Microbial Profile and Safety of Selected Street Vended Foods and Antibiotic Susceptibility Patterns of Isolates in Adama City, Oromia Regional State, Ethiopia

Dagmawit Mekuria Desalegn

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Microbiology

Office of Graduated Studies

Adama Science and Technology University

November, 2022

Adama, Ethiopia

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Declaration

I hereby declare that this Master's Thesis entitled "Microbial Profile and Safety of Selected Street Vended Foods and Antibiotic Susceptibility Patterns of Isolates in Adama City, Oromia Regional State, Ethiopia" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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Recommendation of Advisor

I, the advisor of this thesis, hereby certify that I have read the revised version of the thesis entitled "Microbial Profile and Safety of Selected Street Vended Foods and Antibiotic Susceptibility Patterns of Isolates in Adama City, Oromia Regional State, Ethiopia" prepared under my guidance by Dagmawit Mekuria submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Biology (Micro Biology). Therefore, I recommend the submission of revised version of the thesis to the department following the applicable procedures.

Major-Advisor	Signature	Date
Co-advisor	Signature	Date

Approval of Advisor

We, the advisors of the thesis entitled “Microbial Profile and Safety of Selected Street Vended Foods and Antibiotic Susceptibility Patterns of Isolates in Adama City, Oromia Regional State, Ethiopia” and developed by Dagmawit Mekuria hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

Approval of Board of Reviewer

We, the undersigned, members of the Board of Examiners of the thesis by Dagmawit Mekuria have read and evaluated the thesis entitled "Microbial Profile and Safety of Selected Street Vended Foods and Antibiotic Susceptibility Patterns of Isolates in Adama City, Oromia Regional State, Ethiopia" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Biology (Micro Biology).

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LIST OF ACRONYMS AND ABBREVIATIONS

ACA Adama City Administration

ASTU Adama Science and Technology University

CLSI Clinical and Laboratory Standards Institute

FAO Food and Agriculture Organization

MALDI-TOF MS Matrix Assisted Laser Desorption Ionization-Time of Flight Mass spectrometry

NAHDIC National Animal Health Diagnostic and Investigation Center

NCCLS National Committee for Clinical Laboratory Standards

NSW New South Wales Food Authority

WHO World Health Organization

ABSTRACT

Street vended foods are ready to consume foods prepared and serve on the street. Health risks related to such kinds of foods are thought to be common. Thus, the aim of this study was to determine microbial quality, safety and antimicrobial susceptibility patterns of isolates from selected street vended foods in Adama city. A total of 96 food samples (32 from each food type) were collected aseptically from four vending sites (Amede gebeya, ASTU, Franko and around Hailemariam Mamo-Hospital) where there are a number of street food vendors and their customers. Food samples were collected using vendors' serving utensils and placed into sterilized plastic zipper bags and transported (using ice box) to Bacteriology Laboratory, Adama Public Health Research and Referral Laboratory. Identification of isolates was done using MALDI TOF technique at Sebeta. Socio-demographic data and knowledge about food safety and hygienic nature of street-vended food venders were collected using a structured questionnaire. The result showed that more than half (52.1%) of vendors were women, 10 (10.4%) had no formal education, 69 (71.9%) of the vendors didn't have protective cloth, more than half of the vendors (51%) had no frequent hand washing habit, and 58.3% wore no hair covering. Of 96 food samples of street vended food analyzed 53 tested positive for microbial count. In this study E. coli 44(56.4%) and staphylococcus aureus 34(43.6%) were isolated frequently. No Salmonella was detected in all food samples. E. coli isolates were resistant to maximum of six antibiotics and Staphylococcus aureus showed resistance to seven antibiotics. The data showed the E. coli strains did not harbor Enterotoxigenic Lt (ETEC) and St (ETEC). The data also showed S. aureus isolates did not harbor blaZ and mecA resistant genes. Generally, the microbial quality of street-vended food in Adama city was below the acceptable level and calls for special attention.

Keywords: Antibiotic susceptibility patterns, Microbial profile, Safety, Street vended foods, Venders.

CHAPTER ONE

1. INTRODUCTION

1.1 Background Study

Street foods are food and beverage prepared and expose for sell on the road side or prepared in home and sold along street without further treatment by vendors and handlers (FAO, 1990). All over the world, people are gradually being habituated to consuming street-vended foods due to their relatively low price and easily availability along the streets for immediate consumption, or consumption at a later stage without further processing or preparation (Hanashiro *et al*., 2005). They are usually contaminated with various microorganisms during processing and harvesting using machines (Alam *et al*., 2015). Most of the street vended foods harbor a huge range of bacteria (Debroy *et al*., 2011). Potential health risks are associated with contamination of food by *E. coli*, *Salmonella typhi*, *Pseudomonas species*, *Staphylococcus aureus*, *Bacillus*, *Vibirio* and other species during preparation, post-cooking and various handling stages (Ghosh *et al*., 2007).

According to a 2015 WHO report, the global burden of food borne diseases states that each year as many as 600million, or almost 1 in 10 people in the world, fall ill after consuming contaminated food. Of these, 420,000people die (WHO, 2015). Food borne diseases can be classified into two main types, food infections and food poisoning. Foodborne infection occurs as a result of ingestion of food together with pathogenic microorganisms such as *Salmonella* and *Listeria* which grow and establish themselves in the host causing illness manifested in diarrhea, vomiting, etc. (Kalinowski *et al*., 2003). Likewise, Food poisoning is caused by eating food containing preformed bacterial toxin, such as the toxins produced by *Staphylococcus aureus* and *Clostridium botulinum*- when ingested develops the same symptoms as food borne infections.

In countries where street food vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to street vended foods (WHO, 2011). Due to lack of proper knowledge and guidance on street food vending, vendors prepare their foods in explicitly unhygienic and sanitary conditions (Muinde and Kuria, 2005). In Ethiopia, several studies showed that street foods are contaminated with *Bacillus cereus*, *E. coli*, *Salmonella*, *Shigella*, *Staphylococcus aureus* and other food-borne pathogens were identified in similar studies on street-

vended foods in this country (Getu *et al*., 2013). A study conducted in Gonder town also showed that, the majority of street-vended food items were contaminated with one or more different pathogenic bacteria (Amare *et al*., 2019). A similar study conducted in Jijiga also revealed that almost all the samples are unacceptable microbiologically and safety levels (Bereda *et al*., 2016).

Street foods are everywhere in Adama City particularly, in the busy economic activity center and heavy population concentration areas. It becomes common practices to observe many people involved in the preparation and sale of street foods around markets, school, taxi station, and other busy places. Street vendors are selling various types of ready-to-eat food items such as *Ambasha, Bombolino, Bread, Cookies, Donat, Ertib, Koker, Sambusa, Penchera, and Potato chips.* However, there is dearth of scientific report about the microbial quality and safety of street vended foods particularly Chips, Penchera and Sambusa in the city.

The hot climate type and environmental conditions in which street food vendors in Adama operate provide favorable conditions for bacterial growth and fortification (Kok and Balkaran, 2014). Consequently, this could pose a serious threat to the health of the people who consume ready-to-eat foods from street side vendors. Therefore, this study was initiated to provide information about microbial quality and safety of street vended foods in Adama city. Such information is useful to appreciate the safety problems related to street foods so that regulatory agencies may take appropriate steps to improve safety and sanitation with respect to this growing economic sector.

1.2 Statement of the Problem

Street food trade is a growing sector in many developing countries today. Its expansion is linked with urbanization and the need of urban populations for both employment and food (Sharmila, 2011). The street food industry offers a significant amount of employment, often to persons with little education and training (Latham, 1997). In countries where street food vending is prevalent, there is commonly a lack of information on the incidence of food borne diseases related to street vended foods (WHO, 2011). Due to lack of proper knowledge and guidance on street food vending, vendors prepare their foods in explicitly unhygienic and sanitary conditions (Muinde and Kuria, 2005). Consumers who depend on such food are more interested in its convenience and usually pay little attention to its safety, quality, and hygiene (Mensah *et al*., 2002). According to a 2015 WHO, report the global burden of food borne diseases states that each year as many as 600 million,

or almost 1 in 10 people in the world, fall ill after consuming contaminated food. Of these, 420,000 people die (WHO, 2015).

On the other hand, according to Adama city administration report (2019) the climate condition of Adama city is hot and dry climate type. It is one of the sunniest and windiest cities in the Rift Valley. High wind mostly blows from east to west year-round and moderate the devastating effects of scorching sun. Wind still wobbles trees' boughs and causes great dust from dusty areas of the city (ACA, 2019). The hot climate type and environmental conditions in which street food vendors in Adama operate provide favorable conditions for bacterial growth and fortification (Kok and Balkaran, 2014). Consequently, this could pose a serious threat to the health of the people who consume ready-to-eat foods from street side vendors. However, information on the microbial profile, bacterial load and antimicrobial susceptibility patterns of isolates from street foods sold in Adama city are lacking.

1.3. Objectives

1.3.1. General Objective

- ✓ To determine the microbial quality and safety of street vended foods (Chips, Penchera and Sambusa) and antibiotic susceptibility patters of the isolates in Adama City, Oromia Regional State, Ethiopia.

1.3.2 Specific Objectives

- ✓ To investigate the microbial quality of street food with respect to, total viable counts, total coliforms count, total *Enterobacteriaceae count and yeast and mold count.*
- ✓ To evaluate antibiotic susceptible patterns of the isolates for different antibiotics.
- ✓ To examine the presence of antibiotic resistant genes in E. coli and staphylococcus aureus obtained from the food samples using molecular technique.
- ✓ To determine if the street vended foods meet the required microbiological standards of ready to-eat foods.

- ✓ To assess the relationship between the state of hygiene of street vended foods, food safety practices of street food vendors and the microbial quality of the street food.

1.4 Hypothesis

- ✓ *E. coli* isolated from street food (Chips, Penchera, and Sambusa) in Adama City possess genes coding for virulence/pathogenicity for *Enterotoxigenic E. coli*.
- ✓ *S. aureus* isolated from street food (Chips, Penchera, and Sambusa) in Adama City harbor antibiotic resistant genes.
- ✓ Microbial safety is influenced by the hygiene practices during preparation and handling of street food in Adama City.

1.5 Research Questions

In view of the threat to microbial quality and safety, research questions listed below were identified.

- ✓ What is the level of food safety knowledge among the street food vendors?
- ✓ What kinds/types of bacteria can be isolated through analysis of street food samples?
- ✓ Are the levels of bacteria load within or above acceptable limits?
- ✓ How much diversity is there in the range of prepared foods sold on the streets of Adama city?
- ✓ How diverse the microbes were from street-vended foods sold on the streets of Adama City?
- ✓ Are vendors of street foods in Adama aware of the microbial quality and safety hazards associated with these foods?

1.6 Significance of the Study

Sale of food in the streets is very controversial from a health standpoint. The main health hazard associated with street foods is microbial contamination (Getu *et al*., 2013). Knowing the microbiological quality of street vended foods is important to solve problems related to street foods so that concerned bodies may take appropriate measurement to improve quality and sanitation with respect to this economic sector (Deriba Muleta and Mogessie Ashenafi, 2001). Ready-to-eat street foods are also subjected to cross-contamination from various sources such as flies that sporadically landing on the foods, knives, occasional food handling by consumers, raw foodstuffs, utensils and

vendors bare hand serving (Nicolas *et al*., 2007). The majority of the street-vended food consumers seem to have little confidence in the safety of street-vended foods; however, this does not affect their preference for such foods (Asiegbu *et al*., 2016).

Bacterial food-borne diseases pose considerable problems in countries like Ethiopia and thus call for marked concern (Amare *et al*., 2019). Hence, there is strong need to generate data on the microbial load and safety of street foods in large cities such as Adama. The result of this study provides information about the microbial quality status of street foods sold in Adama city. It offers information about the character and nature of isolated pathogenic microorganism with their antibiotic susceptibility patterns. Besides, it gives awareness to prevent food contaminants and suggest the suitable mechanisms to decrease pathogenic microorganisms. It also provides information for responsible organizations to take actions to provide regular training and to create awareness on food handling and personal hygiene among street food venders as well as consumers. Such information is useful to address the safety problems related to street foods so that regulatory agencies may take appropriate steps to improve safety and sanitation with respect to this economic sector.

1.7 Delimitations and limitations of the study

The survey sample size (96) may not have been large enough to yield a true representation of the actual situation in the designated areas at present. Furthermore, because this research measured information on the food safety knowledge and awareness of microbial risk of street-food vendors through their own responses, these self-reported claims might be prone to bias. Hence, there is need for validation through further testing and observation. In addition, the number of street food samples analyzed were 96 insufficient, for various technical reasons. However, this study provided an insight on the bacteriological quality of street-vended foods in Adama city.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Street Foods

2.1.1 Diversity of Street Foods

The diversity of street foods is extensive, as they vary widely not only from country to country, but also from vendor to vendor. Street food ingredients are country specific and mostly undocumented. There are so many varieties that is impossible to provide a menu of all the different street foods consumed around the world (Chapman, 1984). Street foods can be grouped in various ways: by meal (constituents of meals, snacks, and drinks), by number and type of ingredients (simple and complex foods that contain more than one main ingredient), and by level and type of processing (minimally processed foods, such as fruit which may only have been peeled or sliced, traditionally processed foods made by the vendor or another informal sector operative, and centrally processed foods) (Powell *et al*.,1990). The range of foods sold by vendors in specific contexts varies. Most countries vendors sell more than one kind of product, although many specialize in certain food types or product line (Cohen, 1985; Powell *et al*., 1990). As ready-to-eat foods vary widely, the decision on what to test will vary from product to product. Some of the factors influencing the choice of test include; type of ingredients in the finished product, status of ingredients (cooked vs raw), type of cooking/processing undertaken, level of handling after cooking or processing, presence of preservatives, including acids and salt, presence and type of packaging, distribution and storage of finished product (NSW food Authority, 2009).

2.1.2 Cost and Availability of Street Foods

The cost of street foods is usually cheap compared with that of foods purchased from larger food establishments, such as restaurants and hotels. Also, due to high costs of fuel and ingredients in urban contexts, economies of scale can create a street food cheaper than the same food prepared at home (FAO ,1989). Competition between vendors may also keep prices low, although, as FAO has pointed out, this can lead to purchasing of lower raw materials that may have implications for food safety (FAO, 1989). Many vendors operate elaborate pricing systems in which they may give regular customers or fellow traders a discount (Owens and Hussain, 1984).

2.1.3 Preparations and Handling of Street Food

Food borne diseases causes high morbidity mainly in developing countries due to poor hygienic condition during food preparation and the lack of awareness about food safety (Ram *et al*., 2007). Upon preparation, the food is not reheated to high temperature before serving. Often, stalls where Street vended foods are sold are poorly constructed and built in the open thereby increasing their exposure to contamination by dust and combustion from vehicles on the road side (Muinde and Kuria 2005). Street foods are not reliable, they also carry diseases originating from food sources in many countries. One of the reasons for spreading of diseases originating from food sources is that street food sellers do not have sufficient information about food safety (Omemu and Aderoju, 2008). Street food vendors are often unlicensed, untrained in food hygiene and sanitation, and work under crude unsanitary conditions (FAO, 1997). Most street food vendors undergo little or no training and lack the required knowledge on food safety and hygiene (Aluko *et al*. 2014; Liu *et al*. 2014; Asiegbu *et al*., 2016). This makes them develop their own way of handling foods which they perceive to be hygienic and conventional (Gitahi *et al*., 2012).

According to WHO (2011), food handling personnel play an important role in ensuring food safety throughout the chain of food production and storage. Poor personal hygienic like handling and serving practices, contaminated hands and lack of knowledge, improper waste disposal system, unsafe food storage temperatures and inadequate water supply attract houseflies resulting the increases food contamination (Tambekar *et al*., 2008; Chumber *et al*., 2007). Street vendors use tap water supplied from the municipal council or buy from shops (Mwangi, 2002; Mwadime, 2001). In other instances, water is ferried from home of the food vendors because there is no portable water available in their area of operation. This water is not enough for dish washing and food preparation and vendors do not wash fresh foods properly (Muinde and Kuria, 2005).

Unsanitary handling of street foods by the some of the vendor has been commonly found to be the source of contamination (Akinyele, 1987; Dawson and Canet ,1991). The vendors can be carriers of pathogens like *E. coli, Salmonella, Shigella, Campylobacter* and *S. aureus* who eventually transfer this food borne hazards to the consumers. The hands of the food handlers are the most important vehicle for the transfer of organisms from faeces, nose, skin to the food (WHO,2011). In some cases, even after washing, supports the reports of contamination of street vended food

with toxigenic *S. aureus*, the major being suppurative lesions of human beings and the environment (Mohapatra *et al*.,2002).

Street food vendors prefer to take their products to their customers, they often operate from places such as bus terminals, industrial areas, schools, market places and streets. Such locations usually do not meet food and safety requirements. Sale of foods in the streets is very controversial from a health point of view. The main health hazard associated with street foods is microbial contamination. Most of the vendors have no formal education or few years of schooling. Therefore, they are uninformed of proper food handling and their role in transmission of pathogens (Tesfaye *et al*., 2016). According to Charles *et al*., (2011) street food vendors in Kampala, Jinja and Masaka districts in Uganda were surveyed to assess risk factors, practices and knowledge of street food vendors with respect to food safety and hygiene. About 87.6% street vendors are women and with low education level. Vendors had access to tap water within 5 min walk. Use of soap and cold water for washing utensils was common practice. Wash water recycled several times and only changed when very cloudy and soapy. Street vendors had some knowledge about diarrhea and its associated risk factors (Charles *et al*., 2011).

Few vendors congregate in overcrowded areas where there are high numbers of potential customers, which usually provide limited access to basic sanitary facilities. Hence, the contamination of street foods is often linked to the waste generated by food processing, that is usually dumped near the vending site (Ekanem, 1998). The lack of facilities for liquid drainage and wastewater and garbage disposal encourages wastes to be thrown into nearby streets and gutters. Such areas act as habitats for rodents, breeding points for flies and media for growth of microorganism. A study done in Africa revealed that 85% of the vendors prepared foods like fish, fruit salads, roasted maize and chips in unhygienic conditions, given that garbage and dirty waste were conspicuously close to the stalls (Muinde and Kuria, 2005). In these areas large amounts of garbage accumulates which provide harborage for insects and animal pests that are linked to enteric disease transmission (Barro *et al*., 2007 and El-Sherbeeny *et al*., 1985).

2. 2 Importance of Street Vended Foods

If prepared under hygienic and regulated conditions, play a significant role in feeding the urban population with cheap, accessible and nutritious diet. Street foods are appreciated for their unique flavours and convenience as well as for maintaining the nutritional status of the population street

vended foods also assure food security for low-income urban population in many developing countries (Kumar *et al*.2006; Nonga *et al*. 2015). In most developing countries with high rate of unemployment, street foods serve as a veritable source of employment and income generation (Riet 2002).

According to the Food and Agriculture Organization, 2.5 billion people worldwide eat street food every day (FAO, 2007). Increased reliance of street food has been identified as one of the characteristics of urban food distribution systems driven by changes in the urban way of life and poverty in developing countries (FAO, 1998). Street foods have already become a common feature of urban life. The increasing poverty and time constraints to survive in developing countries indicate that the street food phenomenon will only increase (Hilda, 2002). With the increasing pace of globalization and tourism, the safety of street food has become one of the major concerns of public health, and a focus for governments and scientists to raise public awareness of (FAO, 2007). Street foods play significant nutritional role for consumers, particularly for middle and low-income sectors of the population, who depend on street foods for their main food intake (Mensah *et al*., 2002).

2. 2.1 Nutritional Benefits

Street vended foods are not only appreciated for their unique flavors, convenience and the role which they play in the cultural and social heritage of societies, but have also become important and essential for maintaining the nutritional status of the populations (Ekanem, 1998). The street food industry plays an important role in developing countries in meeting the food demands of the urban dwellers (Latham, 1997). Street foods play significant nutritional role for consumers, particularly for middle and low-income sectors of the population, who depend on street foods for their main food intake (Mensah *et al*., 2002). FAO reports that street foods provide nutritionally balanced diets, sufficient in quantity and presenting options for variety and choice for consumers, particularly from middle and low-income sectors of the population, who depend heavily on them (FAO, 1997). The contribution to the daily food intake of poor urban dwellers is scarcely quantified in energy and nutrients (Hilda, 2002). Their nutritional value however depends on the ingredients used and how they are prepared, stored and sold (Owusu-Darko and Ablordey, 2002).

2.2.2 Economic Benefits of Street Food

Besides offering business opportunities for developing entrepreneurs, the sale of street foods can make a sizeable contribution to the economies of developing countries. In India, the National Policy for Urban Street Vendors/Hawkers stated that street vendors constitute approximately 2% of the population of a metropolis (Bhowmik, 2005). The street food industry offers a significant amount of employment, often to persons with little education and training (Latham, 1997). Street food in Nairobi provides a substantial amount of income for most vendors, with most of them earning an income above the official minimum wage while some of them earn twice or more of this amount (Mwangi, 2002). Street food operations sometimes involve the entire family in the procurement of raw materials, preparation and cooking of the meals. The role of women in the sector is significant, as they control a large share of market activity and commodity trading. Street food vendors benefit from a positive cash flow, often evade taxation, and can determine their own working hours (Mensah *et al*., 2002). In selling snacks, complete meals, and refreshments at relatively low prices, they provide an essential service to workers, shoppers, travelers, and people on low incomes. However, the people who depend on such food are often more interested in its convenience than in questions of its safety, quality and hygiene (Mensah *et al*., 2002; Muinde and Kuria, 2005).

Street foods are sold in almost every country in the world. But it is very common in developing country because of its convenient availability and low cost (Getu, *et al*., 2013). The street food industry plays an important role in developing countries in meeting the food demands of the urban dwellers. In a survey conducted in poorer households in Bangkok it was observed that 67 percent of households cooked only once a day and bought one to two meals of ready-to-eat food from street food vendors. The households interviewed explained that street food was more economical than home cooking, was readily available with a large number of vendors at their doorsteps and was convenient as time for cooking was scarce. The survey also showed that street foods provide economic opportunities for low and middle-income people, especially for women (Chung *et al*., 2010).

In Tanzania, urbanization coupled with low salaries offered to employees has led to proliferation of street food vendors who offer commercial meals but of high microbial contaminants due to poor hygiene and handling methods (Kinabo, 2003). In Ethiopia, street foods are flourishing in major

towns of the country and it becomes common practices to observe many people who get involved in the preparation and sale of street foods around school, markets, taxi station, and other places where several people found (Getu, *et al*., 2013). In Gondar town manual workers and other low-income individuals use street food as it is both available and affordable. However, the quality of these foods and their safety for human health is not well known (Amare *et al.,* 2019).

2. 3 Microbial Contamination and Food Borne Disease

Food is an excellent medium for microbes to flourish and expand their number quickly (Ghosh *et al*., 2007). Pathogens present in street vended foods come from different sources and practices. Improper food handling can lead to transfer of pathogens such as *Salmonella, E. coli* and *S. aureus* from human body and environment into foods (Rane, 2011). Improper waste disposal has been associated with transmission of enteric pathogens like *Salmonella*, *Shigella* and *E. coli.* Contaminated water has been associated with pathogens such as *E. coli*, fecal *Streptococci*, *Salmonella* and *Vibrio cholera* while vegetables and spices are associated with introduction of spore formers like *Bacilli* and *Clostridium* and pathogens like *L. monocytogenes*, *Shigella*, *Salmonella* etc. (Rane, 2011). Improper storage temperature and reheating of food have been associated with production of heat stable toxins produced by pathogens like *C. perfringens* and *Bacillus cereus* (Rane, 2011). Food borne illnesses are defined as diseases, usually either infectious or toxic in nature, caused by agents that enter the body through the ingestion of food (WHO, 2011). The serving utensils used at the vending site are often contaminated with *Micrococcus* spp. and *Staphylococcus* spp. which may have originated from the vendors hands when they touched the food preparation areas, dish cloths, or the water during dish washing or hand washing which indicates cross contamination between dishwater, food preparation surfaces, and the food itself (Cardinale *et al*., 2005).

Street foods are prepared, processed and handled by a non-professional personnel in developing countries tend to be vulnerable to microbial contamination propagated from the surrounding environment (Hassan *et al*., 2013). Microbiologically contaminated street food is considered a global problem, which is liable to be a significant contributor to the transmission of food borne diseases (Mamun *et al*., 2013). Food-borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food (Doyle and Evans, 1999). These are the result of ingesting contaminated foodstuffs, and range from diseases caused by a multitude of microorganisms to those caused by chemical hazards (Okareh and Erhahon, 2015).

Today, millions of people catch diseases originating from food sources and thousands of deaths occur in the world. From these *Salmonella*, *E. coli* and *Staphylococcus aureus* can cause food poisoning and other such as *cholera, tuberculosis* and *typhoid fever* causes food infection (Foskett *et al*., 2003). Illness resulting from the consumption of contaminated food has become one of the most widespread public health problems in contemporary society (Notermans *et al.*, 1995). The most common clinical presentation of food-borne diseases takes the form of gastro-intestinal symptoms, but such diseases can also lead to chronic, life-threatening symptoms including neurological, or immunological disorders as well as multi-organ failure, cancer and death (Okareh and Erhahon, 2015). Food-borne illnesses impose a substantial economic and quality of life burden on society by way of acute morbidity and chronic squeals (Duff *et al*, 2003). Food contamination with antibiotic resistant bacteria can also be a major threat to public health, since the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially comprising the treatment of severe bacterial infections (Oladipo and Adejumobi, 2010).

2.4 Approaches to Ensure Safe Street Food Practice

Food safety is the assurance that food will not cause harm to the consumer when prepared and consumed according to its intended use (Gitahi *et al*., 2012). (Ackah *et al*., 2011) also defined food safety as the conditions and measures that are necessary during the production, processing, storage, distribution and preparation of food to ensure that it is safe, healthy and suitable for human consumption. Regulation of food safety principally depends on the knowledge on hygienic handling of foods, types and modes of food borne illnesses, the virulence traits of the food contaminating microorganisms; and the demands of implementation of food protection measures including the microbiological quality assurance of the associated waters used to clean the food items or utensils (Alam *et al*., 2015; Sarker *et al*., 2013).

The hygiene of street foods is a major concern for food control officers (Mensah *et al*., 2002). Wide consumption of street food around the world increases the importance of safety and health issues. Regulation of food safety principally depends on the knowledge on hygienic handling of foods, types and modes of food borne illnesses, the virulence traits of the food contaminating microorganisms; and the demands of implementation of food protection measures including the microbiological quality assurance of the associated waters used to clean the food items or utensils (Alam *et al*., 2015; Sarker *et al*., 2013).

Product standards and code of practice assist manufacturers to produce foods that meet minimum specifications for quality and safety. Standardization is a process of ensuring uniformity in products and services by use of appropriate standard. In any country, food standards are established by regulatory authorities and enforced by governments, food companies and retailer. Also, Food handlers play an important role in ensuring food safety throughout the chain of production, processing, storage, and preparation (WHO, 1998). A logical step towards reducing the risks of food borne illness from street foods would be controlling the steps in food preparation and sale that may contribute to the contamination, growth and survival of the microbes responsible for food borne illness. The efforts made should focus on educating the food handlers, improving the environmental conditions under which the trade is carried out and providing essential services to the vendors to ensure safety of their commodities (WHO, 1996).

Malaysia, Philippines and India are the three countries which have regulations for protecting street vendors. Malaysia is the only country where licensed street vendors are provided facilities for conducting their trade. An initiative has been taken in Africa, where a coalition between local and national authorities, explored the food laws associated with street vending and developed strategies that could be used to control identified food hazards (Natural Resources Institute (NRI, 2004). Another policy was framed in Durban, Africa, where the street vendors were allocated specific areas to operate, issued certificate of acceptability and were also given training on essential food hygiene practices (Holy and Makhone, 2006). In India, CII Institute of Quality's Food Safety and Quality (FSQ), has taken an initiative to create awareness among the consumers and street food vendors and it has issued a simple informative checklist of hygienic practices, called the ''CII-14-point checklist on food safety for street vended food'' which emphasizes on implementation of good hygiene standards by the street vendors (CII, 2008). The scheme to upgrade hygiene and quality of street food has also been undertaken by the Ministry of Food Processing Industries, India. Under the proposed program, 10,000 street food vendors will be identified, and the majority of stake-holders will be upgraded in terms of quality and hygiene and efforts would be made to make it mandatory for the vendors to register with the local authorities (MFPOI, 2009).

2.5 MALDI-TOF-MS

2.5.1 Background of MALDI-TOF-MS

Mass spectrometry (MS) is an analytical technique that relies on measuring mass-to-charge ratios of the analyzed samples for the purpose of identifying the amount and type of the chemical

compounds that exist inside the molecules. Old MS techniques took advantage from the ionization of the sample by bombardment of the analyte with electron beams. This method of ionization typically results in breaking the sample into thousands of charged fragments Molecules in the analyzed sample could then be identified by correlating the known masses to the resultant patterns of the fragments. Although conventional MS techniques were quite common and applicable in many different areas of research, they were only capable of offering "hard ionization". Therefore, there was no available technique at the time to preserve the molecules during the ionization or to minimize the chance of fragmentation (Sparkman, 2000).

In 1985, soft ionization technique was established by two scientists from Germany named Hillenkamp and Karas (Karas *et al*.,1985). These researchers discovered that amino acid alanine could easily be ionized when mixed with amino acid tryptophan and irradiated by a 266 nm laser pulse. They have also found that other types of peptides could be ionized after mixing with same kind of "matrix" (Karas *et al*., 1987). More importantly they have found that in this technique, the molecules are protected from fragmentation during the ionization procedure by the presence of the matrix. For that reason, the technique was named soft ionization. Soon after their discovery opened its way to the scientific community, Koichi Tanaka and his colleagues from Japan could successfully soft ionize a much bigger molecules by using nitrogen laser for ionization. Later at 2002, Tanaka won one-quarter of the Nobel Prize in Chemistry for his contribution in development of the soft desorption ionization technique for mass spectrometric analysis of biological macromolecules (Markides and Gräslund, 2022). Karas and Hillenkamp have subsequently improved the technique based on the concept of soft ionization and, as a results, first matrix assisted laser desorption ionization (MALDI-MS) instrument was commercially available in the early 1990s (Hillenkamp and Karas, 2000).

Four decades before the birth of MALDI, in late 1950s, the technology of time of flight (TOF) had already been invented; a technique in which charged ions were forced to fly from a source to the detector so that they could be identified based on the time of their flight to the ion detector. But TOF was suffering from its poor resolution and did not find its application in MS techniques. Only later on, it was realized that the combination of MALDI-MS and TOF technology can results in a highly sensitive technique that today we know as MALDI-TOF-MS. The application of TOF

technology, in particular, plays an important role when the reflection mode is applied for analyzing the specimen (Samira and Sergio, 2017).

2.5.2 Application of MALDI-TOF

MALDI has opened numerous windows to the future of the mass analysis domain. The valuable information that can be extracted from this single fast analytical technique enables researchers to develop their understanding of different materials for variety of applications (Samira and Sergio, 2017). MALDI analysis has rapidly found variety of applications in many different scientific areas. In particular, MALDI analysis provides essential information about important parameters such as molecular weight (Mw) and polydispersity of the compound that can be effectively used for variety of applications such as studying the synthesis pathways, verifying degradation mechanisms, measurement of the additives and impurities, product formulations, and identification of compositional variations (Samira and Sergio, 2017). In the area of microbiology, MALDI-TOF-MS can be applied for identification of microorganisms such as bacteria or fungi. This technique has a great potential for species identification in the field of medical microbiology in its future developments (Seng et al., 2009). The other applications of MALDI that may have impact on management of infectious diseases include antimicrobial susceptibility testing and epidemiological typing. Antimicrobial susceptibility testing targets a) modification and degradation product of antimicrobial after exposure to microbe, b) proteomic changes in resistant versus susceptible microbial species, c) shift of protein peak after growth of microbe in presence of antimicrobials, d) semi-quantitative estimation of intracellular/extracellular antimicrobials. The techniques require further standardization before getting regulatory approval (Chakrabarti, 2016).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted in Adama city which is located in East Shoa Zone, Oromia Regional State, at 99km south east of Addis Ababa, the capital city of Ethiopia. The city is found in altitude ranges from 1,442 meters in the eastern part to 2,048 meters asl in the north. The average elevation is 1,745 maters. The city experiences its most of the rain during summer season. The average maximum and minimum annual rainfall record as 1106.2 mm and 492.5 mm respectively and the mean annual rain fall of 866.25mm. The average maximum and minimum annual temperature of the city is 32.8℃ and 11.6℃ respectively and the mean annual temperature of the city is around 21.9℃. The climate of this area is hot and dry climate type (ACA, 2019). The city has an area of 31,185.05 hectares and Adama administrative district fully demarcates Adama city in four corners and divided into 14 kebeles with an estimated 338,940 population size. Total population is currently growing at a rate of 5.3% annually from the projection for 2016 by using the 2007 census data (ACA, 2019).

3.2 Study Population

Selected street vended foods and vendors that are counted by direct census at the four selected major street vending sites of Adama city were considered as the study population.

3.3 Study Design

A cross-sectional observational, purposive sampling study was conducted from January 2022 to August 2022 to assess the microbiological quality and safety of street vended food in Adama city. The study included four vending sites (ASTU, Amede-gebeya, Franko and Hailemariam Mamo-hospital) in Adama City where the numbers of street food vendors and their customers are high.

For the study to arrive at a sample size of 96, Yamane **(**1967**)** method of sample size determination formula was used. The simplified formula for calculation of sample size from a population for a 95% confidence level and p = 0.05, size of the sample should be

$$n = \frac{N}{1+N(e^2)}$$

Where, N is the population size and e is the level of precision.

The study subjects (96) were calculated from a total of 127(N) street food venders counted by direct census from four major vending sites. Using the above formula for N=127 and 5% level of precision (0.05), the result 96 considered as the sample size of street food vendors who sells common street foods (Chips, Penchera and Sambusa).

3.4 Sample Size and Sampling Technique

A purposive sampling method was used in this study. The number of the study samples included in this study were 96 which means 32 sample from each food item (Chips, Penchera, Sambusa). A systematic random sampling technique was used to select representative street food vendors in the study area. Systematic sampling is a statistical method involving the selection of elements from an ordered sampling frame (Frankline, 2021).

3.5 Socio-Demographic Data Collection

Data about general socio-demographic characteristics of street food users were collected from the street vendors using a structured questionnaire. Hygiene and sanitation status of vending sites were determined by pretested structured questionnaire, interview and observations. A total of 127 vendors were registered from those four places, and 96 participants (24 from each vending site) were selected randomly from the list. The native language of the city is Afan Oromo and Amharic; hence, the 1questionnaire was translated into those language. Then, it was back-translated blindly into English to check its validity. Ninety-six pre-structured questionnaires were used to obtain preliminary information on the demographic data of street food vendors and to determine the state hygiene of street vended foods and food safety practice of food handlers by recording all observation. In this study various factors and practices were observed and measured such as, nail cutting habit of vendors, protective clothing worn by food handlers, frequent hand washing habit, source of water for preparation and washing, frequency of changing the vending utensil, the way uses to dispose-off the wastes generated during food preparation, methods of packaging used for take away, source of raw material for food preparation and hair cover culture during preparation and selling of foods.

3.6 Sample Collection and Transport Procedure

Food samples were purchased from vendors between 5 to 6 pm during the period and all samples were collected from vendors using vendors' serving utensils and placing into labeled sterilized plastic zipper bags. The samples were transported on Ice box to Adama Referral and Regional Laboratory for sample preparation and analysis. The food samples were kept in the refrigerator at 4^{o}c until microbial analysis was conducted. To avoid further contamination and overgrowth of the microorganisms in the samples, analysis was conducted within fourteen hours of purchase. Analysis and process were performed as per the Laboratory Manual of Food Microbiology for Ethiopian Health and Nutrition Research Institute (Kiiyukia, 2003). Aseptic technique was used throughout all sampling and handling procedures by using sterile materials, flaming and refrigeration. To avoid unpredictable changes, samples were analyzed without delay and samples were analyzed two times in order to confirm the contamination levels. For significant studies of microorganism, pure culture was used. Receptacles containing all essential nutrients were free prior sterilized; solution and equipment containing water was autoclaved at 121℃ for 15 to 20 min. Icebox was used during sample collection and transportation.

3.7 Microbial Analysis of Food Samples

3.7.1 Sample Preparation

Approximately 100 g of each food sample was collected from street vendors to be analyzed for microbiological quality analysis. Accordingly, 10 g of each well-mixed food sample was weighed and mixed with 90 ml buffered peptone water at 7.2 pH and 25℃ (10g/L Peptone and 5g/L Sodium chloride), and vortexed for three minutes to dislodge adhered bacteria (Kiiyukia 2003). After homogenization, 10- fold dilutions were made by adding 1ml of the supernatant to 9ml of blank solution (distilled and sterile water) to 10^{-6} . All dilutions were mixed thoroughly before transferring to the next in order to ensure uniform distribution of the microorganisms. A 0.1 ml aliquot of dilution was spread-plated on pre-solidified in duplicate petri dishes and incubated at appropriate temperature and time.

All plates were incubated under aerobic conditions. For characterization of predominant isolates, colonies from each duplicate plate of the highest dilution showing growth for each sample were randomly picked and purified on blood agar. Average counts obtained were expressed as colony

forming units per gram of food (cfu/g). These isolates were then characterized by biochemical test and MALDI-TOF to genus level.

3.7.2 Total Plate Count

For detection of total plate count, approximately 10 g of sample was weighed and put into 90 mL peptone water and vortexed for 2 minutes. Subsequent 10-fold dilutions were made by adding 1ml of the supernatant to 9ml of distilled water up to 10^{-6}. A 0.1ml diluted samples from each serial dilution was pour plated on 12-15ml tempered plate count agar in duplicate and was mixed by rotating. When it becomes solidified, plates were incubated at 37^{o}C for 48h and duplicate plates containing between 20 and 200 colonies were counted. Average counts obtained were expressed as colony forming units per gram of food (cfu/ g) (Kiiyukia, 2003).

3.7.3 Total Coliform Count

For detection of the coliform count, approximately 10 g of sample was weighed and put into 90 mL peptone water and vortexed for 2 minutes. Subsequent 10-fold dilutions were made by adding 1ml of the supernatant to 9ml of distilled water to 10^{-6}. A 0.1 ml of the samples from each serial dilution was spread plated on plate containing Violet Red Bile Agar media in duplicate. Then the plates were incubated at 32°C for 18-24 h. After this, purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms (Weil *et al*., 2006). Then, the coliform count was compared with the standard coliform count level set for microbiological quality of ready-to-eat foods (NSW, 2009).

3.7.4 Total *Enterobacteriaceae* Count

For the detection of *Enterobacteriaceae* count, about 10 g of sample was weighed and put into 90 mL peptone water and vortexed for 2 minutes. Subsequent 10-fold dilutions were made by adding 1ml of the supernatant to 9ml of distilled water to 10^{-6}. A 0.1 ml of the sample from each serial dilution was spread plated on MacConkey agar (3g/L proteose peptone; 10g/L Lactose monohydrate; 1.5g/L Bile salt; 5g/L Sodium chloride; 0.03g/L Neutral red; 0.001g/L Crystal Violet; 13.5g/L Agar) in duplicate and incubated at 32°C for 24 h after which, pink to red purple colonies were counted as member of the family *Enterobacteriaceae* (Spencer *et al*., 2007). Then, the total *Enterobacteriaceae* count was compared with the standard total *Enterobacteriaceae* count level set for microbiological quality of ready-to-eat foods (NSW, 2009).

3.7.5 Total *Staphylococci* Count

For detection of *Staphylococci* count, approximately 10 g of sample was weighed and put into 90 mL peptone water and vortexed for 2 minutes. Subsequent 10-fold dilutions were made by adding 1ml of the supernatant to 9ml of distilled water to 10^{-6}. A 0.1 ml of the samples from each serial dilution were spread plated on pre-solidified surfaces of Mannitol Salt Agar in duplicate and incubated at 32 °C for 48 h (Acco *et al*., 2003). Then, the total *Staphylococci* count was compared with the standard total *Staphylococci* count level set for microbiological quality of ready-to-eat foods (NSW, 2009).

3.7.6 Yeasts and Molds Counts

For detection of yeasts and molds counts, approximately 10 g of sample was weighed and put into 90 mL peptone water and vortexed for 2 minutes. Subsequent 10-fold dilutions were made by adding 1ml of the supernatant to 9ml of distilled water to 10^{-6}. About 0.1 ml of samples from each serial dilution were spread-plated on pre-solidified surfaces of Potato Dextrose Agar (PDA) supplemented with 0.1g chloramphenicol to suppress bacterial growth and incubated at 25°C for 5-7 days. Chloramphenicol was used due to its broad-spectrum nature to most bacterial species. The pH was adjusted to about 5.6 using HCL. Non-hairy colonies without extension at margin were counted as yeasts whereas hairy colonies with extension at margin were counted as molds (Spencer *et al*., 2007).

3.8 Physiological and Morphological Characterization of Isolates

Colonies with distinct morphological differences such as colony color, size, elevation, margin and action on media were randomly picked from MacConkey Agar and Mannitol Salt Agar and aseptically transferred in to Sheep Blood Agar media for purification. Microscopic examination was also performed to identify for their morphological type as rod, cocci and spiral in shape.

3.8.1 Gram's Staining Procedures

Gram's staining was performed on the bacterial isolates to differentiate between Gram positive and Gram negative species. Bacterial colonies were smeared on a drop of water on a clean glass slide then were left to air dry and heat fixed. Then the slides were flooded with crystal violate dye for 1 min followed by rinsing by tap water. Then the slides were flooded with Gram's iodine for 1 min as a mordant and again rinsed by tap water. Thirdly the slides were rinsed with 70% (v/v) ethanol for 15 sec and then rinsed with tap water. Lastly the slides were flooded with safranin as a counter stain for 1 min and rinsed with tap water. Next the slides were allowed to dry and

were viewed under microscope x100 with oil immersion. The results were photographed and recorded (Gram, 1884).

3.9 Biochemical Characterization of Bacteria

Different biochemical tests were carried out to differentiate bacterial isolates. The biochemical tests such as catalase test, citrate utilization test, H_2S production, indole production, oxidase test, motility test and urease test were carried out using standard methods (John, 2012). The characterizations of isolates were done based bacterial classification manual. To determine the morphology and biochemical characteristics of the bacterial isolates, bacterial cells were Gram-stained (Gram, 1884). Motility test was conducted according to Shields and Cathcart (2012). Oxidation-reduction properties were determined according to Hugh and Leifson (1953).

3.9.1 Catalase Test

Slide method was used for catalase test. For the test fresh 18-24 hours old pure bacterial colonies were used. A 3-4 ml of 3% (v/v) H_2O_2 briefly a loop full of the isolated bacteria was smeared on a clean microscope slide and 2-3 drops of 3% (v/v) H_2O_2 was added directly on the smear, the formation of visible bubbles due to conversion of H_2O_2into H_2O and O_2 was indicates positive result and results were recorded and photographed (MacFaddin, 1980).

3.9.2 Urease Test

For urease test urea agar slants were prepared. Fresh 18-24 hours old pure bacterial colonies were streaked on the slants and incubated at 35℃ for up to 5 days. The bacterial colonies that can produce urease and convert the urea into CO_2 and NH_3 will convert the slant colors from yellow to bright pink (Vashist *et al*., 2013).

3.9.3 Citrate Utilization Test

Simmons Citrate agar, a medium containing citrate as the sole carbon source and ammonium salts as the sole nitrogen source was used to test for the ability of the bacterial isolates use organic compounds other than sugars as their sole source of carbon. Fresh 18-24 hours old pure bacterial colonies were streaked on the slants and the test tubes were incubated at 37℃ for 2-4 days. If the bacteria metabolize citrate the color of the Simmons Citrate agar slants will change from green to deep blue because the bromothymol blue indicator changes from green to blue due to shift of pH as a result of production of NH_3 from $NH_4\ H_2PO_4$ (Woodward and Bartel, 2005).

3.9.4 H_2S Production, Motility and Indole Production (SIM) Test

In order to find out the isolated organism's ability to reduce sulfur, produce indole and for motility, SIM media was used. SIM media is a differential medium that tests for the three characteristics simultaneously. For this experiment SIM medium was prepared and poured into test tubes. After solidifying the test tubes were inoculated by stabbing in the center to half an inch. Then the test tubes were incubated for 24 hours at 37℃. After 24 hours the test tubes were checked for the formation of a black precipitate (H_2S production) and fuzzy area surrounding the stab line (motility) and then 2-3 drops of Kovac's indole reagent was added to the tubes and the formation of red ring was checked (Vashist *et al*., 2013).

3.9.5 Coagulase Test

Both slide and tube coagulase tests were done. A drop of coagulase test reagent was dropped on a clean microscope slide and a loop full fresh 18-24 hours old pure bacterial colonies were smeared on it. The formation of coagulation record as positive.

3.10 Identification of Isolates Using MALDI TOF MS

The bacteria were investigated using the Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) which analyzes the protein or peptide constituents of a sample microorganism and enables identification of the microorganisms up to genus and species level. MALDI TOF-MS identification of the bacterial isolates was carried out at the National Animal Health Diagnostic and Investigation Center (NAHDIC) found in Sebeta.

3.10.1 Preparation of Pure Culture for MALDI TOF Analysis

Pure culture preparation was carried out in Adama Referral and Regional Laboratory. Each selected bacterial colony was carefully picked and purified separately in blood agar (supplement with 5% sheep blood per L) and incubated at 37 ℃ for 24 hours. From the purified colony a loop full of each selected bacteria colony was carefully picked and grown separately in Brain heart infusion agar and incubated at 37 ℃ for 24 hours for further purification. The overnight grown bacteria were carefully covered by Aluminum foil and transported to Sebeta using ice free glass box.

3.10.2 Characterization Process of the Isolates

The extended direct transfer (eDT) procedure per manufacturer's instruction was used to analyze the bacteria (Daltonics, 2012). The extended direct transfer method uses 70% formic acid to lyse

the cell membrane of the bacteria for simple extraction of the microbial proteins or peptides and makes for easier characterization of the samples. The target plate which contains 94 wells was cleaned thoroughly and well dried before starting the procedure. A distinct colony from the overnight grown bacteria was smeared directly on the wells as a thin film and left to dry. Then the spot was overlaid with 1µl of 70% (v/v) formic acid and allowed to dry at room temperature. Then immediately the spot was overlaid with 1µl of α-cyano-4 hydroxycinnamic acid (HCCA) solution and allowed to dry at room temperature. Then after the samples dried the target plate was placed inside the MALDI biotyper and analyzed. The samples are to be said adequately identified to the species level when the logarithmic score value (LSV) is greater than or equal to 1.9 (Toubal *et al*., 2018).

3.11 Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing was investigated on Mueller Hinton Agar (Oxoid) plates following the standard disk diffusion techniques. Characterized bacterial isolates were inoculated on pre solidified Mueller Hinton Agar (Oxoid) plates carefully. McFarland standards (1% BaCl_2 and 1% H_2SO_4) are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria was within a given range to standardize microbial testing. The antibiotic discs were placed on the medium by using sterilized needles and incubated at 35℃ for 18 h and the zone of inhibition was measured manually with Caliper. The results of the antimicrobial susceptibility were interpreted based on the guidance of National Committee for Clinical Laboratory Standards (NCCLS, 2007).

The following standard discs (Oxoid) and their potency (µgml^{-1}) were used depending up on the antibacterial spectrum, toxicity, effectiveness and availability (Vlkova *et al*., 2006): Azithromycin (30 µgml^{-1}), Ciprofloxacin (5 µgml^{-1}), Clindamycin (10 µgml^{-1}), Erythromycin (15 µgml^{-1}), Gentamycin (10 µgml^{-1}), Oxacillin (1 µgml^{-1}), and Penicillin G (10 µgml^{-1}) were used for *Staphylococcus aureus*. Amoxycillin (2 µgml^{-1}), Cefazolin (30 µgml^{-1}), Ceftazidime (5 µgml^{-1}), Ceftriaxone (30 µgml^{-1}), Cefuroxime (30 µgml^{-1}), Ciprofloxacin (5 µgml^{-1}), Meropenem (10 µgml^{-1}), Nalidixic acid (30 µgml^{-1}), and Piperacillin (85 µgml^{-1}) were used for *E. coli*. These antimicrobial agents were selected based on the availability and frequency of prescription for the treatment of bacterial infections in Ethiopia. The reference strain *E. coli*

(ATCC 25923) and *Staphylococcus aureus* (ATCC33591) were used as control to check the potency of antimicrobial disks.

3.11 Profiling and Identification of *Enterotoxigenic E. coli* (ETEC), Methicillin *and* Beta Lactam Resistant *Staphylococcus aureus.*

3.11.1 Preparation of Pure Culture

Using a sterile inoculation loop, each selected bacteria colony was carefully picked and grown separately in blood agar (supplement with 5% sheep blood per L) and incubated at 37 ℃ for 24 hours. Subsequently, using a cotton swab the grown bacteria culture was inoculated in Tryptone Soya broth media and stored in deep freezer up to the next step.

3.11.2 DNA Extraction

Bacteria genomic DNA extraction was done at the National Animal Health Diagnostic and Investigation Center (NAHDIC) found in Sebeta. Extraction was carried out according to Qiagen DNeasy DNA extraction protocol for bacterial cultures (2006). About 1.75ml of bacterial culture was added to appropriately labeled tubes for each bacterial sample and tubes were spin at 20,000 rpm for 5 minutes in centrifuge. After decanting the liquid 180µl of enzymatic lysis buffer (ATL) was added to tubes and vortexed for about 15s and incubate at 37℃ for 30 min in water bath. About 25 µl of proteinase K was added to the tube and vortexed briefly. Tubes were incubated at 56℃ for 30min and 200µl of 100% ethanol was added to the tubes and vortexed briefly. Using a micropipette entire content (~600µl) of tube were transferred to labeled spin column and centrifuge the column at 10,000rpm for 1min. Column was removed from the collection tube and placed in new collection tube. About 500µl of buffer AW1 was added to the column and centrifuge at 10,000rpm for 1minute. Column was removed from collection tube and placed in new collection tube. About 500µl of buffer AW2 was added to the column and centrifuged at 20,000rpm for 3 minutes. Tubes were carefully removed from centrifuge and the column transferred to 1.5 ml tubes and 200µl of AE buffer added to the column and was left at room temperature for 1 minute then centrifuged at 10,000 rpm for 1 minute. The column was discarded and the DNA was stored at 4℃.

3.11.3 Polymerase Chain Reaction (PCR) Technique

The DNA extract from bacterial isolates were used as template and primers used in the amplification process are listed in the table below.

Table 1. Primers used for PCR amplification

Strain	Locus	Primer sequence
ETEC	*Lt*	F:5´GGC GAC AGA TTA TAC CGT GC3´ R:5´CGG TCT CTA TAT TCC CTG TT3´
ETEC	*St*	F:5´ATT TTT CTT TCT GTA TTG TCT T3´ R:5´CAC CCG GTA CAA GCA GGA TT3´
BlaZ	*BlaZ*	F:5´TACAACTGTAATATCGGAGGG3´ R:5´CATTACACTCTTGGCGGTTTC3´
MecA	*MecA*	F:5´GTAGAAATGACTGAACGTCCGATGA3´ R:5´CCAATTCCACATTGTTTCGGTCTAA3´

Two sets of primers were used for amplification Enterotoxigenic *E. coli* gene. These genes are *Lt* (ETEC) and *St* (ETEC), are commercially synthesized by Eurofin genomics India Pvt. Ltd. For each primer 10.95 µl RNase free water, 5 µl of PCR buffer, 1.25 µl 25mM of $Mgcl_2$,0.8 µl dNTPs, 1 µl enzyme mix (Taq polymerase enzyme) and 5 µl of extended DNA. Sterile water was used as a negative control. *E. coli* (ATCC 25923) strain was used as a positive control for the two selected primers. The PCR tubes containing the mixture were tapped gently and the PCR tubes with all the components were transferred to (Flex cycler, Biometra Gmbh, Germany) thermal cycler. The PCR program included denaturation at 95℃ for 1 min, 52℃ for 1 min, 72℃ for 1min for 30 cycles, and a final extension at 72℃ for 10min used to complete the amplification. Two sets of primers were also used for amplification of targeting antibiotic resistance genes. These genes are *blaZ* and *mecA*. These primers are commercially synthesized by Eurofin genomics India Pvt. Ltd. Oligonucleotide primers *mecA*-F and *mecA*-R was used to target oxacillin resistant genes (Hoque *et al*., 2014). The *mecA* PCR program included denaturation at 95℃ for 5 min followed by (37) cycles at 95℃ for 30sec, 55℃ for 30sec, 72℃ for 60sec, and a final extension at 72℃ for 10min has completed the amplification (Salisbury *et al*., 1997). Primers used for amplification of Oligonucleotide primer *blaZ gene of staphylococcus isolates blaZ-F and blaZ*-R (Okiki *et al*., 2020). The *blaZ* amplification program was performed under the following conditions: Initial denaturation 95℃ for 5min, 37cycles of 95℃ for 30 sec, 55℃ for 30sec, 72℃ for 1min with a final extension of 72℃ for 10 min.

3.11.4 Agarose Gel Electrophoresis and Visualization

The PCR products were separated on 1.5% (w/v) agarose gel containing ethidium bromide (2µl) that intercalate into the DNA and used for visualization. About 8 µl amplification product of each sample with 2 µl of loading dye was applied into the slots of agarose gel. DNA marker of 100 bp (Qiagen; Germany) was used to estimate the molecular weight of the DNA fragments. Electrophoresis (Apelex Midigel XL, France) was performed in 1X in Tris- TBE buffer at 100v for 1 hr. The products were visualized with UV illumination (Gel Doc^{TM}XR, BioRad; Germany) and imaged with a gel documentation system.

3.12 Data Analysis

The data obtained from the respondents, frequency and mean values of microbial count from food samples were analyzed using Statistical Package of Social Science Software SPSS software version 26.0. Descriptive statistics was used for analysis and the results were interpreted. Mean values of food samples from different sites were compared using one way ANOVA and the significance of differences were considered at 95% confidence interval ($P < 0.05$).

CHAPTER FOUR

4. Results and Discussions

4.1 Socio-Demographic Characteristics of Street-Vended Food Venders in Adama City.

In this study, a total of 96 street venders were participated. As indicated in Table 2, the socio-demographic characteristics of street vendors shows that more than half 50 (52.1%) of the street food venders were female while the rest were male 46 (47.9%). The finding is in line with other studies carried out in Ethiopia and elsewhere (Getu *et al*., 2013) in Gondar, Muleta and Ashenafi (2001) in Addis Ababa, Tafesse *et al*. (2014) in Jigjiga, and Abdalla *et al*. (2009) in Sudan reported that (95, 80, 78.8 and72%) of the street vendors were females respectively. Among the participated vendors 78 of them were younger than 30 years with the majority (62.5%) falling within the 21-30 years range. Similarly, Nemo *et al*. (2015) from Jima, Ethiopia reported that 39.1% of street vendors belonged to the age group less than 30. Of the 96 street food vendors recruited in this study, 58 (60.4%) of them had less than five years of experience in street food vending. These, result was higher than what was reported by Nemo *et al*. (2015) who reported 26.4% had an experience of less than five years. Up to 39 (40.6%) of the respondents had primary education, 22(22.9%) secondary school completed, 25 (26.1%) had diploma and degree. In contrast to Amare *et al*. (2019), who report all vendors were literate in this study 10 (10.4%) had no formal education. Most (58.3%) of respondents have no alternative source of income (Table 2).

Table 2: Biographic information of respondents (n=96)

Variables		**No of respondents(n=96)**	**Frequency (%)**
Gender	Female	50	52.1
	Male	46	47.9
Age	Below 20	18	18.8
	21-30	60	62.5
	31-40	16	16.7
	Above 40	2	2.1
Level of education	No formal education	10	10.4
	1-8	39	40.6
	9-12	22	22.9
	Diploma	19	19.8

	Degree	6	6.3
Experience in food vending	Below 5 years	58	60.4
	6-10 years	35	36.5
	Above 10 years	3	3.1
Alternative source of income?	Yes	40	41.7
	No	56	58.3

4.2 Food Safety Knowledge and Hygienic Nature of Street-Vended Food Venders in Adama City.

In this research food vendors habits and their general cleanliness status were assessed (Table 3). Of the 96 street food vendors enlisted in this study, majority70 (72.9%) were displayed foods on fixed location. As shown in Table 3, 69 (71.9%) of the vendors did not have protective clothing and could not protect the foods they handle from any contamination from their bodies and clothing. Muinde and Kuria (2005) also reported that 81.3 % of the vendors did not use protective cloths. In contrast to a report by Tafesse *et al*. (2014) 33.3%, about 56 (58.3%) of the food vendors had no hair covering practice during preparing and serving the foods for customers. In line with Amare *et al*., (2019), more than half of the vendors (51%) had no frequent hand washing habit with soap and water during the preparation, collecting and displaying of food. The hands of the food handlers are the most important vehicle for the transfer of organisms from faces, nose, and skin to the food. The vendors can be carriers of pathogens like *E. coli*, *Salmonella* and *S. aureus* eventually can transfer these foodborne pathogens to the consumers (WHO, 1989). Among the participants, 58(60.4%) of them had no regular nail cutting practice. This result was higher than what was reported by Amare *et al*. (2019) where 11 (45.8%) had a frequent nail cutting. Training-wise, the majority 66 (68.8 %) of the study participants had no training in food safety which is similar to a study in the Cameroon by Shojaei *et al*. (2006) where 97% of the respondents had not received any training on food safety. This was also as noted in India by Tambekar *et al*., (2011) where food vendors were unaware of food regulations and were untrained on food hygiene. About 88.5% of street venders get raw materials from open market while 11.5% of them buy raw materials from super markets.

Table 3: Vendors Personal Hygiene Practice and Methods

Variables		Frequency	Percent
Vending site	On fixed location	70	72.9
	Mobile	26	27.1
Protective cloth wearing practice?	Yes	27	28.1
	No	69	71.9
Hair covering practice?	Yes	40	41.7
	No	56	58.3
Hand washing practice?	Yes	47	49
	No	49	51
Regular nail cutting practice?	Yes	38	39.6
	No	58	60.4
Training on food safety?	Yes	30	31.3
	No	66	68.8
Source of raw materials?	Open market	85	88.5
	Super market	11	11.5
Sources of water	Tap	96	100
	River	0	0
	Ground	0	0
Way of cleaning utensils	Using water only	54	56.3
	Using soap and warm water	40	41.6
	Using soap and cold water	2	2.1
Frequency of changing utensils	Weekly	3	3.1
	Monthly	17	17.7
	Yearly	40	41.7
	Not changed	36	37.5
Method of packaging for takeaway	Polythene bag	23	24.0
	Paper sheet	14	14.6

	Aluminum foil	10	10.4
	Polythene bag and Paper sheet	49	51.0
Practice of blowing air into polythene bag?	Yes	52	54.2
	No	44	45.8
Practice of licking hands to separate papers?	Yes	63	65.6
	No	33	34.4
Method of waste disposal?	Dump into nearby garbage	62	64.6
	Selling for others	34	35.4

Similar to a study conducted in Jigjiga (60.6%), about 54 (56.3%) vendors use only water for cleaning utensils. As shown in the above table, all (100%) of the street vendors found in Adama city use tap water for preparation of food, washing utensils and for personal hygiene. Again, 36 (37.5%) of the venders' utensils had not changed for long time, (40) 41.7% of respondent change their utensils yearly, 17 (17.7%) of venders change utensils monthly while the rest 3 (3.1%) of food venders had a frequency of changing utensils weakly. More than half, 49 (51%) of the respondents use both polythene and paper sheets as a method of packaging for take away. Among the vendors 52 (54.2%) had practice of blowing air into polythene bag and 63 (65.6%) had practice of licking hands to separate papers during packaging. On the other hand, this hand licking practice may be prone to spread COVID19 and other related disease. Majority 62 (67.7 %) of vendors collect all wastes together till the end of vending process and dump it into nearby waste bins after finishing their work. they also drain the waste water into the drainage nearby. This was in line with study conducted in Nairobi, Kenya, Muinde and Kuria (2005) reported that 92.5 % of street food venders did not have garbage receptacles; hence they disposed their garbage near the stalls. Some vendors (35.4 %) sell the wastes to other person who need it for other purpose.

4.3 Microbial Analysis of the Food Samples (Chips, Penchera and Sambusa)

This study tried to examine the microbial quality and safety of ready-to-eat street foods at selected four vending places in Adama city, Ethiopia. The pictorial representation, constituents and

description of the three street foods included in this study (Chips, Penchera and Sambusa) are presented Figure 1 and Table 4 respectively.

Figure 1: (A): Chips, (B): Penchera, (C): Sambusa

Table 4: The ingredients and description of local street foods analyzed from Adama City

Food items	Ingredients	Preparation
Chips	Chopped potatoes with salt, catchup (optional) and cooking oil	Piece of potato which have been sliced extremely thin and then fired or baked until they become crisp and ready to eat (Tambeker *et al*., 2011)
Penchera	Peeled boiled potato, chopped carrot, avocado (optional), onion, oil, pepper and salt	Boiled potato then peeled and served with uncooked chopped carrot, avocado, pepper and onion mixtures with other ingredients such as 'Senafich', salt and oil.
Sambusa	Wheat dough, lentils, chopped onions, cooking oil	Triangle of wheat dough stuffed with lentils, chopped onions and pepper fried deeply (Tesfaye *et al*., 2016)

Accordingly, 54/96 (56%) of the food samples were contaminated with food borne pathogens and indicator organisms including *E. coli* and *S. aureus*. The bacterial load results are put together and presented in Table 5.

Table 5: Microbial mean count result

Sample Name	Bacterial loads									
	Total Plate Count		Total Coliform Count		Total Enterobacteriaceae Count		Total *S.aureus* Count		Total *E. c* Count	
	TPS (n=32)	Mean count (CFU/g)	TPS (n=32)	Mean count (CFU/g)	TPS (n=32)	Mean count (CFU/g)	TPS (n=32)	Mean count (CFU/g)	TPS (n=32)	Mean count (CFU)
Penchera	26(81%)	$7.0*10^7$	22(68%)	$1.6*10^7$	19(59%)	$7.5*10^5$	15(49%)	$7.1*10^4$	17(53%)	$3.8*10^5$
Chips	17(53%)	$1.8*10^7$	12(37%)	$2.9*10^6$	14(43%)	$2.0*10^5$	13(40%)	$1.0*10^4$	16(50%)	$1.3*10^4$
Sambusa	10(31%)	$1.1*10^7$	7(21%)	$1.8*10^6$	9(28%)	$3.6*10^5$	6(18%)	$2.6*10^3$	11(34%)	$3.8*10^3$
NSW acceptable level	$< 10^5$		10^2 to $< 10^4$		10^2 to $< 10^4$		10^2 to $< 10^3$		3 to $< 10^2$	

Key; TPS: **T**otal **P**ositive **S**ample out of 32 food samples.

There is no standard set for the permissible level of microbes for street-vended food being served in Ethiopia. However, NSW (2009) set the recommended microbial quality guideline for street-vended food. In this study 55% of street vended foods showed high mean total plate count ($1.8*10^7$ CFU/g to $7.0*10^7$ CFU/g) compared to what was set for microbiological quality of ready-to-eat street foods (NSW, 2009). The mean total plate count was the highest in Penchera ($7.0*10^7$ CFU/g) followed by Chips ($1.8*10^7$ CFU/g). In line with study conducted in Jima (Nemo *et al*., 2015), the lowest Plate count was observed in Sambusa. The mean Plate counts of all tested foods were found to be beyond the acceptable level set by NSW (below 10^5CFU/g). Similarly, the mean coliform counts of all tested food items were beyond the acceptable range (10^2 to $< 10^4$ CFU/g) (NSW, 2009). Relatively much higher coliform count was seen in Penchera ($1.6*10^7$ CFU/g) than in other food types. The mean *Enterobacteriaceae* count was the highest in Penchera ($7.5*10^5$

CFU/g) whereas it was lower in Chips ($2.0*10^5$ CFU/g) and Sambusa ($3.6*10^5$CFU/g). Likewise, the mean counts of *Enterobacteriaceae*, yeast and mold were the highest in Penchera ($7.5*10^5$ CFU/g and $1.4*10^5$ CFU/g respectively). As it was suggested by Oluyege *et al*. (2009), Enteropathogens are known to survive on the hands for three hours or longer so that isolation of these organisms in this study is an indication of fecal contamination of the foods. In this study, no *Salmonella* species were encountered. There were other studies in Zimbabwe (Kwiri *et al*., 2014), Kenya (Githaiga *et al*., 2012) and Gondar (Getu *et al*., 2013) which are in agreement with this finding. However, considering the low sample size together this study cannot prove that street foods are free from *Salmonella* species rather it indicates that the prevalence of *Salmonella* in street food may be very low.

Amare *et al*., (2019) in a study conducted in Gonder, Ethiopia, reported that seventy-two food samples of street vended food were analyzed for bacterial pathogens and 44/72 tested positive which is a bit lower than 54 total positive food sample result of this study. As shown in Figure 2, Penchera were more contaminated followed by Chips. This may be due to the uncooked ingredients in it. The quality of raw materials for preparing those foods, personal hygiene of the venders, cleanness of the vending environment and method of exposing those foods along street side could increase degree of microbial fortification. Of the total positive food samples about 26/53 were Penchera, 17/54 were Chips and 11/54 were Sambusa (Figure 2).

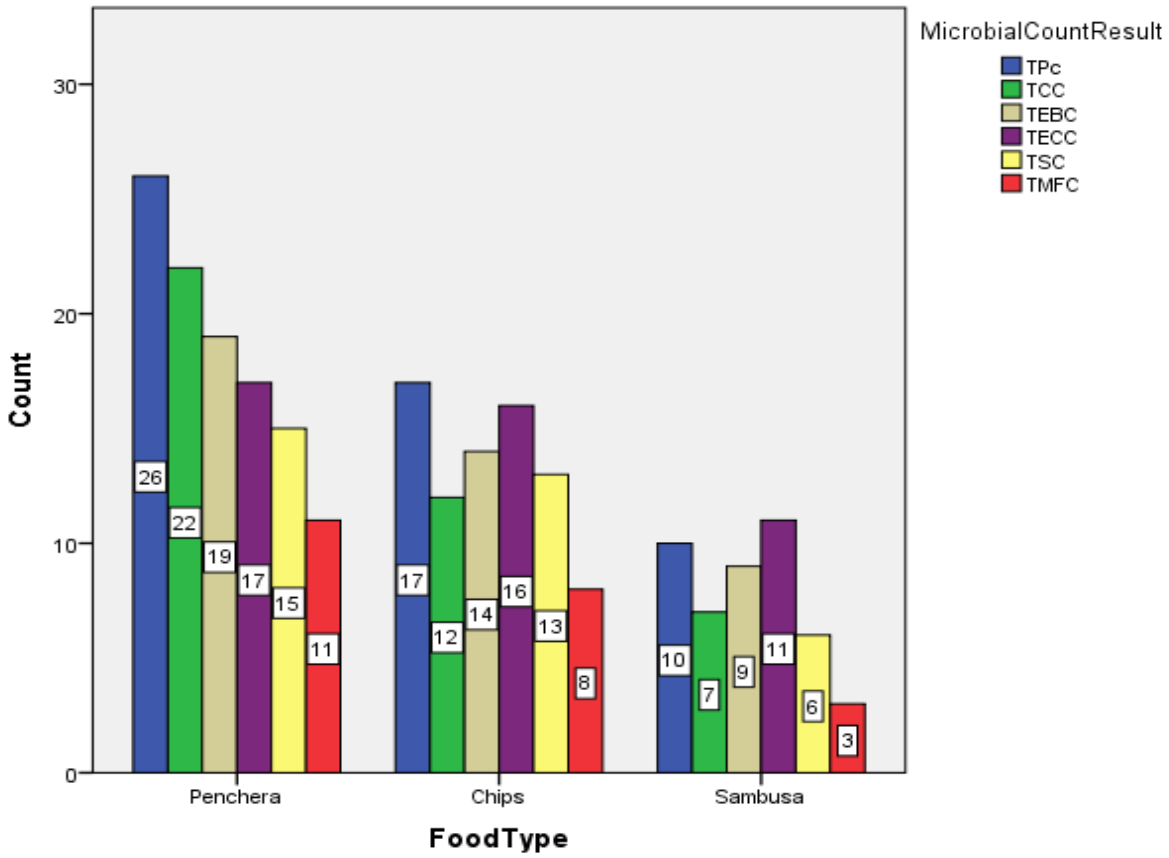

Figure 2: Number of positive samples from total food sample analyzed in the study. TPC, TCC, TEBC, TECC, TSC and TMFC indicates total plate count, total coliform count, total *Enterobacteriaceae* count, total *E. coli* count, total *Staphylococcus* count and total mold and fungi count respectively.

Comparison of food samples analyzed in this study referring to the total microbial count record at 95 confidence level and P= 0.05 can be summarized as illustrated below in Table 6.

Table 6: Multiple Comparisons

Dependent Variable: Bacteria Count
Tukey HSD

(I) Food Type	(J) Food Type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Penchera	Chips	15642352.173*	5458937.143	.013	2765865.23	28518839.12

	Sambusa	17108807.881*	6523184.618	.025	1721984.52	32495631.24
Chips	Penchera	-15642352.173*	5458937.143	.013	-28518839.12	-2765865.23
	Sambusa	1466455.709	6874387.237	.975	-14748780.92	17681692.34
Sambusa	Penchera	-17108807.881*	6523184.618	.025	-32495631.24	-1721984.52
	Chips	-1466455.709	6874387.237	.975	-17681692.34	14748780.92

*. The mean difference is significant at the 0.05 level.

As shown in Table 6, there is a significant difference in total microbial count of Penchera with Chips and Sambusa. Because the mean deference of Penchera was higher than the mean deference of Chips. But there is no significant difference between Chips and Sambusa.

Of the isolated bacteria from each food, *E. coli* was the most frequent isolate 44 (56.4%) followed by *S. aureus* 34 (43.6%) which is similar with the study conducted in Hawasa on bacteriological quality of street foods and antimicrobial resistance (Temesgen *et al*., 2016), Furthermore, the mean counts (log CFU/g) of *E.coli* and *Staphylococci* were the highest ($3.8*10^5$ and $7.1*10^4$ respectively) in Penchera followed by Chips ($1.3*10^4$ and $1.0*10^4$ respectively). *E. coli* was the most frequent isolate followed by *Salmonella* species (12.7%) and *S. aureus* (9.9%). However, it was relatively lower in Sambusa ($3.8*10^3$ and $2.6*10^3$ respectively). This indicates that *E. coli* is the most predominant bacteria in selected food items followed by S. aureus. It is known that *E. coli* and *S. aureus* are the most common pathogens found on hands (Shojaei *et al*., 2009). The hands of the food handlers are the most important vehicle for the transfer of organisms from feces, nose and skin to the food (Rane, 2011). As a result, the food and food related materials, skin, clothes, displaying sites and other materials which are related with poor hygienic condition can be contaminated. Food handler may be cause contamination by poor food handling practices contributing to cross contamination or by being carriers. In this study, *Salmonella* and *Shigella* were not isolated. The results of this survey showed that street food consumption is commonly consumed in Adama City of Ethiopia. This study, undertaken showed that the food items most

commonly available for sale were Chips, Penchera and Sambusa. Contamination of Penchera could result from pre- and post-cooking contamination and contamination of Chips and Sambusa could result from post-cooking contamination from the food handlers The observed significant differences reported in the total plate count between Penchera and the other two may be attributed to the fact that the Penchera contains raw ingredients which may contribute to the higher microbial load and serve on an open space and exposed to contamination from gaseous emissions and atmospheric gases such as oxygen which supports the growth of aerobic microorganisms (Suneetha *et al*., 2011). The total plate count result record indicates that raw materials in perishable products were contaminated and might also indicate unsuitable time and temperature storage conditions. The working site remain uncleaned after preparation of food that will increase the chance of cross contamination during the making of next meal. Conducting such epidemiological studies are of great importance in researching the links between food borne sickness and its causes.

4.4 Morphological and Biochemical Characterization of Isolates

From the total screened food samples 108 bacterial isolates were identified. Of these bacterial isolates 61 (56.5%) were gram negative and 47 (43.5%) were gram positive.

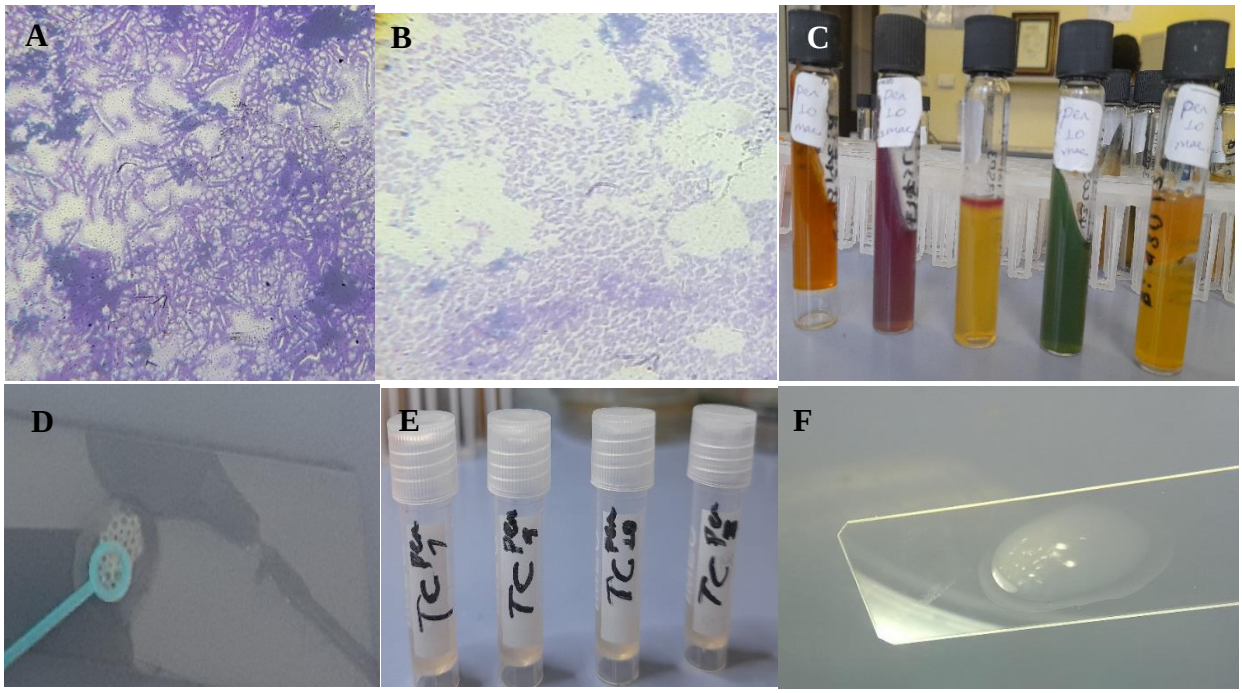

Figure 3: Gram staining and biochemical test, (**A):** gram negative rod shaped bacterial structure, **(B):** gram positive cocci structure (**C):** biochemical tests, **(D):** catalase test, **(E):** tube coagulase, **(F)**: slid coagulase of bacterial isolates.

Table 7: Morphological Characterization and Biochemical Test Results

Bacterial code	Gram staining	Shape	Urease	Citrate	Catalase	Coagulase	Motility	H2S	Indole	Suspected bacterial sp.
BFPI05	-	R	-	-	_	_	+	-	+	*Escherichia coli*
BMCI17	+	R	-	+	+	_	-	+	+	*Bacillus cereus*
BMCI01	-	R	-	+	+	_	+	+	+	*Aeromonas hydrophila*
BAPI26	+	C	_	_	+	-	_	_	_	*Staphylococcus sciuri*
BHPI08	+	R	+	+	+	_	-	-	-	*Enterobacter cloacae*
BFPI08	+	C	_	_	+	+	_	_	_	*Staphylococcus aureus*
BFSI07	-	R	-	-	_	_	+	-	+	*Leclercia adecarboxylata*
BMCI27	-	R	+	+	+	_	-	-	+	*Klebsiella oxytoca*
BMPI07	-	R	-	-	_	_	+	-	+	*Escherichia coli*

Key words: bacterial code indicates, B: **B**acteria, second letter indicates vending sites (**H**/mariam, **F**ranko, **A**STU, a**M**ede), third letter indicates food samples (**C**hips, **P**enchera and **S**ambusa), I: **I**solate and numbers indicates isolate number: + (Positive), - (Negative) and _ (not done).

4.5 Identification of Bacteria Isolates by MALDI-TOF MS

From 61 total gram-negative bacteria isolates, Urea positive, no H2S production, motile, indole positive and oxidase negative were considered as a best candidate for *E. coli* and were prepared for analysis by the extended direct transfer (eDT) method. The analysis was done by the MALDI-TOF MS as a confirmatory test. Among total 47gram positive bacterial isolates, catalase positive, tube and slide coagulase test positive were prepared for analysis by the MALDI-TOF MS at NAHDIC, Sebeta. The analysis was done three times. In this study *Aeromonas caviae, Aeromonas hydrophila, Acinetobacter calcoaciticus, Bacillus cereus, Citrobacter fruendii, Enterobacter cloacae, Escherechia coli, Exiguobacterium auranticum, Enterococcus casseliflavus, Klebsiella*

oxytoca Leclercia adecarboxylata, Staphylococcus aureus and Staphylococcus sciuri were identified less frequently (Table 8).

Table 8: MALDI-TOF MS analysis for bacterial isolate species obtained from Chips, Penchera and Sambusa

S.N.	Sample Id	Species	Score value	S.N.	Sample Id	Species	Score value
1	BFSI07	*Leclercia adecarboxylata*	2.05	14	BHPI23	*Aeromonas caviae*	2.07
2	BMCI01	*Aeromonas hydrophila*	2.12	15	BHCI25	*Exiguobacterium auranticum*	2.09
3	BHPI02	*Citrobacter freundi*	2.26	16	BAPI26	*Staphylococcus sciuri*	2.06
4	BAPI04	*Bacillus cereus*	2.28	17	BMCI27	*Klebsiella oxytoca*	2.22
5	BACI05	*Exiguobacterium spp*	2.27	18	BACI28	*Exiguobacterium auranticum*	2.12
6	BFCI07	*Aeromonas caviae*	2.02	19	BFSI30	*Bacillus cereus*	2.20
7	BHPI08	*Enterobacter cloacae*	2.20	20	BFPI05	*Escherichia coli*	1.97
8	BMPI11	*Leclercia adecarboxylata*	2.42	21	BMPI07	*Escherichia coli*	2.20
9	BHSI14	*Acinetobacter calcoaciticus*	2.06	22	BAPI02	*Enterococcus casseliflavus*	2.01
10	BFPI16	*Leclercia adecarboxylata*	1.19	23	BFPI08	*Staphylococcus aureus*	2.12
11	BMCI17	*Bacillus cereus*	2.32	24	BHPI03	*Staphylococcus Pasteuri*	2.00
12	BAPI18	*Bacillus cereus*	2.36	25	BFCI09	*Staphylococcus hominis*	2.15
13	BFPI29	*Bacillus cereus*	2.27	26	BAPI16	*Staphylococcus aureus*	2.25

Key words: bacterial code indicates, B: **B**acteria, second letter indicates vending sites (**H**/mariam, **F**ranko, **A**STU, a**M**ede), third letter indicates food samples (**C**hips, **P**enchera and **S**ambusa), I: **I**solate and numbers indicates isolate number.

As described by Neelja *et al.* (2015) MALDI based identification of microbial isolates are very accurate. A total of 26 microbial isolates were identified by MALDI-TOF technique of which *Bacillus cereus, Escherichia coli* and *Staphylococcus aureus* dominated the quantity of isolates next to opportunistic human pathogen *Leclercia adecarboxylata.*

4.6 Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was carried out using standard disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2020) on Mueller Hinton agar with standard antibiotic discs. McFarland standards were used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria was within a given range to standardize microbial testing. If the bacterial suspension is more turbid than that of McFerland solution implies to much bacterial growth. In such condition sensitive bacteria may be observed as resistant. If bacterial suspension is less turbid than McFarland solution implies less bacterial growth so resistant bacteria may be seen as sensitive which is false result. There for, to get true positive result in the course of this study McFarland standards were used as a reference to adjust the turbidity of bacterial suspension. Antimicrobial susceptibility test was conducted for *E. coli* and *S. aureus* isolates.

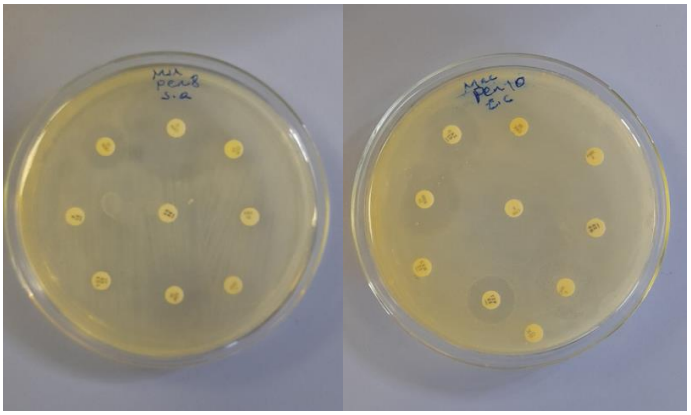

Figure 4: The antibiotic susceptibility test result of *Staphylococcus aureus* and *E. coli*

As shown in Table 9 all *E. coli* isolates were 100% sensitive to ceftazidime which is in contrast to result of Temesgen *et al*., (2016) and show resistance for cefuroxime 21(47.73%), nalidixic acid 20 (45.45%), ceftriaxone 15 (34.1%), amoxycillin and ciprofloxacin 14(31.82%). Since, these drugs are known to be highly effective against *E. coli* (Mazumdar *et al*., 2006). On the other hand, *S. aureus* evolved resistance to azithromycin and cefoxitin 14 (41.18%), erythromycin 13(38.24%) and other tested antimicrobial drugs which would make the treatment of *S. aureus* infections difficult. *S. aureus* infection is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis. Although most staph infections are not serious, *S. aureus* can cause serious infections such as bloodstream infections, pneumonia, or bone and joint infection About 23.53% of *S. aureus* isolates were also resistant to oxacillin which is a bit higher than 15% resistance reported in Benin (Sina et al., 2011). At this point, it is important to remark that bacterial

isolates resistant to oxacillin eventually become resistant to chloramphenicol, cloxacillin, doxycycline, erythromycin and penicillin G which may make the treatment of *S. aureus* infection more difficult.

Table 9: Antibiotics susceptibility test results of *Staphylococcus aureus and E. coli*

Name of antibiotic disks	**Disc concentration (µg/ml)**	**Code**	***Staphylococcus aureus***		***E. coli***	
			Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Amoxycillin	2	AMX	-	-	30(68.1%)	14(31.8%)
Azithromycin	15	AZM	20(58.8%)	14(41.1%)	-	-
Ceftazidime	30	CAZ	-	-	44(100%)	-
Clindamycin	10	CLN	34(100%)	-	-	-
Ciprofloxacin	5	CPR	34(100%)	-	30(68.1%)	14(31.8%)
Ceftriaxone	5	CTR	-	-	29(65.9%)	15(34.1%)
Cefoxitin	30	FOX	20(58.8%)	14(41.1%)	-	-
Cefuroxime	30	CXM	-	-	23(52.2%)	21(47.7%)
Erythromycin	15	ERY	21(61.7%)	13(38.2%)	-	-
Gentamicin	10	GEN	27(79.44%)	7(20.56%)	-	-
Meropenem	10	MER	-	-	27(61.36%)	17(38.64%)
Nalidixic acid	30	NA	-	-	24(54.55%)	20(45.45%)
Oxacillin	1	OXC	26(76.47%)	8(23.53%)	-	-
Tetracycline	30	TE	28(82.35%)	6(17.65%)	27(61.36%)	17(38.64%)
Penicillin G	10	P	26(76.47%)	8(23.53%)	-	-

4.7 Molecular Identification of Bacterial Strains Isolated from Street Foods In Adama City

Molecular typing was carried out in the Molecular laboratory of the Department of NAHDIC, Sebeta. The analysis was done three times. Seven isolates of *E. coli* spp were analyzed by convectional PCR technique. There were 3 isolates from Penchera, 2 from Chips and 2 from Sambusa. All the isolates were negative for the *LT* and *ST*. On the other hand, 8 isolates of

Staphylococcus aureus were analyzed to determine the pathogenicity of *Staphylococcus aureus* strains. There were 5 isolates from Penchera, 2 from Chips and 1 from Sambusa. All the isolates were negative for *blaZ* and *mecA*. In contrast with study on Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers food samples were not contaminated with methicillin resistant *Staphylococcus aureus* (Seid *et al*., 2020).

Beta-lactam antibiotics are one of the most commonly prescribed drug classes with numerous clinical indications. These resistance to antimicrobial drugs would make the treatment of *Staphylococcus aureus* infections difficult. As explained by Zhang *et al*. (2011), the emergence of methicillin resistant *S. aureus* strains has become a major concern in medical practice.

5. Conclusions and Recommendations

5.1 Conclusions

In this study Penchera was the most contaminated food with highest mean count of total plate count followed by Chips. As the result indicates the required quality and safety levels of the foods was not acceptable and do not meet required quality and levels of ready to eat foods compared to standards set by the NSW guide. Pathogenic microorganisms like *S. aureus, Bacillus cereus* and *E. coli* were isolated and their detection at unacceptable level indicates the foods do not meet required safety standard. All isolates were negative for Enterotoxigenic *E. coli* and *S. aureus for* methicillin and beta lactam antibiotic resistance gene. The identified foodborne bacteria and antibiotic resistance isolates could pose a public health problem in Adama city. Source of contamination can be the unhygienic manner of venders, vending sites and the food during preparing, handling and displaying for sell. Therefore, regular inspection, health education and training of vendors on food handling and safety practices are needed.

5.2 Recommendations

Adama is a vast city with increasing number of vendors day to day, so it's not daring to say that the sample size of the vendors included in this study was adequate. Therefore, we recommend to do further assessment by making a sample size that is higher than the number included in this study. Additionally, due to lack of basic requirements, it was impossible to conduct MALDI TOF analysis for all isolates. Thus, further investigation using MALDI-TOF and other molecular technique required.

6. REFERENCES

Abdalla, M., Suliman, S. and Bakhiet, A. (2009). Food safety knowledge and practices of street food vendors in Atbara City (Naher Elneel State Sudan). *African Journal of Biotechnology*.

ACA (2012). Socio economic profile of ACA. Adama city administration. 2008-2012.

Acco, M., Ferreira, F., Henriques, J. and Tondo, E. (2003). Identification of multiple strains of *Staphylococcus aureus* colonizing nasal mucosa of food handlers. *Journal of Food Microbiology.* 20:489-493.

Ackah, M., Gyamfi, E., Anim, A., Osei, J., Hansen, J., and Agyemang, O. (2011). Socio-economic profile, knowledge of hygiene and food safety practices among street-food vendors, *Hygiene and Food Safety*,13: 191-197.

Akinyele I. (1987). Study on street foods in Ibadan, Nigeria. Characteristics of food vendors and consumers: implications for quality and safety. Food and Agriculture Organization of the United Nations/Department of Human Nutrition, University of Ibadan, Rome/Ibadan

Alam, S., Feroz, F., Rahman, H., Das, K. and Noor, R. (2015). Microbiological contamination sources of freshly cultivated vegetables. *Journal of Nutrition & Food Science.* 45(4);646.

Aluko, O., Ojeremi, T., Olaleke, D., and Ajidagba, E. (2014). Evaluation of food safety and sanitary practices among food vendors at car parks in Ile Ife.

Amare Azanaw, Tifito Worku, Birukitait Ashagirie, Marie Adugna, Alem Getaneh and Mulat Dagnew. (2019). Bacteriological profile, antimicrobial susceptibility patterns of the isolates among street vended foods and hygienic practice of vendors in Gondar town, Northwest Ethiopia: a cross sectional study. *BMC microbiology.*19(120);2-9

Asiegbu, C., Lebelo, S., and Tabit, F. (2016). The food safety knowledge and microbial hazards awareness of consumers of ready-to-eat street-vended food.

Authority NF. Microbiological quality guide for ready-to-eat foods: A guide to interpreting microbiological results. NSW/FA/CP028/0906. 2009:1-9.

Balaban, N. and Rasoonly, A. (2000). *Staphylococcal enterotoxins. Int J Food Microbiol*

Barro, N., Razack, B., Yollande, I., Aly, S., Tidiane, O., Philippe, N., Comlan, D.and Sababenedjo TA. (2007). Street vended foods improvement: contamination mechanisms and application of food safety objective strategy: critical review.

Bhowmik, S. (2005). Street vendors in Asia: a review. Econ Political Wkly, pp 2257–2264

Cardinale, E., Claude, J., Tall, F., Gueye, E. and Salvat, G. (2005). Risk factors for contamination of ready-to-eat street vended poultry dishes in Dakar, Senegal. Int J Food Microbiol 103:157–165.

Chakrabarti, A. (2016). The Future of Infectious Diseases Diagnostics. *International Journal of Infectious Diseases* 45: 1–477.

Chapman, B. (1984). Street foods in Indonesia: Vendors in the urban food supply. Chevy Chase, MD: Equity Policy Center.

Charles, M., Leontina, N., Namugumya, B., George, N. (2011). Practices, knowledge and risk factors of street food vendors in Uganda. *Journal of Food Control*, 22 (2011) 1551-1558.

Chumber, S., Kaushik, K. and Savy, S. (2007). Bacteriological analysis of street foods in Pune. *Indian journal of Public Health*. 51(2);114-6.

Chung, C., Ritoper, S. and Takemoto, S. (2010). Bangkok and access to food for low-income residents. *Journal of Massachusetts Institute of Technology*.

CII. (2008). CII-14-point checklist on food safety for street vended food, version 1.0.

CLSI. (2020). Performance Standards for Antimicrobial Susceptibility Testing. *Clinical and Laboratory Standards Institute.*

Cohen, M. (1985). The influence of the street food trade on women and children. In Advances in maternal and child health. Oxford: Oxford University Press.

Dawson, R.and Canet, C. (1991). International activities in street foods.

Daltonics, B. (2012). MALDI Biotyper 3.1 User Manual. 1(May), 1–212.

Debroy, C., Roberts, E., Valadez, A., Dudley, E. and Cutter, C. (2011). Detection of shigela toxin-producing E. coli O26, O45, O103, O111, O113, O121, O145, and O157 serogroups by

multiplex polymerase chain reaction of the wzx gene of the O-antigen gene cluster. *Journal of Food borne pathogens and disease.*8(5);651-652.

Deriba Muleta and Mogessie Ashenafi. (2001). Bacteriological profile and holding temperatures of street vended foods from Addis Ababa. *International Journal of Environmental Health Research*, 11: 1, 95 — 105.

Doyle, M., and Evans, P. (1999). Food borne pathogens of recent concern. *Annual Revised Nutrition.* 5; 25–41.

Duff, S., Scott, E., Mafilios, M., Todd, E., Krilov, L., Gedded, A. and Ackerman, S. (2003). Cost-effectiveness of targeted disinfection program in household kitchens to prevent food-borne illnesses in the United States, Canada and the United Kingdom. *Journal of Food Protection.* 2: 2103-2115.

Ekanem, E. (1998). The street food trade in Africa: safety and socio-environmental issues.

El-Sherbeeny, M., Saddik, M., Bryan, F. (1985). Microbiological profile and storage temperatures of Egyptian rice dishes.

FAO (1989). Street foods. Report of an FAO expert consultation Jogjakarta. *FAO food and nutrition paper* no.46. Rome: FAO

FAO (1990). Street foods. Report of an FAO expert consultation. Jogjakarta, Indonesia, 5-9 December, 1998. FAO Nutr. 1990; 46:3-30.

FAO. The state of food and agriculture, Rome. 1998

Food and Agriculture Organization (1997). Street Foods. Report of an FAO technical meeting on street foods Calcutta India, 6-9 Nov. 1995. FAO Food and Nutrition Paper **63**, Rome.

Foskett, P., Ceserani, V. and Kinton, R. (2003). The theory of catering 10th ed. Hodder and Stoughton.

Frankline, K. (2021). How to determine sample size for a research study.

Galane, P., Le Roux, M. (2001). Molecular epidemiology of *E. coli* isolated from young South African children with diarrhoeal diseases. Journal of Health Population and Nutrition 19 (1), 31-38.

Getu Derbew, Samuel Sahle and Mengistu Endris (2013). Bacteriological Assessment of Some Street Vended Foods in Gondar, Ethiopia. *Internet Journal of Food Safety*. 15;33-38.

Ghosh, M., Wahi, S., Kumar, M., Ganguli, A. (2007). Prevalence of enterotoxigenic Staphylococcus aureus and Shigella spp. in some raw street vended Indian foods. *International Journal of Environmental Health Research*. 17;151-6

Gitahi, M., Wangoh, J., Njage, P. (2012). Microbial safety of street foods in industrial area, Nairobi. Research Journal of Microbiology **7**; 297-298.

Githaiga, G. (2012) Microbial quality, strain distribution and Enterotoxigenicity of selected food borne pathogens in relation to the hygienic practices in industrial area, Nairobi, Kenya: Faculty of Agriculture, College of Agriculture and Veterinary Sciences, University of Nairobi.

Gram, H. (1884). Uber die isolierte Färbung der Schizomyceten in Schnitt-und Trockenpräparaten". Fortschritte der. Medizin. 2: 185–189.

Hanashiro, A., Marisa, M., Glavur, R., Matt-e, Maria, H., Matt-e, E. and Torres, A. (2005). Microbiological quality of selected street foods from a restricted area of Swao Paulo city. Brazil. *Food Control*, 16, 439-444.

Hassan, M., Acharjee, M., Das, E., Das, K., Ahmed, T., Akond, M., Fatema, K. and Noor, R. (2013). Microbiological study of sea fish samples collected from local markets in Dhaka city, *International Food Research Journal.*

Hilda, V. (2002). The role of street foods in the diet of low-income urban residents, the case of Nairobi.

Hillenkamp, F.and Karas, M. Matrix-assisted laser desorption/ionisation, an experience. Int J Mass Spectrom. 2000; 200:71–7.

Holy, A. and Makhone, F. (2006). Improving street food vending in South Africa: achievements and lessons learned.

Hoque, S., Islam, M., Amin, M. and Alim, S. (2014). Microbial loads and association of Enteropathogenic bacteria in ice-creams sold by street vendors at Dhaka city in Bangladesh, *International Journal of Pharmaceutical Sciences and Research*. **5** (1)2436-2440.

Hugh, M., Leifson, E. (1953). The taxonomic significance fermentative versus oxidative gram-negative bacteria. *Journal of Bacteriology*. 66:24-26.

John, L. (2012). An introduction to bacterial identification. Di chotkey handout.

Kalinowski, R., Tompkin, R., Bodnaruk, P., and Pruett, W. (2003). Impact of cooking, cooling, and subsequent refrigeration on the growth or survival of Clostridium perfringensin cooked meat and poultry products. *Journal of Food Protocol*. 66: 1227-1232.

Karas, M., Bachmann, D., Bahr, U. and Hillenkamp, F. (1987) Matrix-assisted ultraviolet laser desorption of non-volatile compounds. *Int J Mass Spectrom Ion Processes*,78:53–68.

Karas, M., Bachmann, D., Hillenkamp, F. (1985). Influence of the wavelength in high-irradiance ultraviolet laser desorption mass spectrometry of organic molecules. Anal Chem.;57:2935–9.

Kiiyukia, C. (2003). Laboratory Manual of Food Microbiology for Ethiopian Health and Nutrition Research Institute.

Kinabo, J. (2003). Impact of globalization on food consumption, health and nutrition in urban areas: a case study of Dares Salaam, United Republic of Tanzania.

Kok, R. and Balkaran, R. (2014). 'Street food vending and hygiene practices and implications for consumers. Journal of Economics and Behavioural Studies, 6 (3): 188-193.

Kumar, M., Agarwal, D., Ghosh, M., Ganguli, A. (2006). Microbiological safety of street vended fruit chats in Patiala City. Indian J Med Microbiol, 24(1): 75-81.

Kwiri, R., Winini, C., Tongonya, J., Gwala, W., Mpofu, E. and Mujuru F. (2014). Microbiological safety of cooked vended foods in an urban informal market: a case study of Mbare Msika, Harare, Zimbabwe.

Latham M (1997). Human nutrition in tropical Africa. FAO. 329-437

Liu, Z., Zhang, G., Zhang, X. (2014). Urban street foods in Shijiazhuang city, China: Current status, safety practices and risk mitigating strategies.

MacFaddin, J. (1980). Biochemical tests for identification of medical bacteria. Williams and Wilkins, Baltimore. USA

Mamun, M., Rahman, S., Turin, T. (2013). Microbiological quality of selected street food items vended by school-based street food vendors in Dhaka, Bangladesh. International Journal of Food Microbiology.166(3):413–8.

Markides, K., Gräslund, A. 2002). Advanced information on the nobel prize in chemistry 2002. Royal Swedish Academy of Sciences. p. 1–13. Retrieved 2013-08-28.

Mazumdar, K., Dutta, N., Dastidar, S.G., Motohashi, N.and Shirataki, Y. (2006). Diclofenac in the management of *E. coli* urinary tract infections. In Vivo 20(5), 613-9.

Mensah, P., Yeboah, D., Owusu, K., Ablordey, A. (2002). Street foods in Accra, Ghana: how safe are they? Bulletin of World Health Organization.546-554.

MFPOI (2009) Upgradation of the quality of street food.

Mohapatra, A., Rath, C., Dash, S., Mishra, R. (2002) Microbiological evaluation of street foods in Bhubaneshwar. J Food Sci Technol 39(1):59–61.

Muinde, O. and Kuria, E. (2005). Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. Afr J Food Agric Nutr Dev, 5(1): 1-14.

Mwadime, E. (2001). Nutritional and Safety Quality of street foods in Korogocho and Industrial area of Nairobi, Kenya. Page 39-54

Mwangi, A. (2002). Nutritional, hygienic and socio- economic Dimensions of Street Foods in Urban Areas: The Case of Nairobi. Wageningen University.

National Committee for Clinical Laboratory Standards (NCCLS) (2007). Performance standards for antimicrobial susceptibility testing 17th informational supplement.

Natural Resources Institute (NRI). (2004). Developing food safety strategies and procedures through reduction of food hazards in street-vended foods to improve food security for consumers, street food vendors and input suppliers.

Neelja, S., Manish, K., Pawan, K. K., and Jugsharan, S. (2015). MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. 6(August), 1–16. https://doi.org/10.3389/fmicb.2015.00791.

Reda Nemo, Ketema Bacha and Tsige Ketema (2015). Microbiological quality and safety of some-street-vended foods in Jimma Town, Southwestern Ethiopia. *African Journal of Microbiology Research.*

New South Wales Food Authority NSW. (2009). Microbiological quality guide for ready-to-eat foods. A guide to interpreting microbiological results. 1-9.

Nicolas, B., Razack, B., Yollande, I., Aly, S., Tidiane, O., Philippe, N. (2007). Street Vended Foods Improvement: Contamination Mechanisms and Application of Food Safety Objective Strategy. *Pakistan Journal of Nutrition*.;6(1).

Nonga, H., Ngowi, H., Mdegela, RH., Mutakyawa, E., Nyahinga, G., William, R., Mwadini, M. (2015). Survey of physicochemical characteristics and microbial contamination in selected food locally vended in Morogoro Mu nicipality, Tanzania. BMC Res Notes, 8: 727

Notermans, S., Gallhof, G., Zweitering, M., and Mead, G. (1995). Identification of critical control points in the HACCP system with a quantitative effect on the safety of food products. *Food Microbiology*, **12** (5): 93–98.

Okareh, O. and Erhahon, O. (2015). Microbiological Assessment of Food and Hand-Swabs Samples of School Food Vendors in Benin City, Nigeria.

Oladipo, I. and Adejumobi, O. (2010). Incidence of Antibiotic Resistance in Some Bacterial Pathogens from Street Vended Food in Ogbomoso, Nigeria. Pakistan Journal of Nutrition.9(11):1061-8.

Oluyege, A., Dada, A., Ojo, A. and Oluwadare, E. (2009). Antibiotic resistance profile of bacterial isolates from food sold on a university campus in south western Nigeria. African Journal of Biotechnology. 8(5883-5888).

Omemu, A. and Aderoju, S. (2008). Food safety knowledge and practices of street food vendors in the city of Abeokuta, Nigeria.

Owens, N. and Hussain, N. (1984). Street foods in Bangladesh. Chevy Chase, MD: Equity Policy Center.

Owusu-Darko, K. and Ablordey, A. (2002). 'Street food in Accra, Ghana: How safe are they?' Bulletin of the World Health Organisation Vol 80: 546-554 Tent H. Research on food safety in 21stcentury. Food Control. 1999; 10: 239-247

Pethers, J., Gilbert, R., (1971) Survival of *Salmonella* on finger tips and transfer of the organism to foods. J Hyg (Camb) 69:673–681.

Powell, D., Brodber, E., and Campbell, V. (1990). Street foods of Kingston. Kingston, Jamaica: Institute of Social and Economic Research, University of the West Indies.

Qiagen. (2006) .DNeasy handbook,.

Ram, P., Naheed, A., Brooks, W., Hossain, M., Mintz, E., Breiman, R. (2007). Risk factors for typhoid fever in a slum in Dhaka, Bangladesh. Epidemiol Infect. 135(3):458–65.

Rane, S. (2011). Street vended food in developing world: hazard analyses. Indian J Microbiol. Jan;51(1):100-6. doi: 10.1007/s12088-011-0154-x. E0pub 2011 Jan 26

Rappelli, P., Maddau, G., Mannu, F., Colombo, M.M., Fiori, P.L., Cappuccinelli, P. (2001) Development of a set of multiplex PCR assays for the Simultaneous identification of enterotoxigenic, enteropathogenic, enterohemorrhagic and enteroinvasive *E. coli*.

Riet, H. (2002). The Role of Street Foods in the Diet of Low-Income Urban Residents, The Case of Nairobi. Wageningen.

Samira, H. and Sergio, O. (2017). Martinez-Chapa. Principles and mechanism of MALDI-ToF-MS analysis. Research Gate

Sarker, N., Islam, S., Hasan, M., Kabir, F., Uddin, M. and Noor, R. (2013). Use of multiplex PCR assay for detection of diarrheagenic. *E. coli* street vended food items.

Seid, K.,Amal, G,,Fatma, M. and Abdelazeem, M.(2020). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. *International journal of food microbiology.*

Seng, P., Drancourt, M.and Gouriet, F. (2009). Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

Sharmila, R. (2011). Street vended food in developing world: Hazard analyses. *Indian Journal of Microbiology.*

Shields, P., Cathcart, L. (2012). Motility test medium protocol. Motilitytest-medium-protocol.

Shojaei H, Shooshtaripoor J, Amiri M. Efficacy of simple hand-washing in reduction of microbial hand contamination of Iranian food handlers. Food Res Int. 2006;39(5):525 –9.

Sina, H., Baba-Moussa, F., Kayodé, A., Noumavo, P. and Sezan, A. (2011). Hounhouigan Characterization of Staphylococcus aureus isolated from street foods: Toxin profile and prevalence of antibiotic resistance. Journal of Applied Biosciences.;46:3133-43.

Sparkman,O. (2000). Mass spectrometry desk reference. Pittsburgh: Global View. Pub ISBN 0-9660813-2-3.

Spencer, K., John, F. and Spencer, A. (2007). Food Microbiology Protocols, Homana Press, Totowa, New Jersey, India

Suneetha, C., Manjula, K.and Baby, D. (2011). Quality assessment of street foods in Tirumala. Asian J Exp BiolSci, 2(2): 207-211.

Tafesse F, Desse G, Bacha K, Alemayehu H. Microbiological quality and safety of street vended raw meat in Jijiga town of Somali regional state, Southeast Ethiopia. Afr J Microbiol Res. 2014;8(48):3867 –74.

Tambekar D. H., Kulkarni R. V., S. D. Shirsat and D. G. Bhadange. 2011. Bacteriological quality of street vended food Manipuri. A case study of Amravati city (ms) India. Bioscience discovery Vol 2 (3):350-354.

Tambekar, D., Jaiswal, V., Dhanorkar, D., Gulhane, P. and Dudhane. M, (2008). Identification of microbiological hazards and safety of ready-to-eat food vended in streets of Amravati City, *India Journal of Applied Bioscience.*

Temesgen Eromo, Haimanot Tassew, Derese Daka, Gebre Kibru (2016). Bacteriological Quality of Street Foods and Antimicrobial Resistance of Isolates in Hawassa, Ethiopia. *Ethiopian Journal of Health Science.*26(6); 533-542.

Tesfaye Wolde, Bereda Yohannes, Mekonnen Emerie, Melese Abate, Reta Henok, Sileshi Asfaw. (2016). Microbiological Safety of Street Vended Foods in Jigjiga City, Eastern Ethiopia. *Ethiopian journal of health sciences.*26(2);162-170.

Toubal, S., Bouchenak, O., Elhaddad, D., Yahiaoui, K., Boumaza, S., & Arab, K. (2018). MALDI-TOF MS detection of endophytic bacteria associated with great nettle (urtica dioica l.),

grown in Algeria. Polish Journal of Microbiology, 67(1), 67–72. https://doi.org/10.5604/01.3001.0011.6145

Vashist, H., Sharma, D. and Gupta, A. (2013). A review on commonly used biochemical test for bacteria innovare journal of life science, 1(1), 1–7. https://doi.org/10.1109/icdm.2013.109

Veras, J., Carmo, L., Tong, L, Shop J.W., Cummings, C., Santos, D., Cerqueira, M., Cantini, A., Nicoli, R., and Jett, M. (2008). A study of the enterotoxigenicity of coagulase negative and coagulase positive staphylococcal isolates from food poisoning outbreaks in Minas Gerais Brazil. J Infect Dis Vol 12:410-415.

Vlkova, E., Rada, V., Popelarova, P., Trojanová, I. and Killer, J. (2006). Antimicrobial susceptibility of bifidobacteria isolated from gastrointestinal tract of calves. Live st. Sci. 105: 253-259.

Weil, Q., Hwang, S., Chen, T. (2006). Microbiological Quality of Ready-to eat Food Products in Southern Taiwan. *Journal of Food Drug Analysis*. 14: 68-73.

WHO (1996) Essential safety requirements for the street vended foods (revised edition). World Health Organization, Food Safety Unit, Geneva

WHO (2003) estimates of the global burden of food borne diseases Food borne diseases burden epidemiology reference group. *WHO technical estimation report*.

WHO (2011). Food safety and food borne illness; Fact sheet No 237. Reviewed March 2007. Accessed 12th February 2011.

WHO (2015). WHO's first ever global estimates of foodborne diseases find children under 5 account for almost one third of deaths. Departmental news.

WHO (2021). WHO steps up action to improve food safety and protect people from disease. Departmental news.

Woodward, A. and Bartel, B. (2005). Auxin: Regulation, action, and interaction. Annals of Botany, 95(5), 707–735. https://doi.org/10.1093/aob/mci083

Yamane, T. (1967). Statistics: An Introductory Analysis. 2nd Edition, Harper and Row, New York.

Yarwood, J., McCormick, M., Paustian, P., Orwin, V., Kapur, and Schlievert, P. (2002). Characterization and expression analysis of *Staphylococcus aureus* pathogenicity island J Biol Chem Vol 277:13138–13147.

Zhang, Y., Cheng, S., Ding, G., Zhu, M., Pan, X., Zhang, L. (2011). Molecular analysis and antibiotic resistance investigation of *Staphylococcus aureus* isolates associated with *staphylococcal* food poisoning and nosocomial infections. *African Journal of Biotechnology*.;10(15):2965-72.

7. Appendixs

Appendix 1: Questioner to Asses Demographic Data and Hygienic Nature of Vendors

My name is Dagmawit Mekuria. I am a master's student of microbiology from Adama Sience and Technology University and I am working my research on microbial quality and safety of street vended foods in Adama city and I need your help to study my research by answering the following questions.

Thank you for your help!

Mark an x in the column containing your response

1. **Gender**

□ Male □ Female

2. **Age**

□ Below 20 □ 21-30 □ 31- 40 □ Above 41

3. **Education level**

□ No formal education □ Primary □ Secondary

□ Diploma □ Above

4. **Vending site**

□ In fixed locations on the streets □ Mobile

5. **Experience of food vending (in years)**

□ <5 □ 5-10 □ >10

6. **Do you have alternative source (s) of income?**

□ Yes □ No

7. **Where do you obtain your raw material?**

□ Informal market □ Municipal market/supermarket

8. **Safety of food from dust, sun and winds?**

□ Yes □ No

9. **Do you have training on food safety?**

□ Yes □ No

10. **Do you have frequent hand washing practice with soap and water during the preparation, collecting and displaying of food?**

□ Yes □ No

11. Do you have regular nail cutting practice?

□ Yes □ No

12. Do you cover your hair during preparation and selling of foods?

□ Yes □ No

13. Do you wear protective cloth? (If the answer is No, jump question 12 and 13)

□ Yes □ No

14. Is protective clothing clean?

□ Yes □ No

15. How often are they washed?

□ Every day □ Once within a week □ Once within a month □ Not washed

16. Where do you obtain water for cleaning and other activities?

□ Tap □ River □ Ground water

17. How you wash the utensils

□ By hand using water only □ With warm water and soap □ With cold water and soap

18. Frequency of changing the vending utensils

□ Daily □ Weekly □ Monthly □ Yearly □ Not changed

19. Which method(s) of packaging do you use for take away?

□ Polythene bag □ Paper sheets □ Aluminum foil □ Polythene bag and Paper sheets

□ Other

20. If you used polythene bags, do you blow air into polythene bag for serving food

□ Yes □ No

21. If you used paper sheets, do you lick hands to separate paper sheets for serving food

□ Yes □ No

22. How do you dispose-off the wastes generated during food preparation?

……

Appendice 2: Comparison of Bacteria Type Frequency

Multiple Comparisons

Dependent Variable: Bacteria Count

Tukey HSD

(I) Bacteria Type	(J) Bacteria Type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
TPc	TCC	32875660.589*	7091186.519	.000	12496274.85	53255046.33
	TEBC	42173899.178*	7043427.810	.000	21931767.36	62416031.00
	TECC	42507879.503*	6953547.717	.000	22524054.40	62491704.60
	TSC	42626788.438*	7491470.576	.000	21097024.95	64156551.93
	TMFC	42544113.139*	8647014.786	.000	17693427.10	67394799.17
TCC	TPc	-32875660.589*	7091186.519	.000	-53255046.33	-12496274.85
	TEBC	9298238.589	7485267.370	.816	-12213697.49	30810174.66
	TECC	9632218.914	7400755.241	.784	-11636837.46	30901275.28
	TSC	9751127.849	7908317.374	.820	-12976613.11	32478868.81
	TMFC	9668452.550	9010561.414	.892	-16227031.41	35563936.51
TEBC	TPc	-42173899.178*	7043427.810	.000	-62416031.00	-21931767.36
	TCC	-9298238.589	7485267.370	.816	-30810174.66	12213697.49
	TECC	333980.325	7355006.948	1.000	-20803599.87	21471560.51
	TSC	452889.261	7865521.772	1.000	-22151861.27	23057639.79
	TMFC	370213.961	8973024.354	1.000	-25417392.11	26157820.04
TECC	TPc	-42507879.503*	6953547.717	.000	-62491704.60	-22524054.40
	TCC	-9632218.914	7400755.241	.784	-30901275.28	11636837.46
	TEBC	-333980.325	7355006.948	1.000	-21471560.51	20803599.87
	TSC	118908.936	7785138.616	1.000	-22254828.16	22492646.03
	TMFC	36233.636	8902646.606	1.000	-25549113.58	25621580.85

TSC	TPc	-42626788.438*	7491470.576	.000	-64156551.93	-21097024.95
	TCC	-9751127.849	7908317.374	.820	-32478868.81	12976613.11
	TEBC	-452889.261	7865521.772	1.000	-23057639.79	22151861.27
	TECC	-118908.936	7785138.616	1.000	-22492646.03	22254828.16
	TMFC	-82675.299	9328848.918	1.000	-26892886.83	26727536.23
TMFC	TPc	-42544113.139*	8647014.786	.000	-67394799.17	-17693427.10
	TCC	-9668452.550	9010561.414	.892	-35563936.51	16227031.41
	TEBC	-370213.961	8973024.354	1.000	-26157820.04	25417392.11
	TECC	-36233.636	8902646.606	1.000	-25621580.85	25549113.58
	TSC	82675.299	9328848.918	1.000	-26727536.23	26892886.83

*. The mean difference is significant at the 0.05 level.