



Addis Ababa University
School of Graduate Studies
Center of Food Science & Nutrition

Microbial Safety and Quality of Fresh Beef Supplied to
Gullele Sub-City Market, Addis Ababa

By:

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Addis Ababa

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of Science in Food Science & Nutrition

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ACRONYMS AND ABBREVIATIONS

AAAE:	Addis Ababa Abattoir Enterprise
AAU:	Addis Ababa University
CFU:	Colony forming unit
CLSI:	Clinical and Laboratory Standards Institute
CSA:	Central statistics Agency
EB:	Enterobacteriaceae
EC:	European Commotion
FQ:	fore quarter
HQ:	Hinde quarter
GMP:	Good manufacturing practices
LIA:	Lysine Iron Agar
MoA:	Minister of Agriculture
PCA:	Plate Count Agar
SC:	Selenite Cysteine
SPSS:	Statistical Package for Social Sciences
TAMC:	total aerobic mesophilic count
TSC:	Total staphylococci count
TSIA:	Triple Sugar Iron Agar
UN:	United Nations
XLD:	Xylose lysine deoxycholate
YM:	Yeasts and molds

Microbial Safety and Quality of Fresh Beef Supplied to Addis Ababa Market

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ABSTRACT

Food-borne pathogens are the leading cause of illness and death in developing countries. The widespread habit of raw beef consumption is a potential cause of food-borne illnesses in Ethiopia. Therefore, this study was initiated to evaluate the microbiological quality and safety of beef samples supplied to Gullele sub-city Addis Ababa and also to assess the associated risk factors in handling the product. A questionnaire survey was used to answer some questions concerning the current status of food hygiene and sanitation practiced in abattoir and butchery shops. Fresh beef samples from different sources and swab samples from contact surface were collected for microbial analysis, following standard methods. This study revealed that the mean microbial counts of total aerobic mesophilic, staphylococci, Enterobacteriaceae, total coliform, fecal coliform, aerobic spore and yeasts and molds of the abattoir were 4.788, 3.287, 4.882, 4.763, 4.072, 1.476, 4.024 log cfu/100cm², respectively. Likewise, the mean microbial counts of total aerobic mesophilic, Staphylococci, Enterobacteriaceae, total coliform, fecal coliform, aerobic spore and yeasts and molds of the butcher shops were as great as 6.4, 4.8, 5.98, 5.9, 5.5, 1.84 and 5.3log cfu/g, respectively. Microbial load on meat was found to be low in the carcass sample compared to the morning and afternoon samples from butcher shops. The mean microbial count of total aerobic mesophilic, staphylococci, Enterobacteriaceae, total coliform, fecal coliform, aerobic spore and yeasts and molds of the contact surface samples were > 6.3, 3.7, 4.8, 5.3, 4.4, 1.8 and 4.9 log cfu/cm², respectively. The aerobic mesophilic flora was dominated by Enterobacteriaceae (29.5%) followed by Staphylococci ssp (26.67%) and Bacillus spp (17.4%). The prevalence of E. coli, Salmonella and S. aureus in meat samples were 29.55%, 3.4% and 28.41%, respectively. The isolated food-borne pathogens were resistant mostly to Penicillin (>60%), Amoxicillin (>40%) and Ampicillin (>40%). The overall facilities, slaughtering techniques, cold chain management, handling of slaughter cattle in the abattoir and personal hygiene, environmental sanitation, knowledge on food handling and educational status of the butcher shops 'workers were among the predominant factors of contamination of the beef. Therefore, implementations of GMP and HACCP principles as strategy should be adapted to control pathogenic microbes which pose public health risks.

Keywords: beef samples, dominant microflora, microbial counts, microbial contamination

1. INTRODUCTION

1.1 Back ground

Meat refers to animal tissue used as food, mostly skeletal muscles and associated fat but it may also refer to organs including lungs, livers, skin, brains, bone marrow, kidney and a variety of other internal organs as well as blood (Mohamed *et al.*, 2010). It is the major source of protein and valuable qualities of vitamins for most people in many parts of the world and is essential for the growth, repair and maintenance of body cells and necessary for our everyday activities (Tesfay Kebede *et al.*, 2014). On the other hand, the global livestock sector is growing faster than any other agricultural sub-sector. Global meat production has been projected to more than double from 229 million tons in 1999/2001 to 465 million tons in 2050 (FAO, 2010).

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 microorganisms. Nutrient and moisture contents are adequate to support the growth of all organisms. Although the Oxidative Reduction potential of whole meats is low, O/R conditions at the surfaces tend to be higher so that strict aerobes and facultative anaerobes as well as strict an aerobe can generally find conditions suitable for growth. Of the extrinsic parameters, temperature of storage stands out as being of utmost importance in controlling the types of microorganisms that develop on meats, as these products are normally held at refrigerator temperatures (James *et al.*, 2005). In addition to the nature of meat and cross-contamination from cattle's hind and gastrointestinal track, microbial safety and quality of beef could be compromised due to poor slaughtering techniques, practices and distribution systems. This includes pre-slaughtering handling, slaughtering equipment, handling and storage environment and hands of handlers.

Fresh meat is highly prone to contamination regardless of its nutritional values. In mild to severe illness, hospitalization or even death can be caused due to ingestion of infected food (Ayehu Gashe *et al.*, 2014). Recent data from either developing or developed countries indicate that at least 10% of the population may experience a food -borne diseases. The situation is equally serious in developing countries, with obvious economic consequences (Nel *et al.*, 2004). In

industrialized countries, infected food handlers are important source of food- borne diseases. In 2002, the Centers for Disease Control in the United States reported 76000 cases of food- borne illness, of which the majority being of bacterial origin (CDC, 2003). As indicated by Haeghebaert *et al.* (2002) out of 559 cases,64% of food-borne diseases reported in France, were due to salmonellosis.

The economic growth of developing countries has been compromised due in part to illness and death of young labor force caused by food-borne diseases (WHO, 2004). Changes in eating habits, mass catering, unsafe food storage conditions and poor hygiene practices are major contributing factors to food associated illnesses (Tesfay Kebede *et al.*, 2014). Contaminated raw meat is one of the main sources of food-borne illnesses. The risk of the transmission of zoonotic infections (Anthrax, Avian influenza, Leptospirosis etc.) is also associated with contaminated meat. Relevant study done in Morocco (Nozha *et al.*, 2006) has reported that food-borne diseases still represent one of the main causes of morbidity. This study has also reported that, during the previous years, 7118 cases of food- borne diseases have been reported among which 86% was of bacterial origin. According to the same report, 21.3% of the bacterial food- borne diseases was caused by red meat and meat products.

In Ethiopia, like other developing countries, it is difficult to evaluate the burden of food-borne pathogens because of the limited scope of studies and lack of coordinated epidemiological surveillance systems. In addition, under-reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of food- borne pathogens (Oosterom, 1991 and Edget Abayneh *et al.*, 2014).

The widespread habit of raw beef consumption is a potential cause for the spread of food-borne illnesses in Ethiopia (Edget Abayneh *et al.*, 2014). Some common factors such as overcrowding, poverty, inadequate sanitation conditions, poor general hygiene, mass production, inadequate knowledge on food handling and environmental factors were reported to play significant role in the spread of food-borne illness in Ethiopia (Nozha *et al.*, 2006 and EdgetAbayneh*et al.*, 2014).

A study conducted in Dire-dawa, Ethiopia shows that out of the 384 meat samples collected from the two abattoirs, a total of 61 (15.89%) meat samples were found to be positive for *E. coli* (Ousman Mohamed *et al.*, 2014). Similarly, Haimanot Tassew *et al.* (2010) have showed that from a total of 165 meat samples collected from hotels, butchers and an abattoir, various food-borne pathogens were isolated in Jimma, Ethiopia.

1.2 Statement of the problem

Ethiopia is estimated to have 53.99 million heads of cattle, 25.5 million heads of sheep and 24.06 million head of goat (CSA, 2013). Simultaneously, as per capital income of the population is increasing, annual per capital consumption of meat is also continuously growing. Consequently, in 2020 meat per capital consumption is estimated to be 30kg (FAO, 2009).

On the other hand, even though there is no precise data, the incidence of food -borne outbreaks in Ethiopia seems to be higher compared to developed countries (Tavakoli *et al.*, 2008). Even if data regarding meat-borne diseases in Ethiopia are extremely scarce, a few studies conducted in different parts of the country showed that pathogenic organisms like *Campylobacter* spp, *Salmonellas* pp, *Taenia* spp, *Toxoplasma* spp, *Mycobacterium* spp, *Brucella* spp, *Escherichia coli*, *Echinococcus/hydatid* cysts were identified as causes of food-borne illness (Tesfay Kebede *et al.*, 2014)

In developing countries like Ethiopia, food-borne diseases occur because of the prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, lack of financial resources to invest in safer equipment and lack of education for food-handlers (WHO, 2004). Of the foods intended for humans, those of animal origin tend to be most hazardous unless the principles of food hygiene are employed. Animal products such as meats, fish and their products are generally regarded as high risk commodity with regard to pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al.*,2008).

Microbial contamination of raw meat results from processing and starts during slaughter, when the carcass becomes contaminated with microorganisms residing on external surfaces, the

gastrointestinal tract and lymph-nodes of the animal and from environment (Ousman Mohamed *et al.*, 2014). Because of the nature of meat, the means by which it has to be obtained from the animal and the means of distribution to the consumer are prone to microbial contamination including pathogenic bacteria (Nozha *et al.*, 2006).

The demand for meat product in the country has been increased dramatically; especially the consumption of raw meat becomes a status of symbol. It is clearly stated that, around 30% of the national meat consumption is in Addis Ababa City (Tesfay Kebede *et al.*, 2014). However, the full value chain of meat supply from abattoirs, distribution, butchery shops to final consumer are not properly handled to ensure the microbial quality, safety, soundness, wholesomeness and hygiene. There is also a limited study on investigation of microbial safety, quality and safe handling procedures of meat processed in Addis Ababa abattoirs. In addition, there is no adequate information regarding the assessment on food safety practice, food-borne diseases and microbial load of meat contact surface in Addis Ababa abattoirs and butchery shops. These factors could hinder government and other stockholders to accurately apply measures on the impact of food contamination problems on public health. The final consumer has also limited information on quality and safety of the meat consumed regularly.

Therefore, this study was focused on identifying the predominant factors and steps at which the quality and safety of meat supplied to Gullele sub-city market, Addis Ababa have compromised.

1.3 Objectives

General objective

The general objective of this study was to examine the microbiological quality and safety of beef samples supplied to Addis Ababa market and to assess the associated risk factors in handling the product.

Specific objectives

The specific objectives were:

- To determine the microbial load (aerobic mesophilic bacteria, Enterobacteriaceae, staphylococci, aerobic spore formers, yeasts, molds, total coliform, fecal coliform,) and identify of food-borne pathogens (*Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*) from selected meat cuts (Neck and topside) of beef carcass collected from the abattoir.
- To examine and compare the microbial load and the prevalence and distribution of food-borne pathogens of raw beef from selected meat cuts (Top side, Neck and Minced meat)of specific butchery shop and among butcher shops from samples collected at the morning and at late afternoon
- To determine the microbial load and identify food-borne pathogens in meat contact surfaces of butcher shops
- To evaluate antimicrobial susceptibility of the bacterial pathogens isolated from meat samples
- To characterize the dominant micro-flora of meat samples to various bacterial groups/genera
- To assess the hygienic condition of slaughtering practices and knowledge of workers in the butcher shops

2. LITERATURE REVIEW

2.1 Microbial contaminants of fresh meat

Originally meat was a term used to describe any solid food, but has now come to be applied almost solely to animal flesh. Since the days of hunting and gathering, and animals (sheep) were first domesticated at the beginning of the Neolithic revolution around 9000 BC, meat has contributed substantial role in the human diet. Though denied by some on moral or religious grounds, meat eating still remains widely popular. This is due to the fact that it has desirable texture and flavor characteristics; beside meat protein does also have a high biological value (Adams and Moss, 2000). Though numerous species are used as a source of meat around the world, they range from flying foxes to frogs and from kangaroos to crocodiles. Meat from cattle, pigs, sheep, goats and poultry are among animals of principal importance in economic. Meat consumption is often something of a status symbol and is generally far greater in wealthy societies. Meats are the most perishable of all major foods for the reasons listed in Table 1. Meat contains an abundance of all nutrient required for the growth of bacteria, yeast and molds and an adequate quantity of these constituents exist in fresh meats (Firew Tafesse *et al.*, 2010).

In general, the biota is reflective of the slaughtering and processing environments with Gram-negative bacteria being predominant. Among Gram-positives, the enterococci are the biota most often found along with lactobacilli. Because of their ubiquity in meat-processing environments, a rather large number of mold genera may be expected, including *Penicillium*, *Mucor*, and *Cladosporium*. The most ubiquitous yeasts found in meats and poultry are members of the genera *Candida* and *Rhodotorula* (Adams and Moss, 2000). Delmore *et al.* (1999), noted that, when spoiled meat products are examined, only a few of the many genera of bacteria, molds, or yeasts are found, and in almost all cases, one or more genera are found to be characteristic of the spoilage of a given type of meat product. The presence of the more-varied biota on non-spoiled meats, then, may be taken to represent the organisms that exist in the original environment of the product in question or contaminants picked up during processing, handling, packaging, and storage.

When microbial numbers of fresh meat reach around 10^7 cfu cm^{-2} , it can be detected due to production off odors. This is signal of spoilage of fresh meat. At this point it is believed that the micro-organisms switch from the diminishing levels of glucose in the meat to amino acids a substrate for growth. In meat with lower levels of residual glucose this stage is reached earlier (10^6 cfu cm^{-2}) and this accounts for the earlier onset of spoilage in high pH meat. At final spoilage stage form of a visible slime surface on the meat is one sign. This stage is happened when microbial numbers reach levels of around 10^8 cfu cm^{-2} (Adams and Moss, 2000).

Table: 1 Chemical Composition of Typical Adult Mammalian Muscle after Rigor Mortis but Before Degradative Changes Postmortem (% , w/w)

Water	75.5%
Protein	18.0
Myofibrillar	
Myosin, tropomyosin, X protein	7.5
Actin	2.5
Sarcoplasmic	
Myogen, globulins	5.6
Myoglobin	0.36
Hemoglobin	0.04
Mitochondrial—cytochrome C	ca. 0.002
Sarcoplasmic reticulum, collagen, elastin, “reticulin,” insoluble enzymes, connective tissue	2.0
Fat	3.0
Soluble nonprotein substances	3.5
Nitrogenous	
Creatine	0.55
Inosine monophosphate	0.30
Di- and triphosphopyridine nucleotides	0.07
Amino acids	0.35
Carnosine, anserine	0.30
Carbohydrate	
Lactic acid	0.90
Glucose-6-phosphate	0.17
Glycogen	0.10
Glucose	0.01
Inorganic	
Total soluble phosphorus	0.20
Potassium	0.35
Sodium	0.05
Magnesium	0.02
Calcium	0.007
Zinc	0.005
Traces of glycolytic intermediates, trace metals, vitamins, etc. ca.	0.10

Source: Modern Food microbiology, (James *et al.*, 2005)

2.2 Food- borne illness and its type

Most cases of food borne illness are a result of pathogens in food. **Pathogens** are microorganisms that can cause illness in humans. The pathogens that cause food borne illness do not necessarily cause undesirable changes in food. Many times, pathogens cause a food to be unsafe to eat before there are any visible signs of spoilage (Adams and Moss, 2000). Pathogens can cause illness in one of three ways: intoxication, infection or toxic infection (Firew Tafesse *et al.*, 2010).

Food Intoxication

Some microbes can give off a by-product that causes illness. Substances released by microbes that are harmful to humans are called **toxins**. In this case, it is not the microbe that makes people sick but the toxin it produces. A food borne illness caused from a toxin released by microbes is called a **food intoxication**. It is important to remember that killing the microbes may not be enough to prevent cases of food intoxication. If the toxin is still present and has not been damaged or altered, the person will still become ill. The severity of the illness will depend on the amount and/or type of toxins present in the food eaten. It will also depend on how susceptible the person is to illness. A number of microbes' cause food intoxication some of the most important includes *Clostridium Perfringens*, *Staphylococcus Aureus*, *Clostridium botulinum* and *Escherichia coli* (James *et al.*, 2005)

Food Infection

The second major cause of food borne illness is the microbes themselves. The increase in the number of these microbes and their metabolic activities damage body tissue and cause illness. This type of food borne illness is called food infection. A food infection cannot occur if the microbes are killed. Food infections may be caused by bacteria, parasites, fungi and viruses. A large number of living organisms is usually required to cause these types of illness. Symptoms are related to damage caused by the organisms affect their hosts. Some of these microbes include *Listeriamonocytogenes* and *Salmonella* spp. Texico-infection results when bacteria present in food, such as *Clastridium perfringens*, are ingested and subsequent produce as toxin in the host (Firew Tafesse *et al.*, 2010).

Food consumers in developing countries including Ethiopia suffer from food-borne bacterial illness especially from those of *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Bacillus cereus* (Bean *et al.*, 1990). In Addis Ababa, (Molla *et al.*, 2004) reported 5.1% prevalence of *Listeria* from retail meat and milk products. The *Listeria* spp were detected in 69.8%, 47.5%, 43.5%, 18.6%, 15.4% and 1.6% of the pork, minced beef, ice cream, fish, chicken and cottage cheese samples, respectively.

A study conducted in Modjo town export abattoir, Ethiopia (Zelalem Ayana *et al.*, 2015) revealed occurrence and proportion of *E. coli* O157:H7 from feces 4.7%, skin swabs 8.7%, carcasses before washing 8.1% and after washing 8.7% and on water samples 4.2%. In their report, the proportion of carcasses contaminated with *E. coli* O157:H7 was strongly associated with those recovered from fecal and skin samples. And in Addis Ababa, the overall prevalence of *Salmonella* isolated from minced meat beef, mutton and pork from retail supermarkets were 14.7%. *Salmonella* was detected in 14.4% minced beef, 14.1% mutton and 16.4% pork samples subjected to isolation and identification.

2.3 Causes of Foodborne disease and its outbreak

Food borne disease has been defined by the World Health Organization as: '*Any disease of an infectious or toxic nature caused by or thought to be caused by, the consumption of food or water.*' This definition includes all food and waterborne illness and is not confined to those primarily associated with the gastro-intestinal tract and exhibiting symptoms such as diarrhea and/or vomiting (Adams and Moss, 2000). Food borne disease outbreak is the occurrence of two or more cases of a similar food borne resulting from the ingestion of a common food (WHO, 2008).

More than 40 food borne microbial pathogens are known to cause human illness, including bacteria, parasite, viruses, fungi and their toxins. Several pathogens were recognized only recently as a cause of food borne illness. Some food borne pathogens have not yet been scientifically identified (Firew Tafesse *et al.*, 2010). Epidemiological investigations of food borne

illness provide some information about the specific pathogens and foods that causes illness. Contaminated animal products such as meat, poultry, sea foods dairy products and eggs are the foods most likely to cause outbreak of human illness in United States (Jean *et al.*, 2001).

The World Health Organization (WHO) reports that hundreds of millions of people worldwide suffer from diseases caused by contaminated food and those products of animal origin rank at the top of the list of causes (WHO, 1997). More than 3 million children under five years of age die each year because of diarrhea. Contaminated water and food- borne pathogens cause much of this diarrhea (Chris *et al.*, 1999).

About two third of all outbreaks involve bacteria. The rest are caused by viruses, parasite, fungi and chemicals. Despite long-established food quality assurance systems in developed countries, new food contamination risks have now been emerging. According to WHO, seven food- borne pathogens (*Campylobacter jejuni*, *Clostridium perfringens*, *E. coli* O157:H7, *Listeria-monocytogenes*, *Salmonella-typhimurium* ,*Staphylococcus aureus*, and *Toxoplasma gondii*) are responsible for an estimated 3.3 to 12.3 million infections and 3,900 deaths annually in the United States (WHO 1997). Furthermore, global surveys by WHO indicate that food- borne diseases may occur 300- 350 times more frequently than reported (Chris *et al.*, 1999).

Salmonella spp are blamed for more than 50,000 cases of bacterial food poisoning in the United States every year (WHO 1997). Transmission of this microbe usually occurs through in sufficiently cooked meats and eggs. Chickens are a major reservoir of *Salmonella*. Ingesting foods contaminated with significant amounts of salmonella can cause intestinal infection.

Beef that was contaminated in the slaughterhouse is the principal cause of *E. coli* infection in humans. Bacteria are transmitted when contaminated meat is consumed raw or undercooked. Ground meats, such as ham burgers, are particularly associated with infections because the infected material is mixed throughout the product during the grinding process (Chris *et al.*, 1999). The author also indicated that, hygienic slaughtering practices will reduce contamination of carcasses but will not guarantee the absence of microbial contamination from products. The

only effective method of eliminating dangerous microbes is to heat (thoroughly cook or pasteurize) or irradiate food.

Transmission of intestinal parasites and enteropathogenic bacteria is effected directly or indirectly through objects contaminated with feces. These transmission tools include food, water, nails, and fingers, indicating the importance of fecal-oral human-to-human transmission (WHO, 1987). Accordingly, food-handlers with poor personal hygiene working in food-serving establishments could be potential sources of infections of many intestinal helminths, protozoa, and enteropathogenic bacteria. Food-handlers who harbor and excrete intestinal parasites and enteropathogenic bacteria may contaminate foods from their feces via their fingers, then to food processing, and finally to healthy individuals. Compared to other parts of the hand, the area beneath fingernails harbors the most microorganisms and is most difficult to clean (Gashaw Andargie *et al.*, 2008).

2.4 Prevention and control of food-borne pathogens

Food born bacterial illness by bacteria are most commonly prevented and controlled by proper cooking and preparing of food as well as storing. The control method or measures includes; a) education of those who prepare the food at home and other food handlers, so that they have to take proper personal measures; b) prohibiting individuals with absences or other skin lesions from handling food; c) placing of food in cold place at 4 degree centigrade or lower of all food in order to prevent bacterial multiplication and the formation of toxin. Foods must be kept at room temperature for as little time as possible (WHO, 2008).

The prevention/avoidance of foodborne illness caused by *E. coli* can be prevented by the same method as prevention of other food borne illness caused by bacteria. However, in the cases of ground beef, the recommendation is that it cooked to 160°F or that the core temperature be brought to a minimum of 155°C for at least 15 second and that the juices are clear (Mokonnen Addis and Desta Sisay, 2015).

There is a critical need to develop method to control the spoilage or poisoning of food by *Salmonella* ordinary farms by instituting bio-security and bio-containment practices in addition

to enhanced food processing method, preparation and storage practices (Quinn *et al.*, 2001). Effective heat processing of food of animal origin, which includes pasteurization of milk and eggs, irradiation of meat and poultry thermal processing; good hygiene practices during production of food; vaccination of egg-producing flocks and food producing animals (Mokonnen Addis and Desta Sisay, 2015).

2.5 Antibiotics susceptibility pattern of food borne bacterial isolates

Antibiotics used in intensive animal production especially in poultry have led to the emergence of *Salmonella*, *Listeria*, and *E. coli* resistant to a number of antibiotics those are used from human bacterial diseases. There is increasing concern about new strains of influenza developing in pigs and chickens that could be transmitted to humans, such as the *avian flu* strain that forced eradication of chickens in Hong Kong in 1997 (Chris *et al.*, 1999). At present the occurrence of antibiotic resistant strains are great problems worldwide even though the use of antibiotics has been proven to be an effective means for the prevention and control of bacterial infection (Tesfay Kebede *et al.*, 2014).

During the past decade, bacteria that causes human disease have developed resistance to many of the antibiotics commonly used for treatment. The incidence of zoonotic foodborne *Salmonella* infection has increased in most industrialized countries. In recent years, testing of *Salmonella* isolates has shown that an increasing proportion of isolates are resistant to several antimicrobial agents both in developing and developed countries (Endrias Zewdu, 2014). Colonization of patients with methicillin- resistance or/and reduced vancomycin-sensitivity in *Staphylococcus aureus* is a crucial factor in health care. Genes expressing antibiotic resistance in *S. aureus* can be either in chromosomic or in plasmidic DNA (Pesavento *et al.*, 2007).

Drug resistance in *Salmonella* increases the frequency and severity of infection with this pathogen, limits treatment options, and raise health care costs. These effects may be related to enhanced shedding and augmented virulence of resistant strains, increased rates of transmission of this strain, and the ineffectiveness of initial regimens of antimicrobial therapy against such strains (Gorbach, 2001).

Inappropriate antibiotic treatment of a septicemic patient infected with a resistant *Salmonella* strain could lead to a fatal outcome if the therapeutic drug administered upon hospitalization subsequently proved to be identical to the resistance phenotype. Such an occurrence would likely stem from the antibiotic-dependent inactivation of resident microflora in the intestinal tract and from the rapid colonization, growth, and dissemination into deeper tissues of antibiotic-resistant *salmonella* spp to a point beyond therapeutic management. Recent increases in the rates of human isolation of chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole-resistant *Salmonella* have necessitated a major shift in the antibiotic treatment of human systemic salmonellosis and the sanitation of chronic carriers (Endrias Zewdu, 2014).

There is evidence that patients infected with antibiotic-resistance strains suffer worse outcome (invasive illness or death) than those infected with sensitive strains. There is growing scientific evidence that the use of antibiotics in food animals leads to the development of resistance pathogenic bacteria that can reach humans through the food chain. This underlines the need to limit the use of antimicrobials in veterinary practice to limit the occurrence of resistance (Lemma Dadi and Daniel Asrat 2008).

2.6 Slaughtering techniques and its source of contamination

Pre-slaughter handling of livestock and post-slaughter handling of meat play an important part in deterioration of meat quality. The glycogen content of animal muscles is reduced when the animal is exposed to pre-slaughter stress which changes the pH of the meat, to higher or lower levels, depending on the production level of lactic acid. Lactic acid is produced due to the breakdown of glycogen content of animal muscles via an anaerobic glycolytic pathway. Higher levels of pH (6.4-6.8) result in Dark, Firm and Dry (DFD) meat. Long term stress causes DFD meat which has a shorter shelf life. Severe short term stress results in a Pale, Soft and Exudative (PSE) meat. PSE meat has a pH lower than normal ultimate value of 6.2 which is responsible for the breakdown of proteins, providing a favorable medium for the growth of bacteria (David and Gihaly, 2011).

It is generally agreed that the internal tissues of healthy slaughter animals are free of bacteria at the time of slaughter, assuming that the animals are not in a state of exhaustion. When one examines fresh meat and poultry at the retail level, varying numbers and types of microorganisms are found (Delmore *et al.*, 1999). According to James *et al.* (2005), the following are the primary sources and routes of microorganisms to fresh meats with particular emphasis on red meats:

1. *The stick knife.* After being stunned and hoisted by the hind legs, animals such as steers are exsanguinated by slitting the jugular vein with what is referred to as a “stick knife.” If the knife is not sterile, organisms are swept into the bloodstream, where they may be deposited throughout the carcass.

2. *Animal hide.* Organisms from the hide are among those that enter the carcass via the stick knife. Others from the hide may be deposited onto the dehaired carcass or onto freshly cut surfaces.

Some hide biota becomes airborne and can contaminate dressed out carcasses.

3. *Gastrointestinal tract.* By way of punctures, intestinal contents along with the usual heavy load of microorganisms may be deposited onto the surface of freshly dressed carcasses. Especially important in this regard is the paunch or rumen of ruminant animals, which typically contains $\sim 10^{10}$ bacteria per gram.

4. *Hands of handlers.* This is a source of human pathogens to freshly slaughtered meats. Even when gloves are worn, organisms from one carcass can be passed on to other carcasses.

5. *Containers.* Meat cuts that are placed in non-sterile containers may be expected to become contaminated with the organisms in the container. This tends to be a primary source of microorganisms to ground or minced meats.

6. *Handling and storage environment.* Circulating air is not an insignificant source of organisms to the surfaces of all slaughtered animals.

7. *Lymph nodes.* In the case of red meats, lymph nodes that are usually embedded in fat often contain large numbers of organisms, especially bacteria. If they are cut through or added to portions that are ground, one may expect this biota to become prominent. In general, the most significant of the above are non-sterile containers. When several thousand animals are

slaughtered and handled in a single day in the same abattoir, there is a tendency for the external carcass biota to become normalized among carcasses, although a few days may be required. The practical effect of this is the predictability of the biota of such products at the retail level (Dillon and Board 1991).

Carcass sanitizing/washing

Just prior to slaughter, the outer surfaces of meat animals are laden with dust, dirt, and fecal matter. It is inevitable that some of the microorganisms from these sources will be found on the carcasses of slaughtered animals, and although most are non-pathogens, pathogens may be present. In an effort to reduce the number and types of pathogens on dressed carcasses and finished products, a number of methods have emerged:

1. Trimming—the excising of skin or outer tissue
2. Washing—the use of plain water at varying temperatures and hose pressures
3. Organic acids—the addition to wash water of acetic, citric, or lactic acid at concentrations of 2% to 5%
4. Other chemicals—the addition to wash water of hydrogen peroxide, chlorine dioxide, or chlorhexidine
5. Steam vacuum treatments—the application of steam for 5 to 10 seconds at 80°C or higher as the final carcass preparation step
6. Combinations—the use of two or more of the above

In the USDA's pathogen reduction program for beef carcasses, 1 out of every 300 carcasses is to be examined by sponging 100-cm² sections from three carcass areas (rump, flank, and brisket) for *E. coli*, which should be <5 cfu/cm². The sponging method is one of six that were compared for beef carcasses (Dillon, 1998).

2.7 Good manufacturing practices and personal hygiene

Employees are the largest contamination source. Especially those, who do not follow sanitary practices properly, contaminate food that they touch with spoilage and pathogenic microorganisms. Employees hands, hair, nose and mouth, harbor micro-organisms that can be transferred to food during processing, packaging, preparation and service by touching, breathing, coughing or sneezing (Tofick Kedir, 2013).

Many good manufacturing practices focus directly on reducing contamination by food handlers. Examples of personal hygiene include washing hands, removing jewelry, and maintaining personal cleanliness. Also, the food processor should provide training for new employees in personal hygiene based on creating good manufacturing practices. Equipment's for food processing should be free from dirt to reduce contamination of food (Keener, 2009).

HACCP focuses on three types of hazards: biological hazards, chemical hazards and physical hazards. Biological hazards are the type of hazards that receive the most attention in the HACCP system and which also present the greatest risk of severity and occurrence. Biological hazards include hazards from pathogens such as bacterial, viruses, yeast, and molds. Bacteria that receive the greatest attention include *E. coli* 0157:H7, *Listeria Monocytogenes*, *Salmonella*, *Staphylococcus aureus* and *Campylobacter*. Chemical hazards in meat products could result from the misuse of antibiotics in production, contamination with sanitizers or cleaning agents, or environmental contamination from hydraulic fluids. Physical hazards are probably the most recognized by consumers as they usually find this hazard. Glass, metal and plastic are physical hazards that can occur in meat products (Pearsen and Dutson, 1999).

2.8 Food regulations

Food regulation, which subjects' suppliers of goods and services to behavioral control and which penalize those who fail to perform in accordance with the specified standards are the dominant form of social regulation (Nadvi and Waltring, 2003). Since ancient time producers of food products have attempted to alter their wares in an effort to obtain dear prices for cheaper goods by adding water to wine and by skimming cream from milk. This shows that adding worthless substance to food and taking valuable substance from food product was experienced in ancient society. Hence regulation governing what could or could not be added to food products and regulations that require the use of official weights and measures have been introduced to protect consumers from fraudulent and/or unsafe food products (Codex standards, 2009).

Food regulation in Ethiopia is a shared responsibility of Ministry of Health, Ministry of Agriculture, Ministry of Trade and Industry, and Ethiopian Standards Authority. Moreover, the

existing laws and regulations are outdated and could not respond to contemporary food quality and safety issues. Hence for the purpose of identifying the problems and challenges associated with food quality regulation in Ethiopia, international food standards guidelines and selected countries experience serve as useful instruments. (Mulat Abegaz, 2004).

As information obtained from ESA reveals there are still food and food products such as meat, baby foods, bottled mineral water, whose compliance with the standards are not still made compulsory. This indicates that there is a need to revise the existing compulsory Ethiopian standards to prepare compulsory standards for those foods the absence of which may have an impact on health and safety of the public (Erkyihun. 2010).

Though Ethiopian Food Medicine and Health Care Administration and Control Authority established as an autonomous government office having its own legal personality cannot bring improved food regulatory system in the country (FMHACA, 2010). The Ethiopian food inspection and control system is mainly divided between the Health sector responsible for the safety of food products to be consumed and strongly oriented to consumers and to food processing factories, and the Agriculture sector responsible for the animal and plant health. It might seem paradoxical to discuss on the subject of food control when millions are suffering from lack of food and the most inferior quality. At a national level, however, both food shortage and lack of appropriate food regulation assurance systems are problems that have become obstacles to the Ethiopian economic development and public health regulation. Though the country is endowed with enormous potential for the production of agricultural and industrial food products, its competitiveness in the world market has been so far very low (Melese Temesgen, 2015).

2.9 Value chain of meat supplied to Addis Ababa Market

Beef Cattle Value Chain

The Ethiopian meat and live animal value chains have developed over the years into a series of complex constituents involving various actors that include producers, collectors, small private and cooperative fatteners/feedlots, various (and in some places, numerous) middlemen, livestock trading cooperatives, individual traders and exporters. In general, there has not been historically a reliable, sustained relationship among actors within this value chain. Most relationships are casual and change often to suit the situation and the actors. Although value chain relationships work best when they are on a strict business basis, such relationships in the highlands can be characterized as “clannish”. Although these relationships are not all clan-based, trust is built through such relationships and being native to an area gives one a significant advantage. There is very few well-developed backward-linked relationship from processors to traders and producers (Harko, 2015).

According to the Trade and Industry Bureau (LIVES, 2014), the supply of cattle, to Addis Ababa market, is mainly from Oromia, Southern Nation Nationalities People Region (SNNPR) and Amhara. The supply of sheep to *Berchuko* is dominated by Arsi, west Shoa and North Shoa zones. The livestock supply to the markets varies from season to season. The largest number of livestock is sold from April to September, followed by October to January. The lowest market is in the months of February and March (during Ethiopian Orthodox fasting months). To serve these consumers, there are five regional livestock market centers around Addis Ababa: *Berchuko*, *Karalo*, *Kerra*, *Shogolle* and *Akaki*, and established in 1988, 1987 1949, 1995 and 2004, E.C., respectively.

The live animals purchased, from the indicated livestock markets, by butcheries or supermarkets are sent directly to the official Abattoirs, and sent through the Orthodox, Muslim, or European slaughter facilities (Semeneh Seleshi *et al.*, 2014). After slaughtering the meat products will be distributed to the butcher shops and supermarkets.

2.10 Ethiopian meat consumption habits

The success in understanding the culture of other countries or ethnic groups lies in understanding their rituals in food consumption customs. In developing countries, culture plays a crucial role in determining food patterns (Semeneh Seleshi *et al.*, 2014). Cultural diversity is the unique feature of Ethiopia; the country's population composed of about 80 ethnic groups whose cultures are diverse from one another. Each ethnic group has its own culture manifested to the widely-practiced diet (national foods), way of living, celebrations, dressing and dances at the cities and the cultural fabric intertwining is still continuing (Getu Kebede, 2010).

Differences in religious affiliations tend to influence the way people live, the choices they make, what they eat and whom they associate with; the beliefs play significant parts in sculpting social behavior and are inbuilt to dictate what a person can eat and what he cannot. The consumption of meat and meat products in Ethiopia has very tight association with religious beliefs, and are influenced by religions. On holidays and social ceremonies of Ethiopia, meat products are eaten and the stews are also made mainly from chicken, beef, lamb and mutton (Getu Kebede, 2010).

3. MATERIAL AND METHODS

3.1 Description of the Study area

The study was conducted in Addis Ababa, Gulele sub-city. Gulele sub-city, covered 30.18km², is one of the ten sub cities in Addis Ababa, capital of Ethiopia. The district is located in northern suburban of the city, near the Mount Entoto and Entoto Natural Park. It borders with the districts of Kolfe Keranio, Addis Ketema, Arada and Yeka (Fig. 1). According to CSA (2012), the number of population in the sub-city was 284,865. The population density per sq. meter of the sub-city was 9,438.9. GuleleSub-City is located at 9°3'46.8"N and 38°44'36.96"E, it is divided into 10 Weredas.

According to UN report, accessibility and distribution of potable water in Gulele sub-city was estimated to be 40.1-47.5% and 35-48.3%, respectively and 40% of the water is sourced from public tap. Only 20-25.1% of the households of this sub-city have adequate toilet facility. About 85% of households use shared toilet facilities (UN, 2013).

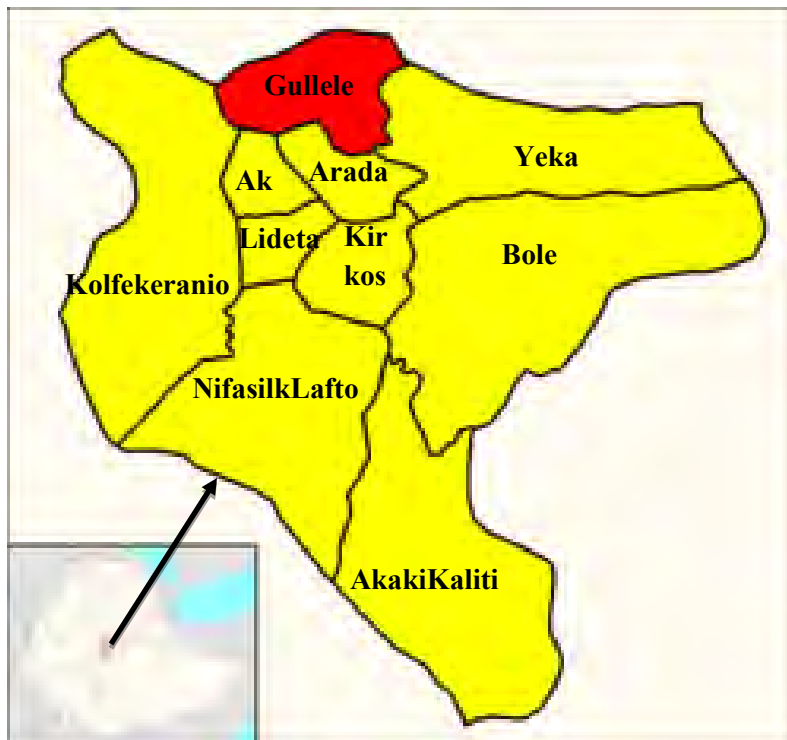


Fig: 1. Map of the study area (Gulele is shown in red)

Cattle are brought to the city from Oromia, Southern Nation Nationalities People Region (SNNPR) and Amhara. The butcheries, supermarkets and restaurants purchase the certified and slaughtered animals from this Addis Ababa market. When the cattle have arrived to the abattoirs, they hold in lairage for a given time. Then the slaughtering process will undergo. The main slaughtering steps that the abattoirs follow was ante and post Mortem inspection, bleeding, de-hiding, evisceration, splitting and transportation (Semeneh Sileshi *et al.*, 2014). After transportation, the customers' (butchers, supermarkets and restaurants) of the abattoirs will prepare the quarters as per the final consumer's preference. For this study, Addis Ababa abattoirs enterprise (AAAE) and 8 subsequent butcher shops (on its own route) were selected.

3.2 Study design

Across-sectional study was carried out to collect data from multiple cases at a given point in time. Systematic random sampling technique was employed to assess the beef quality, safety and the source of contamination through the whole value chain of Addis Ababa beef market. For this study, samples of beef from different sources namely abattoir and butcher shops were collected from selected meat cuts from neck (FQ), topside (HQ) and from minced meat for microbial analysis of beef with the aim of enumerating viable colonies, identifying pathogenic bacteria and investigating their antimicrobial susceptibility profiles.

A questionnaire survey was used to answer questions concerning facilities, equipment, current status of food hygiene and sanitation practices in abattoir and butcher shops. Hygiene and sanitation were determined by the use of structured interview and through direct observations of the hygienic status and practices of butcher shops' workers. Bacteriological analysis of swabs taken from contact surface and identification of pathogenic bacteria were conducted to supplement the sanitary survey.

3.3 Sample collection and preparation

Swab Samples from 16 beef carcass (8x2) were taken from AAAE. The samples were collected by systematic random sampling techniques during two operation days. The swab samples were taken from 100 cm² area of the carcass parts (hind and fore quarter). The samples were contained in sterile containers and transported to Center for Food Science and Nutrition laboratory, AAU for immediate analysis (Arse Gebeyehu *et al.*, 2013).

Beef meat samples (500g from each cut) were also collected from 8 randomly selected butcher shops in Gullele sub-city, Addis Ababa which are sourced from the AAAE. The beef samples were taken from neck (FQ), topside (HQ) and minced meat in separate sterile plastic bags. The samples were placed in ice box (4⁰C) and transported to Center for Food Science and Nutrition laboratory, AAU for immediate analysis. The samples were collected in the morning (8:00-9:00 a.m.) and late in the afternoon (5:00-6:00p.m). Therefore, a total of 48 (8x3x2) meat samples from butcher shops were considered for the study. Furthermore, swabs sample were randomly collected aseptically from butcher's knives, cutting tables and weighing balance. A total of 24 samples (8x3) were collected from butchers' shops. The samples were taken simultaneously with the beef samples. An area of 1cm² was used for swabbing with sterile swabs soaked into 10 ml 0.1 % saline water. The swab samples were kept in sterile broth in icebox cooler and taken to the laboratory for further study (Endale Balacha *et al.*, 2013 and Obeng *et al.*, 2013).

3.4 Microbial enumeration

For microbiological analysis, 25 g of meat sample from selected meat cuts were transferred aseptically into a sterile stomacher bag containing 225 ml of sterile distilled water and homogenized for 1-3 minute using a stomacher lab blender (Depofen, France). On the other hand, each tube containing swab samples (10ml of 0.1% saline water) was vortexed for 10 s to ensure mixture of the sample. A tenfold serial dilution was prepared by transferring 1 ml of the homogenized sample (both, meat and swab) to 9 ml diluents. From appropriate serial dilutions, a 0.1 ml aliquot was plated on various types of media for microbial counts. The number (N) of cfu/g of the test sample was calculated using the following formula (Tofik Kedir 2013):

$$N = \frac{n}{1/d \times v}$$

where;

N = total number of bacteria (cfu) per gram of the sample

n = average number of bacterial colonies, from different dilutions in Petri-dish that contained 30-300 colonies

v = volume of sample for plating

1/d = dilution factor of the specimen/food sample

Total aerobic mesophilic count

Total viable aerobic mesophilic counts of all samples were determined using standard plate count agar (PCA) (HIMEDIA). A 0.1 ml of aliquot from appropriate dilution was pipetted and spread on standard pre-solidified plate count agar medium. Inoculated plates were incubated at 32°C for 48-72 hrs. After incubation, plates with colonies between 30- 300 were counted (ISO, 2009).

Total and fecal coliforms count

A 0.1 ml of homogenate from appropriate dilution was pipetted and spread on Violet Red Bile Agar (SRL) after incubating inoculated plates at 32°C and 44.5°C for 18 – 24 hrs. for total and fecal coliform, respectively. Purple-red colonies that are 0.5 mm or larger in diameter and surrounded by zone of precipitated bile acids were counted. The purple-red colonies were counted and then confirmed by transferring the selected five colonies to Escherichia coli Broth by their gas production. (American Public Health Association, 2012).

Enterobacteriaceae count

To count the members of Enterobacteriaceae, 0.1 ml from appropriate serial dilution of the samples was spread plated on MacConkey agar (SRL) supplemented with glucose and was incubated at 35°C for 24 hrs. All reddish purple/pink colonies were counted as members of Enterobacteriaceae (American Public Health Association, 2012).

Staphylococci count

For staphylococci count, Mannitol Salt Agar (MSA, OXOID) was surface plated with 0.1ml of the homogenate from 10^{-1} – 10^{-2} . The inoculated plates were incubated at $35^{\circ}\text{C}\pm 2$ for 36 hrs. Then, golden yellow color colonies were counted as *staphylococci*. Typical colonies of *Staphylococci* spp (golden yellow colonies shining and convex), diameter 1.0-1.5 mm after 24 hours' incubation and 1.5- 2.5 mm after 48 hours' incubation were isolated, purified and tested for coagulase positive as a confirmatory test (American Public Health Association, 2012).

Aerobic spore count

Bacterial spores were counted after heating the suspension of meat samples for ten minutes in water bath adjusted at 80°C . A 0.1 ml of the homogenate was spread plated on the pre-solidified surface of plate count Agar (PCA) plates. Colonies were counted after incubation at 35°C for 36 to 72 hrs.

Yeasts and molds counts

The yeasts and molds count of all samples were done by direct plate count using Potato Dextrose Agar (PDA; SRL) supplemented with 0.1g Chloramphenicol. A 0.1 ml of the homogenate from appropriate serial dilution was spread on PDA that contained Chloramphenicol. Total Yeasts and molds were counted after incubation at 25°C for 3-5 days (United States Pharmacopeial Convention, 2007).

3.5 Characterization of dominant microflora

After enumeration, from aerobic mesophilic bacteria, about 10 -20 colonies were picked randomly from countable plates and inoculated into tubes containing about 5ml Nutrient Broth(HIMEDIA). These were incubated at 30°C overnight. The cultures were purified by repeated plating and were characterized to the genus level and/or various bacterial groups using the following tests (Bergey, 1994).

Morphotyping

Wet mount

From over-night, pure broth culture, wet mount was prepared on a microscope slide. The preparation was observed under light microscope using oil immersion objective (x1000). The morphological criteria considered during the observation were cell shape (spherical, rod, spiral, etc.) and cell arrangement (single, pair, chain, clusters, tetrads, etc.).

Gram staining

Young colonies were spread on clean glass slide using sterile loop. The slide was exposed to air drying. The slide with smear was heat fixed by passing it several times through a flame. After the heat fixing, the slide was flooded with crystal violet for 60 seconds. Thereafter, the slide was rinsed several times under gently running tap water and flooded with Gram's iodine for about 1 minute. After a minute, the slide was rinsed with tap water and carefully decolorize with 95% ethanol for about 10-20 seconds. Lastly, the slide was counter stained with safranin for 60 seconds. The slide was washed carefully under gently running tap water and allowed to air-dry. Finally, the stained slide was examined under light microscope (x1000) to assign the cells to their respective groups. Organisms that retained the primary color (the violet-iodine complex) after washing in ethanol were termed as Gram-positive cells, but those that lost this complex and stained red were designated as Gram-negative.

Spore staining

Bacterial smear was heat fixed and covered by pieces of absorbance paper saturated with 5% of malachite green for 1 minute. The slide was allowed to be heated by steam for 5 minutes (stain was added repeatedly). Thereafter, the slide was cooled and rinsed several times with distilled water. The slide containing smear was stained counter stained with safranin for one minute. The slide was rinsed by water and examined using light microscope (x1000). The vegetative cells appeared as red but endospores stained green color.

3.5.1 Biochemical tests

Oxidation Fermentation (O/F)

The utilization of glucose by each isolate was assessed by O/F test to identify microorganisms that metabolized glucose fermentative or oxidative or that do not utilize glucose by either way. The freshly prepared medium (SLR India) was immediately cooled under tap water to avoid dissolution of oxygen in the medium and paired tubes were inoculated by stab with a sterile straight wire to the bottom (one of the tubes were covered by paraffin oil). Color change due to acid formation was interpreted after 2 and 5 days of incubation, at 37°C (Bergey, 1994).

Cytochrome Oxidase test

Filter paper was misted by freshly prepared reagent (1%N, N-dimethyl- p-phyneylenediammonium chloride (99.5%)), speck of culture was rubbed on it. Appearance of a blue color on the colonies within 30 seconds to 2 minutes was considered as positive reaction. Any very weak or dubious reaction after 2 minutes was ignored (Bergey, 1994).

Catalase test

Young colonies were flooded with a 3% solution of hydrogen peroxide. The formation of bubbles was considered as presence of catalase (Bergey, 1994).

3.7 Detection of Pathogenic microorganisms

3.7.1 Detection of *Escherichia coli*

Suspected colonies of the fecal *coliform* were inoculated into *Escherichia coli* Broth (CONDA) and incubated at 44°C for 24 hours. The cultures were checked for production of gas (NMKL, 1993).

Biochemical tests for *Escherichia coli* spp

Indole test

From gas forming E.C broth cultures, a loopful was transferred into Tryptone water (Uni-chem, UK) and incubated at 44°C for 48 hours. Thereafter, 3 drops of Kovac's reagent was added to the test culture and observed for any reaction. Formation of red ring color considered as indole positive reaction (NMKL, 1993 and Bergey, 1994).

MRVP test

Young colonies of suspected isolates were inoculated into MRVP broth(HIMEDIA)and incubated at 37°C aerobically for 48hrs. After incubation, drops of (6-12) MR and VP reagents (KOH and alpha-naphthol)were added separately. Formation of reddish pink color and no color change for MR and VP were recorded, respectively (NMKL, 1993 and Bergey, 1994).

Citrate test

The slant was streaked with suspected isolates and the tube was incubated at 37 °C for 24 hrs.to determine citrate utilization as a sole source of carbon and incubated at 37°C for 24 hrs. The absence of color change (negative result) was considered (NMKL, 1993 and Bergey, 1994).

Triple sugar iron test

The butt was stabbed and the slant was streaked with suspected isolates of genus *Escherichia* and incubated at 37°C for 24 hrs.to detect fermentation of glucose, sucrose and lactose as well as production of H₂S. The presence of yellow slant and butt with gas production was considered as positive (NMKL, 1993 and Bergey, 1994).

3.7.2 Detection of *Staphylococcus aureus*

For isolation and identification of *S. aureus* a loopful of sample from the homogenate was inoculated onto Mannitol salt agar (MSA; OXOID) and golden yellow colonies on MSA which were catalase positive and coagulase positive isolates were identified as *S. aureus*. The biochemical tests (catalase and coagulase) were done as confirmatory (American Public Health Association, 2012).

Coagulase test:

Slide and tube coagulase test was done. Accordingly, slide coagulase test was done first, two drops of human plasma (sourced from Black Lion Hospital) was dropped on one end of clean glass slide (on the other end two drops of plasma was considered as negative control) and inoculum of presumptive of *S. aureus* was added and emulsified. After 10-15 seconds' formation of clump due to coagulase enzyme was considered. During tube coagulase test, a tube containing 0.5ml of plasma was inoculated with inoculum of presumptive of *S. aureus* and placed in 37°C. After every 30 min, for four hours, formation of clump was checked. The negative results were re-observed after 24 hrs (American Public Health Association, 2012).

3.7.3 Detection of *Salmonella* spp

A 25g of meat sample (minced by stomacher) or 1ml of swab sample, was transferred to 225 or 9 ml of Buffer Peptone Water (BPW), respectively and incubated at 37°C for 24 hrs. An aliquot of 1 ml from pre-enrichment was pipetted to 10 ml tetrathionate broth (supplement with iodine) (SRL). Another 1 ml of the pre-enrichment was transferred with a sterile pipette to 10 ml Selenite Cysteine broth (HIMEDIA) and incubated at 44°C for 48 hrs. and 37°C for 24 hrs., respectively. A loopful sample from tetrathionate and SC broth cultures were separately streaked onto XLD (OXOID) and SS (HIMEDIA) agar plates. The plates were incubated at 37°C for 24 hrs. After 24 hrs, formation of colonies with slightly transparent zone of reddish color and black centers on XLD or with gray colors on SS agar considered as presumptive *Salmonella* spp. (NMKL, 1991 and ISO, 6579).

Biochemical tests for *salmonella* spp

Triple Sugar Iron Agar

The butt was stabbed and the slant was streaked and incubated at 37⁰C for 24 hrs.to detect fermentation of glucose, sucrose and lactose as well as production of H₂S. The presence of alkaline (red) slant and acid (yellow) butt, with or without production of H₂S was considered as presumptive for *Salmonella*.

Lysine Iron Agar

The butt was stabbed and the slant was streaked and incubated at 37 ⁰C for 24 hrs. *Salmonella* produces the enzyme lysine decarboxylase that produces an alkaline reaction (purple color) throughout the medium. Due to the production of H₂S, an intense blackening of the medium was seen that is a positive reaction for *Salmonella* (NMKL, 1991 and ISO, 6579).

Simmons Citrate Agar

The slant was streaked and the tube was incubated at 37⁰C for 24 hrs.to determine citrate utilization as a sole source of carbon. The presence of growth and color change from green to blue was considered as presumptive for *Salmonella*.

Urease test

Suspected isolates were inoculated on urea broth and was incubated at 37⁰C for 24 hrs. The absence of color change (negative result) was considered (NMKL, 1991; ISO, 6579).

Serological test

Salmonella colonies which fulfill all the above biochemical tests were finally confirmed by serogroup with slide agglutination using polyvalent antisera. *Salmonella* polyvalent antisera (DENKA SEIKEN) was dropped on one end of a circle (1cm diameter) of clean glass slide and 85% NaCl solution was dropped on the other end of the slide (as negative control). Presumptive *Salmonella* spp. Suspension was dropped on both circles, after emulsification formation of agglutination was considered as positive result (ISO,6579; Reeves *et al.*,1989).

3.8 Antimicrobial Sensitivity Test:

Antimicrobial susceptibility testing for *Salmonella*, *E. coli* and *S. aureus* was examined against selected (Oxoid) antibiotics discs (Amoxicillin (10µg), Ampicillin (10 µg) Ciprofloxacin (5µg), Oxytetracycline (30µg), Penicillin (10µg) Chloramphenicol (30µg) and Vancomycin (30µg). The criteria used to select the antimicrobial agents were based on the availability and frequency of prescription for the management of bacterial infections in Ethiopia.

This test was done by standard disk diffusion method. In this test, three to five well-isolated colonies of the same morphological type were selected from an agar plate culture and were transfer to saline water (0.9% NaCl) and the turbidity of the broth was adjusted by measuring the optical density using ultraviolet visible spectrophotometer (7804, China) until it achieves or exceeds the turbidity of the 0.5 McFarland standard (mixture of 0.05 mL of 1.175% barium chloride dihydrate (BaCl. 2H₂O) and 9.95 mL of 1% sulfuric acid (H₂SO₄)) (equivalent to a growth of 1-2 x10⁸ CFU/mL for *E. coli* ATCC 25922). Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The dried surface of a Mueller-Hinton Agar (Oxoid) plates was seeded with the culture laden swab over the entire sterile agar surface. After the inoculation, predetermined batteries of antimicrobial discs were dispensed onto the surface of the inoculated agar plate. The plates were inverted and placed in an incubator set to 37°C within 15 minutes after the discs were applied. After 16-24hrs, the plates were removed from the incubator and the inhibition zone diameter was measured using a plastic ruler to the nearest whole millimeter. The results (susceptible, resistant or intermediate) were interpreted using the criteria of the CLSI (2014). The quality of medium and the potency of the antibiotic were checked by using control strains (*E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923). In addition, like other microbial enumeration, each test was done by duplication.

3.9 Measurement of pH

The minced beef samples (from top-side, neck and minced meat) were homogenized in distilled water in the ratio 1:10 (W/V) and the pH was measured using a calibrated digital pH meter(MP511, China).

3.10 Questionnaire and interview survey

On site observation and face to face interviews were conducted. Based on this, AAAE was observed and cross checked against a given standard check list (Appendix I).Lay out of the abattoir, toilet ration (no. of workers: no. toilet), water and power supply, chilling facility, slaughtering techniques, distribution system and others were checked weekly for two successive months.

A structured questionnaire was prepared to assess the knowledge of workers in butcher shops regarding the hygienic practices during processing of meat. The respondents(two workers from each butchery shops) were given with the questionnaire to respond accordingly. Educational status, exposure and frequency of training, hair cover, jewelry; handling money practice; frequency of washing and effectiveness of training were included in the questionnaire. Before this survey, pilot test was conducted in order to decide how to manage the questionnaire.

3.11 Statistical analysis

The quantitative data were entered in to Microsoft excel spread sheet and coded properly. Following coded the data entry; the data were analyzed using Statistical Package for Social Sciences (SPSS) version 20 (One way ANOVA). Qualitative data were analyzed by percentile. For means separation, LSD test was used at 5% level of significance.

4. RESULTS

4.1 Observational survey

4.1.1 Physical observation of Addis Ababa abattoir Enterprise

Addis Ababa abattoir enterprise (Table 2) has two separated lines for cattle slaughtering (for Christian and Muslim). This abattoir has a slaughtering capacity of more than 4,000 heads of cattle per week. And more than 500 workers are assigned in beef carcass preparation. This makes it the biggest local abattoir in the country. The Lairage of the abattoir has enough pens for daily slaughtering, but the maximum fasting period practiced is not more than 3 hours prior to slaughtering.

In this abattoir, there was no stunning box; the cattle were stunned by sharp knife on the floor (Table 2). Most of the slaughtering processes are done manually by the butchers and none of butchers uses gloves during this survey. The carcasses are washed by potable water only before dispatching. Most of the abattoir workers wore unlearned coats and hair covers. And ‘one man one job’ principle was not applied. The slaughtering house was air ventilated but there was no controlled air conditioner in the production line and chilling facilities were not available. Moreover, the distribution tracks had no chilling facilities and the carcasses were transported together with the offal.

Table 2. Survey of Addis Ababa Abattoir Enterprise

Variables	Standard	Actual
Cross contamination due to lay out	No**	Yes
Ratio of number of toilet and number of workers at AAAE	1:05**	>1:20
Cattle washing before slaughtering	Optional*	No
Fasting period/resting time	12-24hrs*	<3hrs
Stunning techniques	Bolt**	Knife
Slaughtering house room air conditioner	Must*	Not available
General personal hygiene of the butchers	Good*	Poor
Sanitation and sterility of the equipment	Good *	Poor
Frequent use of clean water for washing	Must*	Not used
Chilling facility	Must*	Not available
Cold chain management	Sustainable*	Broken
Butchers 'renewal of their health certificate	6 Months*	6 Months
Know-how of the butchers about personal and food hygiene	Good	Good
Training about personal hygiene and food-borne diseases	Good	Good
Refrigerated distribution track	Must*	Not available
Separate transportation for beef and offal	Must*	Not used
Vehicles washing program	Each shipment*	Each shipment
Method of loading and unloading	Over rail	Manual

*FAO (2010), **MoA (2008),

4.1.2 Observation survey of butcher shops

The survey result shows that 14 out of 16 of the meat handlers were male and 12 of them were elementary school completed (Table 3). Out of these, ten of those meat handlers took sanitation training. However, only 8 of 16 of them had renewed their health certificate. Only 6 out of 16 of the meat handlers wore clean working coat and 12 of them did not wear hair cover and their hands' nails were not clean. And 12 of 16 of the butchers handled money while serving meat.

The overall sanitation of the butcher shops was poor. The meat products were not separated from offal (internal organs) and were displayed uncovered for more than 5 hours with dozens of visiting houseflies. It was also observed that, only two of the shops had refrigerator.

Table 3. Survey on knowledge of butchers on hygienic practices in Gullele sub-city Addis Ababa

Variables	Value	Frequency
Sex	Male	14
	Female	2
Education status	Literate	6
	1-6	6
	7-10	4
Training	Yes	10
	No	6
Health Certificate	Certified	8
	Not	8
Clean over coat	Yes	6
	No	10
Hair cover	Yes	4
	No	12
Nail shortness and clean	Yes	6
	No	10
visible skin rash, skin boils, cut or wound	Yes	4
	No	8
	Difficult	4
Paper money handling	Separate cashier	1
	Butcher	7
Refrigerator usage	Yes	1
	No	7
General sanitation of the shop	Better	0
	Good	2
	poor	6
Location of the toilet and possibility of contamination	Yes	3
	No	2
	Difficult	3

4.2 Microbiological analysis

4.2.1 Microbial quality of fresh meat samples

The microbiological quality of analyses of samples of carcass from abattoir showed that the mean total aerobic mesophilic (TAM) count was 4.79 log cfu /100cm²(Table 4). Likewise, TAM counts of meat samples from the butcher shops were 6.44 and 6.76 log cfu /g in samples collected during the morning and afternoon, respectively. On the other hand, mean total staphylococci counts (TSC) of the carcass samples collected from abattoir was 3.29 log cfu/100cm². In butchery beef samples, TSC was ranged from 4.85 to 5.91 log cfu /g (Table 4).

The microbial quality examination of abattoir meat samples indicated that, the mean enterobacteriaceae and total coliform counts were 4.82 and 4.76 log cfu/100cm². Similarly, the mean count in the butcher shops were 5.9 and 6.5 log cfu /g during the morning and afternoon respectively. Moreover, the mean fecal coliform of carcass samples in this study was 4.07 log cfu /100cm² and in butcher samples 5.52 and 6.03 log cfu /g during the morning and afternoon, respectively. The mean aerobic spore count in both carcass and butchery samples was as greater as 1.4 cfu log. Similarly, the mean yeast and mold count was ranged from 4.03 to 5.69cfu log/g.

In this study, all except aerobic spores, microbial counts of carcass samples were statistically significantly different ($p < 0.05$) from the butchery beef samples. Except TAMC and yeasts and molds count, all microbial count of samples taken in the morning and in the afternoon, were statistically significantly different ($p < 0.05$) (Table 4).

Table 4: Comparison of microbial count cfu_{log10}(± SD) of beef samples collected from abattoir and butcher shops (morning and afternoon)

	Distribution location and period		
	Abattoir/carcass	Butchery	
		Morning	After noon
Bacteria	(n =16)	(n = 24)	(n = 24)
Total Mesophilic aerobic	4.79±0.780 ^b	6.44±0.847 ^a	6.76±0.857 ^a
Total Staphylococci	3.29±1.79 ^c	4.86±0.818 ^b	5.92±0.865 ^a
Enterobacteriaceae	4.82±0.530 ^c	5.99±0.788 ^b	6.53±0.717 ^a
Total coliform	4.76±0.485 ^c	5.96±0.863 ^b	6.49±0.870 ^a
Fecal coliform	4.07±0.826 ^c	5.53±0.747 ^b	6.03±0.823 ^a
Aerobic spore	1.48±1.39 ^a	1.84±1.56 ^a	2.12±1.64 ^a
Yeasts and molds	4.02±0.76 ^b	5.36±0.87 ^a	5.69±0.91 ^a

Means in the same row with different superscript letters are different (p < 0.05).

The TAM count of meat samples (Table 5) with pH of < 5.8 was 5.87log cfu /g. On the other hand, meat samples with pH values 5.81-6.2 and >6.21 (category two and three) had TAM counts of 6.38 and 6.70 log cfu /g. Mean enterobacteriaceae, total coliform and fecal coliform counts of meat samples with pH values <5.8 (category one) were less than 5.4 log cfu /g. And on category two and three the microbial count reached greater than 6 log cfu /g.

Mean AS count of the meat samples was below detectable, 2.27 and 2.35 log cfu /g in category one, two and three, respectively. Yeasts and molds counts of these samples were 4.97, 5.69 and 5.43log cfu /g in category one, two and three, respectively. For all microbial counts, except YM, pH on category one was statistically different (p<0.05) from counts on category three. In addition, enterobacteriaceae, total coliform and fecal coliform counts classified on of category one were statically different from counts on category two.

Table 5. Comparison microbial load (cfu log₁₀ (± SD)) of butcher shop beef samples in three pH categories

Bacteria	pH category ^X		
	One (<5.80)	Two (5.81-6.20)	Three (>6.21)
Total Mesophilic aerobic	5.87±0.946 ^{bc}	6.63±0.605 ^{ab}	6.70±0.778 ^a
Total Staphylococci count	4.16±0.707 ^{bc}	4.87±0.66 ^{ab}	5.29±0.692 ^a
Enterobacteriaceae count	5.35±0.75 ^b	6.40±0.521 ^a	6.17±0.729 ^a
Total coliform	5.15±0.750 ^b	6.46±0.374 ^a	6.20±0.795 ^a
Fecal coliform	5.13±0.706 ^b	5.24±0.461 ^b	5.93±0.731 ^a
Aerobic spore count	0.66±1.150 ^b	2.27±1.23 ^a	2.35±1.63 ^a
Yeasts and molds counts	4.97±0.710 ^a	5.69±1.059 ^a	5.43±0.85 ^a

Means in the same row with different superscript letters are different (p < 0.05).

^XLowiwers *et al.* (2010)

The mean TAM counts were 5.83, 6.09 and 6.69 log cfu /g from HQ, FQ and minced meat, respectively (Table 6). In this study, the mean total staphylococci count ranged from 4.36 to 5.17 log cfu /g. Enterobacteriaceae and coliform counts of the meat cuts were greater than 5.4 log cfu /g.

Low (5.05 log cfu / g) mean fecal count was obtained from HQ but higher count was recorded from minced meat (5.78 log cfu /g). Mean counts of all yeasts and mold were greater than 4.8 log cfu /g. In this study, all except TSC microbial counts of minced meat were statistically significantly (p< 0.05) different from other two beef cuts. In addition, TAM and fecal coliform count of HQ were significantly different from minced meat.

Table 6. Comparison of microbial count log cfu/g of three beef cuts collected from abattoir and butcher shops

Bacteria	Mean log cfu/g counts from beef cuts		
	HQ/topside (n=24)	FQ/neck (n=24)	Minced meat (n=16)
Total Mesophilic aerobic	5.84±1.19 ^{bc}	6.09±1.20 ^{ab}	6.69±0.82 ^a
Total Staphylococci	4.36±1.66 ^a	5.15±1.61 ^a	5.17±0.96 ^a
Enterobacteriaceae	5.45±0.94 ^b	5.94±0.88 ^b	6.52±0.79 ^a
Total coliform	5.39±0.99 ^b	5.90±1.03 ^b	6.50±0.73 ^a
Fecal coliform	5.06±1.09 ^{bc}	5.36±1.20 ^{ab}	5.78±0.84 ^a
Aerobic spores	1.087±1.35 ^b	2.28±1.47 ^a	2.37±1.56 ^a
Yeasts and molds	4.86±1.05 ^b	4.99±0.93 ^b	5.82±1.11 ^a

Means in the same row with different superscript letters are different ($p < 0.05$).

The mean TAM count of the butcher shops (Table 7) was ranged from 5.02 to 7.21 log cfu /g. The minimum count was on butchery 5, 7 and 8. The maximum mean value of total staphylococci count in this study was on butcher 3(6.25 log cfu/g) and minimum was on butcher 8 (4.38 cfu log).

The highest mean count of enterobacteriaceae and coliforms of the eight butchers (from butcher 6) was 6.8 and 7.11 log cfu /g, respectively. In all the butcher shops included in this study, the count of fecal coliform was >5 log cfu /g with maximum count on butcher 4 (6.8 cfu log/g). Count of yeast and mold (Table 7) was ranged from 4.87 to 6.86 log cfu /g from butcher 8 and butcher 2, respectively. High mean count of aerobic spore 3.57 log cfu /g recorded from butcher 3, but the lowest was 1.54 log cfu/g from butcher seven.

Table 7: Microbial load (log cfu /g (+ SD) of meat samples of eight butcher shops

Bacteria	Butchery 1 (n =6)	Butchery 2 (n =6)	Butchery 3 (n =6)	Butchery 4 (n =6)	Butchery 5 (n =6)	Butchery 6 (n =6)	Butchery 7 (n =6)	Butchery 8 (n =6)
TMAC	7.02+0.16 ^a	7.07+0.20 ^a	7.15+0.20 ^a	7.21+0.18 ^a	6.57+0.75 ^a	7.19+0.25 ^a	5.54+0.16 ^b	5.02+0.20 ^b
TSAC	5.21+1.02 ^{bc}	5.68+1.01 ^{ab}	6.25+0.77 ^a	6.06+0.86 ^a	4.68+0.60 ^c	4.99+0.68 ^{bc}	5.82+0.78 ^{ab}	4.38+0.73 ^c
EBC	6.67+0.35 ^a	6.11+0.69 ^a	6.68+0.26 ^a	6.32+0.99 ^a	6.33+0.504 ^a	6.80+0.25 ^a	6.23+0.914 ^a	4.89+0.24 ^b
Total coliform	6.31+0.90 ^{cd}	6.61+0.63 ^{bc}	7.05+0.23 ^{ab}	6.27+0.90 ^{cd}	5.73+0.65 ^d	7.11+0.21 ^a	5.72+0.71 ^d	4.98+0.26 ^e
Fecal coliform	5.18+0.27 ^b	6.47+0.77 ^a	6.74+0.40 ^a	6.83+0.34 ^a	5.18+0.27 ^b	5.46+0.31 ^b	5.23+0.41 ^b	5.10+0.28 ^b
ASC	2.41+1.86 ^{cb}	3.20+1.58 ^{ab}	3.57+0.49 ^a	BDL*	3.00+0.21 ^{ab}	2.13+0.11 ^{cb}	1.54+1.20 ^c	BDL*
YMC	5.08+0.23 ^c	6.86+0.31 ^a	5.49+0.71 ^{bc}	4.88+0.24 ^c	5.66+1.15 ^{bc}	6.54+0.22 ^{ab}	4.79+0.26 ^c	4.87+0.29 ^c

Means in the same row with different superscript letters are different (p < 0.05).

* BDL: Blew detectable level

4.2.3 Microbial quality of contact surface of butcher shops

The TAM count of these contact surfaces was ranged from 4.95 to 7.3 log cfu /cm² (Table 8). Mean Staphylococci count was recorded as greater as 3.72cfu /cm². Enterobacteriaceae and total coliform counts of the contact surfaces were ranged from 3.77 to 6.9log cfu / cm². Mean of fecal coliform counts were 4.61, 4.42 and 4.44log cfu /cm²from knife, table and balance, respectively. The maximum count of aerobic spore was on balance (4.1 log cfu / cm²). Minimum (3.5log cfu / cm²) count of Yeasts and molds was on cutting table, but maximum (6.84 log cfu / cm²) count was on knife. Generally, there were no significant (p>0.05) differences in all counts except aerobic spore count (Table 8).

Table 8: Microbial load (log cfu /cm²) of samples from contact surface of eight butcher shops

	log cfu/cm ² counts from contact surfaces								
	Knife (n=8)			Cutting Table (n=8)			Balance (n=8)		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
TAM	6.31 ^a	4.95	7.1	6.32 ^a	5.18	7.47	6.43 ^a	5.27	7.3
ST	3.85 ^a	BDL	4.95	3.99 ^a	3	5.13	3.72 ^a	BLD	5.9
EB	4.93 ^a	4.3	5.47	4.85 ^a	3.77	5.67	4.93 ^a	4.2	5.2
TC	5.51 ^a	4.39	6.87	5.34 ^a	4.4	6.9	5.70 ^a	4.4	5.9
FC	4.61 ^a	4.3	5	4.42 ^a	3.5	5.1	4.44 ^a	3	5.1
AS	1.96 ^{ab}	BDL	3.77	1.87 ^{bc}	BDL	3.33	3.27 ^a	2	4.1
YM	5.03 ^a	3.47	6.84	4.92 ^a	3.5	6.01	5.17 ^a	4.1	6.16

Means in the same row with different superscript letters are different (p < 0.05).

* BDL: Blew detectable level

4.2.4 Microbial quality of samples from different categories

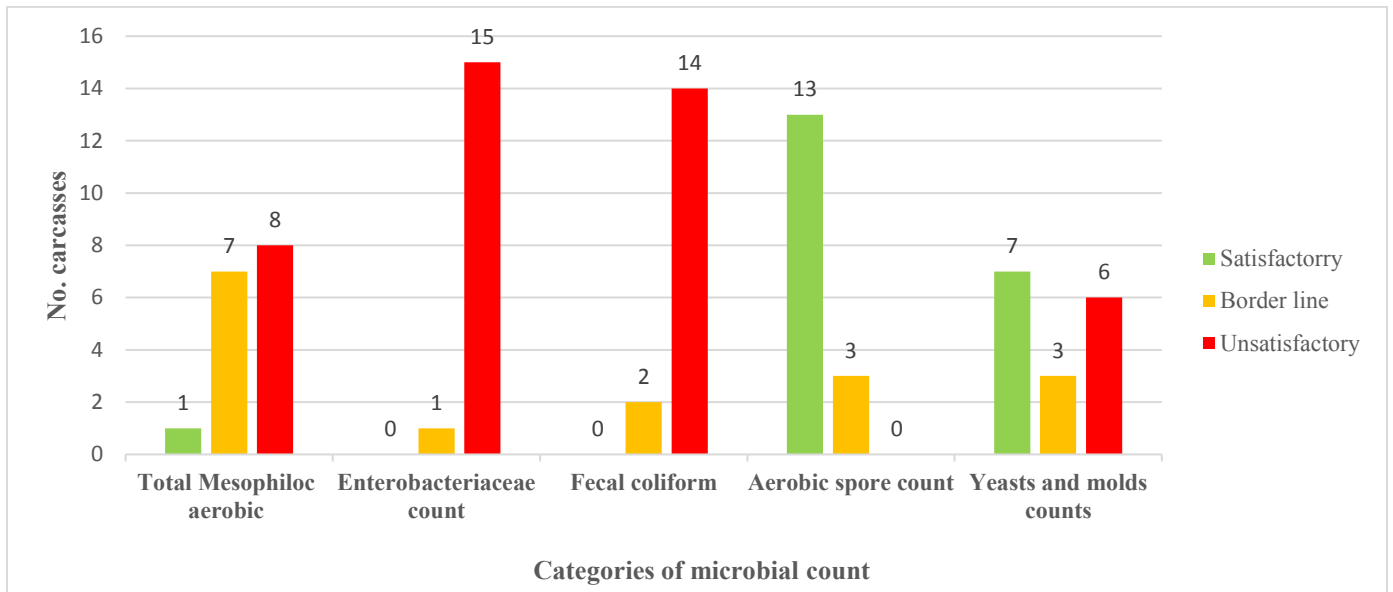
Out of the total 88 samples, TAM count of only 3 samples were classified on 10²- 10⁴log cfu/g and 32 samples were > 10⁷(Table 9). The highest STC count was found on 10⁴- 10⁶ which were 54 samples. FC, TC and EB counts of 62, 52 and 52 samples were classified on 10⁴- 10⁶logcfu/g, respectively. On the other hand; 5, 5 and 6 samples were found exhibiting counts > 10⁷log cfu/g of FC, TC and EB, respectively. In this study, ASC 54 samples were observed with counts between 10²- 10⁴log cfu/g. On the other hand, yeasts and molds count of 57 samples were showed between on 10⁴- 10⁶ log cfu/g and only three samples were found exhibiting counts > 10⁷cfu.

Table 9: Microbial load in cfu of the total samples categorized in different levels

	10^2-10^4	10^4-10^6	10^6-10^7	$>10^7$	Total
TAMC	3	35	18	32	88
STC	16	54	10	3	83
EB	1	52	29	6	88
TC	1	52	30	5	88
FC	10	62	11	5	88
ASC	54	4	0	0	58
YM	11	57	17	3	88
Total	96	316	115	54	581

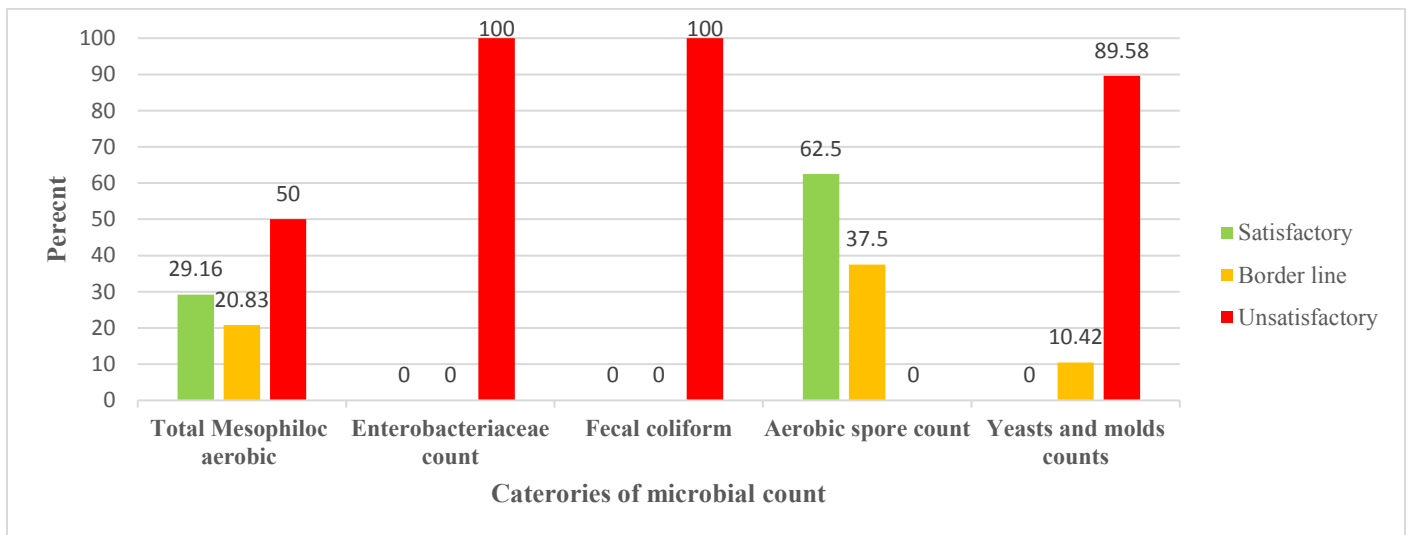
According to many country standard (EC,2005), based on TAM count, 8 carcass sample out of 16 were classified on unsatisfactory and only one carcass was satisfactory. On the other hand, 50 % of the meat samples were classified on unsatisfactory and 29.13% and 20% was categorized on satisfactory and boarder line, respectively. Based on Enterobacteriaceae and Fecal coli form count, 15 and 14 carcass samples were classified on unsatisfactory and none of the carcass samples were on satisfactory category. Similarly, all the meat samples were categorized on unsatisfactory (Fig. 2 and 3).

According to EC (2005) and regulation (2011), based on aerobic spore count, 13 of the carcass samples were on satisfactory and the rest three samples were on border line. Similarly, 62.5% of the meat samples were classified on satisfactory and 37.5 of them were on border line. Based on Yeast and mold count, 6 out of 16 carcass sample were classified on unsatisfactory and 7 on them were on satisfied. On the other hand, 89.58% of the meat samples were classified on unsatisfactory and 10.42% of the meat samples were on border line (Fig. 2 and 3).



* Categories are based on: New Zealand Food Regulations (1995), EC (2005), and Regulation (2011)

Fig 2: Quality category of Carcass samples by different microbial loads



* Categories are based on: New Zealand Food Regulations (1995), EC (2005), and Regulation (2011)

Fig 3: Quality category of beef samples by different microbial loads

4.2.5 Dominant microflora

A total of 105 bacterial isolates were recovered and characterized to various genera and bacterial groups (Fig. 4). Accordingly, the aerobic mesophilic flora was dominated by *Enterobacteriaceae* (29.5%) followed by *Staphylococci* spp (26.67%) and *Bacillus* (17.4%). *Streptococci* (7.619%), *Micrococcus* (6.667%), *Pseudomonas*(4.762), *Acinetobacter* (2.856%) and *Aeromonas* (2.856%) were among the aerobic mesophilic bacteria isolated from beef and contact surface samples.

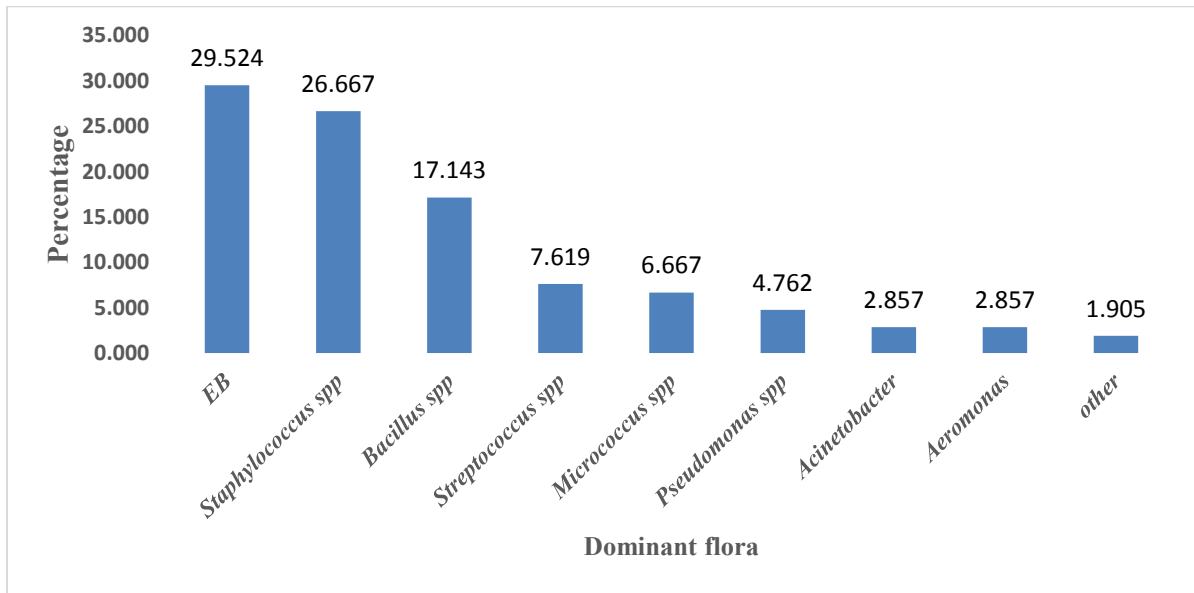


Fig 4: Frequency distribution of mesophilic bacteria in meats collected from abattoir and butcher shops

4.2.6 Prevalence of pathogenic microorganisms and distribution

In this study (Table 10), from the total 88 samples, 26 (29.55%) of them were presumptively designated as *E. coli*. Out of the total samples, higher frequency of *E. coli* (16) was retrieved from butcher shop samples followed by contact surface samples (7) and only 3 samples were positive from the abattoir. Only three (3.42%) samples were suspected for *Salmonella* species. In addition, from the total samples, 28.41% suspected for *Staphylococcus aureus*. Accordingly, samples from contact surface showed the highest frequency(9 out of 24) of the suspected strains of *S. aureus* followed by butcher shop (14 out of 48) and abattoir samples (2 out of 16).

Table 10: Prevalence of suspected pathogenic bacteria

Site	<i>E. coli</i>			<i>Salmonella</i>		<i>S. aureus</i>	
	Tested samples	Positive	Percentage	Positive	Percentage	Positive	Percentage
Abattoir	16	3	18.75	0	0.00	2	12.5
Contact surface	24	7	29.17	1	4.17	9	37.5
Butcher shops	48	16	33.33	2	4.17	14	29.17
Total	88	26	29.55	3	3.41	25	28.41

4.2.7 Antimicrobial susceptibility profile of pathogenic microorganisms

Antimicrobial susceptibility tests (Table 11) were done on total of 33 isolates (20 *E. coli*, 3 *Salmonella spp.* and 10 *S. aureus*). *E. coli* isolates were observed to be the most resistant to penicillin (60%) followed by Amoxicillin (40%) and Ampicillin (40%) and none of the isolates were resistance for chloramphenicol.

All isolates of *Salmonella* (100%) were resistant to penicillin and Vancomycin. And 66.67% of the isolates were resistance to Ampicillin. None of the isolates were resistance to Ciprofloxacin.

S. aureus isolates were resistant to penicillin (60%), Amoxicillin (40%) and Ampicillin (40%) and none of the isolates were resistant to Ciprofloxacin (Table 11).

Table 11: Antimicrobial profile of *E. coli*, *Salmonella* and *S. aureus*

Antibiotics	µg	Antibiotic resistance of different bacterial genera								
		<i>E. coli</i>			<i>Salmonella</i>			<i>S. aureus</i>		
		R	I	S	R	I	S	R	I	S
Amoxicillin	10	8(40)	2(10)	10(50)	1(33.33)	1(33.33)	1(33.33)	5(50)	2(20)	3(30)
Ampicillin	10	8(40)	6(30)	6(30)	2(66.67)	1(33.33)	0	5(50)	1(10)	4(40)
Ciprofloxiline	5	1(5)	1(5)	18(90)	0	0	3(100)	0	0	10(100)
Oxytetracycline	30	5(25)	2(10)	13(75)	1(33.33)		2(66.67)	3(30)	3(30)	4(40)
Chloramphenicol	30	0	2(10)	18(90)	1(33.33)	1(33.33)	1(33.33)			
Vancomycin	30				3(100)					10(100)
Penicillin	10	12(60)	0	8(40)	3(100)	0	0	6(60)	0	4(40)

*R- Resistance, I- Intermediate, S- Sensitive

5. DISCUSSION

5.1 Observational survey

5.1.1 Physical observation of Addis Ababa abattoir Enterprise

AAAE was used municipal water as water supply with reserve tanker in the compound and it has its own electric power supply as a reserve. These facilities help to reduce water and power interruption in the abattoir. However, most slaughtering house standards recommends to have its own raw water treatment (MoA, 2008) which can minimize water borne contamination.

Actual fasting period of the abattoir deviated from the slaughtering standards. A 12-24 hrs. fasting period prior to slaughtering reduces the volume of gut contents and hence bacteria and therefore reduces the risk of contamination of the carcass during dressing (FAO, 2010). AAAE had very small amount of number of toilets for the slaughtering workers, the ratio of number of toilets to the number of the workers was one to more than twenty and there was no hand washing facility in the toilet. Besides, the sanitation of the toilets was poor which can be one potential source of contamination.

The severing/sticking and the bleeding process were done on the floor. This stunning and bleeding technique did not make the cattle unconscious immediately and this delays the bleeding process. Due to this, slaughtered animal would be exposed to unnecessary stress, this may lead to glycogen depletion and the quality of the meat would be affected (Dave and Ghaly, 2011). In addition, the bleeding time was not kept as the standard; the objectives of bleeding are to kill the animal with minimal damage to the carcass and to remove quickly as much blood as possible as blood is an ideal medium for the growth of bacteria (FAO, 2010).

In order to reduce cross contaminations from skin and viscera, each carcass was supposed to be rinsed by potable water or organic acid before dispatching (James *et al.*, 2005). However, in this abattoir the carcasses were only rinsed by potable water and were not done at each stage.

Even though all the slaughtering workers wore coat and hair cover, the coat was not clean and the general personal hygiene of the slaughtering workers were poor. And during slaughtering they were not washing their hands at each stage and after toilet. This indicates that there was no

GMP implemented in the abattoir. In the slaughtering line the clean and unclean area was not clearly separated and the movement of workers was not limited in one specific place in other word “one man on job” principle was not implemented. Due to these slaughtering practices, cross contaminations are more likely to happen.

Even though the carcass dispatched with in less than an hour, but there was no chiller in AAEE and the distribution trucks had no chilling facilities. This indicates the cold chain management was poor. Chiller retard bacterial growth and extend the shelf-life, provided that if high standards of hygiene were observed during slaughter and dressing (FAO, 2010). In the abattoir, personal hygiene of the loading and unloading workers was poor. Moreover, during distribution, the carcass and other offal were loaded in the same truck. In the other hand, WHO (1996) stated that, to prevent cross-contamination the vehicle used for transport should be clean and should not carry other materials and should maintain the cold chine.

5.1.2 Observation survey of butcher shops

It is clearly indicated that, 12 out of 16 of the handlers have not completed elementary school (Table 3). Even though, 10 of them had taken training on food handling and personal hygiene, the practice of the meat handlers towards good manufacturing practices was poor, rather they were focusing on the managerial aspects. Similarly, Endale Balcha *et al.* (2013) has reported that only 7 out of 12 of the meat food handlers in Mekelle, Ethiopia had taken trainings concerning personal hygiene and food handling. On the other hand, Ayehu Gashe *et al.* (2014) have showed that gender and knowledge of food handling was directly related to food handling practices. Furthermore, according to WHO (1996) food handlers should be educated, encouraged or supervised to stop their business promptly if at any time, they suffer from jaundice, diarrhea, vomiting, fever, sore throat with fever, discharges from ear, eye or nose or have visibly infected skin lesions.

In this study showed that, only 6 out of 16 of the handlers worn clean working coat and;12 of them did not wear hair cover and their hands nails were not clean (Table 3). This result is well supported by Endale Balcha *et al.* (2013) which reported that, 5 out of 12 (41.7%) did not wear overall and 7 (58.3%) of them did not put hair cover and Frew Tafesse *et al.* (2010) has reported

none of the food handler wore hair cover. In addition, regulation of WHO Division of Food and Nutrition (WHO,1996) stated that, Food handlers should wear clean and proper clothing and should wash their hands with soap and water after engaging in any activities that are likely to introduce hazards.

In developing countries, most of the trades are done using hard currency. When used as medium of exchange, paper currencies could be handled under poor hygienic conditions and possibly contaminated with many microorganism, this makes it a prime multiplication medium for various microorganisms and could constitute a major health hazard (Gosa Girma *et al.*, 2014). Nevertheless, in this study 14 out of 16 of the butchers handled money while serving meat. Present study has supported by Endale Balcha *et al.* (2013) which reported that 11out of 16 (91.7%) of the food handlers handled money frequently while serving food.

The overall sanitation of the butcher shops included in this study, was poor: the meat products were not separated from offal; moreover, only one of the butcher shops had refrigerator. This poor sanitation, condition for cross contaminations and poor cold chain management may cause favorable condition for multiplication of microorganisms. In this study, most of the meat products, in the butcher shops, were held on hanger or on tables for more than 5 hours. This practice may give sufficient time for microbial growth. In agreement with this, Deriba Muleta and Mogessie Ashenafi (2002) reported that, microbial duplicates dramatically, if food waits as greater as 4 hours at temperatures of 15-45°C.

5.2 Microbiological analysis

5.2.1 Microbial quality and microbial comparison of the abattoir and butcher shops

Most of the carcass samples (8 out of 16) were classified on unsatisfactory (Fig. 2). The mean TAM count of carcass was similar with a study conducted in Morocco (Nozha *et al.*, 2006) and Tofick Kedir (2013) which was reported 5 cfu log. Another study in Northern Ethiopia (Tesfay Kebede *et al.*, 2014) has reported higher count of TAM (6.24cfu log/100cm²) than our study.

Moreover, this high mean TAM count of carcass samples may be due to poor slaughtering techniques, cross contamination from environment and poor cold chain management.

Mean TAM count of butcher shops was 6.42 and 6.75 cfu log/g in the morning and afternoon respectively, which has increased roughly by 1.7 and 2 log of carcass samples. This study is strongly supported by a study conducted on Northern Ghana (Obeng *et al.*, 2013) who reported that there were 2 log differences between morning and afternoon. Besides, more than 50% of meat samples of the butcher shops were classified as unsatisfactory (Fig. 3). Moreover, based on observational survey (Table 3), unsuitable storage, frequent handling of paper currency, prolonged display of meat, poor personal hygiene and environmental sanitation could be the major reasons for high TAMC.

In this study mean total Staphylococci count of carcass samples was 3.28cfu log/100cm², which was lower than with study conducted by Tofick Kedir (2013). On the other hand, mean TSC of the butcher shop has increased by 1.6 cfu log/g and 2.6 cfu log/g in the morning and afternoon, respectively. And this count was lower than a study of Firew Tafesse (2010) which was ranged from 5.8-7.5cfu log/g. Meanwhile, the study of Firew Tafesse (2010) had not specify the sampling period, this sampling period may cause the count difference between the counts. The high count of staphylococcus count could be due to improper personal hygiene of employees' unskilled manpower, cross contamination from skin and utilities (Table3).

Mean EB and coliform count of carcass sample (4.82 and 4.76cfu log/100cm²) was higher than a study conducted by Atnafu Wendmu (2012). This difference may be due to method difference, Atnafu Wendmu (2012) had used pour plating, however our study was done by spread plating. As shown in (Fig. 2), according to many country standards, none of the samples were satisfactory, rather 15 of them were on unsatisfactory.

The EB and coliform count of butcher meat samples were 5.9 and 6.49 cfu log/g in the morning and in the afternoon. This result is higher than comparative study of Firew Tafesse (2010) which was reported 4.71 and 4.45cfu log/g. Difference between these studies could be due to the method difference and environmental factors. The entire meat samples (100%) of butcher shops were classified as unsatisfactory (Fig. 3). This high count could indicate broken cold chain

management and poor transportation system. As they are safety indicators presence of high count may indicate possible presence of pathogens Adams and Moss, (2000).

As shown in Fig. 2 and stated on EU and other countries standards (EC, 2005 and New Zealand Food Regulations,1995) based on fecal coliform count, almost all of carcass samples were classified as unsatisfactory. Similarly, all meat samples (100%) from the butcher shops were unsatisfactory. This high fecal count could be due to cross contamination from the gut of the animals and immediate fecal contamination. As fecal coli form is safety indicator, presence of pathogen could be expected.

Yeast and mold count obtained from carcass sample were lower than result obtained by Firew Tafesse (2010) and higher than that of butcher sample counts. In addition, According to Indian standards (Regulation, 2011) half of the carcass samples were categorized on unsatisfactory, and most of the meat samples were classified on unsatisfactory.

This study has recorded pH of beef ranged from 5.68 to 6.93 (mean of 6.2). Comparative study done by Atnafu Wendmu (2012) and Firew Tafesse (2010) reported a mean pH of 5.94 and 6 respectively, which was lower than our report .All of the meat samples were above the ideal standard of ultimate pH range, 5.4 to 5.6, (Węglarz,2010). Moreover,19of the meat samples were above the practical standard seat by New Zealand standard (Lowiwres *et al.*, 2010) ranged from 5.4 to 5.7. According to this survey (Table 3) and this high ultimate pH measure was believed to happened due to; poor cold chain management, slaughtering of stressed animals before slaughtering and due to lack of proper stunning system (the animals were highly miss treated and become stressed).

A study conducted by Mari'a *et al.* (2003) clearly revealed that, pH and toughness are two of the main parameters used to judge meat quality on an industrial level. Moreover, with the accumulation of lactic acid the pH falls from 7 to 5.5. The drop-in pH is a desirable feature as a low pH slows down growth of micro-organisms and enhances flavor, juiciness and color of the meat to give an attractive saleable product (Węglarz ,2010).

In this study, TAM, enterbacteraece, total coliform and fecal coliform counts which showed a pH recode < 5.8 , had make one log cfu difference from category two and three (pH > 5.8) (Table 5). On aerobic spore, staphylococcus and YM the difference was two, one and less than one log respectively. On all microbial counts, except YM, pH on category one was statistically different ($p < 0.05$) from counts on category three. In addition, enterbacteraece, total coliform and fecal coliform counts on category one were statically different from counts on category two.

Saleh *et al.* (2013) has reported that, the mean TAMC of topside (HQ) and neck (FQ) was 5.9 and 6.9 cfu log/g which was higher than our findings. But Gill *et al.* (1998) and Moreno *et al.* (1997) have reported lower than this study. This difference among these studies may be due differences on animal handling and slaughtering technics. On the other hand, TAMC of minced beef samples of our study was almost comparable with a study done by Tesfay Kebede (2004) which has reported 6.81 cfu log/g.

A study conducted in Egypt (Saleh *et al.*, 2013) has reported TSC of topside (HQ) and neck (FQ) was 5.2 and 4.7 cfu log/g, respectively, which was higher on topside (HQ) and lower on neck (FQ) than our result. Similarly, Mogessie Ashenafi (2002) has reported minced meat (raw 'kitfo' 's) TSC (6 cfu log/ g) was higher than this study.

Mean count of EB, coliform and fecal coliform of HQ and FQ done by comparative study Saleh *et al.* (2013) and Gill *et al.* (1998) was reported lower than our study. Similarly, a study conducted in Islamabad (Ömer *et al.*, 2008) and Kammenou *et al.* (2003) have reported lower minced meat count of EB, coliform and fecal coliform than this study. The differences are may be due to sampling period, method and environmental factors. In our study mean aerobic spore and yeast and mold count of HQ and FQ was comparable with a study conducted by Salehe *et al.* (2013). Regarding minced meat, counts of AS were similar with Mogessie Ashenafi(2002)report and YM count was higher than this study. Study of Tizta Bekele (2014) has reported lower YM and AS count of minced meat than this result.

In almost all of the microbial counts, minced meat had greater count than the other two beef cuts. This might be due to the mincing/ size reduction adds higher microorganism to the surface of exposed tissue which was supported by Mogessie Ashenafi (2002). Even though, there was no significant difference between HQ and FQ in all count except aerobic spore count, FQ count had

slight higher counts. Muscles around the neck and foreleg of cattle undergo relatively extra movement which leads to decrease meat acidity. This can be increase the microbial load. In addition, during slaughtering forequarter (FQ) had higher chance of being contaminated by blood and viscera (Gihanan, 2004).

Generally, the overall microbial count of butchery no. 5, 7 and 8 had relatively lower count than the rest of the butcher shops (Table 7), this result was supported by our survey observation (Table3) which shows these butchereries also had relatively good general sanitation, one of them had refrigerator and the toilet location was far enough from the shop. In addition, workers of these butcher shops had taken food handling training; their education status were also better than the others and one of them had separated cashier to handle the paper currency.

5.2.2 Microbial quality of contact surface of butcher shops

Mean TAMC on our findings was slightly lower than a comparable study conducted in northern Ethiopia Endale Balcha *et al.* (2013) (6.56 and 6.78 cfu/cm² on table and knife, respectively) (Table 8). Similar study reported by Henok Ayalew *et al.* (2015), showed that the mean TAM count of knife and cutting table was 6.01 and 6.03 respectively, which was lower than this study. Enumeration of higher TAM count on the contact surface indicated insufficient cleaning material and mechanism and its frequency (Table 3). The mean TS count of meat cutting table and weighing balance of our report was 3.99 and 3.75 log cfu/cm², which was higher than a study conducted on UK's (Little and Louvois 1998) which was 3cfu/cm². The difference may indicate this country's personal hygiene practices were lower than that European. In addition, as in this survey indicated cross contamination from other animal products may contribute to this count.

In this study, mean total coliform of knife and working table were 5.51 and 5.34 cfu log/cm² which was higher than comparative study in UK (Little and Louvois 1998). Fecal coliform count from cutting board and knife of study done in Jigjiga, Ethiopia (Henok Ayalew *et al.*, 2015) was 5.80 and 5.83cfu/cm², this was similar to present study's finding. Occurrence of cross and immediate contamination may increase this count. Mean yeast and mold count of contact surface in our study was around 5cfu/cm², which was higher than a comparative study done by

Henok Ayalew *et al.* (2015). This high microbial load, on the contact surface, in all counts may indicate presence of pathogenic microbes and may contribute to the contamination of meat products of the butcher. Except aerobic spore, the microbial load of the contact surface (among three of them) were not significantly ($p < 0.05$) different, the same was reported by Henok Ayalew *et al.* (2015), This may indicate there was high cross contamination among the contact surfaces during operation.

5.2.3 Dominant micro flora

Domination of *Enterobacteriaceae*, *Staphylococci* and *Bacillus* spp in the meat and contact surface samples indicates that the microbial quality and safety was highly compromised and may lead to pathogen infection (James *et al.*, 2005 and Anbesa Dabessa, 2013). Deriba and Mogessie (2001) reported that the micro-flora of “kitfo” was also dominated by the same spp. Similarly, Anbesa Dabessa (2013) reported that micro flora Jima house hold meat samples were also dominated by *Bacillus*, *Staphylococcus* and *Enterobacteriaceae* spp.

5.2.4 Prevalence of pathogenic microorganisms and distribution

The prevalence of *E. coli* in this study was 33.33%, 29.17% and 18.75% on butcher shops, contact surface and abattoir samples. Comparative study done in northern Ethiopia (Tesfay Kebede *et al.*, 2014) has reported that, prevalence of *E. coli* in butcher shops was 30% and abattoir was 15% which was lower than our study. In the side, Ousman Mohamed *et al.* (2014) and Nozha *et al.* (2006) also reported that 25% prevalence of *E. coli* from municipal abattoir, which was 7.25% higher than our report. Moreover, prevalence of *E. coli* of contact surface reported by comparative study (Endale Balcha *et al.*, 2013) was 32% which was higher than our study. This difference may be due to worker’s personal hygiene and environmental factors.

Even though presence of *E. coli* in foods are not always alarming because most strains are harmless and opportunistic in nature (Nozha *et al.*, 2006), the harmfully strains can cause gastroenteritis and toxins. Tizeta Bekele *et al.* (2014) had isolated shiga-toxin producing *E. coli* O157 at a frequency of 21.9% from meat samples of retailers.

Salmonella was not detected in abattoir samples which was included in this study. This was in agreement with Nozha *et al.* (2006), and Atinafu Wondimu (2012). However, in studies done by Ejeta *et al.* (2004) and Gizachew and Mulugeta (2015) prevalence of salmonella were 4% and 7.6%, respectively. Method and environmental difference may cause this difference among the above reports.

In present study, 4.17% of *Salmonella* was detected from meat samples of butcher shop. A comparative study done by Ejeta *et al.* (2004) and Anbessa Dabassa *et al.* (2012) had reported 14.1% and 13.3% prevalence of *Salmonella*, which was higher than our study. Similarly, 4.17% of *Salmonella* was also detected from our contact surface samples. This result was far away from a study done by Endale *et al.* (2013) which has reported 20% of the contact surface samples were *Salmonella* positive. The overall prevalence of *Salmonella* (3.41%) of our sample could be due to poor hygiene and sanitary practices through over the value chain of the meat supply. In addition, animal fecal contamination could also contribute. This *Salmonella* contamination could be high public health risk for a country like Ethiopia where consumption of raw meat is common (Beshatu Ferede *et al.*, 2015).

The overall prevalence of *S. aureus* of our study was 28.41% (37.5%, 29.17% and 12.5% on contact surface, butcher shops and abattoir samples, respectively). Comparative study (Tofick Kedir, 2013) has reported that 31% isolates were detected as *S. aureus* positive, which was higher than our report. In the other side, study of Tesfay Kebede *et al.* (2014) reported none of the abattoir meat samples were positive for *S. aureus*.

A study done by Pesavento *et al.* (2007) and Tesfay Kebede *et al.* (2014) have reported (29.4% and 30% prevalence, in a sequence) comparative result, on the other side, report given by Lubna *et al.* (2015) was higher than present study which was 50%, whereas our report was lower than a study conducted by Nozha *et al.* (2006).

Comparative study conducted by Endale *et al.* (2013) has reported 40% of the contact surface samples were positive for *S. aureus* which was higher than our study. Similar done by Henok

Ayalewet *et al.* (2015) has reported lower (35%). High contamination of food with *S. aureus* has been related to improper personal hygiene of employees during handling and processing of meat products Nozha *et al.* (2006).

5.2.5 Antimicrobial susceptibility profile

In the present study, *E. coli* isolates were observed to be the most resistant to Penicillin, Amoxicillin and Ampicillin (60%, 40% and 40%, in that order) and none of the isolates were resistance for Chloramphenicol. Comparative study (Zare *et al.*, 2014) reported that, resistance of *E. coli* for Ampicillin, Amoxicillin and Chloramphenicol (52.5%, 45% and 35%) were higher than our study. Another study done by Tesfay Kebede *et al.* (2014) showed that all (100%) *E. coli* isolates were resistance for Penicillin. Spices and method used may cause the susceptibility difference among the above reports. In this study, *S. aureus* isolates were resistant to penicillin (60%), Amoxicillin (40%) and Ampicillin (40%). In the other hand, Tesfay Kebede *et al.* (2014) reported that, *S. aureus* isolates were resistance to Penicillin (66.6%) and Ampicillin (100%), which were higher than our study.

Salmonella spp of Beshatu Ferede *et al.* (2015) were resistance for Ampicillin (54.5%) and Amoxicillin (45.5%) which were similar to our findings. Moreover, studies in other developing countries have shown that the trend in enteric pathogens is toward increasing antibiotic resistance Ousman Mohamed *et al.* (2014). The reason for the high antibiotic resistance could be the wide use of antibiotics in therapy, to promote feed efficiency in animal production and human medicine (Tefay Kebede *et al.*, 2014). When humans consume food, particularly those of animal origin, resistant bacteria can be transmitted (Anbessa Dabassa *et al.*, 2012).

6. CONCLUSION AND RECOMMENDATION

Based on the result obtained from this study, the following conclusions and recommendations were drawn:

6.1 Conclusions

1. The overall facility and layout of AAAE; water supply, toilet ratio and waste water treatment were not to the standard. The sanitation and hygiene facility was poor which can be one potential source of contamination
2. Low educational status, unhygienic practices, frequent handling of paper currency, broken cold chain and poor sanitation of the butcher shops are among the predominant factors of contamination of the beef.
3. Due to the above reasons the quality of the meat products has been compromised: TAMC, TSC, EB, coliform, fecal coliform, AS and YM count of carcass samples from the abattoir had high count and according to many countries standards; 50%, 93.75%, 87.5% and 37% of TAMC, EB, fecal coliform and YM, respectively were beyond safety limit. Moreover, out of butcher shops samples; 50%, 100%, 100% and 89% were categorized as unsatisfactory for TAMC, EB, fecal coliform and YM counts respectively
4. Microbial load on meat was found to be low in the carcass sample compared to the morning and afternoon samples from butcher shops. This shows, displaying of the beef samples for prolonged period at room temperature can increase the microbial load. In addition, minced meat had highest microbial counts than hide quarter/topside and fore quarter/neck
5. All the microbial count of the contact surface of the butcher shops was high. This high microbial load may indicate presence of pathogenic microbes and may contribute to the contamination of meat.
6. Due to poor microbial quality of the processing environment and the product itself, the prevalence of pathogenic microbes of this study is alarming; prevalence of *E. coli*, *Salmonella* and *S. aureus* were 29.55%, 3.4% and 28.41%, respectively

6.2 Recommendations

1. In-order to reduce potential cross contaminations, major lay-out, facilities and machinery modification is recommended (such as spraying of carcass by organic acids).
2. Chilling of beef carcass before distribution and distribution of beef carcass using separate track is highly recommended.
3. In AAAE and butchery shops, GMP and HACCP principles should be implemented to reduce the risk contaminations of pathogenic microorganisms. Moreover, contamination prevention rather than end-product testing to ensure the safety of meat is needed
4. Careful use of antibiotics in animal husbandry is advisable which are essential for the control of further emergence of multidrug resistance.
5. Finally, it is advisable to cook any meat product before consumption, rather than consuming as a raw.

7. REFERENCES

- Adams, M.R. and Moss, M.O. (2000). **Food Microbiology**, 2nded. Royal Society of Chemistry, Guildford, UK.
- Adem Hiko, Daniel Asrat, Girma (2008) *Escherichia coli* O157:H7 in retail raw meat products of Ethiopia. *J Infect Devel. Countries*. 2: 389-393.
- American Public Health Association (2012). **Standard Methods for the Examination of Water and Wastewater**. 22nd ed. APHA, Washington, D.C.
- Anbesa Dabessa (2013). Evaluation of Home Slaughtered Meat Quality used for Human consumption at household and food seller house in Jimma. *J. Med. Sci.* **13**: 779-784.
- Anbesa Dabassa and Ketema Bacha (2012). The Prevalence and Antiprogram of *Salmonella* and *Shigella* Isolated from abattoir, Jimma town, South West Ethiopia. *Int. J. Phar. and Bio.Res.* **3**: 881-890.
- Arse Gebeyehu, Mohammed Yousuf and Ameha Sebsibe (2013). Evaluation of Microbial Load of Beef of Arsi Cattle in Adama Town, Oromia, Ethiopia. *J. Food Process Technol.* **4**:6.
- Aslam, A. Mariam, I. Haq, I. Ali, S. (2000). Microbiology of Raw Minced Beef. *Pak. J. Biol. Sci.* **8**: 1341-1342.
- Atinafu Wodimu (2012). **Customer's Beef preference, Safety Perception and Bacterial load in the carcass of Addis Ababa and kara abattoir**. MSc. Thesis, Addis Ababa University, Addis Ababa.
- Ayehu Gashe, Kassahun Alemu and Daniel Haile (2014). Factors affecting food handling Practices among food handlers of Dangila town food and drink establishments, North West Ethiopia. *BMC Public Health* **14**:571.
- Bean, N. H. Griffin, P. M. Goulding, J.S. Ivey, C.B (1990). Food-borne Disease Outbreaks, 5 Year Summary, CDC Surveill. Summ. *J. Food Prot*, **53**: 711-728.
- Bergey, D. H. Holt, J.G and Krieg, N.R (1994). **Bergey's Manual of Determinative Bacteriology**, 9thed. Lippincott Williams & Wilkins. MI, USA.

- Beshatu Ferede, Fanta Desissa, Aklilu Feleke, Getachew Tadesse and Nebyu Moje (2015). Prevalence and antimicrobial susceptibility of Salmonella isolates from apparently healthy slaughtered goats at Dire-Dawa municipality abattoir, Eastern Ethiopia *J. Micr. Andantimic.* **7**: 1-5.
- CDC (2003). Preliminary food net Data on the incidence of food borne illness-selected CDC or the US Department of Health and Human Service. Washington DC, US Food and Drug Administration, Cent. Food Safety App. Nutr.
- Chris, P. Mark, R. Henning, S. Simeon, E. Claude,C. (1999). Livestock to 2020 The Next Food Revolution. *Food, Agri. Envir.* **30**: 27–29
- CLSI (2014). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement M100-S24.**34**.
- CSA /Central Statistics Agency/ (2013). Ethiopia- Livestock sample survey 2012-2013 (2005 E.C). Minister of finance and Economic Development. Retrieved from: <http://192.168.100.13/nada4/index.php>
- Dave, D. and Ghaly, A. E. (2011). Meat Spoilage Mechanisms and Preservation Techniques: A Critical Review. *Amer. J. Agri. Bio. Sci.***6**: 486-510.
- Delmore, R.J. Sofos, J.N. Belk, K.E. Lloyd, W.R. Bellinger, G.L. Schmidt, G.R. and Smith, G.C. (1999). Good manufacturing practices for improving the microbiological quality of beef variety meats. *Dairy Fd. Environ. Sanit.* **19**:742–752.
- Deriba Muleta and Mogessie Ashenafi (2002). Some street vended foods from Addis Ababa: microbiological and socio-economic considerations. *Ethiop. J. Health Sci.* **10**: 89-100
- Diez-Gonzalez, F. (1999). **Detection and enumeration of pathogens and indicator organisms**. Application of microbiology to food systems monitoring. McGraw Hill, New York.
- Dillon, VM and Board, R.G. (1991). *Yeasts* associated with red meats. *J. Appl. Bacteri.* **71**:93–108.
- Dillon, VM. (1998). Yeasts and molds associated with meat and meat products. In: **The Microbiology of Meat and Poultry**, pp85–117 (A. Davies and R. Board,ed): Kluwer Academic Publishers, Inc. New York.

- Edget Abayneh, Dafnar N. and Biruhtesfa Asrade, (2014) Review on common foodborne pathogens in Ethiopia. *Afri. J. Micro. Rese.*,**8**: 4027-4040.
- Ejeta G. Molla, B. Alemayehu, D. and Muckle, C. A. (2004). *Salmonella* serotypes isolated from minced meat beef, mutton and pork in Addis Ababa, Ethiopia. *Revue Méd. Vét.*,**155**: 547-551.
- Endale Balcha and Hailay Gebretinsae (2013). Assessment of bacteriological quality of meat contact surfaces in selected butcher shops of Mekelle city, Ethiopia. *J. Env. Occu. Sci.* **2**:61-66.
- Endrias Zewdu (2014). **Prevalence, distribution and antimicrobial resistance profile of salmonella isolated from food items and personnel in Addis Ababa, Ethiopia.** MSc. Thesis Addis Ababa University, Debrezeyt Ethiopia.
- European Commission regulation (EC) (2005). Microbiological criteria for food stuffs. No 2073/2005 Retrieved, from: https://scholar.google.de/scholar?q=COMMISSION+REGULATION+%28EC%29+No+2073%2F2005&btnG=&hl=en&as_sdt=0%2C5.
- FAO (2009). Food and Agriculture Organization of the United Nations, the state of food and agriculture, livestock in the balance, Retrieved from: <http://www.fao.org/catalog/inter-e.htm>.
- FAO (2010). Techniques and hygiene practices in slaughtering and meat handling. Retrieved from: <http://www.fao.org/docrep/004/T0279E/T0279E04.htm>
- Firew Tafesse, Gulelat Desse, Ketema Bacha and Haile Alemayehu (2010). Microbial quality and safety of street vended raw meat in Jijiga town: south east Ethiopia. *African J. Micro. Res.***8**: 3867-3874.
- Food Safety Authority of Ireland (2016). **Guidance Note**: Guidance Note No. 3 Guidelines for the Interpretation of Results of Microbiological Testing of Ready-to-Eat Foods Placed on the Market (Revision 2) Food Safety Authority of Ireland. Dublin, Ireland
- Fratamico, P.M., Bhunia, A. K and Smith, J.L. (2005). **Foodborne Pathogens in Microbiology and Molecular Biology.** Caister Academic Press, Wymondham, Norfolk.

- Gashaw Andargie, Afework Kassu, Feleke Moges, Moges Tiruneh and Kahsay Huruy (2008). Prevalence of Bacteria and Intestinal Parasites among Food-handlers in Gondar Town, Northwest Ethiopia. *J. Heal. Pop. Nut.* **26**:451-455.
- Getu Kebede (2010). **Branding Ethiopia: opportunities and challenges**. MSc. Thesis, University of South Africa, South Africa.
- Gill, C.O. McGinnis, J.C. Bryant, J. (1998). Microbial contamination of meat during the skinning of beef carcass hindquarters at three slaughtering plants. *Inter. J. Food Micr.* **42**:175–184.
- Gihan Anwar Eid Abdallah (2004). **Quality management of meat in a slaughterhouse in Khartoum state**. MSc. Thesis, University of Khartoum, Sudan.
- Gosa Girma, TsigeKetema and Ketema Bacha (2014). Microbial load and safety of paper currencies from some food vendors in Jimma Town, Southwest Ethiopia. *BMC Research Notes*. **7**:843.
- Gizachew Muluneha and Mulugeta Kibretb (2015). *Salmonella* spp. and risk factors for the contamination of slaughtered cattle carcass from a slaughterhouse of Bahir Dar Town, Ethiopia. *Asi. Pac. J.Trop. Disease*. **5**: 130-135
- Gorbach, S.L. (2001): Antimicrobial use in animal feed – time to stop. *N. Engl. J. Med.* **345**: 1202 – 1203.
- Haeghebaert, S. Le Querrec, F. Bouvet, P. Gallay, A. Espie', E.Vaillant, V. (2002). Les toxi-infections alimentaires collectives en France. *B.E.H.* **50**: 249–254.
- Haimanot Tassew, Alemseged Abdissa, Getenet Beyene, Solomon Gebre Selassie (2010). Microbial flora and food borne pathogens on minced meat and their susceptibility to antimicrobial agents. *Ethiop J Health Sci.* **20**:137-143.
- Hammer GF (1997). **Meat processing: ripened products Fleischwirtschaft**. Consequences of packaging on bacterial growth. Meat as an ecological niche. *Meat Sci.* **52**: 299–305.
- Harko Halala. (2015). Review of Beef Cattle Value Chain in Ethiopia. *Indus. Enginee. Letters* **5**:11-22.
- Henok Ayalew, Amare Berhanu, Berhanu Sibhat and Biresaw SerdaHenok (2015). Microbiological assessment of meat contact surfaces at abattoir and retail houses in

- Jigjig a town Somalia national regional state of Ethiopia. *J. food and agricultural sci.* 5:21-26.
- ISO/TS 11133-1 (2009). Microbiology of food and animal feeding stuffs. Guidelines on preparation and production of culture media. Part 1: General guidelines on quality assurance for the preparation of culture media in the laboratory.
- International Organization for Standardization 6579 (1998): **Microbiology of food and animal feeding stuff-horizontal method for the detection of Salmonella**, ISO, Geneva.
- Livestock and Irrigation Value chain for Ethiopian Small holders (LIVES). (2014). Retrieved from: <http://lives-ethiopia.org/2014/05/12/addis-livestock-markets>.
- James M, Martin J and David A. (2005). **Modern Food microbiology**, 7thed. Springer, New York.
- Jean C. Buzby, Paul D. Frenzen and Barbara Rasco (2001). Product Liability and Microbial Foodborne Illness: Product and Microbial Food borne Illness, Food and Rural Economics Division, *Econ. Resea. Service/USDA, Agricultural Economic Report 799*.
- Kammenou, M. Metaxopoulos, J.Drosinos, E.H.(2003). Microbiological quality of minced beef from butcher shops and supermarkets. *Ital. J. Food Sci.* **15**: 95.
- Keener, K., (2009). Safe food guidelines for small meat and poultry processors, SSOP and GMP and programs. Retrieved from https://scholar.google.com/scholar?cluster=4503496702796543309&hl=en&as_sdt=2005&scioldt=0,5.
- Lemma Dadi and Daniel Asrat (2008). Prevalence and antimicrobial susceptibility profiles of thermotolerant *Campylobacter* strains in retail raw meat products in Ethiopia. *Ethiop. J. Health Dev.* **22**:198-200.
- Little, CL. and Louvois, J De. (1998). The microbiological examination of butchery products and butchers' premises in the United Kingdom: Environmental Surveillance Unit, Public Health Laboratory Service (CDSC), *UK J. Appl. Micro.* **85**: 177–186.

- Lowiwers, D. Farouk, M.M. Frost, D.A. Dobbie, P.M and Young, O. A. (2010). **The biochemical basis of tenderness in Beef**. Ph.D. Project, Austria University.
- Lubna, S.A. Harrington, W. and Mohamed, K.F. (2015). *Staphylococcus aureus* is More Prevalent in Retail Beef Livers than in Pork and other Beef Cuts. *Pathogens* **4**:182-198.
- Mari'a, G.A.Villarroel, M.Sañudo, C.Olleta, J.L. and Gebresenbet, G. (2003). Effect of transport time and ageing on aspects of beef quality. *Meat Scie.* **65**: 1335–1340.
- Molla, B., Mohammed, A. & Salah, W. (2004). Salmonella prevalence and distribution of serotypes in apparently healthy slaughtered camels (*Camelus dromedarius*) in Eastern Ethiopia. *Ethio. Trop. Animal Heal. Prod.* **36**: 451-458.
- Meat Standards Australia (2013). **Meat Standards Australia Beef Information Kit**. Meat & Livestock Australia. Australia 1-9.
- Melese Temesgen (2015). Food Standards, Food Law and Regulation System in Ethiopia: A Review. *Pub. Poli. And Admini. Res.* **5**:58-72.
- MoA (2008). Constriction guideline 2. Ministry of Agriculture and Rural Development Animal and Plant Health Regulatory Directorate. Addis Ababa, Ethiopia.
- Mogessie Ashenafi (2002). The microbiology of Ethiopian Foods and Beverages: A Review, *SINET: Ethiop. J. Sci.* **25**: 97-140.
- Mohamed, A.A.Rajput, I.R.Khaskheli, M.Faraz, S.DevrajaniK. andFazlani, S.A. (2010). Microbial Quality of Goat Meat at Local Market of TandoJam. *Paki. J. Nutrition* **9**: 287-290.
- Mokonnen Addis and Desta Sisay (2015). A Review on Major Food Borne Bacterial Illnesses. *J. Trop. Dis.* **3**: 176.
- Moreno, P. Pla, S. Fagoag, F. Garcia, M. and Torregrosa A. (1997). Microbiological quality of meat products from the Alcoi and Xativa (Spain): health areas during 1993-1995 Alimentary. Retrieved, from: https://scholar.google.com/scholar?q=Microbiological+quality+of+meat+products+from+the+Alcoi+and+Xativa+%28Spain%29&btnG=&hl=en&as_sdt=0%2C5.
- Mulat Abegaz (2004.) UNIDO Food Safety and Technology Projects Coordinator and Consultant, Gap Analysis Report, Recommendations & Proposals on Food Control System in Ethiopia, UNIDO's Regional Programme on Harmonization of Food

- Control System in East-Africa, Retrieved from https://scholar.google.com/scholar?q=Mulat+Abegaz%2C+%282004.%29+&btnG=&hl=en&as_sdt=0%2C5.
- Nel, S., Lues, J. F. R., Buyus, E. M., Venter, P. (2004). Bacterial populations associated with meat from the deboning room of a high throughput red meat abattoir. *Meat Sci.*, **66**: 667-674.
- New Zealand Food Regulations (1995). Food Administration Manual: Microbiological reference criteria for food. *Microbiological Criteria: S. 11. 2*:1-12
- Nordic Committee on Food Analysis (NMKL) (1991). Salmonella bacteria, detection in foods. NMKL method no. 71, 4th ed. Nordic Committee on Food Analysis, Oslo, Norway.
- Nordic Committee on Food Analysis (NMKL) (1993). Coliform bacteria and Escherichia coli in foods. NMKL method no. 68, 4th ed. Nordic Committee on Food Analysis, Oslo, Norway.
- Nozha, C. Hayat, E. Mohamed, H. and Hakim, K. (2006). The bacterial quality of red meat and offal in Casablanca (Morocco). *Mol. Nutr. Food Res.* **50**: 557 – 562
- NSW Food Authority (2009). **Microbiological quality guide for ready-to-eat foods: A guide to interpreting microbiological results**: Australia New Zealand.
- Obeng, A.K. Johnson, F.S and Appenteng, S.O. (2013). Microbial Quality of Fresh Meat from Retail Outlets in Tolon and Kumbungu Districts of the Northern Region of Ghana. *Int. J. Sci. and Tech.***2**: 423-427.
- Ömer,Ç. Enver Barış, B. Hila, Ç. Özer E. and Can, D. (2008).The microbiological, serological and chemical qualities of mincemeat marketed in İstanbul. *Turk. J. Vet. Anim. Sci.***34**: 407-412.
- Oosterom, J. (1991). Epidemiological studies and proposed preventive measures in the fight against salmonellosis. *Int. J. food Microb.* **12**:41-52
- Ousman Mohammed, Dawit Shimelis, Petros Admasu and Teka Feyera(2014).Prevalence and Antimicrobial Susceptibility Pattern of E. Coli Isolates from Raw Meat Samples Obtained from Abattoirs in Dire Dawa City, Eastern Ethiopia. *Int. J. Microb. Research***5**: 35-39.

- Pearson A.M. and Dutson T.R. (1999). **Advance in Meat Research: HACCP in Meat, Poultry and Fish Processing**. Springer- Science + Business Media. B.V.UK
- Pesavento,G. Ducci, B. Comodo, N. NostroA. Lo (2007). Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus aureus* (MRSA). *Food Control***18**: 196–200
- Quinn,P. J. Markey, B. K. Leonard, F. C. Hartigan, P. Fanning, S. and Fitzpatrick, E. S. (2011).**Veterinary microbiology and microbial disease**. (2nded.) Blackwell publishing, oxford, UK.
- Regulation (2015). **Microbiological Standards for Meat and Meat Products**: Food products standards and food additives. Food safety and standard authority of India. New Delhi.
- Reeves, M.W. Evins, G. M.Heiba, A. A.Plikaytis, B D and Farmer, J. J. (1989). Clonal Nature of Salmonella typhi and Its Genetic Relatedness to other Salmonellae as Shown by Multilocus Enzyme Electrophoresis, and Proposal of Salmonella bongori comb. nov.. *J. Clin. Microbiol.* **27**: 313-320.
- Saleh,E.A. Ibrahim, H.A. EL-kewaiey, I.A. and Zaqzouq G.S.(2013).Microbiological aspects of sheep and cattle meats in el-beheria province. *Assuit.vet. med. J.***59**: 192-202.
- Semeneh Seleshi, Cheorun J., and Mooha L. (2014). Meat Consumption Culture in Ethiopia. *Korean J. Food Sci.* **34**: 7-13.
- Tavakoli HR, Razipour M. (2008). Microbial quality of cooked meat foods in Tehran Universities Restaurants. *Paki. J. Medical Sci.***24**:595-599.
- Tesfay Kebede, Berihun Afera, Habtamu Taddele and Abrha Bsrat(2014). Assessment of Bacteriological Quality of Sold Meat in the Butcher Shops of Adigrat, Tigray, Ethiopia. *Applied J. Hygiene***3**: 38-44.
- Thomas, N. Kiros, A. Pal, M. Aylate, A.(2015). Bacteriological Quality of Raw Beef Collected from Municipality Slaughterhouse and Local Markets in and around Wolaita Soddo Town, Southern Ethiopia. *Int. J Vet. Health. Sci. Res.* **3**: 75-81.

Tizeta Bekele, Girma Zewde, Genene Tefera, Aklilu Feleke and Kaleab Zerom Bekele et al., (2014). *Escherichia coli* O157:H7 in Raw Meat in Addis Ababa, Ethiopia: Prevalence at an Abattoir and Retailers and Antimicrobial Susceptibility. *Int. J. Food Contam.* 1:4.

Tofick kedir (2013). **Prevalence of *staphylococcus aureus* in beef carcass and its source of contamination at Addis Ababa abattoir, Ethiopia.** MSc. Thesis. Haramaya University, Haramaya.

UN (2013). Urban inequality report; Addis Ababa: Cities and citizen's series. Retrieved from https://www.google.ch/search?q=Addis+ababa,+gulelle+subcity+water+suply+and+sanitation&ie=utf-8&oe=utf-8&client=firefox-b&gfe_rd=cr&ei=391cWJ2DNYqh8we1pegAQ&gws_rd=cr

United States Pharmacopeial Convention (2007). **The United States pharmacopeia**, 31st ed., Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention, Rockville, MD.

Węglarz (2010). Meat quality defined based on pH and color depending on cattle category and slaughter season. *Czech J. Anim. Sci.*, 55: 548–556.

WHO (1996). Essential Safety Requirements for Street-vended Foods: Food Safety Unit Division of Food and Nutrition. Retrieved from: https://www.google.ch/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwiCq6r48YnRAhVMOFAKHQJ1DYQFggaMAA&url=http%3A%2F%2Fapps.who.int%2Firis%2Fbitstream%2F10665%2F63265%2F1%2FWHO_FNU_FOS_96.7.pdf&usg=AFQjCNG86uqm87HY0DByzNZN4Qwhzdr0qA&sig2=jAY2erBCwjT61JO1s5KHsQ&bvm=bv.142059868,d.ZWM.

WHO (2008). Foodborne Disease Outbreak: Guidelines for investigation and control. WHO Library Cataloguing-in-Publication Data. Retrieved from: https://www.google.ch/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=0ahUKEwjajoKL84nRAhWdcFAKHSNIAWUQFggeMAA&url=http%3A%2F%2Fwww.who.int%2Ffoodsafety%2Fpublications%2Ffoodborne_disease%2Foutbreak_guidelines.pdf&usg=AFQjCNGZUG1drEwwdZ8Tb8I3IqsroZ_bg&sig2=aRV0B5mpFsB5dppbUvavgw&bvm=bv.142059868,d.ZWM

- WHO. (2004). Developing and Maintaining Food Safety Control Systems for Africa Current Status and Prospects for Change: Second FAO/WHO Global Forum of food Safety Regulators Regional Office for Africa, Bangkok, Thailand. 12-14.
- WHO. (1987). Prevention and control of intestinal parasitic infections. Geneva: World Health Organization, 7-18. (Technical report series no. 749).
- Yousuf. A.H.M. Ahmed, M.K. Yeasim, S.Ahsan, N.Islam, M.M. (2008). Prevalence of microbial load in shrimp, peanuts modonon and prawn, Macro brachium Rosen Berger from Bangladesh. *World J. Agri. Sci.*; **4**: 852-855.
- Zare, P. Ghorbani, C.H. Jaber, S. Razzaghi, S.Mirzae, M. and MafuniK (2014). Occurrence and Antimicrobial Resistance of *Salmonella* spp. and *Escherichia coli* Isolates in Apparently Healthy Slaughtered Cattle, Sheep and Goats in East Azarbaijan Province. *Int. J. Entric. Pathog.* **2**:1-4.
- Zelalem Ayana, Moti Yohannis and Zelalem Abera (2015). Food-Borne Bacterial Diseases in Ethiopia *Acad. J. Nutr.* **4**: 62-76.

8. APPENDIX I

1. Plate count agar

Typical Formula*	gm/ litre
Tryptone	5.0
Yeast extract	2.5
Glucose	1.0
Agar	

1. VRBA

Peptic digest of meat	7.0 g
Yeast extract	3.0 g
Lactose	10.0 g
Bile salts	1.5 g
Sodium chloride	5.0 g
Neutral red	30.0 mg
Crystal violet	2.0 mg
Bacteriological agar	12.0 g

2. MSA

Beef Extract	1.0 g
Pancreatic Digest of Casein	5.0
Peptic Digest of Animal Tissue	5.0
Sodium Chloride	75.0
D-Mannitol	10.0
Phenol Red	0.025
Agar	15.0

3. TT

	gm/ liter
Casein peptone	2.5
Meat peptone	2.5
Bile salts	1.0
Calcium carbonate	10.0
Sodium thiosulphate	30.0

4. SC

5. XLD

Sodium Phosphate	10.0gm
Tryptone	5.0gm
Lactose	4.0gm
Sodium Selenite	4.0gm
L-Cystine	0.01gm

Typical Formula	gm/liter
Yeast extract	3.0
L-Lysine HCl	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
Sodium desoxycholate	1.0
Sodium chloride	5.0
Sodium thiosulphate	6.8
Ferric ammonium citrate	0.8
Phenol red	0.08
Agar	12.5

6. MRVP

Typical Formula	gm/liter
Peptone	7.0
Glucose	5.0
Phosphate buffer	5.0

7. OF Basal Medium

Pancreatic Digest of Casein	2.0 g
Agar	2.5 g
Sodium Chloride	5.0 g
Bromthymol Blue	0.03 g

Dipotassium Phosphate

0.3 g

8. Simmon citrate

	gm/liter
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	0.2
Sodium ammonium phosphate	0.8
Sodium citrate, tribasic	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0

9. TSI

	gm/liter
'Lab-Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0

10. LIA

typical Formula	gm/liter
Bacteriological peptone	5.0
Yeast extract	3.0
Glucose	1.0
L-lysine	10.0

Ferric ammonium citrate	0.5
Sodium thiosulphate	0.04
Bromocresol purple	0.02
Agar	14.5

11. Hugh and Leifson's OF basal medium

Peptone (tryptone)	2.0 g
Sodium chloride	5.0 g
Glucose (or other carbohydrate)	10.0 g
Bromthymol blue	0.03 g
Agar	3.0 g
Dipotassium phosphate	0.30 g

12. Kovac's reagent

p-dimethylaminobenzaldehyde	10 gm
isoamyl alcohol	150 ml
concentrated hydrochloric acid	50 m

13. MR reagent

methyl red	0.1 g
ethyl alcohol (95%)	300 ml
distilled water to make	200 ml.

14. VP reagent

A. Alpha-Naphthol, 5%	50.0gm
Absolute Ethanol	1000.0ml
B. Potassium Hydroxide	400.0gm

Deionized Water	1000.0ml
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9. APPENDIX II

Survey questions

I. Check list for the abattoir observation

1. Is the layout of the slaughtering house does cause cross contamination? **A. Yes** B. No
2. What is the average cattle slaughtered each day? >4000 head
3. The ratio of no. of toilet and no. of butchers
A. 1:5 B. 1:10 C. 1: 15 **D. 1:20**
4. Is there reserve electric power and water supply? A. Yes B. No
5. Does the slaughtering house have enough amount/size of Lairage for it daily slather capacity?
A. Yes B. No
6. Do they have enough water line and supply to the Lairage? A. Yes B. No
7. Do the cattles wash before slaughtering?
A. Yes B. No
8. The time of fasting period/resting time
A. < 12 hours B. 12-24 C. >24 **no resting period**
9. Stunning techniques they use
A. Bolt **B. sticking knife** C. other
10. The temperature of the slaughtering house
A. 20-25⁰C B. 25-30⁰C **C. >30⁰C**
11. Do they have hot water supply? A. Yes B. No
12. General personal hygiene of the butchers
A. **Poor** B. Good C. better
13. Sanitation and sterility of the equipments they use
A. **Poor** B. Good C. better
14. Do they frequently use clean water to wash/clean slaughtering wastes and their hands at every steps of the process?
⇒ After bleeding A. Yes **B. No**
⇒ After skinning A. Yes **B. No**
⇒ After evisceration A. Yes **B. No**
- 15. The temperature of the chillers**
A. 0⁰C B. 0-2⁰C C. >2⁰C D. not specified
16. The time takes from stunning to chiller
A. <45 min. B. 45-60 min. C. > 60 min.
17. The cold chin management
A. **Poor** B. Good C. better
18. Do they use carcass washing/sanitizing chemicals A. Yes B. No

If yes, specify _____

19. Do the butchers wash their hands at each steps and after each cattle? A. Yes B. **No**
20. Do the butchers wash their hand when they visit rest room/toilet? A. Yes B. **No**
21. Is there any missing step from a standard slaughtering procedure? A. Yes B. No
If yes, _____
22. How often the butchers renew their health certificate?
A. **6 months** B. annually C. they don't have at all
23. Know-how of the butchers about personal and food hygiene
A. Poor B. Good C. better
24. Do the butchers have been given any training about personal hygiene and food borne diseases
A. Yes B. No

II. Check list for transportation observation

1. Do the vehicles which transport the beef carcass possess refrigerated environment for the product? A. Yes **B. No**
2. Is the capacity of the vehicles specified with regards to the distance they cover?
A. Yes B. No
3. Is there a standard space among the hanged caresses inside the vehicles?
A. Yes **B. No**there fore the is cross contamination
4. Do they transport the beef carcass with other red and green offals?
A. Yes B. No
5. How often the vehicles washed?
A. **After each shipment** B. daily C. not specified
6. The sanitation of the loading area of the vehicle
A. Poor **B. Good** C. better
7. Method of loading and unloading of the beef carcass from and/or to the vehicle
A. **Manually** B. through conveyer C. other
8. Do the workers who transport the carcass to the vehicle wear clean cloths and safety materials
A. Yes **B. No**

10. APPENDIX III

Questioners for butcher shop workers

I. General information

1. Sex/ጾታ : A. Male/ወንድ B. Female/ሴት
2. Age/ እድሜ: _____
3. Marital status/ የጋብቻሁኔታ:-----
4. Educational Status/ ትምርት ደረጃ: A. Illiterate/ መደበኛ ት/ት ያልተማረ B. Grade/ክፍል _____
5. For how long have you been working in butcher shop? _____
በሉካንዳብቱየቆየህበት ጊዜ
6. Had you been given any training about sanitary handling of food? A. Yes/ አለ B. No/ የለም
ስለምግብ እያያዝየተሰጠህስልጠናካለ?
7. If you had been given any training, who gave you the training?
ስልጠናከተሰጠኝ የሰጠህካልማነው?
 - A. From approved organization/ እውቅናካለው ድርጅት
 - B. Cite administration health office/ ጤናቢሮ
 - C. Other/ ሌሎች _____
8. Have you ever suffered from disease symptoms like vomiting, diarrhea, skin infection and continuous coughing during the period of your food handling? A. yes/ አዎ B. no / አያውቅም
በዚህስራሰት ሰራሽ ለሚከተሉ የበሽታ ምልክቶች ታይቶብህ ያወቃሉ: ቀጣይነት ያለው ተቆማት፣ ትውከት፣ የቆዳ ማመርቀዝ፣ ማያቃርጥሳል?
9. If you have suffered from any of these disease symptoms status of sick leave and resting after suffer of the disease
ከላይ የተጠቀሱ የበሽታ ምልክቶች ሲሰተዋሉ በህዩሚሰጥህ የ እረፍት ካለ?
 - a. Taken rest till become free of sign and symptoms/ ከ በሽታ ምልክቶች እስከ ሚድል አረፍሰሁ
 - b. Not taken rest and have been on my duet/ ምንም አላርፍም
 - c. Others/ ሌሎች _____
10. How do you being supervised with health experts on normal working days
የጤና ጥበቃ ባለሙያዎች ቁጥጥር ምን ያህል ነው?
 - A. Regular / ቀጣይነት ባለው
 - B. Intermittently/ አልፎ አልፎ
 - C. Never supervised/ ጨራሽ ከትትል የለም
11. How often the butchers renew their health certificate?
የጤና ሰርተፍኬት በየስንት ጊዜ ታላድሳለህ?
 - A. 6 months/ 6 ወር
 - B. annually/ በየአመቱ
 - C. they don't have at all/ ይለኝም

III, Observation for butcher shop and the workers

1. Does the worker wear appropriate over coat? A. Yes B. No
2. Does the worker wear appropriate hair cover? A. Yes B. No
3. Are nails short and clean? A. Yes B. No
4. Discharge from nose, eye, ear and cough during visit A. not observed B. Observed
5. Any visible skin rash, skin boils, cut and wound at time of visit A. not observed B. Observed
6. Is there a refrigerator in the shop A. Yes B. No
7. General sanitation of the shop A. Poor B. Good C. better
8. Is the location of the toilet can cause contamination?

A. Yes

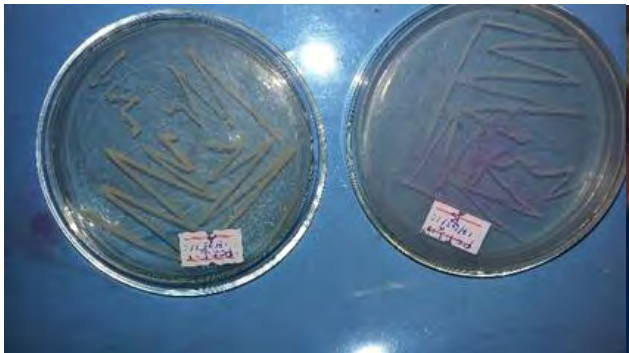
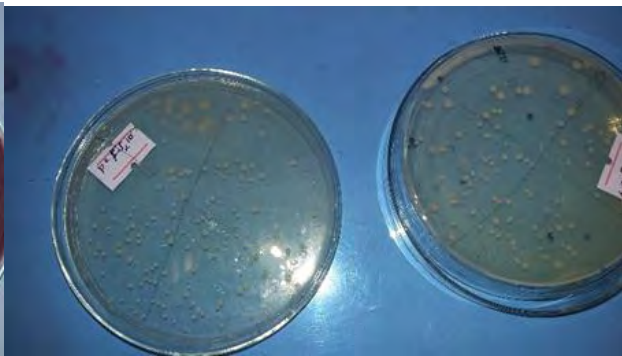
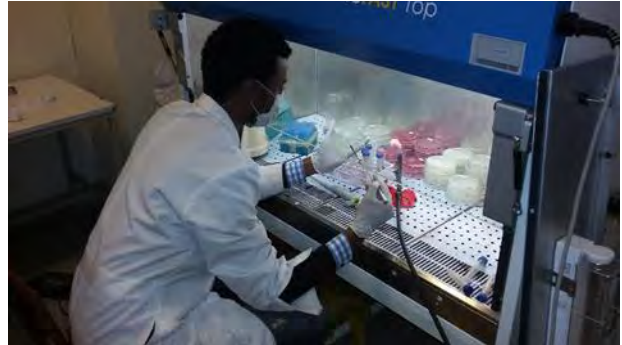
B. No

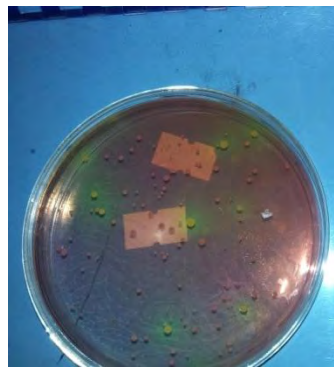
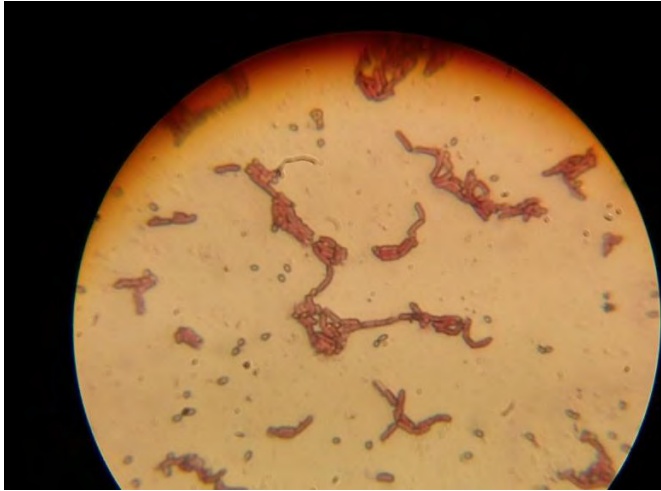
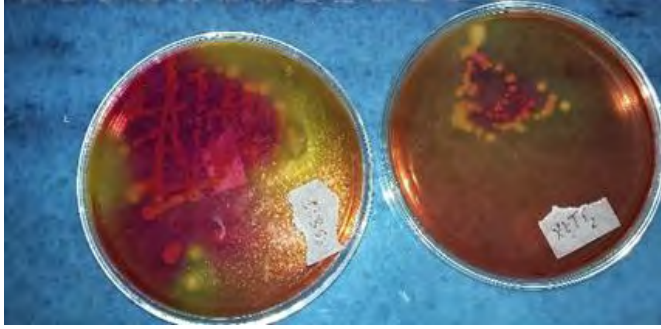
11.PHOTO GALLERY

1, Slaughtering techniques and practices



2, Enumeration and characterizations





3, Biochemical tests





4, Microbial susceptibility test

