

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

**BACTERIOLOGICAL QUALITY OF RAW BEEF COLLECTED FROM
MUNICIPALITY SLAUGHTERHOUSE AND LOCAL MARKETS IN AND
AROUND WOLAITA SODDO TOWN, SOUTHERN ETHIOPIA**

BY

NAOD THOMAS MASEBO

JUNE, 2012

DEBRE ZEIT, ETHIOPIA

ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

**BACTERIOLOGICAL QUALITY OF RAW BEEF COLLECTED FROM
MUNICIPALITY SLAUGHTERHOUSE AND LOCAL MARKETS IN AND
AROUND WOLAITA SODDO TOWN, SOUTHERN ETHIOPIA**

**A thesis submitted to the School of Graduate Studies of Addis Ababa University in
Partial Fulfillment of the Requirements for the Degree of Master Science in
Tropical Veterinary Public Health**

BY

NAOD THOMAS MASEBO

JUNE, 2012

DEBRE ZEIT, ETHIOPIA

**BACTERIOLOGICAL QUALITY OF RAW BEEF COLLECTED FROM
MUNICIPALITY SLAUGHTERHOUSE AND LOCAL MARKETS IN AND
AROUND WOLAITA SODDO TOWN, SOUTHERN ETHIOPIA**

BY

NAOD THOMAS MASEBO

Board of External Examiners

Signature

1. Dr. Dereje Gudet

2. Dr. Genene Tefera

3. Prof. Getachew Tilahun

Academic Advisor

Professor Mahendra Pal (PhD, DSc)

TABLE OF CONTENTS

	PAGES
TABLE OF CONTENTS.....	I
ACKNOWLEDGEMENTS	III
LIST OF TABLES	IV
LIST OF FIGURES	V
LIST OF ANNEXES	VI
ABBREVIATIONS	VII
ABSTRACT.....	VIII
1. INTRODUCTION	1
2. LITERATURE REVIEW	1
2.1. Microbial quality of meat.....	1
2.2. Sources of meat contamination	2
2.2.1. Pre-harvest sources of meat contamination	2
2.2.2. Harvest sources of meat contamination.....	4
2.2.3. Post-harvest sources of meat contamination	6
2.3. Microorganisms contaminating meat.....	6
2.4. Indicator organisms.....	7
2.5. Spoilage organisms	8
2.6. Staphylococcus aureus	9
2.7. Informally slaughtered meat.....	11
3. MATERIALS AND METHODS.....	13
3.1. Study area.....	13
3.2. Study design	14
3.3. Sampling methods	15
3.5. Collection of raw beef samples	15

3.6. Bacteriological examination of collected samples	16
3.6.1. Sample preparation	16
3.6.2. Enumeration of aerobic plate count.....	16
3.6.3. Enumeration of coliform counts	17
3.6.4. Isolation and identification of <i>Staphylococcus aureus</i>	17
3.6. Observation	19
3.7. Statistical analysis	19
4. RESULTS	20
4.1. Aerobic plate count	20
4.2. Coliform count	22
4.3. Identification of <i>Staphylococcus aureus</i>	24
4.4. Observations.....	25
5. DISCUSSION	27
6. CONCLUSIONS AND RECOMMENDATIONS	33
7. REFERENCES	35
9. SIGNED DECLARATION SHEET	51

ACKNOWLEDGEMENTS

I thank my advisor Professor Mahendra Pal for his intellectual advice, giving me important guidance through the whole my postgraduate studies, during my project work and for correcting and improving these research paper.

I am highly indebted to members of Soddo Regional Veterinary Laboratory, especially Dr. Ergate, Ato Endashaw and W/ro Mulumebete for their kind reception, preparing equipments and materials for the project work and good willing to use their laboratory with unfavorable condition.

My thanks also extend to Ato Alexander and Ato Solomone member of Soddo Christian Hospital Laboratory for their support in providing me consumable materials, reagents, different bacteriological media and for their advice.

My heartfelt gratitude also goes to my beloved family for their overall support throughout my study. God bless you all.

I would like also to express my appreciation to my friends in School of Veterinary Medicine and in Wolaita Soddo Agricultural College for their support. God bless you all.

LIST OF TABLES

	PAGES
Table 1: Total aerobic bacteria counts from various raw beef from different locations...	21
Table 2: Coliform counts from the various raw beef from different locations.....	23

LIST OF FIGURES

	PAGES
Figure 1: Map of Wolaita zone depicting the study areas	14
Figure 2: Prevalence of <i>Staphylococcus aureus</i> in raw beef of different location	24
Figure 3: The raw beef selling environment in local markets	26
Figure 4: Salemen and their raw beef in rural retail markets.....	26

LIST OF ANNEXES

	PAGES
Annex 1: Chick list used for recording in observation	48
Annex 2: Medias used for bacteriological examination	49
AOAC	Association of Official Agricultural Chemists
APC	Acidified Plate Count
BAC	Bristol, Neno Corporation
CFU	Colony Forming Units
CPS	Coagulase Positive Staphylococci
DED	Dark Firm Dry
EDC	Enzyme Unit
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point
ICMSF	International Commission on Microbiological Specifications for Food
MAA	Mineral Salt Agar
SPC	Standard Plate Count
SPSS	Statistical Package for Social Science
TVC	Total Viable Count
UK	United Kingdom
USA	United States of America
WHO	World Health Organization
WFPED	World Food Finance and Economic Development Department

ABBREVIATIONS

AMC	Mesophilic Count
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
APC	Aerobic Plate Count
BBC	British News Corporation
CFU	Colony Forming Unite
CPS	Coagulase Positive Staphylococci
DFD	Dark Firm Dry
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
GMP	Good Manufacturing Practices
HACCP	Hazard Analysis Critical Control Point
ICMSF	International Commission on Microbiological Specification for Food
MSA	Mannitol Salt Agar
SPC	Standard Plate Count
SPSS	Statistical Package for Social Science
TVC	Total Viable Count
UK	United Kingdom
USA	United States of America
WHO	World Health Organization
WZFFDD	Wolaita Zone Finance and Economic Development Department

ABSTRACT

The study was conducted from October, 2011 to May, 2012 in Wolaita Soddo town and surrounding areas with the objective to determine the microbiological quality, source of contamination and sanitary and hygienic condition of meat selling environment. A total of 260 raw beef samples were randomly collected which comprised of 55 from Soddo Municipality Slaughterhouse, 55 from restaurants/hotels, 50 from Boditte local retail market, 50 from Humbo local retail market and 50 from Baqullo Sagno market. The samples were analyzed using aerobic plate count, coliform count and the presence of *Staphylococcus aureus* was also evaluated. The mean aerobic plate count in beef from slaughterhouse, from butchers shop (restaurants/hotels), from Boditte local retail market, from Humbo local retail market and from Baqullo Sagno market were 2.28×10^6 , 2.82×10^6 , 6.35×10^6 , 6.12×10^6 and 7.45×10^6 cfu/g (colony forming unit per gram), respectively. And the mean coliform count From raw beef sample of slaughterhouse, from butchers shop (restaurants/hotels), from Boditte local retail market, from Humbo local retail market and from Baqullo sagno market were 3.36×10^4 cfu/g, 3.73×10^4 cfu/g, 5.83×10^4 cfu/g, 6.13×10^4 cfu/g and 5.64×10^4 cfu/g (colony forming unit per gram), respectively. There were no significant ($p > 0.05$) variation in the means of aerobic plate and coliform count found in raw beef from different locations. The prevalence of *Staphylococcus aureus* in raw beef was 12.7%, 21.8 %, 18 %, 28 % and 22 %, respectively. The results indicated that the microbiological quality of the raw beef samples analyzed was unsatisfactory, and could be an important cause of food poisoning. Good manufacturing practices (GMP) for slaughtering and processing of raw beef should be accepted as strategies to control pathogenic microbes which pose health risks to humans or which pose public health risks.

Key words: Aerobic plate count, Coliform counts, Raw beef, *Staphylococcus aureus*, Wolaita Soddo.

1. INTRODUCTION

Food security is a complex issue, where animal proteins such as meats, meat products, fish and fishery products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al.*, 2008). Food borne infections and illnesses is a major international health problem with consequent economic reduction. It is a major cause of illness and death worldwide (Adak *et al.*, 2005). Recognizing this, the World Health Organization (WHO) developed its Global Strategy for Food Safety (Adak *et al.*, 2005). In the developing world, food borne infections lead to the death of many children and the resulting diarrheal diseases can have long-term effects on children's growth as well as on their physical and cognitive development (Adak *et al.*, 2005). According to Clarence *et al.* (2009), food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin (Clarence *et al.*, 2009).

Meat is the most perishable of all important foods since it contains sufficient nutrient needed to support the growth of microorganisms (Magnus, 1981). Meat is considered to be spoiled when it is unfit for human consumption. Meat is subjected to changes by its own enzyme, by microbial action and its fat may be oxidized chemically microorganisms grow on meat causing visual, textural and organoleptic change when they release metabolites (Jackson and McGowan, 2001). In fact, tissue from healthy animal are sterile however, it has been pointed that during slaughter, dressing and cutting, microorganisms came chiefly from the exterior of the animal and its intestinal tract but that more added from knives, cloths, air, carts and equipment in general (Pal, 2012). External contamination of meat is a constant possibility from the moment of bleeding unit consumption (Lawrie, 1984). Among the factors that affect microbial growth in meat are

intrinsic properties (physical and chemical properties of meat) and extrinsic (environmental factors) (Rombout and Wout, 1994). However, the factors having the greatest influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture and oxygen availability (Forest *et al.*, 1985).

Food borne microbiologic hazards may be responsible for as many cases of illness as possible each year and are thus an important food safety challenge. To lower the incidence of food borne disease, many experts and stakeholders urge the development of a science- and risk-based food safety system, in which decision makers prioritize hazards and interventions using the best available data on the distribution and reduction of risks (Batz *et al.*, 2005). Such a system requires an understanding of the many risk factors between the point of production and the point of consumption and the ability to systematically target intervention efforts along this "farm-to-fork" continuum (Batz *et al.*, 2005). It has been reported that Gram negative bacteria account for approximately 69% of the cases of bacterial food borne disease (Clarence *et al.*, 2009). Turtura (1991) reported that the most frequently coliform identified on meat are *C. freundii*, *Escherichia coli*, *En. agglomeram* and less frequently strains are of the genera *Klebsiella*, *Shigella sonnie* and *Proteus*. *Escherichia coli* and *S. aureus* are normal flora in human and animals, their presence in foods are indications of excessive human handling (Clarence *et al.*, 2009). Members of the gram negative bacteria e.g. *Escherichia coli* are widely distributed in the environment contaminated food and water are the major sources by which the bacteria are spread (Clarence *et al.*, 2009). *Escherichia coli* is commonly used as surrogate indicator, its presence in food generally indicate direct and indirect fecal contamination (Clarence *et al.*, 2009). Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs (Ternhag *et al.*, 2008).

The possible sources of these bacteria are likely to come from the skin of the animal from which the meat was obtained. Other potential sources of microbial contaminations are the equipment used for each operation that is performed until the final product is eaten, the clothing and hands of personnel and the physical facilities themselves are all implicated (Rombouts and Nouts, 1994). Retail cut could also result in greater microbial load because of the large amount of exposed surface area, more readily available water, nutrient and greater oxygen penetration available (Forest, *et al.*, 1985). Hence retail cuts displayed are conducive for microbial growth and proliferation which leads to spoilage of the meat (Ayres, 1995). Meat and meat products are sometimes contaminated with microbes after leaving the manufacture plant. In most cases the contamination is due to inadequate manipulation. Hygiene conditions are poor when foods are produced in non-industrial establishments, mainly due to lack of required equipment for adequate processing. The children, elderly and immunosuppressed individuals are more susceptible to food infections and or intoxications (Stagnitta *et al.*, 2006). While food borne diseases remain an important public health problem worldwide, one of the most significant food safety hazards is associated with foods of animal origin (Mead, 1994).

In Ethiopia, the wide spread habit of raw beef consumption is a potential cause for food borne illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor general hygiene. Raw meat is available in open-air local retail shops without appropriate temperature control and this is purchased by households; and also minced meat (Kitfo) is served at restaurants as raw, slightly-cooked or well-cooked. In addition, outbreaks of infections somehow related with poor hygiene and consumption of contaminated food were reported in Ethiopia (Mache *et.al.*, 1997). Ethiopia produces approximately 300,000 million tons of beef, 37,000 million tons of mutton, 25,000 million tons of goat meat and 50,000 million tons of poultry meat (FAOSTAT, 2004). In spite of such a huge production of meat, quality control is either lacking or poorly developed (Avery, 2004). Most of the abattoirs are traditionally

operated without quality control systems. Meat processing units are also generally not equipped with quality control system in the country.

There is limited information on the health challenges from food borne diseases from fresh raw meat retailed within a highly populous community. Therefore, the present study was contemplated with the following general and specific objectives

General objective was

- To ascertain the safety of raw beef at the study area in regarded to bacteriological quality.

Specific objectives were

- To assess the bacteriological quality of raw beef at the study area by using total aerobic viable bacteria count, coliform count and by identification of *Staphylococcus aureus* in fresh ready to eat raw bovine meat
- To determine the bacterial load of raw beef and to assess hygienic situation and condition of beef marketing, handling facilities, the selling environment and to identify the possible sources of contamination.

2. LITERATURE REVIEW

2.1. Microbial quality of meat

The prime objective of a meat hygiene and safety programme is the assurance of wholesomeness and the supply of quality meat sold to the consumer. The presence of a meat inspection system examines grossly apparent abnormalities during the ante-mortem and post mortem examination, but does not recognize complex microbial contamination, which could later precipitate major public health hazards and economic loss in terms of food poisoning and spoilage of meat (Ahmed *et al.*, 2002). Meat quality is dependent on the entire meat production chain from the farm where animals are conceived to the consumer. It covers sensory and microbiological properties (color, tenderness, smell taste, microbial load and shelf-life) (Monin and Ouali, 1991).

Surface contamination of carcasses during slaughter and processing can be reduced by ensuring good manufacturing practices such as hygiene and sanitation of the floor, equipment, and carcasses, with suitable disinfectants and sanitizers (FAO, 2006). To enable risks involved to be estimated and appropriate measures to be taken, analysis of the slaughtering process has to be complemented by collection of abattoir-specific microbiological monitoring data, in accordance with hazard analysis critical control point (HACCP) principles (Zweifel *et al.*, 2005). According to Zweifel and Stephan (2003), a regular microbiological examination of carcasses allows reliable conclusions to be drawn with regard to long-term hygienic conditions in abattoirs.

The United States Department of Agriculture has been in the fore in efforts to improve the microbiological safety of meat in general, through the implementation of HACCP systems during the slaughtering process (Katchayanad *et al.*, 2007). According to Brown

et al., (2000), in Australia and New Zealand, the microbiological testing programmes have been jointly developed by the industry and regulatory agencies, where the primary objective of testing is to verify the control of the processes. Microbiological data are required to correlate microbiological contamination with visible soiling, to identify the microbiological effects of individual operations or processes and to confirm or reject suspected sources of microbiological contaminants on products. Failure to meet microbiological standards precipitates investigative activities aimed at improving control over processes.

Sources of food contamination may be primary, coming directly from an infected food animal or its secretions, or excretions; or secondary, resulting from contamination in handling of food (Marriot and Gravini, 2006). Many of the procedures involved in stages of breeding and fattening meat animals to processing them into meat for the table, serve to spread the micro-organisms from animal to animal and from carcass to carcass. The spread of contamination can be divided into several stages: on the farm, during transport and holding prior to slaughter, during slaughter and post-slaughter. The level of contamination of the carcass depends on the cleanliness of the animal before slaughter, the number of bacteria introduced during slaughter and processing, as well as the temperature, the time and the conditions of storage and distribution (Nortje *et al.*, 1990).

2.2. Sources of meat contamination

2.2.1. Pre-harvest sources of meat contamination

On the farm, heavy soil and poor drainage often result in animals arriving at the abattoir with muddy feet and abdomens, thus the state of the animal at slaughter is important. Dirty skins provide major sources of microbial contamination for the carcass. Soiling can be influenced by many factors including the prevalence of diarrhea in animals, climatic conditions on the farm and the length of time spent in the lairage. The design of transport

trucks and abattoir lairages can also make a significant contribution to the level of soiling (Edwards *et al.*, 1997, Hadley *et al.*, 1997). Animals from feedlots frequently carry variable amounts of manure, bedding and soil on their skins when they enter the abattoir. Mud, bedding and manure adhere to the skin of the animal and may contribute to microbiological contamination of carcasses during skin removal. Microbial contamination from the skin normally includes staphylococci, micrococci, pseudomonads, yeasts and moulds. Skins may also carry as many as log 9 bacteria of the soil or faecal origin per cm² of skin. Mud and faeces may contain food-borne pathogens like *E. coli*, *Clostridium perfringens* and *Salmonella* spp. (Van Donkersgoed *et al.*, 1997).

Stress before slaughter also contributes to meat contamination in the live animal. Transport stress may lead to increased frequency of defecation and discharge of caecal contents resulting in shedding of bacteria in the faeces, with increased risk of contamination of hides and subsequently of carcass meat. Meat from animals which have undergone prolonged muscular activity or stress before slaughter, with consequent depletion of glycogen reserves in muscles, undergoes spoilage at low cell (bacterial) densities (10 /cm²)⁶. This meat contains little or no glycogen and, therefore, spoilage bacteria growing on such meat immediately attack amino acids, so that spoilage odors and ammonia are detected (Nortje *et al.*, 1985).

Pre-slaughter handling of animals influences to a large extent the rate of pH decline in the muscles after slaughter. The mean ultimate pH of beef muscles suggest that beef meat may be classified as an extreme dark firm dry meat (pH >6.2). DFD is a condition normally associated with pre-slaughter stress. It occurs mostly in beef, if muscle glycogen reserves are depleted before slaughter, with subsequent production of meat with a low shelf- life (Lawrie, 1990, Gracey *et.al.*, 1992). Symptomless carriers of pathogenic infections are also of particular significance in meat contamination. In symptomless carriers, the pathogens are generally found in the gastrointestinal tract, but they may also be confined to the mesenteric lymph nodes and the gallbladder. It has been recognized for

decades that pigs and poultry are major reservoirs of *Salmonella* spp. (Roberts, 1982).

2.2.2. Harvest sources of meat contamination

The slaughter process inevitably involves some degree of meat contamination, whether from the animals themselves, the abattoir environment or through contact with personnel and equipment as carcasses move through the process (Hudson *et al.*, 1996). Before slaughter, meat and other edible organs without contact with the exterior of healthy and physiologically normal animals may be regarded as sterile with the exception of the gastrointestinal tract and the tongue. Usually, meat contamination occurs during the slaughter process due to contact with the skin, hair, wool or feathers and the gastrointestinal tract contents. Contamination of carcasses during the slaughter process depends on care taken during flaying and evisceration. The skin and viscera are both reservoirs of human pathogens and spoilage microorganisms (Gracey *et al.*, 1999).

During the flaying process, when an incorrect technique is used, most of the carcass bacterial contamination is acquired on the first incision, when the knife being used for slaughter penetrates a heavily contaminated skin and comes into contact with the underlying tissue. Further contamination occurs, if the skin or workers' hands come into contact with the carcass. During the evisceration process contamination occurs if there is puncture or spillage of intestinal or bile content on the carcass. The operations involved in the freeing of the anal sphincter and the rectal end of the intestine constitute an important source of contamination for the carcass. The perianal region of the carcasses is often heavily contaminated with *E. coli* and *Salmonella* spp. The incision of the gallbladder, lymph nodes and bile ducts may contribute to contamination of the carcass with *Salmonella* spp. and *Campylobacter* spp. (Samuel *et al.*, 1980).

Other sources of meat contamination during the slaughter process include clothing of workers, processing equipment such as saws, boning tables, conveyors and mincers, and the water used to wash carcasses, hands and equipment. It has been demonstrated that

2.2.3. Post-harvest sources of meat contamination

Contamination may come from infected humans or live-animal carriers of pathogens, soil, equipment, excreta and hands, nasal discharges, contaminated wounds, contaminated water, insects or feed additives. Infected humans may be the source of contamination at any point in the food chain but are most frequently implicated when preparing food for the table (Hubbert *et al.*, 1996). The contamination of meat during storage in chillers has also been shown. Organisms like *Pseudomonas* spp. were found on structural surfaces in the chillers. It was demonstrated that contamination during chilling was also airborne. The presence of spoilage flora in chillers indicated that the disinfection and cleaning routines were inadequate with regard to removal of spoilage microorganisms (Gutavson and Borch, 1993).

2.3. Microorganisms contaminating meat

To get a reliable indication on the hygienic quality of meat and meat products, microorganisms on the meat surfaces must be enumerated. One would want to know the identity and numbers of all the micro-organisms on the carcass, but this is impractical. The best way is to make separate estimates of a few organisms or groups of particular significance for hygiene. The microbes on carcasses and primal cuts will usually be most numerous on the surfaces. Exceptions to this do occur from time to time as in the case of bone taint, but it is rare. Routine sampling of whole joints is usually confined to the surface of the meat (Kilsby, 1982).

A number of infectious microorganisms associated with food have been identified. These include *Aeromonas hydrophilia*, *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium perfringens*, *E. coli* 0157:H7, *Klebsiella pneumonia*, *Listeria monocytogenes*, Norwalk virus, *Plesiomonas shigelloides*, *Salmonella enteritidis*, *Serratia marcescens*, *Toxoplasma gondii*, *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Yersinia enterocolitica* (Geonaras

et al. 1996; Mortimore and Wallace 1994; Pal, 2012). A large number of organisms belonging to several genera such as *Bacillus* spp., *Aeromonas* spp., *Corynebacterium* spp., *Staphylococcus* spp., *Alcaligenes* spp., *Proteus* spp., *Alteromonas* spp., *Psychrobacter* spp., the *Moraxella/Acinetobacter* group, *Kingella* spp., Micrococcaceae and lactic acid bacteria are associated with the contamination of meat. Studies in the events taking place and their influence on meat when *Pseudomonas* spp. and *Brochothrix* spp. contaminate meat were undertaken by Dainty and co-investigator (1985).

2.4. Indicator organisms

The term "indicator organisms" can be applied to any taxonomic, physiological or ecological group of organisms whose presence or absence provides indirect evidence concerning a particular feature in the past (usually recent) history of the sample. An indicator organism is a micro-organism or group of micro-organisms that indicate 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 to pathogen growth (Buchanan, 2000).

Assessment for various groups and individual indicator organisms has been used to obtain information about the microbiological quality and safety of meat. 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 an indication of faecal contamination and the possible presence of *Salmonella typhi* (Jay, 1992). 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.

Jay (1992) elaborated on some criteria for the use of indicator organisms. An indicator

bacterium 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 quality and the indicator bacteria should be easily and rapidly detected and counted. The indicator bacteria should be easily distinguishable from other bacteria. Other bacteria normally present in food should not inhibit the growth of the indicator bacteria. 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 slaughter and processing, and the keeping quality of the product. Indicators are used to monitor meat hygiene at various stages of processing and distribution to forewarn of potential microbiological problems. The economic incentive accompanying longer shelf life has led industry to also use indicators to try and assess the keeping quality of the meat.

2.5. Spoilage organisms

The keeping quality of meat and poultry products can be predicted by monitoring for spoilage organisms, e.g. pseudomonads. Pseudomonads have been identified as important organisms in the aerobic spoilage of meat. Other important spoilage bacteria are Enterobacteriaceae, lactic acid bacteria (vacuum-packed meats) and *Alteromonas* spp. *Pseudomonas* spp. usually dominate on aerobically cold stored meat, while lactic acid bacteria usually dominate in vacuum and modified atmospheres packages having elevated carbon dioxide concentrations. The number of spoilage bacteria is usually low in cold storage meat, and spoilage bacteria therefore constitute a minor part of the total APC (Gustavson and Borch, 1993). Spoilage is caused predominantly by organisms capable of growth at refrigeration temperatures (of $<5^{\circ}\text{C}$). Such spoilage organisms are all psychrotrophs; i.e. they are capable of growth at temperatures close to zero and an optimum temperature of 20°C - 30°C (Jay, 1992).

The members of the genus *Pseudomonas* are common inhabitants of the soil, fresh water

and marine environments, where their activities are important in mineralization of organic matter. Pseudomonads are unaffected by pH in the range that occurs in meat at chill temperatures and therefore grow faster than competing species. The *Moraxella/Acinetobacter* group is inhibited by the combined effect of a low pH and a low temperature in chilled meat. At slightly higher temperatures they can overcome the effects of a low pH and are able to compete more successfully with other organisms. Good control of the carcass chilling process will limit the growth of spoilage organisms, and low microbial numbers can be maintained during the cutting of carcasses, if equipment and surfaces are properly cleaned to eliminate material that may harbor high populations of spoilage bacteria (Gerats, 1987).

2.6. *Staphylococcus aureus*

Staphylococci are ubiquitous in nature and human carriers of these organisms are numerous and are often the source of food poisoning outbreaks (Pal, 2001). *S. aureus* occurs with considerable frequency on the skin and nasal membranes, in the intestinal tract, and as the causative agent of a variety of cutaneous infections in human populations, meat animals and poultry (Gracey *et.al.*, 1999, Pal, 2007). The primary, and almost exclusive reason for concern about staphylococci on meat is the potential ability of many strains to produce heat-resistant enterotoxins that are a major cause of food poisoning. So far, six serologically distinct staphylococcal enterotoxins have been identified and are designated as SEA, SEB, SEC₁₋₃, SED, SEE and SEF. SEF is the most common cause staphylococcal food poisoning (Isigidi *et al.* 1992, Pal, 2001).

S. aureus is the most enterotoxigenic *Staphylococcus spp* causing food-borne disease. In the food processing industry, it is usual to identify only this group to the level of coagulase-positive staphylococci (CPS), as most enterotoxigenic strains of *S. aureus* produce coagulase. However, there are exceptions. Other staphylococcal species, such as *S. hyicus* and *S. intermedius* also produce coagulase and may produce staphylococcal

enterotoxins, and some *enterotoxigenic S. aureus* do not produce coagulase (Jablonski and Blohach, 1997). Even though the enumeration of CPS in foods is not highly specific, it has been proven to be an effective indicator of the degree of contamination with potentially pathogenic strains, particularly from human sources. Human isolates of CPS are reported to produce staphylococcal enterotoxins more frequently than meat isolates (Desmarchelier *et al.*, 1999). Contamination of food with *S. aureus* may be traced to food handlers with minor septic hand infections, or severe nasal infections, with subsequent heavy growth on the food medium and production of sufficient enterotoxin to cause vomiting and diarrhoea (Pal, 2001). The nasal mucous membrane, particularly, is another likely source of staphylococci of human origin. Consequently, they constitute part of the flora on meat products although normally as a minor component (Gracey *et.al.*, 1999).

In one study Notermans *et al.* (1982) found *S. aureus* population on the skin of broilers before processing to be less than 10/g, but after processing the counts had increased to more than 1000/g. The plucking and eviscerating operations were the major source of the increased *S. aureus*. Desmarchelier and co-investigators (1999) tested 126 CPS isolates from beef carcasses for staphylococcal enterotoxin production. Staphylococcal enterotoxin was detected from 70% of isolates examined. Isigidi *et al.*, (1992) examined human biotypes of *S. aureus* strains and found that 77% produced staphylococcal enterotoxins, so the human biotype may be considered the most dangerous biotype of public health significance. Staphylococci grow vigorously under anaerobic conditions and even better under aerobic conditions. Strains have generally a minimum growth temperature a few degrees below 10°C and maximum growth temperature only a few degrees above 45°C. Since staphylococci cannot grow under normal refrigeration conditions, they do not represent a spoilage problem for fresh meat and poultry. High counts of *S. aureus* in a foodstuff indicate a potential health hazard due to staphylococcal enterotoxins. *S. aureus* is also an indicator of questionable food sanitation, especially in processes of handling food by human handlers (Banwart, 1989).

2.7. Informally slaughtered meat

Illegal slaughter, according to the Environmental Health Directorate (Department of Health-Government of Western Australia, 2008), is referred to as slaughtering and processing of food animals and the sale of carcasses and meat and meat products without the approval of local government, or compliance with food and safety standards. In many cases, illegal slaughter is informal; however, informal slaughter for home consumption is legal. Grandin and Regenstein (1994) are of the view that illegal slaughter involves an increased proportion of meat from diseased animals as well as emaciated carcasses not fit for human consumption, entering the food chain.

In developing countries, regulations concerning meat inspection and/or control may be inadequate, insufficiently implemented, or non-existent, allowing consumers to be exposed to pathogens, including zoonoses. Slaughter normally takes place in remote farm outbuildings or even outdoors, where the premises usually do not have the necessary facilities for hygienic meat preparation and slaughter men are not even aware of hygiene requirements (SFELC, 2004). Those performing the slaughter, as well as those handling the meat, are also exposed to zoonoses such as anthrax, brucellosis, leptospirosis, toxoplasmosis, cowpox, contagious-ecthyma, pseudocowpox, and Rift Valley fever to name but a few of the most dangerous (WHO, 1995; Pal, 2007).

The problem of informal slaughter is not restricted to Africa alone. In Brazil it has been reported that 40% of the meat originates from informal slaughtering, a fact that constitutes a major problem for food safety. Meat consumption without sanitary care may cause diseases such as *E.coli*, tuberculosis, salmonellosis and cysticercosis in consumers (Azevedo and Bankuti, 2003; Pal, 2007). The problem of illegal slaughter is experienced even in developed countries like the United Kingdom. Informal slaughtering of livestock often takes place at unlicensed premises such as farms, under unsanitary and unhygienic conditions. According to the BBC News (2002) eight farmers in western Wales had

pleaded guilty to possessing meat unfit for human consumption, after slaughtering sheep illegally on the farm with the intention of selling the meat.

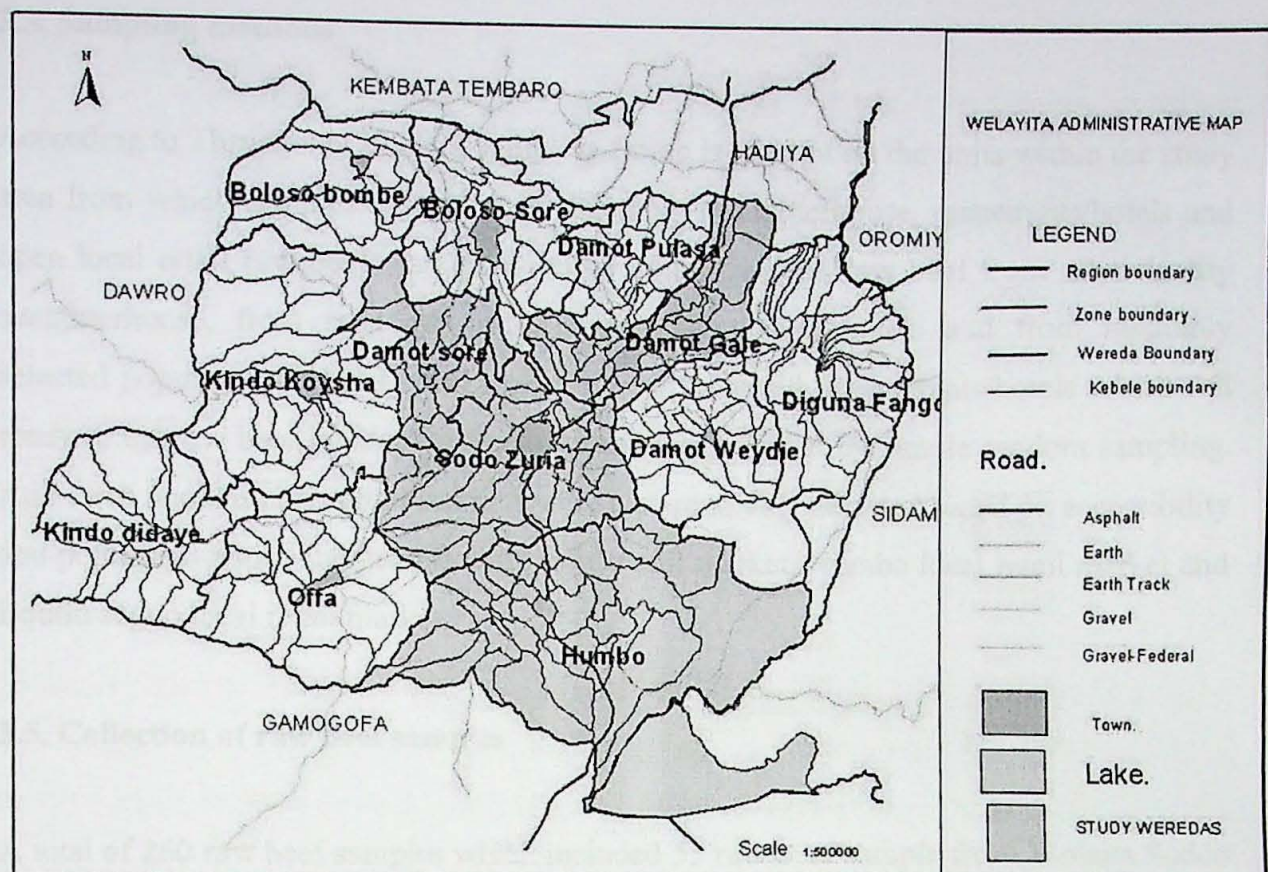
Food of animal origin is derived from animals that live in close association with the soil, water, air, and other environmental sources of microorganisms, such as insects, rodents, and birds (Unc and Goss, 2004). Microorganisms including bacteria are an expected and natural occurrence in the environmental and will therefore, establish themselves on the hide, hair, hooves, skin, feathers, and feet and in the gastrointestinal tract of live animal's. There are few, if any, bacteria in the muscle tissue of normal healthy live animals (Gill *et al.*, 1978). Klinger (2004) stated that the reasons for illegal and/non-inspected slaughtering of animals in developing countries included: The eating habits of the population: people are used to eating meat only from their own animals and trust no one else to slaughter them; Both Jewish and Islamic religious laws require that animals be slaughtered according to a prescribed method; and Illegally or home-slaughtered meat is cheaper than inspected meat.

In Ethiopia it is difficult to find accurate data on the number of livestock slaughtered in abattoirs and outside of abattoirs. In Addis Ababa as readily available data, Irungu and Alemayehu (2002) suggested that only one third to one-half of the cattle slaughtered at the Kéra abattoir are supplied through the terminal markets of Addis Ababa. Furthermore, according to some estimates, an almost equal number of cattle are slaughtered outside of the designated abattoirs, which are not recorded. It is known that backyard illegal slaughtering of cattle is practiced in Ethiopia.

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted at Wolaita Soddo town and surrounding areas of Wolaita zone, Southern Ethiopia. Wolaita zone is located about 390 km south of Addis Ababa at an altitude of 900-2600 m above sea level with a total land area of 4537.5 square kilometers, is located between 6°4'N to 7°1'N and 37°4'E to 38°2'E (Figure 1). It has got an average annual rain fall ranging from 450-1446 mm. The rain fall over much of the areas is typically bimodal with the major rainy season extending from June-September and the short rainy season occurs from February-April. The mean annual maximum and minimum temperature of the area is 34.12 and 11.4°C, respectively. The area has 51.7% cultivated land, 6.4% cultivable land, 11.9% grazing land and 30% others. The average crude population density is 342 Person/Km². The predominant farming system is a mixed crop-livestock production. The livestock population of Wolaita zone is estimated to be 886,242 bovine, 117,274 ovine, 99,817 caprine, 41,603 equines and 442,428 poultry. The zone consists of twelve Woredas /districts (WZFEDD, 2005; CSA, 2006).



Source: Ayele, T (2008)

Figure 1: Map of Wolaita zone depicting the study areas

3.2. Study design

A cross sectional survey was conducted at Wolaita Soddo town and surrounding area of the southern nation's nationalities regional state from October, 2011 to May, 2012. The microbial quality raw beef and the general hygienic and sanitation of the beef selling environments were evaluated through observation. The microbial evaluation of the raw beef sample was conducted at Regional Veterinary Laboratory which is located at Wolaita Soddo town, South Ethiopia. This study was conducted on raw beef collected from different locations in Wolaita Soddo town and surrounding local markets.

3.3. Sampling methods

According to Thrusfield (2005), a sampling frame is a list of all the units within the study area from which samples are taken. In this study slaughterhouse, restaurants/hotels and open local retail raw beef markets were sampling frames. Raw beef from municipality slaughterhouse, from randomly selected restaurants and hotels and from randomly selected popular open local retail markets were collected. Restaurants/hotels which sell ready to eat raw beef in Wolaita Soddo town were selected by simple random sampling. And three popular open retail markets were also randomly selected based on accessibility and popularity which includes Boditte local retail market, Humbo local retail market and Bqullo sagno local retail market.

3.5. Collection of raw beef samples

A total of 260 raw beef samples which included 55 raw beef sample from Wolaita Soddo Municipal Abattoir, 55 raw beef samples from restaurants/hotels of Wolaita Soddo town which sell raw beef (ready to be eaten), 50 raw beef samples from Boditte retail market, 50 raw beef samples from Humbo retail market and 50 raw beef samples from Bqullo sagno retail market were collected. Raw beef samples from butchers shop (restaurants/hotels) from Wolaita Soddo town and raw beef from popular local retail markets of surrounding rural markets were randomly collected during the study period. The samples from local retail markets were obtained from the surrounding areas of Wolaita Soddo in local market days which were practiced by the local peoples. This is because in these local market days known as “yegebeya keni” the raw beef was displayed in open markets for sell and raw beef in some rural areas originated from back yard slaughtering operation. The samples from slaughterhouse, restaurants/hotels and from local markets were obtained in the morning as much as possible within few hours of post-slaughter in order to minimize the microbial changes due to environmental temperatures and post-slaughter timings. Each raw beef sample was chopped by the butcher and treated

as any food purchased by a consumer. Samples were placed in a sterile polyethylene bags and stored in an ice box. Then the samples were transported to the Microbiology Laboratory of Regional Veterinary Laboratory of Wolaita Soddo where they were processed within short time after the sample collection. Due to resource, financial and laboratory facility limitations the samples are analyzed using aerobic plate count, coliform count and evaluated for the presence of *staphylococcus aureus* only.

3.6. Bacteriological examination of collected samples

3.6.1. Sample preparation

At the laboratory from each beef samples in polyethylene bag 10 gram of each sample was weighed into a mortar (that had been previously sterilized) and ground with a sterile pestle until it became smooth and 90 ml of sterile distilled water was poured into the mortar. After maceration, appropriate serial dilutions was made, which was transferred to a test-tube followed by serial dilution up to 10^{-7} dilution. Serial dilutions will be prepared in the usual way to a dilution of 10^{-7} using 9 ml aliquots of sterile distilled water. This dilution was made to express microbial counts as CFU/g or $\log_{10}$ CFU/g. For bacteriological examination of raw beef samples, the methods used by Uzeh and co-workers (2006) and Rindhe and co-workers (2008) were used.

3.6.2. Enumeration of aerobic plate count

To determine total viable counts, 1 ml of pre prepared test sample of each of 10^{-5} and 10^{-7} dilutions were pipetted and plated on nutrient agar plates in triplicates using the spread plate method. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile spreader. Spreader was always flamed and cooled in between different dilutions when spreading. The plates were incubated at 37°C for 24 hours.

Following incubation, plates exhibiting 25-250 colonies were counted. The result was calculated using the following formula:

$$N = \frac{\sum c}{(n_1 + 0.1 * n_2) d}$$

$\sum c$: Sum of colonies counted on all 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.

3.6.3. Enumeration of coliform counts

To determine coliform counts, 1 ml of pre prepared test sample of each of 10^{-3} and 10^{-5} dilutions were pipetted and plated on MacConkey agar plates in triplicates using the spread plate method. The diluted samples were spread as quickly as possible on the surface of the plate with a sterile spreader. Spreader was always flamed and cooled in between different dilutions when spreading. The inoculated plates were kept at 37°C for 24 hours and plates exhibiting 20-200 colonies were counted. The result was calculated using formula as mentioned in section 3.5.2

3.6.4. Isolation and identification of *Staphylococcus aureus*

For isolation and identification of *S. aureus* from aerobic plate count, suspected colonies were purified on nutrient ager.

Gram's staining

All suspected cultures from nutrient plate were subjected to gram's stain and observed under a microscope for gram's reaction, size, shape and cell arrangements. The gram stained smears from the typical colonies that showed gram-positive cocci occurring in bunched, grape like irregular cluster were taken as presumptive *staphylococcus* species.

Catalase test

Purified culture also were picked using sterile loop and mixed with a drop of 3% H₂O₂ on a clean glass slide. If the organism was positive, bubbles of oxygen were liberated within a few seconds and the catalase negative did not produce bubbles. The catalase positive cocci were considered as *staphylococcus* species.

Mannitol salt agar

The colonies that were identified by Gram staining and catalase test as *staphylococcus* were streaked on mannitol salt agar (MSA) plates and incubated at 37⁰c examined after 48hour for growth and change in the colour of the medium. Fermentation of Mannitol by *staphylococcus aureus* causes yellow discoloration of the medium. Golden yellow colonies on MSA which were gram positive and catalase positive isolates were identified as *staphylococcus aureus*.

Coagulase test

Staphylococcus aureus is known to produce coagulase, which can clot plasma into gel in tube or agglutinate cocci in slide. This test is useful in differentiating *S. aureus* from other coagulase-negative staphylococci. Dense suspensions of Staphylococci from culture were made on two ends of clean glass slide. One was labeled as "test" and the other as

“control”. The control suspension serves to rule out false positivity due to auto agglutination. The test suspension was treated with a drop of citrated plasma and mixed well. Agglutination or clumping of cocci within 5-10 seconds was taken as positive.

A sterile test tube was taken and filled with 0.5 ml of 1 in 10 diluted rabbit plasma. And to the test tube, 0.1 ml of overnight broth culture of test bacteria is added. The tube was incubated at 37°C and observed up to four hours. Positive result was indicated by gelling of the plasma, which remains in place even after inverting the tube. If the test remains negative until four hours at 37°C, the tube is kept at room temperature for overnight incubation.

3.6. Observation

To determine the general hygiene, sanitation and possible source of contamination observations were made. Practices such as meat handling by the salesmen, the meat selling and marketing environments situation (sanitation) and the hygienic condition where the beef was sold and also the personal hygiene of the men who sold the raw beef in local markets and restaurants/hotels and the location of the markets were observed and recorded through an observation checklist (Annex 1).

3.7. Statistical analysis

Data was evaluated using descriptive statistics and all counts were converted to log values to enable statistical analysis and to express count to log₁₀ CFU/g, and to compare the results. The data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 16, SSPS). One way ANOVA was performed to compare the mean of bacterial counts obtained from beef sample based on their location. Statistical significance was set at a P value of < 0.05.

4. RESULTS

4.1. Aerobic plate count

Raw beef samples obtained from different locations were analyzed and the results are summarized in Table -1. The mean concentration of aerobic plate count (APC) detected from raw beef sample of Soddo municipality slaughterhouse, Soddo town restaurants/hotels, Boditte local retail market, Humbo local retail market and Baqulo Sagono local retail market were 2.28×10^6 , 2.82×10^6 , 6.35×10^6 , 6.12×10^6 and 7.45×10^6 cfu/g (colony forming unit per gram), respectively and range between 1.27×10^5 to 1.05×10^7 cfu/g in raw beef collected from Soddo municipality slaughterhouse, 1.08×10^5 to 2.00×10^7 cfu/g in raw beef from Soddo town restaurants/hotels, 1.62×10^5 to 3.02×10^7 cfu/g in raw beef from Boditte local retail market, 1.04×10^5 to 3.00×10^7 cfu/g in raw beef from Humbo local retail market and range between 2.35×10^6 to 3.20×10^7 cfu/g in raw beef from Baqullo sagno retail local market. The overall aerobic plate count (APC) in raw beef computed ranged between 1.04×10^5 and 3.20×10^7 cfu/g (mean, 5.91×10^6). Furthermore, the results showed that there were no significant differences ($p > 0.05$) in means of aerobic plate counts detected from raw beef samples of different sampling location.

Table 1: Total aerobic bacteria counts from various raw beef from different locations.

Beef Sample location	Number of sample	Mean bacterial count (cfu/g)	Rang		Mean log value (cfu/g)
			Minimum (cfu/g)	Maximum (cfu/g)	
Municipality slaughterhouse	55	2.28×10^6	1.27×10^5	1.05×10^7	6.35
Restaurants/hotels	55	2.82×10^6	1.08×10^5	2.00×10^7	6.45
Boditte local retail market	50	6.35×10^6	1.62×10^5	3.02×10^7	6.80
Humbo local retail market	50	6.12×10^6	1.04×10^5	3.00×10^7	6.78
Baqullo sagno local market	50	7.45×10^6	2.35×10^6	3.20×10^7	6.87
Total	260	5.91×10^6	1.04×10^5	3.20×10^7	6.77

4.2. Coliform count

The coliform counts of beef sample from Soddo municipality slaughterhouse, Soddo town restaurants/hotels, Boditte local retail market, Humbo local retail market and Baqullo Sagno local retail market were analyzed and the results are presented in Table-2. The coliform count varied from 1.26×10^3 to 2.10×10^5 cfu/g (average, 3.36×10^4 cfu/g) in raw beef collected from Soddo slaughterhouse, varied from 1.01×10^3 to 1.81×10^5 cfu/g (average, 3.73×10^4 cfu/g) in raw beef from Soddo town restaurants/hotels, varied from 2.3×10^3 to 1.91×10^5 cfu/g (average, 5.80×10^4 cfu/g) in raw beef from Boditte local retail market while 1.22×10^3 to 1.91×10^5 cfu/g (average, 6.13×10^4 cfu/g) in raw beef from Humbo local retail market and from 1.22×10^3 to 2.11×10^5 cfu/g (average, 5.64×10^4 cfu/g) in raw beef from Baqullo sagno local retail market. The overall mean of coliform count in raw beef computed in ranged between 1.01×10^3 and 2.11×10^5 cfu/g (mean, 4.88×10^4). Furthermore, the results showed that there were no significant ($p > 0.05$) variation in the means of coliform count found in raw beef collected from different locations and market.

Table 2: Coliform counts from the various raw beef from different locations.

Beef Sample location	Number of sample	Mean bacterial count (cfu/g)	Rang		Mean log value (cfu/g)
			Minimum (cfu/g)	Maximum (cfu/g)	
Municipality slaughterhouse	55	3.36×10^4	1.26×10^3	2.10×10^5	4.52
Restaurants/hotels	55	3.73×10^4	1.01×10^3	1.18×10^5	4.57
Boditte local retail market	50	5.80×10^4	2.30×10^3	1.19×10^5	4.76
Humbo local retail market	50	6.13×10^4	1.22×10^3	1.19×10^5	4.78
Baquallo sagno local market	50	5.64×10^4	1.22×10^3	2.11×10^5	4.72
Total	260	4.88×10^4	1.01×10^3	2.11×10^5	4.68

4.3. Identification of *Staphylococcus aureus*

Examination of 260 raw beef samples collected from municipality slaughterhouses, different restaurants/hotels and local retail market for identification of *Staphylococcus aureus* revealed an overall prevalence of 20.3 % (n= 53). The specific prevalence rate of *Staphylococcus aureus* based on the origin and the location of the raw beef is shown` in Figure 2. Soddo municipality slaughterhouse ,Soddo town restaurants and hotels, Boditte local retail market, Humbo local retail market and Baqullo Sagno local retail market revealed the prevalence of 12.7% (n=7), 21.8% (n=12), 18% (n=9), 28 % (n=14) and 22% (n=11), respectively.

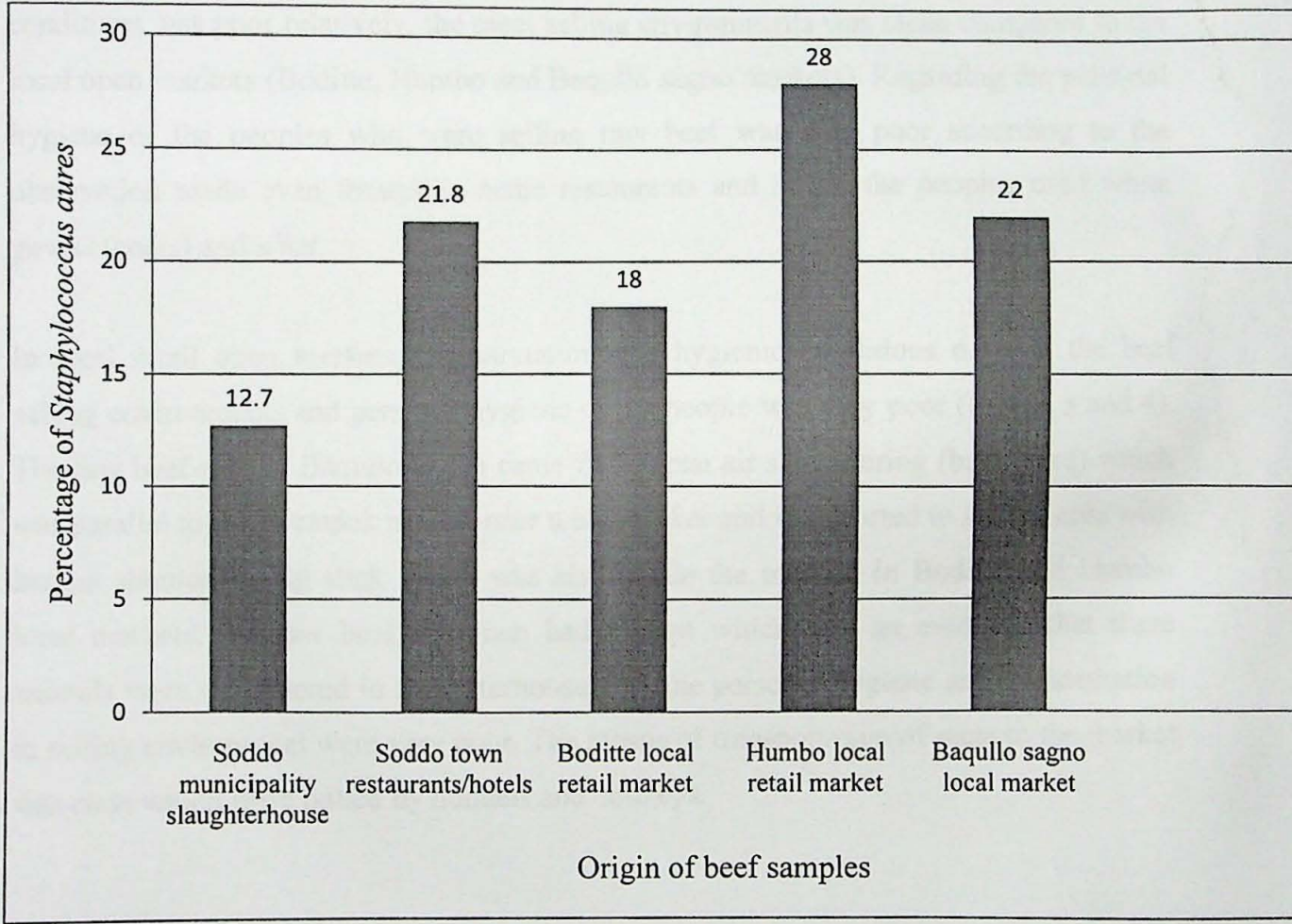


Figure 2: Prevalence of *Staphylococcus aureus* in raw beef of different location

4.4. Observations

Observation was made to determine the general sanitation and hygienic raw beef handling condition, selling environment, transporting condition and personal hygiene of the beef salesmen during the study period and recorded in separate sheet of papers. According to the observations made, the raw beef handling and selling environment both in Soddo town restaurants/hotels and local market were very poor. The raw beef which was sold in restaurants /hotels came from the Soddo town municipality slaughterhouse and was transported with meat transporting van. The raw beef was kept in separate room which was only used for hanging of raw beef and displayed to the consumer which was in some hotels/restaurants covered with glass window. Even though the meat selling conditions was poor relatively, the meat selling environments was clean compared to the local open markets (Boditte, Humbo and Baqullo sagno markets). Regarding the personal hygiene of the peoples who were selling raw beef was also poor according to the observation made even though in some restaurants and hotels the peoples used white gowns (coats) and a hat.

In local retail open markets, the sanitation and hygienic conditions of both the beef selling environments and personal hygiene of the people was very poor (Figure 3 and 4). The raw beef sold in Baqullo sagno came from open air slaughtering (back yard) which was parallel to the livestock market near a big market and transported to selling area with human shoulder using stick which was also inside the market. In Boditte and Humbo local markets, the raw beef salesmen had receipt which was an evidence that there animals were slaughtered in slaughterhouse. But the personal hygiene and the sanitation in selling environment were very poor. The means of transportation of meat to the market was carts which were pulled by humans and donkeys.



Figure 3: The raw beef selling environment in local markets



Figure 4: Salesmen and their raw beef in rural retail market

5. DISCUSSION

The current study revealed a high aerobic plate count from raw beef samples of different sources. The specific mean aerobic plate count obtained from different sources were 2.28×10^6 cfu/g from raw beef collected from Soddo municipality slaughterhouse, 2.82×10^6 cfu/g from Soddo town restaurants/hotels, 86.35×10^6 cfu/g from Boditte local retail market, 6.12×10^6 cfu/g from Humbo local retail market and 7.45×10^6 cfu/g from Baqullo sagno local retail market.

Raw beef samples from all locations yielded marked growth of bacteria. The presence of these organisms on meat parts could be attributed to the fact that meat contains an abundance of all nutrients required for the growth of bacteria in adequate quantity (Pal, 2012). The high total viable counts recorded in this study showed the microbial diversity (differences in form or species) in these locations and markets, condition of the market and the hygienic practice employed by meat sellers and butchers. This determined the variation of bacterial contamination. On comparing the bacterial contamination of these locations, the results obtained were on the high side. This is an indication of recontamination in food handling and hygiene techniques (Clarence *et al.*, 2009).

The highest mean aerobic plate count (APC) was observed from beef of Baqullo sagno local retail market which was 7.45×10^6 cfu/g. Even though the highest number of aerobic bacteria was enumerated from this market, the count was below 10^7 - 10^8 for which spoilage of meat was apparent (Warriss 2001; Teye and Okutu, 2009). Nevertheless raw beef sold on Baqullo sagno local retail market can be said to be near spoilage. It was observed that in Baqullo sagno local retail market, the meat originated from back yard slaughtering process which was in an open air that only had fence with woods and the process of slaughtering took place without any infrastructure and without any inspection with qualified veterinary inspector. It was observed that in Baqullo Sagno local retail

market, the meat was transported by human shoulder hanging with a wood stick to the market and the meat was sold inside huge market and the meat sellers displayed and advertised their meats on wooden tables which were not clean, and the environment was littered with dirt and the meat sellers (butchers) themselves appeared dirty. This could be the reason for high aerobic plate count detected in this market.

Boditte local retail market and Humbo local retail market had mean aerobic plate count (APC) 6.35×10^6 cfu/g and 6.12×10^6 cfu/g, respectively which was also second highest aerobic plate count recorded. These areas had also big markets where butchers sold their meat. Even though the cattle's were slaughtered in slaughterhouses the transportation and meat holding environment condition is on the worst side. The raw fresh meats were displayed on wooden table which was covered with banana leaf and advertised by the butchers. The local market of Boditte is located near the main road. The hygienic conditions of the meat selling environment in both markets were poor and contaminated with dirt and dust which was not appropriate environment for handling of meat.

Poor personal hygiene of the butchers and retailers, possible cross contamination of between adjacent raw meat through unclean hands of the handlers and or/flies, contamination with the dust, handling of the carcass and the money with the same unwashed hands and careless sneezing and coughing among butchers can lead to a contamination of the raw beef. This could be the reason for high aerobic plate count detected in all of the local retail markets. Unhygienic handling of meats can affect the ultimate quality of fresh product (Biswas *et al.*, 2011). Salihu *et al.* (2010) indicate that the hygienic conditions are poor when foods are produced in non industrial establishments, mainly because of the lack of required equipment for adequate processing.

Raw beef which was collected from Soddo municipality slaughterhouse had mean

aerobic plate count (APC) 2.28×10^6 cfu/g and Soddo town restaurants/hotels 2.82×10^6 cfu/g. The raw beef in Soddo town restaurants was placed in building (room) separate from the other rooms of the restaurants and the meat was hanged over on metal structure and covered with glass which was like windows. This seems to reduce the number of flies within the meat holding area. The meats sold here were originated from Soddo municipality slaughterhouse. The main source of contamination may be the tables and handling of meat with unsterilized instruments such as knives. The beef collected from Soddo municipality slaughterhouse had the least mean aerobic plate (APC) (2.28×10^6 cfu/g). Even though their numbers were below the spoilage causing limit, the meat was unwholesome.

The higher incidence of microbial load in raw beef obtained from raw beef of slaughterhouse and hotels/restaurants in this study might be attributed to unhygienic and improper handling of animals during slaughter, dressing, evisceration, transportation and marketing. The usual practice of washing the carcass with the same water in which intestines and offal had been washed was considered as one of the predominant reasons for increased microbial counts of the carcasses. A complete ignorance on the part of the meat handlers/ butchers in hygienic handling of carcasses during slaughter and retailing processes might be the main factors for producing meat with high microbial load. Hot and humid climate of this study area would have contributed in increasing the microbial load. A high APC usually related to poorer quality and reduced shelf life (Eisel *et al.*, 1997). High APC can be dangerous for the consumers, as some of the microorganisms could be pathogenic (Berry and Foegeding, 1997).

Cattle and carcass handling coupled with microbial contamination of beef samples have also been reported in Ethiopia and other developing countries. In Ethiopia, Kumar *et al.* (2010) found a high total aerobic plate (APC) count of 75.91 % in beefs produced and marketed in some parts of Tigray region (ranged 2.5×10^5 cfu/g to 1.63×10^8 cfu/g). They also reported that a high percentage of samples (varying from 38.56 % – 84.34 %) were

of unsatisfactory quality. In Oja-ghoro, Nigeria, Raji (2006) reported a total bacterial count of 3.5×10^4 cfu/g. In Ghana Tamale Metropolis in the Northern Region Adzitey *et.al.* (2011) described a high APC (1.67×10^6 cfu/cm²) in beef from different market. In Nigeria Ukut *et.al.* (2010) recorded 5.01×10^5 cfu/g APC from fresh meat sold in Calabar metropolis. The mean count of $\log_{10} 4.93$ cfu/g in Cote d'ivoire from beef offered for retail sale was recorded by Rose and co-investigators (2011).

Aerobic plate count, also known as the total viable count (TVC), aerobic mesophilic count (AMC) or standard plate count (SPC), is the most common method of assessing the number of organisms in raw meat (Gracey *et al.* 1999), and has been employed as a test to evaluate the sanitary quality of meat sold in the market (Lasta *et al.*, 1992, Nassar and Emam 2002, Haque *et al.*, 2008). Since the safety, shelf life and keeping quality of fresh foods are related to microbial content, microbiological standards have been proposed for a variety of foods. Such standards usually specify a total count as well as indicator maxima (Hubbert *et al.*, 1996). There are no available regulatory standards for the microbiological safety criteria for locally (nonindustrial) prepared read to eat foods to compare with this study findings. As per the recommendations of ICMSF (1985), viable count of fresh meat tissue generally should be less than log 6.0 per gram. Microbiological specifications for fresh meat in developing countries like India is 1×10^6 and the values greater than 1×10^6 are not considered as good (Bachhil *et al.*, 2004). Considering these specifications, 205 (78.8%) of 260 beef samples were not of good quality. The results demonstrated that the sanitary conditions under which the beef was produced and sold in the study areas were not of acceptable standards. The undesirable level of bacterial load might have originated from sources like abattoir or meat processing, meat selling environment, contaminated instruments, from meat handlers, from meat salesmen and from transportation methods used.

The current study also revealed a high coliform count from raw beef samples of different sources. The specific mean coliform count obtained from different sources were $3.36 \times$

10^4 cfu/g in raw beef collected from Soddo slaughterhouse, 3.73×10^4 cfu/g in raw beef collected from Soddo town restaurants/hotels, 5.80×10^4 cfu/g in raw beef collected from Boditte local retail market, 6.13×10^4 cfu/g in raw beef collected from Humbo local retail market, and 5.64×10^4 cfu/g in raw beef collected from Baqullo sagno local retail market. The concentration of coliform count is higher in the raw beef meat which is assumed to be an indicator of fecal contamination. According to Pace (1975) and Solberg *et al.* (1986), coliform count higher than 10^2 /g in delicatessen food products are indicative of dangerous contamination. High coliform count also has been reported from different developing countries. In Nigeria, 3.72×10^3 cfu/g coliform count from fresh meat sold in Calabar metropolis was recorded by Ukut *et al.* (2010). Rose and co-investigators (2011) reported fecal coliform count which ranged between 1.83 to 4.73 $\log_{10}$ cfu/g from Cote d'ivoire beef offered for retail sale.

Fresh meats sold to the public in open markets are grossly contaminated with coliform bacteria. The possible sources of contaminants are due to the unhygienic manner of handling meat from the slaughtering facility to the markets. This also implies that these meats are viable source of various diseases. High coliform count indicated poor hygienic quality of raw beef. The contamination of meat with coliforms may occur during slaughtering, cutting or dressing of carcasses, soiled hands, from the marketing areas or knives used for handling and cutting besides the use of contaminated water.

Evaluation of 260 raw beef sample collected from different restaurants and market for identification of *St. aureus* revealed an overall prevalence of 20.3 % (n=53/260). The specific prevalence rate of *S. aureus* based on the origin and the location of the beef sample was 12.7 % (n=7/55), 21.8 % (n=12/55), 18 % (n=9/50), 28% (n= 14/50), 22 % (n=11/50) from Soddo municipality slaughterhouse, from Soddo town restaurants and hotels, from Boditte local retail market, from Humbo local retail market and Baqullo Sagno local retail market respectively. The presence of high percentage of *S. aureus* was also reported from Ethiopia and other developing country. Haimanot and co-workers

(2010) reported 12% of *S. aureus* in unprocessed slices of meat and abattoir from Jimma town of Ethiopia. Raw beef sample originated from Osogbo, Nigeria, yielded 28% *S. aureus* (Adesiji *et al.*, 2011).

A high percentage of raw meats, including beef carcasses become, however, contaminated with staphylococci due to the excessive handling of the product (Doyle, 1989). Since bacteria are the causative agents of two thirds of food-borne disease outbreaks with *Staphylococcus aureus* being one of the leading causes of gastroenteritis resulting from the consumption of contaminated food (Le Loir *et al.*, 2003). The high isolation rate of *S. aureus* could be due to poor personal hygiene of the workers and the technique used for opening the abdomen, and the presence of cross contamination, which is usually related to human skin and clothing. As the carrier rate varies with different populations ranging from 10 to 40% in adults outside the hospital environment, carriage may be intermittent or continuous over a long period of time (Gabriel *et al.*, 2010). With the technique of hand evisceration predominantly practiced in the traditional system and markets under study and with infrequent hand washing, a high prevalence of bacteria related to human contact was expected in these samples. Such a high level of contamination with *S. aureus* has been associated with increased risk of staphylococcal food poisoning (Peiffer, 1999; Pal, 2001). The high isolation rate of *S. aureus* in this study indicates poor hygiene and working practices of the meat handlers during the processing stage as well as lack of sterilization of utensils and working surfaces. Enterotoxin-producing *S. aureus* is the most common cause of food-borne human illness throughout the world in which red meat and poultry products have been implicated (Normanno *et al.*, 2005).

6. CONCLUSIONS AND RECOMMENDATIONS

The high aerobic bacteria count, coliform count and isolation of *Staphylococcus aureus* from the raw beef samples is an indication of its low bacteriological quality, and this can make it a potential source of food infection. Fresh meats sold to the public in open markets are grossly contaminated with high count of bacteria. The finding of this study revealed that fresh beef meat sold at different restaurants and local markets around Wolaita Soddo area are contaminated by various bacterial species including *S. aureus*. The possible source of contaminants are due to the unhygienic manner of handling meat from the slaughters to the markets and contamination during slaughtering in slaughterhouses and also during backyard slaughtering which includes unhygienic environment for marketing of meat. The general sanitary conditions at the meat shops and local markets in addition to poor hygienic practices by the butchers and person who sell beef are probable contributors to the microbial contamination on the beef. This also implies that these meats are viable source of various diseases. Some diseases could spread and acquire epidemic status which poses serious public health hazards. Since improper handling and improper hygiene might lead to the contamination of fresh meats and this might eventually affects the health of the consumers. Based on the above conclusion the following recommendations are forwarded:

- It is a well known practice of eating raw meat in Ethiopia, Therefore for the production of safe and wholesome meat good manufacturing practices (GMP) must be adopted.
- Ensuring good hygienic standard at the various meat shops, restaurants and markets in the study area should be a shared responsibility between government, consumers and retailers.

- Meat handlers and sellers should be educated on the adverse effect of lack of proper personal and environmental hygiene and sanitation.
- Raw beef consumers should also be educated about the hazard associated with consumption of unhygienic and unwholesome raw beef.
- Raw beef which are collected from local markets to be used for consumption purposes should be adequately cooked before use for home consumption.
- It is emphasized that hazard analysis critical control point (HACCP) should be implemented in the abattoirs to identify hazards during slaughtering and processing of meat.
- Establishment of quality standards concerning consumer safety and shelf life of meat helps in production of quality meat and meat products and their evaluation. Therefore to measure the quality of meat available in the market, it is essential to have a comprehensive code or document listing national meat standards.

7. REFERENCES

- Adak, G.K., Meakins, S.M., Yip, H. Lopman, B. A., O'Brien S.J. (2005): Disease risks from foods in England and Wales, 1996–2000. *Emerging Infectious Diseases*, Accessed online May, 2012 from <http://www.cdc.gov/ncidod/EID/vol11no03/04-0191.htm>
- Adesiji, Y.O., Alli, O.T., Adekanle, M.A and Jolayemi, J.B.(2011): Prevalence of *Arcobacter*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* species in retail raw chicken, pork, beef and goat meat in Osogbo, Nigeria. *Sierra Leone journal of Biomedical Research*, **3**: 8-12
- Adzitey, F. Teye, G.A., Kutah, W.N and Adday, S. (2011): Microbial quality of beef sold on selected markets in the Tamale Metropolis in the Northern Region of Ghana. *Live Research for Rural Development*, **23**: 18-25
- Ahmed, S. N, Chattopadhyay, U. K., Sherikar, A. T., Waskar, V. S., Paturkar, A. M., Latha, C, Munde, K.D. and Pathare, N.S (2002): Chemical sprays as method for improvement in microbiological quality and shelf-life of fresh sheep and goat meats during refrigeration storage (5-7 degrees C). *Journal of Meat Science*, **18**: 339-344.
- Avery, A. (2004): Project Report on red meat and poultry production and consumption in Ethiopia and distribution in Addis Ababa, International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia, Retrieved from online database, www.worldfoodprize.org/assets/YouthInstitute/BRinternship/2004/papers/AveryAbbey.pdf
- Ayele, T. J. (2008): Livelihood Adaptation, Risks and Vulnerability in Rural Wolaita, Ethiopia, PhD Thesis, Norwegian University of Life, Norway.

- Ayres, C.P. (1995) Microbiology of spoilt food and food stuffs. *Food Microbiology Journal*, **16**: 266-280.
- Azevedo, P.F and Bankuti, F.I (2003): When food safety concern decreases safety: evidence from the informal meat market, Accessed online April, 2012. From http://www.pensa.org.br/anexos/biblioteca/73200716331_.pdf.
- Bachhil, V.N., Malik, S.V.S. and Singh, D.K. (2004): Microbiological specifications and food safety. In: A.T., Sherikar, V.N., Bachhil and D.C. Thapliyal (eds), Elements of Veterinary Public Health, 2004 published by Indian Council of Agricultural Research, New Delhi, India, Pp.259–284.
- Banwart, G. J. (1989): Basic Food Microbiology. An avi book. Van Nostrand Reinhold, New York, Pp. 204.
- Batz, M. B., Doyle, M. P., Morris, J. G., Jr. Painter, J. Singh, R. Tauxe, R.V. (2005): Attributing illness to food. *Emerging Infectious Diseases*, **19**: 145-149.
- BBC News (2002): Farmers admit illegal meat trading. Accessed online April, 2012 from: <http://news.bbc.co.uk/1/low/wales/2296951.stm>
- Berry, E.D. and Foegeding, P.M. (1997): Cold adaptation and growth of microorganisms. *Journal of Food Protection*, **60**: 1583– 1594.
- Biswas, A. K., N. Kondaiah, A.S.R., Anjaneyulu and Mandal, P. K. (2011): Causes, concerns, consequences and control of microbial contaminants in meat. A review. *International Journal of Meat science*, **1**: 27-35

- Brown, M.H., Gill, C.O., Hollingsworth, J., Nickelson, R., Sewrda, S., Sheradin, J.J., Stevenson T., Sumner J.L., Theno, D.M., Usborne, W.R. and D. Zink (2000): The role of microbiological testing in systems for assuring the safety of beef. *International Journal of Food Microbiology*, **62**: 7-16.
- Buchanan, R.L. (2000): Acquisition of microbiological data to enhance food safety. *Journal of Food Protection*, **63**: 832-838.
- Clarence, S. Y, Obinna, C. N, Shalom, N. C. (2009): Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. *African Journal of Microbiology Research*, **3**: 390-395.
- CSA (Central Statistical Authority) (2006): Statistical Abstract 2006, Addis Ababa.
- Dainty R. H., Edwards R. A. and Hibbard C. M (1985): Time course of volatile compounds formation during refrigerated storage of naturally contaminated beef. *Journal of Applied Bacteriology*, **59**: 303-309
- Department of Health Government of Western Australia: Environmental Health Directorate (2008): Website of the Environmental Health Directorate. Accessed online April, 2012 from: <http://www.health.wa.gov.au/envirohealth/food/slaughter.cfm>.
- Desmarchelier, M.P., Higgs, M.G., Mills L., Sullivan M.A. and Vanderlin B.P. (1999): Incidence of coagulase positive *Staphylococcus* on beef carcasses in Australian abattoirs. *International Journal of Food Microbiology*, **47**: 221-229.
- Doyle, P. M. 1989. Foodborne Bacterial Pathogens. Marcel Dekker, Inc, New York. Chapter 11, 475.

- Edwards, D., Johnston, A.M., and Mead, G.C. (1997): Meat Inspection: An overview of present practices and future trends. *The Veterinary Journal*, **154**: 135-147.
- Eisel, W.G., Linton, R.H. and Muriana, P.M. (1997): A survey of microbial levels for incoming raw beef, environmental sources and ground beef in a red meat processing plant. *Food Microbiology*, **14**: 273-282.
- FAO (2006): Animal Production and Health Manual: Good Practices for the Meat Industry. FAO, Rome, Italy.
- FAOSTAT, (2004): Food and Agricultural Organization of the United Nations. Accessed from FAOSTAT online database, <http://faostat.fao.org/faostat/default.jsp?language=EN&version=ext&hasbulk=0>
- Forest, D.C, Harold, D. A., Judge B. A., Robert E. A. (1985): Different types of meat and meat product consumed by Nigerian. Principle of Meat Science. W.A. Freeman and Co. publishers, pp 4-178.
- Gabriel, R., Estifanos, and Nasal, K. (2007): Carriage and drug sensitivity of *Staphylococcus aureus* among health workers of Jimma University specialized Hospital, Southwestern Ethiopia. *Ethiopian Journal Health Science*, **17**:73-79.
- Geornaras, I., De Jesus, A. E., Van Zyl, E. and Von Holy, A. (1996): Bacterial populations associated with poultry processing in a South African abattoir. *Food Microbiology*, **13**: 457-465.
- Gerats, G. E. (1987): What hygiene can achieve - how to achieve hygiene. In: Smulders F.G.M. (Ed), Elimination of Pathogenic Organisms from Meat and Poultry, Elsevier, Amsterdam, the Netherlands, Pp 269-283.

- Gill, C.O., Penney, N and Nottingham, P.M (1978): Tissue sterility in un-eviscerated carcasses. *Applied Environmental Microbiology*, **36**: 356-359.
- Gracey, J.F., Collins, D.S., and Huey, R.J. (1999): Meat Hygiene, 10th ed. W.B. Saunders Company Ltd., London, pp 354-367.
- Grandin, T and Regenstein, J.M. (1994): FAWC report on the welfare of farmed animals at slaughter or killing Part 1: Red meat animals. Accessed online May, 2012 : <http://www.mcb.org.uk/library/DEFRA.pdf>.
- Gustavson, P. and Borch, E. (1993): Contamination of carcasses by psychrophilic *Pseudomonas* and *Enterobacteriaceae* at different stages along the processing line. *International Journal of Food Microbiology*, **20**: 67-83.
- Hadley, P. J., Holder, J. S. and Hinton, M. H. (1997): Effects of fleecing and skinning method on the microbiology of sheep carcasses. *Veterinary Record*, **140**: 570-574.
- Haimanot, T., Alemseged A., Getenet, B. Solomon, G.S. (2010): Microbial flora and food born pathogen on minced meat and their susceptibility to antimicrobial agents. *Ethiopian Journal of Health Science*, **20**:137-143.
- Haque, M. A., Siddique, M. P., Habib, M. A., Sarkar, V. and Choudhury, K. A. (2008) Evaluation of sanitary quality of goat meat obtained from slaughter yards and meat stalls at late market hours. *Bangladesh Journal of Veterinary Medicine*, **6**:87-92.
- Hubbert, W. Hagstad, H.V., Spangler, E, Hinton, M.H and Hughes, K.L (1996): Food Safety and Quality Assurance- Food of animal origin. 2nd edition, Iowa State university press, Iowa, USA: pp127- 128.

- Hudson, W. R., and Mead, G. C. and Hinton, M. H. (1996): Relevance of abattoir hygiene assessment to microbial contamination of British beef carcasses. *Veterinary Record*, **139**: 587-589.
- ICMSF (1980): Microbial Ecology of Foods, Volume 2, Food Commodities, Silliker, J.H., Elliot, P., Baird-Parker, A.C., Olson, J.C. and Roberts, T. A. Academic Press, New York, Pp. 340.
- ICMSF (1985): Microorganism in foods; samples for Microbiological Analysis: Principles and specific applications. Recommendation of the International Commission on Microbiological Specification for Foods Association of Microbiological Societies Toronto University of Toronto Press, Pp. 23-32.
- Isigidi, B. K., Mathieu, A. M., Devriese, L. A., Godard, C. and Van Hoof, J. (1992): Enterotoxin production in different *Staphylococcus aureus* biotypes isolated from food and meat plants. *Journal of Applied Bacteriology*, **72**: 16-20.
- Jablonski, L.M., and Blohach, G.A. (1997): *Staphylococcus aureus*. In: Doyle M .P., Beuchat L. R., Montville T. J., (Eds), Food Microbiology: Fundamentals and Frontiers, ASM Press, Washington, D C, Pp. 353-375.
- Jackson, D. and McGowan, C. H. (2001): Diet management effects on carcass attributes and meat quality of young goats. *Small Ruminant Research*, **28**: 93-98.
- Jay, J. (1992): Modern Food Microbiology. 2nd edition, Chapman and Hall, New York, Pp. 354-364.
- Katchayanad, N. Arthur, T.M., Bosilevac, J.M., Brichta-Harhay, D.M., Guerini, M.N., Shackelford, S.D., Wheeler, T.L and Koohmaraie, M (2007): Microbiological

characterization of lamb carcasses at commercial processing plants in the United States. *Journal of food Protection*, **70**: 1811-1819.

- Kilsby, D.C. (1982): Sampling schemes and limits in meat microbiology. In: Brown M.H. (Ed), *Meat Microbiology*, Applied Science Publishers, London: Pp. 387-421.
- Klinger, I. (2004): FAO/WHO/OIE Electronic Conference on Veterinary Public Health and Control of Zoonoses in Developing Countries. Accessed online April, 2012 from, <http://www.vphasia.org/electronicconferences.htm>.
- Kumar, A., Kebede, E and Kassaye, E. (2010): Evaluation of quality of beef produced and sold in parts of Tigray Region of Ethiopia. *Tropical Animal Health Production*, **42**: 445 – 449.
- Lasta, J.A., Rodriguez, H.R. and Zanelli, M. (1992): Bacterial count from bovine carcasses as indicator of hygiene at slaughtering places, *Journal of Food Protection*, **55**: 271–278.
- Lawrie, R. A. (1984): The preservation effect of smoke on meat. In: *Meat Science* Pergamon Press Inc. Maxwell House Fair View Park -Elmford, New York, Pp. 49-52.
- Lawrie, R. A. (1990): The conversion of muscle to meat. In: *Meat Science*. 4th edition, Pergamon Press, Oxford, UK, Pp. 136.
- Le Loir, Y., Baron, F. and Gautier, M. (2003): Review: *Staphylococcus aureus* and food poisoning. Online journal: *Genetics and Molecular Research*, **1**: 63-76.
- Mache, A., Mengistu, Y. and Cowley, S. (1997): *Salmonella* serogroups identified from adult diarrheal out-patients in Addis Ababa, Ethiopia: Antibiotic resistance and plasmid profile analysis. *East African Medical Journal*, **74**:183-187.

- Magnus, P. (1981): Meat Composition. Food Science and Technology, 4th edition. Gohumunary Pub. London, pp. 108- 215.
- Marriot, N.G. and Gravani, R.B. (2006): Principles of Food Sanitation-Food Contamination Sources. Accessed online May, 2012, from: www.spirnerlink.com/content/v05322609481pox.
- Mead, G.C. (1994): Microbiological hazards from red meat and their control. *Britain Food Journal*, **96**: 33-36.
- Monin, G. and Ouali, A. (1991): Muscle differentiation and meat quality *In*: Lawrie R (Ed) Developments in Meat Science, Elsevier Applied Science, London, Pp. 89-157
- Mortimore, S and Wallace, C. (1994): HACCP A Practical Approach. Chapman and Hall, London, Pp. 36-41.
- Nassar, A.M. and Emam, W.H. (2002): Biogenic amines in chicken meat products in relation to bacterial load, pH value and sodium chloride content, *Nahrung*, 46, No.3, 2002, Retrieved from online database on April, 2012 from: http://d.wanfangdata.com.cn/NSTL_QK_NSTL_QK5880644.aspx.
- Normanno, G., Firinu, A., Virgilio, S., Mula, G., Dambrosio, A., Poggiu, A., Decastell,i L., Mioni, R., Scuota, S., Bolzoni G., Di Giannatale, E., Salinetti, A.P., La Salandra, G., Bartoli, M., Zuccon, F., Pirino, T., Sias, S., Parisi, A., Quaglia, N.C., Celano, G.V. (2005):Coagulase-positive Staphylococci and *Staphylococcus aureus* in food products marketed in Italy. *International journal of Food Microbiology*, **98**: 73-79.
- Nortje, G. L., Nauman, H. D., Laubsher, A., Grobler, I., Naude, L., Oosthuizen, W., Jordan E. and Naude, R. D. (1985): Effects of exercise, electrical stimulation and vacuum

packaging on bacterial counts and tenderness of fresh beef primal cuts. *Journal of Food Protection*, **48**: 1036-1039.

Nortje, G. L., Nel, L., Jordaan E., Badenhorst, K., Goedhart, G., Holzapfel, W. H. and Grimbeek, R.J. (1990): A quantitative survey of meat production chain to determine the microbial profile of the final product. *Journal of Food Protection*, **53**: 411-417.

Notermans, S. Dufresne, J. and Van Leeuwen, W. J. (1982): Contamination of broiler chickens by *Staphylococcus aureus* during processing; incidence and origin. *Journal of Applied Bacteriology*, **52**: 275.

Pace, P.J. (1975): Bacteriological quality of delicatessen foods: are standards needed? *Journal of Milk and Food Technology*, **38**: 347-353.

Pal, M. (2001): Epidemiology of staphylococcal food poisoning. *Beverage food world*, **28**:11-13.

Pal, M. (2007): Zoonoses. 2nd ed. Satyam publishers, Jaipur, India. Pp. 296.

Pal, M. (2012): Raw meat poses public health risks. The Ethiopian Herald, May 15, 2012 Pp. 2-3.

Peiffer, B. (1999): Toxi-infections alimentaires a` staphylocoques. Actualite' TIAC, Paris, France. Pp. 413.

Prescott, L.M., Harley, J.P and Klein, D.A. (2002): Food and Industrial Microbiology. In: Microbiology 5th Edition. The WCB McGraw-Hill companies, Boston, USA. Pp 125 – 964.

Rahkio, T. M. and Korkeala, H. J. (1997): Airborne bacteria and carcasses contamination in

slaughterhouses. *Journal of Food Protection*, **60**: 38-42.

Raji, A. I. (2006): Bacteriological Quality of Dried Sliced Beef (Kilishi) Sold In Ilorin Metropolis. *Journal of Applied Science and Environmental Management*, **10**: 97-100.

Rindhe, S. N., Zanjad, P.N., Doifode, V.K., Siddique, A. and Mendh, M.S. (2008): Assessment of microbial contamination of chicken products sold in parbhani city. *Veterinary world*, **7**: 208-210

Roberts, D. (1982): Bacteria of public health significance. In: Brown M H (Ed), *Meat Microbiology*, Applied Science Publishers London UK. Pp. 331.

Rombouts, F. M and Nout, R. (1994): Food Microbiology and Hygiene. Encyclopedia of Human Biology Academic Press. New York, Pp. 661-665.

Rose, K.N., Marina, K and Seydou, O.C. (2011): Bacteriological quality of beef offered for retail sale in Cote d'ivoire. *American Journal of Food Technology*, **6**: 835-842.

Salihu, M.D., Junaidu, A.U., Magaji, A.A., Aliyu, R.M. and Yakubu, Y. (2010): Bacteriological quality of traditionally prepared fried ground beef (*Dambun nama*) in Sokoto, Nigeria. *Advanced Journal of Food Science and Technology*, **2**: 145-147

Samuel, J. L., O'Boyle, D. A. and Mathers, W. J. (1980): The contamination with *Salmonella* of bovine livers in an abattoir. *Australian Veterinary Journal*, **56**: 526-528.

SFELC (2004): Safer food, better business: illegal meat and smokies guidance notes Scotland. Accessed online, May, 2012, from: <http://www.food.gov.uk/scotland/regsscotland/regsguidscot/illegalmeatguidrscot>.

- Solberg, M., Miskinuri, D.K. Martin, B.A. Page, G., Golderer, S. and. Libfield, M (1986): What do microbiological indicator tests tell us about safety of foods? *Food Production Development*, **10**: 27-30.
- SPSS (Statistical Procedures for Social Science) (2007): SPSS (Version 16) BI survey tips. Statistical procedure for social science (SPSS) Inc. Chicago, USA.
- Stagnitta, P.V., B. Micalizzi and A.M. Stefanini de Guzman, (2006): Prevalence of some bacteria, yeast, and molds in meat foods in san luis, Argentina. *Central Europe Journal of Public Health*, **14**: 141-144.
- Ternhag A., Törner A., Svensson Å., Ekdahl K., Giesecke, J. (2008): Short- and long-term effects of bacterial gastrointestinal infections. *Emerging Infectious Diseases*, Accessed on May, 2012 from: <http://www.cdc.gov/EID/content/14/1/143.htm>.
- Teye, G.A., and Okutu, I. (2009): Effect of ageing under tropical conditions on the eating qualities of Beef. *African Journal of Food Agriculture and Nutrition Development*, **9**: 1903-1904.
- Thrusfield, M. (2005): *Veterinary Epidemiology*. 3rd ed. Blackwell Science Ltd., Oxford, Great Britain. Pp. 182-198.
- Turtura, G. C. (1991): Enterobacteriaceae and other gram negative bacteria in slaughtered poultry. *Microbiology Ailments and Nutrition*, **2**: 139-146.
- Ukut I.O.E., Okonko I.O., Ikpoh IS1, Nkang A.O., Udeze A.O., Babalola T.A., Mejeha O.K., Fajobi, E.A. (2010): Assessment of bacteriological quality of fresh meats sold in calabar metropolis, Nigeria. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **9**: 89-100.

- Unc, A. and M. J., Goss. (2004): Transport of bacteria from manure and protection of water resources. *Applied Soil Ecology*, **25**: 1-18.
- Upmann, M., Jakob P. and Reuter, G. (2000): Microbial transfer during cutting and deboning of pork in a small-scale meat processing plant. *Dairy Food and Environmental Sanitation*, **20**: 14-23.
- Uzeh, R.E., Ohenhen R.E. and Adeniji, O.O. (2006): Bacterial Contamination of Tsire-Suya, a Nigerian Meat Product. *Pakistan Journal of Nutrition*, **5**: 458-460
- Van Donkersgoed, J. Jericho K.W.F., Grogan, H. and Thorlakson, B. (1997): Preslaughter hide status of cattle and the microbiology of carcasses. *Journal of Food Protection*, **60**: 1502-1508.
- Warriss, P. W. (2001): Postmortem changes in muscles and its correction into meat. In: Meat science An Introductory Text, CABI Publishing, Wallingford, UK. Pp. 100-161.
- WHO (1995): Report of the WHO consultation on emerging food borne disease, Berlin, German, 20-24th March, 1995. Accessed online May, 2012 from: <http://who.int/zoonoses/resources/foodborne/en/index.htm>.
- WZFEDD, Wolaita Zone Finance and Economic Development Department (2005): Socioeconomic profiles of Wolaita Zone. Pp.1- 97.
- Yousuf, A. H. M., Ahmed, M. K., Yeasmin, S., Ahsan, N., Rahman, M.M., Islam, M. M. (2008): Prevalence of microbial load in shrimp, *Penaeus monodon* and prawn, *Macrobrachium rosenbergii* from Bangladesh. *World Journal of Agricultural Science*, **4**: 852-855.

Zweifel, C and Stephan, R. (2003): Microbiological monitoring of sheep carcasses, contamination in three Swiss abattoirs. *Journal of Food Protection*, **66**: 946-952.

Zweifel, C, Baltzer, D and Stephan, R. (2005): Microbiological contamination of cattle and pig carcasses at five abattoirs determined by swab sampling in accordance to EU decision 2001/471/EC. *Meat Science*, **69**: 559-566.

8. ANNEXES

Annex 1: Chick list used for recording in observation

1. Name of the market/restaurant/hotels _____

2. Location o the market/hotel/restaurant

A) Road side

B) Inside the market

C) Other _____

3. General sanitation and hygienic condition of meat selling environment

4. Origin of the meat

A) Slaughterhouse B) Backyard slaughter

5. Means of transport of the meat to selling market

A) Vehicle (Van)

B) By people

C) Other means _____

5. Personal hygiene of the people who sell raw beef

6. Other observations (if any)

Annex 2: Medias used for bacteriological examination

1. Nutrient agar (Oxoid, England)

Typical formula (g/l):

Lab-Lemco powder.....	1.0
Yeast extract	2.0
Peptone.....	5.0
Sodium chloride.....	5.0
Agar.....	15.0

pH: 7.4 ± 0.2

Directions: Suspend 28 g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

2. MacConkey agar (Oxoid, England)

Typical formula (g/l):

Peptone.....	20.0
Lactose.....	10.0
Bile salts.....	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar.....	12.0

pH: 7.4 ± 0.2

Directions: Suspend 52g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Dry the surface of the gel before inoculation.

3. Mannitol salt agar (Oxoid, England)

Typical formula (g/l):

Lab-Lemco' powder.....	1.0
Peptone	10.0
Mannitol	10.0
Sodium chloride	75.0
Phenol red	0.025
Agar.....	15.0
pH: 7.5 ± 0.2	

Directions: Suspend 111g in 1 liter of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

9. SIGNED DECLARATION SHEET

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any University and that all sources of material used for the thesis have been duly acknowledged.

Name: Naod Thomas: _____

Date of submission: June, 2012.

This thesis has been submitted for examination with my approval as University advisor:

Professor Mahendra Pal: _____