

**Microbiological Quality and Safety of Street Vended Raw Meat  
in Jijiga Town of Somali Regional State, Ethiopia.**

**By**

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## LIST OF ABBREVIATIONS

ALIPB	-	Aklilu Lemma Institute of Pathobiology
ANOVA	-	Analysis of Variance
AOAC	-	Association of Official Analytical Chemists
APC	-	Aerobic Plate Count
$a_w$	-	Water activity
BAM	-	Bacteriological Analytical Manual
BPW	-	Buffered Peptone Water
CDC	-	Center for Disease Control
CFU	-	Colony Forming Units
cm	-	Cent meter
CV	-	Coefficient of Variance
ECSA	-	Ethiopian Central Statistics Authority
FAO	-	Food and Agriculture Organization of the United Nations
FDA	-	U.S. Food and Drug Administration
g	-	Gram
GI	-	Gastrointestinal
GMP	-	Good Manufacturing Practices
H <sub>2</sub> O <sub>2</sub>	-	Hydrogen peroxide
HACCP	-	Hazard Analysis and Critical Control Points
HPA	-	Health Protection Agency
ICMSF	-	International Commission for Microbial Specifications of Foods
IDF	-	International Dairy Federation, Brussels
IOM	-	Institute of Medicine
IFT	-	Institute of Food Technology
K <sub>2</sub> HPO <sub>4</sub>	-	Dipotassium hydrogen phosphate
KOH	-	Potassium hydroxide
LAB	-	Lactic Acid Bacteria
LIA	-	Lysine Iron Agar

log <sub>10</sub>	- Logarithm of ten
ml	- Mili liter
mm	- Mili Meter
MPN	- Most Probable Number
NaCl	- Sodium chloride
NAS	- National Academy of Sciences
NPN	- Non protein nitrogenous compounds
O/F	- Test of Oxidation Fermentation
O/R	- Oxidation -reduction Potential
pH	- Power of hydrogen
PDA	- Potato Dextrose Agar
RTE	- Ready-to-eat
RV	- Rappaport – Vassiliadis
SD	- Standard Deviation
SPSS	- Statistical Products and Service Solutions
USDA	- United States Department of Agriculture
USDHHS	- United States Department of Health and Human Services
WHO	- World Health Organization
XLD	- Xylose Lysine Desoxycholate medium
<sup>0</sup> C	- Degree Celsius
<sup>0</sup> F	- Degree Fahrenheit

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## Abstract

*The study was aimed to assess the microbial quality and safety of street vended raw meats in Jijiga town of Ethiopia. Questionnaire was used to assess the socio-demographic characteristics and knowledge of raw meat vendors about food safety from randomly selected 33 vendors. Visual assessment was also made. A total of 60 raw meat samples containing 30 raw beef and 30 raw goat meat samples were collected for microbiological analysis. The pH and holding temperature of the meat were measured. The microbial load was analyzed in terms of counts of aerobic mesophiles, Enterobacteriaceae, Coliforms, Staphylococci, Lactic acid bacteria, Yeasts and Moulds using standard methods. Furthermore, the aerobic mesophilic flora of the samples was characterized to different genera and bacterial groups. The presence Salmonella was also determined. Survey indicated that 78.8% of vendors were females and only 30.3% of the vendors were literate. The majority (58%) were in the age of 26-41. None them had working garment. 27% and 33% had untrimmed (unclean) nails and unclean clothing (body parts) respectively. Wear of jewelries (70%) and finger nail painting (76%) were also observed. Vendors had different degree of awareness about food safety, sources of contamination and food borne diseases. The sanitary condition of the vending environment was not also satisfactory. Basic sanitary facilities were not available. The mean pH values were 6.03 and 5.2 for beef meat and goat meat samples respectively the samples were held in a temperature range of 17.5-27.5°C at the time of vending. Beef meat and goat meat samples had mean aerobic mesophilic counts >7logcfu/g. Enterobacteriaceae and coliforms were frequently encountered in all samples and both of the varieties had mean counts for both microbial groups > 4logcfu/g. Both species of meat had mean staphylococci counts > 6logcfu/g. Both meat types had mean counts of lactic acid bacteria, yeasts and moulds 4logcfu/g. The aerobic plate counts were dominated by Staphylococcus spp. followed by Enterobacteriaceae and other Gram positive rods. Salmonellae were also isolated from 5(8.3 %) raw meat samples. One-way ANOVA revealed as there were significant differences ( $P<0.05$ ) between goat and beef samples in aerobic plate count and staphylococci counts. In general the samples harbored high counts of microorganisms. This might be attributed to unhygienic and improper handling, processing and storage. Training and inspections are important. More over, provision of basic infrastructures and establishment of code of practice for the sector are also recommended.*

**Key words:** *Jijiga, raw meat, street vendors, quality, safety.*

## 1. INTRODUCTION

Food is essential for survival. However, in spite of gaining good nutrition and satisfaction from eating food, occasionally human beings consume undesirable chemical and biological agents and toxins resulting in food borne illness. Consequently, in many countries food safety and quality is becoming a matter of increasing concern.

Safe food can be defined as a food being free from chemical and biological danger or from anything else which may generate adverse health effects (Unnevehr and Hirschhorn , 2000). Health hazards from food can arise from the raw materials used, from handling and through the other stages involved in the processing, transportation, storage and the sale of the food (Abalanka, 1999).

Food quality has dimensions related to both production process and final product. Its determinants can be grouped in to four as hygienic properties, nutritional properties, functional properties, and organoleptic properties (Abalanka, 1999). Briefly, it can be defined as the subjective or objective valuation of food with respect to any or all of these four properties.

Food safety problems are becoming an increasingly serious threat to public health in developing countries. Lack of adequate regulations related to food safety as reflected in many unrecognized cases of food borne illnesses puts especially children and infants at high risk (Unnevehr *et al.*, 2000). Biological contaminants, largely bacteria, viruses, and parasites constitute the major cause of food-borne diseases (Kaferstein, 2003). In developing countries, contaminants are responsible for a wide range of diseases, including cholera, campylobacteriosis, *E. coli* gastroenteritis, salmonellosis, shigellosis, typhoid and paratyphoid fevers, brucellosis, amoebiasis, and poliomyelitis. According to a WHO study in 1996, 70 percent of the approximately 1.5 billion global incidents of diarrhea occurring annually, which resulted in 3 million deaths among children under five, had been estimated to be caused by biologically contaminated food (WHO, 1996). As stated in the same study contaminated food had been playing a major role in the epidemiology of cholera and other forms of epidemic diarrhea, substantially contributing to malnutrition. Street vended foods include raw foods, ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers, especially in streets around the market and other public places (FAO, 1988). Vending foods on the street is a common aspect of lifestyle both in industrialized as well as countries in which there are high unemployment, low salaries, limited work

opportunities and limited social programmers (Bryan *et al.*, 1988). Street vendors provide an essential service to people of all walks of life by selling raw foods, complete meals, refreshing drinks and snacks to them ( WHO,1996). Generally street foods provide a source of readily available, inexpensive, nutritional meals, while providing a source of income for the vendors (Bryan *et al.*, 1988; Dawson and Canet ,1991; Mosupye and Von Holy , 1999).

In spite of numerous advantages offered by street vended foods, there are also several hazards associated with this sector of the economy. Multiple line evidence revealed that foods exposed for sale on the roadside may become contaminated by either spoilage or pathogenic microorganisms (Mogessie Ashenafi, 1995). This constitutes serious health hazards, particularly in economically disadvantaged countries where food surveillance are undeveloped or not there at all. Evidently, street vended foods have shown epidemiological link with illness (Van Kappel *et al.*, 1998; Mogessie Ashenafi, 1995) and laboratory result also have shown alarming high counts of microorganisms and presence of food borne pathogens in street vended foods (Mogessie Ashenafi, 1995; Umoh *et al.*, 1984, 1985). Some foods like meats, rice, fish, and fruits have been frequently identified as vehicles in outbreaks of food borne diseases in countries where food-borne surveillance data are available (Bryan, 1988; Davey, 1985).

Among the most common street vended foods, meat and meat products were known to be the major in either processed or unprocessed form (WHO, 1996). WHO (1996) further indicated that retailing unprocessed raw meat in the street or in an open air market for the public was common in Africa as well as in some parts of Asian countries. Studies made in Africa, Asia and Latin America (FAO, 1995) pointed out that the important aspect of street vended food is their safety and understanding the possible ways of contamination.

According to Deriba Muleta and Mogessie Ashenafi (2001), microbial contamination of street vended foods could occur due to different possible reasons such as raw materials of doubtful quality, storing food in cheap utensils, holding food at a temperature that would permit bacterial growth, utilization of water of questionable hygienic quality, using packing materials that were not of food-grade quality, vending site that had no facilities for waste disposal and utilization of unclean utensils. In 1996, WHO also undertook a survey on 100 countries to assess hazards posed by street vended foods and contributing factors for the hazards. It was found that infrastructure developments were relatively limited in those countries with restricted access to potable water (47%), toilets (15%), refrigeration

(43%), hand washing facilities (32%), dish /utensils washing facilities (48%) and waste disposal facilities (47%) (WHO,1996). Deriba Muleta and Mogessie Ashenafi,(2001) described that most of the time street food vendors are unaware of the basic importance of personal cleanliness thus their products are usually vulnerable to gross contamination by flies, insects, rodents, other dirt and dust. Bryan *et al.* (1988) also indicated that street-food vendors are often poor and uneducated and lack appreciation for safe food handling.

Although vending raw meat is not practiced in most parts of Ethiopia, there are some areas in which vending raw meat in an open market is common. Jijiga town is one of these areas where raw meat street vendors are available throughout the town. In Jijiga, raw meats from different animals (such as sheep, goat, camel and cattle) are vended in open-air local retail sections out- door everyday in a week particularly in a large market at the center of the town.

Studies concerning various street vended foods in Ethiopia showed the presence of pathogens or the presence of good conditions in street foods to allow growth of pathogens in them (Mogese Ashenafi, 1994; Deriba Muleta and Mogessie Ashenafi, 2002). However, there is no information on the microbial quality and safety of street vended raw meats sold particularly in Jijiga town. Poor food handling practices of most of the raw meat street vendors (exposing the meat to open air, use of uncleaned utensils for displaying the meat, lack of access to water) as well as poor sanitary condition of the vending environment makes the safety and quality of these street vended raw meats questionable( doubtful) . Therefore, the aim of this study was to evaluate the microbiological quality of raw beef and goat meats as these types of meats were the most familiar vended meats in the study area.

## 1.2 OBJECTIVES OF THE STUDY

The study has the following general and specific objectives:

### 1.2.1 General objectives

The general objectives of this study are to determine the microbial safety and quality of street vended raw meat and to assess the knowledge of the street food vendors about food safety issues in Jijiga town.

### 1.2.2 Specific objectives

1. To assess knowledge and food handling practices of street raw meat vendors with respect to food safety,
2. To determine the microbial load of street vended raw beef and raw goat meat,
3. To isolate, characterize and identify the dominant microbial groups,
4. To evaluate the bacteriological safety of the meats with regard to *Salmonella*.

## 2. LITRATURE REWIEW

### 2.1 PUBLIC HEALTH RISKS FROM STREET VENDEED FOODS

Most handlers of street-vended foods in Africa, and the developing world at large, are largely ignorant of basic food safety issues. Consequently, street foods are commonly exposed to dangerous abuses, often at all stages of handling. Products (from the raw material to the finished stage) are often exposed to sources of contamination like soil, dust and sand. Other common real risk factors include time temperature abuses involving, for instance, under roasting street meat snacks to minimize shrinkage, handling prepared foods under unsafe storage temperatures and serving such foods cold or without sufficient reheating (Bryan *et al.*, 1988).

Food handlers may be a major source of food contamination and ultimate sources of health risks either as carriers of pathogens or through poor hygienic practices. Workers can carry microbial pathogens on their skin, in their hair, on their hands, and in their digestive systems or respiratory tracts. Unless workers understand and follow basic food protection principles; they may unintentionally contaminate foods, water supplies and equipment, and thereby create the opportunity to transmit food borne illness (Dugassa Guteta, 2007).

A number of direct concerns also exist about the sanitation of street food vending operations. As previously noted by (Bryan *et al.*, 1988) for example, street vendors often use stands and carts of crude and inefficient construction, and running water is seldom available at the locations. Hand, dish and utensil washing is usually done in one or more buckets or pans, and sometimes without soap. Disinfection is rarely carried out. Waste water is usually discarded in the street and garbage is at times discarded near the food stand. In addition to all these, toilet facilities are seldom readily available. Akobundu, (1996) also indicated that most traditional street foods are presented and delivered in the open, without proper protective packaging.

It has been recognized that in countries where street vending of foods are common, there is usually a deficiency of epidemiological data on the incidence of food borne diseases, and outbreaks of such diseases are frequently not investigated. (Bryan *et al.*, 1988; Bryan *et al.*, 1992b). Tjoa *et al.*, (1977) indicated that persons of all ages commonly experience gastrointestinal diseases, the

extent of implication of street foods in these diseases and in the outbreak of food borne illnesses, in particular, is not known. (Ericsson *et al.*, 1980) and (Mogessie Ashenafi, 1995) have, however, reported epidemiologic associations between street-vended foods and sicknesses.

Moreover, foods such as poultry, pork, beef, fish and rice, which are sold in the street, are frequently identified as vehicles of disease transmission in countries that have surveillance programmes. In Nigeria, available data indicate that street foods carry high levels of microorganisms, including pathogens. For instance, Igene (1983) and Igene and Abulu (1984) have reported mean total plate count and coliform MPN levels of  $6.24 \times 10^7$ - $1.4 \times 10^9$  and  $8.5 \times 10^2$ - $2.0 \times 10^3$  /g, respectively in suya meat products. Similarly, Idio (1995) has reported total plate count and coliform levels of  $6.5 \times 10^6$  -  $8.0 \times 10^6$  and  $3.0 \times 10^6$  -  $3.62 \times 10^6$  cfu/g, respectively, in Nigerian ready-to-eat (street) clam meat. These levels all exceed the recommended tolerable levels of  $10^6$ /g total count and  $10^2$ /g coliforms in delicatessen products (Pace, 1975; Solberg *et al.*, 1976) and therefore suggest contamination with pathogens like salmonellae, *shigellae*, *staphylococci* or enter pathogenic *Escherichia coli* (Harris *et al.*, 1975).

## 2.2. SOURCES OF RAW MEAT MICROBIAL CONTAMINATION

Food for human consumption is expected to be clean, wholesome and sanitary, and free from noxious materials (Dugassa Guteta, 2007). But food can be contaminated by, physical, chemical and microbiological agents (Stewart, 1997). The sources of food contamination are diverse. It may be contaminated by polluted water, flies, animals and pets, unclean utensils and pots, dust and dirt (Dugassa Guteta, 2007). Contamination can occur at any point in the food chain, in processing plants, at supermarkets and restaurants or in the consumer's kitchen in short, from the farm to fork (USDA, 2009). Hazards can be introduced into foodservice operations in numerous ways: by employees, food, equipment, cleaning supplies and customers (Bryan *et al.*, 1988).

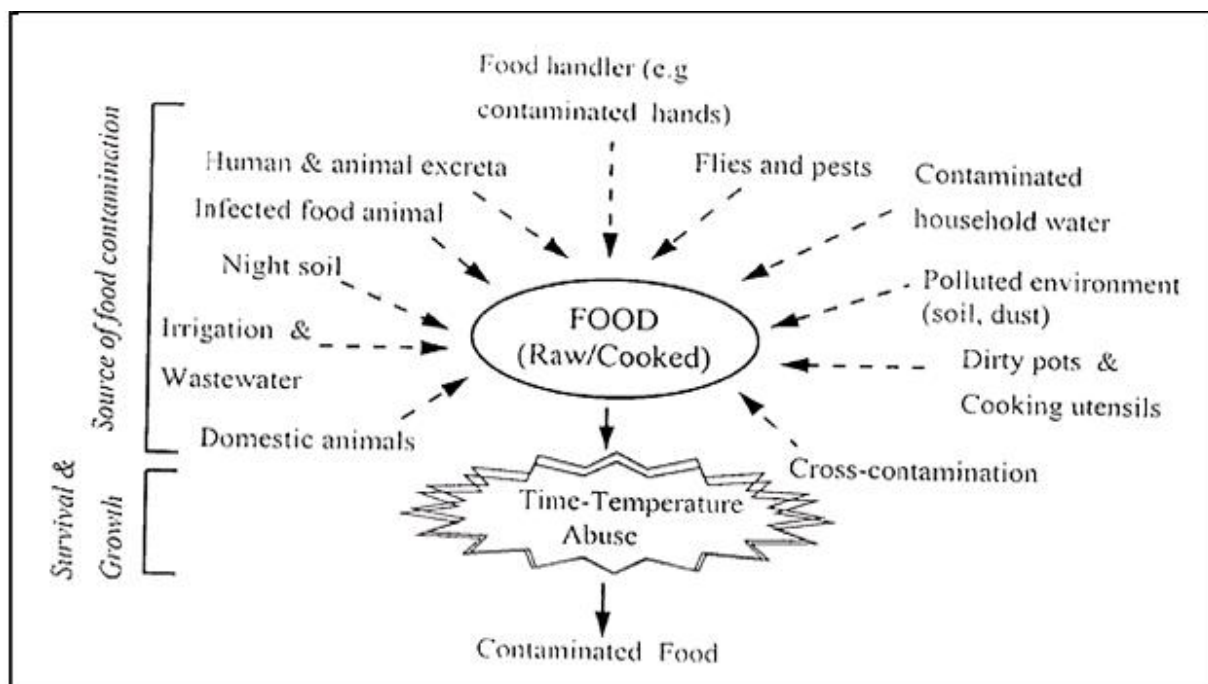


Figure 1: Sources of food contamination

Source: USDHHS (2007)

In the healthy animal, tissues that ultimately become meat (muscle, fat and edible offal) are usually sterile (Gill, 1979). Animal carcasses and meat cuts are easily contaminated with bacteria during the slaughter, dressing, cutting and packing process (Koutsoumanis and Sofos, 2004). If not properly handled, processed and preserved, meat will support the growth of these organisms thereby creating a significant health risk. Sources of contamination include faeces, ingesta, hide, Lymph nodes and intestines of the animals themselves, and air, water, soil, processing equipment, utensils and personnel from the abattoir environment (Gill, 1979). For instance after dressing, lamb carcasses typically carry a total mesophylic bacteria between  $10^2$  and  $10^4$  bacteria/cm<sup>2</sup> (Lovatt *et al.*, 2006). The initial flora is very diverse, although it is predominantly mesophilic, with *Micrococci*, *Staphylococci*, *Bacillus*, oryneforms, Enterobacteriaceae, *Flavobacteria*, Pseudomonads, lactic acid bacteria (LAB) and *Brocothrix* likely to be present (Dainty and Mackey, 1992).

Meat is an ideal medium for many organisms to grow because it is high in moisture, rich in nitrogenous compounds (e.g. amino acids, peptides, proteins) and plentiful in minerals and

accessory growth factors. Furthermore it has some fermentable carbohydrates usually glycogen and keeps favorable pH for growth of most microorganisms (Frazier and Westhoff, 1978). Comminuting also adds microbial contamination to meat (Sachindra *et al.*, 2005).

It is quite normal and unavoidable to find bacterial counts of “total plate count” (TPC) of the order of several thousand per cm<sup>2</sup> on meat surfaces in commercial slaughtering and meat handling. However, the principle must be to keep bacterial counts as low as possible through adequate hygienic measures. Total plate count numbers exceeding 100,000 per gram (10<sup>5</sup> per cm<sup>2</sup>) on fresh meat are not acceptable and alarm signals and meat hygiene along the slaughter and meat handling chain must be urgently improved (FAO, 2010) (Table 2.2 ).

Table 2: FAO, 2010 recommended microbiological criteria for fresh meat.

Microbial count per cm <sup>2</sup> Or per gram	Good microbiological standard	Critical microbiological condition	Not acceptable
Total plate count	<10 <sup>4</sup>	>10 <sup>4</sup> - <10 <sup>5</sup>	>10 <sup>5</sup>
Enterobacteriaceae	<10 <sup>2</sup>	>10 <sup>2</sup> - <10 <sup>3</sup>	>10 <sup>3</sup>

Source: FAO (2010)

Poor sanitary practices in food storage, handling, and preparation can create an environment in which bacteria such as *campylobacter*, *salmonella*, and other infectious agents are more easily transmitted (Murat *et al.*, 2006).

Food handlers may transmit pathogens passively from a contaminated source, for example, from raw poultry to a food such as cold cooked meat that is to be eaten without further heating. They may also, however, themselves to be sources of organisms either during the course of gastrointestinal illness or during and after convalescence, when they no longer have symptoms. During the severe stages of gastroenteritis large numbers of organisms are excreted and by the nature of the disease are likely to be widely dispersed; clearly, food handlers who are symptomatically ill may present a real hazard and should be excluded from work. Good hygiene,

both personal and in food handling practices, is the basis for preventing the transmission of pathogens from food handling personnel to consumer (Murat *et al.*, 2006).

Moreover, transmission of intestinal parasites and enteropathogenic bacteria is affected directly or indirectly through objects contaminated with faeces. These include food, water, nails, and fingers, indicating the importance of faecal-oral human-to-human transmission. Accordingly, food-handlers with poor personal hygiene working in food-serving establishments could be potential sources of infections of many intestinal helminthes, protozoa, and enteropathogenic bacteria. Food-handlers who port and excrete intestinal parasites and enteropathogenic bacteria may contaminate foods from their faeces via their fingers, then to food processing, and finally to healthy individuals (Gashaw Andargie *et al.*, 2008). In some instances, transmission occurs through close contact between infected and uninfected individuals as in infected food handlers and consumers, respectively (Donato *et al.*, 2003).

### 2.3. SPOILAGE MICROORGANISMS

Microbiological examination of foods is focused not only on causative agents of human diseases, but also on microorganisms causing spoilage and affecting the quality or shelf life (Napravnikova *et al.*, 2002). Food products serve not only as sources of nutrition for humans and other animals, but also as substrates for the growth of microorganisms. The uncontrolled growth of microorganisms in food causes spoilage, a serious problem accounting for sizable losses of food products. Spoilage microorganisms are those that can grow in food and cause undesirable changes in flavor, consistency (body and texture), color, or appearance (IOM, 1985; Hayes, 1995). Bacterial enzymes may also affect foods during long time storage. These changes diminish the quality characteristics of foods and may render ultimately unfit for human consumption. Most prone to spoilage are foods with high protein content, such as meat, poultry, fish, and milk, which have a high dietetic value, neutral, or lightly acid pH, and a high water content providing favorable conditions for bacterial growth (Huis and Veld, 1996).

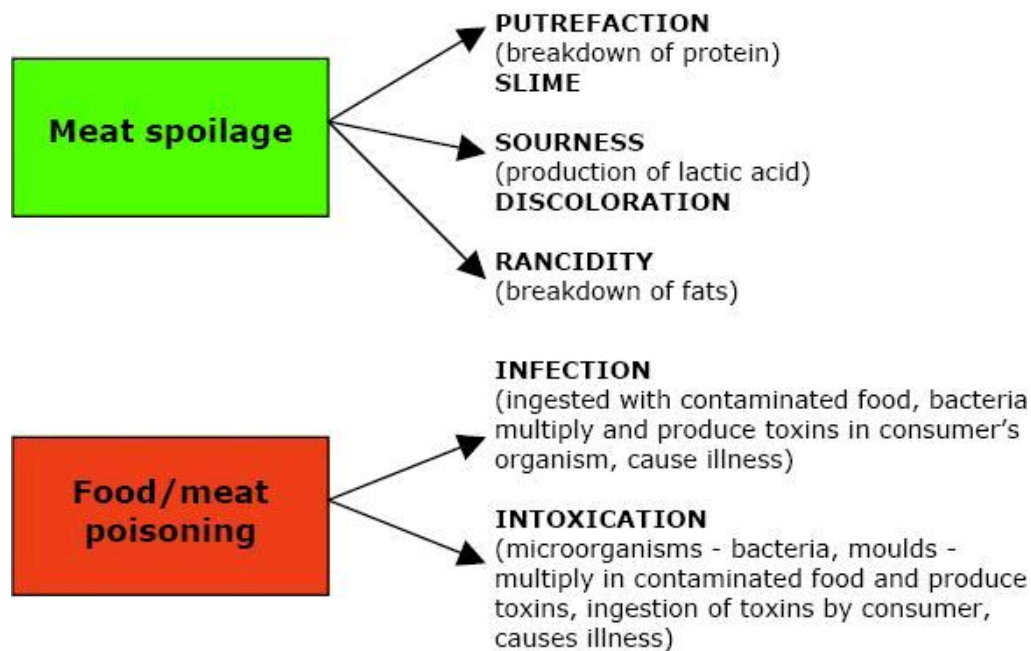


Figure 2: Impacts of bacteria on meat.

Source: FAO (2010)

The chemical composition of meat (approximate - water 71-76%, protein 20-22%, lipid 3-8%, carbohydrate ~1.2%, and soluble non-protein substances ~2.3%) provides an ideal substrate for microbial growth (Samelis, 2006). The muscles of healthy animals are essentially sterile but become contaminated at slaughter or at handling. Avoiding microbial contamination is practically impossible. For instance, (Ho, 2004) indicated that the shelf life of unpreserved meat, such as is sold in the warm “wet markets” that dominate meat trading in Asia is very short, measurable in hours, compared to days for unpreserved refrigerated meat. Long time storage of meats either in factories or in shops may also contribute to an increase of aerobic mesophilic counts (Korkeala *et al.*, 1985).

No. of microorganisms per gram (total plate count)

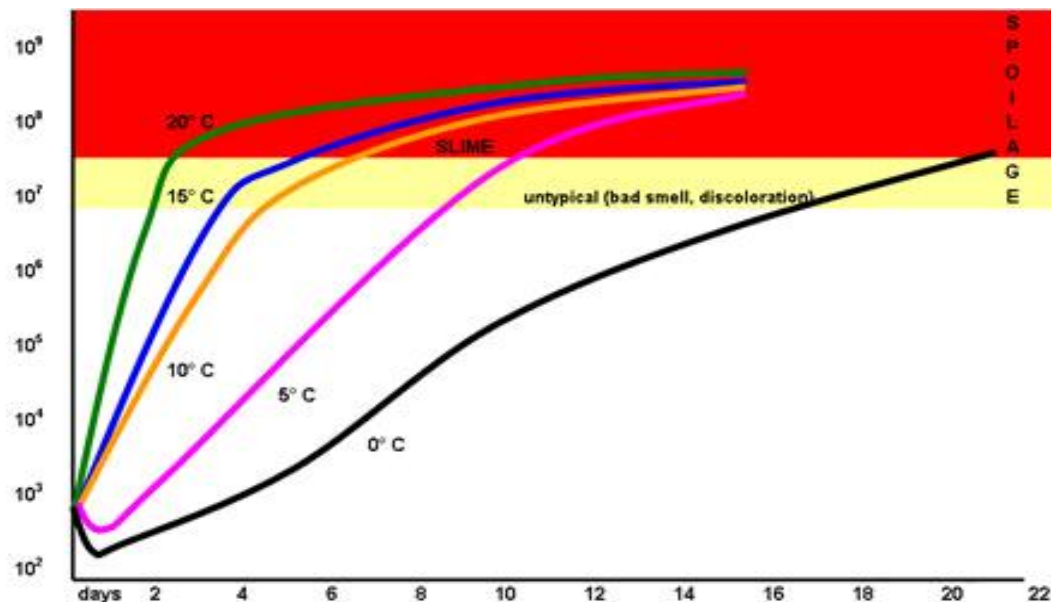


Figure 3: Growth of microorganisms on meat (starting from same initial bacterial loads/approx. 1000 per gram meat , but different storage temperatures, 0°C, 5°C, 10°C, 15°C).

Source: FAO (2010)

Meat is generally considered spoiled when one or a combination of undesirable organoleptic (sensory) changes makes them unacceptable to the consumer (Ellis and Goodacre, 2006). These changes can be purely visible (e.g. discolouration, surface colonies), malodorous (e.g. sulphurous smell) or due to slime formation (“sticky” texture). Whilst endogenous enzymatic activity post-mortem contributes to biochemical changes during storage, it is generally accepted that detectable organoleptic spoilage of meat is a result of the decomposition and subsequent metabolite formation caused by the growth and catabolic activity of microorganisms (Warris, 2001).

The predominant spoilage flora in a meat is determined by nutrient availability, oxygen availability, storage temperature, pH, storage time of the product, and generation time of the

microorganisms present in a given environment. Fresh meats such as beef, pork, and lamb, as well as fresh poultry, seafood, and processed meats, have pH values within the growth range of most of the organisms. Nutrient and moisture contents are adequate to support the growth of most organisms (Jay, 2005).

## 2.4. PATHOGENS

Ensuring the safety of food products depends on minimizing the initial contamination with pathogenic microorganisms and inhibiting their development during handling and storage (Stekelenburg, 2003). Pathogenic microorganisms can render foods harmful to humans in a variety of ways.

Foods may serve as vehicle of introduction of infectious microorganisms in to the gastrointestinal tract, e.g., *Salmonella* and *Shigella* (IOM, 1985). Multiplication of certain microorganisms in foods prior to consumption may result in production of toxins e.g. *Clostridium botulinum*, *S.aureus* and *B. cereus*. Microbial pathogens in food cause between 6.5 million and 33 million cases of human illness and up to 9,000 deaths in the United States each year, and the estimated annual cost of human illness caused by food-borne pathogens ranges from \$5.6 billion to \$9.4 billion (Buzby and Roberts, 1995).

### 2.4.1. Prevalence of *Salmonella* in Meat and Meat products

*Salmonella* contamination could be from the actual infection of food animals at the farm, cross-contamination during slaughtering, distribution and subsequent handling and processing. Cross-contamination may arise from cutting board, knives and hands of the meat handlers. (Nyeleti *et al.*, 2000) reported a low prevalence of *Salmonella* in faeces (2.2%) and mesenteric lymph nodes (4.2%) of slaughter cattle. On the contrary, the same authors also reported a high prevalence of *Salmonella* in diaphragm (11.9%) and abdominal muscles (9.8%). This suggests the presence of severe cross-contamination during slaughtering process as a result of poor hygienic conditions during subsequent dressing operations. The other probable source of cross contamination could be from *Salmonella* carrier slaughter house personnel (Nyeleti *et al.*, 2000 and Bayleyegn Molla *et al.*, 2003).

Food borne salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the carcasses or edible organs (Daniel Alemayehu *et al*, 2002; Arroyo and Arroyo, 1995). *Salmonella* infection in meat animals, including cattle, pig and sheep, also arises from intensive rearing practices and the use of contaminated feeds (D'aoust, 1989). Cross-contamination of carcasses with *Salmonella* can also occur during slaughtering operations. Stress associated with transport of animals to abattoir augments shedding of *Salmonella* by carrier animals and this may contribute to the spread of the organism to other animals in the slaughter plant (Baird-Parker, 1990; Isaacson *et al.*, 1999).

Slaughtering procedures potentially involve many risks of both direct and cross-contamination of carcasses and meat surfaces. During slaughter, fecal contamination of edible organs with subsequent contamination of the carcass may occur. This can be carried through all slaughter procedures up to the processing of the raw products, which are important sources of *Salmonella* in the human food chain (Baird-parker, 1990; Edwards *et al*, 1997). Contamination of equipment utensils and hands of workers can spread *Salmonella* to uncontaminated carcasses and parts, which can occur in subsequent handling, processing, transport, storage, distribution and preparation for consumption (Bryan *et al.*, 1992b).

The true incidence of salmonellosis both in humans and animals is difficult to evaluate because of lack of an epidemiological surveillance system in place, which is particularly true in developing countries. However, the number of outbreaks particularly in humans has increased considerably in recent years (Acha *et al.*, 2001; Mache *et al.*, 1997). Carrier states of humans are of concern to the food manufacturing and food service industries because of the perceived risk of contamination of food by infected food handlers and the risk of foodborne disease outbreaks (D'aoust, 1994). Meat processing and packaging at the wholesale or retail levels are likely to contribute to the higher levels of contamination in minced beef and pork products compared to beef and pork carcasses. The presence of even small numbers of *Salmonella* in carcass meat and edible offal may lead to heavy contamination of minced meat (Deriba Muleta and Mogessie Ashenafi, 2001).

When meat is cut into pieces, more microorganisms are added to the surfaces of exposed tissue. Raw meats, particularly minced meats have very high total counts of microorganisms and *salmonellae* are likely to be present in large numbers (Mezgebu Tegegne and Ashenafi Mogessie, 1998). Previous studies conducted in Addis Ababa indicated the occurrence of *Salmonella* in different food animals, meat and meat products (D'aoust, 1994; Bayleyegn Molla and Ayele Mesfin, 2003; Bayleyegn Molla *et al.*, 1999; Mezgebu Tegegne and Mogessie Ashenafi, 1998).

The catalogue of pathogenic bacteria that have been found on red meat is extensive. However, only some of these bacteria are implicated in food-poisoning disease, notably *Salmonellae*, *Escherichia coli* O157:H7, *Campylobacter*, *Clostridium botulinum*, *Aeromonas hydrophila*, *Yersinia enterocolitica* in pork, and *Listeria monocytogenes* in processed meats (Fung *et al.*, 2001). All of these pathogens are considered adulterants in high risk “ready-to-eat” foods (that require no additional cooking).

Despite the fact that raw, fresh meat will be subjected to cooking as a final hygienic control step prior to consumption; many countries have now adopted a “zero tolerance” approach to the presence of some pathogenic micro-organisms. In the USA, a well-publicised outbreak associated with undercooked hamburgers (501 recorded cases of food poisoning, 151 hospitalizations, 45 cases of hemolytic uremic disease (HUD) and 4 deaths), led to the causative organism *Escherichia coli* O157:H7 being confirmed an adulterant of meat. A large-scale surveillance programme was established to monitor all beef destined for the US burger market, including most of New Zealand export beef (FSIS, 2004; Fung *et al.*, 2001).

A more intensive programme to effect control of Salmonellosis in Sweden and Denmark, included “top-down” eradication of infected flocks of poultry and a continual test programme in pork (Wegener *et al.*, 2003). The hygienic objectives of post-slaughter carcass and product management are, therefore, to first minimize contamination. As total eradication is impractical, the second objective is to restrict subsequent proliferation (Bell *et al.*, 1998; Borch *et al.*, 1996).

Most of the meat-borne pathogens described above (*Salmonellae*, *Escherichia coli* O157:H7 and *Campylobacter*) are mesophilic. Whilst there is significant risk of these organisms proliferating (with associated hazard to health) at ambient temperatures in the “wet market” scenario, they will

not multiply during chilled or frozen storage. Refrigeration therefore provides a simple technology to safely extend shelf-life (Bell, 2001).

Whilst non-proteolytic *Clostridium botulinum*, *Aeromonas hydrophila*, *Yersinia enterocolitica* and *Listeria monocytogenes* can grow at lower temperatures (*Cl. botulinum* cannot produce toxin below 2-3 °C, minimum growth temperature for the remainder is 0-4°C).

## 2.5. INDICATOR MICROORGANISMS

To estimate food sanitary quality, the classic approach is based on the search for not only pathogenic microorganisms but also indicator microorganisms (Leclercq *et al.*, 2002). An indicator organism is a microorganism that indicates that a food has been exposed to conditions that pose an increased risk, that the food may have been contaminated with a pathogen or held under conditions conducive for growth of pathogens (Buchanan, 2000).

The concept of testing for indicator bacteria rather than pathogenic bacteria dates back to 1892, when Shardingerm instituted the practice of testing water for *E. coli* as indication of fecal contamination and the possible presence of *Salmonella typhi* (Banwart, 1989). It has traditionally been preferred to search for the more numerous and more readily determined indicator organisms. When one tries to recover pathogenic bacteria, they can be so few that they often escape detection because of problems of sampling and recovery. However, indicator organisms only give an indication that the pathogen may be present and not necessarily that they are present (Roberts, 1982).

### 2.5.1 What do indicator organisms indicate?

Indicator organisms are important components for microbiological tests. They may signify the potential presence of pathogens, a lapse in sanitation as required in good manufacturing practices (GMPs), or a process failure. They may reflect quality attributes that can influence consumer acceptability of a product. Sometimes the presence of an indicator organism alone is cause for concern; in other cases, it is the quantity that is significant. Many foods provide an environment conducive to microbial growth, and indicator counts in such foods may reflect the time and conditions of storage. The microbiological snapshot that is the indicator test must always be

assessed in an appropriate context, taking into account the natural microbial ecology, intrinsic and extrinsic chemical and physical factors that might influence microbial growth, process history, and storage conditions of the product (Jay, 1996).

The dual goals of safety and quality often overlap in the water, food, and environmental arenas, and it is important to choose the type of indicator organism that best fits a particular system. This is not an easy task, and the question of indicator selection has generated much discussion and debate. Perhaps adding confusion to the discussion are attempts that have been made over the years to apply various terms so as to distinguish the different functions of indicators, e.g., index, marker, model, sentinel, and surrogate organisms (IFT, 2001).

It seems reasonable to view two general categories of indicators, i.e., safety and quality indicators (Smoot *et al.*, 1997; Jay, 2000). Safety indicators suggest that a microbial hazard may exist, and their use is intended to minimize the risk of exposure to the hazard. Quality indicators are used to assess issues important to product acceptability, e.g., shelf life, organoleptic characteristics, spoilage, etc. The International Commission on Microbiological Specifications for Foods (ICMSF, 2002) has noted that selection of an indicator must be considered carefully with an understanding of how to interpret the results of indicator testing.

Jay (1996) elaborated on some criteria for the use of indicator organisms for the determination of food quality. An indicator organism should be detectable in all foods whose quality is to be evaluated. Growth and numbers of indicator bacteria should have a direct negative correlation with product quality and should be easily and rapidly detected and counted. The indicator bacteria should be easily distinguishable from other bacteria. Other bacteria normally present in the food should not inhibit the growth of the indicator bacteria.

According to Tompkin (1983), the choice of an indicator is product and process specific, when evaluating the microbiological quality of food. Indicator organisms have been used in meat and poultry products to assess three factors: microbiological safety, hygiene during processing, and the keeping quality of the product.

### 2.5.2 Safety Indicators

Buchanan (2000) has noted that safety indicators signify exposure “to conditions that pose an increased risk (of contamination) with a pathogen.” Coliforms have had the longest history of use as indicators, having been recommended since the early 1900s for water and food testing.

### 2.5.3 Quality Indicators

The microbial composition of a product significantly determines its quality. The types and number of microorganisms present influence the sensory properties (taste, aroma, texture, color) and shelf life of the product. Among these microbial populations, a particular one may be useful as an indicator to reflect quality changes in the product. Such quality indicators are often used to ensure that the product is microbiologically stable and aesthetically acceptable (Smoot and Pierson, 1997).

The primary attribute of a quality indicator is that its growth and numbers should be inversely related to acceptable product quality. The indicator should be present in all products whose quality is to be assessed, its growth unaffected by other microbial populations presents, and there should be relatively simple methods available for detection, differentiation, and quantitation (Jay, 2005).

### 2.5.4 Commonly Used Indicator Organisms

Many different types of indicators have been advocated for use in particular applications; the most common indicators used for foods and drinking water, are the aerobic plate count; coliforms; *E. coli*; *Enterobacteriaceae*; *Listeria* spp.; and the yeasts and molds. Microbial groups which may have use as indicators in certain applications, e.g., enterococci, *Staphylococcus*, endospore-formers, lactic acid bacteria, and others (Jay, 2000).

#### ***Aerobic Plate Count***

The aerobic plate count (APC) is among the more popularly used nonpathogenic microbiological indicators of food quality (FEHD, 2001; FSAI, 2001; Vandereit, 1985). It is generally used for descriptive evaluation of microorganisms on nonselective media under mesophilic and aerobic

conditions of incubation (FEHD, 2001; FSAI, 2001). This plate method serves as an indicator of food quality and as a measure of the effectiveness and maintenance of procedural integrity of food preparation protocols (Shapton and Shapton, 1991). It is generally believed that high aerobic mesophilic counts in foods indicate greater risks of pathogens being present in consumable products, poor implementation of sanitation procedures or problems in process controls to which a test food item has been subjected (Miskimin *et al.*, 1976).

### ***Coliforms and E. coli***

The coliform group is not a valid taxonomic distinction, but is defined functionally, that is by the fermentation of lactose in the coliform test (Kornacki *et al.*, 2001). Coliforms may be defined as Gram-negative, oxidase-negative, aerobic or facultative anaerobic non-spore-forming rods, able to grow in the presence of bile salts, and which ferment lactose to produce acid and gas within 48 h at 37°C (Rompere, 2002). Genera that fit this description are *Escherichia*, *Enterobacter*, *Klebsiella*, and *Citrobacter*. Coliforms are also one of the typical indicator organisms of food quality. Currently, tests for the presence and number of coliforms are used to assess the rate of total contamination of foods and the hygienic standard of food manufacture (Hitchins *et al.*, 1998).

Enumeration of coliforms has been adopted as a more convenient standard of sanitary significance by U.S. Public Health Service in 1914 (Anon 2004). Increased counts of coliform bacteria are indicative of failures in sanitation and very high counts can be dangerous to human health (Napravnkova1 *et al.*, 2002). This does not mean, however, that all foods that are free from coliform bacteria are safe. According to Smith and Schaffner (2004), high numbers of coliforms in meat products may be representative of improper handling or storage, which allows for the multiplication of any coliforms present.

### ***Enterobacteriaceae***

The detection of any member of the Enterobacteriaceae family present in raw meat and its product has been used to imply the presence of enteric pathogens and high levels of Enterobacteriaceae on meat products suggest processing contamination or microbial proliferation due to inadequate storage conditions (Smith and Schaffner, 2004).

The family Enterobacteriaceae encompasses approximately 20 genera, including *E. coli* and the other members of the coliform group; foodborne pathogens *Salmonella*, *Shigella*, *Yersinia*; and other related genera (Murray, 1998). The family was originally proposed as an indicator alternative to the coliform group, because testing for the entire family would be more inclusive for the pathogenic genera. Lactose, the carbohydrate specified in the coliform test, is not fermented by *Salmonella*, *Shigella*, or *Yersinia*, so their presence would not be detected by the test. But substituting glucose for the lactose in the test would allow detection of all members of the *Enterobacteriaceae*, including the pathogens, as well as variant strains that do not show the typical lactose fermentation trait (Leclerc *et al.*, 2001).

The rationale for the use of the *Enterobacteriaceae* as indicators was advanced by reports noting low or negative coliform test results despite detection of *Salmonella* in certain foods (Mossel *et al.*, 1963), by a shigellosis outbreak in a nursing home in which *Enterobacteriaceae* tests might have indicated a cause for concern (Kayse *et al.*, 1984), and by a cheese-associated outbreak caused by an enteropathogenic *E. coli* strain that was a slow lactose fermenter (Marier *et al.*, 1973). These reports notwithstanding, the *Enterobacteriaceae* are no more indicative of fecal contamination in foods than are the coliforms that are not indicative at all. Nevertheless, they are useful, like the coliforms, as process integrity indicators (NAS, 1985). The Enterobacteriaceae may be superior to the coliforms as indicators of sanitation GMPs because they have collectively greater resistance to the environment than the coliforms, can colonize where sanitation has been inadequate, and are sensitive to sanitizers. Thus, the *Enterobacteriaceae* are useful for monitoring sanitation in food manufacturing plants (Kornacki, *et al.*, 2001).

### ***Yeasts and Molds***

Yeasts and molds are commonly enumerated in foods as quality indicators (Baross, 2001). They have no predictive value for the occurrence of toxigenic fungi or other pathogens. As a group, the yeasts and molds are diverse and can grow on virtually any type of food. They survive a wide range of environmental conditions: pH 2–9; temperatures of 5–35 °C; and water activity ( $a_w$ ) of 0.85 or less (Beuchat and Cousin, 2001). As quality indicators, they can be used to assess ingredient acceptability, organoleptic characteristics, stability, and shelf life of a product (Beuchat and Cousin, 2001).

If the growth is above the limited range, affects the food with an unpleasant “mildew” smell develops (Deibel and Swanson,2001).Growth of yeasts and molds in foods is generally not influenced by the presence of many other microorganisms, which would be inhibited by the low water activity of the commodity. A number of standard methods using antibiotic-supplemented media have been validated for performing yeast and mold counts. Thus, the yeast and mold population possesses the desirable attributes of an appropriate quality indicator for many foods (Tournas *et al.*, 1998).

### 3. MATERIAL AND METHODS

#### 3.1. LOCATION OF THE STUDY SITE

The study was conducted at Somali region, Jijiga town. Jijiga (Somali: *Jigjiga*) is a town in eastern Ethiopia and the capital city of the Somali Regional state. It is located in the Jijiga Zone approximately 80 km East of Harar and about 620 km far from Addis Ababa. Its geographical coordinates are 9° 21' 0" North, 42° 48' 0" East.

In Jijiga towns, vending food in the street is common and street fixed food vendors are distributed haphazardly in all parts of the town with the highest density at the center of the of a large market. On the other hand, mobile food sellers are found everywhere in the town.

#### 3.2. SURVEY ON FOOD SAFETY KNOWLEDGE AND FOOD HANDLING PRACTICE OF VENDORS

The survey to evaluate the food safety knowledge and practices of street food vendors within Jijiga town was carried out between Decembers, 2010 to March, 2011. For this study, those of hawkers vending raw meat of the two species-goat and beef within the area of study were included. From the total of 44 raw meat vendors recognized (legally known) by the city administration office (tax payers) and operating in the major, open air market in a fixed place only 33 food vendors were randomly recruited for this study.

Initially, data on street vended food and street food vendors were collected by preliminary survey and informal observation. Secondary data was collected from concerned bodies. Consultations and interviews were also made to key informants. Based on the background information, a questionnaire was developed and pre-tested for clarity and validity on 7 randomly selected street food vendors of the area. These vendors were not included in the final survey.

The questionnaire was prepared originally in English and then translated into Amharic in order to obtain content validity. Finally, the questionnaire was administered in Amharic. Results of the pre-test were used in the revision of the initial survey tool. The final version of the survey tool contained 49 (some of them were observational type) questions which were divided into four

sections: (i) socio-demographic characteristics of the respondents, health and personal hygiene (ii) food handling practices of vendors (hand washing, bathing, smoking cigarette, jewelries, overcoat and/ or gloves) ; (iii) vendors food safety knowledge (source and occurrence of food related illnesses, factors that can affect food safety) (iv) Sanitary facilities and water supply (toilet , receptacles and sanitation of the vending site ).

#### 3.2.1 Ethical Consideration

Informed consent was obtained by reading a statement to prospective respondents seeking permission for the data gathering and affirming that the data would be treated confidentially. Data was collected only after getting willingness of the respondents.

### 3.3. SAMPLE COLLECTION

A total of 60 meat samples (30 from each species) were collected from 30 vendors. A unit of 60-75 g of meat sample from individual vendor was aseptically picked from different groups of meat (trimmings, final ground meat and hooked/clipped meat) to make it as representative as possible at the height of sale and hand touch (from 12:00am - 15 pm) using the vendors' own utensils and hand. Samples were aerobically packed into sterile plastic bags and transported within 15 hrs period after collection to Akilu Lemma Institute of Pathobiology (ALIPB) using an ice box for microbial analysis. Collection of meat samples were within the period from December 2010 to March 2011.

### 3.4. SAMPLE PREPARATION

Twenty-five grams of each meat sample was cut into small pieces using sterile scissors and forceps and then homogenized in a sterile stomacher bag with 225ml of 0.1% peptone water (Oxoid) (AOAC, 1990; Mukhopadhyay *et al.*, 2009). This homogenate was considered as the first dilution. Following, serial 10 fold dilutions ( $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  and /or  $10^{-7}$ ) were made by transferring 1 ml of the homogenized sample to 9 ml diluents. Appropriate dilutions were spread plated in duplicate on various pre-dried solid media for enumeration of microbial groups described in the following section (section 3.5).

### 3.5. MICROBIAL ENUMERATION and ISOLATION OF *SALMONELLA* SPP.

The enumeration of microorganisms in meat was as described in (Diane *et al.*, 2003). Different microbial groups analyzed in this study includes total aerobic mesophilic , coliform , Enterobacteriaceae, staphylococci spp, Lactic acid bacteria, yeasts and molds and the presence of *salmonella* spp. were also assessed.

#### 3.5.1. Aerobic mesophilic count

Total viable counts were enumerated according to the method of (IDF, 1991). Prepared test sample (1 ml) of  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$  and/or  $10^{-7}$  dilutions was transferred separately into sterile petri dishes in duplicate using sterile graduate pipette and/or dispensing pipette (1000  $\mu$ l) with sterile plastic tips and spread plated in duplicates on pre-dried surfaces of Plate Count Agar (Oxoid) plates (*Casein Peptone.5.00g, Yeast Extract.2.50g, Dextrose .1.00g, Bacteriological Agar.15.00g, Final pH  $7 \pm 0.2$  at  $25^{\circ}\text{C}$* ). The culture media were incubated at  $30\text{-}32^{\circ}\text{C}$  for 48 hours. Parallel to that, control plates were also prepared using similar medium (15-20 ml) to check the sterility of media. The dishes containing more than 30 and/or fewer than 300 colonies were selected and counted using colony counter. The result was calculated using the following formula:

$$N = \frac{C}{v (n_1 + 0.1 \times n_2) d} \quad \text{where ...}$$

C: Sum of colonies counted on the dishes retained.

$n_1$  : Number of dishes retained in the first dilution.

$n_2$  : Number of dishes retained in the second dilution.

d : Dilution factor corresponding to the first dilution.

v : The volume added per plate, v , is 0.1 mL

#### 3.5.2. Counts of Enterobacteriaceae

From appropriate dilutions, 0.1 ml aliquots were spread plated in duplicates on pre-dried surfaces of Violet Red Bile Glucose Agar (Oxoid) plates (*Glucose Monohydrate 10.00g, Lactose Monohydrate.10.00g, Gelatin Pancreatic Digest 7.00 g, Sodium Chloride.5.00g, Yeast Extract 3.00g, Bile Salts N° 3 1.50g, Neutral Red. 0.03g, Crystal Violet .0.002g, Bacteriological Agar*

15.00g, Final pH  $7.4 \pm 0.1$  at  $25^{\circ}\text{C}$ ). The seeded culture plates were incubated at  $30\text{-}32^{\circ}\text{C}$  for 20-24 hours after which pink to red purple colonies with or without haloes of precipitation were enumerated as members of Enterobacteriaceae.

#### 3.5.3. Counts of Coliforms

Coliform counts were made according to the method of (Diane *et al.*, 2003). From appropriate dilutions, 0.1 ml aliquots were spread plated in duplicates on pre-dried surfaces of Violet Red Bile Agar (Oxoid) plates (*Yeast Extract* .3.00 g, *Gelatin Peptone* 7.00g, *Bile Salts n° 3* 1.50 g, *Lactose*.10.00g, *Sodium Chloride* 5.00 g, *Bacteriological Agar*15.00g, *Neutral Red* .0.03 g, *Crystal Violet* 0.002g, Final pH  $7,4 \pm 0,2$  at  $25^{\circ}\text{C}$ ). The seeded culture plates were incubated at  $30\text{-}32^{\circ}\text{C}$  for 20-24 hours after which purplish red colonies surrounded by reddish zone of precipitated bile were counted as Coliforms.

#### 3.5.4. Counts of staphylococci spp.

From appropriate dilutions, 0.1 ml aliquots were spread plated in duplicates on pre-dried surfaces of Mannitol Salt Agar (Oxoid) plates (*Sodium Chloride* 75.00 g, *Peptone Mixture* 10.00g, *D-Mannitol* 10.00 g, *Beef Extract* 1.00g, *Phenol Red* 0.025 g, *Bacteriological Agar* 15.00g, Final pH  $7.4 \pm 0,2$  at  $25^{\circ}\text{C}$  ). The culture media were incubated at  $30\text{-}32^{\circ}\text{C}$  for 36 hours after which yellow colonies were counted as staphylococci.

#### 3.5.5. Counts of lactic acid bacteria

From appropriate dilutions, 0.1 ml aliquots were spread plated in duplicates on pre-dried surfaces of MRS (De man, Rogasa, Sharpe) (Oxoid) agar plates (*Dextrose* 20g, *Bacteriological Peptone* 10g, *Beef extract* 8g, *Sodium acetate* 5g, *Yeast extract* 4g, *Dipotassium phosphate* 2g, *Ammonium citrate* 2g, *Tween 80* 1g , *Magnesium sulfate* 0.2g , *Manganese sulfate* 0.05g, *Bacteriological agar* 10g and Final pH  $6,2 \pm 0,2$  at  $25^{\circ}\text{C}$  ). The inoculated plates were placed in anaerobic jar (BBL, Anaerobic Gaspak System) and incubated at  $30\text{-}32^{\circ}\text{C}$  for 48 hours. All colonies were counted as lactic acid bacteria.

### 3.5.6. Yeasts and moulds

Yeasts and moulds were enumerated according to the method of IDF (1990). Pre prepared test sample (1 ml) of  $10^{-1}$ ,  $10^{-2}$  and/or  $10^{-3}$  dilution was transferred into sterile Petri dishes through dispensing pipette with sterile plastic tips and each dilution was spread-plated in duplicates on pre-dried surfaces of potato dextrose agar (PDA) (Oxoid) plates (*Potato Infusion ;solids 4g, Dextrose 20 g, Bacteriological Agar 15g and Final pH 5.6 ± 0.2 at 25°C*) and incubated at 25°C for 5 days.

## 3.6. FLORA ANALYSIS

Flora analysis was performed for the purpose of isolating the dominant genera on the meat samples. After enumeration of aerobic mesophilic bacteria, about 10 to 20 colonies were picked randomly from countable plates and inoculated in to tubes containing about 5 ml Nutrient Broth No 2 (Oxoid). These were incubated at 37°C over-night. Cultures were purified by repeated plating and were characterized to the genus level and various bacterial groups (Aneja, 1993) using the following tests.

### 3.6.1. Cell morphology

From an overnight pure culture, wet mount was prepared on a microscope slide. The preparation was observed under light microscope using oil immersion objective. The morphological criteria considered during the observation were:

Cell shape: -Regular: rods, cocci, Cocco-bacilli , Irregular: branched, coryneforms, pleomorph

Cell arrangement: -Singles, pairs, clusters, chains, and tetrads

Motility: -Motile, non-motile

### 3.6.2. KOH Test

This test was done according to Gregerson (1978). One or two drops of 3 % KOH solution were placed on a clean microscope slide. A colony was picked with a sterile bacteriological wire loop and stirred in the KOH solution for 10 seconds to 2 minutes and the inoculating loop was then raised slowly from the mass. When KOH solution became viscous, the thread of slime followed

the loop for 0.5 to 2 cm or more. Typically, this was observed in Gram negative bacteria. In cases of no slime, the watery suspension did not follow the loop, the reaction was negative and this was seen in Gram-positive bacteria.

#### 3.6.3. Oxidation Fermentation (O/F) test

The utilization of glucose by each isolate was assessed by O/F test as suggested by Hugh and Leifson (1953) to identify microorganism that metabolize glucose fermentatively or oxidatively or that do not utilize glucose by either way.

Ingredients (g/l): Peptone, 2g; yeast extract, 1g; NaCl, 5g; K<sub>2</sub>HPO<sub>4</sub>, 0.2 g; glucose, 10g; bromophenol blue, 0.08g; agar, 2.5g; distilled water, 1000ml, pH, 7.10.

The freshly prepared medium (15ml amounts in 18 x 180 mm test tubes) was immediately cooled under tap water to avoid dissolution of oxygen in the medium and inoculated by stabbing with a sterile straight wire to the bottom. Acid formation and growth regions were interpreted after 2 and 5 days of incubation at 37 °C.

#### 3.6.4. Catalase Test

Young colonies were flooded with a 3% solution of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). The formation of bubbles indicated the presence of catalase.

#### 3.6.5. Cytochrome oxidase test

This test was conducted following the method outlined by Kovacs (1956). Freshly prepared reagent A and B were mixed in the ratio of 2:3 immediately before use. reagents:

A) 1% - naphthol in absolute ethanol

B) 1% N, N- dimethyl -p-phenylene diammonium chloride in distilled water .After flooding the young (24 hours) colonies with the mixture on Nutrient Agar plates, appearance of a blue color on the colonies within 30 seconds to 2 minutes indicated a positive reaction. Any very weak or dubious reaction that occurred after 2 minutes was ignored.

### 3.7. ISOLATION AND CHARACTERIZATION OF *SALMONELLA*

#### 3.7.1. Primary enrichment

To test for the presence of *Salmonella*, 25g of sample was mixed with 225ml Buffered Peptone Water (BPW) for the sake of homogenization. The homogenized solution was incubated at 37°C for 18-24 hours for the metabolic recovery and proliferation of cells which could have been injured during processing or to bring the number of target organisms to a detectable level (Diane *et al.*, 2003).

#### 3.7.2. Secondary enrichment

After pre- enrichment in buffered peptone water, 0.1 ml of culture was transferred in to a tube containing 10 ml of Rappaport – Vassiliadis (RV) broth (Oxoid) and incubated at 42°C for 24 hours. The pre-enriched culture was suspended in the RV broth to inhibit non – targeted microorganisms like Gram – positive bacteria and coliforms and permit the rapid multiplication of *Salmonella*.

#### 3.7.3. Solid Media

Xylose Lysine Desoxycholate (XLD) medium (Oxoid) was used for plating purpose. A loop ful of culture from selective enrichment broth was streaked separately on this medium and incubated at 37 °C for 18 -24 hours. Characteristic colonies from the medium were picked and further purified and tested biochemically. Uninoculated culture plates were incubated to check for sterility of the solid media.

#### 3.7.4. Biochemical Identification

##### **3.7.4.1. Lysine Iron Agar (LIA) (Oxoid)**

The butt was stabbed and the slant was streaked and incubated at 35°C for 24±2hrs to assess oxidative deamination of lysine on the slant and decarboxylation of lysine in the butt. The presence of alkaline (purple) reaction in both butt and slant of the tube was considered presumptive for *Salmonella*.

#### **3.7.4.2. Triple Sugar Iron Agar (TSI) (Oxoid)**

The butt was stabbed and the slant was streaked and incubated at 35°C for 24+2hrs to detect fermentation of glucose and lactose as well as production of H<sub>2</sub>S. The presence of alkaline (red) slant and acid (yellow) butt, with or without production of H<sub>2</sub>S was considered as presumptive for *Salmonella*.

#### **3.7.4.3. Urea Agar (Oxoid)**

The slant was streaked and the tube was incubated at 35°C for 24+2hrs to assess the hydrolysis of urea. The presence of no color change in the slant was considered as negative and thus presumptive for *Salmonella*.

#### **3.7.4.4. Simon's Citrate Agar (Oxoid)**

The slant was streaked and the tube was incubated at 35°C for 24+2hrs to investigate utilization of citrate as a sole source of carbon. The presence of growth and color change from green to blue was considered as presumptive for *Salmonella*.

#### **3.7.4.5 Motility test medium (Oxoid)**

The media in the test tube was stabbed in the center and to 2/3 the depth of motility test medium. Diffuse circular bacterial growth from line of stab was considered as a positive test for presumptive *Salmonella* spp. after 24 h incubation at 35°C, with tightly closed test tube (FDA's Bacteriological Analytical Manual, BAM 1998)

### **3.8. MEASUREMENT OF pH**

The pH value of each meat sample was determined using digital laboratory pH meter (CG843P, Schott Germany). Ten gram of meat sample was sliced and chopped into pieces using sterile scissors. Chops were blended with 90 ml distilled water in a stomacher bag. The probe of the digital pH meter was inserted to the geographical center of the suspension and pH reading was recorded.

### 3.9. MEASUREMENT OF HOLDING TEMPERATURE

The holding temperatures of each meat sample were measured at the spot of collection. A sensing bulb of a laboratory thermometer was inserted at the geographical center of the meat sample and awaited until the temperature of the food item was stabilized. The reading was recorded as holding temperature in °C.

### 3.10. STATISTICAL ANALYSIS

Data from the questionnaire were analyzed using SPSS for window (version 15.0). All Microbial counts were converted to  $\log_{10}$  cfu  $\text{g}^{-1}$  values. Difference in microbial counts among meat samples of the two varieties was analyzed by analysis of variance (ANOVA). Significance was determined at the 5% level.

## 4. RESULTS and DISCUSSION

Vending foods in the street is common in many parts of Ethiopia. Consumers are entitled to quality products, which are safe and wholesome. However, there is no information regarding the quality and safety of street vended raw meat in Ethiopia. In general, the majority of samples investigated in our study yielded high microbial load. The food handling and processing operations, personal hygiene of the vendors and sanitary condition of the vending environment may contribute for the presence of high microbial load in the meat samples. Furthermore, the lack of municipal (running) water, waste disposal and cooling facilities at the vending sites worsened the situation.

### 4.1. SOCIO-DEMOGRAPHIC CHARACTERISTIC OF STREET VENDORS

Survey results indicated that the majority of the food vendors were females (78.8 %). The age of both sex vendors was in the range of 26-40 years with majority (58 %) fell between 26 and 40 years (Table 2). With regard to education, only 30.3% of the vendors were literate (elementary school) (Table 2). More than 80% of street vendors included in this study were married and most of them (58%) were involved with vending meat for 5-10 years (Table 2). Idowu and Rowland (2006) reported that in countries like Nigeria, Ghana, Uganda, and Botswana, the majority of vendors are women who balance the income-generating opportunities of street vending with traditional household and child care duties. On the other hand, Muinde and Kuri (2005) have reported that 60% of the vendors surveyed in Nairobi were male. Although the quality and safety of raw meats sold by males and females was not assessed in our study, however, Ohiokpehai (2003) reported that female vendors sold food of better quality than their male counterparts. Klontz *et al.*, (1995) also reported that in the United States, safer food preparations were consistently reported by persons who were female, at least 40 years old, with at least high school education and experienced with the sector. In this survey the experience and the age consistent with as indicated by Klontz *et al.*, (1995). However, there were significant percentage of youngsters under the age of 16-25 and inexperienced (0-4 yrs) vendors had also participated at vending activities in addition, their higher percentage of illiteracy would influence the good handling practice so does the safety of raw meat.

Table 2 : Socio-demographic characteristics of raw meat vendors in Jijiga Town.

<b>Characteristics</b>	<b>Frequency</b>	<b>Percent</b>
Sex - Male	7	21.2
- Female	26	78.8
Age Range 16-25	5	15
26-40	19	58
41-53	6	18
51-60	3	9
Educational Status		
Illiterate	23	69.7
Literate - Elementary	10	30.3
Marital Status		
Single	6	16.2
Married	27	81.8
Service Year in meat Vending		
0-4	14	42
5-10	19	58
>10 Years	0	0

#### 4.2. VISUAL ASSESMENT

On the survey, it was observed that the sanitary condition of the vending environment was poor as it was dusty and full of remains of slaughtered animals such as bones, horn, head and other body parts. House flies were also very prevalent throughout the vending area and even on the raw meats displayed for sale by street vendors. The presence of animals, insects, liquid waste and solid waste in all of food vending areas is similar to a study conducted elsewhere (FAO, 1988). The linkage between houseflies and diarrheal diseases has been also documented (Smith, 1998).

All street vendors included in our study had no access to clean potable water. Obligated by the situation, they simply reused the water that they brought from their home. Reused water would have dissolved organic material in it to serve as a 'culture medium' favoring the growth of array of microorganisms including pathogens (FAO, 1995; Bryan *et al.*, 1992c). For instance, in Ibadan, Nigeria, water was considered to be the major source of food contamination (FAO, 1995).

During the survey, it was also observed that the raw meats were displayed for more than 6 h for sale at ambient temperature in a table or a carton which would be used again with out cleaning. Moreover, it was also observed that the raw meats were displayed uncovered. It has been mentioned that holding foods for more than 4–6 h is one of the main contributing factors of high possible microbial counts (El-Sherbeeny *et al.*, 1985; Bryan *et al.*, 1992a, b, c). Deriba Muleta and Mogessie Ashenafi (2001) also indicated that foods that are held at ambient temperatures of 15–45°C for more than about 4 hours present a considerable public health risk.

#### 4.3. FOOD HANDLING PRACTICES OF RAW MEAT VENDORS

Survey conducted showed that levels most variables of the study were not satisfactorily accorded by the vendors (Table 3). Most vendors (79%) had hair covers but none of them wore appropriate over coat. Only 67% of the vendors had good personal hygiene with respect to cleanness of their cloths and visible body parts. About 27% of the vendors had fingernails which were not trimmed and cleaned. The majority (70%) of the food handlers wore jewelry on their hands, ear and different body parts. Painting of finger nails was also as high as 76% among street vendors.

Table 3: Food handling practice of raw meat vendors in Jijiga Town.

Practices	Number	Percent
Wear of over coat		
- yes	0	0
- no	33	100
Wear of hair cover		
- yes	26	79
- no	7	21
Personal hygiene		
- clean	22	67
- dirty	11	33
Trimmed and cleaned nail		
- yes	24	73
- no	9	27
Wear of jewelry		
- yes	23	70
- no	10	30
Nail painting		
- observed	25	76
- not observed	8	24
Hand washing		
- yes	25	76
- no	8	24

All food handlers have a basic task to maintain a high degree of personal cleanliness and observed hygienic and safe food handling practices. Keeping hands clean, shortening fingernails, wearing clean working garment and hair cover (hair net and cap) are some of the precautions that a food handler must maintain (Kinfu Zeru and Abera Kumie, 2005). However, none of raw meat street vendors evaluated in our study wore appropriate working garment (over coat). The majority (70%) of street vendors wore jewelry on their hands, ear and different body parts. This number was very high compared to street food vendors assessed in other areas of Ethiopia such as Mekele

(35.7%) and Awassa (28.7%) (Kinfe Zeru and Abera Kumie, 2005). Thus the culture might have also its own effect for food safety in relation to jewelries and clothing.

Several studies have shown that skin under rings is more heavily colonized by microorganisms compared to fingers without rings (Jacobson *et al.*, 1985).

About 27% of street vendors assessed in our study had dirty hands with untrimmed finger nails. Hands are the most important vehicle for the transfer of organisms from faeces, nose, skin or other sites to food (WHO, 1984). Epidemiological studies of *Salmonella typhi*, *non-typhi salmonellae*, *Campylobacter* and *Escherichia coli* have demonstrated that these organisms can survive on finger tips and other surfaces for varying periods of time and in some cases after hand washing (Pether and Gilbert, 1971; WHO, 1984). Hands are important agents when it comes to transmitting microorganisms and intestinal parasites to food. Therefore they should always be washed before starting work, immediately after using the bathroom, after handling contaminated material or any other material that could possibly transmit diseases, and whenever necessary (Goh *et al.*, 1993). WHO (1984) also indicated that food vendors should wash their hands in hot soapy water before preparing or touching foods and after using bathroom. However, washing hands was not a common practice by raw meat street vendors in Jijiga town. Absence of clean water and washing facilities in the vending environment and lack of awareness of the vendors about food handling and safety might be possible reasons for the poor handling practice of vendors observed in this study. Van-Kampen *et al.* (1998) reported that the lack of available hand washing facilities and poor knowledge concerning hygiene were correlated with improper food handling practices of street food vendors in Jakarta, Indonesia. On the other hand, a study conducted by Azanza *et al.* (2005) in Philippines showed that street vendors had good practice of washing hands during handling foods due to the relatively high level of knowledge in hand washing and the availability of a number of hand washing facilities within the area.

#### 4.4. KNOWLEDGE OF STREET RAW MEAT VENDORS ABOUT FOOD SAFETY

With regard to knowledge of street vendors about food safety, it was found that only 27 % of food vendors are familiar with the term – food borne illness (Table 4). Mass-media was the first source of information for 5(56%) of the respondents and the remaining get informed from health

professionals (Table 4). Street vendors were also asked about the possible ways food contaminated and about 36% of them responded that food can be contaminated if exposed to flies (Table 4). Handlings in contaminated environment or air and use of dirt equipment during processing were mentioned as sources of contamination by 24% and 21% of the respondents respectively (Table 4). However, none of the respondents were aware of using of the same container for cooked and raw foods as a possible source of food contamination (Table 4).

Table 4: Food contamination knowledge exhibited by street raw meat vendors in jijiga Town.

Knowledge	Frequency	Percent
Aware of forborne diseases		
- yes	9	27
- no	24	73
If yes, source of information		
- mass media	5	56
- health professionals	4	44
- formal training and written display	0	0
How can foodborne diseases be transmitted?		
- contaminated foods	3	33
- contaminated hands	2	22
- contaminated water	1	11
- vectors	2	22
- I do not know	1	11
How food can be contaminated?		
- when exposed to flies	12	36
- handling in contaminated environment or air	8	24
- using of dirt equipment	7	21
- using of contaminated water for cleaning and food preparation	5	15
- dirt hands	1	3
- using of the same container for cooked and raw foods	0	0

Awareness of food handlers about food born illness, food safety and possible sources of food microbial contamination was also very low as all the vendors indicated only one possible source of contamination out of six possible sources of microbial contamination listed in the questionnaire.

Dugassa (2007) indicated that controlling the overall sanitary condition of the food handling area, health status of workers and raising the awareness of food handlers has great roles in improving food safety and prevention of food borne illness and its transmission.

## 4.2. MICROBIOLOGICAL ANALYSIS

### 4.2.1. pH and Holding Temperature of Meat Samples

The pH values of raw beef samples measured varied between 5.20 and 7.1 with a mean of  $6.03 \pm 0.51$  and pH value of raw goat meat varied from 5.2 up to 7.0 (with a mean  $5.98 \pm 0.51$ ) (Table 5). Mean pH values for all meat sample types ranged between 5.98 and 6.03.

The mean temperature reading at the sampling point was  $23.9^{\circ}\text{C}$  and  $24.0^{\circ}\text{C}$  for the raw beef and raw goat meat samples respectively (Table 5).

Table 5: pH and holding temperature ( $^{\circ}\text{C}$ ) values of street vended raw beef and goat meats samples in Jijiga Town.

Species	pH Units			Temperature ( $^{\circ}\text{C}$ )		
	Range	Mean	SD	Range	Mean	SD
Raw beef	5.20-7.10	6.03	0.51	19.30-27.50	23.98	2.26
Raw goat	5.20-7.00	5.98	0.51	17.50-27.00	24.06	2.55

These mean pH values might make these products susceptible to bacteria as well as mold and yeast spoilage (Jay, 1996) and could allow the multiplication of several bacterial pathogens (Ferrari *et al.*, 2002). Freese *et al.*, (1998) also indicated that pH above 4.4 and 5.0 would promote growth of pathogens.

The raw meat samples analyzed in this study were within a temperature range of  $17.5-27.5^{\circ}\text{C}$  during the time of vending and the raw meats could be displayed for more than 6 hours. Food that is not maintained within the safety temperature zone acts as an incubator for pathogenic bacteria whether the food is raw, partially cooked, or fully done (Roller, 1999). According to Van Kampen

*et al.* (1998) and Joseph and Doser (1999) time–temperature abuse was considered particularly potentially hazardous and initiate microbial proliferation. Freese *et al.* (1998) also indicated that storing foods at a temperature range of 15–47°C could promote growth of pathogens. A reduction of temperature of raw meat to below 7°C is required to prevent proliferation of pathogenic bacteria (Lovatt *et al.*, 2006).

#### **4.2.2 Microbial spectrum of street vended raw beef and raw goat meat samples**

The mean values of aerobic mesophilic counts of street vended raw meat obtained in this study were 8.07 log cfu/g (ranged from 6.20 to 9.40 logcfu/g) and 7.59 log cfu/g (rang 6.00-9.00 log cfu/g) for raw beef and raw goat meat, respectively (Table 6). These mean values were relatively higher than that reported by Okonko *et al.* (2009) for fresh meats sold in Calabar metropolis, Nigeria which had a mean aerobic mesophilic count of 4log cfu/g. Comparable results with our study were reported by Kumar *et al* (2010) for raw beef meat marketed in some parts of Tigray region as samples had areobic mesophilic counts >7logcfu/g. According to Jay (2005) foods kept at ambient temperature, will stimulates the growth of aerobic mesophilic organisms, including most of the pathogens. Thus, high aerobic mesophilic count recorded in this study might reflect the time temperature abuse during displaying the meats for sale. (ICMSF, 1980) also indicated that high total bacterial count might be attributed to the contamination of the product from different sources or un-satisfactory processing as well as it may be due to un-suitable temperature during storage.

Although, there are no standards or guidelines regarding the microbial contamination of street vended raw meat in Ethiopia, HPA (2009) indicated that aerobic mesophilic count must be < 7 log cfu/g for raw meats. However, in this study the mean counts of raw beef and raw goat meat samples had 8.07 and 7.59 log cfu/g respectively. These mean values, thus exceeded the typical guideline for aerobic mesophilic count. Total bacterial count is considered an index of quality, which gives an idea about the hygienic measures during processing and helps in the determination of the keeping quality of the product (Aberle *et al.*, 2001). Comparable results were also reported by Mukhopadhyay (2009) as most of goat meat and beef meat samples showed aerobic plate counts above 7.00 log cfu/g. Thus, it can be also said that most of the meat samples analyzed in

this study were in a condition at which spoilage of meat can occur since they had aerobic mesophilic counts greater than 7logcfu/g (Warriss, 2001). Thus the city administration and /or the regional health office should take appropriate measures so as to protect the consumers from possible health risks.

Table 6: Microbial counts (log cfu/g) of street vended raw beef and goat meat samples in Jijiga town.

Microbial Groups	Raw beef meat			Raw goat meat		
	Mean	S.D.	Range	Mean	S.D.	Range
Aerobic Mesophilic Bacteria	8.07	0.75	6.20-9.40	7.59	0.76	6.00-9.00
Enterobacteriaceae	4.45	1.31	2.00-6.90	4.10	1.14	2.30-6.60
Coliforms	4.71	1.32	2.30-7.70	4.31	1.12	2.00-6.10
Staphylococci	6.74	0.37	5.80-7.50	6.23	0.40	5.30-7.00
Lactic Acid Bacteria	5.16	0.88	3.30-7.40	4.82	0.81	2.90-6.40
Yeasts & Moulds	4.62	1.06	2.70-7.00	4.66	0.87	2.30-6.10

S.D.-standard deviation

Enterobacteriaceae and coliforms were frequently encountered in the samples. The mean counts of Enterobacteriaceae and coliforms in raw beef samples were 4.45 and 4.71 log cfu/g respectively. And the mean counts of Enterobacteriaceae and coliforms in raw goat meat samples were 4.10 and 4.31 log cfu/g respectively (Table 6). The mean counts of raw beef and raw goat meat samples had counts of Enterobacteriaceae and coliforms as high as log 4 cfu/g (Table 6). Comparable Enterobacteriaceae counts were also reported by Khalafalla *et al.* (1993) for ground beef meat samples. However, the mean values of our samples were higher than that reported by Mehmet *et al.* (2005) for ground beef samples in Turkey which had mean count of Enterobacteriaceae and coliforms as low as 3 log cfu/g. Similarly, Westhoff and Feldstein (1976) reported an average coliforms count of 2 log cfu/g for ground beef meat. According to Cathy (1997) and HPA, 2009 a raw meat is categorized as unacceptable if the count of Enterobacteriaceae and coliforms is  $> 4 \log \text{cfu/g}$ . Based on this, it can be said that both species of meat samples were found to be unacceptable as they had counts of these microbial groups  $> 4 \log \text{cfu/g}$ . The presence of such high counts in the investigated samples could indicate time/temperature abuse during handling or inadequate storage and displaying conditions during sale. As these microbial groups are safety indicators, the presence of high counts may indicate possible presence of pathogens (Jay, 1996).

Both raw meat species samples analyzed in this study had staphylococci counts  $\geq 6 \log \text{cfu/g}$ . The mean counts of staphylococci obtained from raw beef and raw goat meat samples were 6.74 log cfu/g (range 5.80-7.50 log cfu/g) and 6.23 log cfu/g (range 5.30-7.00 log cfu/g) respectively. Comparable results were obtained for ground beef by Tekinsen *et al.* (1980). However, the mean values of our samples were by far greater than that reported for ground meat obtained at retail (2 log cfu/g) (Mehmet *et al.*, 2005). Khalafalla *et al.* (1993) also reported lower counts of staphylococci (3 log cfu/g) for ground beef meat samples. Staphylococci are common in unprocessed animal products and in products handled by bare hands. The high count of staphylococci in our meat samples indicates the presence of cross contamination, which is usually related to human skin, hand touch, discharge from human and clothing because of faulty

handling activities, as they are typical contaminants from hands, clothes and utensils (Postgate, 2000).

Counts of lactic acid bacteria recorded in this study were also high with mean counts as high as log 5.16 cfu/g for raw beef meat and 4.82 log cfu/g for raw goat meat. The presence of such high counts of lactic acid bacteria (LAB) in this study might indicate improper handling of the meats and inadequate storage conditions. Since lactic acid bacteria (LAB) are spoilers (Jay, 2005) the presence of such high counts in the samples may limit their keeping quality.

The mean count of yeasts and moulds from the two species of raw meat was recorded as log 4.62 cfu/g (range 2.70-7.00 log cfu/g) for raw beef meat and log 4.66 cfu/g (rang 2.30-6.10 log cfu/g) from raw goat meat ( Table 6). A study on microbial quality of fresh goat meat and beef meat in retail outlets in Pondicherry, India by Mukhopadhyay *et al.* (1998) found that yeast and mould counts as low as 2logcfu/g. Lower counts of yeasts and molds (1logcfu/g) were also reported by Mehmet *et al.* (2005) for ground beef samples in Turkey. Jay (1996) described yeasts and moulds as spoilage organisms of meat which are usually contributed by dryness of outer parts of meat. Therefore, the presence of such high counts of yeasts and moulds in the raw meats analyzed in this study may indicate limited keeping quality of the meats which might be either in the market or in consumers' home.

#### 4.2.3. Comparison of microbial quality of raw beef and raw goat meat samples

Statistical analysis with one-way ANOVA revealed that there were significant differences ( $P < 0.05$ ) between goat and beef samples regarding aerobic mesospheric count and *staphylococci* spp (Table 7). These differences may be explained by personal hygiene, individual difference in awareness to safe food handling practice, displaying period and intrinsic characteristics of the two meat species.

It was found that raw goat meat samples had relatively lower mean counts of aerobic mesophiles and staphylococcus. Although statistical analysis of the data didn't show variations in the counts of other microbial groups ( $P > 0.05$ ) from the raw data it can be also said that goat meat samples had relatively lower counts of Enterobacteriaceae, coliforms and yeasts and moulds compared to beef samples. In contrast to our finding, Selvan *et al.* (2007) reported that the mean total viable

count was significantly greater in goat meat than other products (chicken and beef) studied in Chennai City, India. Another study in India by Mukhopadhyay *et al*, (2009) also indicated that coliform count was slightly lower in beef than goat meat samples (mean 5.84 logcfu/g and mean 6.40 logcfu/g). The presence of low microbial counts in raw goat meat samples compared to raw beef samples in this study can be explained by the relatively short display time of goat meat at retail due to consumer preference for goat meat. In addition to this, trimming and cutting which usually enhance microbial contamination was minimized during sale of goat meat compared to beef meat.

Table 7: ANOVA for microbial counts (cfu/g) of raw beef and raw goat meat samples collected from street vendors in Jijiga Town.

<b>Bacterial Groups</b>	<b>Log cfu/g (Mean+S.D.)</b>	
	Raw beef meat	Raw goat meat
Total Mesophilic Bacteria	8.07±0.75 <sup>a</sup>	7.59±0.76 <sup>b</sup>
Total Coliform	4.45±1.31 <sup>a</sup>	4.10±1.14 <sup>a</sup>
Enterobacteriaceae	4.71±1.32 <sup>a</sup>	4.31±1.12 <sup>a</sup>
Staphylococci	6.74±0.37 <sup>a</sup>	6.23±0.40 <sup>b</sup>
Lactic Acid Bacteria	5.16±0.88 <sup>a</sup>	4.82±0.81 <sup>a</sup>
Yeasts and Moulds	4.62±1.06 <sup>a</sup>	4.66±0.87 <sup>a</sup>

In rows followed by the same letters are not significantly different ( $P > 0.05$ )

#### 4.2.4 Frequency distribution of dominant bacterial groups in raw beef and goat sample

A total of 302 bacterial groups (149 isolates from raw beef and 153 isolates from raw goat meat) were isolated and characterized to various genera and bacterial groups. Generally, in both types of meats, the aerobic mesophilic flora was dominated by *staphylococci* followed by enterobacteriaceae and other gram positive rods (Table 8). Specifically the aerobic mesophilic bacterial flora of raw beef meat was dominated by *Staphylococcus spp.* (52 %) followed by *Enterobacteriaceae* (22.2 %), other gram positive rods (7.4%) and *Micrococcus spp.* (6.7 %) (Table 8). The aerobic mesophilic flora of raw goat meat was also dominated by *Staphylococcus spp* (47.7%) followed by *Enterobacteriaceae*, (23.5 %) and other gram positive rods (10.5 %) (Table 8). *Pseudomonas spp*, *Alcaligenes spp*, *Acinetobacter spp*, *Aeromonas spp* were also among the aerobic mesophilic bacterial groups isolated in beef and goat meat samples although they were not significant in their number (Table 8).

Generally, the aerobic mesophilic flora of both types of raw meat samples evaluated in our study were dominated by staphylococci spp followed by Enterobacteriaceae and other gram positive rods. Deriba Muleta and Mogessie Ashenafi (2001) reported that the microflora of 'kitfo' a traditional Ethiopian spiced, minced meat samples collected from street vendors in Addis Ababa were also dominated by various bacterial genera, *Staphylococcus spp.*

Isolation of *Staphylococcus spp.* and Enterobacteriaceae from the street vended meat can be worrying because certain strain of these bacteria cause food-borne infections (Mogessie Ashenafi, 1994). Thus, the raw meat samples investigated were under question from food safety point of view.

Table 8: Frequency distribution of dominant bacteria in meats collected from street vendors in Jijiga town.

<i>Meat type</i>	<i>No. Of isolates</i>	<i>Staphylococcus spp</i>	<i>Micrococcus Spp</i>	<i>Other gram + ve rods</i>	<i>Enterobacteriaceae</i>	<i>Pseudomonas spp</i>	<i>Alcaligenes spp.</i>	<i>Acinetobacter spp.</i>	<i>Aeromonas spp.</i>
Beef	149	77(52)	10(6.7)	11(7.4)	33(22.2)	4(2.7)	7(4.7)	4(2.7)	3(2.0)
Goat	153	73(47.7)	11(7.2)	16(10.5)	36(23.5)	3(2.0)	10(6.5)	3(2.0)	1(0.7)
Sum	302	150(49.7)	21(7)	27(8.9)	69(22.8)	7(2.3)	17(5.6)	7(2.3)	4(1.3)

Numbers in parentheses represent percentage and number outside indicates the number of isolates.

#### 4.2.5. Isolation and identification of *Salmonella* spp.

From a total of 60 (30 beef and 30 goat) meat samples examined in this study, five (8.3 %) samples (3 beef and 2 goat meat) were found positive for *Salmonella* (Table 9).

Table 9: Prevalence of *Salmonella* in raw beef and goat meat samples in Jijiga town.

Meat type	Number of Samples	Number of positive Samples	Incidence (%)
Raw beef meat	30	3	10
Raw goat meat	30	2	6.7
Total	60	5	8.3

*Salmonella* was isolated from 5 meat samples (8.3%) (3 from goat meat and 2 beef samples). This was higher than that reported in Jimma town by (Tasew Haimanot *et al.*, 2010) for minced meat in which rate of salmonella isolation was 2(1.2%). However, our samples had lower prevalence of salmonella compared to other findings where rate of isolation from raw meat at retail was 20% in Gaborone, Botswana (Mrema *et al.*, 2006), 9% in raw meat obtained from butchers shop in Awassa, Ethiopia (Mogessie Ashenafi ,1994) and 42 % from raw “kitfo” (minced meat) in Addis Ababa (Mezgebu Tegegne and Mogessie Ashenafi, 1998). The variation in the prevalence of *Salmonella* contamination could be partly due to differences in sample type, sampling techniques, distribution of salmonellae in a lot examined and the detection methods employed (Bryan and Doyle 1995; Dominguez *et al.*, 2002). Low *salmonella* detection in our study could be due to the fact that *salmonella* usually contaminates chicken and water than beef and goat meat (Maharjan *et al.*, 2006). ICMSF (1980) also indicated that salmonellae are found more often in pork and in veal from young calves than in other meats. More over consumers in the study area are not much acquainted for eating raw meat; this in turn might play its role in reduction of the prevalence by minimizing *salmonella* carrier food handlers (Nyeleti *et al.*, 2000 and Baylegnege Molla *et al.*, 2003).

In general, the majority of raw meats considered in this study had high microbial load and even pathogens like *Salmonella* were isolated. Time/temperature abuse during vending on the street or cross contamination due to improper handling of meat or inadequate displaying conditions or a combination of these factors might contribute for the presence of high microbial counts. Furthermore, the absence of clean potable water and receptacles, and also the poor sanitary condition of the vending area revealed inadequacies concerning quality and safety of the meats analyzed in this study.

## 5. CONCLUSIONS and RECOMMENDATIONS

Based on the finding of this study, the following conclusions and recommendations are forwarded:

### 5.1. CONCLUSIONS

1. The study revealed that the majority of vendors do not have the required knowledge about food safety and possible sources of food microbial contamination. Personal hygiene of food handlers and the sanitary condition of the vending environment was generally poor. This may cause food borne disease outbreaks as many of them did not know how these diseases can be transmitted.
2. The high microbial load of raw meats recorded in this study indicated that the level of contamination was very high. This might be attributed to unhygienic and improper handling of animals during slaughter, dressing and evisceration, displaying the meats in open air, processing of the meats using unclean knives, cutting boards, lack of infrastructures (potable water, refrigerators, hand washing facilities, etc.), and the poor hygienic status of food handlers as well as unsanitary condition of the vending environment.
3. Raw meat samples analyzed in this study had pH values greater than 6 and their temperature at the time of vending was in the range of 17.5-27.5°C. These conditions could favor the rapid growth of different microorganisms on these meat samples.
4. Although the prevalence of salmonella recorded in this study was low, the presence of these organisms in meat should receive particular attention, because their presence indicate public health hazard and give warning signal for the possible occurrence of food borne intoxication. The poor food handling practice of street raw meat vendors would also favor multiplication of the pathogen and cross contamination to other cooked or uncooked food items.
5. The aerobic mesophilic flora of raw meats investigated in this study was dominated by *staphylococci* spp. followed by Enterobacteriaceae and other gram positive rods. Although these genera (*Micrococci*, *Staphylococci*, *Bacillus*, oryneforms, Enterobacteriaceae, *Flavobacteria*,

Pseudomonads, lactic acid bacteria (LAB) and *Brocothrix* ) are abundant in livestock, the high prevalence rate *staphylococci* in raw meat indicates the presence of cross contamination, which is usually related to human skin, hand touch, discharge from human and clothing because of faulty handling activities, as they are typical contaminants from hands, clothes and utensils

## 5.2 RECOMMENDATIONS

1. Periodic training about basic principles of hygiene and food safety (possible sources of microbial contamination and food borne diseases) should be given to raw meat street vendors.
2. Food safety cannot be reasonably assured unless basic infrastructures and services are available for street vendors. Therefore the government needs to avail basic infrastructures (such as potable water, hand washing facilities, refrigerators, electricity, waste disposal services, good drainage, and toilets) for street vendors.
3. The vendors also should be organized to clean and sanitize the vending environment.
4. Periodical inspection of the raw meats should be practiced by concerned bodies to protect the public from possible health hazards from eating either raw meat or undercooked meat. More over establishment of code of practice for the street food industry is also recommended.

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APPENDIX –A

***English Version Questionnaire for Data Collection on the Overall Hygienic Practices, Food Safety knowledge & Facilities of Selected Raw meat vendors in Jigjiga town.***

***Identification*** \_\_\_\_\_

***Verbal consent form before conducting interview***

***Introduction;***

My name is \_\_\_\_\_ I am a post graduate student in Addis Ababa University & working a research, entitled Microbiological quality and safety of street vended raw meat in Jigjiga town. The finding of the study will be used as a basis for better planning of sanitary conditions and safe food vending in the market. Therefore, I am requesting you to respond honestly for interview questions and I need your willingness to take the data and to observe the conditions and the equipments used for food preparation and service; your response is completely confidential; your name will not be written and the result will not be given to a third body.

Your participation is voluntary and you are kindly requested to answer every question and you may stop at any time you want however your honest answers to interview questions and willingness will help us better understand at what condition that the raw meat vendor are found in Jigjiga town.

Are you willing to participate?

If yes, proceed to the next page,

Name of interviewer \_\_\_\_\_ signature \_\_\_\_\_

Date of interview \_\_\_\_\_ time started \_\_\_\_\_ time finished \_\_\_\_\_

Supervisors name \_\_\_\_\_ signature \_\_\_\_\_

## INSTRUCTION

The questionnaires have interview and observational types.

### For selected/ sampled raw meat vendors

#### 1 - General information

101 – Sex 1. Male 2. Female

102–Age year\_\_\_\_\_

103 -Marital status

1. Single 2. Married 3. Divorced 4. Widowed 5. Separated

104 - Educational status 1. Illiterate 2. Literate (grade\_\_\_\_\_)

105 - For how long have you been practicing raw meat vending?

Year\_\_\_\_\_month\_\_\_\_\_

106 - Had you been given any training about sanitary handling of food?

1. Yes 2. No

107 - If you had been given any training, who gave you the training?

1. Formal certification from approved organizations

2. Town health office

3. Others (specify\_\_\_\_\_)

108 - Have you ever suffered from disease symptoms like vomiting, diarrhea, skin infection?

And continuous coughing during the period of your food handling?

1. Yes 2. No

109 - If you have suffered from any of these disease symptoms Status of sick leave and resting after suffer of these diseases.

1. Taken rest till become free of sign & symptoms

2. Not taken rest and have been on their normal work

3. Others\_\_\_\_\_

110 - How do you supervised with health experts on normal work of food handling

Practice? 1. Regularly supervised 2. Intermittently Supervised

3. Never supervised

## **2 - Observational type of practice related question for selected raw meat vendors**

111 - Does the worker wear appropriate over coat? 1. Yes 2. No

112 - Does the worker wear appropriate hair cover? 1. Yes 2.No

113 – Are nails short, trimmed and clean? 1. Yes 2.No

114 -Cleanness of over coat and visible body during visit 1.Kept 2. Not kept

115- Discharging from nose, eye, ear and cough during visit

1. Not observed 2. Observed

116 - Any visible skin rash, skin boils, cut and wound at time of visit

1. Not observed 2. Observed

117 - If any visible cut and wound seen,

1. Plastered with water impermeable bandage

2. Openly left

3.Others (specify\_\_\_\_\_)

118 -Wear of any jewelry or ring at time of visit

1. Observed 2. Not observed

119 - Is nail paint observed? 1. Yes 2. No

### **2.1 - Practice related interview**

120 - Have you washed your hands before starting work today?

1. Yes 2 . No

121- Where is the source of the food?

1. From carcass delivering bodies

2. From individual slaughters

3. From our home slaughtering

122 – When you deliver the food to the market?

1. Soon after the slaughter

2. after one day

3.Others specify\_\_\_\_\_

123 – How could you transport the food to the market?

1. by people

2. by animals back

3. by track

4. Others specify\_\_\_\_\_

124. Have you ever faced with any left over foods?

1. Yes 2. No

125. If yes, what do you do for the left over foods?

1. Redisplayed again for the coming day

2. Throw away

3. Eaten at home

4. No answer/no left-over

3. Specify if other\_\_\_\_\_

126. Do you smoke cigarette? 1. Yes 2. No

127. Do you smoke at the time of food vending? 1. Yes 2. No

128 - If yes, after smoking, do you wash your hands before handling food during your food handling practice? 1. Yes 2. No

### **3 - Knowledge related questions to raw meat vendors**

129 - Have you ever heard about food borne disease? 1. Yes 2. No

130 - If you heard about food borne disease, what is your source of information?

1. Formal training certification

2. Health professionals

3. Written display.

4. Mass Medias

5. Others (specify\_\_\_\_\_)

131 - How can food borne disease be transmitted? (Circle all responses)

1. Contaminated food 2. Contaminated hand

3. Contaminated water 4. Vectors

5. I don't know 6. Other (specify \_\_\_\_\_)

132 - How can food be contaminated (what factors can affect cleanness /safety of foods)?

(Circle all responses)

1. Exposure to flies

2. Handling in contaminated environment (area)

3. using of dirt equipment
4. using of contaminated water for equipment washing and preparation
5. Dirt hands
6. Using the same container for cooked and raw foods.
7. Other (specify\_\_\_\_\_)

**4 –Sanitary facilities and water supply - water supply**

133 – Is there any water for vendor’s usage?

1. Yes
2. No

134- If yes what is the source of water?

1. Privately instilled from municipal supply
2. from communal distribution
3. Buy from privately instilled pipe
4. Others (specify\_\_\_\_\_)

**4.1 – Toilet**

135. Is there any toilet near by the market? 1. Yes      2. No

136 – If yes the types of toilet

1. Flush type
2. Dry pit latrine
3. No latrine
4. Other (specify\_\_\_\_\_)

**4.2 - Utensils and conditions of equipment**

137 -Way of Cleaning and sanitizing of utensils

1. Hot and cold water and detergent used for cleaning
2. Only cold water with detergent used
3. Only hot and coldwater used
4. Only cold water used
5. Only local soap and cold water used

138 - Are utensils and equipment stored in containers, on shelves under conditions which can protect against contaminations? 1. Yes      2. No

**4.3 - Solid waste handling and disposal**

139 - Are appropriate refuse receptacles placed in appropriate place?

1. Yes
2. No

140 - If refuse receptacles placed, is it durable type? 1. Yes 2. No

141 - Are the receptacles fit to cover and tight? 1. Yes 2. No

142 - Are the receptacles filled and splashed in the area in a manner that can aid spreading of flies?

1. No 2. Yes

143- Is the refuse transported to final disposal before over filling?

1. Yes 2. No

144 -Where is the refuse disposed at final?

1. Supplied to municipal service 2. Burn at site (open burn)

3. Disposed on street 4. Thrown in to rivers

5. Other

#### **4.5 - Liquid waste collection and disposal system**

145 - Is there installation of drainage system for collection and handling of liquid waste?

1. Yes 2. No

146 - If drainage system present, what type?

1. Closed type which can collect all generated liquid waste

2. Open trench that can collect fraction of generated waste

3. Other (specify\_\_\_\_\_)

147 – Where is liquid waste disposed at final?

1. Open dumping in the area 2. To septic tank

3. Dump in Latrine 4. Discharge in to the river

5. Other (specify\_\_\_\_\_)

149 - Is there stagnation of liquid waste due to blockage or careless handling which can aid fly?

Breeding and can affect sanitary condition of the market?

1. Yes 2. No

**Remarks**\_\_\_\_\_

\_\_\_\_\_

## APPENDIX – B

### One way ANOVA Table and Descriptive statistics

**Table 1- One way ANOVA**

Bacterial groups	Differences	Sum of Squares	df	Mean Square	F	Sig.
Total mesophylic bacteria	Between Groups	3.408	1	3.408	5.927	.018
	Within Groups	33.354	58	.575		
	Total	36.762	59			
Total coliform	Between Groups	.267	1	.267	.177	.675
	Within Groups	87.219	58	1.504		
	Total	87.486	59			
Enterobacteriaceae	Between Groups	.060	1	.060	.052	.821
	Within Groups	67.190	58	1.158		
	Total	67.250	59			
Staphylococci	Between Groups	4.004	1	4.004	26.279	.000
	Within Groups	8.838	58	.152		
	Total	12.842	59			
LAB	Between Groups	1.768	1	1.768	2.437	.124
	Within Groups	42.080	58	.726		
	Total	43.849	59			
Yeast & mould	Between Groups	.024	1	.024	.025	.874
	Within Groups	54.883	58	.946		
	Total	54.907	59			

Table 2- DESCRIPTIVE STATISTICS

Bacterial groups	Species	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower bound	Upper bound		
Total mesophilic bacteria	beef	30	8.0667	.75079	.13707	7.7863	8.3470	6.20	9.40
	goat	30	7.5900	.76580	.13982	7.3040	7.8760	6.00	9.00
	Total	60	7.8283	.78936	.10191	7.6244	8.0322	6.00	9.40
Total coliform	beef	30	4.7367	1.36848	.24985	4.2257	5.2477	3.00	7.70
	goat	30	4.6033	1.06528	.19449	4.2056	5.0011	3.00	6.40
	Total	60	4.6700	1.21771	.15721	4.3554	4.9846	3.00	7.70
Enterobacteriaceae	beef	30	4.6133	1.09693	.20027	4.2037	5.0229	3.00	6.90
	goat	30	4.5500	1.05528	.19267	4.1560	4.9440	3.20	6.60
	Total	60	4.5817	1.06763	.13783	4.3059	4.8575	3.00	6.90
<i>Staphylococci</i>	beef	30	6.7467	.37668	.06877	6.6060	6.8873	5.80	7.50
	goat	30	6.2300	.40356	.07368	6.0793	6.3807	5.30	7.00
	Total	60	6.4883	.46654	.06023	6.3678	6.6089	5.30	7.50
LAB	beef	30	5.1667	.88992	.16248	4.8344	5.4990	3.30	7.40
	goat	30	4.8233	.81184	.14822	4.5202	5.1265	2.90	6.40
	Total	60	4.9950	.86209	.11130	4.7723	5.2177	2.90	7.40
Yeast & mould	beef	30	4.6233	1.06405	.19427	4.2260	5.0207	2.70	7.00
	goat	30	4.6633	.87197	.15920	4.3377	4.9889	2.30	6.10
	Total	60	4.6433	.96469	.12454	4.3941	4.8925	2.30	7.00

## **DECLARATION**

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

Name: Firew Tafesse Mamo

Signature: \_\_\_\_\_

Date: \_\_\_\_\_

The thesis has been submitted with my approval as a supervisor.

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Signature: \_\_\_\_\_

Date: \_\_\_\_\_

Dr. Gulelat Desse

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Dr. Ketema Bacha

Signature: \_\_\_\_\_

Date: \_\_\_\_\_