamination rates of 45.5%, 29.1%, and 6.9% for *S. aureus*, *E. coli*, and *Salmonella*, respectively, lower than findings in Arba Minch, Ethiopia (67.71%, 50%, and 41.67%) [65]. These discrepancies may be attributed to cross-contamination during processing, poor water quality, or inadequate hygiene standards.

The emergence of drug-resistant pathogens presents a significant public health issue in developing countries. In an analysis of 86 *S. aureus* isolates, resistance rates were observed at 90.7% for penicillin and 63.3% for oxacillin. These findings are consistent with earlier study indicating high levels of penicillin resistance in Pakistan [36], yet they contrast with the lower resistance rates documented in Axum, Ethiopia [69]. A study conducted in Jimma reported similar results, with oxacillin resistance at 56.9% and a penicillin resistance rate of 90% [71]. In contrast to our findings, which indicated higher resistance levels in Hawassa [72]. Furthermore, 58.1% of the isolates exhibited resistance to cefoxitin, indicating the potential presence of methicillin-resistant *S. aureus* (MRSA) [73]. Our investigation revealed that 41.9% of *S. aureus* isolates demonstrated multi-drug resistance (MDR) to three or more antibiotics, which is lower than the 62.5% MDR rate reported in another study [74].

The resistance of isolated *E. coli* strains to ampicillin and tetracycline was recorded at 58.2%, which is lower than the resistance levels found in a similar study conducted in India [35]. However, these isolates were fully susceptible to gentamicin, sulfamethoxazole-trimethoprim, and other antibiotics. Furthermore, the prevalence of multidrug-resistant (MDR) strains was noted at 10.9%, which is lower than that reported in Southern Ethiopia [75]. Additionally, 21.8% of the *E. coli* isolates were positive for extended-spectrum beta-lactamases (ESBL), higher than some previous reports from Nigeria (14.29%) [76] and 11.8% prevalence documented in another study from Southern Ethiopia [75].

All *Salmonella* isolates exhibited resistance to ampicillin and tetracycline, while demonstrating complete susceptibility to ciprofloxacin, meropenem, norfloxacin, and other antibiotics. This finding aligns with multiple studies conducted in Mekelle and Addis Ababa, Ethiopia [52, 53]. The isolates displayed a multidrug resistance (MDR) rate of 15.38%, which is notably higher than that reported in another study conducted in Ethiopia [52]. However, our finding is lower than the 98.06% resistance observed in a study from Bangladesh, where isolates were resistant to two to seven different antibiotics [77].

Differences in these results could be influenced by in appropriate antimicrobial usage patterns, food safety protocols, surveillance mechanisms, and sanitation standards across different countries. All these factors can affect the resistance levels in bacteria found in food [78]. These findings emphasize the growing threat of antimicrobial resistance and the need for ongoing surveillance and tailored interventions.

6 Strength and limitation if the study

Strength of the study

- This study seeks to conduct an evaluation of antimicrobial susceptibility tests, offering detailed and comprehensive information regarding the bacteriological quality of ready-to-drink juice.

Limitation of the study

- The study doesn't assess other potential pathogenic bacteria due to shortage of re-sources.
- It was conducted in a restricted location, which may limit the generalization of the findings to other settings or populations.

7 Conclusion

The study shows serious hygiene and safety issues among juice vendors in Yeka Sub-City, Addis Ababa, with over half of the samples exceeding permissible bacterial levels. *E. coli*, *S. aureus*, and *Salmonella* were detected in over 98% of juice samples, raising significant public health concerns. Factors include poor hand-washing, inadequate use of protective equipment, untreated water, and unsanitary preparation areas. Notably, multi-drug resistance was prevalent in most isolates. The findings highlight the urgent need for interventions to improve hygiene standards and address antimicrobial resistance.

8 Recommendation

To enhance the bacteriological quality of ready-to-consume juices in Yeka Sub-City, Addis Ababa, it is essential to improve hygiene practices among vendors. This includes

- Promoting regular hand washing with soap, using gloves, aprons, and hair covers, and maintaining clean utensils.
- Public health education campaigns should raise awareness about food safety, while regulatory bodies enforce strict sanitation standards and conduct periodic microbial inspections.
- Ensuring vendors have access to treated water and proper waste disposal systems, as well as setting up hand washing stations, can further enhance hygiene.
- Monitoring antibiotic resistance patterns in bacterial isolates will support public health interventions and improve food safety outcomes.

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10 Glossary of terms

Aerobic Colony Count (ACC): The total quality of microbiology is determined by the number of bacteria that can grow and form colonies in an oxygen-rich environment.

Antibiotic Susceptibility Testing (AST): A laboratory test used to assess a bacterium's susceptibility to different antibiotics.

Antimicrobial Resistance (AMR): The ability of a microorganism to withstand the effects of drugs that previously were effective against it.

Bacteriological Quality: The evaluation of a sample's bacterial content to determine its safety and hygienic requirements.

Beta-Lactam Antibiotics: A class of antibiotics that interfere with the synthesis of bacterial cell walls, including cephalosporins and penicillins.

Coliforms: A class of rod-shaped, Gram-negative bacteria frequently found in the environment and often used as indicators of food and water quality.

CFUs (Colony-Forming Units): A measurement of a sample's viable bacterial or fungal cells based on their capacity to form observable colonies in a controlled environment.

Combination Disk Test (CDT): A diagnostic method used to identify bacteria capable of producing extended-spectrum beta-lactamases.

Contaminated Water: Water containing harmful microorganisms or chemicals that can cause illness.

Durham Tubes: Small inverted tubes used in microbiology to detect gas production by microbes.

Escherichia coli: A bacterium commonly found in the intestines of humans and animals. Certain strains can cause serious foodborne illness.

Extended-Spectrum Beta-Lactamase (ESBL): Enzymes produced by certain bacteria that confer resistance to beta-lactam antibiotics, including penicillins and cephalosporins.

Fecal Coliforms: A subset of coliform bacteria found in the intestines of warm-blooded animals, used as indicators of fecal contamination.

Food Safety Practices: Steps taken to ensure food is prepared, handled, and stored in ways that minimize the risk of food borne illnesses.

Food borne illnesses: Conditions caused by consuming contaminated food or drink, often due to

bacteria, viruses, or parasites.

Foodborne Pathogens: Microorganisms that cause illness when ingested through contaminated food or water.

Fruit Juice: The liquid or tissue extracted from one or more fruits, usually in an aqueous state.

Hygienic Risk Factors: Conditions or practices that increase the likelihood of contamination and associated health risks during food preparation.

Indicator Organisms: Non-pathogenic organisms used to assess the presence of pathogenic microorganisms.

MDR (Multi-Drug Resistance): The resistance of bacteria to multiple antibiotics, complicating treatment.

Methicillin-Resistant Staphylococcus aureus (MRSA): A type of bacteria resistant to many antibiotics, including methicillin, making infections challenging to treat.

Microbial Growth: The increase in the number of microorganisms, such as bacteria, fungi, or viruses, often facilitated by favorable environmental conditions.

Microbiological Counts: The measurement of microbial populations in a sample, typically expressed as colony-forming units per milliliter (CFU/ml).

Pathogens: Microorganisms, including bacteria, viruses, or fungi, that cause disease.

Personal Hygiene: Practices undertaken to maintain cleanliness and reduce the spread of infections.

Polluted Equipment: Tools or devices used in food preparation that are unclean and may harbor harmful microorganisms.

Prevalence: The proportion of a population with a specific condition or characteristic at a given time.

Public Health Concern: An issue affecting population health that requires collective action to address.

Public Health Interventions: Actions taken to prevent or control health issues within a population, such as improving food safety standards.

Resistance Profile: The pattern of resistance exhibited by a microorganism to various antimicrobial agents.

Salmonella spp: A genus of bacteria that commonly causes food borne illnesses, often associated with contaminated meat, eggs, or produce.

Sanitation Practices: Activities undertaken to maintain cleanliness and prevent contamination in food handling and preparation environments.

Secondary Metabolites: Organic compounds not directly involved in growth, development, or reproduction but often involved in defense and signaling.

Shigella spp.: A genus of bacteria known to cause dysentery through contaminated food or water.

Standard Operating Procedure (SOP): Step-by-step instructions designed to ensure consistent and high-quality task performance.

Statistical Package for the Social Sciences (SPSS): Software used for statistical analysis in social science research.

Thermotolerent Coliforms: A subset of coliform bacteria that survive at elevated temperatures, indicating fecal contamination.

Total Viable Count (TVC): A measure of the number of live microorganisms in a sample, used to assess food and water quality.

Xylose Lysine Deoxycholate (XLD) Agar: A selective growth medium used for isolating Salmonella and Shigella species.

Zone of Inhibition: The clear area around an antibiotic disk where bacterial growth has been prevented, used in susceptibility testing

11 Annexes

ANNEX I: English version of Participation information sheet

Principal Investigator: Hana Mekonnen, College of health sciences, department of medical laboratory sciences.

Title of the research project: Bacteriological quality of ready-to-consume juices associated with hygienic practice in Yeka sub-city

First of all, I would like to thank you in advance for your cooperation for the permission . Please read the following statements and ask any unclear points before you agree. Participation in this study is exclusively voluntarily.

Purpose of the study; This study will primarily help to consumer to have better understanding and awareness about bacteriological quality ready-to-consume fresh fruit juices and for the vendors to stay competent in the market and finally to benefit consumer by preventing disease spreading through consumption of fresh juices. Government regulatory bodies (Ethiopian Standard authority and Ethiopian Food, Medicine and Health Care Administration and Control Authority) can also use the study result for designing appropriate disease prevention strategies.

Confidentiality

The information recorded is strictly confidential. The information about your identity will be put away after recording your file and kept in secure place. Only the principal investigator will be able to link your identity with the code number. Information will be only disclosed for the study area and publication purpose.

Assurance of Principal investigator

I put my signature below to confirm you that I will take over the responsibility for the scientific ethical and technical conduct of the research project and for provision progress reports for all stockholders of the research project.

Hana Mekonnen (PI) College of health sciences, school of allied health sciences, department of medical laboratory sciences.

Signature: _____ Date: _____

If you have any question about this study, you should feel free to ask now or anytime throughout the study by contacting:

PI Address: Hana Mekonnen, College of health sciences, school of allied health sciences, department of medical laboratory sciences.

Email: hanmekonnen804@gmail.com Tell- 0988247005

ANNEX II: Consent Form

I have been informed about the objective of the study entitled "Bacteriological quality of ready-to-consume juices associated with hygienic practice in Yeka sub-city, Addis Ababa, Ethiopia." I am also agreed to answer questions about my personal hygiene and my processing habits during preparation of fruit juice. I have also been assured that I can withdraw my consent at any time without any negative effect. The proposal has been explained to me in the language I understand. I_____ here by give my consent for

giving of the requested information.

Participant's name_____ Sign. _____Date____

Name of Principal Investigator: ----------

Principal Investigator signature: ----------

Participant's ID: ……

ANNEX III: English Version Questionnaires for study participant

Addis Ababa University, College of health sciences, school of allied health sciences, department of medical laboratory sciences. Questionnaire for data collection to determine the Bacteriological quality of ready-to-consume juices associated with hygienic practice in Yeka sub-city.

Participant's ID: _____

Please thick the box for your correct answer!

Section 1: Socio-demographic Information

1. Age: _____year.
2. Gender

☐ Male ☐ Female

3. Education level:

☐ No formal education

☐ Grade1- Grade 8

☐ High school and above

Section 2: Risk Factors

4. Where do you preserve the fruit prior to juice processing?

☐ In a refrigerator ☐ on the floor

☐ On a Shelf ☐ In a bucket

☐ Others: Specify ----------

5. Where do you get water used for washing of fruits?

☐ From tap ☐ purified water

☐ stored water

6. Where do you get water used for dilution of the juice?

☐ From tap ☐ purified water

☐ Stored water

7. Frequency of Cleaning of hand after using toilet?

☐ Either before and after ☐ Both before and after

8. If yes for No 4, what cleaning agent you use to clean your hand after using toilet?

☐ with water and soap ☐ With water only

9. Have you a habit of washing utensils before re preparing of juice?

☐ Yes ☐ No

10. Where do you store the prepared juice?

☐ Shelf ☐ Refrigerator

Thank you for your cooperation!!

ANNEX IV: ለጥናቱ መረጃና ተሳታፊነት መግለጫና የስምምነት ማረጋገጫ ቅጽ

Principal Investigator: ሃ ና መኮ ን ን ፣ College of health sciences, department of medical laboratory sciences.

የ ምር ምር ፕ ሮ ጀ ክ ቱ ር ዕ ስ : Bacteriological quality of ready-to-consume juices associated with hygienic practice in Yeka sub-city

በ መጀ መሪ ያ ለ ፈ ቃ ዱ ለ ምታ ደ ር ጉ ት ት ብ ብ ር አ ስ ቀ ድ ሜ ላ መሰ ግ ና ች ሁ እ ወ ዳ ለ ሁ። እ ባ ክ ዎ ከ መስ ማማት ዎ በ ፊ ት የ ሚከ ተ ሉ ት ን መግ ለ ጫዎ ች ያ ን ብ ቡ እ ና ግ ል ጽ ያ ል ሆ ኑ ነ ጥ ቦ ች ን ይ ጠ ይ ቁ። በ ዚ ህ ጥ ና ት ው ስ ጥ መሳ ተ ፍ በ ፈ ቃ ደ ኝ ነ ት ብ ቻ ነ ው.

የ ጥ ና ቱ ዓ ላ ማ ; ይ ህ ጥ ና ት በ ዋ ነ ኛ ነ ት ሸ ማ ቹ የ ት ኩ ስ ፍ ራ ፍ ሬ ጭ ማ ቂ ዎ ች የ ባ ክ ቴ ሪ ያ ጥ ራ ት ን በ ተ መ ለ ከ ተ የ ተ ሻ ለ ግ ን ዛ ቤ እ ን ዲ ኖ ራ ቸ ው ፣ ሻ ጮ ች በ ገ በ ያ ላ ይ ብ ቁ ሆ ነ ው እ ን ዲ ቆ ዩ እ ና በ ሽ ታ ን በ መ ከ ላ ከ ል ሸ ማ ቹ ን ተ ጠ ቃ ሚ ለ ማ ድ ረ ግ ይ ረ ዳ ል። የ መ ን ግ ስ ት ቁ ጥ ጥ ር አ ካ ላ ት (የ ኢ ት ዮ ጵ ያ ስ ታ ን ዳ ር ድ ባ ለ ስ ል ጣ ን እ ና የ ኢ ት ዮ ጵ ያ የ ም ግ ብ ፣ የ መ ድ ሃ ኒ ት እ ና የ ጤ ና ክ ብ ካ ቤ አ ስ ተ ዳ ደ ር እ ና ቁ ጥ ጥ ር ባ ለ ስ ል ጣ ን) የ ጥ ና ት ው ጤ ቱ ን ተ ገ ቢ ው ን በ ሽ ታ የ መ ከ ላ ከ ል ስ ል ቶ ች ን በ መ ን ደ ፍ ሊ ጠ ቀ ሚ ት ይ ች ላ ሉ።

የ ጥ ና ቱ መ ረ ጃ ዎ ች ሚ ስ ጥ ራ ዊ ነ ት

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የ ዋ ና ው መር ማ ሪ ማ ረ ጋ ገ ጫ

ለ ምር ምር ፕ ሮ ጀ ክ ቱ ሳ ይ ን ሳ ዊ ስ ነ ም ግ ባ ር እ ና ቴ ክ ኒ ካ ል ስ ነ ም ግ ባ ር የ ሂ ደ ት ሪ ፖ ር ቶ ች ን ለ ማ ቅ ረ ብ ሃ ላ ፊ ነ ቴ ን እ ን ደ ም ወ ስ ድ ለ ማ ረ ጋ ገ ጥ ፊ ር ማ ዬ ን ከ ዚ ህ በ ታ ች አ ስ ቀ ም ጫ ለ ሁ።

ሃ ና መኮ ን ን (PI)

ፊ ር ማ፡ _____ ቀ ን ፡ _____

ስ ለ ዚ ህ ጥ ና ት ም ን ም አ ይ ነ ት ጥ ያ ቄ ካ ሎ ት አ ሁ ኑ ኑ ወ ይ ም በ ማ ን ኛ ው ም ጊ ዜ በ ጥ ና ቱ ወ ቅ ት በ መ ገ ና ኘ ት ለ መ ጠ የ ቅ ነ ፃ ነ ት ሊ ሰ ማ ዎ ት ይ ገ ባ ል ::

የ PI አድራሻ፡ -ሃና መኮንን፣ College of health sciences, school of allied health sciences, department of medical laboratory sciences.

ኢሜል፡ hanmekonnen804@gmail.com ይደውሉ - 0988247005

ANNEX V: ስለ ስምምነቱ ማረጋገጫ ፊርማ

በ የ ካ ክ ፍ ለ ከ ተ ማ፣ አ ዲ ስ አ በ ባ ፣ ኢ ት ዮ ጵ ያ ከ ን ጽ ህ ና አ ጠ ባ በ ቅ ጋ ር የ ተ ቆ ራ ኙ ባ ክ ቴ ሪ ያ ዎ ች ጥ ራ ት በ ፍ ራ ፍ ሬ ጭ ማ ቂ ዎ ች ”የ ሚ ለ ዉ የ የ ጥ ና ት ር ዕ ስ ዓ ላ ማ ተ ነ ግ ሮ ኛ ል ። በ ተ ጨ ማ ሪ ም የ ፍ ራ ፍ ሬ ጭ ማ ቂ በ ሚ ዘ ጋ ጅ በ ት ጊ ዜ ስ ለ ግ ል ን ጽ ህ ና እ ና ስ ለ ማ ቀ ነ ባ በ ር ል ማ ዶ ቼ ጥ ያ ቄ ዎ ች ን ለ መ መ ለ ስ ተ ስ ማ ም ቻ ለ ሁ ። ፈ ቃ ዴ ን በ ማ ን ኛ ዉ ም ጊ ዜ ያ ለ ም ን ም አ ሉ ታ ዊ ተ ጽ እ ኖ መ ሰ ረ ዝ እ ን ደ ም ች ል ተ ረ ጋ ግ ጦ ል ኛ ል ። ፕ ሮ ፖ ዛ ሉ በ ም ረ ዳ ዉ ቋ ን ቋ ተ ብ ራ ር ቶ ል ኛ ል ::

እ ኔ _____በ ጥ ና ቱ ተ ሳ ታ ፊ መ ሆ ኔ ን በ ፊ ር ማ ዬ እ ያ ረ ጋ ገ ጥ ሁ ነ ዉ::

የ ተ ሳ ታ ፊ ዉ ስ ም _____ ፊ ር ማ _____ቀ ን ____

የ ዋ ና መ ር ማ ሪ ስ ም፡ ----------ፊ ር ማ----------

የ ተ ሳ ታ ፊ መ ለ ያ ኮ ድ :----------

ANNEX VI: በአማርኛ ቋንቋ ለጥናት ተሳታፊዎች የተዘጋጀ መጠየቅ

አዲስ አበባ ዩኒቨርሲቲ፣ የጤና ሳይንስ ኮሌጅ፣ የሕብረት ጤና ሳይንስ ትምህርት ቤት፣ የሕክምና ቤተ ሙከራ ሳይንስ ክፍል። በየካ ክፍለ ከተማ ከንፅህና አጠባበቅ ጋር የተያያዘ የባክቴሪያ ጥራትን ለመጠጣት ዝግጁ በሆኑ ጭማቂዎች ለማወቅ የመረጃ አሰባሰብ መጠይቅ።

የተሳታፊ መታወቂያ _____

እባክዎ ከመልስዎ አጠገብ በሚገኝ ውሳጥን ውስጥ የ "X" ምልክት ያስቀምጡ።

የመላሹ መለያ _____.

ክፍል 1፡ የስነ ሕዝብ አወቃቀር መረጃ

1. ዕድሜ: _____አመት

2. ፆታ

☐ ወንድ ሴት ☐

3. የትምህርት ደረጃ፡ -

☐ ያልተማረ

☐ ክፍል 1 - ክፍል 8

☐ ከ 9 በላይ

ክፍል 4፡ የአደጋ መንስኤዎች

4. ጭማቂ ከማዘጋጀትዎ በፊት ፍሬውን የት ያቆዩታል ?

☐ ወለሉ ላይ ☐ በማቀዝቀዣ ውስጥ

☐ በመደርደሪያ ላይ ☐ በ ባልዲ ውስጥ

☐ሌሎች፡ ይግለጹ ----------

5. ፍራፍሬዎችን ለማጠብ የሚያገለግል ውሃ ከየት ታገኛለህ ?

☐ ከቧንቧ ☐ የተጠራ ውሃ

☐ የተቀመጠ ውሃ

6.የ ጭማቂ ውን ለማሟሟት የሚያገለግል ውሃ ከየት ታገኛለህ ?

☐ ከቧንቧ ☐ የተጠራ ውሃ

☐ የ ተ ቀ መ ጠ ውሃ

7. ሽ ን ት ቤ ት ከ ተ ጠቀ ሙበ ኋ ላ እ ጅ ን የ ማጽ ዳ ት ል ማድ አ ለ ዎ ት ?

☐ አ ዎ ☐ አ ይ ደ ለ ም

8. አ ዎ ከ ሆ ነ ፣ ከ መጸ ዳ ጃ ቤ ት በ ኋ ላ እ ጅ ዎ ን ለ ማፅ ዳ ት ምን ዓ ይ ነ ት የ ጽ ዳ ት ወ ኪ ል ይ ጠቀ ማሉ ?

☐ በ ውሃ እ ና በ ሳ ሙና ☐ በ ውሃ ብ ቻ

9 ጭማቂ ን እ ን ደ ገ ና ከ ማዘ ጋ ጀ ት ዎ በ ፊ ት እ ቃ ዎ ች ን የ ማጠብ ል ምድ አ ለ ዎ ት ?

☐ አ ዎ ☐ አ ይ ደ ለ ም

10 .የ ተ ዘ ጋ ጀ ውን ጭማቂ የ ት ነ ው የ ሚያ ከ ማቹ ት ?

☐ በ መደ ር ደ ሪ ያ ላ ይ ☐ በ ማቀ ዝ ቀ ዣ ውስ ጥ

ስ ለ ት ብ ብ ር ዎ እ ና መሰ ግ ና ለ ን !!

ANNEX VII: Observational checklist

Parameter		Observation
Type of juice house	☐ Congested ☐ Non congested	
Clothing of juice makers	☐ Neat and appropriate ☐ Sloppy and inappropriate	
Are there adequate hand washing facilities inside the kitchen	☐ Yes ☐ No	
Is there food disposal facilities available	☐ Yes ☐ No	
Hygiene of the Kitchen	☐ Hygienic ☐ Less hygienic	
Is vending stall protected from sunlight, dust, and wind	☐ Yes ☐ No	
Hair cover	☐ Covered ☐ Uncovered	
Use of kitchen gloves	☐ Yes ☐ No	

ANNEX VIII: LABORATORY PROCEDURES, PRINCIPLES AND MATERIALS

I. PLATE COUNTS METHOD:

The spread plate approach includes applying a small amount of bacteria suspended in a solution to a plate using a sterile spreader with a smooth surface made of metal or glass. The plate must be dry and at normal temperature in order for the agar to absorb the bacteria more effectively. A countable number of isolated bacterial colonies are equally scattered on a successful spread plate.

Procedures:

1. Decimal dilutions of 10^-1, 10^-2, and 10^3 will be prepared using separate sterile pipets. This will be done by transferring 10 ml of the previous dilution to 90 ml of diluent or 1 ml of dilution to 10 ml of diluent.
2. All dilutions will be shaken 25 times in a 30 cm (1 ft.) arc within 7 seconds. Then, 1 ml of each dilution will be pipetted into separate, duplicate, appropriately marked petri dishes.
3. If the dilution bottle stands for more than 3 minutes before being pipetted into the petri dish, it will be re-shaken 25 times in a 30 cm arc within 7 seconds.
4. Plate count agar (cooled to 45 ± 1°C) will be added to each plate within 15 minutes of the original dilution.
5. Agar will be immediately added to the petri dishes when the sample diluent is poured. Agar and dilution water control plates will be prepared for each series of samples. The sample dilutions and agar medium will be thoroughly and uniformly mixed by alternate rotation and back-and-forth motion of the plates on a flat level surface.
6. The agar will be allowed to solidify. Then, the solidified petri dishes will be inverted, and they will be promptly incubated for 48 ± 2 hours at 35°C. Plates will not be stacked during agar pouring or solidification.
7. The CFU/ml value of the sample will be calculated. Once the colonies are counted, they will be multiplied by the appropriate dilution factor to determine the number of CFU/ml in the original sample

TOTAL COLIFORM DETECTION

Dilution Preparation:

1. Nine milliliters of sterile saline solution will be used as the diluent.
2. One milliliter of juice sample will be transferred to prepare 10-2 to 10-5 ten-fold serial dilutions.

Agar Preparation and Layering:

1. Five milliliters of tryptone soya agar, previously cooled to 45.0 ± 1.0°C, will be added to appropriately labeled petri dishes.
2. The petri dishes will be pre-incubated for 1-2 hours at 20 – 25°C.
3. Subsequently, they will be covered with 10 - 15 ml of violet red bile agar at a temperature of 45.0 ± 1.0°C.

Inoculation and Incubation:

1. One milliliter aliquot from each dilution (10-2, 10-3, 10-4, 10-5) will be aseptically transferred to each Petri dish.
2. The Petri dishes will be incubated at 37°C for 24 ± 3 hours in an inverted position.
3. Typical colonies will be counted, and selected colonies will be confirmed by testing for gas production in Brilliant Green Lactose Bile broth.

Confirmatory Test for Coliforms:

1. A loop full of inoculum from all presumptive-positive violet red bile agar petri dishes will be inoculated into tubes containing 5 ml of Brilliant Green Lactose Bile broth with inverted Durham tubes.
2. These tubes will be incubated at 37°C for 24 hours.
3. A Brilliant Green Lactose Bile broth tube will be observed for gas formation in the Durham tubes.
4. All positive BGLB broth tubes will be considered positive for coliform confirmation .

FECAL COLIFORM DETECTION

Procedure:

1. Approximately 5 ml of tryptone soya agar is prepared and cooled to 45.0 ± 1.0 °C.
2. Appropriately labeled petri dishes are arranged.
3. The petri dishes are pre-incubated for 1-2 hours at 20 – 25 °C (room temperature).
4. The pre-incubated petri dishes are covered with 10 - 15 ml of violet red bile agar at a temperature of 45.0 ± 1.0 °C.
5. One ml aliquots from each dilution (10-2, 10-3, 10-4, 10-5) are aseptically transferred to each Petri dish.
6. The inoculated Petri dishes are incubated at 44.5°C for 24 hours.
7. After incubation, the colonies are counted using a colony counter.
8. Confirmatory Test for Fecal Coliforms: Five colonies from each presumptive-positive violet red bile agar petri-dish are taken.
9. These colonies are inoculated into tubes containing 5 ml of EC broth with inverted Durham tubes.
10. The tubes are incubated at 44.5°C for 24 hours.
11. Confirmation is obtained by gas production.

ANNEX IX: The recommended microbiological standards for any fruit juice (Gulf standard, 2000)

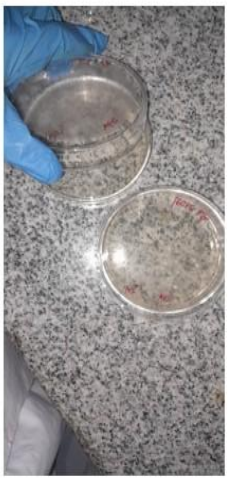
Table 3: Recommended microbiological standards for any fruit juice

Standard	Level	Viable count	Total Coliform	Fecal Coliform
Gulf	Maximum Bacterial load anticipated	5.0×10^3	10	0
	Maximum Bacterial load permitted	1.0×10^4	100	0

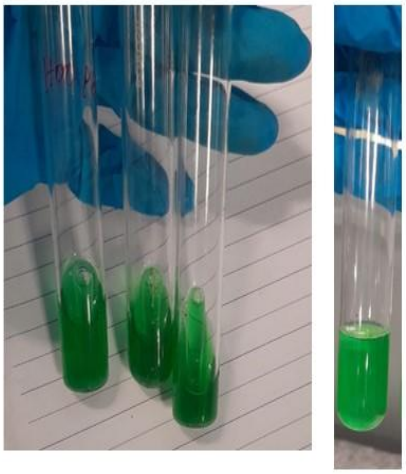
ANNEX X: Laboratory Analysis Result

Media preparation

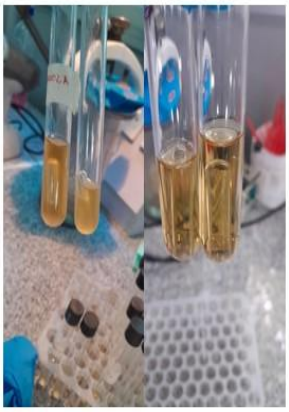
Sample preparation and processing



Aerobic plate count



A,Positive BGBLB B,Negative BGBLB

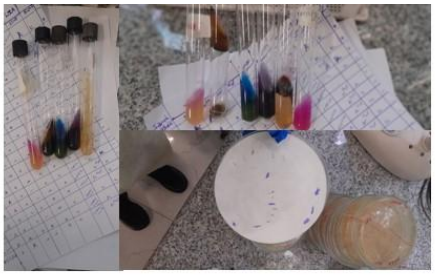


A,Positive EC B, Negative EC

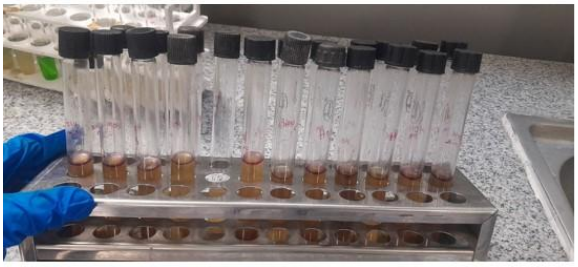
A, Rappaport Vassiliadis broth(RVB) B, Growth on RVB

Suspected *salmonella* on XLD agar

Manitol salt agar



Uria , Lysine iron , Triple sugar iron .citrate test. motility test and oxidase .



Indo test (positive)

AST

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university, and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Hana Mekonnen (B.Sc.)

Signature _____

Date: _____

This thesis has been submitted with our approval as advisors.

Advisor: Dr. Melese Hailu (Ph.D)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mr. Gebreab T/birhan

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.