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DEPARTMENT OF MEDICAL LABORATORY SCEINCES

Identifications of Intestinal Parasites, *Salmonella and Shigella* Species and Knowledge, Attitude and Practice of fruit juice makers in selected Sub cities of Addis Ababa, Ethiopia.

By :

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A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology)

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This is to certify that the thesis prepared by Henok Tsegaye Woldegebreal, entitled:

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and submitted in partial fulfillment for the requirement of Master of Science degree in Clinical Laboratory Sciences (Diagnostic Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABBREVIATIONS

ALIPB: Aklilu lemma institute of Pathobiology

ATCC: American Type Culture Collection

BAP: Blood Agar plate

BPW: Buffer Phosphate Water

CLSI: Clinical and Laboratory Standards Institute

EPHI: Ethiopian Public Health Institute

FDA: Food and Drug Administrations

GMP: Good Manufacturing Practices

H_2S: Hydrogen Sulphide

KAP: Knowledge Attitude Practice

LIA: Lysine iron agar

MAP: MaCconkey agar plate

MDR: Multidrug resistance

MIC: Minimum Inhibition Concentration

Nacl: Sodium Chloride

RVB: Rappaport Vasiliadis broth

SCB: Selenite Cysteine broth

SFB: Selenite Fecal Broth

UK: United Kingdom

XLD: Xylose-Lysine Deoxycholate

ABSTRACT

Background: Unpasteurized fruit juices known to cause food poisoning from harmful germs such as *Salmonella, Shigella* and gastrointestinal parasites. Fruits are susceptible to different microbes if didn't prepared practice good hygiene in overall process. Due to that Food borne diseases are the main public health concerns in the world, especially in developing countries.

Objective: To identify Intestinal Parasites, *Salmonella* and *Shigella* species from consumed local fruit juices sample and juice makers and assessing their Knowledge, Attitude and Practice of fruit juice makers.

Methods: A Cross-Sectional study was conducted at selected sub cities of Addis Ababa, Ethiopia. Knowledge, Attitude, Practice assessment questionnaire was taken to evaluate the factors to microbial quality and safety of local fruit juices. Different varieties of fruit juices and stool samples from juice makers was collected and examined for microbiological tests from February 2021 to June 2021.Juice samples were pre-enriched in Buffer Phosphate Water followed by selecting enrichment for isolation, culture, biochemical tests, Becton Dickinson phoenix analyzers, serotyping were used for confirmation of those pathogens. Antimicrobial susceptibility tests were also carried out using 10 antibiotic discs on each isolate by using Kirby-Bauer disc diffusion method. The data was entered into Epi info version 3.5.4 and analyzed using SPSS version 23. A p value ≤ 0.05 at 95% CI was considered as statistically significant.

Results: A Total of 100 fruit juice sample, 100 fruit juice makers fecal samples, 120 KAP assessments were enrolled in the study. The majority study participants were female 108(90%). About 27 (27%) and 17(17%) of juice makers and fruit juice samples were positive for different intestinal parasites with the most abundant parasite of *A. lumbricoides* 7(7%) and *E.histolytica* 5(5%) from juice samples and juice makers respectively. Stool and juice cultures revealed 6% and 5% of *Salmonella* isolates. While *Shigella* species was not isolated from both sample types. The participants level of knowledge and attitude score were 95.8% and 63.1% and the practice level score were 39.5%. *S.Dublin* were the only serotype found in both fruit juices and stool specimen. All isolates of *Salmonella* were sensitive to Ciprofloxacin, Chloramphenicol, Sulfamethoxazole trimethoprim, Nalidixic acid, Ceftriaxone and only Gentamycin and Tetracycline shows the highest intermediate. But resistant to Erythromycin, Ampicillin, Amoxicillin Clavunilic acid.

Conclusion: The results clearly illustrated the fresh juices preparations didn't meet public health standards. They know their work but poor practice indicated. Such foods are responsible to causes food borne diseases specially carrier to many kinds of intestinal parasites and enteropathogenic bacterias. Therefore, policy makers and implementers must adopt strict measures and supervision to the vendors about food safety and hygienic practices to update the guidelines and standards for fruit products preparations. Certain antibiotics for *Salmonella* from fecal and non-fecal source gives exceptional result for further study since multiple drug resistance of many antibiotics still differentiated that affect drug viability to *Salmonella* species.

Key words: Fruit juice, Contaminations, *Salmonella* species, *Shigella* species, Antimicrobial resistance, Knowledge, Attitude, Practice

1. INTRODUCTION

1.1. BACK GROUND

Unpasteurized fruit juices is a type of fruit juices that produced by pressing or squeezing of fruits mechanically fleshing without heating or adding solvents (1,2). Food borne pathogens and their infection is highly linked with one of the major global public health problems (2,3).

However, Freshly squeezed fruit juices are naturally susceptible to different microorganisms and specially harmless bacteria such as saprophytic. Unsafe fruits pathogens occurred primarily due to proliferation of acid tolerant and osmophilic micro flora. Many microorganisms such as acid tolerant bacteria and fungi (molds, yeasts), the main flora of fruits used them as a substrate for their growth because of acidic pH that has been documented and identified as the vehicle of food borne pathogens globally (3,4).

Contamination of fruit can occur anywhere in the growing, harvesting, cleaning and transportation chain from orchard to processor, in preparation up to final consumer consumption. Water used in farming and diluting pesticides, irrigation, and washing different varieties of represents a possible source of contamination. Fruits in raw agricultural commodities also be exposed to contamination from animals, birds, insects and from domestic and agricultural waste. The harvesting and use of drop fruit can increase the risk of contamination. The contact surfaces of equipment used in storage, and packing of the fruit may also be contaminated with rodent or animal manure. Other possible sources of contamination include workers hygiene's status when they handling the fruit, store and shipping condition. Therefore, Detection and identification of those pathogens found in fruit juices are giving a structural intervention to solve the public health problems of all process and preparation play an important role to prevent food out breaks (5,6).

The problem is exacerbated in countries, where lack of overall practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment, and lack of food safety training are common. The safety of street fruit products, on the other hand, becomes the major source concern for food control officers (7).

Nowadays, Food borne disease is affecting about one-third world population annually. specially Intestinal parasites, *Salmonella*, *Shigella* species is mostly caused by unsafe food preparation, handling and processing due to poor hygienic practice and contamination with bare hands.

Recently, it is a worldwide burden. The world health organization (WHO) estimated that in developed countries up to 30% of population suffer from food-borne diseases each year. Where as in developing countries up to 2 million deaths are estimated per year because of the poor sanitation, food handling, preparation, processing and practice. National Hygiene and Sanitation Strategy program reported that about 60% of the disease burden is related to poor hygiene and sanitation in Ethiopia (8,9).

In Ethiopia the cases of food-borne illnesses are not sufficiently investigated but few reports even if diagnosed in the form of outbreak or illness shows that highly linked to Intestinal parasite, *Salmonella* and *Shigella.* Center for disease control and prevention (CDC) are attributed that Bacteria and intestinal parasites are commonly found in soil, water, plants and animals including humans. People can also be exposed to some bacteria through inhalation, contaminated drinking water and fruit juices (10). Due to that, Fruit Contamination by pathogenic bacteria is a one of significant public health concern for consumer's worldwide. The economic consequences also significant for the producers and the industry. Therefore, Detection and identification of those microorganisms in fruit juices are giving a structural intervention to solve the public health problem of all process and play a significant role for preventing food borne outbreaks (11).

Recent studies shown that most of the fruit juices selected by customer preferences have higher microbial load than the fruit juices sets specification standard in some parts of the world. the products could be the cause of health problems and potential vehicle of food borne out breaks, high levels of works hygiene should be enforced and the use of disinfectant better practiced to improve the microbial quality, safety,storage condition and shelf life of the final products also noted (12).

In addition, the use of unhygienic fruit preservation without properly storage of refrigeration, unhygienic surroundings often with swarming house flies, fruit flies and airborne dust can also acts as sources of contamination. Various juices have shown the potential sources of bacterial pathogens basically *E.coli 0157:H7, Salmonella, Shigella and Staphylococcus aureus* (13).

Intestinal parasites still remain considerable public health problems in low-income countries where poor food hygiene practice is common. people involved in preparing and serving food, working with poor personal hygiene could pose a potential threat of spreading intestinal parasites to the public in a community.

Salmonellosis and *Shigellosis* are one of major pathogens affecting nutrients contents (Food safety) and causes disease to human by various microorganisms worldwide caused by consumption of various food stuff, mainly fruit juices contaminated with vegetative form of the pathogen and their toxins and are one of Gram negative rods bacteria causative agents of two-third of human borne diseases worldwide with high burden in developing countries. As a result most of fruit juices the ability to cause diseases by various bacteria causing agents that play a significant role on both public health and economic sectors (14).

Globally, *Salmonella* remains a major cause of foodborne infection in humans, which leads to approximately 93 million infections every year. The World Health Organization (WHO) estimates that there are around 16 million new cases and 600,000 deaths due to typhoid fever each year worldwide. It causes bacterial bloodstream infections with a fatality rate of 20–25% (15,16).*Shigella* continues to play a major role in the etiology of inflammatory diarrhea and dysentery in food handlers. The annual incidence of *Shigella* is estimated to be 164.7 million people, with 69% of all deaths attributable to *Shigellosis* worldwide (17,18,19).

Globally economic contribution of fruit production also beneficial to farmers, merchant's and others related peoples gets more money. Nowadays freshly squeezed juices are selected by consumer because of its nature flavor, tests, no addition of preservatives, no artificial color, sweetness characteristics due to that preferred over pasteurized fruits (20, 21).

Hence the main objectives of this research are to detect and identify those microbes associated with fruit juice samples and fruit juices makers.

1.2 STATEMENT OF PROBLEM

Freshly squeezed fruit juices are very important for human being which contributes vital nutrients important to health diet. However, unpasteurized juices which are contaminated during processing from raw materials, poor hygienic conditions, using of tap water in all processes and unsafe environmental conditions in processing result in different food- borne illnesses. When fruits and vegetables are made into fresh-squeezed juice, harmful bacteria may be present and become part of the finished product (19).

Globally, according to different studies shown that unpasteurized orange juice has been linked to several outbreaks of disease caused by Intestinal parasites, *Salmonella*, *Shigella* or viruses in different countries. Although food borne pathogens can be destroyed by pasteurization and consumption of unpasteurized juice occurs frequently due to consumer preferences (20,21).

The main challenges associated with fruit products quality and safety are seasonal production, manufacturing challenges homogenization, extraction, filtration, processing, miscellaneous additives like appearances changes, flavor, color deterioration, shelf life, water quality and bottling issues microbiological problems specially simple carbohydrates, sugar content found in fruits and marketing challenges like cost, consumer assessment and complaints. Additionally bio solids, workers health hygiene, Sanitary status of facilities, cleaning habit of fruits and equipment's used related to hygienic status, transportation, storage conditions of fruits in all processes are the most known risk factors and gaps responsible for microbiological spoilage of fruits and vegetables. Contamination of intestinal parasites, *Salmonellosis* and *Shigellosis* remain a major public health problem across the globe, particularly in developing countries like Ethiopia, where hygiene standards and food safety issues are still below the required standards (22,23).

Until this study conducted, In Ethiopia the knowledge, attitude, practice of fruit juice makers and venders faces poor hygienic challenges with in different assessments. different unpasteurized fruit juices still consumed with their different bacterial loads, therefore, different researchers are determined different bacterial loads in those unpasteurized fruit juices vender in juice bars, café and restaurants of Ethiopia (1,12,59,90).

Finally, due to all the above mentioned problem this study was done to fill the gaps of different contaminant factors by identifying in Ethiopia context the cause for spoilage of locally prepared fruit juices quality to deliver safe fruit juices products by maintaining nutritional values to consumers.

1.3. SIGNIFICANCE OF STUDY

- ✓ To identify basic hygienic problems that affect quality of locally prepared unpasteurized fresh juices.
- ✓ To implement better hygienic conditions by creating awareness among juice makers to use disinfectants, microbiological safety and quality in juice processing conditions.
- ✓ This study also help to create awareness in juice houses by maintaining prepared juices to be matched with customer desire to minimize fruit products spoilage.
- ✓ This study also help for policy makers in order to identify which juice types are easily contaminated, to recommend remedial action for identified problems.
- ✓ To give baseline information for further study to responsible bodies on how source of contamination affect the quality of locally prepared fruit juices.
- ✓ To know the amount of prepared juices to be matched with customer desire in order to minimize fruit products spoilage
- ✓ The result and my recommendations soon given to responsible government planning bodies that helps to build additional standards and how to maintain fruit safety and health of the consumers.

2. LITERATURE REVIEWS

2.1. FRUIT JUICES AND UNPASTEURIZED FRUIT JUICES

Fruit juice is the aqueous liquid that can be obtained from a fruit expressed or extracted from one or more fruits or vegetables, the edible portions of one or more fruits or vegetables or any concentrates of such liquid or puree. Drink produced by squeezing or crushing fruit. Fruit juices include apple, grape, orange, and pineapple (24). Unpasteurized fruit juices is a liquid formed without any additive/ treatment. Furthermore it can be freshly buys from venders juice bars, cafeterias, grocery stores, road side stations and farmer markets. local juices are stored in ice box, in refrigerator ceases and in different sections. However, its Shelf life is shorter than canned fermented juices (25).

2.2. PASTEURIZED JUICES

Is a liquid formed with adequate treatment/additive and preservative to kill harm full bacteria and to extend shelf-life. Global study shows that both locally prepared fruit juices and imported pasteurized juices indicate significant increase bacterial load and responsible to cause food borne illness to humans. Known to Stay for long period of time in shelves because of adding preservatives and can be heated to high temperatures for a short time in order to kill any bacteria or other microorganisms that may be present. When fruits and vegetables are made into fresh-squeezed juice, harmful bacteria may be present and become part of the finished products (26,27). The lack of adequate consumption of fruit and its products has become a worldwide concern since different juice products play a crucial components which has been associated with a reduced risk of developing a number of chronic diseases (28).

In developing countries various studies shown that, fruit and vegetables juices sold by street venders are widely consumed by millions of people. These juices provide a source of readily available and affordable sources of nutrients to many sectors of the populations, including the urban poor (29).

2.3. HANDLING AND PROCESSING

Handling and processing of fresh fruit juices are the main source associated risk factors to spread water borne and febrile illness to community especially in developing countries. According to studies conducted in Dhaka, Bangladesh. The number and type of the pathogens from the freshly squeezed fruit juices found them unsafe for drinking. Therefore, they confirmed that sanitary and hygienic conditions are important to prepare safe fruit juices and to prevent consumers health (30).

2.4. CONTAMINATION

Bacterial contamination of fresh or minimally processed fruits and vegetables can occur any time of the processing stages (harvest, trimming, washing, slicing, soaking, dehydrating, blending, and/or packaging) use of unhygienic water for dilution, long storage without refrigerator, unhygienic environment in preparation and poor handling of prepared juices. Contamination of juices sold in different restaurants, cafes and even road side stalls are sometimes un acceptable for human consumption and create significant health problem. Different juice products can be contaminated with harm full bacteria like *Salmonella, Shigella* and *E.coli.* Although fruits naturally does not contain harm full bacteria, viruses or parasites. but through all the above risk factors unpasteurized juices and its products can potentially pose a serious health risk from acute to chronic food borne disease to consumers (31,32,33).

2.5. ENTRANCE AND SURVIVAL SYSTEM

Those pathogenic organisms can enter to fruits and vegetables through damaged surfaces, cuts, splits and punctures. This damage can occur by insects, birds during maturation, harvesting, handling and processing (34).After the entrance of a specific pathogens able to survive in the fruit juices until it reaches to consumer in order to give a public health hazard. Therefore, most fruit juices are acidic by nature to inhibit the growth of pathogenic organisms. Studies conducted on the survival or growths of microorganisms in juice have showed a number of pathogenic organisms can be present and survived in a wide range of fruit and vegetables (35).

2.6. EQUIPMENT

Equipments including lubricants and surfaces coming into contact with galvanized buckets, pipes of sheeting materials possibly lead to unacceptable migration or attachments of chemicals into juice (36,37).

2.7. WATER SOURCE

Water potability is important for safe preparation of fruit juices. hot and cold water under pressure can be used for fruit juice preparation and transportation. There are so many possible source of water contamination such as sanitary conditions in street vended juice shops, waste disposal system, overcrowding. According to research conducted in Visakhapatnam city, India, Over the results of the study indicates lack of sanitary conditions in street vended juice shops the occurrence of pathogenic *E.coli, S.typhimurium and Shigella* (38).The quality of drinking water and juice were found to be good in Bangladesh but proper hygiene should be maintained more strictly to avoid the contamination by other gram negative bacteria which are also capable of causing disease (39).

In Ethiopia, Particularly in large urban areas, fruit juices are available in super markets in canned or bottled forms. In addition in fruit juices vending houses serving different types of fruit juices in fresh forms are proliferating. However, very few studies shows that almost 100% fruit venders uses tap water for preparation of fruit juices (40).

2.8. PERSONNEL

All workers must be set frequent medical checkup and free from communicable diseases. They should be trained on hygienic issues, built standard requirements for personal hygiene should be available. According to study conducted in Gondar, Ethiopia food-handlers with poor personal hygiene working in food-service establishments could be potential sources of infection due to pathogenic organisms (40,41).

2.9. STORAGE CONDITIONS

Generally fruit juices are prepared as soon as possible after picking to avoid increases of pH because of their nature favored the growth of survival of pathogens. Therefore as much as

possible fruit juices needs to be stored from(0^{o} to 4^{o}C).storage facilities must be clean, secure from rodents insects and other possible interferences when storing food should be avoided.

2.10. OUTBREAK DIRECTLY ASSOCIATED TO LOCAL(UN PASTEURIZED FRUIT JUICES)

According to study conducted in the united states of America, un pasteurized orange juice from one company was the main source of a wide spread out break of salmonellosis and additionally critical control point regulation likely contributed to this out break. Therefore, pasteurization or other reliable treatment of fruit juices could have similar prevention of out breaks caused by fruit and vegetables spoilage .Another survey study report conducted by CDC suggested that unpasteurized fruit juices highly linked to several outbreaks of human food borne disease from various microorganisms (42).

2.11. FRUIT MICROORGANISMS

The sources of fruit that can be made in to juices are many in plants and its parts are appropriate for juice. However, the fruit producers and juice processor must adhere to some very important guidelines, in fruit selection and juice manufacture. Spoilage of fruits are usually happen due to their low P^{H} (<4) and their products is linked to yeasts, molds and aciduric bacteria.

Fresh fruits are susceptible to rot by different types of yeasts like from the *genera Candida,Saccharomyces,Torulopsis and Hansenula.Molds* such as *pencillium, alternaria, Botrytis,Rhizopus,Aspergillus,Cladosporium,Tricothecium,Phytophthora,Aureobasidium,Colleto trichum* and many others are associated with fermentation of fruits. Bacterial Spoilage associated with the souring of berries and figs has been attributed to the growth of lactic and acetic acid bacteria. Pathogens on fresh fruits and vegetables are *Salmonella, Shigella, Listeria monocytogenes, E.Coli 0157:H1*.Many Viruses like *gastrointestinal viruses* and *Entamoeba histolytica, Ascaris species* are predominantly reported cases to parasitic spoilage of fruits and its products. usually all pathogens incorporated by polluted irrigation of waters (43,44).

In other hand specially in sub-Saharan Africa, the burden of intestinal parasites is dramatically high. About a third of (26million), one quarter (21 million), one in eight (11 million) Ethiopian people harbor Ascaris lumbricoides, Trichuris trichiura and Hook worm respectively. Consequently, Ethiopia bears the second, the third, and fourth highest burden of *ascariasis, hookworm* and *trichuriasis* respectively (45).

Fruits and juice production in many regions mainly Avocado, Mango, Apple, Banana, Lemon, Orange, Papaya, Pineapple, Grapes and Spris are dominant. Most microorganisms that are members of a very large and diverse community of microbes that collectively responsible for maintaining a dynamic ecological balance within most agricultural systems. Most bacteria and fungi that arrive on the developing crop plant are completely benign to the crop's health or in many instances, provide a natural biological barrier to infestation by the subset of microorganisms responsible for crop damage (44,45,46).

2.12. RAPPAPORT-VASSILIADIS, SELENITE FECAL, SELENITE CYSTINE SELECTIVE ENRICHMENT BROTHS

Even if difficulties to get study reviews by juice samples. Various studies findings done in food samples shows Rappaport –Vassiliadis(RV) medium involves in expanded uses for food with both high and low levels of competitive micro flora. Selenite Cystine and Tetrathionate(TT) broths continued to be used as the second selective enrichment broths. However, SC and SF broths is incubated at 37^{o}C (for the analysis of high microbial load foods and at 35^{o}C for the analysis of low microbial load foods. RV and TT have similarities and effective to incubate at 43^{o}C for the analysis of many microbial load foods. On the other hand incubation hour for both SF and SC are 24hrs.Whereas, 48hr to 72hr depending on the food types the incubation times was prolonged in RV and other enrichment medias like Tetrathionate (47,48,49).

The efficacy and sensitivity of Selenite Fecal Broth (SFB) enrichments for the isolations of *Salmonella* from fecal specimens were much higher and confirmed by using both clinical and artificially infected (artificial) fecal specimens. Therefore, for recovery of *salmonella* from fecal specimens with SFB broth is increased 3.3-fold over the SC,RV,TT due to the P^{H} ,nutrient contents and typical formulas (50,51).

2.13. OCCURRENCE OF INTESTINAL PARASITES, *SALMONELLA* AND *SHIGELLA* FROM FOODS HANDLERS

Globally several studies indicated that Food handlers (makers) playing a significant role in transmission of food borne infections mainly intestinal parasites and enteric pathogens like *Salmonella* and *Shigella.* In Ethiopia recent studies indicated that the occurrence of those enteric pathogens associated with food are recently increased. This may be due to water quality used, environmental condition, personal hygiene status of food handlers, the sanitary conditions of service houses and equipments have been mentioned to favor the disease. Different studies found that asymptomatic carriers are recently linked with those enteric pathogens (52,53,54).

2.14. KNOWLEDGE, ATTITUDE, PRACTICE OF FOOD HANDLERS

Foods are affected by work experience, training, education level, medical check up,personal hygiene, cross contamination, source of foods and water, storage temperature are found the main factor that foods spoiled due to lack of knowledge, attitude, practice of handlers because human bodies are the most inhabitants for microorganisms growth and multiplication.in Uganda and Rubaga in particular being a highly populated area, diseases related to food contamination are recoginized an important health problems they create big social and economic burden to the communities and to the general health system.in Ethiopia the prevalence of intestinal and bacterial parasites among university and public cafeterias indicates contamination rate is presented everywhere through feco oral transmission of food handlers led to outbreaks of food borne illnesses(55,56,57).

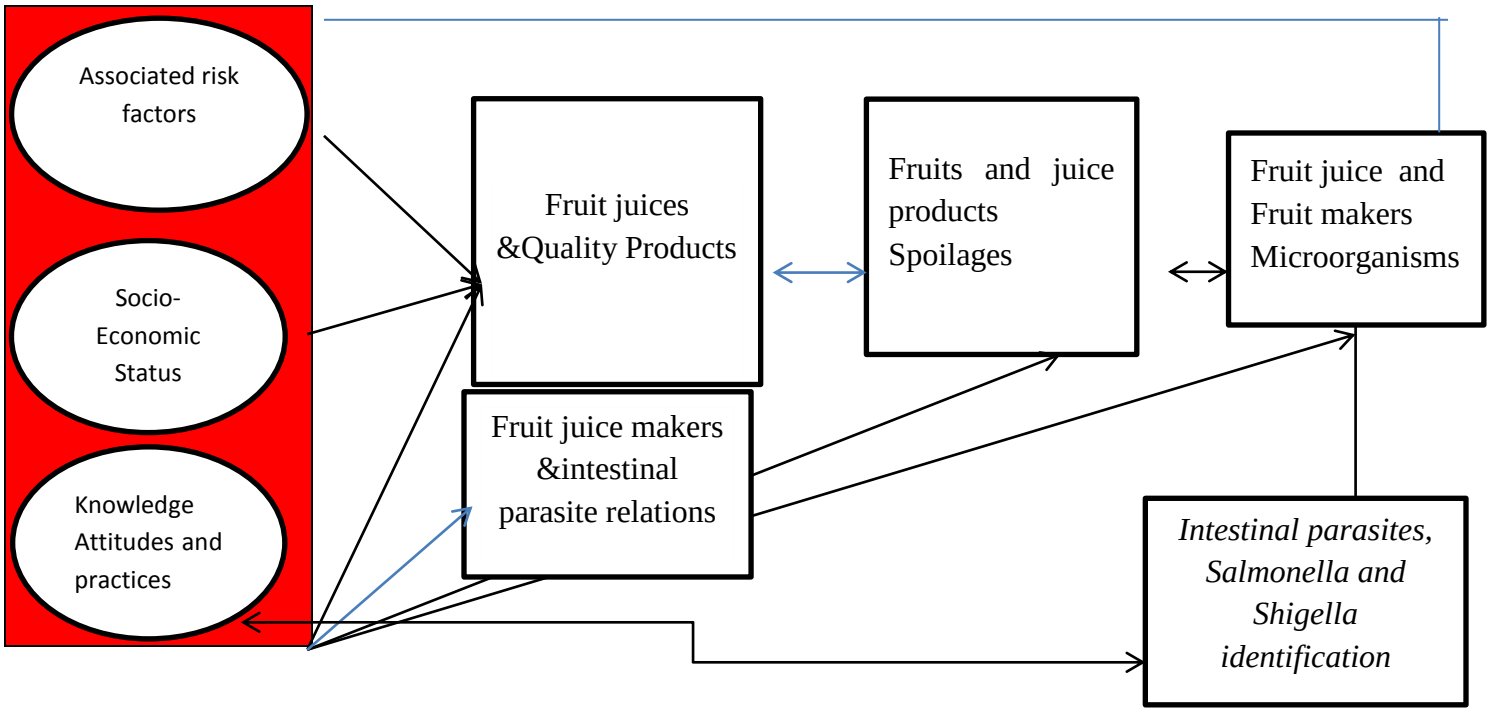


Figure 1. Conceptual frame work on factors affecting fruit juices

3. OBJECTIVES

3.1. GENERAL OBJECTIVE:-

- To identify intestinal parasites, *Salmonella, Shigella* Species from locally consumed fruit juices and juice makers by assessing the hygienic conditions of fruit juice houses in selected sub cities of Addis Ababa, Ethiopia.

3.2. SPECIFIC OBJECTIVES:-

- To determine *Salmonella, Shigella* species and Intestinal parasites from different Fruit juice samples and stool samples of juice makers.
- To assess the knowledge, attitude and practice of fruit juice workers by their work environment on hygienic status and food safety.
- To determine the antimicrobial susceptibility pattern of isolated pathogenic microorganisms (*Salmonella and Shigella*).
- To comparisons the yield of *Salmonella* and *Shigella* species using Rapport vasiliadis and Selenite F and Selenite Cysteine selective enrichment broths.

4. HYPOTHESIS

The Identifications of *Salmonella* and *Shigella* Species from different varieties of fruit juice samples and intestinal parasites from stool samples of fruit juice makers may could be similar to the previous studies.

5. MATERIALS AND METHOD

5.1. STUDY AREA

The study was conducted from locally prepared unpasteurized fruit juices in the Addis Ababa city, is a capital and largest city of Ethiopia. The city was founded in1886 and named Addis Ababa (New Flower) by the emperors Tayetu Betul. According to 2008 census a total of 3,384,569 populations. Currently, Addis Ababa Restructured into 11 sub cities which contain an aggregate of more than 116 woredas under their structure**.** Currently, in Addis Ketema(81),Gulele(75),Arada(69),Yeka sub city(53) number of juice houses are registered. A total of 278 juice houses is found in selected four sub cities. approximately from 856-900 fruit juice workers are found.

5.2. STUDY DESIGN AND STUDY PERIOD

A Cross Sectional Study Design was carried out involving structured KAP assessment questionnaire to evaluate the factors related to bacteriological quality and safety of local fruit juice and laboratory investigation was followed to identify and characterize the bacterial and parasitic load of the pathogenic microbes. Different varieties of fruit juices and stool sample from juice makers was examined for microbiological tests from February 2021 to June 2021 in selected sub cities of Addis Ababa, Ethiopia.

5.3. POPULATION

5.3.1. SOURCE POPULATION

- All Fruit Juice house vender that prepare and sell unpasteurized fruit juices in Addis Ababa selected sub cities.

5.3.2. STUDY POPULATION

- Fruit juice makers directly involved in preparing fruit punch, washing, cutting within the study period in selected sub cities of Addis Ababa, Ethiopia. In general those makers interested to participate in KAP Assessment questionnaire, to give stool sample and unpasteurized fruit juices were collected as a sample for further advanced microbiological tests respectively.

5.4. INCLUSION AND EXCLUSION CRITERIA

5.4.1. INCLUSION CRITERIA

- Fresh unpasteurized fruit juice samples and Fruit juice makers who voluntary agrees to participate in KAP assessment and to give their stool in selected sub cities of Addis Ababa with in the study period were included in the study.

5.4.2. EXCLUSION CRITERIA

- Any samples that is different from fruit juice sample and fecal specimen
- Food handlers not working and making in fruit juices
- Fruit makers not voluntary to participate in KAP assessment interview questionnaires
- Pasteurized or any other canned fruit juices were excluded in the study.

5.5. STUDY VARIABLES

5.5.1. DEPENDENT VARIABLES

- Magnitude of Intestinal Parasites, *Salmonella and Shigella* from Locally prepared fruit juices and Knowledge, Attitude, Practice of Fruit juices makers on juices contaminations.

5.5.2. INDEPENDENT VARIABLES

- Sex, Age, Personal hygiene, Environmental hygiene, Water quality, Educational status of juicer, Health status of juicer, Storage condition of juices, training certificate.

5.6. SAMPLE SIZE CALCULATION AND METHODS

5.6.1. SAMPLE SIZE CALCULATIONS

The number of fruit juices and juice makers in the study areas were adequate, For isolation and identification of Intestinal parasites, *Salmonella* and *Shigella*, the sample size is calculated based on 45.3%(58),3.6%(59) and 0.8%(60), expected prevalence in fruit juice samples respectively, and for KAPs assessments questionnaire sample size is calculated based on 27.4%(i.e.108 food handlers) were found to have good food hygiene practices is selected(61)expected prevalence in fruit juice makers respectively.to increase the precision of estimate, for the KAP assessment sample size was inflated approximately 120 participants was considered.

Where n is required sample size, Z is 1.96, P_{exp} is expected prevalence, and d is desired absolute precision of 0.05. Accordingly, the minimum sample size calculated are 53 and 12. To increase the precision of the estimate, the sample size was inflated and a total of 200 samples was considered. 95% confidence interval using the formula recommended by Thrusfield(62).

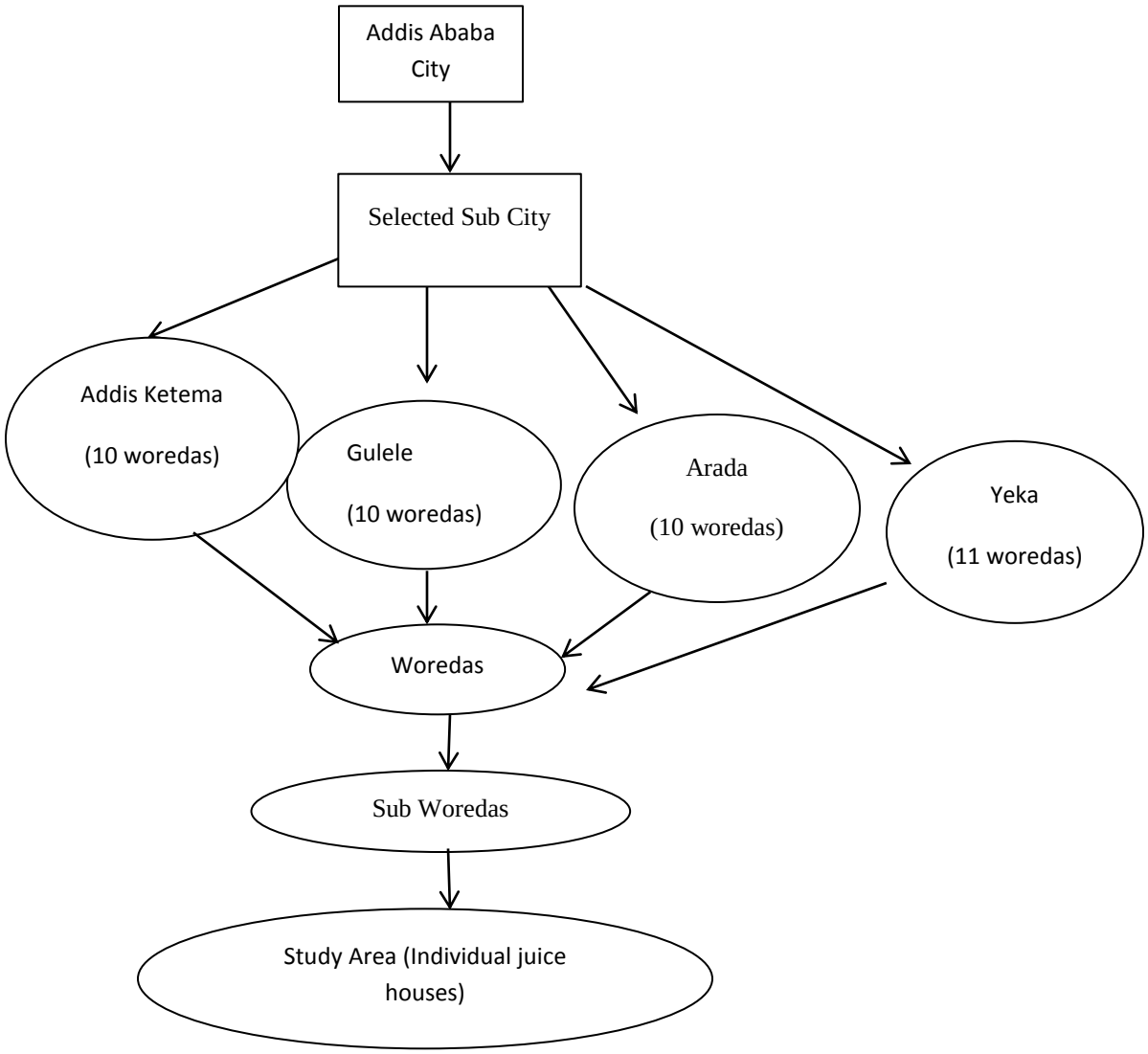
$$n = \frac{Z^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

Therefore, The sample size for KAP assessments is 120 and from Fruit Juice Sample 100 and from Juice Makers collected stool samples is 100 was taken. a total of 320 was considered.

5.6.2. SAMPLING METHODS AND PROCEDURE

Multi-stage sampling method was used to select study site(units) by classifying different stages starts from selected sub city to woredas then sub woredas finally select the individual fruit juice venders from sub woredas randomly due to the limited resource to include all venders. Complete list of fruit juice makers those directly involved in the preparation was taken for knowledge assessment interview after that from juice makers approximately 1 gm stool sample and 25ml fruit juice sample was collected to identify a specific microbes by convenient standard laboratory methods.

Multi-stage sampling Procedures to select individual sample juice houses as follows



Note:- The selected sub cities where done based on number of juice houses, populations and consumer flows

5.7. MEASUREMENT AND DATA COLLECTION

5.7.1. INTERVIEW METHODS

The normal interview were conducted from the KAP data of which 120 juice makers was participated in selected different juice house of Addis Ababa sub city. This face to face KAP assessment questionnaire was very important to gather the real information from the respondents on socio demographic charactestics of fruit juice venders, hygienic quality, storage conditions, source of fruit, water source of juice preparation, cleaning habit of juice makers, practice of washing the fruits before making juices, the practice of equipment's cleaning, training certificate in food safety and hygiene, contaminants and its consequences will be assessed.

5.7.2. DATA COLLECTION PROCEDURE

The sample were collected by using primary data collection method. A total 100 of locally prepared unpasteurized fruit juice samples and 100 of stool specimen from fruit makers were collected after interview. The questionnaire was structured into four distinctive parts; demographic (5questions), knowledge (10 questions), attitude (10 questions), and practices (10 questions). Fruit makers that obtained a total score ≥ mean (2.90) were considered to have "Good knowledge" and those that had scores < mean were considered to have "poor knowledge. The attitude and practice of fruit handlers' towards food safety was measured using 10 questions that have five likert scales response. The scores for knowledge, attitude, practice were normally distributed. Therefore, i add all scores together and divide by the number of scores to obtain the mean. Multi-stage sampling method was used to select individual juice houses randomly from different fruit juice houses of Addis Ababa, Ethiopia in February, 2020. All the samples was collected on a voluntary basis from participating juice houses in a clean beaker (250 ml) container aseptically, labeled and brought to the laboratory for microbiological analysis after processed it immediately.

A wide range of local juice varieties and stool was collected equivalently in order to ensure the precision issues. four out of eleven sub cities were selected based on their number of juice houses in the sub cities and namely: Gulele, Addis Ketema, Arada, Yeka sub cities.

5.8. LABORATORY ANALYSIS

Laboratory procedures such as sample collection, sample processing, bacterial culture, microscopically examination and biochemical tests was used for isolation and identification of selected pathogens.

5.8.1. SPECIMEN COLLECTION

5.8.1.1. STOOL EXAMINATION

The stool specimen were collected from related fruit juice makers in labeled wide-mouthed plastic container and clean wooden applicator stick based on standard procedure.

5.8.1.1.2. TRANSPORTATION

Stool specimen was immediately placed in to carry-blair transport medium using swabs and transported to Addis Ababa university EPHI nutrition laboratory within 2-6 hours using ice-box.

5.8.1.1.3. SAMPLE PROCESSING

Approximately 1g of stool sample was inoculated in Selenite F Broth (a liquid medium used as selective enrichment for *Salmonella* and *Shigella* in food, environmental and clinical samples) enrichement broth at 37^{o}c for 24hr and subculture on to selective media (63).

5.8.1.2. FRUIT JUICES

Different varieties of unpasteurized fruit juice types were collected randomly from fruit juice vender houses located in Addis Ababa sub cities. All samples was gathered on a voluntary basis from participating fruit juice houses in a sterilized wide mouth graduated bottles of 250ml container aseptically, labeled and immediately transported to Addis Ababa University, EPHI nutrition laboratory in an icebox to processed immediately.

Microbiological Analysis were done using appropriate media designed for identification and characterization of different microbial groups by following standard procedures. For primary enrichment of *Salmonella* and *Shigella*, 25ml of fruit juice homogenate is mixed with 225ml of Buffer phosphate water (BPW) (CM 0509,Oxoid,Uk)were used and incubated at 37^{0}C for 18-24hrs for metabolic recovery and proliferation of injured cells in the juice samples and to raise the population of target organisms to a detectable level (64).

After 24 hrs incubation, Approximately 10µl of loop full of fruit sample were inoculated in Selenite Cystine Broth (familiar to use as a selective enrichment of *Salmonella* and *Shigella* in food and environmental samples) at 37^{o}c for 24hr (64,65).

5.8.2. CULTURE

5.8.2.1 ISOLATION AND CHARACTERIZATION OF STOOL

After Following the incubation of Selenite F broth(CM 0395,Oxoid,Uk), a loop full of samples was streaked to XLD (CM 0469,Oxoid,Uk) and MaCconkey agar (MAP) (CM 0115,Oxoid,Uk) was incubated at 37°C for 24 h. The growth of *Salmonella* and *Shigella* species was detected by their characteristic appearance on XLD agar (*Salmonella*: Red with black center, *Shigella*: red/pink colonies without Black center) For confirmation, at least 1–3 presumptive colonies was selected and purified by streaking on to MAP (CM 0115,Oxoid,Uk) plates and incubated at 37°C for 24 hr. pure colonies with white/colorless colonies grows was used for biochemical tests. The biochemical tests used for final identification were followed using standard methods (66,67).

5.8.2.2. ISOLATION AND CHARACTERIZATION OF JUICE SAMPLE

After pre-enrichment in BPW(CM 0509,Oxoid,Uk) then employed for secondary enrichment purpose. From pre-enrichment sample 0.1 ml was mixed with 9.9 ml of Rappaport-Vasiliadis Broth (RVB) (CM 0866, Oxoid, Uk) and at the same time, 1 ml of the suspension was also transferred to 10 ml of Selenite Cystine Broth (SCB) (SM 034,SRL,India Ltd). The RV was incubated at 42°C and The SCB was incubated at 37°C for 24 hrs. A loop full from selective enrichment streaked and subculture on Xylose lysine Deoxycholate (XLD) (CM 0469,Oxoid,Uk) were incubated at 37°C for 24 hours for isolation of *Salmonella* and *Shigella* species. A loop full of culture from selective enrichment broth were streaked separately on to each of the solid media and incubated at 37^{0}C for 18-24hr. un inoculated culture plates are inoculated and rechecked for sterility of the solid media (68).

Finally typical colonies for *Salmonella* or *Shigella* was picked from each plate and analysis for characterization biochemically following standard methods.

5.8.2.3. XYLOSE LYSINE DEOXYCHOLATE AGAR (XLD)

5.8.2.3.1. PRINCIPLES OF XLD

After 24hr Of Incubation, Sub Cultured On Xylose Lysine Deoxycholate (XLD) Agar(CM0469,Oxoid, Ltd Uk).After Overnight Incubation At 37°C The Growth Of *Salmonella* and *Shigella* was Diff erentiated by their colony Characteristic appearance on XLD agar (*Salmonella* red with a black center, *Shigella*: Red Colonies with out black colonies).colony with or without black centered on XLD isolates was picked and preserved by *TSB* plus glycerol. Further identification were done by biochemical tests and automated **BD PHOENIX™ M50 INSTRUMENT** for identification and antimicrobial susceptibility test panel and finally serotyped by polyvalent antisera slide agglutination test for *Salmonella* species differentiation was done (68,69).

5.8.2.3.2 INTERPRETATION OF XLD CULTURE RESULTS

After incubation followed pink colonies with and without black centers from selective medium was picked, purified and tested biochemically (69).

5.8.3. BIOCHEMICAL TESTS FOR IDENTIFICATION OF SALMONELLA AND SHIGELLA

Detection of *Salmonella* and *Shigella* species as clearly shown from table 2 below, from the overnight cultured broth, taking a one loop full organisms was sub cultured in to the biochemical tests . The suspected colony from selective medias was slightly touched and stabbing with sterile loop to the butt and streaking the slants to the specific prepared tests then incubated at 37^{o}C for 24 hours. The isolated colonies was differentiated and identified based on colony charactestics, pigmentation, color, carbohydrate fermentation,H_2S production and specifically SIM(CM 0435,Oxoid,Uk,Ltd),Lysine decarboxylase(Cat No 21044,Deben Diagnostics Ltd, Uk), urease(CM0053,Oxoid,Uk),Simmons citrate utilization(B319,Biomark,India Ltd), Methyl red-Voscus proscure tests(0417,Biomark,India Ltd) were used.

5.8.3.1 INTERPRETATIONS OF BIO CHEMICAL TESTS

In KIA,(CM 0033,Oxoid,Uk) A yellow butt (acid) and red or pink (alkaline) slope indicates the fermenting of glucose only. Cracks and bubbles in the medium indicate gas production from glucose fermentation. A yellow (acid) butt indicates the fermentation of lactose. A red or pink

(alkaline) slope and butt indicates no fermentation of glucose or lactose. After overnight incubation, the clear pinkish color will observed in the Urea agar (CM 0053,Oxoid,Uk) and yellow color, black spots as well as CO_2 bubbles was clearly observed in KIA for *Salmonella*. Where as in *Shigella* blue color was successfully observed by using Simmons citrate agar.

In brief colonies producing an alkaline slant with acid butt and hydrogen sulfide production on Kilgler iron agar, Positive for lysine, negative for urea hydrolysis ,negative for indole test, positive for citrate utilization and motility test considered to be *Salmonella* species and in other way urease negative, indole positive(Varieties) ,produced a pink red slopes and yellow butt with no blackening on Kilgler iron agar, lysine decarboxylase negative and citrate negative was be considered to be *Shigella* species (70,71).Finally, all of the confirmed *Salmonella* isolates were reported and analyzed with current advanced technology by **BD PHOENIX™ M50 INSTRUMENT** and **SEROTYPE DIFFERENTIATIONS** further to know antimicrobial susceptibility test patterns of each isolates (72,73).

5.8.4. *SALMONELLA* SEROTYPING

Salmonella serogrouping were done by using slide agglutination technique using poly O (AI),polyA-S,polyP^H1-2 and monovalent(O2,O3,O4,O5,O6,O7,O8,O9, O15, O34, Ha, Hgm, Hb, Hc, Hd, Hi, Henx) antigens for identification of *Salmonella* serogroups A–E (74,75).

The isolates were serotyped at Ethiopian Public Health Institute, according to the Kauffmann-White classification scheme using a battery of somatic and flagellar antigenic formula (76,77).

5.8.5. WET MOUNT FOR PARASITOLOGICAL EXAMINATION

From carry blair transport media(CM1169,Oxoid,Uk) approximately 1 g of stool and fruit juice sample was emulsified in the center of the applicator stick therefore to a possible detection of parasite a drop of 0.85% Nacl (normal saline) were added to a microscope slide. Take a small amount of stool with a wooden stick mix the stool sample with saline then put cover slide. Examine all fields to detect the presence of protozoa and heliments first by 10x and then 40x. Objective lenses of a light microscope for detection of intestinal protozoan trophozoite, cysts and ova of nematodes. The finding was registered.

5.8.6. ANTIMICROBIAL SUSCEPTIBILITY TESTING

Antimicrobial susceptibility tests were performed on the Mueller-Hinton agar (M173, Himedia, India Ltd) using the modified disk diffusion technique on Mueller–Hinton agar Kirby-Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) 2021 guideline. Approximately 20 mL of medium was poured into 90 mm diameter sterile Petri dishes to a depth of 4 mm and left at 37◦ C overnight to check for sterility. Five ml tryptic soya broth (CM 0876,Oxoid, Uk) was inoculated with test isolates and incubated at 35◦ C for 4 hr. Culture of each isolate was be compared with 0.5 McFarland turbidity standards (also adjusted by adding sterile saline into tubes). In brief three to five pure colonies was be picked with a sterile loop and mixed with sterile normal saline (0.85% NaCl) to prepare a uniform suspension equivalent to 0.5 McFarland standards. The suspension was uniformly spread onto a Mueller-Hinton agar plate using a sterile cotton swab. The plate was left at room temperature for 5-30 minutes to dry. Only *Salmonella* and *Shigella* isolates were tested for susceptibility to the following 10 antibiotics using an automatic disk dispenser (ST6090, Oxoid, Ltd, UK). The antimicrobial disks (Oxoid, Ltd) it includes ampicillin (10µg), amoxicillin clavunilic acid (30µg), chloramphenicol (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), gentamicin (10µg), nalidixic acid (30µg), sulfamethoxazole-trimethoprim (23.75/1.25µg), and tetracycline (30µg), erythromycin (15µg).After incubating the plates at 37°C for 24hours, the diameters of the zone of inhibition including the disks were measured using a ruler and finally recorded to the nearest whole millimeters and interpreted as sensitive, intermediate or resistant based on CLSI 2021 interpretive breakpoints by established interpretive chart (78,79,80).

5.9. DATA QUALITY ASSURANCE

The Quality of the study was collected by trained persons prepared using standard operational procedures for laboratory investigation and media preparation. Structured Questionnaire was followed. It contains clear objectives, logically sequenced questions which are free from scientific terms. Sample collection and processing was carried out using aseptic techniques. The samples also labeled properly. Culture and biochemical results were cross checked by other experienced and trained laboratory personnel. The following quality control strains used: *Escherichia coli* ATCC 25922 for positive control and *Proteus mirabilis* ATCC 35659 for negative control in the LDC and indole tests; un inoculated for negative control in the citrate and urease tests for *Salmonella Typhimurium* ATCC 13311 was used. *K. pneumonia* ATCC 700603 and E. coli were used as positive and negative controls, respectively. for the citrate tests. E. coli ATCC 25922 and *K. pneumonia* ATCC 700603 were used as positive and negative controls in the motility test. The performance and sterility of prepared media checked by incubating some randomly selected plates was inoculating with specific control strain organisms respectively for 24 hrs at 37◦ C.

5.10. DATA ANALYSIS AND INTERPRETATION

Data was entered and analyzed using SPSS version 23. All the Data Reliability was checked by Cranach alpha. Results were analyzed carefully to keep their accuracy, reliability and validity using descriptive statistics, Frequency distributions and percentages were computed for categorical variables. Bivariate logistic regression was applied to determine the crude association (using crude odds ratio) between the socio-demographic variables and the occurrence of *Salmonella, Shigella* or intestinal parasites among the study participants. chi square test were used to determine association between dependent, independent variables,fruit juice, fruit makers. Results of the analysis were displayed by using tables, graphs, charts. A p-value ≤ 0.05 was considered to indicate statistically significant differences. 0.05 at 95% confidence level was considered as statistically significant association. The result was presented using tables and charts.

5.11. ETHICAL CONSIDERATION

The Study was conducted after getting full approval by scientific and ethical committee of Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences. Informed written consent was kept confidential and every data regarding the study participants were coded. Participants will have a full right to continue or withdraw from the study. Participation was taken on a strictly voluntary basis and all personal identifiers were removed to avoid linking responses to specific individuals, those preserving confidentiality. Fruit makers who have found to be positive for enteric pathogens were referred to their respective medical center for appropriate antimicrobial treatments. All information were coded and kept confidential.

5.12. DISSEMINATION OF THE RESULTS

After conducting the research, the result of this study were submitted to the school of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University Research and Publication Office and finally the Manuscript will be submitted to Different journals for publication.

5.13. OPERATIONAL DEFINITIONS

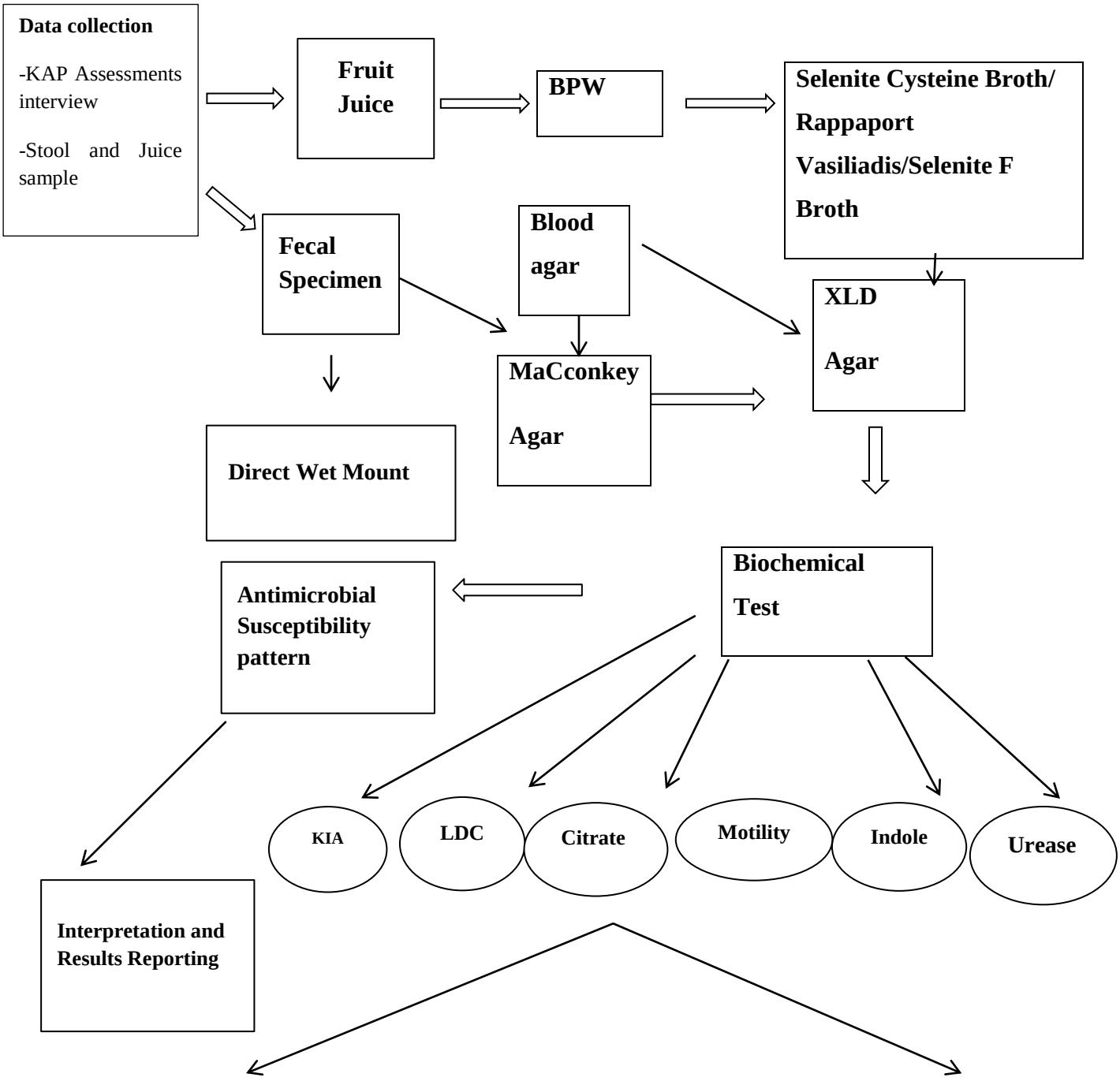
Shigellosis: is an acute invasive enteric infection caused by bacteria belonging to the genus *Shigella*, it is clinically manifested by diarrhea that is frequently bloody.

Salmonellosis: is an infectious disease of humans and animals caused by organisms of the species of *Salmonella*.

Unpasteurized fruit juice: is a fruit obtained by mechanically squeezing of the fruit or vegetable flesh without heating or adding solvents or any preservatives.

Multidrug resistance (MDR): is defined when isolates are resistant to two or more antimicrobials of different group.

5.14. WORK FLOW



Conformation by BD PHOENIX™ M50 INSTRUMENT ID and AST panel analyzer and Serotype species differentiation by Polyploidy and monovalent *Salmonella* specific antisera

Figure 2. Over all brief plan of the research

6. RESULTS

6.1. SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE STUDY PARTICIPANTS

Overall, 120 fruit makers were participated in this study, majority of them were females 108(90%) with the mean age of 23.5 and standard deviation of 0.941.42(35%) of juice makers were in age group of 26-30 years. 65(54.2%) participants had completed elementary school.45(37.5%) were working for greater than 2 year's experience in juice maker. About 21(17.5%) and 106(88.3%) of the fruit makers have training certificates and periodical medical checkups at least within six months respectively(Table1).

Table 1. Socio-demographic characteristics of fruit juice makers in selected sub cities of Addis Ababa, Ethiopia,2021 (N=120).

Sr. No	Sociodemographic Characteristics	Variable	Frequency	Percent
1	Gender	Male	12	10
		Female	108	90
2	Age	18-21	26	21.7
		22-25	39	32.5
		26-30	42	35
		>31	13	10.8
3	Educational Status	Illiterate	23	19.2
		Elementary	65	54.2
		High School	32	26.7
4	Medical Check up (from September 2020 to February 2021)	Yes	106	88.3
		No	14	11.7
5	Training Certificate on food preparation	Yes	21	17.5
		No	99	82.5
6	Year of service	≤1	31	25.8
		1 up to 2	44	36.7
		≥2	45	37.5

6.2. MICROBIAL FINDINGS FROM FRUIT JUICE MAKERS STOOL SPECIMEN IN SELECTED SUB CITIES OF ADDIS ABABA, ETHIOPIA

6.2.1. IDENTIFICATION OF INTESTINAL PARASITES FROM FRUIT JUICE MAKERS

A total of 100 fruit juice makers 10(10%) were males and 90(90%) females. However, in Figure 3(Fig 3a and Fig 3b) showed that the overall intestinal parasite distribution of juice makers was found to be 27 (27%).

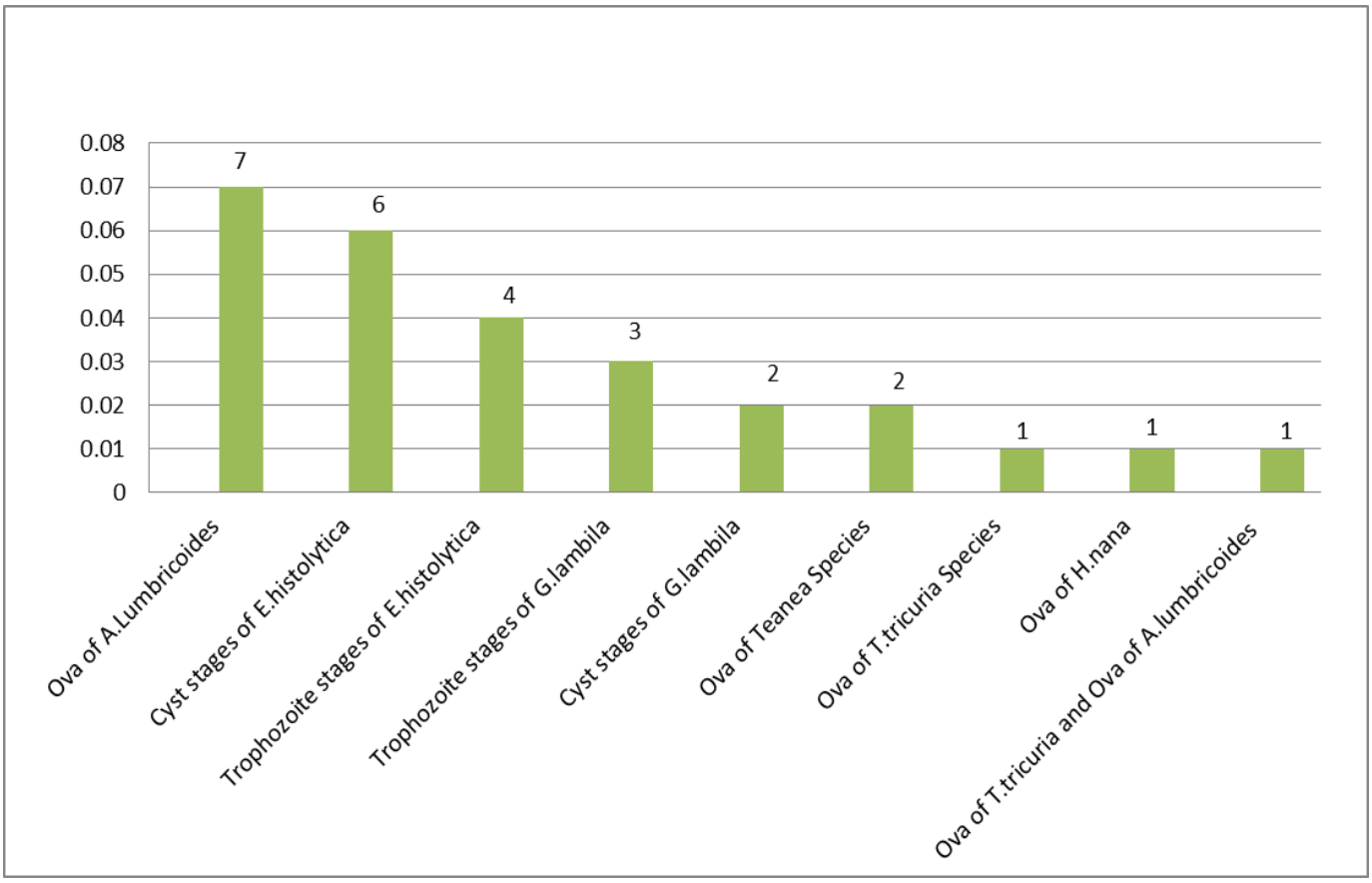


Figure 3a:-Identifications of intestinal parasites from fruit juice makers in selected sub cities of Addis Ababa, Ethiopia, 2021.

The above (Figure 3a) showed that from the fecal samples of fruit juice makers the most dominant detected intestinal parasite found was *A. lumbricoides 7(7%).Followed by cyst of E.histolytica 6(6%),Trophozoite stages of E.histolytica 4(4%),Trophozoite stages of G. lambila 3(3%),cyst of G.lambila 2(2%),Ova of T.tricuria 2(2%),Ova of Taenia species 2(2%),Ova of H.nana 1(1%)* and *only 1(1%) Ova of T.tricuria* and *A.lumbricoides* was identified from a single juice maker fecal specimen.

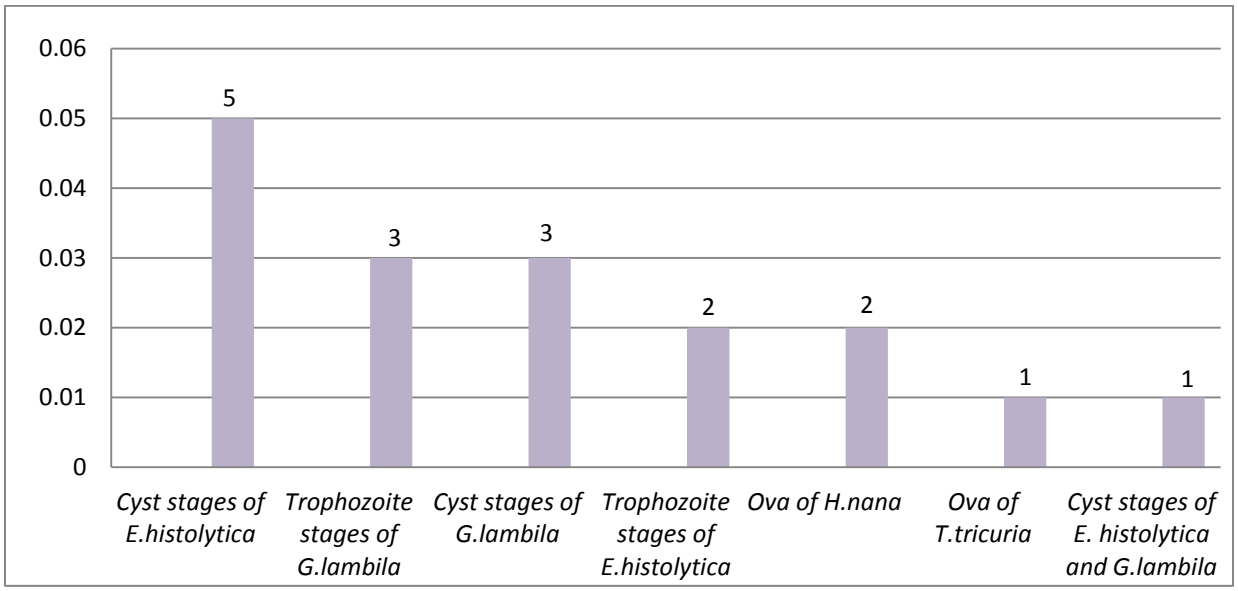


Figure 3b:- **Identifications of intestinal parasites from local fruit juice samples in selected sub cities of Addis Ababa,Ethiopia,2021.**

Where as (Figure 3b) showed that From the total of 100 unpasteurized fruit juices samples was 17 (17%). The most identified intestinal parasite identified was *Cyst stages of E.histolytica 5(5%).*and *Cyst* and *Trophozoite stages of G.lambila 3(3%) for each. Trophozoite stages of E.histolytica 2(2%),Ova of H.nana 2(2%),Ova of T.tricuria 1(1%)* and *only Cyst of E.histolytica* and *G.lambila 1(1%)* mixed parasites were identified from a single avocado fruit juice.

Table 2. Shown that only an association between socio demographic and stool *Salmonella*, stool *Salmonella* and Fruit *Salmonella*. There is no association between socio demographic and Stool *intestinal parasites*, socio demographic and fruit identified among fruit makers engaged in fruit juice houses. There are statistically significant association between socio demographic and Stool salmonella culture(X1=182.747, p=0.01), stool *Salmonella* and fruit *Salmonella*(X1=46.791, p=0.004). The strongest positive association between socio demographic and stool *Salmonella* result. Sociodemographic charactestics of juice makers are the key element to influence the outcome of juice among the fruit handlers. This association shows fruit makers and their juice products have a strong impact to the consumers. However, with good attitudes can give more impact to their practices in food safety. Therefore, giving good training among the fruit makers can improve their quality products throughout all processes hence in fruit juice safety and quality is the main concern.

Table 2. Association between socio demographic characteristics, intestinal parasites, stool culture identification of *Salmonella,* fruit juice identification of *Salmonella* in selected sub cities of Addis Ababa, Ethiopia,2021.

Variable	Chi-square	P-value	Significance(P≤0.05)
Sociodemographic α Intestinal Parasites	19.133	0.603	No
Socio Demographic α Stool *Salmonella* culture	182.747	0.001	Yes
Socio Demographic α Fruit identified	60.380	0.587	No
Stool intestinal parasites α Stool *Salmonella* culture	7.685	0.475	No
Stool *Salmonella* α Fruit *Salmonella*	46.791	0.004	Yes

From a total of 100 stool samples only 6 isolates were identified as *Salmonella*. Isolation and identification of S*almonella* species by microbiological analysis and its further confirmation test was employed using BD phoenix and serotype identification by slide agglutination polyploidy ag tests. Briefly, On microbiological analysis of 100 samples 12 samples revealed presumptive *Salmonella* and *Shigella* colonies on XLD agar plate. Biochemical tests were done on *Salmonella* and *Shigella* species. All the 12 suspected isolates were KIA positive, Urease positive, indole negative, methyl red positive, vogesproskauer negative and citrate test positive, lysine decarboxylase positive/negative. but only *Salmonella* isolates was identified. Therefore, the identified *Salmonella* isolates were confirmed using BD-Phoenix analyzer and serogrouping was done by slide agglutination test using poly and monovalent antigen tests. There is no confirmed *Shigella* isolates that was detected in this study (Table 3).

Table 3. Effect of BD phoenix and Serotyping with microbiological tests on identified *Salmonella* isolates from stool samples of fruit juice makers in selected sub cities of Addis Ababa,Ethiopia,2021(N=100).

Stool Sample	Source of sample	No of sample screened	Positive samples on microbiological analysis	Positive samples on biochemical analysis	BD Phoenix	Serotype by Agglutination
	Male Juice makers	5	12	10	6	6
	Female juice makers	95				

6.3. MICROBIAL FINDINGS FROM FRUIT JUICE SAMPLES IN SELECTED SUB CITIES OF ADDIS ABABA, ETHIOPIA.

6.3.1. SAMPLE SOURCE DISTRIBUTION OF *SALMONELLA* SPECIES

Of which the total of 5(45.5%) fruit juices *Salmonella* identified samples avocado(18.2%), spris (9.1%),papaya(9.1%),others(9.1%) were contaminated by *Salmonella* species identified

respectively. (Table 4) shows the distribution of *Salmonella* samples distribution. The remaining 6(54.5%) was identified from juice makers fecal specimen.

Table 4. Distributions of *Salmonella* isolates from different fruit juice samples in selected sub cities of Addis Ababa,Ethiopia,2021 (N=5).

Type of Fruit juice	Frequency	Percent
Avocado	2	18.2
Spris(Mixed Juices)	1	9.1
Papaya	1	9.1
0thers(Ananas+Zeytuna)	1	9.1
Total	5	45.5

6.3.2. IDENTIFICATION OF *SALMONELLA* AND *SHIGELLA* FROM FRUIT JUICE SAMPLES

Among a total of 100 samples examined for bacteriological status, 5 samples were positive for *Salmonella* by culture, biochemical test and confirmed by BD Phoenix (but the number of true positive samples was reduced). Ninety five samples were negative for *Salmonella* and *Shigella* Species. In this study period non of local fruit juice samples were contaminated by *Shigella* species by all microbiological works that was rechecked by *BD* Phoenix automatic analyzer. The highest number of *Salmonella* species were occurred from avocado sample(Table 5).

Table 5. *Salmonella* species and type of fruit juices distribution in selected sub cities of Addis Ababa Ethiopia,2021(N=5).

Types of fruit juices	Frequency	Percentages	*Salmonella* isolates	
			Frequency	Percentage
Avocado	28	28	2	18.2%
Spris	34	34	1	9.1%
Papaya	24	24	1	9.1%
Others(Ananas+Zeytuna)	14	14	1	9.1%

6.3.3. IDENTIFICATION OF PRESUMPTIVE ISOLATES USING BD PHOENIX AUTOMATIC ANALYZER

A single pure colony of each isolate on XLD agar was refreshed on MaCconkey agar and Blood agar and incubated at 37 °C for 24 hr. Add organism to ID Broth. Make 0.50 - 0.60 McFarland prepare purity plates (Standard inoculum). Make .20 - .30 McFarland (Low inoculum).Prepare Purity Plate. Using a sterile loop, recover a small drop from the inoculum fluid either before or after inoculating the panel. ID is based on 45 chromogenic and fluorogenic substrates. Isolates from the 24 hrs colonies without extraction according to manufacturer instructions (Becton, Dickinson). A broth based micro dilution method that utilizes a redox indicator (colorimetric oxidation-reduction) to enhance detection of organism growth. The degree of spectral pattern matching is expressed as a logarithmic identification score and interpreted according to the manufacturer's instructions. BD-Phoenix identification results were recorded as score values (SV),graph, table and used to determine the identity of the organism as proposed by the manufacturer. Highly probable species identification were confirmed.

In this study even if no *Shigella* species were encountered. *S. aureus, E.coli, Enterobacter and Citirobacter* species, *Pseudomonas aeroginosa*, *Serracia marcescens* were detected from different fruit varieties (Table 6).

Table 6. Bacterial isolates identified by BD phoenix automated analyzer other than *Salmonella* species.

Identified organisms	Sample Source	Frequency	Percentages
Fruit juices	*P. aeroginosa*	2	100
Fruit juices	*S. Marcescens*	1	100
Fruit juices	*K. pneumonia*	1	100
Fruit juices	*E.coli*	1	100
Fruit juices	*Enterobacterclocae*	1	100
Fruit juices	*Citrobacter Species*	1	100
Fruit juices	*S.aureus*	1	100
Stool	*E.coli*	2	100
Stool	*S.aureus*	1	100

6.4. SALMONELLA SEROVAR DISTRIBUTION

A total of 11 different *Salmonella enterica serovars* was identified and 6(54.6%) of them were from juice makers fecal specimens and 5(45.4%) from fruit juices specimen. From fecal specimen *S.Newport 2(18.2%), S.Thyphi 2(18.2%)* predominantly identified. *S.Dublin 1(9.1%),S.Anatum1 (9.1%)*was followed respectively. Where as from fruit specimen *S.Gaminara 1(9.1%), S.Enteritidis 1(9.1%), S.Thyphimurium 1(9.1%), S.Montevideo 1(9.1%)* and the two serovars namely *S.Dublin and S.Saintpaul 1(9.1%),* from each identified from single avocado samples (Figure 4). However, *S.Dublin* were the only serotype identified in both fruit juices and stool specimen. All the confirmed *Salmonella* isolates from fruit juice was enriched with convenential methods RV and SCB. Figure 4 shows the distribution of *Salmonella* serovars and their antigenic formula.

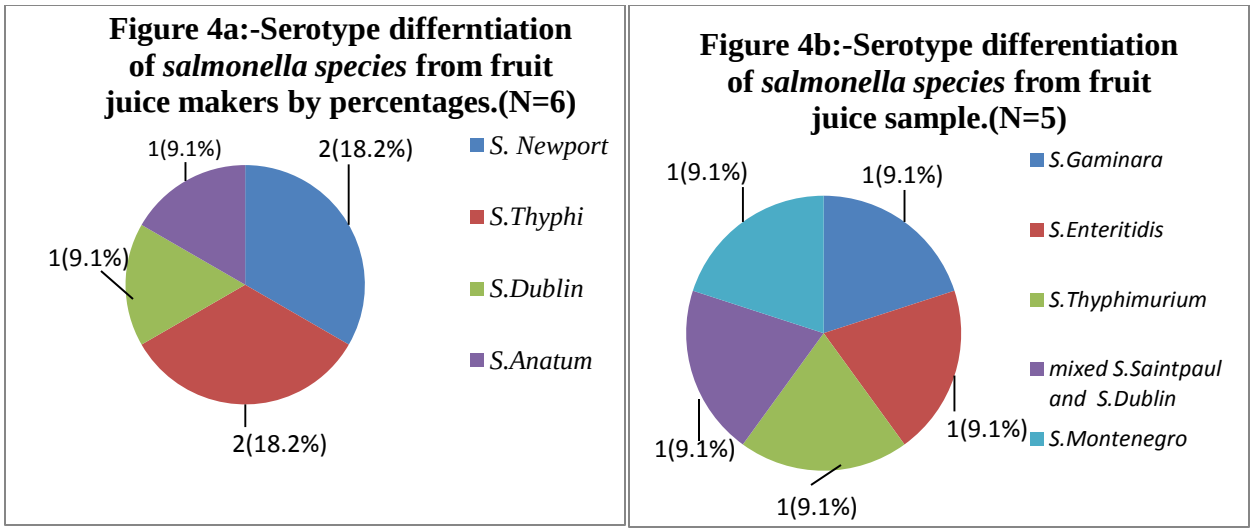


Figure 4:-Pie chart on serovars distribution of *Salmonella* from fecal specimen of fruit juice makers and different juice samples in selected sub cities of Addis Ababa, Ethiopia,2021,(N=11).

Salmonella serotyping by using slide agglutination technique poly O (AI), polyA-S, poly P^{H}1-2 and monovalent (O2,O3,O4,O5,O6,O7,O8,O9,O15,O34,Ha,Hgm,Hb,Hc, Hd,Hi,He,Hh, Hp,Hg,Henx) antigens for identification of *Salmonella serogroups* A–E were used.

6.5. Comparisions of Rappaport vasiliadis with Selenite F and Selenite Cystine Selective enrichment broths

In total of 100 fruit juice samples from juice houses and 100 fecal samples from juice makers were tested. Depending on the selective media used as followed in Table 7,from fruit juices 8(6.7%) and 6(5%) were found contain *Salmonella* in Rappaport vasiliadis and Selenite cysteine selective enrichment media of 100 sample for each after 48hr and 24hr incubation respectively. Statistically significant results were found in both selective medias. Using RV,6.7%(Mean 1.92,SD 0.239).95% of the total number of the isolate. From fruit juice sample were found to contain *Salmonella*. Whereas, from fruit juice makers fecal specimen 4(3.3%) and 12(10%) were shown positive for *Salmonella* in RV and SF selective enrichment media of examined samples. After 48hr and 24hr incubation respectively. A statistically significant difference is shown using Rv,(Mean1.89,SD 0.345).Using SF,(Mean1.96,SD 0.197) from fecal specimens contain *Salmonella*.

Table 7. Comparisions between Rappaport vasiliadis, Selenite F, Selenite Cysteine enrichment broth medias from fruit juice and juice makers fecal specimen in selected sub cities of Addis Ababa,Ethiopia,2021(N=200).

Types of Specimen	Names of Enrichment Medias	Mean	SD	Frequency(%)	Chi-square	P-value	R(odds ratio)
Fruit Juices (N=100)	Rappaport Vasiliadis	1.92	0.273	8(6.7)	29.849	0.001	0.546
	Selenite Cysteine	1.94	0.239	6(5)			
Stool Specimen (N=100)	Rappaport Vasiliadis	1.89	0.345	4(3.3)	15.664	0.044	0.380
	Selenite F	1.96	0.197	12(10)			

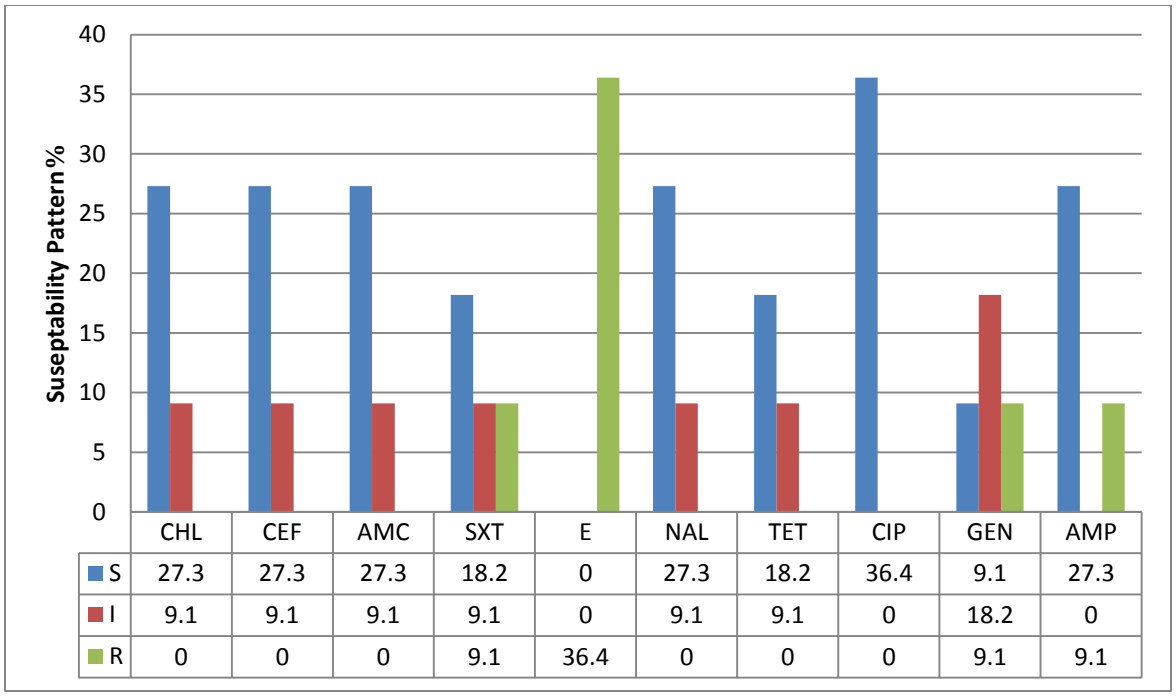
6.6. ANTIMICROBIAL DRUG SUSCEPTIBILITY TEST

From the identified *Salmonella* species tested against10 selected antimicrobial agents as followed in Table 8 and Bar graph 5. From juice makers fecal specimen antimicrobial susceptibility pattern of *Salmonella* isolates showed multi drug resistance to ampicillin 7(63.6%) and erythromycin 7(63.6%), amoxicillin- clavunilic acid 6(54.5%).Where as ciprofloxacin 6(54.5%),sulfa methoxazole trimethoprim 6(54.5%),ceftriaxone 5(45.5%), chloramphenicol 5(45.5%), naldix acid 5(45.5%) were susceptible isolate detected in 6 sample respectively. On the other hand, the antimicrobial susceptibility test pattern of *Salmonella* isolates identified from 5 fruit juice samples showed highest multi resistance to erythromycin 4(36.4). However, among all only gentamycin 2(18.2%) shows the highest intermediate. Whereas ciprofloxacin 4(36.4%), chloramphenicol 3(27.3%), ampicillin 3(27.3%) and amoxacilin 3(27.3%) were detected susceptible respectively (line graph 4).

Table 8. Antibiotic susceptibility pattern of *Salmonella* isolates from fruit juice makers fecal specimen in selected sub cities of Addis Ababa, Ethiopia,2021 (N=6).

No	Antibiotics	*Salmonella* Isolates		
		Susceptible (%)	Intermediate (%)	Resistance (%)
1	CHL(30µg)	5(45.5)	1(9.1)	1(9.1)
2	CEF(30µg)	5(45.5)	1(9.1)	1(9.1)
3	AMC(30µg)	0	0	6(54.5)
4	SXT(25µg)	6(54.5)	0	0
5	E(15µg)	0	0	6(54.5)
6	NAL(30µg)	5(45.5)	1(9.1)	1(9.1)
7	TET(30µg)	2(18.2)	2(18.2)	3(27.3)
8	CIP(5µg)	6(54.5)	0	0
9	GEN(10µg)	3(27.3)	3(27.3)	1(9.1)
10	AMP(10µg)	0	0	6(54.5)

CHL=Chloramphenicol(30µg)CEF=Ceftriaxone(30µg) AMC=Amoxicillin Clavunilic acid(30µg) SXT=Sulfamethoxazoletrimethoprim(23.75/1.25µg)E=Erythromycin(15µg)NAL=NalidixicAcid(30µg)TET=Tetracycline(30µg)CIP=Ciprofloxacin(5µg)GEN=Gentamicin(5µg) AMP=Ampicillin(10µg)



	CHL	CEF	AMC	SXT	E	NAL	TET	CIP	GEN	AMP
S	27.3	27.3	27.3	18.2	0	27.3	18.2	36.4	9.1	27.3
I	9.1	9.1	9.1	9.1	0	9.1	9.1	0	18.2	0
R	0	0	0	9.1	36.4	0	0	0	9.1	9.1

CHL=Chloramphenicol(30µg)CEF=Ceftriaxone(30µg) AMC=Amoxicillin Clavunilic acid(30µg) SXT=Sulfamethoxazoletrimethoprim(23.75/1.25µg)E=Erythromycin(15µg)NAL=NalidixicAcid(30µg)TET=Tetracycline(30µg)CIP=Ciprofloxacin(5µg)GEN=Gentamicin(5µg) AMP=Ampicillin(10µg)

Figure 5:-Antimicrobial susceptibility test pattern of *Salmonella* species from fruit juices in selected sub cities of Addis Ababa, Ethiopia,2021,(N=5).

6.7. KNOWLEDGE, ATTITUDE, PRACTICE OF FRUIT JUICE MAKERS ON FOOD HYGIENE AND SAFETY OF POSSIBLE MICROORGANISMS

6.7.1. Knowledge of fruit juice makers on Food hygiene and safety

Table 9. Highlights are almost the majority of fruit juice makers knowledge correctly answered with a mean percentage score average of 95.8% knowledge in the categories of self-hygiene, washing, storing, possible safety and quality of all fruits products. this shows that their level of knowledge is in excellent conditions since they answer well for all questions with a score nearer to 95.8%.

Fruit makers in Addis Ababa show good knowledge in categories the way they wash fruits when blending, cutting to prevent microbes (99.2%), frequently washing different fruits and equipment's using tap water (98.3%), the time they should clean their equipment and work area (93.3%) and the time to wash their hand after touching unhygienic materials any time (97.5%). the fruit makers show good knowledge in categories of fruit storage of fruits and varieties products temperature (96.7%). Even if, almost all the average participants answered it correctly the results indicates personal awareness, discussions evaluations must be needed on how stored and monitor different varieties of fruit types. This study demonstrates fruit juice makers have the awareness of the need for personal hygiene and contamination regardless of possible microbes and fruits juices quality, the crucial aspect linked to temperature values as it is needed to control the growth of microbes in fruits.

Table 9. Knowledge assessment of fruit juice makers on hygiene and safety of fruit juices in selected sub cities of Addis Ababa,Ethiopia,2021 (N=120).

Topic	Correct (%)	Incorrect (%)	Not Sure (%)
Fruit Juices after preparation can be safe when we store at 2-8 degree Celsius	102(85.0)	6(5.0)	12(10.0)
Washing different fruit products frequently is adequate	113(94.2)	7(5.8)	0
Better to wash fruits before Blending/Cutting are the safest way to prevent any contamination	119(99.2)	1(0.8)	0
Always washing all the equipments, work area used to prepare juices	112(93.3)	8(6.7)	0
Frequently washing customer used equipment's all the time using tap water is not enough	118(98.3)	0	2(1.7)
Unclean water leads to cause water borne disease	117(97.5)	1(0.8)	2(1.7)
Do you think microbes may contaminate fruit juice any time during preparation	118(98.3)	1(0.8)	1(0.8)
Do you think During preparation of fruit juices following proper hand washing microbes can't causes illness	118(98.3)	1(0.8)	1(0.8)
Proper storage of fruits will help in preparation of getting different microbes	116(96.7)	1(0.8)	3(2.5)
Juice makers should wash their hands always after touching unhygienic materials any time	117(97.5)	0	3(2.5)

6.7.2. Attitudes of Fruit juice makers on Food Hygiene and Safety

The overall attitudes of fruit makers to ward to fruit hygiene and safety were at the high level of satisfaction. Most respondents strongly agree with all 8 questions regarding food hygiene and safety attitudes as followed in Table 10. Most of the food handlers strongly agreed that they should to wash hands properly after unhygienic practice on (80.8%), to use a clean hand towel to wipe their hands after washing them(77.5%),personal cleanness and wearing gloves, cloths, cap before start preparation(69.2%) for each respectively. and Fruit makers suffering from food borne diseases should not be allowed to work(65%), but there is slight decrease of the percentage in using filtered/distilled water when prepare juices to consumers is important to prevent food borne diseases (59.2%) and a marked decreasing of results says strongly agreed in using diluted detergents and chemicals only(34.2%),wearing gloves when touch ready to eat juice varieties(46.7%),in having proper short nails and clean hands(59.2%).

Table 10. Attitude assessment of fruit juice makers on hygiene and safety of fruit juices in selected sub cities of Addis Ababa,Ethiopia,2021 (N=120).

TOPIC	Strongly Agree(%)	Agree(%)	Uncertain(%)	Disagree(%)	Strongly Disagree(%)
Always personal cleanness is highly important in preparation of fruit juices one main responsibility of my jobs is to handle fruit safely	83(69.2)	32(26.7)	5(4.2)	0	0
Fruit makers suffering from food borne diseases should not be allowed to work	78(65.0)	34(28.3)	6(5.0)	2(1.7)	0
Fruit Juice makers if get accident their wounded fingers and hands can handle food only if they correctly cover their cuts	84(70.0)	28(23.3)	8(6.7)	0	0
fruit juice makers must wear gloves, cloths,cap before start preparations	83(69.2)	35(29.2)	2(1.7)	0	0
fruit makers should have proper short nails and clean hands are important to fruit juice preparation	71(59.2)	47(39.2)	2(1.7)	0	0
fruit juice makers should wear gloves when they touch ready to eat juice varieties	56(46.7)	49(40.8)	14(11.7)	1(0.8)	0
i think fruits must be washed first with diluted detergent chemicals then rinse well with water thoroughly before used in juice preparation to prevent different microbes	41(34.2)	34(28.3)	20(16.7)	23(19.2)	2(1.7)
fruit juice makers should make sure to use filtered/distilled water when prepare juices to consumers is important to prevent food borne diseases	71(59.2)	44(36.7)	4(3.3)	1(0.8)	0
It is important to wash hands right after unhygienic practice	97(80.8)	22(18.3)	1(0.8)	0	0
Fruit juice makers should use a clean hand towel to wipe their hands after washing them	93(77.5)	26(21.7)	1(0.8)	0	0

6.7.3. Practices of fruit juice makers on Fruit Hygiene and Safety

Table 11. The level of fruit makers' practices shows low with a percentage score of 39.5% of the respondents but the practices hygienic and safety status of respondents 5 out of 10 questions shows low percentage score of 39.5%. Three aspects are being evaluated which are hand washing, prevention of contamination in waste disposal and safety issues were included. Hand washing aspects have the highest score as they always practice right-hand washing procedure (45%), always washing your hands after returning from bathroom (73.3%), always wash hand after doing unhygienic practice (84.3%), always wash hands after break session (82.4%) wash your hands after handling fruit waste/dealing with rubbish(55%), wash your hands after rubbing your nose/scratching your body(52.5%).even if the practice score level is significantly decreased in wearing any items of jewellery when you handle fruit juices(5.8%). Washing in the right hand procedures (45%).in our country, few studies showed that poor hygiene practices among food handlers are explained by food handlers' lack of knowledge.

Table 11. Practice assessment of fruit juice makers on hygiene and safety of fruit juices in selected sub cities of Addis Ababa,Ethiopia,2021 (N=120).

TOPIC	Always(%)	Often(%)	Sometimes(%)	Rarely(%)	Never(%)
Do you wash your hands after returning from bathroom	88(73.3)	26(21.7)	5(4.2)	1(0.8)	0
Do you wash your hands after rubbing your nose/scratching your body	63(52.5)	53(44.2)	4(3.3)	0	0
Do you wash your hands after handling fruit waste/dealing with rubbish	66(55.0)	43(35.8)	11(9.2)	0	0
Do you make sure that your hands are dry and clean every time you are handling of fruits	64(53.3)	40(33.3)	14(11.7)	2(1.7)	0
Do you wear any items of jewellery when you handle fruit juices	7(5.8)	10(8.3)	27(22.5)	47(39.2)	29(24.2)
Do you follow the right hand washing procedures	54(45.0)	23(19.2)	13(10.8)	12(10.0)	4(3.3)
Do you touch fruit when you cut your fingers and the cut is not well covered	16(13.3)	24(20.0)	25(20.8)	23(19.2)	32(26.7)
Do you wear proper clean suitable uniform before working	65(54.2)	35(29.2)	14(11.7)	2(1.7)	4(3.3)
Do you eat, drink or chew gum as you prepare juices	37(30.8)	29(24.2)	15(12.5)	17(14.2)	22(18.3)
What's your cleaning habits of fruits during preparation	14(11.7)	16(13.3)	14(11.7)	32(26.7)	44(36.7)

6.7.4. FRUIT HYGIENE, QUALITY, SAFETY ON KNOWLEDGE, ATTITUDE AND PRACTICE LEVELS OF FRUIT JUICE MAKERS

From the total 120 respondents, 103 (95.8%) had a good level of knowledge and 76 (63.1%) had a good level of attitude about the food safety whereas only 47 (39.5%) fruit juice makers had a good level of food safety practice and 53 (60.5%) had a poor level of self-reported practice (Figure 6).

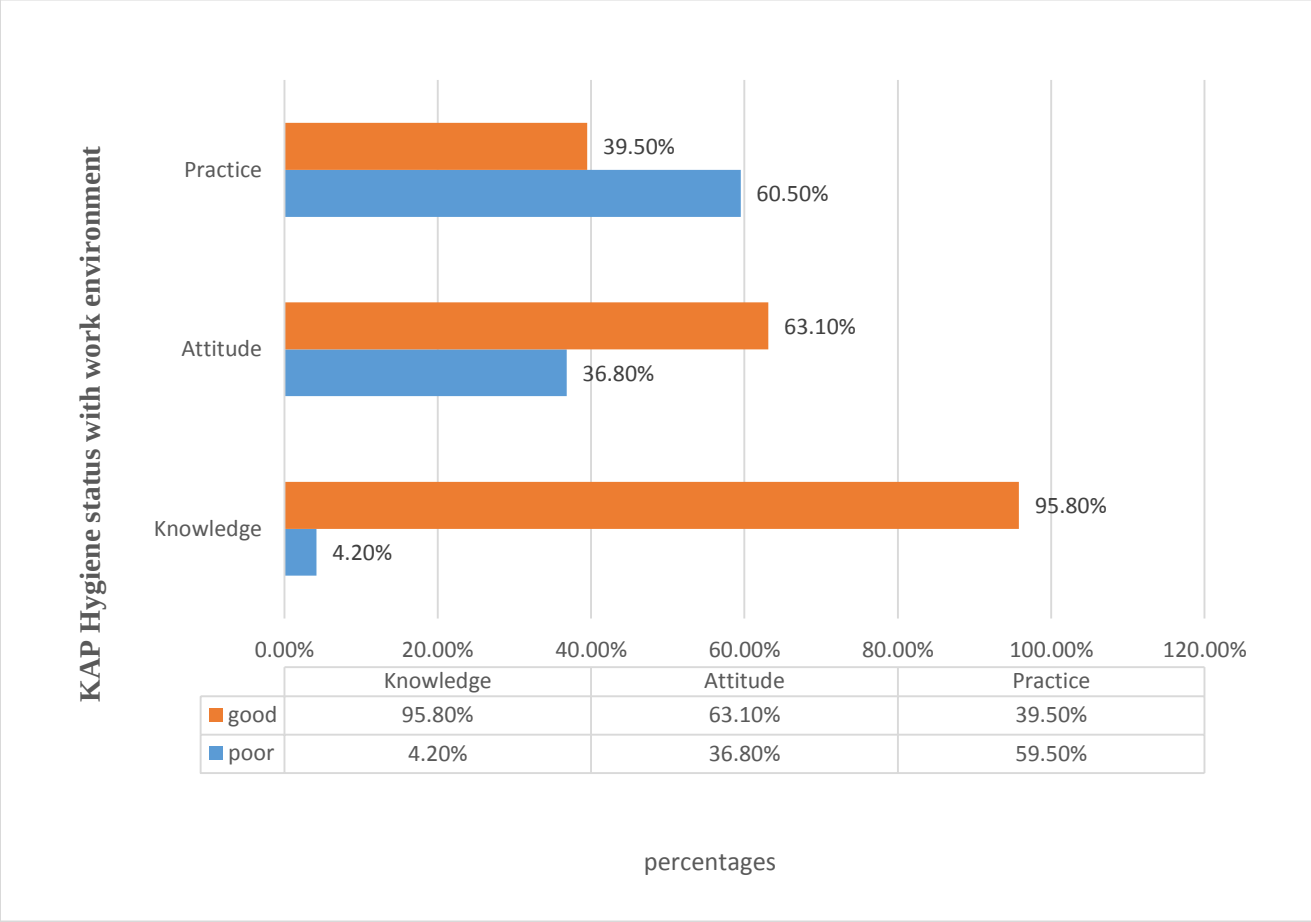


Figure 6. Average score/cut off values of fruit juice makers by KAP analysis using likert scale

OVERALL ASSESSMENT OF SOCIO DEMOGRAPHIC CHARACTERISTICS OF FRUIT JUICE MAKERS BY THEIR KNOWLEDGE, ATTITUDE, PRACTICE

Association between Knowledge on Fruit Safety Based on Sociodemographic Characteristics of Participants

Statistically significant differences in the fruit safety knowledge of fruit vendors were found on the basis of year of Service ($X1 = 24.037$; p value = 0.046) as indicated in Table 13 . No statistical difference was found between the food safety knowledge of food vendors based on their training certificate ($X1 = 4.99$; p value =0.958), sex (X1= 5.48; p value =0.940), age group ($X1 = 33.68$; p value =0.580), education status ($X1 = 27.45$; p value =0.285), and medical check up ($X1 = 12.66$; p value =0.394)(Table 12).

Association between Attitude on Fruit safety Based on Sociodemographic Characteristics of Participants

No statistically significant differences in the fruit safety attitude of fruit vendors were found on the basis of Sex (X1= 14.40; p value=0.346), age group (X1=43.74; p value=0.28), educational status (X1 = 27.482; p value=0.384),medical checkup (X1 = 16.130; p value=0.242), training certificate (X1= 15.81; p value=0.260), and year of service (X1 = 21.03; p value =0.742) as indicated in (Table 12).

Association between Practice on Fruit safety Based on Sociodemographic Characteristics of Participants

Statistically significant differences in the fruit safety practice of fruit vendors were found on the basis of age group (X1=65.022; p value=0.036) and No statistical difference was found between the fruit safety of fruit vendors based on their training certificate (X1= 21.682; p value=0.358), year of service (X1 = 50.073; p value =0.132), sex (X1= 8.92; p value=0.984), educational status (X1 = 39.64; p value=0.486), medical checkup (X1 = 16.60; p value=0.492) as indicated in (Table 12).

Table 12. Summary table on association between knowledge,attitude,practice on fruit safety based on socio demographic characteristics of fruit juice makers at selected sub cities of Addis Ababa,Ethiopia(N=120).

Name of Variables	Socio demographics Characteristics	Chi Square	P. value
Knowledge	Sex	5.48	0.940
	Age	33.68	0.580
	Education Status	27.45	0.285
	Medical Check up	12.66	0.394
	Training Certificate	4.99	0.958
	Years of Service	24.037	0.046
Attitude	Sex	14.40	0.346
	Age	43.74	0.28
	Education Status	27.482	0.384
	Medical Check up	16.130	0.242
	Training Certificate	15.81	0.260
	Years of Service	21.03	0.742
Practice	Sex	8.92	0.984
	Age	65.02	0.036
	Education Status	39.64	0.486
	Medical Check up	16.60	0.492
	Training Certificate	21.68	0.358
	Years of Service	50.073	0.132

7. DISCUSSION

Fruit juice makers may be a potential carrier to a wide range of enteric pathogens and they have been responsible in the transmission of many microorganisms. The spread of those Intestinal Parasites, *Salmonella, Shigella* species are come from various risk factors. Therefore, this study was undertaken to identify Intestinal Parasites, *Salmonella, Shigella* species from locally consumed fruit juices and juice makers by assessing the hygienic conditions of fruit juice houses in selected sub cities of Addis Ababa, Ethiopia. Among fruit makers working in selected sub cities of Addis Ababa, Ethiopia.

The present study has attempted to identify the level of contamination caused by intestinal parasites and enteric bacterias mainly *Salmonella* and *Shigella* from different local fruit juices and fruit juice makers prepared in selected sub cities of Addis Ababa, Ethiopia.

In this study identified intestinal parasites from fruit juice and fruit juice makers was 17% and 27% Respectively. Even if higher findings found in Jimma, South west Ethiopia collected from fruits and vegetables parasitic contamination of collected from selected local markets was 57.8% (81). The high prevalence of intestinal parasites(27%) among fruit juice makers was in line with findings of other studies conducted in Ethiopia like Mota town 27.6% (82).

In the present study, from different fruit juice samples *cyst of E. histolytica* (5%) was the most frequently detected parasite followed by trophozoite, *cyst* of *G. lamblia* (3%) which is in line with previous finding from Nigeria, Sudan (83,84). However it varies among many other studies (85,86,87).

Ova of A.lumbricoides (7%) were the predominant contaminants in fruit makers according to previous similar study done in ArbaMinch (88). Also 1% *Ova of T.tricuria* and *A.lumbricoides* identified from single fruit makers, this finding gives an indication to say juice makers are carrier to gastrointestinal parasites and other enteric pathogens. The national deworming program against soil transmitted helminthes might bring this shift. In addition to this, variation in the season ability of the protozoan parasite to survive in any environment, kind of fruits and vegetables considered also matters.

Fruits directly supplied by farmers to vendors were 3.5 times more likely to be contaminated with parasites as compared to fruits supplied by large scale vendors. Large scale vendors receive fruits on the farm land, pack and transport to Addis Ababa Atkilt tera/Haile Garment town using vehicle, store it properly and distribute to small scale vendors.

In the present study,6% of fruit makers and 5% of fruit juice samples had *Salmonella* species. indicating that fruit juice makers could be potential source of Salmonellosis and acts as carrier. infections to the community. This finding found similar to previous studies conducted in Ethiopia Gonder 5.9% (89), ArbaMinch 6% (90), Wolita Sodo 8.8% (91) and Jordan 6.3% (92), Similar comparative study in Bangladesh also reported that unpasteurized fruit juices were 7.89% positive for *Salmonella* species respectively. Even if this study not identify *Shigella* species, similar studies found in Addis Ababa (93) and Jordan (94) have an agreement with this study report. On the other hand, this result was lower than the studies reported from Ethiopia, Addis Ababa (10.5%) (95) and Nigeria (42.3%) (96). The variation might be attributed to poor personal hygiene and environmental sanitation differences among the study areas.

The discrepancy between all the above mentioned with this study and previous other studies might be as a result of the variations in geographical area, climatic and level of environmental conditions, epidemiologic distribution of pathogens, the kind of sample and sample size examined, the sampling techniques, methods used for detection of the Intestinal parasites, *Salmonella, Shigella* and Socioeconomic status of the respondents. So as long as these factors differ, consequently the discrepancy of the results would be expected.

In the present study, from different varieties of fruit juice samples 5% of local juices identified *Salmonella*. which implies that juices are a potential sources to cause salmonellosis and spreaded to the community.

In the current study, the incidence of *Salmonella* isolates from avocado,spris,papaya and others(ananas with mango) was 2(18.2%),1(9.1%) and 1(9.1%) to each respectively. However highest and lowest study results was found in addis ababa (97).The main reason to this pathogen is during the preparation of juices, proper washing of hands, equipments which don’t careful about utensil washing in all process. among various factors to acquire this pathogens of possible contaminations for the freshly squeezed street vended fruit juices can be from the fruit (98),

prolonged storage outside refrigerator (99), type of water used in all process (100).A previous study revealed that fruit juices under these conditions are might be contaminated with many enteric pathogens like *Salmonella* and *Shigella* species (101).

The performance of Rv broth in fruit juice samples was significantly higher compared with Selenite cysteine broth for detection of *Salmonella* from different varieties of fruit juices. Approximately 6.7% of the samples containing *Salmonella* from fruit juices were contain these bacteria. In this study findings there is a significant difference between the results using RV and Sc. on other hand from fruit juice makers stool samples Selenite Fecal broth(SFB) shows better performance when we compared with Rv broth. For detection of *Salmonella* from stool (fecal) samples of fruit juice makers. Approximately 10% of the total samples have *Salmonella* from juice makers fecal samples identified.

Even if in the aim of this study to identify *Salmonella* and *Shigella* species and only *salmonella* detected others pathogenic bacteria's like *P.aeroginosa, S.marcescens, K.pneumonia, E.coli, Enterobacterclocae, Citrobacter, S.aureus also* detected by BD phoenix from fruit juice sample and fruit juice makers. this finding have an agreement with so many research published papers.

The emergence and re-emergence of antibiotic-resistant food borne bacteria in recent times calls for interesting efforts to research globally, especially for developing countries like Ethiopia. MDR is still tackling all diagnosis (102).

Antibiotic resistance of *Salmonella* isolates from fruit juice samples revealed same similarities and differences in resistance percentages and patterns. The overall resistance is shown by erythromycin 4(36.4%),intermediate resistance predominantly only by gentamycin 2(18.2%).but susceptibility shown by ciprofloxacin, Ampicillin, chloramphenicol, nalidix acid, ceftriaxone, amoxacilinclavunilicacid,tetracycline(tetracycline)and sulfonamide(suphamethoxazole/trimethoprim) susceptibility showed similar trend with previous studies except resistance to erythromycin (103).

Differences and Similarities in resistance patterns can occur among foodborne isolates from the same or different sources were observed (104,105,106,107). These differences have been reported to be widely due to the differences in in geographical locations, the bacteria species involved, the production systems employed, the extent to which antibiotics are used, sampling

techniques and period of sampling (108,109). Stated that, the presence of residual antibiotics in foods constitute an important health risk because of the increased microbial resistance detected in latest years.

On the other hand the antibiotic susceptibility profile of *Salmonella* isolates identified from fecal samples of juice makers was interestingly erythromycin, ampicillin and amoxacilin clavunilic acids 100% and 85% found resistance. This finding was found in agreement with previous findings from Addis Ababa that reports indicated 100% (110) and 82.3% (111) resistant in Addis Ababa isolated from dairy farm attendant and febrile patients respectively.

Interestingly sulfamethoxazole trimethoprim, ciprofloxacin, ceftriaxone, chloramphenicol, nalidix acid discs susceptible 60% of discs found susceptible to *Salmonella* isolates. still gentamycin, tetracycline found intermediate (110,111,112).

In developing countries like Ethiopia, antibiotics are carelessly used by patients and medical personnel's. It is thus a common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics by the public contributing to the emergence and spread of antimicrobial resistance (112).

Resistance to two or more drugs was observed in 100% of the isolates in this study. The organisms seem to have increased their resistance to the drugs from lower levels reported earlier to levels of more than 90% in reports by Asrat. This is similar to the pattern across the globe where the organisms have consistently increasing their resistance to these commonly used first line drugs(111,112,113).

Moreover, in this study even if no *shigella* species were encountered. From different fruit juice varieties. Further, *S.aureus, E.coli, Enterobacter* and *Citirobacter* species, *Pseudomonas aeroginosa, Serracia marcescens* were identified by BD phoenix automated analyzer. Few similar results were reported from the study that was conducted in Harare Zimbabwe, Nairobi Kenya which are in agreement with this findings (114,115,116,117).

A total of 11 isolates consisting of 9 different serotypes were isolated from the various un pasteurized (local) juices types using 17-25 specific antigens, among all the predominant were *Salmonella enterica serotype Newport, S.enterica serotype Dublin.* Therefore, juice makers

seems carrier to many kinds of intestinal parasites and enteropathogenic bacterias. However, *S .enterica serotype Antum, S .enterica serotypes Gaminara, S. enterica serotype Saintpaul, S .enterica serotype Enteritidis, S. enterica serotype Montevideo, S .enterica serotype Enteritidis, S .enterica serotype Typhimurium, S .enterica serotype Saintpaul* were followed respectively. Few previous studies like *S.Newport, S.Enteritidis, S.Thyphimurium* found in fruits and vegetable cause food borne disease (118). In a 2008 outbreak of *Salmonella saintpaul* in the United States, jalapeño peppers imported from Mexico were implicated (119). *Salmonella enterica subsp. enterica serotypes Anatum, Enteritidis* also identified (120).Geographical distribution, season, age groups, and gender were variables considered to analyze *S. enterica* incidence.

Serogroups CDEO is identified from fruit makers fecal specimen. Whereas Serogroups BCDI was identified from local juice samples. In this study 1 mixed cases namely *S.Dublin and S. saintpaul* was detected from a single avocado sources.

The level of fruit makers knowledge is excellent with a mean percentage score of 95.8%.Fruit makers score from all 10 questions out of ten. This shows that their level of knowledge is in excellent conditions because they answer all questions with average score of 95.8%. It implies that fruit juice makers have the awareness of the need for personal hygiene and contamination regardless of possible microbes and fruits juices quality, the crucial aspect linked to temperature values as it is needed to control the growth of microbes in fruits. a study done on fruit juice makers shows that a high mean percentage score of 95.8% achieved with excellent knowledge in the categories of self-hygiene, washing, storing, possible safety and quality of all fruits products.

However, among fruit juice makers respondents related to storages 85% of them know how store different fruits products but on the average10% and 5% of them not sure and give incorrect answer how store different fruits products basically. it shows that they must get proper training on how to handle different varieties of fruits and stored as well but their level of understanding with hygiene related question is so confidential.

Those are important for food safety knowledge, beliefs that give positive impacts on fruit juice preparation. Hence, training is vital to ensure that fruit handlers have all the required amount of awareness and education to meet the fruit hygiene requirements, although this does not necessarily lead to a positive change in the management and handling, processing of the overall

fruit juice makers status. This study demonstrates that although fruit makers are aware of the need for personal hygiene, they do not know crucial aspect linked to temperature values as it is needed to control the growth of microbes in food. However, this study could be the same with previous finding done by Firdaus et al., and among food handlers in Putrajaya shows a high mean percentage score of 84.1% with excellent knowledge in the categories of food storage temperature, storage of foods, self-hygiene, and high-risk foods (121,122).

Regarding to their attitudes their level of attitude is in good conditions since fruit juice makers mean percentage score of 63.10%.which implies that they have awareness on hygiene and possible contaminants of juice products. Even if most respondents strongly agree with 8 questions from ten. Surprisingly, only 46.7% (56/120) of the food handlers responded that handling fruits with gloves could reduce the risk of contamination of fruit. This contradicts with a previous study, in which 77.9% of participants knew the importance of gloves in food handling.34.7% of the juice makers only accept to wear gloves when they touch ready to eat juice varieties, washing fruits by diluted detergents chemicals. This may indicates possible microbes still survive in the surface. However, this finding contradicts with study found in (123). Which 77.9% of participants knew the importance of gloves in food handling. This finding have pointed out that the fruit handlers in the study areas need health education or training programs on fruit safety and on the common pathogens that cause foodborne diseases.

Now a days due to globalization access of information is updated from time to time and food handlers can develop good knowledge and positive attitude towards food handling so that they could perform good handling practice relatively better. This is also evidenced by other studies Gondar (124),Dire Dawa (125),Addis Ababa (126) and FAO (127).

The WHO recommends wearing white coats during preparing and serving food to ensure that food is not exposed to any clothes worn underneath. Individuals engaged in food handling are also supposed to wear white caps or aprons to protect the food from hair (128).

The level of fruit makers practice is low with a percentage score of 39.5%. Only 5 questions is answered out of 10.based on the three aspects that evaluated the result shows mal practicing which is potential to cause food borne diseases due to poor washing, handling, preparations of

fruits. The result of this study is lower than studies conducted in Dangla 52.5% (129) and Diredawa 52.4% (130).

The discrepancy might be due to the mean cut off point to determine food handling practice and the percentage they used. However, the results of this study is higher than other studies conducted in Gondar 30.3% (131), Gamogofa 32.6% (132).This might be due to difference in year of study and cut off points used. The study conducted in Gondar town got around 5 years long. The result is found consistent with the study conducted in Nigeria 36.5% (133).

In the current study, there were significant association between knowledge to wards attitude and also knowledge level and food safety practice (Table 12). This finding was supported by other studies (134,135).which revealed that there was a significant association between knowledge level and attitude level score. However, this result was contradicted by another study (137,138).Additionally, the level of knowledge and practice there were also a significant association between knowledge and practice. This finding was in line with another study conducted in Malaysia (135,136).On the other hand, this study shown there was no significant association between attitude and food safety practice score. This finding have an agreement with another study conducted in Jigjiga town, Ethiopia (137,138,139).

In the present study, there were significant association between knowledge to wards year of service and fruit safety practices to wards age (Table13).This finding have an agreement with previous studies(140,141).However, this result was contradicted by another study (142,143,144).

On other hand, None of the respondent's socio-demographic data was statistically significant in the attitude section of fruit safety $p < 0.05$. This finding was in line with another study conducted in Malaysia, Iran, and Ghana (145,146,147).However, this result was contradicted by another studies conducted in Putrajaya and Gondar (148,149).

7.1. LIMITATIONS OF THE STUDY

- ✓ Can't perform Concentration method for intestinal parasite because of difficulty to get Modified Formol ether to sedimentation techniques and Zinc sulphate for flotation techniques.
- ✓ The lack of generalizability to the large population as this study was conducted among fruit makers at selected sub cities of Addis Ababa. Finally, cross-sectional designs lack the capacity to definitely demonstrate cause-effect relationships because of the inherent limitation of the cross sectional designs.

7.2. STRENGTH OF THE STUDY

- ✓ Adding of serotype tests and confirmatory advanced BD Phoenix automatic analyzers Machines.
- ✓ Comparisions of Rappaport vasiliadis, Selenite Fecal, Selenite Cysteine enrichment medias that could increase the isolation of *Salmonella* species.
- ✓ On site face to face interview allows the researcher to see the overall status of fruit makers and juice houses.
- ✓ Study participants that have positive findings for enteric pathogens was instructed and referred to their respective medical center for appropriate treatments and check up.

7.3. CONCLUSION AND RECOMMENDATION

In the current study, fruit juices and fruit makers prepared for human consumption in selected vendor shops were assessed for hygienic status of the preparation; microbiological load. Generally, the results in this study clearly indicate the poor hygienic conditions of juices to consumers such as luck of sanitary conditions of juice bars, swage system juices wastage, Lack of training (orientation) including improper storage and preparation of fruit juices may aggravate the contamination. Fecal contamination was also observed in both fruit juice and stool samples.

Out of 11 fruit positive samples, 18.2% Avocado, 9.1% Mango, 9.1% Mixed juice were contaminated with *Salmonella* and other microbes this indicates poor hygiene practice. Finally, based on the antibiogram resistance of the isolates on 10 commonly used antibiotics, the effective drugs for *Salmonella* from fecal source were cotrimoxazol(SXT) and ciprofloxacin and from non-fecal samples (fruit juices in this study) ciprofloxacin, chloramphenicol and ceftriaxone for *Salmonella*. Gentamycin showed intermediate to resistance and Erythromycin showed resistance to *Salmonella* in both samples.

The results of the present findings clearly demonstrated that the fresh juices did not meet public health standards and many kinds of intestinal parasites and enteropathogenic bacteria were found. Such foods lead to hazardous effects to the consumers.

Even if this study can't perform concentration test to detect more parasite detections and can't cover all sub cities Based on the findings of the present study, the following recommendations were given: The importance of personal hygiene, washing fruits always with diluted detergents before preparation, wearing gloves when handles the prepared fruit juices to consumers, storage of fruit at cold temperature, using sterilized/filter water for diluting the juice/cleaning equipment better use boil water should be informed to people involved in preparing and handling of fruit juices. Government agencies must adopt measures to educate the vendors about food safety and hygienic practices and enforce adequate guidelines for juice preparations, especially street vended fruit juice houses. Further studies should be done to identify more species and to know the quality of fresh juice by increasing the sample size because the current study was carried out only on very small number of bacteria and small sample size.

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APPENDEX

ANNEX-I: INFORMATION SHEET AND CONSENT FORM

Title of the Research:- Identifications of Intestinal Parasite, Salmonella and Shigella Species and their Knowledge, Attitude and Practice from fruit juice and fruit juice makers in selected Sub cities of Addis Ababa, Ethiopia.

Name of Investigators:- Henok Tsegaye

Name of Medical University:-Medical Laboratory Sciences, College of Health Science, Addis Ababa University.

Introduction:- The above named researchers is Master of Diagnostic and Public Health Microbiology student at Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. They will carrying out a research project as part of their graduation requirements(Master Science),you are invited to participate in the study which ties to asses your knowledge, attitude, practice of your jobs and taking 25 ml sample of fruits juices for diagnosis.

Your Participation:-it is on voluntary basis. If you refuse to participate it will not involve any penalty and punishment related to your work. Thus your rights will be respected of whether you choose to participate or not.Even if you have decided to participate,you will be free to change your mind and you may with draw your consent and dis continue participation in the study at any time.

In order for you to decide if you want to participate or not, this brief information sheet has been prepared to help you understand the purpose of the research. Please read it and if you have questions you may contact any of the investigators whose names are provided above.Their contact information has also been included in the information sheet.

Purpose of the research:-is to identify/evaluate food borne diseases associated with fruit juice makers in selected sub cities of Addis Ababa, Ethiopia .Since fruits and its products are play important role to our health, the research will help us to understand basic hygiene problems, risk

factors how to minimize. if there are areas which need improvement, so that better quality of juice products may be provided to consumers/customers.

Risks associated with the study:-there are no physical, economical psychological risks to you if you agree to participate in the research.

Benefits of the Study:-You will not receive payment for your participation in the research, however your contribution is very valuable because it will serve as an important tools to improve the quality of local juice products at Addis Ababa Ethiopia and will give base line information to ministry of health.

The information which you provide will be treated confidentially. Your participation will be strictly anonymous and no one will be able to identify you from the responses which you provided. The information will not be transmitted to any person or to organization under any circum stances, except within proposal.

The result of this study will be evaluated and summarized and a feed back of the results will be given to policy makers under Addis Ababa Health Bureau and ministry of health.

Researcher Name :-Henok Tsegaye

Phone Number:-0910156806

Consent form for participants

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study to give my stool ☐. I _____here to be a participant in this study regarding to fruit juices possible microorganisms and understand that I have the right to withdraw from the study at any time .

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy)

_____ Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy) _____

I thank you for consenting to take part in the study!!

የአማርኛ መጠይቅ

ስለ ጥናቱ የሚሰጥ ማብራሪያ እና የተሳትፎ ስምምነት ቅፅ

የጥናቱ ርእስ፡-በአዲስ አበባ ከተማ አስተዳደር ስር ከሚገኙ ክፍለከተሞች ውስጥ በተመረጡ የፍራፍሬና ጭማቂ ቤቶች እና ጭማቂዎች ላይ ሳልሞኔላና ሺጌላ የተባሉ ተህዋሲያንን መለየትና/ማግኘት፡፡

ጥናቱን እሚያካሂደው ሰው/ባለጥናቱ፡-ሄኖክ ፀጋዬ

መግቢያ፡- ይህን ጥናት አዲስ አበባ ከተማ ከሚገኙ ክፍለከተሞችና ወረዳዎች ስር በተመረጡ የፍራፍሬ ጭማቂ መሸጫዎች ላይ ከሚሰሩት ስራ ጋር የተያያዙእውቀን፣አመለካከትንና፣ልምድን ለመጠየቅ ፈቃደኝነትዎን ማግኘት አስፈላጊ ነው፡፡የጥናቱን ዓላማና ጥቅሙን በሚገባ ተረድተው መሳተፍ ፍላጎት ካልዎት ዋናው ተመራማሪው ወይም የምርምር ቡድን አባላት ከሆኑት ውስጥ ለሚጠይቅዎት ጥያቄ ተገቢውን መልስ ይሰጣሉ፡፡

በፍቃደኝነት ላይ የተመሰረተ ተሳትፎ፡-ሙሉ በ ሙሉ በተሳታፊዎች ፈቃደኝነት ላይ የተመሰረተ ነው፡፡በተጨማሪ በዚህ ጥናት ላይ መሳተፍ እና አለመሳተፍ በራሳቸው ፍላጎት ብቻ የሚወሰን እንጂ የማንም ጣልቃ ገብነት አይኖረውም፡፡ እንዲሁም ተሳታፊዎች ስራቸውን በማይጎዳና ያለምንም ጫናና ተፅእኖ መቀጠልም ይሁን ማቋረጥ ይችላሉ፡፡

በጥናቱ መሳተፍ ወይንም አለመሳተፍ እንዲወስኑ ይረዳዎት ዘንድ የጥናቱን አላማ የሚገልፅ አጭር ማብራሪያ የተዘጋጀ ሲሆን ይህንኑ ማብራሪያ እንዲያነቡት ወይም እንዲነበብልዎት እንዲፈቅዱ በትህትና እጠይቃለሁ፡፡በማንኛውንም ጥያቄ ቢኖርዎት ለጥናቱ አባል መጠየቅ ይችላሉ፡፡

የጥናቱ አላማና ጥቅም፡-የጥናቱ ውጤት በአዲስ አበባ ከተማ ስር በሚገኙ ክፍለከተሞች እና ወረዳዎች የፍራፍሬዎችን ንፅህና፣ጥራት በመገምገም የተሻለ ንፅህና እንዲተገበር ያግዛል፡፡ጉድለቶች ቢኖሩ ለይቶ በማስተካከል ተጠቃሚው ጥራቱን የጠበቀ አገልግሎት እንዲያገኝ ከፍተኛ አሰተዋፅኦ ያደርጋል፡፡

ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳትም ሆነ ዝርዝር ምርመራ ሂደት ውስጥ ምንም አይነት አካላዊም ሆነ አእምሮዐዊ ጉዳት አይኖረውም፡፡

ለተሳትፎ የሚሰጥ ክፍያ፡-ምንም አይነት የካሳም ይሁን የመስማሚያ ክፍያ የለውም፡፡ነገር ግን የእርሶ ተሳትፎ ከአምራቾቹ ጀምሮ እስከ ተጠቃሚው ደንበኛ ጠቃሚ ግብአቶችን ስለሚሰጥ ጥራትን ለማሻሻል ከፍተኛ ድርሻ ይኖረዋል፡፡

የመረጃ ሚስጥራዊነት፡-ከተሳታፊዎች የሚሰበሰቡ መረጃዎችን በሙሉ በሚስጥር የሚያዙና የሚጠበቁ ይሆናል፡፡በማንኛውም ምክንያት ተሳታፊዎችን ማንነት የሚያሳይ በመጠይቁም ይሁን በሌላ ፅሁፎች አይኖርም፡፡በተጨማሪም መጠይቁ ከተሞላ በኋላ መረጃው ከእኔ አጥኚው

እና ከሚያግዘኝ ባልደረባ እጅ ብቻ ይሆናል፡፡መረጃው በምንም አይነት ለሶስተኛ ወገን ተላልፎ አይሰጥም፡፡የጥናቱ የመጨረሻ ውጤት አዲስ አበባ ጤና ቢሮ የሚቀርብ ይሆናል፡፡በማንኛውም ጊዜ መጠየቅ የሚፈልጉትን ጥያቄዎች ካለ መጠየቅ ይቻላል፡፡

የተመራማሪው ስም፡- ሄኖክ ፀጋዬ

ስልክ አድራሻ፡- 0910156806

የፈቃደኝነት ስምምነት መጠየቂያ

እኔ/ተማሪ/አቶ_____የተባልኩ የአትክልትና ፍራፍሬ ጭማቂዎች ላይ በሽታ አምጪ የሆኑትና በተለይም ተያያዥ ባክቴሪያ የተባሉትን ተህዋስያን ላይ ጥናት ለማድረግ የሚያስፈልጉ መጠይቆችን መረጃ ለመስጠት በሚገባኝ ቋንቋ የተብራራልኝ በመሆኑ በጥናቱ ለመሳተፍ ና የሰገራ ናሙና ለመስጠት በሙሉ ፍቃዴ የተስማማሁ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊዋ/ው ስም_____ የተሳታፊዋ/ው ፊርማ _____

ቀን/ወር/አመት_____

የተመራማሪው ስም_____ የተመራማሪው ፊርማ_____

ቀን/ወር/አመት_____

የተሳትፎሽ/ክ አመሰግናለሁ!!

ANNEX-II:QUESTIONNAIRE
ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY

Name of data collector: _____

Type of vender: _____ Questionnaire number_____

Questionnaire format sheet to asses safety and quality of locally prepared fruit juices to be filled by juice makers.

	PART-I:- Socio Demographic Characteristics of the Worker	
1.	Sex	-Male □ -Female □
2.	Educational status of juice maker	- Illiterate □ -Elementary □ -High school and above □
3.	Year of service	- ≤1 □ - 1–2 □ - > 2 □
4.	Medical Certificate	-Yes □ -No □
5.	Training Certificate	- Yes □ -No □
PART-II:-Knowledge, Attitude and practice based assessment towards their work situation		
Knowledge related Question in Food hygiene and Safety issues		
1.	Do you think Fruit juices can be safe when we store at 2-8^{0}C?	-Correct □ -Incorrect □ - Not sure □
2.	When do you wash different fruits products?	-In arrival immediately □ -Immediately before preparation □ -Never wash □ -Am not Sure □
3.	When do you blending/cutting fruits?	-In morning prepared □ -In the end of the day □ -After order reach □ -No regular time to blending □
4.	When you wash all the equipment used to prepare juice?	-immediately after finished, clean, dry, ready □ -later after preparation clean, dry, ready □ -During time of juicing □ -sometimes □ -never □

5.	When do you wash customers used equipment's?	-Immediately by bleach □ -immediately by water and soap □ -later after collected □ -before making juices □ -At the end of the day work □
6.	Do you think un clean water leads to cause water borne disease ?	-Correct □ -Incorrect □ -Not Sure □
7.	Do you think microbes can contaminate fruit juice any time during preparation?	-Correct □ -Incorrect □ -Not Sure □
8.	When we prepare fruit juice do you think if didn't follow proper hand washing microbes cant causes illness ?	-Correct □ -Incorrect □ -Not Sure □
9.	Do you think proper storage of fruits will help in prevention of getting different microbes?	-Yes, Correct □ -In Correct □ - Not sure □
10.	When do you think juice makers should wash hands after touched unhygienic materials ?	-Correct □ -Incorrect □ -Not Sure □
	1-10Attitude related Question in Food hygiene and Safety issues	
1.	Always personal cleanness is highly important in preparation of fruit juices and One main responsibility of my job is to handle fruit safety	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
2.	Fruit handlers suffering from food borne diseases should not be allowed to go to work	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
3.	Fruit juice makers if wounded fingers and hands can handle food only. if they correctly cover their cuts.	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
4.	Fruit juice makers must wear gloves, clothes, cap before start preparation	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □

5.	Fruit juice makers should have proper short nails and clean hands are important to fruit juice preparation	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
6.	Fruit juice makers should wear gloves when they touch ready to eat juice varieties	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
7.	I think fruits must be washed first with diluted detergent chemicals then rinise well with water thoroughly before used in juice preparation to prevent different microbes.	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
8.	Fruit juice makers should make sure to use filtered/distilled water when prepare juices to consumers is important to prevent food borne disease	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
9.	It is important to wash hands right after unhygienic Practice	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
10.	Fruit makers should use a clean hand towel to wipe their hands after washing them	-Strongly Agree □ -Agree □ -Uncertain □ -Dis Agree □ -Strongly dis agree □
	1-10 Practice related question in Food hygiene and Safety issues	
1.	Do you wash your hands after returning from bathroom?	-Always □ -Often □ -Sometimes □ -Rarely □ -Never □
2.	Do you wash your hands after rubbing your nose/scratching your body?	-Always □ -Often □ -Sometimes □ -Rarely □ -Never □
3.	Do you wash your hands after handling fruit waste /dealing with rubbish's?	-Always □ -Often □ -Sometimes □ -Rarely □

		-Never □
4.	Do you make sure that your hands are dry and clean every time you are handling of fruit?	-Always □ -Often □ -Sometimes □ -Rarely □ -Never □
5.	Do you wear any items of jewellery when you handle fruit juices?	-Always □ -Often □ -Sometimes □ -Rarely □ -Never □
6.	Do you follow the right hand washing procedures?	-Always □ -Often □ -Sometimes □ -Rarely □ -Never □
7.	Do you touch fruit when you cut your fingers and the cut is not well covered?	-Always □ -Often □ -Sometimes □ -Rarely □ -Never □
8.	Do you wear proper clean suitable uniform before working?	-Always □ -Often □ -Sometimes □ -Rarely □ -Never □
9.	Do you eat, drink or chew gum as you prepare juices?	-Always □ -Often □ -Sometimes □ -Rarely □ -Never □
10.	Your cleaning habits of fruits during preparation?	-Always □ -Often □ -Sometimes □ -Rarely □ -Never □

የአማርኛ መጠይቅ

አዲስ አበባ ዩንቨርሲቲ

የምረቃ ት/ቤት

ሜዲካል ላብራቶሪ ት/ቤት

መጠይቁን እሚሞላው ሰው ስም:-_____

የመጠይቁ መለያ ቁጥር፡-_____

ይህ መጠይቅ የተዘጀው የፍራፍሬ ጁስ አዘጋጆችን ስለጁሶች ጥራትና ጠቅላላ መረጃ ለማወቅ የፍራፍሬ ጁስ አዘጋጆችን በቀጥታ የሚመለከት ነው::

ክፍል አንድ - የሰራተኛው ግላዊ መረጃ		
A01.	-ዎታ □ -እድሜ □	-ወንድ □ - ሴት □
A02.	የፍራፍሬ ጭማቂ አዘጋጅዋ/ጁ የትምህርት ሁኔታ?	-ያልተማረች/ረ □ -አንደኛ ደረጃ □ -ሁለተኛ ደረጃና ከዛ በላይ □
A03.	ምን ያህል ጊዜ ሆነሽ?	- ≤1 □ - 1-2 □ - >2 □
A04.	የህክምና ጠቅላላ ምርመራ አለሽ/አለክ?	-አዎን □ -የለኝም □
A05.	ምግብ ነክ ዝግጅት ላይ የስልጠና ሰርተፊኬት አለሽ/አለክ?	-አዎን □ -የለኝም □
	ክፍልሁለት-ከዚህ ቀጥሎ ሰራተኛው ከሚሰራው ስራጋር የተያያዘ እውቀትን፣አመለካከትን፣የስራ ሁኔታና ተያያዥ ጥያቄዎች ላይ እሚያተኩር ይሆናል	
	1-10 ያሉት እውቀትን ንጽህናን ፣አጠቃላይ ተያያዥ ጥያቄዎችን ይመለከታል	
B01.	የተሰሩ ጭማቂዎች ንፅህናቸው ተጠብቀዋል የምንለው ከ 2-8^{0}ሴ ስናስቀምጣቸው ነው?	-ትክክል □ -ትክክል አደለም □ -እርግጠኛ አደለሁም □
B02.	ፍራፍሬዎችን እምታጥቡት መቼ ነው	-ተገዝተው እንደመጡ □ -ተገዝተው እንደመጡና ልናዘጋጅ ስንል □ -አላጥብም ☐ -እርግጠኛ አደለሁም □

B03.	ፍራፍሬዎችን መቼ ነው እምታዘጋጂው/ጀው	-በጠዋት ተዘጋጅቶ ይቆያል -ከስራ ሰአት በኃላ -ከታዘዝን በኃላ -ይሄነው እሚባል ሰአት የለኝም
B04.	ደንበኞች የተጠቀሙበትን እቃዎችን መቼ ነው እምታጥቡት	-ወዲያው በበረኪና በመዘፍዘፍ -ወዲያው በውሃና በኦሞ ወዘተ -በመሰብሰብ ቆየት ብለን
B05.	የፍራፍሬዎችን መስሪያ እቃዎችን መቼ ነው እምታጥቡት	-ወዲያው አጥበን እናደርቃለን -ልናዘጋጅ ስንል -የለቱ ስራ እንዳለቀ -አላውቅም -ትዕዛዝ እንደመጣ
B06.	ንፅህናውን ያልጠበቀ ውሀ መጠቀም በሽታ አምጪ ጀርሞችን ሊያመጣ ይችላል ብለክ\ሽ ታስባለክ\ሽ	-አላውቅም -እርግጠኛ አደለሁም -ይከላከላል
B07.	ፍራፍሬ ጭማቂ ስናዘጋጅ በማንኛውም ሁኔታ በጀርሞች ሊበከል እንደሚችል ታቃለክ\ሽ	-አዎ ይበከላል -አይበከልም -እርግጠኛ አደለሁም
B08.	ፍራፍሬ ጭማቂዎችን ስናዘጋጅ እጅን በአግባቡ አለመታጠብ ለበሽታ አምጪ ተህዋሲያን አያጋልጥም ብለሽ ታስቢያለሽ\ክ	-ያጋልጣል -አያጋልጥም -እርግጠኛ አደለሁም
B09.	ፍራፍሬዎችን ከማዘጋጀታችን በፊት በተገቢው ቦታ ማስቀመጥ በሽታ አምጪ ተህዋሲያንን ይከላከላሉ ብለሽ/ክ ታስባለክ/ሽ	-አዎ ይከላከላል -አይከላከልም -እርግጠኛ አደለሁም
B10.	ፍራፍሬ የሚያዘጋጅ ሰው ማናቸውንም ቆሻሻ ነገር ከነካ/ች ወዲያው አለመታጠብ ችግር ያስከትላል ብለክ ታስባለክ/ሽ	-አዎ ያስከትላል -አያስከትልም -እርግጠኛ አደለሁም
	ከ 1-10 የቀረቡት ጥያቄዎች አመለካከትን ንፁህና ፣አጠቃላይ ደህንነትን ይመለከታል።	
C01.	ዘወትር የፍራፍሬ ጭማቂዎችን ስናዘጋጅ የግል ንፅህናችንን እና አጠቃላይ የጭማቂ እቃዎች ደህንነትን መጠበቅ የኔ የሁልጊዜ ዋነኛ ስራዬ ነው	-በጣም እስማማለሁ -እስማማለሁ -እንደሁኔታ -አልስማማም -በጣም አልስማማም

C02.	የፍራፍሬ አዘጋጅዋ/ዉ ውሃ ወለድ በሽታዎች የተጠቃ ከሆነ የነካቸውን እቃዎች በማፅዳት ስራ እንዳይገባ ማድረግ	-በጣም እስማማለሁ -እስማማለሁ -እንደሁኔታ -አልስማማም -በጣም አልስማማም
C03.	ፍራፍሬ አዘጋጅዋ ቁስል ካላት\ው መስራት እምትችለው ከተሸፈነ ነው	-በጣም እስማማለሁ -እስማማለሁ -እንደሁኔታ -አልስማማም -በጣም አልስማማም
C04.	ፍራፍሬ አዘጋጆች ጭማቂዎችን ሲያዘጋጁ ንንት፣አካልን የሚሸፍኑ ልብሶችን ፣ኮፍያ ማረግ ይኖርባቸዋል	-በጣም እስማማለሁ -እስማማለሁ -እንደሁኔታ -አልስማማም -በጣም አልስማማም
C05.	እጃችንን በስነስርአት መታጠብና ጥፍርን አጭርና ንፁህ ሲሆን ለፍራፍሬው ንፁህና በጣም ጠቃሚ ነው	-በጣም እስማማለሁ -እስማማለሁ -እንደሁኔታ -አልስማማም -በጣም አልስማማም
C06.	የፍራፍሬ ጭማቂ አዘጋጅዋ ያዘጋጀችውን ጭማቂዎችን ስታቀርብ ንንት መጠቀም ይኖርባታል	-በጣም እስማማለሁ -እስማማለሁ -እንደሁኔታ -አልስማማም -በጣም አልስማማም
C07.	ፍራፍሬዎችን ለጭማቂነት ከማቅረባችን በፊት መጀመሪያ በ ማጠቢያ ኬሚካሎች ዘፍዝፎ በማጠብ በመቀጠል በንፁህ ውሃ በደንብ ማለቅለቅ ለጁሱ ንፅህናና ተያያዥ በአይን እማይታዩ ተህዋሲያንን ይከላከላልን	-በጣም እስማማለሁ -እስማማለሁ -እንደሁኔታ -አልስማማም -በጣም አልስማማም
C08.	የፍራፍሬ ጭማቂ አዘጋጆች ጭማቂ ሲያዘጋጁ የተጣራ ውሀና ንፅህናው የተጠበቀ መሆኑን ማረጋገጣቸው ተያያዥ ውሃ ወለድ በሽታዎችን ይከላከላል	-በጣም እስማማለሁ -እስማማለሁ -እንደሁኔታ -አልስማማም -በጣም አልስማማም

C09.	የቆሸሹ ማናቸውንም እቃዎች ከነካን በኃላ አዘውትረን ሁልጊዜ እጅን መታጠብ	-በጣም እስማማለሁ -እስማማለሁ -እንደሁኔታ -አልስማማም -በጣም አልስማማም
C10.	ፍራፍሬ አዘጋጆች እጃቸውን ከታጠቡ በኃላ ማድረቂያ ለስላሳ ወረቀት ቢጠቀሙ ይመከራልን	-በጣም እስማማለሁ -እስማማለሁ -እንደሁኔታ -አልስማማም -በጣም አልስማማም
ከ 1-10 የቀረቡት ጥያቄዎች የስራ ሁኔታን፣ንጽህናን ና አጠቃላይ ደህንነትን ይመለከታል።		
D01.	ከመታጠቢያ ቤት ስትመለሺ\ስ እጅሽን\ክን መቼ ይታጠባሉ?	-ሁልጊዜ -በተደጋጋሚ -አልፎ አልፎ -በፍፁም
D02.	አፍንጫና ሌላ የሰውነት ክፍል ከነካሽ\ክ በኃላ እጅሽን ትታጠቢያለሽ?	-ሁልጊዜ -በተደጋጋሚ -አልፎ አልፎ -በፍፁም
D03.	የፍራፍሬ ቅርፊቶችንና ልጣጮችን፣ቆሻሾችን ከነካሽ \ካክ በሁዋላ እጅሽን/ መቼ ይታጠባሉ?	-ሁልጊዜ -በተደጋጋሚ -አልፎ አልፎ -በፍፁም
D04.	የፍራፍሬ ጭማቂዎቹን ከማዘጋጀትሽ በፊት ሁልጊዜ እጅሽን\ክን ንፁህና ደረቅ ታደርጋለክ?	-ሁልጊዜ -በተደጋጋሚ -አልፎ አልፎ -በፍፁም
D05.	የፍራፍሬ ጭማቂዎቹን በምታዘጋጂ\በት ወቅት ጌጣጌጦችን ታደርጊያለሽ\ክ?	-ሁልጊዜ -በተደጋጋሚ -አልፎ አልፎ -በፍፁም
D06.	እጅን መቼ በአግባቡና በስነስርአት ትታጠቢያለሽ/ክ?	-ሁልጊዜ -በተደጋጋሚ -አልፎ አልፎ -በፍፁም
D07.	በስራላይ እያለሽ/ክ እጅሽ ከተቆረጠ በአግባቡ ሳይድን/ሳይሸፈንና ሳይድን ፍራፍሬንና ጭማቂን መንካት ?	-ሁልጊዜ -በተደጋጋሚ -አልፎ አልፎ -በፍፁም

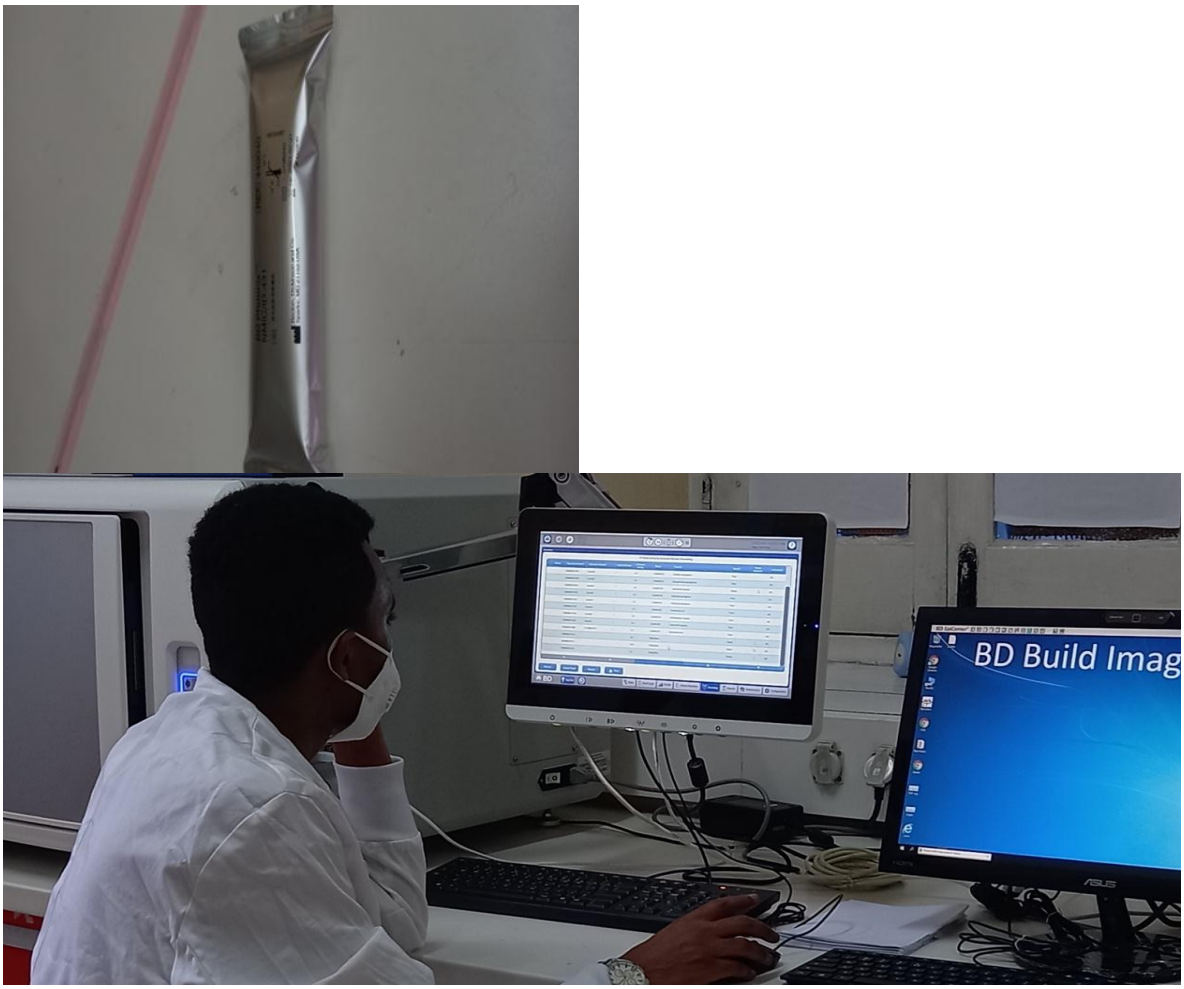
D08.	ስራ ከመጀመርሽ/ክ በፊት ንፅህናውን የጠበቀ የስራ ልብስ ታረጊያለሽ /ክ?	-ሁልጊዜ -በተደጋጋሚ -አልፎ አልፎ -በፍፁም
D09.	ጭማቂዎችን ስታዘጋጂ/እያዘጋጀሽ ምግብ መብላት፣መጠጣት፣ማስቲካ ማኘክ?	-ሁልጊዜ -በተደጋጋሚ -አልፎ አልፎ -በፍፁም
D10.	ዘውትር ለማዘጋጀት የምንጠቀመውን ፍራፍሬዎች የማጠብ ልምድ?	-ሁልጊዜ -በተደጋጋሚ -አልፎ አልፎ -በፍፁም

ANNEX III: PICTURES

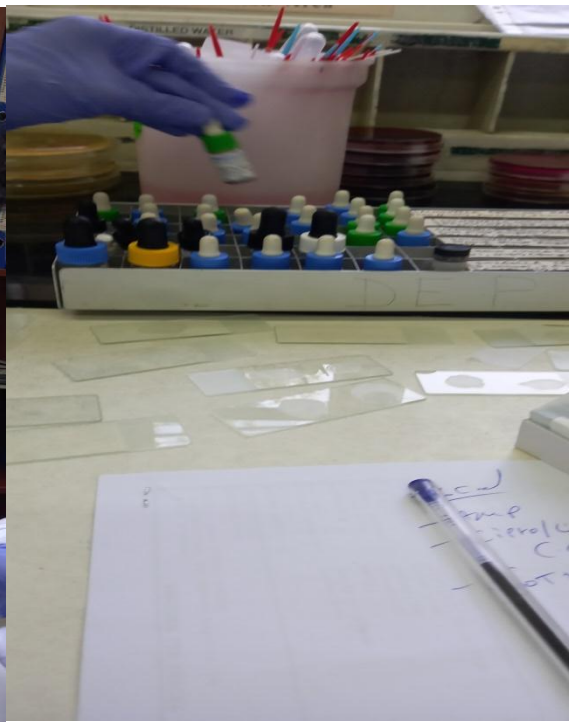
BPW ENRICHMENT MEDIA AND FRUIT JUICE




Sterilization,selective broth and Differential media,Biochemical test inoculations medias

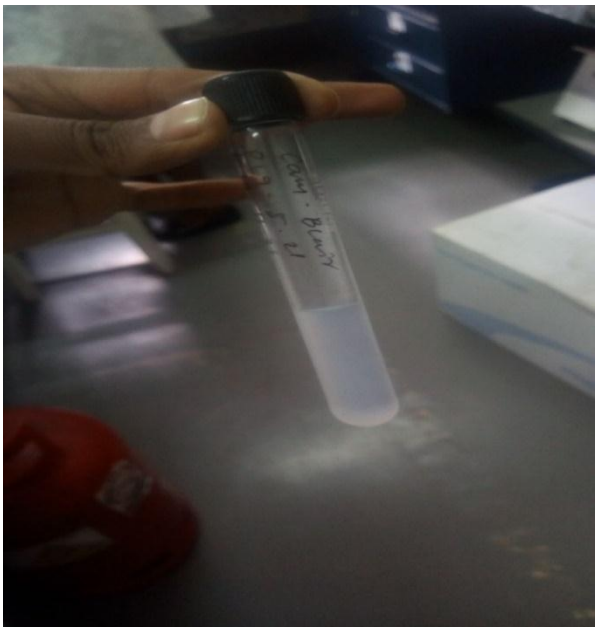


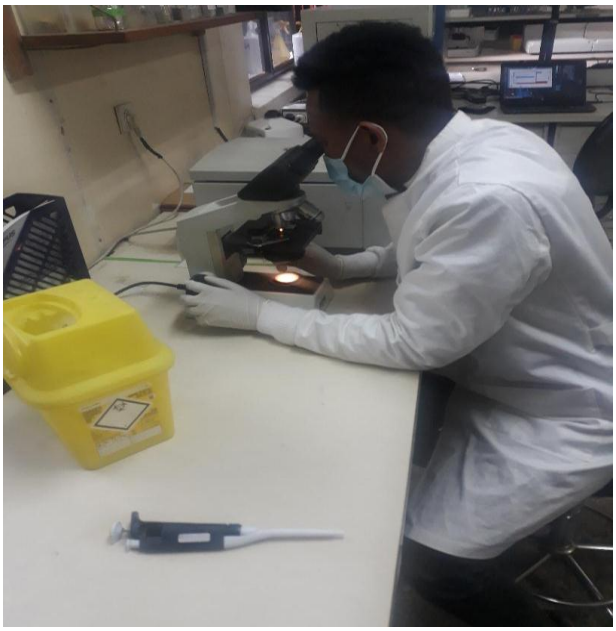
In BD Phoenix M50 ID &AST Automatic Analyzer



Salmonella Serovar Differentiation using slide Agglutination test

AST Result measuring and interpretation





Carry Blair transport media and wet mount Microscopy identification of parasites

ANNEX IV:-BACTERIOLOGY WORK RESULTS INTERPRETATION

1. **Characteristics of *Salmonella and Shigella* species in selective and differential culture medias**

Types of Bacteria	MaCconkey Agar at 37^0C	XLD at 37^0C
Salmonella	Colorless Colonies	Pink–red with a black center colonies or Black center pale Colonies
Shigella	Colorless Colonies	Red Colonies with out black center

2. Identification of *Salmonella and Shigella* by biochemical tests result interpretations

No	Types of tests	Salmonella	Shigella	Salmonella Species	Shigella Species
1.	KIA((Slant)	K	K	Positive	Positive
	KIA(Butt)	A	A	Positive	Negative
	H_2S	Blackening	No blackening	Positive	Negative
	Gas	+	-		
2.	SIM(Indole Motility test)	Growth throughout the medium	Growth along the line of inoculation	Positive	Negative
3.	Urease Test	+	-	Negative	Negative
4.	Lysine Decarboxylase Broth	Purple color	Yellow color	Positive	Negative
5.	Indole test	No Red color	red color	Negative	Varies by species positive
6.	Simmons Citrate	Growth; blue color	No Growth; Green	Positive/variable	Negative
7.	Methyl Red	Red	No Red	Positive/Variable	
8.	Voscus Proscure	Yellow	No Yellow color	Positive	

Note;-A –Acid , K-Alkaline

ANNEX V : LABORATORY INVESTIGATION DATA SHEET

1. Incidence of Salmonella and Shigella from freshly squeezed different varieties of juice juices from fruit juice vendors houses at different sub cities of Addis Ababa, Ethiopia.

Type of fruit samples	Number of samples	Number of samples positive for each pathogens	
		Salmonella	Shigella

2. Fruit Juice Sample Culture Results:_____

Type of Culture media(Agar)	Type of fruit	Number of fruit samples to detected	Salmonella	Shigella
MaCconkey				
XLD				

3. Fruit juices Biochemical Results:_____

No	Type of tests	No of samples detected	No(%) of samples positive for each microbes	
			Salmonella	Shigella

4.Stool sample Culture Results: _____

Type of Culture media(Agar)	Number of stool samples to detected	Salmonella	Shigella
MaCconkey			
XLD			

5. Stool Biochemical Results:_____

No	Type of tests	No of samples detected	No(%) of samples positive for each microbes	
			Salmonella	Shigella

6.AST result Antimicrobial resistance pattern of *Shigella and Salmonella* isolated from fruit juices samples and juice makers fecal specimens in Addis Ababa selected sub city, Ethiopia, February to May 2021 (N=11)

Antimicrobial Discs	Interpretative Criteria (nearest whole mm)			AST result (mm)	Interpretation
	Sensitive	Intermediate	Resistant		
Ampicillin(AMP)(10µg)					
Amoxicillin(Amo) (10µg)					
Chloramphenicol(CHO) (30µg)					
Ceftriaxone (Cef)(30µg)					
Ciprofloxacin(CIP) (5µg)					
Gentamicin(Gen) (10µg)					
Nalidixicacid (30µg)					
Sulfamethoxazole-Trimethoprim (SUT)(23.75/1.25µg)					
Tetracycline (30µg)					
Erythromycin(15µg)					

DECLARATIONS

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: Henok Tsegaye Woldegebreal (B.Sc.)

Signature:_____

Date of submission:_____

This thesis has been submitted with our approval as advisors.

Advisors:

Kassu Desta(MSc, PhD Candidate, Associate Professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Haile Alemayehu (BSc,MSc,Assistant Professor)

Signature:_____

Date:_____

Place: Addis Ababa, Ethiopia.