College of Natural Sciences

School of Post Graduate Studies

Department of Biology

Microbiological quality and safety of some selected street- vended food sat Hagereselam town, Tigray Region, Northern Ethiopia.

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A thesis submitted to the Department of Biology, College of Natural Sciences, Jimma University in partial fulfillment for the requirements of Degree Masters of Science in General Biology

August, 2020

Jimma, Ethiopia

JIMMA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF BIOLOGY

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Acknowledgements

First, I would like to express my sincere gratitude to my advisors Delelegn Woyessa (Associate professor) and Shiferaw Demissie for their willingness to offer critical and constructive comments, excellent scientific guidance and support starting from the approval of the topic up to the completion of my thesis work. I would like also to thank Jimma University for financial support.

I am sincerely thankful to the Department of Veterinary, College of Veterinary, Mekelle University for allowing me to use laboratory, chemicals, media and equipment's for facilitating my research work. I also thank Mr. Haftay Abraha who helped me in laboratory work.

I would like to express my love and gratitude to my beloved family , especially my mother Tsadkan G/meskel , my wife Nigsti fassil and my brothers Tesfay G/her, Dessie G/her and Dr. Halefom G/her for their precious spirit , love ,care and motivation to accomplish my work.

It is my greatest pleasure to thank my best instructor Ketema Bacha (Prof.)for his critical advice as a father and my friends Birhane Hagos, Hadush Kassa, G/hiwot G/yesus,G/wahid Abadi, Tewelde Fisahaye, Kahsay Teklu, Kibrom Belay and G/medhn Tekle for their moral support in the whole work.

Above all, I would like to thank my God who gave me an ease to reach to final work.

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List of Acronyms

AMB Aerobic Mesophilic Bacteria

ATCC American Type Culture Collection

BPW Buffered Peptone Water

CDC Centers for Disease Control and Prevention

MRS de Mann Rogosa Sharpe

MSA Manitol Salt Agar

NCCLS National Committee for Clinical Laboratory Standards

PCA Plate Count Agar

Rpm Rotation per minute

SIM Sulfide Indole Motility

VRBA Violet Red Bile Agar

XLD Xylose Lysine Deoxycholate

Abstract

Street food is defined as "ready-to-eat foods and beverages sold and prepared by vendors or hawkers in streets or other public places .Street- vended food industry has been benefiting both consumers who are in low economic status as well as vendors by creating job opportunity. However, street foods are perceived to be a major public health risk due to contamination by diverse microbes. The aim of this study was to assess microbiological quality and safety of some street-vended foods in Hagereselam town. An experimental cross sectional study design was used to collect samples from street vended foods in Hagereselam town for microbiological quality and safety analysis. A total of 60 street vended food samples (20 bonbolino, 20 sandwiches and 20 sambussa) samples were collected from bus station, Alula and Lsanu vending sites in Hagereselam town using sterile aluminum plates and transported to Mekelle University Microbiology laboratory of Veterinary College. The samples were kept at refrigerator temperature until analysis. Accordingly, 25 g of each of the food samples were homogenized and serial diluted and both morphological and biochemical characterization of the isolates from pure countable colonies was conducted following standard microbiological procedures. Antibiotic susceptibility test for pathogenic bacteria was conducted. Data was analyzed using SPSS software version 20.00. Questionnaire was used to assess the profile of 96 street food vendors. Result of the study showed that 71.9% of vendors were females, 56.3% had primary education, 100 % of the vendors did not use special apparel for their job as street food vendors, 77.1% of the vendors handled food with bare hands, 61.5 % used well water for cleaning of utensils, and 100 % of the vendors wore no hair covering. The mean microbial counts (log $CFUg^{-1}$) of food samples were dominated by aerobic mesophilic bacteria (5.3 ± 1.2), aerobic bacterial spore (4.2 ±0.7), Lactic acid bacteria (4.2±0.5), Enterobacteriaceae (4 ± 0.7), Coli form (3.8 ± 0.5) Staphylococci (3.4 ± 0.6), yeasts (3.4 ± 0.5) and moulds (2.6 ± 0.5) respectively. Of the total seven bacterial isolates characterized, the predominant isolates were Bacillus spp. (31.9%) followed by Staphylococci spp. (26.9%). Out of the food samples, 21.7% were positive for Staphylococcus aureus and 6.7% samples were positive for Salmonella. In morphological test results, 81.9% of the isolates were gram positive, half of them were rod in shape and 51% of them were motile. The antibiotic resistance patterns of the isolates revealed low number of resistance (7.7%) of S.aureus to Chloramphenicol. Generally, the microbial quality and safety of street-vended food in Hagereselam town was poor. Therefore formal training and awareness creations on street food vendors and consumers are recommended.

Keywords: *Hagereselam town. Microbiological quality, Microbiological safety, Street- vended food.*

1.Introduction

According to Food and Agriculture Organization (FAO),street food is defined as "ready-to-eat foods and beverages sold and prepared by vendors or hawkers in streets or other public places"(Lamuka,2014).Street foods are sold in areas of busy economic activity and heavy population concentration. Additionally, since street 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. Street food provides a convenient diet for many people in developing countries (Anandhi *et al*.,2015) and approximately 2.5 billion people eat street food every day, with the consumption supporting the livelihood of millions of low income people and contributing greatly to the economy (Rahman,2013).

According to FAO (2007), street food vendors are common in both developing and industrialized countries with a considerable expansion in developing countries. Extensive Street vending of foods in the world arises from multiple causes. Some of the causes are migration of people to cities and accelerated urbanization leading to enormous urban congestion, long commuting distances between the workplace and home, and a shortage or absence of establishments that serve reasonably priced food close to the work place. Moreover, migration to the cities has given most cities an overpopulation of rural dwellers, while striving for better opportunities, have contributed to the existence of marginal urban areas and unemployment.

According to Zeru (2015), street food vendors trade in a number of commodities in different locations of cities and provide a variety of services such as fried egg, bread, potato chips, sandwich, sambussa, tea, coffee and lunch services, for lower and middle level income customers/consumers. Despite these benefits, (Guven *et al*., 2010) have reported in some developing countries, street food has been associated with outbreaks of food borne diseases. High levels of coli form bacteria have been found in street food in several countries, and street food has been identified as a common medium for transmission of antimicrobial-resistant pathogens. In Ethiopia, almost all categories of people are consuming street foods; while some are protected from using these foods due to fear of contamination (Angaw,2011).As reported by Muleta and Ashenafi (2001b), Street vended foods are predisposed to contaminations because they are sold in the open and are often not covered. Sale of food on the streets is very

controversial from a health point of view. The main health hazard associated with street foods is microbial contamination. Knowing the microbiological safety of street foods is an important factor to appreciate the safety problems related to street foods so that concerned organizations should take proper steps to improve safety and sanitation with respect to this sector. Microbiological studies on street-vended foods in different countries have suggested the presence of high bacteria counts and a high incidence of food-borne pathogens (Rane, 2011). Although governments throughout the world are attempting to improve the safety of the food supply, the occurrence of food borne disease remains a significant health issue in both developed and developing countries.

In Ethiopia, particularly Addis Ababa, Muleta and Ashenafi (2001a) have reported, the presence of *Salmonella* and *Shigella* from street- vended foods. Food borne diseases represent a major concern in developing countries including Ethiopia. Diarrheal diseases represents the second leading cause of death in Ethiopia (Muleta and Ashenafi,2001b). Typhoid fever is responsible for 2.8% of deaths in children aged 5-9 years,8.9% in the 10-14 age group,3.2% in the 15-19 age group and 1.5% in the 20-49 age group (Moyo *et al.*,2007).Food poisoning occurs as a result of consuming food contaminated with microorganisms or their toxins, the contamination arising from inadequate preservation methods, unhygienic handling practices, cross-contamination from food contact surfaces, or from persons harboring the microorganisms in their noses and on the skin. Unhygienic practices during food preparation, handling and storage creates conducive conditions that allow the proliferation and transmission of disease causing organisms. (Greig *et al*.,2007).

In Ethiopia, different studies revealed that pathogens and indicators are enumerated above the acceptable limit from different categories of street vended foods. Study done in Jimma town, highlighted *Staphylococcus* countin street foods was much higher (5.39 log cfu/ml) than the standard and dominated by almost the same load of aerobic mesophilic bacteria (6.13 logcfu/ml) and Enterobacteriaceae (5.96 log cfu/ml) (Solomon and Ketema , 2011).

However, information on the microbial quality and safety of street vended Bonbolino, Sambussa and Lentil sandwich in the study area is scant. Since many people in that locality became patients of diarrhea, pneumonia and skin infections, the researcher initiated to conduct a research in street foods of Hagereselam town.

1.1. Statement of the Problem

Knowing the microbiological quality of street vended foods is an important factor in recognizing the safety problems related to street-vended foods. Evidence of laboratory based diagnosis on the assessment of bacteriological profile and their antimicrobial susceptibility pattern is important for designing and implementing effective treatment,control and prevention strategies to effectively tackle the problem. In Hagereselam town, labourers and other low income individuals eat street food as it is both available and affordable. However, the quality of these foods and their safety for human health is not studied in detail in Hagereselam town. But Hagereselam town is expanding from time to time because of new road construction and becoming center for other cities with road, many people prefer eating street foods for their cheap in cost and easily accessible on streets. This study was tried to assess the food safety and microbiological quality of street vended food at Hagereselam town.

1.2. Objectives of the study

1.2.1. General Objective

The general objective of the study was :

- ✓ To assess the microbiological quality and safety of some selected street vended foods in Hagereselam town, Tigray, Northern Ethiopia.

1.2.2 Specific objectives

The specific objectives of the study were:

- ❖ To determine the microbial load of street vended foods such as Bonbolino,Sambusa and Lentil sandwich.
- ❖ To isolate and characterize bacteria from Bonbolino, Sambusa and Lentil Sandwich.
- ❖ To determine the antibiotic susceptibility patterns of some pathogen isolated from some street vended foods.
- ❖ To assess associated risk factors contributing to safety problems of Sambussa, Bonbolino and Lentil sandwich.

2. Literature Review

2.1. Microbial activities in street vended foods

Street foods are defined as ready to eat foods and beverages prepared and/or sold by vendors, especially on streets and other public places for immediate consumption (WHO, 1996). These foods are well appreciated by consumers, mostly by urban workers because of their taste, low cost, nutrient value, different varieties and availability for immediate consumption (Abdalla *et al*., 2009). They can be found wherever there is a heavy flow of people, since their marketing success depends exclusively on location and word-of mouth promotion (Winarno and Allain, 1991). This is not surprising when one considers that bacteria and fungi are ubiquitous and are plentiful in soil and around us and easily contaminate foods (Bukar *et al*., 2010). Economic situation, social difficulties and urbanization, among other factors, promote the growth of informal sector of the economy including street food vending (Hanashiro *et al*., 2005). These foods may be consumed without heat treatment and cause food-borne illness, a health problem in developing countries (WHO, 1996). Food safety is one of the most important issues in marketing any kind of food, particularly meat and its products (Okonko *et al*., 2010). The most serious safety issues resulting in immediate consumers health problems is associated with bacterial pathogens (Sousa, 2008). The most common bacterial agents are *Campylobacter*, *Salmonella*,*Escherichia coli*, *Shigella*, *Staphylococcus* and *Clostridia* (Agbodaze *et al*., 2005). Street foods displayed on open work area can easily be contaminated by dust, exhaust smoke, insects and hands of the buyers. Thus, food vendor services is on the increase and responsibility for good manufacturing practices of food such as good sanitary measures and proper food handling practices (Clarence *et al.*, 2009). On top of this, availability of tap water is limited to washing of hands, utensils in the vending site, hence, they are forced to be used repeatedly. There is no waste disposal facilities thus garbage is discarded close to the vending site and this attract insects and rodents to double the problem through cross contamination (Tamebaker *et al*., 2008).

2.2. Food Safety Knowledge and Attitude of food vendors

Street food vending activities in most developing countries are mostly outside the regulation and protection of the governments. The economic importance of the activities is not well appreciated due to the informal nature of the enterprise and lack of official data on volume of trade involved

(Alimi and Workneh,2016).Food safety has been defined by Henson and Traill (1993) as the inverse of food risk "the probability of not suffering some hazard from consuming a specific food". If one is not aware of the origin and severity of food borne diseases, it's clear that they will be less motivated to change. It's therefore necessary to believe that someone can get sick by some bad food handling attitudes and by changing the behavior, one can prevent illnesses (Schafer *et al*., 1993). Consequently, food safety knowledge is important in prevention of food borne illnesses although gain of knowledge doesn't necessarily lead to change in behavior. That's why attitude is also very important (Henson and Traill, 1993).

2.3. Overview of Street Foods in Developing Countries

According to Rane (2011), the poor knowledge and improper food handling of street vendors in basic food safety measures and poor knowledge and awareness among consumers on the potential hazards associated with certain foods could explain the health and safety issues that street foods may pose. Moreover, it is important to state that the costs of food-borne illness include the cost of medical treatment, productivity loss, pain and suffering of affected individuals, industry losses, and losses within the public health sector. According to Lianghui (1993), Traditional and indigenous exotic street foods have emerged as a new form of tourist attraction in developing countries. However, street foods have become one of the most common risks associated with the increase in outbreaks of food-borne diseases in developing countries in recent years. There have been several documented cases of food poisoning outbreaks associated to street foods. Street foods were responsible for 691 food poisoning outbreaks and 49 deaths from 1983 to 1992. In 1988, 14 deaths were reported in Malaysia because of food-borne diseases related to street foods (Bryan, 1988). In the same year 300 people became ill in Hong Kong after consumption of street vended foods (Bhat, 2000). An outbreak of cholera in Singapore in 1987 was attributed to the consumption of street foods (FAO, 1990).

In 2011, Vietnam adopted a revised food safety law which for the first time specifically mentioned the problems related to street food. The law states some specific guidelines on how to operate a street food stall. In brief, the five key conditions for street food safety that need to be satisfied by vendors are the stall must be away from a polluted place , clean water must be used to cook food and clean kitchen utensils , the origin of the produce used to make the food must be

clear, vendors must have a waste collection system in place , Vendors can only make use of a specific list of additives.

As a result of its participation in whose international food safety authorities' network (Infosan), Vietnam now has access to food safety authorities worldwide and receives useful information on new international food safety events. Vietnam's food administration (VFA) has made additional efforts to improve the operations of street food vendors. In particular, the VFA has started organizing training on hygiene and food safety for street food vendors. For consumers, the government tries to educate them through a variety of communication channels in order to give them a clear perception of what a hygienic and safe street food stall looks like. In this way, it is hoped that the consumers armed with this knowledge will avoid unsafe food stalls. As a result, the government expects that the improved (food safety) behavior of consumers will put pressure on unhygienic street food vendors to either improve the quality of their stalls or to withdraw from the street food market. In addition, the government has recently started conducting inspections on street food vendors in order to observe if they comply with the regulations or not.

The vendors who adopt the law will be motivated while those who break the rules will be fined or forced to stop their business.
As a member of Infosan, Vietnam has been supported by whom to improve its food safety laws. It has been advised to adopt a participatory method to get all important stakeholders involved in food safety development. In addition, who also supports Vietnam in the development of potential diagnostic techniques for food safety analysis in laboratories and necessary facilities to raise awareness about food safety in consumers through community education and training programs? Moreover, participating in Infosan enables Vietnam to acquire new information from international food safety events and effective consultation to monitor food safety problems at a global as well as local level (WHO, 2015).

The most common food-borne diseases caused by eating street food in Vietnam are intestinal or gastrointestinal complaints, of which the most common symptom is diarrhea. Also, contaminations are the most important cause of these food-borne diseases and are a result of vendors not following the standards for hygienic handling and the five keys to safer food

provided by Vietnam's government (VFA, 2015). the five keys to safer food was widespread not only through mass media channels including television or the national radio station named "voice of Vietnam" but also through other organizations like the farmers association, women's union and veterans association. On the other hand, the government also supplies flyers and broadcasts audio messages (via village speaker systems) so that basic safety knowledge can be reached by consumers who live in remote areas.

Vietnam is a lower middle income country and needs to deal with a lot of challenges of food safety, especially on street foods safety. In particular, the rapid development of industrialization leads to the environmental pollution in most of the big cities, which also negatively affect the safety of street foods. One of the biggest factors that influence street food safety is provision of clean water at street food stalls. The other significant challenge is that the poor knowledge and attitudes on street food of local consumers. In addition, most of street food vendors tend to use unsafe and unhygienic produce or ingredients due to either high profit or limited knowledge.

2.4. Food Handling Practices

Food handlers are definedas "employees who are employed directly in the production and preparation of foodstuffs(Al Suwaidi *et al*., 2015). Those include employees in the manufacturing, catering and retail industries as well as those who are undertaking maintenance or repair of equipment in food handling areas, whether permanent staff, workers on contract or visitors to food handling areas". Food handling comprises all stages of food treatment and storage, from the reception of raw items to the end products and their distribution, i.e., from farm to fork (FAO, 2017).

According to Al Suwaidi *et al*.(2015), Causes of food borne disease by food handlers include cross-contamination between raw and processed food items; storage and cooking of food in adverse conditions; and utilizing contaminated utensils and equipment Food handlers can also be vehicles that carry organisms associated with food borne-illnesses, such as salmonella, staphylococci, and *E. coli* (Rosmawati, 2015). Also, other causes include the probability that food handlers could carry pathogens (while not showing any symptoms) and transmit those pathogens in food (Al Suwaidi *et al*., 2015).

2.5. Safety of Street Foods

According to Muinde and Kuria (2005), the hygienic aspects of street food vending are a major concern for food control officers. Vending stands are often crude structures, and running water, washing facilities and toilettes may not be available. Improved safety of street foods can be achieved through awareness raising programmes involving several partners such as local authorities, the food vendors, government departments, consumer organizations, standard setting bodies, and some nongovernmental organizations. In some instances, the vendors are keen to participate in programmes that provide basic facilities that make it possible for them to work in clean environments. For example, in a survey of street food vendors in Lusaka and Harare, the vendors indicated that they would be willing to pay for basic facilities such as running water and electricity but would want the local authorities to provide the water points, refuse receptacles and washing facilities. As food is biological in nature, it is capable of supporting the growth of microorganisms and food borne diseases result from the ingestion of contaminated foods and food products .More than 250 different types of viruses, bacteria, parasites, toxins, metals, and prions are associated with food borne diseases in humans (Tambekar *et al*., 2008).

In terms of personal Hygiene, purchasing ready-to-eat foods and ingredients from street/market vendors poses a considerable risk to public health, especially due to the poor hygienic practices. In most cases, the vendors do not have adequate washing facilities, and some vendors started their duties without taking a proper bath. Some of the vendors sleep at the vending sites in order to protect their wares. Foods and ingredients are also subjected to repeated contamination from unwashed hands and the materials used for wrapping, such as leaves, old newspapers, and reusable polyethylene bags (Roberts *et al*., 2008).Foods that are cooked immediately prior to consumption are safer than those which have been cooked and stored at ambient temperature (Martins, 2006)has conducted a formative assessment on 200 street food vendors and 800 consumers in greater Jhannesburg investigating the socioeconomic background of vendors and their customers, as well as vendors' facilities and aspects relating to the quality and safety, including microbiological testing, of foods. The author found that street vendors did observe good hygienic practices in preparing, cooking and handling foods, even though they were not aware of the reasons for doing so. Additionally, food was not kept overnight (a potential opportunity for contamination) due to the lack of refrigeration facilities (Martins, 2000).

Mosupye and Von holy (1999) compared the microbiological quality and safety of street foods involving 51 ready to eat street foods, 18 dish water and 18 surface swab samples taken in Johannesburg to those sampled and tested in other countries. The authors concluded that the bacterial counts in Johannesburg were lower than that of other countries. Two studies conducted in India found that the microbial quality of street foods was equivalent to, if not better, than that of foods bought from hotels and restaurants (Bapat, 1992).

2.6. Microbial Quality of Street Foods

The contribution of the street food sector to socio-economic growth is considerable; therefore, the requirement of safety in this sector must be emphasized especially in developing countries. Street food consumption of a large population may increase the burden for public health. Many studies on the microbiological quality of street foods have identified high levels of coliforms and the presence of various pathogens such as *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Vibrio cholerae* (Cho *et al*., 2011). Moreover, street foods have been reported to be an appropriate medium for the transmission of antimicrobial-resistant pathogenic bacteria including *Salmonella* spp., E. coli, and *S. aureus* to people.

Bacterial pathogens pose a great challenge in street foods where they have the ability to grow rapidly from very low numbers in food (Tent, 1999). Furthermore, it is evident that there are many potential health risks associated with the initial contamination of raw foods with pathogenic bacteria as well as subsequent (cross)-contamination by vendors during preparation, improper handling and storage before vending. HACCP studies on street vended foods in many developing countries indicated that there is a high correlation between long holding times at ambient temperatures and high bacteria counts even when the food had been cooked at temperatures high enough to kill harmful vegetative forms of most bacteria (Bryan *et al*. ,1988).

In many developing countries, concern exists as street foods are also consumed by school going children who are at particular risk of food-borne diseases. For many years, vendors normally sell local street foods including light snacks and drinks with attractive and colorful food items near school-based locations under the poor hygienic conditions. Therefore, bacterial contamination of the local food and beverages sold by street food vendors surrounding the schools areas has been a common occurrence in developing countries, and the level of the contamination should be paid more attention by food safety authorities. A study in Dhaka, Bangladesh carried out to assess the

microbiological quality of the food items sold by the school-based street food vendors concluded that nearly half (44.5%) of the tested foods samples unsatisfactory and one third were unsuitable for consumption (Hanashiro *et al*. ,2005). In particular, the food samples were analyzed for coli form counts and followed the coli form criteria for foods for infants and children recommended by ICMSF. Other studies have reported high levels of coliforms in street foods. and also showed many contaminated factors such as contaminated water, unclean towels, dirty water for washing utensils, and cross contamination between raw and processed foods during transportation and storage.

2.6.1. Common pathogenic Microbials of street vended foods

2.6.1.1 *Salmonella*

According to Fàbrega and Vila(2013).**)***Salmonella* is a genus of rod-shaped (bacillus) Gram-negative bacteria of the family Enterobacteriaceae. The two species of Salmonella are Salmonella enterica and Salmonella bongori. S. enterica is the type species and is further divided into six subspecies that include over 2,600 serotypes. Salmonella species are non-spore-forming, predominantly motile enterobacteria with cell diameters between about 0.7 and 1.5 μm, lengths from 2 to 5 μm, and peritrichous flagella (all around the cell body). They are chemotrophs, obtaining their energy from oxidation and reduction reactions using organic sources. They are also facultative anaerobes.

In terms of Pathogenicity, *Salmonella* species are facultative intracellular pathogens. *Salmonella* can invade different cell types, including epithelial cells, M cells, macrophages, and dendritic cells. Most infections are due to ingestion of food contaminated by animal feces, or by human feces, such as by a food-service worker at a commercial eatery. Salmonella serotypes can be divided into two main groups, typhoidal and non typhoidal. Non typhoidal serotypes are more common, and usually cause self-limiting gastrointestinal disease. They can infect a range of animals, and are zoonotic, meaning they can be transferred between humans and other animals (LaRock *et al*., 2015).

2.6.1.2.Escherichia coli O157:H7

As reported by Fotadar *et al*., (2005)**,** *Escherichia coli* is a Gram-negative bacteria. Cells are typically rod-shaped, and are about 2.0 μm long and 0.25–1.0 μm in diameter, with a cell volume of 0.6–0.7 μm ^{3}.E. coli is a facultative anaerobe(that makes ATP by aerobic respiration if oxygenis present, but is capable of switching to fermentation or anaerobic respiration if oxygen is absent) and non sporulating bacterium.

The bacterium is commonly found as a commensal in the human microbiota. However, the plasticity of its genome has led to the evolution of this organism into pathogenic strains able to cause diseases and syndromes of public health importance in humans and animals. Pathogenic E. coli are mainly divided into two groups depending on the disease location: extra intestinal pathogenic E. coli and intestinal pathogenic *E. coli* **(**Croxenand Finlay, 2010).

Most *E. coli* strains do not cause disease, naturally living in the gut, but virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. In rare cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, sepsis, and Gram-negative pneumonia.

Very young children are more susceptible to develop severe illness, such as hemolytic uremicsyndrome; however, healthy individuals of all ages are at risk to the severe consequences that may arise as aresult of being infected with *E. coli***(**Lim *et al*., 2010)**.**

2.6.1.3 *Staphylococcus aureus*

According to Licitra (2013**)**,*Staphylococcus aureus* is a Gram-positive bacterium. Microscopically, *S. aureus* cells are appear in spherical shape. They are often in clusters resembling bunch of grapes when observed under light microscope after Gram staining. Thename '*Staphylococcus*' was derived from Greek, meaning bunch of grapes (staphyle) andberry (kokkos).It is causative agent of wide range ofinfectious diseases such as skin infections, bacteremia, endocarditis, pneumonia and food poisoning. The organism was

originally a leading nosocomial pathogen and after wards epidemiologically distinct clones emerged in community settings. *S. aureus* expresses number of virulence factors which help to establish infection by facilitating tissue attachment, tissue invasion and evading from host immune response *S. aureus* is a commensal and opportunistic pathogen. The anterior nares are the principalecological niche, where the organism colonizes in humans. The nasal carriage of *S. aureus*increases the risk of infection especially in the hospital settings. The average nasal carriage of S. aureus could be at 30% of human population (Kluytmans and Wertheim,2005).

2.6.1.4.*Pseudomonas*

According toMadigan and Martink(2005),*Pseudomonas* is a genus of Gram-negative, Gammaproteobacteria, belonging to the family Pseudomonadaceae and containing 191 validly described species. Their ease of culture in vitro and availability of an increasing number of Pseudomonas strain genome sequences has made the genus an excellent focus for scientific research; the best studied species include *P. aeruginosa* in its role as an opportunistic human pathogen, the plant pathogen *P. syringae*, the soil bacterium *P. putida*, and the plant growth-promoting *P. fluorescens*, *P. lini, P. migulae,* and *P. graminis*(Padda *et al*., 2019).

As reported by Hasset *et al*., (2002), All species and strains of Pseudomonas have historically been classified as strict aerobes. Exceptions to this classification have recently been discovered in Pseudomonas biofilms. A significant number of cells can produce exopolysaccharidesassociated with biofilm formation. Secretion of exopolysaccharides such as alginate makes it difficult for pseudomonads to be phagocytosed by mammalian white blood cells. Exopolysaccharide production also contributes to surface-colonising biofilms that are difficult to remove from food preparation surfaces. Growth of pseudomonads on spoiling foods can generate a "fruity" odor (Ryan and Ray, 2004).

In terms of Pathogenicity, Infectious species include *P. aeruginosa, P. oryzihabitans*, and *P.plecoglossicida. P. aeruginosa* flourishes in hospital environments, and is a particular problem in this environment, since it is the second-most common infection in hospitalized patients(nosocomial infections). This pathogenesis may in part be due tothe proteins secreted by *P. aeruginosa*. The bacterium possesses awide range of secretion systems, which export numerous proteins relevant to the pathogenesis of clinical strains (Hardie ,2009).

Most *Pseudomonas spp*. are naturally resistant to penicillin andthe majority of related beta-lactam antibiotics, but a number are sensitive to piperacillin, imipenem, ticarcillin, or ciprofloxacin. According to Ryan and Ray (2004), Aminoglycosides such as tobramycin, gentamicin, and amikacinare other choices for therapy. This ability to thrive in harsh conditions is a result of their hardy cell walls that contain porins. Their resistance to most antibiotics is attributed to efflux pumps, which pump out some antibiotics before they are able to act.*Pseudomonas aeruginosa* is increasingly recognized as anemerging opportunistic pathogen of clinical relevance. One of itsmost worrying characteristics is its low antibiotic susceptibility. According to Van Eldere(2003), This low susceptibility is attributable to a concerted action of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (Poole, 2004)and the low permeability of the bacterial cellular envelopes. Besides intrinsic resistance, *P. aeruginosa* easily develops acquired resistance either by mutation in chromosomally encoded genes or by the horizontal gene transfer of antibiotic resistance determinants. Development of multidrug resistance by *P. aeruginosa* isolates requires several different genetic events that include acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes.

2.6.1.5.Bacillus

According to Yarza *et al.* (2010*) Bacillus* (Latin "stick") is a genus of Gram-positive, rod-shaped bacteria, a member of the phylum Firmicutes, with 266 named species. The term is also used to describe the shape (rod) of certain bacteria; and the plural Bacilli is the name of the class of bacteriato which this genus belongs. *Bacillus species* can be eitherobligate aerobes: oxygen dependent; or facultative anaerobes: having the ability to be anaerobic in the absence of oxygen. Cultured Bacillus species test positive for the enzyme catalase if oxygen has been used or is present.

Because the spores of many *Bacillus species* are resistant to heat, radiation, disinfectants, and desiccation, they are difficult to eliminate from medical and pharmaceutical materials and are a frequent cause of contamination. *Bacillus species* are well known in the food industries as troublesome spoilage organisms(Xu and Cote, 2003). Ubiquitous in nature, Bacillus includes both free-living (non parasitic) species, and two parasitic pathogenic species. These two Bacillus

species are medically significant: *B. anthracis* causes anthrax; and *B. cereus* causes food poisoning.

Under the microscope, the Bacillus cells appear as rods, and a substantial portion of the cells usually containoval endospores at one end, making them bulge.Two Bacillus species are medically significant: B. anthracis, which causes anthrax; and B.cereus, which causes food poisoning, with symptoms similar to that caused byStaphylococcus. B. cereus produces toxins which cause 2 different set of symptomsemetic toxin which can cause vomiting and nauseadiarrhea (Ryan and Ray,2004).

2.6.1.6*Micrococcus*

Micrococcus is a genus of bacteria in the *Micrococcaceae* family. Micrococcus occurs in a wide range of environments, including water, dust, and soil. *Micrococc*i have Gram-positive spherical cells ranging from about 0.5 to 3 micrometers in diameter and typically appear in tetrads. They are catalase positive, oxidase positive, indole negative and citrate negative.Some species of *Micrococcus*, such as *M. luteus* (yellow) and *M.roseus* (red) produce yellow or pink colonies when grown onmannitol salt agar (Doddamani and Ninnekar, 2001).

According to Yarza *et al.* (2010*)* , In its Pathogenicity, Micrococcus is generally thought to be a saprotrophic or commensal organism, though it can be an opportunistic pathogen, particularly in hosts with compromised immune systems, such as HIV patients. It can be difficult to identify *Micrococcus* as the cause of an infection, since the organism is a normally present in skin microflora, and the genus is seldom linked to disease. In rare cases, death of immune compromised patients has occurred from pulmonary infections caused by *Micrococcus*. *Micrococcus* may be involved in other infections, including recurrent bacteremia, septic shock, septic arthritis, endocarditis, meningitis, and cavitating pneumonia (immune suppressed patients).

2.6.1.7. *Moulds*

As reported by Madigan and Martinko (2005) , a mold (US) or mould (UK) is a fungus that grows in the form of multicellular filaments called hyphae. In contrast, fungi that can adopt a single-celled growth habit are called yeasts. Molds are a large and taxonomically diverse number of fungal species in which the growth of hyphae results in discoloration and a fuzzy appearance,

especially on food. The network of these tubular branching hyphae, called a mycelium, is considered a single organism. Molds are considered to be microbes and do not form a specific taxonomic or phylogenetic grouping, but can be found in the divisions Zygomycota and Ascomycota (Hibbett *et al.,* 2007)..

Some diseases of animals and humans can be caused by certain molds: disease may result from allergic sensitivity to mold spores, from growth of pathogenic molds within the body, or from the effects of ingested or inhaled toxic compounds (mycotoxins) produced by molds(Moore *et al.*,2011)). According to Wareing (2013) Molds can also grow on stored food for animals and humans, making the food unpalatable or toxic and are thus a major source of food losses and illness.

Few molds can begin growing at temperatures of 4 °C (39 °F) or below, so food is typically refrigerated at this temperature. When conditions do not enable growth to take place, molds may remainalive in a dormant state depending on the species, within a large range of temperatures. The many different mold species vary enormously in their tolerance to temperature and humidity extremes. Certain molds can survive harsh conditions such as the snow-Mold on dried Hibiscus sabdariffa Hyphae growing from tomato sauce. Spores from green mold growing on an orange, 1000× wet mount covered soils of Antarctica, refrigeration, highly acidic solvents, anti-bacterial soap and even petroleum products such as jet fuel (Malloch,1981).

Molds can also pose a hazard to human and animal health when they are consumed following the growth of certain mold species in stored food. Some species produce toxic secondary metabolites, collectively termedmycotoxins including aflatoxins, ochratoxins, fumonisins, trichothecenes, citrinin, and patulin. These toxicproperties may be used for the benefit of humans when the toxicity is directed against other organisms; forexample, penicillin adversely affects the growth of Gram-positive bacteria (e.g. Clostridium species), certainspirochetes and certain fungi(Fairey *et al*..,2019).

2.6.1.8. Yeasts

According to Pitt and Hocking(2009),Spoilage fungi, yeasts and moulds can grow on raw and processed foods where the environmental conditions for most bacteria are un favorable (low pH, low water activity, a_w). The nutrients and oxygen available in the food are the main factors determining the kind of fungal spoilage.

In cereals, flours and bakery products ochratoxin A, aflatoxin and the Fusarium toxins: deoxynivalenol (DON), zearalenone and fumonisins can be found . Yeast and mould spoilage results in considerable loss in food supply and enhances food safety problems (Nielsen and Rios ,2000).In yeasts, it has been shown recently that the mechanisms of adhesion, bio film formation, and flocculation have much in common (Verstrepen and Klis, 2006). Each phenomenon is conferred by specific cell surface proteins (adhesins or flocculins), which share a common basic structure but differ between species and strains of the same species. Adhesions are induced by various environmental triggers and regulated by several signaling pathways. In all, adhesionand flocculation are responses of cells to stress factors and allow yeasts to adapt to the changing environment.

In the food processing context, biofilms offer greater resistance to cells, are not easily removed from surfaces by the normal cleaning and sanitizing procedures, and could hence be a continuous source of contamination (Joseph *et al*., 2001). It has been shown, however, that potentially spoiling populations occurred only in 4% of the samples of biofilms formed in breweries (Timke *et al.*, 2005).Few yeast species can be considered genuine pathogens to humans; the most important of theseare *C. albicans*, *Cry. neoformans*, and *Malassezia furfur*.

A number of commensal yeasts can be isolated from the mouth, fingernail, and toenail of healthy hosts, the majority of them belonging to *C. albicans* and *C. parapsilosis* (Kam and Xu, 2002). However, from the food safety point of view, yeasts can be considered harmless organisms in the future too, causing neither food infection nor food poisoning, notwithstanding the sporadic reports that appeared to incriminate some yeasts in diarrhea or allergic responses (Fleet and Balia, 2006).

2.7. Guidelines for the microbial quality of ready-to-eat foods

According to Gilbert *et al*.(2000) the quality and safety of street vended foods for ready to eat food can be categorized based on expected aerobic colony counts, according to the type of food product and the processing it has received. There are four grades to express ready to eat food quality. Satisfactory, when the test results indicating good microbial quality; Acceptable when an index reflecting a border line limit of microbial quality and unsatisfactory when test results

indicating that further sampling may be necessary and that environmental health officers may wish to undertaken a further inspection of the premises concerned to determine whether hygiene practices for food production or handling are adequate or not. Unacceptable /potentially hazardous when test results indicating that urgent attention is needed to locate the source of the problem, a detailed risk assessment is recommended.

The standard plate count also referred to as the aerobic plate count or the total plate count , is one of the most common tests applied to indicate the microbiological quality of food. Three levels of standard plate count are listed in Table 1based on food type and the processing or handling the food has undergone. Level 1:applies to ready-to-eat foods in which all components of the food have been cooked in the manufacturing process or preparation of the final food product and as such microbial counts should be low. Level two: applies to ready-to-eat foods that contain some components that have been cooked and level 3: applicable which applied to foods such as fresh fruits and vegetables.

Table 1. Guideline for determining the microbiological quality of ready –to-eat foods(Gibert *et al*.,2000)

Test	microbial quality (CFUg-[1)]			
	Satisfactory	Acceptable	Unsatisfactory	Unacceptable
Standard plate count				
Level 1	$<10^3$	10^3-$<10^4$	$\geq 10^4$	N/A
Level 2	$<10^4$	$<10^4$ -$<10^5$	$\geq 10^5$	N/A
Level 3	$<10^5$	10^5-$<10^6$	$\geq 10^6$	N/A
Enterobacteriaceae	$<10^2$	10^2- $<10^4$	$\geq 10^4$	N/A
Salmonella spp.	Not detected in 25g			detected in 25g
S.aureus	<20	20-10^2	10^2-$<10^4$	≥ 104
Bacillus cereus and other pathogenic spp.	$<10^3$	10^3-$<10^4$	10^4 -$<10^5$	$\geq 10^5$

Where ; N/A- not applicable

2.8. Street vended foods and public health problems

Pathogens indicators of microorganisms and high numbers of aerobic microorganisms have been isolated from street vended foods. Evidently, therefore, street foods pose a serious danger to public health in general. These are due to lack of basic infrastructure and services, such as potable water supply, difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature, insufficient resources for inspection and laboratory analysis, general lack of factual knowledge about the microbiological status or the precise epidemiological significance of many street vended food.

Rane (2011) reported , vendors' location, utensils and personal hygiene are the most common sources of public health problems(Table 2)

Table 2. Source, type of hazard and microbial risk involved in street foods**.**

Source	Hazard	Risk involved
Vendors' location	improper food handlings	Transfer of pathogens like *Salmonella, E.coli* and *S.aureus* from human body and environment in to foods.
	Improper waste disposal	Transmission of enteric pathogens *Salmonella, Shigella* and *E.coli* via vectors.
Utensils and equipment	Chemical contaminants	Leaching of chemical leading to poisoning cross contamination of food with *Shigella.*
	Microbial contaminants	*E.coli* and *S.aureus* due to contaminated water and handlers
Storage and reheating	Improper storage temperature And reheating food	Likelihood of heat stable toxins. produced by *Bacillus* spp.
Personal hygiene of vendors	Biological hazards	Introduction of *Salmonella*, *Shigella* and *streptococcus* via vectors.

(Source : Rane,2011)

2.9. Benefits of street foods

According to Nago *et al*., (2010), globalization is affecting food systems around the world by means of urbanization, increasing incomes, foreign investment and market liberalization. Due to rapid urbanization taking place in many developing countries, street foods have become increasingly important as an income-generating strategy and as a fast and economical meal

option. Since entry into the field is largely unregulated and does not cost much upfront investment, it has become an increasingly popular way for families, and specifically women, to earn a living. street foods have been sold for numerous decades and provide a source of income to many families. street foods contributed significantly to the diet of children and adults in developing countries, both in terms of energy, protein and micronutrient intakes and in terms of food groups consumed (Namugumya ,B and Muyanja, C(2012).

3. Materials and Methods

3.1. Description of the Study Area

Hagereselam town is located in South West direction 50km far from Mekelle city, the capital city of Tigray Regional state, Ethiopia (Figure 1. Map of the study site).It is located at13° 40’ 0" N latitude and 39° 10’ 0" E longitude. It is found 1,500-2,750m above sea level and has high land of 43.75% ، moderate 37.5% and lowland 18.75%.The temperature of the town is between 15-18^{o}c. It receives 600-800 mm annually. The community of Hagereselam is involving intrade and other related businesses. The total population of the town is 12,601 and out of the total population, 6513 are males and 6088 are females. Street food vendors in the town around main road groceries, taxi terminals and bus station areas are common practices. In the study area, there were 96 street food vendors. The vending environment seemed dirty in the streets. The town has three primary schools, two secondary schools, one preparatory school and one TVET college. Major activities in the town were construction and trade. In Hagereselam town, there are street vended foods such as Bonbolino, Sambusa, Sandwich, Injera and Bread. Many people are consuming during morning and in the evening (Source: Hagereselam town administration office, 2012 E.C).

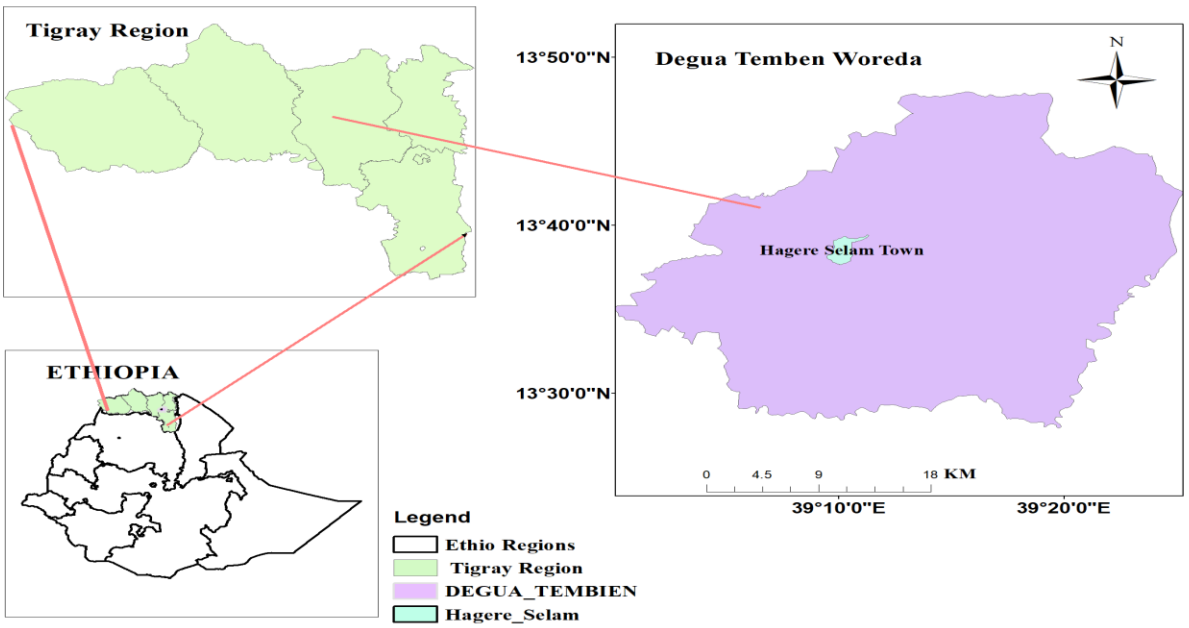

Fig. 1.Map of the study area (source:Hagereselam town administration office, 2012 E.C)

3.2. Study Design and Period

A community based cross sectional and experimental study designs was under taken.This study was conducted between the months of August,2019 to October, 2019.

3.3. Sample Size determination Techniques

The total population of the three sites who engaged in street vending foods was 96 irrespective of their sex and age. Street food vendors of Bonbolino were 44. Sambussa vendors were 24, while Lentil sandwich vendors were 28.Purposive sampling method was used to select samples from the study area since the sample size is small and manageable. So, all the 96 street food vendors were taken as sample size.

3.4. Data collection

Data were collected using pre-tested questionnaire and observation for safety of the foods which are vended in the vending areas. Accordingly, information on sanitary practices, storage, transportation, level of education of vendors and other relevant information were collected. The questionnaires were both self administered and collected by the researcher and data collector for those who can't read and write(Appendix-I).

For microbiological quality, standard microbiological techniques focusing on morphological and biochemical tests were used. Samples were collected and then transported to microbiological laboratory of Mekelle University by covering sterile aluminum foil. All samples were transported within 1-3 hours to Mekelle University, Microbiology laboratory of Veterinary College. Samples were stored in refrigerator until microbiological analysis. Samples were examined for enumeration of total fecal coli- forms.

3.5. Microbiological Methods

3.5.1 Method of Sample collection

A total of 60 commonly used ready-to-eat food samples (200g each) comprising of 20 samples each of bonbolino, sambussa and Lentil sandwich were collected from the three vending sites of Hagereselam town namely Alula, Lsanu and bus station between the months of August,2019 to October, 2019 using simple random sampling technique. In terms of site, bonbolino vendors were 18 from bus station, 15 from Alula and 11 from Lsanu. Sambussa vendors were 12 from bus station, 8 from Alula and 4 from Lsanu. Sandwich vendors were 14 from bus station, 9 from Alula and 5 from Lsanu. the lists of food vendors was taken from town organization then gave number for each vendor from 1-96. Finally to select 60 samples from 96 lottery method was

used. Food samples were collected from vendors using vendors own serving utensils and placed in to sterilized aluminum plates. All samples were transported within 1-3 hours to Mekelle University, Microbiology laboratory of Veterinary College. Samples were stored in refrigerator until microbiological analysis.

3.5.2 Sample preparation

Twenty five grams of food sample was taken and mixed with 225 ml buffered peptone water (BPW), homogenized in a flask for five minutes using a shaker at 160 rpm. After homogenization, one ml of each food sample was aseptically transferred in to 9 ml of BPW and mixed thoroughly by using vortex mixer. The homogenates were serially diluted from 10^{-1} to 10^{-6} and using a pipette and then a volume of 0.1ml aliquot from appropriate dilution factor was spread-plated on pre-solidified plates and incubated at appropriate temperature and time for enumerationof different microbial groups including aerobic mesophilic bacteria, Enterobacteriaceae, fecal Coliforms, Lactic acid bacteria, *Staphylococci*, Yeasts and Moulds. The colonies were counted from plate containing microbial colonies between 30 and 300. The counted colonies were expressed in colony forming units per gram($CFUg^{-1}$) and later converted to log $CFUg^{-1}$.

3.5.3. Microbial enumeration

3.5.3.1. *Enterobacteriaceae count*

From the serial dilutions, 0.1 ml of the aliquot was spread-plated on MacConkey agar (Oxoid) medium and incubated at 32^{o}C for 18 - 24 hrs after which, pink to red purple colonies were counted as member of the family *Enterobacteriaceae*(Spencer *et al*., 2007).

3.5.3.2. *Fecal Coli form count*

From the serial dilutions, 0.1 ml of the aliquot was spread plated on pre-solidified surfaces of Violet Red Bile Agar (VRBA) (Oxoid) plates. Then the plates were incubated at 32^{o}C for 18-24 hrs. After this, purplish red colonies surrounded by reddish zone of precipitated bile were counted as *coliforms* (Weil *et al*.,2006).

3.5.3.3. *Staphylococci* count

From the serial dilutions, 0.1 ml of the aliquot was spread plated on to Mannitol Salt Agar (MSA) (Oxoid) supplemented with 7.5% NaCl and incubated at 37^{o}C for 36 hrs. Golden yellow colonies were presumptive for *S.aureus* (Acco *et al*., 2003)**.**

3.5.3.4. *Lactic acid bacteria count*

From the serial dilutions, 0.1 ml of the aliquot was spreadplated on de Mann Rogosa Sharpe (MRS) agar media (Oxoid)and incubated at 37°C for 48 h under anaerobic condition using anaerobic Jar (Patra *et al*., 2011).

3.5.3.5. *Yeasts and moulds counts*

From the serial dilutions, 0.1 ml aliquot was spread-plated on pre-solidified surfaces of PotatoDextrose Agar (Oxoid) supplemented with 0.1 g chloramphenicol and incubated at 25^{o}C for 3-5 days (Spencer *et al*., 2007). Smooth(non-hairy) colonies without extension at periphery will be counted asyeasts whereas hairy colonies with extension at periphery will be counted as moulds.

3.5.3.6. *Aerobic Mesophilic bacterial count*

From appropriate dilutions, 0.1 ml of the aliquot was spread plated on plate count Agar(PCA) (oxoid) and the plates could be incubated at 32^{o}C for 48 hours (Weil *et al.,* 2006).

3.6. Microbial Analysis and characterization

After enumeration of *Aerobic mesophilic bacteria*, 10 to 15 colonies with distinct morphological differences such as color, size and shape were randomly picked from countable plates and aseptically transferred into a tube containing 5 ml nutrient broth (Oxoid). The inoculated cultures were incubated at 32^{o}C for 24 hours. Cultures were purified by repeated plating and preserved on slants at 4^{o}C for a month. Finally, the obtained organisms were characterized to genus and family levels. The characterizations of isolates were done based on John (2012) 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).

3.7. Morphological Characteristics

3.7.1. Cell Morphology

In order to assess the cell morphology of the pure culture, gram staining and motility test were used. The morphological study includes cell shape and cell arrangement.

3.7.1.1. Gram staining

After culturing of the homogenate at 37^{o}C for 24 hours, a smear of pure isolates was prepared on a clean slide and allowed to air- dry and heat-fix. The heat fixed smear was flooded with crystal violet dye for one minute and rinsed under tap water for three seconds. Then, the slide was flooded with iodine solution for one minute and rinsed under tap water for three seconds. After rinsing, the smear was decolorized with 96% of ethanol for 20 seconds and washed slide gently under tap water for three seconds. Thereafter, the smear was counterstained by safranin and dried by absorbent paper. Finally, the air-dried smear was observed under oil immersion objective. At the completion of the gram staining , gram negative bacteria were stained pink/red and gram positive bacteria were stained blue or purple(Gram,1884).

3.7.1.2. Motility Test

A motility medium was prepared using a test tube. A purified broth culture was taken by sterile needle and stabbed straight vertically in to a test tube containing motility medium to the bottom of the tube and incubated at 35^{o}C for 24 hrs. A positive motility test was indicated by a red turbid area diffusing away from the line of inoculation and a negative test was indicated by red growth along the inoculation line only but no further (Shields and cathcart, 2012).

3.7.1.3. Endospore test

Heat fixed smear taken from aerobic mesophilic bacteria was flooded with 0.5% (w/v) malachite green solution and steamed using cotton dipped in 96% ethanol for 5 minutes. After cooling, the slide was washed with tap water and counterstained with safranin for 30 seconds. The slide was washed with tap water and air-dried /blotted to be observed under the oil immersion lens(x1000) to check the presence of endospores.

3.7.2. Biochemical tests

3.7.2.1. Catalase test

Catalase test was carried out after young colonies flooded with a 3% solution of H_2O_2.The formation of bubbles indicated the presence of catalase (McFadden, 1980).

3.7.2.2. KOH test

Two drops of 3% KOH solution was placed with a clean microscopic slide. A colony was aseptically picked from the surface of nutrient agar using an inoculating loop and was stirred in the KOH solution for ten seconds to two minutes. The inoculating loop was raised slowly from

the mass when the KOH solution become viscous, the thread of slime followed the loop for 0.5 to 2 cm or more in Gram negative bacteria. In case of no slime and a watery suspension did not follow the loop, the reaction was considered negative and the isolate was considered as gram positive bacteria (Gregerson, 1978).

3.8. *Isolation of Salmonella species*

For the detection of *Salmonella spp*, 25 g of food samples were mixed with 225 ml of BPW and was incubate at 37 ^{o} C for 24 h. Then 1ml pre-enrichment broth culture was added to 10 ml of selenite cysteine broth (Oxoid) in order to prevent or eliminate growth of other microbes and again incubated at 37^{o}C for 24 hours. Thereafter, a loopful of suspension from a tube was streaked onto Xylose Lysine Deoxycholate Agar (XLD) (Oxoid). The presumptive Salmonella colonies (black colony surrounded by red color) were picked off.

Sulfide indole motility (SIM) Medium (oxoid): The SIM medium was stabbed and incubated at 37^{o}C for 24 hours for the determination of H_2S production, motility and indole production. Production of indole was investigated by adding Kovac's reagent (HCl,250ml;amyl alcohol ,750ml;and paradimethylamino-benzaldehyde 50g/ml) to growth in this culture medium. The non-utilization of indole and absence of deep red color at the surface of agar was considered as presumptive for *Salmonella species*.

Triple sugar iron Agar(Oxoid): The butt was stabbed and the slant was streaked and incubated at 37^{o}c for 24 hours to detect fermentation of glucose, sucrose and lactose as well as production of H_2S. The presence of alkaline (red) slant and acid (yellow) butt , with or without production of H_2S was considered as presumptive for *Salmonella spp.*

Simmons citrate agar (oxoid): The slant was streaked and the tube was incubated at 37^{o}c for 24 hours to determine citrate utilization as a sole source of carbon. The presence of growth and color change from green to blue was considered as *Salmonella spp.*

3.9. Isolation of *Staphylococcus aureus*

After counting *Staphylococci,* golden yellow colonies on MSA plates were aseptically picked and transferred into 5 ml nutrient broth and incubated at 37^{o}C for 24 hours for further purification. Then, a loop full of culture from the nutrient broth was streaked on nutrient agar

supplemented with 7.5% NaCl and again incubated at 37ºC for 24 hours. Finally, the distinct colonies were characterized using the established microbiological methods(Acco *et al*., 2003).

Coagulase test: It was done using slide test and tube test procedures (Cheesbrough,2006). In slide test, a colony of the purified isolates was emulsified in a drop of distilled water on two ends of clean slide to make thick suspensions. One was labeled as test and the other was as control. A loop full of human blood plasma was added to one of the suspensions and mixed gently. Clumping within 10 seconds was observed for Coagulase positive organisms. On the other hand, coagulase test was done using tube test. Accordingly, three test tubes were taken and labeled as test , negative control and positive control. Each tube was filled with 0.5 ml of 1 in 10 diluted human's plasma. To the tube labeled test, 0.1ml of overnight broth culture of test organisms of *S.aureus* (ATCC 25923) were added. To the tube labeled positive control, 0.1ml of overnight broth culture of known *S. aureus* was added and to the tube labeled negative control , 0.1ml of sterile broth was added. All the tubes were incubated at 37ºC and observed up to four hours. Positive result was indicated by gelling of the plasma, which remains in place even after inverting the tube.

3.10. Antibiotics susceptibility testing for some pathogens

Antibiotics susceptibility testing for pathogens isolated from street vended foods was performed using the disk diffusion method and the results were interpreted as per the criteria of the National Committee for Clinical Laboratory Standards (NCCLS, 2007). A standardized suspension of the bacterial isolates was prepared and the turbidity of the inoculums was matched with the turbidity standard 0.5 Mac Farland in order to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize antibiotics susceptibility testing by measuring of minimum inhibitory concentration (Bauer *et al.,* 1966).

Mac Farland is a Barium sulphate standard against which the turbidity of the test and control inoculums were compared. This standard was prepared by mixing two solutions; solution A and solution B. Solution A was 1% v/v solution of sulphuric acid (H_2SO_4) and solution B was 1% w/v solution of Barium chloride ($BaCl_2$).To get 0.5 Mac Farland standard, concentration equivalents to cell density of about 10^7-10^8 CFU/g , an amount of 99.5ml H_2SO_4 of 1% solution B. A small volume of the turbid solution was transferred to a screw –cap bottle of the same types as used for preparing test and control inoculums. Culture containing test tube with

approximately equal concentration or density with 0.5 Mac Farland standards was used for inoculation of medium. The standard was used after shaking immediately before use and stored in a well sealed container in a dark place at room temperature (20-28^{o}c) when not used. When it matched with the standard, the inocula were confluent growth. Then, the standardized suspension was swabbed by cotton swab onto the Muller-Hinton Agar (Oxoid) and allowed to dry. Thereafter, the antibiotic discs were placed using forceps on the medium and incubated at 37^{o}C for 18 hours and the zone of inhibition was measured manually with a transparent ruler.

The following standardized drug discs (Oxoid) and their potency (µg/ml) were used depending up on the antibiotics spectrum,toxicity, effectiveness and availability (Vlkova *et al.*,2006). As a result , Chloramphenicol (Chl), (30µg/ml); Gentamycin (Gen), (10µg/ml); Ciproflaxin (Cip), (5µg/ml); Kanamycin (Kan),((30µg/ml) were used. Because these drugs are recommended for testing food borne pathogens. Interpretation of readings as sensitive, intermediate or resistant was made according to National Committee for Clinical Laboratory Standards (NCCLS, 2007).The reference strains were *Salmonella Typhimurium*(ATCC13311) , *Staphylococcus aureus* (ATCC 25923) and *E.coli* (ATCC25922).

3.11. Data Analysis

The percentage of coefficient of variation (%CV) was calculated to see if there is significant variation in counts within the food samples analyzed. The data obtained from the respondents were analyzed using SPSS software version 20.0. Mean counts of microbesof food samples from different sites were compared using one way ANOVA and the significance of differences were considered at 95% confidence interval ($P<0.05$).The microbial counts were normalized by transformation to log10 CFU/ml. in order to establish statistically significant microbial loads in different street vended food samples collected from the study sites.

4. Results and Discussion

4.1. Results

4.1.1. Socio-demographic characteristics of the street food vendors

The socio-demographic characteristics of street vendors showed that the majority,69 (71.9%) of the vendors were females and 50(52.1%) of street vendors' belonged to the age group between 21-30 years. Educationally, 54(56.3%) had primary education. Among the vendors 51(53.5%) had an experience of 6-10 years, 31(32.5%) < 5 years and 10(10.4%) had 11-15 years'of experience in street food vending (Table 3).

Table 3**.**Socio-demographic characteristics of street vendors in Hagereselam town, Northern Ethiopia 2019/2020.

Parameter	No of respondents	percentage (%)
Sex		

Male 27	28.1	
Female	69	71.9
Total	96	100
Age		
<20	32	33.3
21-30	50	52.1
31-40	13	13.5
41-50	1	1.0
>51	0	-
Level of education		
Illiterate	10	10.4
Primary school	54	56.3
Secondary school	32	33.3
Experience in vending food (years)		
<5 years	31	32.5
6-10 years	51	53.5
11-15 years	10	10.4
16-20 years	4	4.2
>20 years	0	0

4.1.2. General hygiene of street food vendors and the street vended foods

More than half (57.3%) of the street food vendors usedtap water for preparation of foods while 27.1% used well water. On the other hand, 59(61.5%) of street vendors usedwell water for cleaning utensils. In addition, 51(53.1%) of the vendors cleaned the utensils using dirty water and soap only (Table 4).

In terms of hygiene, 74(77.1%) of the street food vendors handled food with their bare hands. Training-wise, 96 (100%) of the vendors didnot get training on food hygiene. A significant number 66 (68.7%) of the vendors had no information about foodand water-borne diseases whereas 30(31.3%) had information about foodborne and waterborne diseases such as diarrhea and giardia (Table 4).

Table 4.Source of water and Utensils handling by street vendors.

Parameters	No of respondents(n=96)	Percentage(%)
Source of water for preparation of food		
Tap	55	57.3
Well	26	27.1
Spring	15	15.6
Source of water or cleaning utensils		
Tap	22	22.9
well	59	61.5
River	15	15.6
Clean the utensils		
By hands using only water	35	36.5
With warm water and soap	10	10.4
With cold dirty water and soap	51	53.1
Has special cloth for vending		
Yes	-	-
No	96	100
Handling food with bare hands		
Yes	74	77.1
No	22	22.9
Vendors cover their hair		
Yes	-	-
No	96	100
Get training about vending food		
Yes	-	-
No	96	100
Having information about food and water borne disease		
Yes	30	31.3
No	66	68.7

Storage of foods		
Openly in the stalls	57	59.4
In a wheelbarrow	18	18.8
In sealed (transparent or opaque) containers	21	21.8
Maximum days the food stays before selling and/or consumption		
1-2days	83	86.5
3-4days	13	13.5
More than four days	-	-
Materials used to serve food for consumers		
paper	12	12.5
plastic bag	1	1.0
fork/spoon	9	9.4
bare hands	74	77.1

4.1.3. sites observation

The area around food vending and preparing had open and bad smelling drainage system. The water for washing and rinsing the utensils was observed dirty (Figure 2).Based on observation, about 85% of the vendors interviewed prepare their foods in unhygienic conditions given that garbage and dirty waste were obviously close to the stalls (Figure 2). Of the vendors interviewed, 92.5% did not have garbage containers; hence they disposed their garbage just near the stalls. Seventy five percent of vendors threw waste water just beside the stalls making the environment surrounding the eateries somewhat dirty.

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Fig.2.Street food vending area, food preparation practices and utensils used.

4.1.4. Microbial count

The mean count of aerobic mesophilic bacteria (AMB) was the highest (6.5 log CFU/g) in sambussa followed by lentil sandwich (5.2 log CFU/g) whereas the lowest was in bonbolino (4.3 log CFU/g). The mean count of enterobacteriaceae was the highest in sambussa (6.3 log CFU/g) where as it was lower in lentil sandwich (3.0 log CFU/g) and bonbolino (2.7 log CFU/g) (Table 5). Likewise, the mean count of coli form and mould were the highest in sambussa and bonbolino (6.3 and 3.7 CFU/g respectively) and the mean count of lactic acid bacteria was low in sambussa(3.2 log CFU/g) . Furthermore the mean count of *staphylococci* were the highest (4.4 log CFU/g) in bonbolino followed by lentil sandwich(3.1 log CFU/g). (Table 5).However, it was relatively lower in sambussa (2.7 log CFU/g) (Table 5). The difference could occur due to the difference of the sites in PH, water activity of the street foods and awareness variation among vendors.

Table 5.Mean microbial counts (log CFUg-^{1}).

Food type	Sample size	Mean microbial counts (log CFU/g ±SD)																	
		AMB	**%CV**	**Entero**	**%CV**	**Coli**	**%CV**	**ABS**	**%CV**	**Staph**	**%CV**	**LAB**	**%CV**	**Yeast**	**%CV**	**Mould**	**%CV**
Bonbolino	20	4.3±0.9	20.9	2.7±1.2	44.4	3.4±0.5	14.7	4.7±1.1	23.4	4.4±0.8	18.2	4.2±0.5	11.9	2.2±0.6	27.3	3.7±0.4	10.8
Lentil sandwich	20	5.2±1.1	21.2	3.0±0.4	13.3	1.7±0.5	29.4	3.5±0.3	8.6	3.1±0.6	19.4	5.1±0.4	7.8	3.2±0.6	18.8	2.4±0.5	20.8
Sambussa	20	6.5±1.5	23.1	6.3±0.4	6.3	6.3±0.5	7.9	4.3±0.6	14	2.7±0.5	18.5	3.2±0.6	18.8	4.7±0.4	8.5	1.8±0.6	33.3
Total	60	5.3±1.2	21.7	4±0.7	21.3	3.8±0.5	17.3	4.2±0.7	15.3	3.4±0.6	18.7	4.2±0.5	12.8	3.4±0.5	18.2	2.6±0.5	21.6

AMB-Aerobic Mesophilic Bacteria ; Entero- Enterobacteriaceae ; Coli- Coliform

ABS- Aerobic bacterial spore ; Staph - Staphylococci ; LAB- Lactic acid bacteria

4.1.5. Morphological and Biochemical test results

Based on gram reaction, out of 204 isolates, 37 (18.1 %) were gram negative whereas 167(81.9%) were gram positive, and majority (50%) of the isolates were rod in shape (Table 6*).* All of the 204 isolates were identified to genus level based on morphological and biochemical characters (Table 6). Out of 204 isolates, 31.9% were *Bacillus spp*, 26.9% were *Staphylococcus spp*., 23% were *Micrococcus spp.*respectively (Table 6). They dominate because the vending environment was dirty and became favorable for their growth. To suggest the genus both morphological and biochemical and john manual of bacterial classificationwere used(Appendix III).

Table 6. Morphological and biochemical test results of microbes obtained from the selected street-vended foodsamples.

Food type	Sample Code	No of Isolates	Morphological test results				Biochemical test results		Suggested genus
			Cell shape	Endospore Test	Motility test	Gram staining	KOH test	Catalase test	
Bonbolino	MUB_1-MUB_{20}	30	Cocci (cluster)	-	-	G+ve	-	+	*Micrococcus*
	MUB_2-MUB_{20}	14	Cocci(cluster)	-	-	G+ve	-	+	*Staphylococcus*
	MUB_3-MUB_{20}	13	Rod	-	-	G-ve	+	+	*Acinetobacter*
	MUB_4-MUB_{20}	12	Rod	-	+/-	G-ve	+	+	*Pseudomonas*
	MUB_5-MUB_{20}	13	Rod	+	+/-	G +ve	-	+	*Bacillus*
	MUB_6 - MUB_{20}	4	Rod	-	+	G-ve	+	+	*Salmonella*
	MUB_{18} - MUB_{20}	1	Rod	-	+	G-ve	+	+	*Escherichia coli*
Sambussa	MUS_{21}-MUS_{40}	36	Rod	+	+/-	G +ve	-	+	*Bacillus*
	MUS_{22} – MUS_{40}	13	Cocci(cluster)	-	-	G+ve	-	+	*Staphylococcus*
	MUS_{23} –MUS_{40}	10	Cocci (cluster)	-	-	G+ve	-	+	*Micrococcus*
	MUS_{24} –MUS_{40}	1	Rod	-	+/-	G-ve	+	+	*Pseudomonas*
	MUS_{28} – MUS_{40}	2	Rod	-	+	G-ve	+	+	*Escherichia coli*
Sandwich	$MUSA_{41}$- $MUSA_{60}$	28	Cocci(cluster)	-	-	G+ve	-	+	*Staphylococcus*
	$MUSA_{43}$ - $MUSA_{60}$	16	Rod	+	+/-	G +ve	-	+	*Bacillus*
	$MUSA_{47}$ –$MUSA_{60}$	3	Rod	-	-	G-ve	+	+	*Acinetobacter*
	$MUSA_{51}$-$MUSA_{60}$	7	Cocci (cluster)	-	-	G+ve	-	+	*Micrococcus*
	$MUSA_{55}$- $MUSA_{60}$	1	Rod	-	+	G-ve	+	+	*Escherichia coli*

Where: **MU**- Mekelle University **B**- Bonbolino **S**- Sambussa **SA**- Sandwich

4.1.6. Prevalence of Pathogenic bacteria

The overall 43.3% samples were positive for *S. aureus*(Table 7). However, the frequency distribution varied among the food samples. The highest (60%) being found in sambussa and the lowest being found in sandwich (30%). With regard to sites, the prevalence of *S.aureus* was comparably higher in sambussa from bus station, Alula and Lsanu sites. No *S. aureus* was detected in bonbolino from Alula and Lsanu sites and in Lentil sandwich from Alula site. This was due to differences in ecology of the sites. Temperature, PH, water activity and storage practice varied then variation of *S.aureus* happened. For all, only 6.7% samples were positive for *Salmonella* isolates. As a result the prevalence of *Salmonella* species was higher (10%) in sambussa and low in bonbolino and sandwich (5%).

In terms of site, the prevalence of *Salmonella* species was the highest in sambussa from bus station. However, *Salmonella species* were not isolated from Alula and Lsanu sites from bonbolino, sambussa and sandwich food samples (Table 7).

Table 7.Prevalence of pathogenic bacteria from street-vended foods

Food Sample	Sample size	No of *S. aureus* positive	Frequency (%)	No of *Salmonella* positive	Frequency (%)	No of *E.coli* positive	Frequency (%)
Bonbolino	20	8	40	1	5	8	40
Sambussa	20	12	60	2	10	7	35
Sandwich	20	6	30	1	5	5	25
Total	60	26	43.3	4	6.7	20	33.3

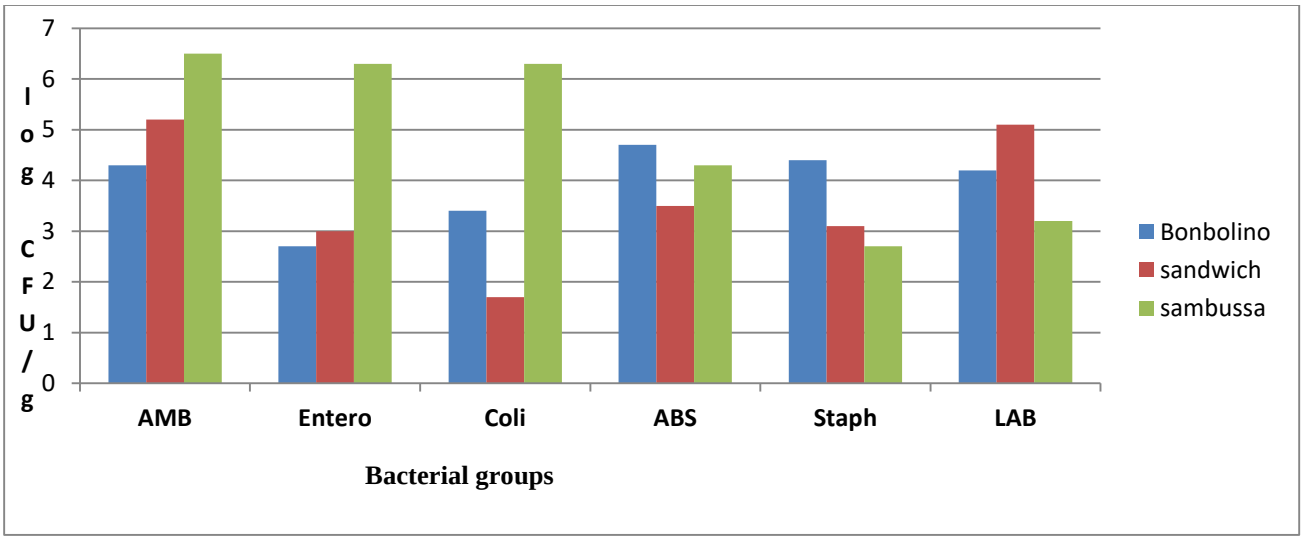

Fig.3. Bacterial load of street- vended foods

4.1.7. Antimicrobial Susceptibility of patterns of pathogenic bacteria

From the result of test isolates (controls), *S. aureus* (15.4%) and *salmonella*(25%) showed resistance to Gentamycin and Kanamycin (Table 8).

From the total of 13*S. aureus* isolates, the majority were susceptible to ciprofloxacin (100%) followed by Chloramphenicol (92.3%) and Gentamycin and Kanamycin (76.9%). However, some isolates of *S.aureus* showed resistant to Kanamycin and Gentamycin (23.1%). Out of the 4 isolates of Salmonella species, 100% were susceptible to Gentamycin followed by Ciprofloxacin and Chloramphenicol (75%) .In contrast to this, 50% of the isolates were resistant to Kanamycin (Table 9).

Table 8.Antimicrobial susceptibility patterns of test isolates.

Antimicrobial agents	Disc concentration(µg/ml)	***S.aureus* (ATCC 25923)**		***Salmonella Typhimurium* (ATCC13311)**		***E.coli* (ATCC25922)**	
		S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Chloramphenicol	30	12(100)	0(0)	4(100)	0(0)	5(100%)	0(0)
Gentamycin	10	11(84.6)	2(15.4)	4(100)	0(0)	5(100%)	0(0)
Kanamycin	30	13(100)	0(0)	3(75%)	1(25%)	5(100%)	0(0)
Ciprofloxacin	5	13(100)	0(0)	4(100)	0(0)	5(100%)	0(0)

S=sensitive **R=** resistance

Table 9**.**Antimicrobial susceptibility patterns of food pathogens isolated from some selected street foods from Hagereselam town, Northern Ethiopia 2019/2020**.**

Antimicrobial agents	Disc concentration(µg/ml)	***S.aureus***		***Salmonella***		***E.coli***	
		S (%)	R (%)	S (%)	R (%)	S(%)	R (%)
Chloramphenicol	30	12 (92.3)	1 (7.7)	3(75)	1(25)	4(75)	1(25)
Gentamycin	10	10(76.9)	3(23.1)	4(100)	0(0)	3(60)	2(40)
Kanamycin	30	10(76.9)	3(23.1)	2(50)	2(50)	5(100)	0(0)
Ciprofloxacin	5	13(100)	0(0)	3(75)	1(25)	3(60)	2(40)

S=sensitive **R=** resistance

4.2. Discussion

The socio-demographic characteristics of street vendors in the present study showed the majority of street vendors, 69(71.9%) were females. Similarly, Mensah *et al*.(2002) in their study in Accra, Ghana, found that 100% of vendors were females. In addition, Adimasu *et al*.(2016) in Gondar also reported that majority of the food vendors, 35 (87.5%) were females. This could be due to the culture of the society provides responsibility for females to process and prepare food.

In the present study, all of the vendors (100%) did not get training about the hygiene of vending food and many had no information about food and water borne diseases. In agreement to the present study, Nemo *et al*.(2017) from Jimma reported that the street vendors (92.7%) had not got training .Training is necessary for street vendors at all ages to prevent cross contamination and mishandling of foods at home and vending site. Safety in foods is critical to the public health, consumers, government and businesses that support those consumers. FAO (1998) also suggested that the food handlers should have the necessary knowledge and skills to handle food hygienically.

In the current study, 59(61.5%) of vendors used well water for cleaning utensils. Water is a critical raw material in many street vending food operations. It could be contaminated with biological, chemical or physical hazards. Contaminated water creates a public health risk if it is used for washing of utensils, incorporated in to foods as an ingredient and used in the preparation and washing of equipments. One of the most critical problems in street food vending is the supply of water of acceptable quality and insufficient quantities for drinking, washing, cleaning and other operations. Therefore, water supply needs cloth attention in street food attentions (WHO,1996).

The food handling practice of the street vendors in the present study revealed that,100% of the vendors served their customers without having special cloth and 77.1% handled food with their bare hands. These numbers were similar with what was reported by Nemo *et al*. (2017)where90.9% served their customers without having a special cloth and 80.9% handled food with their bare hands. . In contrast to this, these numbers were higher than what was reported by Chukuezi(2010) from Owerri, Nigeria. This could be due to lack of training on the handling of food and serving food for customers. The vendors can be carriers of pathogens like *E.coli, Salmonella* and *S.aureus* and eventually can transfer these food borne pathogens to

the consumers. The hands of food handlers are the most important vehicle for the transfer of organisms from faeces, nose and skin to the food (WHO,1989). Salmonella, Campylobacter and *E.coli* can survive on fingertips for different periods of time (Pethers and Gilbert, 1971).

In the current study, the mean count of Enterobacteriaceae was 4 log CFU/g. This agrees with the findings of Mustefa and Abdella(2011) , who reported the counts between 2.3 to 4.4 log CFU/g in Sudanese street-vended traditional foods. According to the guideline, the mean counts of Enterobacteriaceae in bonbolino (4.3), Sambussa (6.5) and Lentil sandwich (5.2) showed unsatisfactory level($\geq$ 4 log CFU/g). Enterobacteriaceae and the high number of Aerobic Mesophilic Bacteria showed that the poor hygiene could be a source of food-borne disease (Motarjemi *et al*., 1993).

The mean count of coliforms in the present study is between 1.7 $x10^6$ -6.3$x10^5$ CFU/g. This result is higher than(1.7 to 4.0 CFU/g) reported by Nemo *et al*.(2017) where the mean count of coliforms were between 1.7 to 4.0 CFUg-1 from Jimma town. This could be due to fecal contamination of food or water after preparation. Heat -processed foods usually have no vegetative microbial contaminants immediately after cooking. However, food could be contaminated later. Most of the time, the source of contamination for food was the water used in washing utensils or directly from hands of vendors. The fecal coliforms have been used as an indicator of the sanitary conditions (Tomkins, 1981).

The mean aerobic bacterial spores(ABS) count(4.2 log CFU/g) of the present study is higher compared to report by Mosupye and van Holy(1999) where the counts ranged between 1.2 to 2.0 log CFU/g and report by Nemo *et al*.(2012)where the counts were 4.0 log CFU/g from Johansburg, south Africa and Jimma, Ethiopia respectively. According to the guideline, mean counts of ABS in bonbolino (4.7 log CFU/g) and sambussa(4.3 log CFU/g) were of unsatisfactory level(>4 log CFU/g) whereas lentil sandwich (3.5 log CFU/g) was in acceptable level(3 to <4 log CFU/g). The higher counts in the present study could be due to the contamination of food by the heat resistant spores, which had survived cooking temperature because of temperature /time inadequacy during preparation of food.

The mean counts of *staphylococci* in the present study were 3.4 log CFU/g. This is in agreement with the microbiological studies made on street vended foods in Jimma (Nemo *et al*.,2017).However, this result is higher than the mean counts of staphylococci on street vended foods conducted in Gondar(0.3$x10^4$ CFU/g)

by Asefa Adimasu *et al*.,2016.The highest detection of *S.aureus* was found in sambussa (30%). The higher presence of *S.aureus* was an indication of contamination from the skin, mouth or nose of food handlers through coughing and sneezing, lack of knowledge of hygiene practices and safety of food products and contaminated hand of vendors (Tambekar *et al.*,2009).According to Mensah *et al.* (2002), the uses of a fork or spoon to serve food reduced the level of contamination, while the use of bare hands resulted in an increase of contamination. Staphylococci exist in air, dust, sewage, water and food or on food equipment and environmental surfaces. *S.aureus* can be found in the nose (50%), on hands (5-30%), in hair, eyes and throat of healthy persons (Hammad, 2004).

In the present study, the mean count of LAB was 4.2log CFU/g. This is higher than that was reported by Nemo*et al.(*2017).In contrast to the present study, Omemu and Omeike (2010) reported the higher count ranging between 4.5 to 9.2 log CFUg -1 in cooked ogi used as weaning food from Nigeria. The high count of LAB in food has significant impact in lowering the counts of pathogens.

The mean counts (log CFU/g) of moulds and yeasts in the present study were2. 6and3.4 respectively. The presence of high count of yeasts and moulds in the present study could be due to litters in the environment. Yeasts do not produce adverse toxins to humans. However, some moulds produce toxic substances that can cause disease or illness when eaten by humans. Moulds growing on foods can be decreased their levels by maintaining hygienic conditions during food processing and storage. Spores of mould can be carried by wind, and hence can easily enter into food sample (Stratford, 2006).

The predominant micro floral of street-vended foods in the present study was generally *Bacillus spp*.(32.5%) followed by *Staphylococcus spp.*(27.5%) and *Micrococcus spp*.(21%). The current study showed higher percentage of isolates than Diriba Muleta and Mogessie Ashenafi (2001b) report where the isolates from street-vended food were dominated by *Bacillus spp*.(29.1%) followed by *Staphylococcus spp*.(22.8%) in Addis Ababa, Ethiopia. The predominance of *Bacillus spp.* among isolates on aerobic plate count was possibly due to the presence of spores in the raw materials. The heat-resistant spores may have survived cooking while vegetative bacteria were eliminated (Mospuye and von Holy,1999). High number of *Bacillus spp*. Could cause food poisoning result food borne disease. *Staphylococcus* and *Micrococcus* spp. were among the dominant isolates possibly due to much litter and dust in vending sites, handling of foods with bare hands and the vendors serving utensils stayed for long period before replacement. They are common environmental bacteria that could be introduced in to the food after cooking through cross

contamination (Cardinale *et al*., 2005).

The prevalence of *S.aureus* and *Salmonella spp*. In the present study were 21.7 % and 6.7% respectively. The higher prevalence of *S.aureus* and *Salmonella spp*. In the present study could be due to washing of the utensils using well water, litter and dust at vending site. The presence of huge number of *S.aureus* in a food may indicate poor handling and lead to cross-contamination from vendors to food(Sina *et al*.,2011).The presence of *Salmonella spp.* Indicated poor food preparation and handling practices such as inadequate cooking(Tunung *et al*.,2007).

The study in Sarab, Iran by Akbarmehr (2012), showed that *Salmonella spp*. were highly susceptible to Chloramphenicol (100%) followed by ciprofloxacin and Gentamycin (91.89% each). In the preset study, high number of *salmonella spp.* was susceptible to Gentamycin (100%) and Chloramphenicol and Ciprofloxacin (75%) lower number to kanamycin and (50%).In agreement to this study, Nemo*et al*.(2017) from Jimma reported that high number of *salmonella spp*. are susceptible to Chloramphenicol(95.24%).Antibiotics such as ampicillin and sulphamethoxazole are the first line antibiotics used for the treatment of salmonellosis. However, Salmonella strains which are resistant to these first-line antibiotics have recently emerged worldwide, and is causing great concern. With that increase, the risk to public health has also increased. It is particularly serious in low-resource countries where bacterial infections remain among the major causes of death (Bartoloni *et al*., 2005).

In the present study, the antibiotic resistance patterns of the isolates revealed low number of resistance (7.7%) of *S.aureus* to Chloramphenicol. Similarly, Alina *et al*. (2011) reported lower number of *S. aureus* isolates resistant to Chloramphenicol (0.5%).However, in the present study, S. aureus was highly susceptible to ciprofloxacin (100%) followed by Chloramphenicol (92.3%).

5. Conclusion and Recommendations

5.1. Conclusion

From the current study, the following conclusions were made.

- The overall bacterial quality of street vended foods assessed in the current study was poor as compared to the guidelines set by other regulatory bodies. This could be due to poor personal hygienic practice of street vendors such as handling food with bare hand, washing the utensils using well water and lack of training for vendors leads to the higher level of microbial load through cross contamination.
- The most predominant microbial groups were *Bacillus spp., Staphylococci spp., Micrococcus spp*. and *Enterobacteriaceae.* Thus, the presence these microorganisms could be a possible prediction for the presence of pathogens. The presence of high number of pathogenic bacteria such as Salmonella spp. and S.aureus could cause food borne diseases like diarrhea, typhoid fever and food poisoning.
- Poor personal hygiene, improper handling and storage practice of foods and poor knowledge of food vendors towards food borne diseases were the associated risk factors to contamination of street vended foods.
- Potential pathogenic bacteria in this study are evident that street foods might contribute a major problem for public health.

5.2. Recommendations

From the present findings, the following recommendations were forwarded.

- Vendors should wash their hands before and after preparation of food.
- There is a need to the government to make more infrastructures available such as potable water, toilets and waste disposal facilities.
- There is also a need formal training in food safety for street foods vendors.
- The Tigray regional Health bureau and Hagereselam Town Health administration ought to create awareness in order to improve street vendors' hygienic conditions during preparation, handling, storing and serving of foods.
- Regular inspection on food vending practices needs to be made.
- Lastly, further study on street-vended foods using other antimicrobials is recommended to produce much more relevant information about antimicrobial susceptibility of microbes status street vended foods in that locality.

References

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

Al Suwaidi, A., Hussein, H.,Al Faisal, W., El Sawaf, and Wasfy, A. (2015).Hygienic practices among food handlers in Dubai. *International Journal of Preventive Medicine Research*. **1(3)**:101 108.

Akbarmehr, J. (2012).Antimicrobial resistance in *salmonella* isolated from broiler chicken carcasses.*African Journal of Microbiological Research*.**6:**1485-1488

.

Ali,H., Bacha,K. and Ketema,T,(2011).Bacteriological quality and antimicrobial susceptibility of Some isolates of well waters used for drinking in Jimma town, Southwest Ethiopia *Ethiopian Journal of Educational Science.***6:**95-108.

Alimi, B., Workneh, S. (2016). Consumer awareness and willingness to pay for safety of street foods in Developing countries: a review *International Journal of Consumers Studies.***40:** 242-248.

Alina, K., Yee-Ann, S., Nicole, D., Daniel, S., William, R., Kourtney, J., Twain, B., Edward, K., Zheng Jie, Z.,Patrick, M. and Jianghong, M. (2011). Antimicrobial susceptibility of Staphylococcus aureus from retail ground meats. *Journal of Food Protection*.**74**:1625-1629.

Anandh,N. and Krishnaveni, N. (2015). Microbiological quality of selected street-vended foods in Coimbatore, India. *African Journal of Microbiological Research*. **9(11**):757–62.

Angaw, M. (2011). Microbiological quality and safety of sambusa and fried fish in bahirdar. Msc. Thesis, Bahir Dar University, faculty of applied microbiology.

Adimasu, A. , Mekonnen, B.,Guadu, T.,Gizaw,Z.andAdane,T.(2016). Bacteriological Quality Assessment of Selected Street Foods and Their Public Health Importance in Gondar Town, North West Ethiopia.

Bapat, M.(1992). Street food vending in Pune. Pune: center of studies in social sciences.India

Bartoloni, A., Benedetti, M., Pallecchi, L., Larsson, M., Mantella, A., Strohmeyer, M., Bartalesi, F.,Fernandez, C., Guzman, E., Vallejos, Y.,Villagran, L., Guerra, H., Gotuzzo, E.,

Paradisi,F., Falkenberg, T.,Rossolini, M. and Kronvall, G. (2005). *Evaluation of a Rapid screening method for detection of antimicrobial resistance in the Commensal microbiota of the gut.*Tropical Medical Hygiene. **100**:119-125.

Bauer,W., Kirby,M.,Sheris,C., and Jurck,M.(1966). Antibiotic susceptibility testing by a standardSingledisc method. *American Journal of Clinical pathology.* **45(4):**493-496.

Bhat, R. and Waghray, K. (2000). Profile of street foods sold in Asian countries. World review of Nutrition and dietetics .53–99.

Bryan, F.(1988). Risks of practices, procedures and processes that lead to outbreaks of food-Borne diseases. *Journal of food protection*. 663–673.

Bryan,.L.,Jermini,M.Schmitt,R.,Chilufya,E.,Mwanza.M.,Matoba,A.,Mfume,E. and Chibiya.(1997).Hazards associated with holding and reheating foods at vending sites in a small town in Zambia. *Journal of food protection*.**60:**391-398

Cardinale,E., Claude, D., Tall, F. Gueye. and Salvat, G. (2005). Risk factors for contamination of Ready-to-eat street-vendedpoultry dishes in Dakar, Senegal. *International Journal of Food Microbiology*. **103**:157-165

Chukuezi, O.(2010). Food safety and hygienic practices of street food vendors in Owerri, Nigeria. *Studies of sociology science.***1**:50-57

Croxen, M. and Finlay, B. (2010). Molecular mechanisms of Escherichia coli pathogenicity. *National Review of Microbiology*. **8**: 26–38.

Doddamani, H. and Ninnekar, H. (2001). Biodegradation of carbaryl by a Micrococcus species.Curr Microbiol. **43 (1):** 69–73.

Fàbrega, A. and Vila, J. (2013). Salmonella enterica serovar Typhimurium skills to succeeding the host: virulence and regulation. *Clinical Microbiology Reviews*. **26 (2):** 308–41.

Fairey, Philip., Chandra, Subrato and Moyer, Neil.(2019). "Mold Growth. Florida Solar Energy Center. University of Central Florida.

FAO(1990).Street foods report of an FAO expert consultation. *Food nutrition*.**46:**50-59.

FAO(1998).Food handling and street food preparation practices, particularly of dairy products in Kathmandu.Technical report project TCP/NEP/6755. Food and Agriculture Organization ofthe United States, Rome, Italy.

FAO(2007).school kids and street food, ViewedMay, 2013,http://www.fao.org/ag/magazine/0702sp1.htm

Fleet, G. and Balia, R. (2006) The public health and probiotic significance of yeasts in foods and beverages. In: Yeasts in Food and Beverages (eds. Querol,A. and Fleet, G.). Springer, Berlin. pp. 381–397.

Food and Agriculture Organization of the United Nations & Pan American Health Organization (2017)**.**Food handlers manual Instructor**.**

Fotadar, U. , Zaveloff. P, and Terracio, L .(2005). Growth of Escherichia coli at elevated temperatures. *Journal of Basic Microbiology*. **45 (5):** 403 - 404.

Guven, K. Mutlu, B., Gulbandilar, A. and Cakir, P.(2010). Occurrence and characterization of Staphylococcus aureus isolated from meat and dairy products in Turkey. *Journal of Food Safety*.**30:**196–212.

Gibert., Louvois,J.,Donovan,T.,Little,C.,Nye,K., Ribeiro,D.,Richards,J., Roberts, D. and Bolton.(2000).Guidelines for the microbiological quality of some ready- to- eat foods Sampled at the point of sale. *Public Health*.**3**:163-167.

Gram, C. (1884). Uber die isolierte Färbung der Schizomyceten in Schnitt-

Und Trockenpräparaten" . Fortschritte der. Medizin. **2**: 185–189.

Gregerson, G. (1978). Rapid method for distinction of gram negative from gram positive bacteria. *European Journal of Applied Microbiology*. **5**:123-127.

Greig, D., Todd, C., Bartleson, A. and Michaels, S. (2007). Outbreaks where food workers have been Implicated in the spread of foodborne disease. Part 1. Description of the problem, Methods and agents involved. *Journal of Food Protection*.**70 (7):**1752–61.

Hammad, M.(2004). Assessment of microbial quality of food samples in Nablus district. Submitted in partial fulfillment of the requirements for the Degree of Master of Public Health, Faculty of Graduate Studies, at An-Najah National University, Nablus, Palestine.

Hanashiro, A. Morita, M., Glavur, R., Maria, H. and Elizabeth, A. (2005). Microbiological quality of selected street foods from a restricted area of S~aoPaulo city, Brazil. Food Control .439–444.

Hassett, D., Cuppoletti, J., Trapnell, B. and Lymar, S (2002). "Anaerobic metabolism and quorum sensing by Pseudomonas aeruginosa biofilms in chronically infected cystic fibrosis airways: rethinking antibiotic treatment strategies and drug targets". Adv Drug Deliv Rev. **54 (11):** 1425–1443.20. Ryan, K. and Ray, C. (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill.

Henson, S. and Traill, B.(1993). The demand for food safety: market imperfections and the role government, food policy, **18**:152-162.

Hardie (2009). "The Secreted Proteins of Pseudomonas aeruginosa: Their Export Machineries, and How They Contribute to Pathogenesis". Bacterial Secreted Proteins: Secretory Mechanisms and Role in Pathogenesis. Caister Academic Press.

Hibbett, D., Binder, M., Bischoff, F., Blackwell, M., Cannon, F. and Eriksson, E. (2007). Ahigher level phylogenetic classification of the Fungi. Mycological Research. **111 (5**):509–547.

John, L. (2012). An introduction to bacterial identification.www.splammo.net,/bac102/dichotkeyhandout.pdf. Last accessed march 27 th 2012.

Joseph, B., Otta, S., Karunasagar, I., and Karunasagar, I. (2001) Biofilm formation by Salmonella spp. on food contact surfaces and their sensitivity to sanitizers. *International Journal of Food Microbiology*.**64**:367–372.

Kam,A. and Xu, J.(2002)Diversity of commercial yeasts within and among healthy hosts. *Diagnostic Microbiology of Infectious Diseases*. **43**:19–28.

Kluytmans,J. and Wertheim, F.(2005).Nasal carriage of Staphylococcus aureus and prevention Of nosocomial infections. *Infection*.**33**:3–8.

Lamuka, P. (2014). Public health measures: challenges of developing countries in management of food Safety.*Encyclopedia of Food Safety*.**4:**20–60.

LaRock, L.,Chaudhary, Miller. And Samuel, I. (2015).Salmonellae interactions with host processes. *Nature Reviews Microbiology*. **13 (4):** 191–205.

Lianghui, x., xingling, s., yuju, c., zhang, l. and haiyan, w. (1993) . Analysis of street food safety in shandong province, abstracts. In: final programme street foods. Epidemiology, management and practical approaches. Beijing,China.

Licitra , G.(2013). Etymologia: Staphylococcus. Emerging Infectious Diseses.**19**:1553.

Lim, J. , Yoon, J. and Hovde, J. (2010). A brief overview of Escherichia coli O157:H7 and its plasmid O157. Journal of Microbiology and Biotechnology. **20 (1):** 5–14.

McFadden, F. (1980). Biochemical tests for identification of medical Bacteria. 2nd edition. Williams and Wilkins, Baltimore. USA.

Madigan, M. and Martink, J. (2005). Brock Biology of Microorganisms (11th ed.). Prentice Hall.

Malloch, D. (1981). Moulds : their isolation, cultivation and identification. Toronto Canada: University of Toronto Press.

Martins, H. and anelich, E . (2000). Improving street foods in SouthAfrica. Unpublished report for the food and agricultural organization of United Nations. Rome.

Martins, H .(2006). Socio-economic and hygiene features of street food vending in Gauteng. *South African journal of clinical nutrition*.**19 (1):** 18-25

Mensah,p., Yeboah,D., Manu,K., Owusu,D and Ablordey,A.(2002).Street foods in Accra,Ghana:How safe are they?Bulletin of the world health organization.**80**:546-554.

Moyo, S., Maselle,M., Matee,N., Langel, B. and Mylvaganam, H. (2007).Identification of DiarrheagenicEscherichia coli isolated from infants and children in Dareselam,Tanzania.BMC infectious diseases .**90(7):**1-7.

Muinde, K. and Kuria, E.(2005). Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. *African journal of food agriculture & nutritional development*.**5**:1–14.

Muleta D, Ashenafi M (2001a). Salmonella, Shigella and growthpotential of other food borne pathogens in Ethiopia street vendedfoods. *East African medical Journal*.**78**:576-580.

Muleta,D.and Ashenafi,M.(2001b). Bacteriological profile and holding temperatures of street-vended foods from Addis Ababa', *International Journal of Environmental Health Research.* **11**(**1):**95- 105.

Mustafa, N. and Abdella, S.(2011).Bacteriological quality of street vended *Um-Jinger:* A traditional Sudanese food.*African Journal of Food and Agricultural Nutrition*.**11:**1684-5374.

Moore, D., Robson, D. and Trinci AP,(2011). 21st Century Guidebook to Fungi (1st ed.).Cambridge University Press.

Mosupye, F. and Holy, A.(1999). Microbiological quality and safety of ready-to-eat street Vended foods in Johannesburg, SouthAfrica. *Journal of food protection.* **62**:1278 – 1284.

Motarjemi,Y.,Kaferstein,F.,Moy, G. and Quevedo, F.(1993).Contaminated waning food: a major risk Factor for diarrhea and associated malnutrition. *Firfitin World Health Organ*.**71**:79-92.

National Committee for Clinical Laboratory Standards(2007). Performance standards for Antimicrobial susceptibility testing 17thinformational supplement.

Nemo, R., Bacha, K., & Ketema, T. (2017). Microbiological quality and safety of some-street-vended foods in Jimma Town, Southwestern Ethiopia. *African Journal of MicrobiologyResearch*,**11(14),** 574-585.

Nielsen, P. and Rios, R. (2000). Inhibition of fungal growth on bread by volatile components from spices and herbs, and the possible application in active packaging, with special emphasis on mustard essential oil. *International Journal of Food Microbiology*.; **60:**219-229.

Omemu, M. and Omeike, O.(2010). Microbiological hazard and critical control points identificationDuring house hold preparation of cooked ogi used as weaning food. *International food research journal*.**17:** 257-266.

Padda, Kiran Preet; Puri, Akshit; Chanway, Chris P. (2018). "Isolation and identification of endophytic diazotrophs from lodgepole pine trees growing at unreclaimed gravel mining pits in central interior British Columbia, Canada". *Canadian Journal of Forest Research.***48 (12):**1601–1606.

Padda, Kiran Preet; Puri, Akshit and Chanway, Chris (2019). "Endophytic nitrogen fixation –a possible 'hidden' source of nitrogen for lodgepole pine trees growing at unreclaimed gravel mining sites"FEMS Microbiology Ecology. 95 (11).

Patra, A., Sil, J. and Das, K. (2011). Isolation and characterization of dominant lactic acid bacteria From Dahi at Medinipur and evaluation of their antibacterial activity. *International Journal of Food Safety*. **13**:157-163.

Pathers, S. and Gilbert, J.(1971).Survival of Salmonella on finger tips and transfer of the organism to Foods.*Journal of Hygiene*.**69**:673-681.

Pitt, J. and Hocking, A.(2009). Primary keys and miscellaneous fungi. In: Pitt JI, Hocking AD eds., Fungi and food spoilage. New York NY: Springer;:1-9; 122-124.

Poole, K. (2004). "Efflux-mediated multiresistance in Gram-negative bacteria" . *Clinical Microbiology Infections*. **10 (1):** 12–26.

Rahman, M. and Turin, T. (2013). Knowledge and awareness of children's food safety among school-Based street food vendors in Dhaka. Bangladesh. *Food borne Pathogenic Distribution*. **10(4):**323–330.

Rane, S. (2011).Street vended food in developing world. Hazard analyses. *Indian Journal of Microbioogyl*.**51**:100-106.

Roberts, R,. Barrett, B., Howells, D., Shanklin, W., Pilling, K. and Brannon, A. (2008). "Food safety training and foodservice employees' knowledge and behavior," *Food Protection Trends*. **28**: 252–260.

Rosmawati, N., Manan, W., Izani, N. and Nurain, N. (2015). Validity and reliability of food safetyKnowledge and practices questionnaire among food handlers**.** *Health and the Environment Journal*.**6 (1):** 11-30.

Ryan, K. and Ray, C. (2004). Sherris Medical Microbiology (4th ed.).McGraw Hill.

Schafer, R., Schafer, E., bultena, G. and hoiberg, E.(1993). Food safety: an application of the health belief model. *Journal of nutritional Education*. **25**:17– 24.

Schaeffer, A, and Fulton, M .(1933). A simplified method of staining endospore. Science 77:194.

Shields, P. and Cathcart, L. (2012). Motility test medium protocol. http://www.microbelibrary.org/library/laboratory-test/3658-motility- test-medium-protocol. Last accessed march 6 th 2012.

Sina, H. , Baba-Moussa, F., Kayoed, P.,Noumavo, A., Sezan, A., Hounhouigan., Kotchoni., Prevost, G. and Baba-Moussa, L. (2011). Characterization of Staphylococcus aureus isolated from street foods: Toxin profile and prevalence of antibiotic Resistance. *Journal of Applied Bioscience*. **46**:3133–3143.

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

Tambekar, D., Kulkarni, S., Shirsat, T. and Bhadange, G. (2009). Bacteriological Quality of Street Vended food Panipuri: A Case Study of Amravati City (MS) India.*Bioscience Discovery*.**2**: 350-354.

Tambekar, H., Jaiswal,V., Dhanorkar, V., Gulhane, B. and Dudhane,N. (2008). "Identification of Microbiological hazards and safety of ready-to-eat food vended in streets of Amravati City, India," *Journal of Applied Bioscience*. **7:** 195–201.

Tekletsadik Solomon and Tsige Ketema (2011).Microbiological safety of street vended ayib in Jimma town, southwest Ethiopia. *Ethiopian Journal of Education and Science*, **7 (1):**81- 91.

Tent, H. (1999). Research on food safety in 21st century. Food Control. 239–247.

Timke, M., Wang-Lieu, N. Q., Altendorf, K., and Lipski, A. (2005) Fatty acid analysis and spoilage potential of bio films from two breweries. *Journal of Applied Microbiology***99**:1108–1122.

Tomkins, J.(1981).Gastroenteritis. *Annual Review Public Health*.**8**:75-81.

Tunung , R., Chai, C ., Usha, R . , Lee, Y., Fatimah, B., Farinazleen. and Son, R. (2007). Characterization of Salmonella enteric isolated from street food.

Van Eldere, J. (2003). "Multicentre surveillance of Pseudomonas aeruginosa susceptibility patterns in nosocomial infections". *Journal of Antimicrobial Chemotherapy*. **51 (2):** 347–352.

Verstrepen, J.and Klis, M.(2006). Flocculation, adhesion and biofilm formation in yeasts. *Molecular Microbiology*. **60**:5–15.

Vlkova,E.,Rada,V.,Popelarova,P.,Trojanova,I. and Killer,J.(2006). Antimicrobial Susceptibility Ofbifido bacteria isolated from gastro intestinal tract of calves.

Livestockscience.**105**:253-259. clinical samples in Malaysia. *Asian Food Journal*. **14**: 161-173.

Wareing, Peter. (2013). The Fungal Infection of Agricultural Produce and the Production ofMycotoxins. European Mycotoxins Awareness Network.

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

WHO(1996).Essential safety requirements for the street vended foods(revised edition).World

Xu, D. and Cote, J. (2003). "Phylogenetic relationships between Bacillus species and related genera inferred from comparison of 3' end 16S rDNA and 5' end 16S-23S ITS nucleotide sequences". International Journal of Systematic and Evolutionary Microbiology. **53 (3):** 695–704.

Yarza, P., Ludwig, W.,Euzéby, J., Amann, R., Schleifer, H., Glöckner, F. and Rosselló-Móra, R. (2010). "Update of the All-Species Living Tree Project based on 16S and 23S rRNA sequence analyses". Systematic and Applied Microbiology. **33 (6):** 291–299. Health Organization, Food Safety Unit, Geneva. Switzerland.

Zeru Birhane (2015). Street food vendors and skill training in Addis ketema sub city. Addis Abeba University College of education and behavioral studies.

Appendix-I

College of Natural Sciences

Department of Biology

Title: Microbiological quality and safety of Bonbolino,Sambusa and lentil Sandwich at Hagereselam town,Tigray,Ethiopia.

This research will be conducted in Hagereselam town concerning microbiological quality and safety of Bonbolino,Sambusa and lentil Sandwich at hagereselam town. You, street food vendors of hagereselam town,are kindly requested to give your genuine response on the following issues.

- Your response is confidential.

Thank youin advance.

Part I. Background information of respondents

Code_____

1. Age< 20 2☐ 31-40 ☐ 41-5☐ ☐ ☐

2. Sex: male ☐ Female ☐

3.Level of education**:** illiterate ☐

Primary education ☐

Secondary education ☐

4. How many years Experience do you have in vending food (years)
A. < 5 B. 6-10 C. 11-15 D. 16-20 E. >20

Part II: Main information

5. Do you useSpecial cloth for vending? yes ☐ No☐

6. Do you Handle food with bare hands ? yes ☐ No ☐

7. Do Vendors cover their hair? yes ☐ No ☐

8. What type of water do you use for preparation of food?

A.tap B. Well C. River D. Spring

E. Other(please specify) _____

9. What type of water do you use for cleaning utensils?

A.tap B. Well C. River D. Spring

E. Other (please specify) _____

10. How do you clean the utensils?

A. by hand using water only B. With warm soapy water

C. With cold soapy water D. others(please specify) _____

11. What material do you use while vending food for customers?

A. using fork/spoon B. Bare hand C. polythene bags D. Piece of clothes E. others.

12.which material do you use for Storage of your foods?

A. Openly in the stalls B. In a wheelbarrow C. In sealed (transparent or opaque) containers

13. Do you have any information about food borne diseases? A. Yes B. No

14. Maximum days the street food can stay before selling and/or consumption:

A. 1-2 days B. 3-4 days C. more than four days

15. Do you get training or education about personal hygiene and handling of vended food?

A. Yes B. No

16. If your answer for question No 15 is yes,

A. Weekly B. Monthly C. Yearly D. others (please specify)_____

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4. ( )

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JIMMA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

DEPARTMENT OF BIOLOGY

Observation check list format

Appendix-II: Observation check list

Non-verbal information such as Environmental conditions, personal hygiene of food vendors , storage place and way of serving foods for consumers was collected through observation check list.

№	**Points considered**	**Standards/ measurements**		
		Very good	**Good**	**Poor**
1	Over all environmental sanitation around the vending site			
2	Food storage sanitation			
3	Personal hygiene of street food vendors			
4	The way of Food handling using gloves			
5	Vendors have long fingernails			
6	Vendors wear neat cloth			

Appendix-III: John's Bacterial classification

Gram reaction	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-
Shape	CCl	CCl	CCh	CT	rod	Rod	IR	Ro	Rod	rod	rod	rod	Rod	Rod	r

								d							od
Aerobic growth	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Anaerobic growth	-	+	+	+	+	-	-	+	+	-	-	-	+	+	+
Endospore	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-
Motility	-	-	-	-	-	-	-	+/-	+/-	+/-	+/-	+/-	-	+	+
Catalase reaction	+	+	-	-	-	+	+	-	+	+	+	+	+	+	+
Oxidase reaction	+	-	-	-	-	-	-	-	+/-	+/-	+	+	-	-	+
Micrococcus	X														
Staphylococcus		X													
Streptococcus			X												
Lactococcus			X												
Enterococcus			X												
Leuconostoc			X												
Pediococcus			X	X											
Aerococcus				X											
Lactobacillus					X										
Acinetobacter						X									
Arthrobacter							X								
Clostridium								X							
Bacillus									X	X					
Alcaligenes											X				
Pseudomonas												X			
Klebseila													X		
Shigella													X		
Salmonella														X	
Escherichia														X	
Other enteric genera														X	
Aeromonas															X

Where : CCl= coccus(clusters) CCh= coccus (chains)

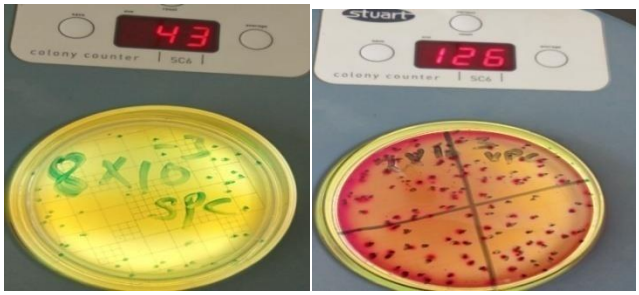
CT= coccus (tetrad) , IR= Irregular rod

Appendix IV: Prevalence of food borne pathogens From street foods of Hagereselam town based on study site.

Food	**Site**	**Sample**	***S.aureus***	***Salmonella***	***E.coli***

sample		size(n)	Frequency	%	frequency	%	frequency	%
Bonbolino	Bus station	10	4	40%	1	10%	4	40%
	Alula	7	-	0	-	0	2	28.6%
	Lsanu	3	-	0	-	0	2	66.7
	To tal	**20**	**4**	**20%**	**1**	**5%**	**8**	**40%**
Sambussa	Bus station	10	3	30%	2	20%	3	30%
	Alula	7	2	28.6%	-	0	2	28.6%
	Lsanu	3	1	33.3%	-	0	2	66.7%
	Total	**20**	**6**	**30%**	**2**	**10%**	**7**	**35%**
Lentil sandwich	Bus station	10	2	20%	1	10%	2	20%
	Alula	**7**	-	0	-	0	2	28.6%
	Lsanu	3	1	33.3%	-	0	1	33.3%
	Total	**20**	**3**	**15%**	**1**	**5%**	**5**	**25%**
All samples	**Total**	**60**	**13**	**21.7%**	**4**	**6.7%**	**20**	**33.3%**

Appendix V: Pictures taken during lab investigation


43
colony counter
SC6
8 X 10
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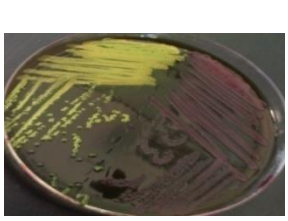
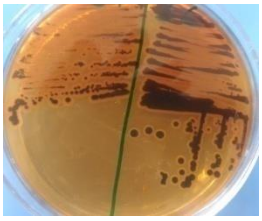
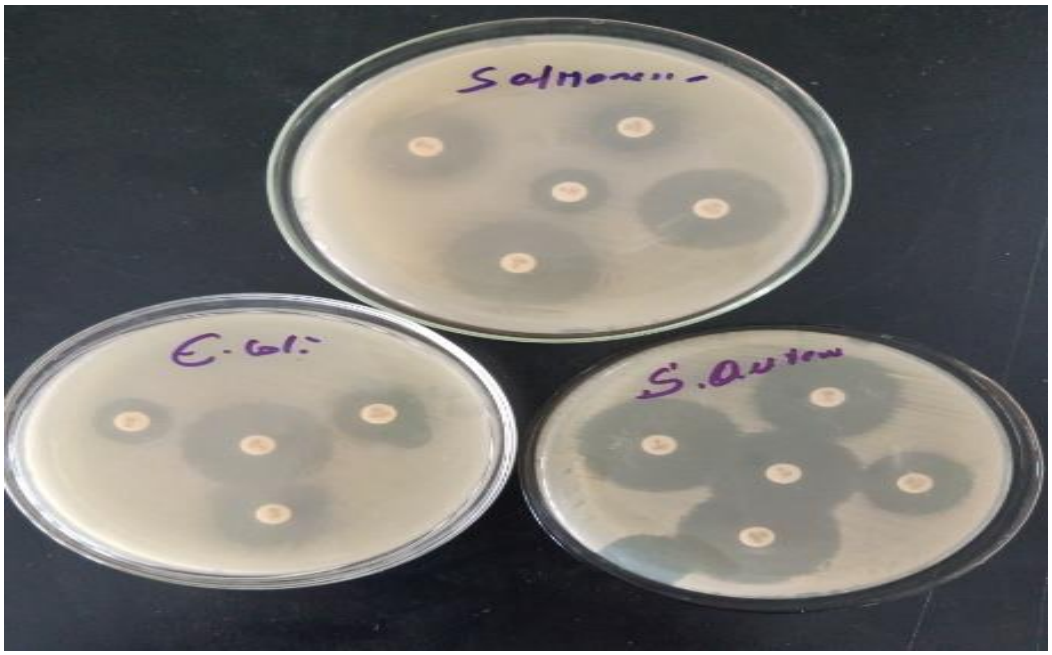
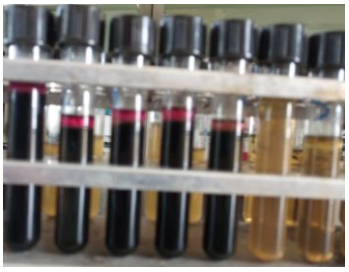
stuart
126
colony counter
SC6






E. Coli
S. Aureus



Declaration

I , the under signed, declare that this is my original work and has not been presented for seeking a degree in any university and that all resources of the materials used for the thesis have been dully acknowledged.

Zenebe Gebreegziabher Tekle _____ _____

Signature Date

The work has been done under supervision

Name	Signature	Date
1. Delelegn Woyessa(Msc., Associate Professor)	———————	———————
2.Shiferaw Demissie(MSc.,Assistantprofessor)	———————	