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STUDIES ON THE PREVALENCE, RISK FACTORS, PUBLIC HEALTH IMPLICATION AND ANTIBIOGRAM OF LISTERIA MONOCYTOGENES IN SHEEP MEAT COLLECTED FROM MUNICIPAL ABATTOIR AND BUTCHER SHOPS IN ADDIS ABABA

MSc Thesis

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MSc Program in Veterinary Public Health

June, 2014

College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia

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A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Department of Public Health

By

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June, 2014

College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia

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As member of the Examining Board of the final MSc open defense, we certify that we have read and evaluate the thesis prepared by: **Selamawit Mulu** Entitled: **Studies on the prevalence, risk factors, public health implication and antibiogram of *Listeria monocytogenes* in sheep meat collected from municipal abattoir and butcher shops in Addis Ababa.** And recommended that it be accepted as fulfilling the thesis requirement for the degree of Masters of Veterinary Public Health

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DEDICATION

This thesis manuscript is dedicated to my families

STATEMENT OF AUTHOR

First I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institute anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ATR	Acid Tolerance Response
CAMP	Christie Atkins Munch Peterson
CCPs	Critical Control Points
CDC	Centers of Disease Control and Prevention
CNS	Central Nervous System
CSF	Cerebro Spinal Fluid
Csps	Cold Shock Proteins
DNA	Deoxyribo Nucleic Acid
ERS	Economic Research Service
FAO	Food and Agricultural Organization
FDA	United State Food and Drug Administration
GAD	Glutamate Decarboxylase
HACCP	Hazard Analysis of Critical Control Points
HIV	Human Immune Deficiency Virus
OXA	Oxford Agar
PALCAM	PolymixinAcriflavin Lithium Chloride CeftazidimeAesculinMannitol
pH	Hydrogen ion concentration
RNA	Ribo Nucleic Acid
RTE	Ready-to-Eat

SA	*Staphylococcus aureus*
Spp.	Species
SPSS	Statistical Package for Social Sciences
SSP	Salt Shock Proteins
TSA	Tryptic Soy Agar
USDA	United States Food and Drug Administration
WHO	World Health Organization
χ^2	Chi-square

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ABSTRACT

Listeriosis is one of the important food-borne bacterial zoonotic diseases caused by Listeria monocytogenes, as a result of food and environmental contamination as well as zoonotic infections. This disease is becoming an emerging bacterial disease, with low incidence but high case fatality rate. The present study was undertaken to determine the presence of Listeriamonocytogenes in raw meat of market and abattoir. A cross-sectional study was conducted from October 2013 to April 2014 to isolate Listeria monocytogenes from swab samples on sheep meat from abattoir, butcher shops, equipments, and to determine antibiotic resistance profiles of the isolates. A total of 873 swab samples comprising of 384 from the abattoir, 384 from butcher shops were collected using systematic random sampling technique and 105swabs were collected from equipments. Questionnaire survey was conducted to assess the hygienic practices of meat production in raw meat of market and abattoir and possible risk factors regarding the contamination of meat. Listeria monocytogenes was isolated and identified using standard bacteriological techniques. Antimicrobial susceptibility test was also conducted on 36 isolates Listeria monocytogenes. The overall prevalence of Listeria monocytogenes identified was 4.1%. And 2.1%, 5.5% and 6.7% from abattoir, butcher shops and equipments respectively. The study also revealed multi-drug resistant isolates in 24/36 (66.7%) for two or more antimicrobials. In addition, the presence of Listeria monocytogenes attributed to unclean working environment and improper handling of meat till it reaches to the consumer. Preventive measures to avoid the presence of pathogenic Listeria monocytogenes in raw meat and meat products should be undertaken, emphasizing the need for improved hygienic practices during meat production and also during distribution and consumption of the final products.

***Key words**: Addis Ababa, Antimicrobial susceptibility, Listeria monocytogenes, Prevalence and Sheep meat*

1. INTRODUCTION

The total number of sheep and goats in Ethiopia is estimated to be nearly 48 million. Sheep and goats are widely adapted to different climates and are found in all production systems. They also have lower feed requirements compared to cattle because of their small body size. This allows easy integration of small ruminants into different farming systems. Economic opportunities exist for small ruminant producers to supply animals to both the export and domestic markets. Taking advantage of these opportunities requires overcoming many barriers to increase productivity (Yami and Merkel, 2008).

Animals are important as producers of meat, milk and eggs, which are part of the food chain and, which provide high value of protein food. They have long played a key role in supplying calories, and protein for human food in virtually all parts of the world, both directly in the form of animal products and indirectly from the contribution of manure and draught power to crop production and generation of income to enable purchase of food (ESAP, 2001). Animals naturally harbor many foodborne bacteria in their intestines that can cause illness in humans, but often do not cause illness in the animals. During slaughter, meat and poultry carcasses can become contaminated if they are exposed to small amounts of intestinal contents(Sheri *et al*., 2008).

Food safety has emerged as an important global issue with international trade and public health implications. *L.monocytogenes* associated with outbreaks have been reported from a wide variety of food types (Pal, 2013).The bacterium has been isolated from meat, milk and milk products in all over the world (Khan *et al*., 2013; Pal, 2014). *L. monocytogenes* is a pathogen of food safety concern as it can induce disease in humans and can be transferred to food products derived from animals (Hassan *et al*., 2000; Pal, 2013).

Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and medical and social costs. Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors (Nafisa*et al*., 2010).The modern food industry aims to decrease the use of preservatives and to increase shelf lives, so food safety is widely based on the cold chain. Even though temperatures lower than optimum decrease growth rates in all bacteria, growth inhibition of *L. monocytogenes*is not complete until temperatures below 0°C (Markkula, 2013).

Food safety is one of the leading issues for the agricultural industry, for both livestock and producers of the property that are influencing consumer demand for meat products, bacteriological safety is the main factor which is considered as very important (Smith, 2000). Listeriosis, a disease of humans and animals, is one of the important emerging bacterial zoonotic diseases worldwide (Pal, 2007; Gebretsadik*et al*., 2011). It is associated with the highest case fatality rate of 30% approximately, unlike infection with other common foodborne pathogens (Khan *et al*., 2013).

Information on the occurrence and distribution of *L. monocytogenes* and other *Listeria* species is very limited both in the veterinary and public health sectors in Ethiopia (Gebretsadik*et al*., 2011). In developing countries most of the times, there have been few or no reports on *L. monocytogenes.* This might be true because no one has given it due attention or were unaware of its occurrence (Molla *et al.,* 2004).

Therefore, this study was contemplated with the following objectives:

- To estimate the prevalence of *Listeria monocytogenes*inmeat of sheep.
- To isolate and identify *Listeria monocytogenes*from sheep meat.
- To conduct the antibiotic susceptibility of the isolates of *Listeria monocytogenes*from sheep meat.
- To determine the risk factor and the public health implications of *Listeria monocytogenes* in butchers and meat handlers.

2. LITERATURE REVIEW

2.1. Meat production and consumption trend in Ethiopia

Meat supply varies enormously from region to region, and large differences are visible within regions. The USA leads by far with over 322 grams of meat per person per day (120 kg per year), with Australia and New Zealand close behind. Europeans consume slightly more than 200 grams of meat (76 kg per year); almost as much as do South Americans especially in Argentina, Brazil and Venezuela. Although Asia's meat consumption is only 25 per cent of the U.S. average (84 grams per day, 31 kg per year), there are large differences. The average meat consumption globally is 115 grams per day (42 kg per year) (UNEP, 2012).

The consumption of sufficient meat is a rare extremity in most developing countries. Many Ethiopians, like residents of other developing countries, do not consume an adequate amount of meat. It remained slightly below the meat intake of all low income countries consuming 9kg per capita annually. The few that do, however, maintain a meat diet of beef, sheep, goat, and poultry. In 1987, 51 percent beef, 19 percent sheep, 14 percent goat, and 15 percent poultry contributed to a meat diet composition. The meat consumption in developing countries, however, increases slightly at a 2.8 percent (Avery, 2004).

2.2. Microbial contamination of meat and meat products

Meat, an excellent source of protein in human diet is highly susceptible to microbial contaminations, which can cause its spoilage and food borne infections in human, resulting in

economic and health losses Although, muscles of healthy animals do not contain microorganisms, meat tissues get contamination during the various stages of slaughter and transportation. A great diversity of microbes inhabit fresh meat generally, but different types may become dominant depending on pH, composition, textures, storage temperature, and transportation means of raw meat. It is a rich source of the protein and fat, low in carbohydrate content and with sufficient water activity, supports the growth of both spoilage and pathogenic bacteria (Ahmad *et al*., 2013).

Contaminated raw meat is one of the main sources of foodborne illnesses. The risk of the transmission of zoonotic infections is also associated with contaminated meat. International food management agencies, especially the WHO, the FAO and the HACCP alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and GMPs (Nafisa*et al*., 2010). Among the food products of animal origin, milk and dairy products like soft cheese, meat and meat products like sausages derived from both animals and poultry and turkey along with sea foods act as important source of *L. monocytogenes* (Dhama*et al*., 2013). Sheep meat is an important food in the human food needed. The world production of about 50 million tons annualy, representing 10% of sheep meat, ranking fourth after beef and veal meat, poultry and pig meat (Christina *et al*., 2008).

The meat, available at retail outlets comes through a long chain of slaughtering, and transportation, where each step may pose a risk of microbial contamination. The sanitary conditions of abattoirs and its surrounding environment are major factors contributing in bacterial contamination of meat. Contaminations can be compounded during transportation, storage and handling of meat at butcher shops(Ahmad *et al*., 2013).

Meat quality is influenced by the slaughter of animals, especially the hygiene conditions in which killing is carried out. During slaughter, sheep carcasses can become contaminated with

microorganisms directly by blowing air during evisceration and storage. The most important sources of contamination of carcasses are the feces, gastric contents, wool or animal from the environment by contact with different surfaces or unhealthy environments (Christina *et al*., 2008).

2.3. Control strategies of meat contamination

The most common sources of *L. monocytogenes*include raw and processed meat, dairy products, vegetables and seafood products. Also, noteworthy are food production facilities and storage environments. Refrigerated foods that may be consumed without prior cooking are of special concern because of the ability of *L. monocytogenes*to grow at refrigeration temperatures (Riemann and Cliver, 2006).

Meat safety challenges involve traceability issues, pathogen and chemical residue detection problems, regulatory issues, addressing consumer concerns, etc. Moreover, meat safety must be regarded as an increasingly global matter due to the increase of meat consumption around the world, exposing higher numbers of consumers to potential hazards. There are reasons to believe there is an information asymmetry between consumers, producers and safety authorities along the supply chain. Producers, sellers and safety authorities have more and better information about the potential hazards and the dimension of risk associated with the consumption of a given food product. The asymmetry can be associated with the intentional or not unavailability of information for consumers, but also with differences between scientific evidence and consumers' perception (Viegas*et al*., 2012).

The incidence of listeriosis can be controlled by controlling our intake of foods contaminated with *L. monocytogenes.* Itinfects mostly immunocompromised and elderly and neonates, lowering the exposure of this vulnerable population should decrease the incidence of listeriosis.

Several basic principles can be adopted to reduce the exposure to *L. monocytogenes*(Riemann and Cliver, 2007).

2.4. *Listeria monocytogenes*

2.4.1. Definition and history of L. monocytogenes

In 1926, Murray and colleagues described symptoms in rabbits and guinea pigs caused by a Gram-positive bacillus organism. They named this organism *Bacterium monocytogenes*, in reference to the mononuclear leucocytosis observed in the affected animals. In 1927, Pirie was able to isolate the same organisms from gerbils and later suggested that the generic name *Listerella*named after the surgeon Lord Lister be changed to *Listeria*, reflecting proper nomenclature. Following its early discovery, the disease caused by this organism (listeriosis) was rare. Only after a Canadian outbreak in the early 1980's where human illnesses were noted, did the organism become a household name in the food industry. Listeriosis is a generic term for a variety of syndromes caused by *L. monocytogenes*, and is a problem to the food industry in part due to the ubiquitous nature of the organism (Riemann and Cliver, 2006).

Since 1981 listeriosis is known to be an important bacterial foodborne disease (Mataragas*et al*., 2010). In recent years, it has emerged as a significant cause of human infection in industrialized countries (Zunabovic*et al*., 2011). This is attributed to the emergence of a vulnerable immunocompromised population and the concomitant development of large-scale agro-industrial plants and refrigerated food (Chitlapilly-Dass, 2011).

Although *L.monocytogenes* has been recognized as an animal pathogen for many years, its significant role as a foodborne human pathogen became evident only in the 1980's, when documented reports of listeriosis outbreaks traced to contaminated food started to appear in the

literature. Nowadays,*L. monocytogenes* is considered to be one of the most important agents of food-borne disease (OIE, 2008).

2.4.2. Classification, morphology and biology

*Listeria monocytogenes*belongs to the family *Listeriaceae*in the class *Bacilli* of the phylum *Firmicutes,* together with *L. innocua, L. ivanovii, L. seeligeri, L.welchimeri, L. grayi, L. murraie*and four novel species *L.rocourtiae*, *L. marthii*, *L.fleischmannii*and *L. weihenstephanensis*(Markkula, 2013).

Listeria monocytogenes is a Gram positive, Catalase positive, Oxidase negative, non-sporeformer (Pal, 2007). The coccoid to rod shaped cells (0.4-0.5 mm - 0.5-2.0 mm) cultured at 20-25°C possess peritrichous flagella and exhibit a characteristic tumbling motility. They are facultative anaerobes that grow best under reduced oxygen and increased carbon dioxide concentration (Fantahun and Firesebehat, 2012). Colonies on tryptose agar viewed under oblique illumination have a characteristic blue-green sheen. *L. monocytogenes* is the only important human pathogen currently recognized within the genus *Listeria* (Martin and Maurice, 2008).

The bacterium can grow over a wide range of temperatures from 0°C - 45°C, with an optimum around 37°C. It can survive below freezing temperature (-7°C) as well as in acidic conditions and high salt concentrations, thus favoring its transmission through food which are chilled, highly processed and have a long shelf life (Ramaswamy*et al*., 2007). They are able to multiply at high salt concentrations (10% NaCl) and at a broad range of pH 4.5-9 and temperature 0-45°C optimum 30-37°C (Kumar, 2011).

The term listeriosis is referred to a group of diseases caused by *L. monocytogenes*in both human and animals (Chitlapilly-Dass, 2011). Listeriosis is one of the important emerging bacterial zoonotic infections worldwide. Among the different species of the genus *Listeria, L.monocytogens*is known to cause listeriosis in humans and animals (Molla*et al*., 2004; Pal, 2007). It is one of the important emerging bacterial zoonotic diseases that occur in humans.And nowadays, it has become recognized as an important opportunistic human food borne pathogen (Liu *et al*., 2005).

Listeria species are ubiquitous in the environment and possess unique physiological characteristics that allow growth at refrigeration temperature that are usually adverse for most pathogenic, foodborne bacteria. The organism can also tolerate a pH between 5.4 and 9.6. Numerous reports implicated food types such as milk and milk products, meat and meat products, raw vegetables and sea foods as sources of food borne listeriosis. Of particular concern are ready-to-eat foods that are refrigerated before consumption and those that do not undergo any substantial heat treatment (Molla*et al*., 2004).

There are thirteen recognized serotypes of *L. monocytogenes*, and about 95% of human infections were caused by serotypes 1/2 a, 1/2b, 1/2 c, and 4b (Heymann, 2008). The illness is caused when the pathogenic strain of *L. monocytogenes*induces the virulence genes after lodging themselves in human cells (Chitlapilly-Dass, 2011).

It is an opportunistic pathogen in humans and various animal species. They are robust bacteria with wide distribution. Despite their poor survival in nutrient-deficient, unpolluted seawater and spring water, they are readily replicate in nutrient rich, contaminated waters, sewage, sludge, soil, foods and animal hosts (Kumar, 2011).

Healthy cows can serve as reservoirs for *L. monocytogenes* and secrete the organism in milk. Almost all cases (about 98%) of human listeriosis and 85 % of animal cases are due to *L. monocytogenes*although; rarely *L. seeligeri* and *L. ivanovii* have also been implicated. The other pathogenic species of genus *Listeria* has been thought to be frequently associated with abortions in sheep and cattle.*L. ivanovii*infection is rare and has been reported from a patient with AIDS (Kumar, 2011).

2.4.3. Characteristics of the organism

i. Growth characteristics

The growth of *L. monocytogenes*in food is dependent on the intrinsic characteristics of the substrate such as pH and water activity the extrinsic characteristics including storage temperature and relative humidity and processing techniques used in the production of food such as cooking and non-thermal processing (Chitlapilly-Dass, 2011).

The primary factors that influence the growth of *L. monocytogenes*in food are temperature, pH and water activity (Zhao *et al.,* 2004). Additionally, it has also been demonstrated that previously stressed cells like exposure to sub-lethal heating before process heating can be more resistant to additional stresses. Although, *L. monocytogenes*has an optimum growth temperature of between 30-37°C at neutral or slightly alkaline pH $\geq$ 7, it can also grow at refrigerated temperatures <5°C (Chitlapilly-Dass, 2011).

ii. Survival characteristics

Survival at low temperatures

*Listeria monocytogenes*has the ability to grow over a wide range of temperatures 2-45 ^{0}C. Survival at these temperatures takes place with changes in *L. monocytogenes*membrane composition. *L. monocytogenes*produces cold shock proteins (Csps) in response to cold acclimation which balances the growth at low temperature (Chitlapilly-Dass, 2011). They accumulate compatible solutes such as glycine, betaine and carnitine during refrigeration temperature. These solutes stimulate growth of cells subjected to cold stress. Under adverse environmental condition, transcription of genes is made possible by the association of alternative sigma factories with the core RNA polymerase (Becker *et al.,* 2000).

Survival under acid stress

*Listeria monocytogenes*encounters a low pH environment in acidic foods, during gastric passage and in the phagosome of the macrophage. The pathogen responds to and survives in these low-pH environments by utilizing a number of stress adaptation mechanisms. Exposure of *L. monocytogenes*to mild acidic pH of 5.5 induces the ATR, where in the cells are resistant to severe acidic conditions. *L. monoytogenes*develops acid tolerance upon exposure to sub lethal acid conditions, a response that has been designated the ATR. The effectiveness of this response appears to be critically dependent upon two principal factors, the pH of the adaptive exposure and the duration of the adaptive period (Chitlapilly-Dass, 2011).

The bacterium further utilizes the GAD system to survive acid stress. The GAD acid resistance mechanism has been found to play a major role in the acid resistance of a relatively small number of bacteria. When the cell is exposed to low pH, the GAD system converts a molecule of extracellular glutamate to extracellular γ-amino butyrate, while consuming an intracellular proton. The net effect is to reduce the proton concentration within the cell, thus alleviating acidification of the cytoplasm. In addition, γ-aminobutyrate is less acidic than glutamate, which

contributes to an alkanization of the environment. A glutamate decarboxylase system protects *L. monocytogenes*in gastric fluid (Chitlapilly-Dass, 2011).

Survival under osmotic stress

The use of salt to lower the water activity is one of the methods of food preservation used by the food industry. However, the ability of *Listeria* to adapt and survive in high concentrations of salt makes it difficult to control the pathogen in foods (Hill *et al.,* 2002). One of the mechanisms used by *Listeria* to tolerate salt stress is a change in its gene expression leading to an increased or decreased synthesis of various proteins (Duche*et al.,* 2002). Two general stress proteins Dna K and Ctc were identified among the SSP, induced in *L. monocytogenes*(Chitlapilly-Dass, 2011).

Resistance to antimicrobial agents

Antimicrobial resistance in the foodborne pathogen *Listeria* is emerging in recent years. Bacteria can acquire resistance by getting a copy of a gene encoding an altered protein or an enzyme like beta lactamase from other bacteria, even from those of a different species. Studies have shown that several species of *Listeria* isolated from humans or from food production or processing facilities are resistant to one or more antibiotics (Ayaz*et al.,* 2010).

There are a number of ways to get a resistance gene: During transformation - in this process, akin to bacterial sex, microbes can join together and transfer DNA to each other, on a small, circular, extra chromosomal piece of DNA, called a plasmid - one plasmid can encode resistance to many different antibiotic, through a transposon - transposons are "jumping genes," small pieces of DNA that can hop from DNA molecule to DNA molecule. Once in a chromosome or plasmid, they can be integrated stably and by scavenging DNA remnants from degraded dead bacteria (Chitlapilly-Dass, 2011).

2.5. Epidemiology

Listeriosis, has emerged as a major foodborne disease during the past 2 decades, since it has a high case fatality rate (Tirumalai, 2013).Listeriosis is an emerging infection with major public health concern worldwide because of occurrence of associated foodborne outbreaks and significant risk of mortality and morbidity (CHP, 2013).

*Listeria monocytogenes*is the causative agent of the disease listeriosis (Pal, 2007). There is evidence that suggest *L. monocytogenes*is a transitory resident of the intestinal tract in humans with 2 to 10 % of the general population being carriers of the organism without any apparent adverse consequences. It is estimated by the CDC that up to 2, 5000 cases of listeriosis occur each year with 500 of these cases resulting in death. FDA and the USDA suggest that as much as 5% of many RTE foods are contaminated with *L. monocytogenes*(Beverly, 2004).

Listeria monocytogenes has been recognized as an animal pathogen for many years. Its significant role as a foodborne human pathogen became evident only in the 1980's, when documented reports of listeriosis outbreaks traced to contaminated food started to appear in the literature. Nowadays,*L. monocytogenes* is considered to be one of the most important agents of foodborne disease (OIE, 2008).

Clinical characteristics of human listeriosis complicating the detection and tracking of outbreaks include a long incubation period (1 to 90 days) in comparison to that of many other foodborne diseases. *L. monocytogenes* has also been shown to persist in food plants and thus can lead to prolonged contamination of food products which may be distributed over a wide geographic range. As a consequence, this organism may cause widespread multistate and possibly multicountry outbreaks with relatively few related cases in each geographic area. Rapid and standardized subtyping methods for *L. monocytogenes* are thus particularly important for effective detection of human listeriosis outbreaks (Cesare*et al*., 2001).

Although the organism is wide spread in nature, clinical diseases in animals occur mainly in the northern and southern latitudes and are much less common in tropical and subtropical than in temperate climates. In the northern hemispheres, listeriosis has a distinct seasonal occurrence, probably associated with seasonal feeding of silage, with the highest prevalence in the months of December through May (Fentahun and Fressebihat, 2012).

Listeriosis is a relatively rare disease. The reported yearly incidence of human listeriosis ranges 3 cases per million people in Australia. The data from the U.S. CDC active food surveillance programme, Food Net, for the years from 1996 to 1998 indicate that there were about 5 reported cases of listeriosis per 1, 000,000 populations annually. Using the CDC 1996-97 surveillance data and extrapolating to the 1997 total United States of America population, estimated that there were 2, 493 cases, including 499 deaths, due to food borne listeriosis (WHO/FAO, 2004).

The overall EU notification rate was 0.32 cases per 100,000 populations with the highest country-specific notification rates observed in Denmark, Finland and Spain; 0.88, 0.80 and 0.79 cases per 100,000 populations, respectively. The lowest notification rates were reported in Romania, Bulgaria and Greece; 0.04, 0.05 and 0.08 cases per 100,000 populations, respectively (EFSA, 2013).The incidence of listeriosis in France during 1999-2000 is ≈3.5 cases/million persons during 2001-2003.The incidence in Sweden was high in 2000-2001 (5.9-7.5 cases/million persons. In Switzerland, an increase has been observed since 2004. In 2005, an outbreak involving 12 cases (16% of cases reported in 2005) was linked to consumption of a cheese (Goulet*et al*., 2008).

In United State, the 121 outbreaks involving dairy products for which pasteurization status was known resulted in 4,413 reported illnesses. Among these illnesses (3%) is caused by *Listeria* species (Langer *et al*., 2012). In Australia during 1997-2007, *L. monocytogenes* infections were reported in 150 human cases. In Spain, the average incidence of human listeriosis was 0.65 per 100,000 populations during 1995-2005. In Germany, the incidence of listeriosis accounted to 0.4% per 100,000population in 2008 (Meeyam, 2010).

Although listeriosis is a relatively rare food borne illness, its severe nature makes it likely that individuals will seek medical care. In the United States of America, where listeriosis is a "reportable" disease, CDC estimates that it recognizes and identifies approximately half of all listeriosis cases, as compared to the 3% identification rate for most other food borne pathogens. Invasive *L. monocytogenes*infections can be life threatening, with fatality rates of 20 to 30% being common among hospitalized patients. In 2000, the CDC reported that, of all the fooborne pathogens tracked by CDC, *L. monocytogenes*had the second highest case fatality rate (21%) and the highest hospitalization rate (90.5%). The reported yearly incidence of human listeriosis in Europe ranges from 0.1 to 11.3 cases per 106 persons (WHO/FAO, 2004).

Large foodborne outbreaks of listeriosis have occurred during the last decade in Europe and the USA. Between 1991-2002, 19 outbreaks of invasive listeriosis infection were reported in nine European countries, with a total of 526 related cases. In 1997, one large outbreak resulting in 1,566 cases of *Listeria* gastroenteritis was reported in Italy and traced to the consumption of contaminated corn salad. A recent nationwide outbreak linked to contaminated packaged meat products occurred in Canada in 2008 resulting in 56 patients, including 20 deaths (CHP, 2013).

In Europe, listeriosis surveillance data is available at the national level in 16 countries. Recent reports based on these national surveillance systems indicate an increase in the incidence of listeriosis in many European countries including England and Wales, Denmark, Belgium, Germany, the Netherlands, Switzerland and Finland during 2000 to 2006. In England and Wales, the incidence of listeriosis has nearly doubled from an annual average of 110 cases in 1990's to 230 per year in 2007. Increased risk of *Listeria* infection is predominantly seen in patients aged 60 years or above. Similar trends are also found in other European countries where the incidence of listeriosis is higher among older persons. In the Asia-Pacific region, Australia's National Notifiable Diseases Surveillance System reported an annual number of listeriosis cases that ranged from 35 to 73 during the period 1991 to 2007. In Singapore, the annual number of administratively required (non-statutory) notifications to the Ministry of Health ranged from 1-9 cases from 2001 to 2007. Recent developments in food processing and food distribution have

increased the risks of listeriosis outbreaks. Epidemiological investigations have revealed that some outbreaks were associated with consumption of contaminated dairy products such as cheese and butter, processed meat and fish products and contaminated salad and ice cream cake (CHP, 2013).

2.5.1. Host Factor

Listeriosis is primarily a disease of ruminants, particularly sheep and the major diseases associated with *L. monocytogenes*are encephalitis and abortion. It also produces syndromes of septicemia, spinalmyelitis, uveitis, gastroenteritis and mastitis(Pal, 2007; Fentahun and Fressebihat, 2012).

The development of listeriosis seems to be dose-dependent. Prolonged consumptionof food contaminated with as few as 14-2.2 x 10^3 colony forming units of *L. monocytogenes*per day may be sufficient to infect susceptible people(Markkula, 2013).

2.6. Susceptibility

The elderly, pregnant women, neonates, and immunocompromised individuals are at greatest risk of severe listeriosis (Pal, 2007). There are studies showing that the risk of infection increases with age, with higher incidence among persons of older ages, especially those above the age of 65. As for pregnancy, it was reported in the United States that pregnant women are about thirteen times more likely than other healthy adults to become infected by *L. monocytogenes*, and approximately one in six (17%) cases occurred in pregnancy. There has been a strong association between decreased T-cell mediated immunity and invasive listeriosis (Goulet*et al.*, 2012).

Listeriosis may occur at any time but usually in the third trimester when cell-mediated immunity is at its lowest. The disease is also often superimposed on other debilitating illnesses or conditions such as malignancy, organ transplantation, diabetes, liver disease, renal disease, heart disease, HIV infection, and use of immunosuppressant (Goulet*et al*., 2012). Pregnant women naturally have a depressed cell-mediated immune system. In addition, the immune systems of fetuses and newborns are very immature and are extremely susceptible to these types of infections. Other adults, especially transplant recipients and lymphoma patients, are given necessary therapies with the specific intent of depressing T-cells, and these individuals become especially susceptible to *Listeria* as well (Bennett, 2000).

According to the CDC, individuals at increased risk for being infected and becoming seriously ill with *Listeria* include pregnant women: They are about 20 times more likely than other healthy adults to get listeriosis. About one-third of listeriosiscases happen during pregnancy, newborns rather than the pregnant women themselves suffer the serious effects of infection in pregnancy, Persons with weakened immune systems, Persons with cancer, diabetes, or kidney disease, Persons with AIDS they are almost 300 times more likely to get listeriosis than people with normal immune systems and persons who take glucocorticosteroid medications such as cortisone and the elderly (CDC, 2011).

2.7. Source of infection and reservoir host

*Listeria monocytogenes*is universally found in the environment, particularly in soil, vegetation, animal feed, and in human and animal faeces (CHP, 2013).The ability to form biofilm may assist in its survival in the environment and in perpetuating its presence in water troughs on infected farms (Pal *et al*., 2013). The risk for contamination of silage with *Listeria* is higher when it contains soil. Infected animals can also serve as a source of infection from their urine, feces, aborted fetuses, uterine discharges and the milk(Fentahun and Fressebihat, 2012).

The bacterium can contaminate a variety of foods such as meat, milk, fish and vegetables, and is frequently isolated from the environment of food processing plants (Barros *et al*., 2007). There have been epidemiological investigations showing that a large range of RTE food such as deli meats, soft cheese, sandwich, sprouts, and hot dogs have been implicated in outbreaks of listeriosis worldwide (CHP, 2013).

Changes in food production and demands of a growing society have increased the number of incidences of food borne illness. *L. monocytogenes*has also developed as a foodborne pathogen due to several other factors. One factor is the development of technology, which provides cold storage. This new storage condition provides adequate temperatures for the growth and survival of *L. monocytogenes*. A second factor is the achievements and hindrances of the medical community with the increase in the population of elderly, immunocompromised, AIDS, transplant and cancer patients. However, one of the main factors for the increase of the emergence of *L. monocytogenes*is the production of minimally processed foods, mainly ready-to-eat products, which may or may not be heated to temperatures to properly destroy the pathogen(Beverly, 2004).

2.8. Mode of transmission

Soil contamination and ingestion of contaminated feed are the primary modes of transmission of *Listeria* (Fentahun and Fressebihat, 2012). Except for the transmission of mother to fetus, human-to-human transmission of *Listeria* is not known to occur.Infection is caused almost exclusively by the ingestion of the bacteria, most often through the consumption of contaminated food (Pal, 2007). The most widely accepted estimate of food borne transmission is 85-95% of all *Listeria* cases. The mode of transmission of *Listeria* to the fetus is either transplacental via the maternal blood stream or ascending from a colonized genital tract. Infections during pregnancy can cause premature delivery, miscarriage, stillbirth, or serious health problems for the newborn (Bennett, 2000; Pal, 2007).

Listeriosis is primarily a foodborne disease. Adults can get listeriosis by eating food contaminated with *Listeria*. The disease can also be acquired from an infected mother transplacentally, or during passage through the birth canal (Pal, 2007). Some cases of nosocomial transmission in hospital have also been reported (CHP, 2013).

2.9. Disease in humans and animals

2.9.1.Disease in humans

Listeria monocytogenes is a pathogen that causes a severe human foodborne disease (Cesare, *et al*., 2001). During the early stages of infection, human listeriosis often displays non-specific flu-like symptoms like chills, fatigue, headache and muscular and joint pain and gastroenteritis. However, without appropriate antibiotic treatment, it can develop into septicaemia, meningitis, encephalitis, abortion and in some cases death (Pal, 2007). The incubation period between exposure and onset of listeriosis varies between 1 day and 3 months. The majority of cases in adults and juveniles occur amongst the immunosuppressed i.e., patients receiving steroid therapy. Other at risk groups includes patients with AIDS, diabetics, elderly people and individuals with alcoholic liver disease. Listeriosis in pregnancy manifests as severe systemic infection in the unborn or newly delivered infants. Infection can occur at any stage of pregnancy (Kumar, 2011).

As such, this human pathogen has the potential to cause disease in all aspects of the farm-to-fork continuum. In farm animals such as sheep, abortion, encephalitis and/or septicemia can occur when listeriosis strikes (Riemann and Cliver, 2006; Pal, 2007). It arises mainly from the consumption of contaminated food products (Acha and Szyfres, 2001).However, the majority of human listeriosis infections arise via the consumption of contaminated food (Riemann and Cliver, 2006).

The disease manifestations include septicaemia, meningitis or meningoencephalitis and encephalitis, usually preceded by influenza-like symptoms including fever. In pregnant women, intrauterine or cervical infections may result in spontaneous abortion or stillbirths (Pal, 2007). It has also been associated with gastroenteric manifestations with fever. Although the morbidity of listeriosis is relatively low, the mortality of the systemic/encephalitic disease can be very high with values in the vicinity of 30%. A number of molecular and cellular determinants of virulence have been identified for this intracellular pathogen and although there is evidence of polymorphism among different strains of *L. monocytogenes* for some of these virulence determinants, this heterogeneity cannot be correlated with the ability or inability of the organism to produce disease. Therefore, all *L. monocytogenes* strains are considered to be potentially pathogenic (OIE, 2008).

Possible explanations for the emergence of human foodborne listeriosis as a major public health concern include major changes in food production, processing and distribution, increased use of refrigeration as a primary preservation means for foods, changes in the eating habits of people particularly towards convenience and RTE foods and an increase in the number of people considered to be at high risk for the disease (OIE, 2008).

2.9.2. Disease in animals

A wide variety of animal species can be infected with *L. monocytogenes* (Pal, 2007). But clinical listeriosis is mainly a ruminant disease, with occasional sporadic cases in other species (OIE, 2008). Listeriosis has been detected in nearly all domestic animals (Pal, 2007). Most listeriosis cases have been reported in sheep (Markkula, 2013). The disease in animals is broad ranging from asymptomatic infection and carriage to uncommon cutaneous lesion such as conjunctivitis, urethritis, endocarditis and severe disturbance of gait followed by death. Mastitis, abortion, repeat breeding, infertility, encephalitis and septicaemia are common in the cattle (Pal, 2007). *Listeria ivanovii* has also been implicated as a cause of abortion in cattle and sheep but occurs

less frequently than *L. monocytogenes*. *Listeria* infection and abortions usually develop in the winter or early spring. Abortions are most commonly recognized in the last trimester of pregnancy in the absence of other clinical sign (Kumar, 2011).

Among these, *L. monocytogenes*is the most pathogenic species to small ruminants. The other two species, the so called *L. ivanovii*and *L. innocua*are less frequently implicated in disease of animals. Listeriosis is mostprevalent during spring and winter seasons. Soil contamination and ingestion of contaminated silage are theprimary modes of transmission. Poor quality silage, a pH greater than 5.5 is commonly implicated and accounts for listeriosis often being referred to as "silage disease"(Pal, 2007).The clinical manifestations of listeriosis in small ruminants are usually severe and include abortion, meningoencephalitis and neonatal septicemia.Especially in sheep,listeriosis leads to head tilt and permanent circling movement (Fentahun and Fressebihat, 2012).

The main clinical manifestations of animal listeriosis are encephalitis, septicaemia and abortion (Pal, 2007), and the disease is often associated with stored forages, usually silage. Postmortem findings and histopathology depend on the clinical presentation. The evidence indicates that animal listeriosis is predominantly associated with stored forage and with the environment as the main source of contamination. Silage is the most frequent source. The intestinal mucosa is the main route of entry, after oral ingestion, in the case of septicaemic or abortive listeriosis. The incubation period can be as short as 1 day. The incubation period for the encephalitic form is usually 2-3 weeks, and the course of the disease is usually short in sheep and goats; 1 - 4 days, although it can be more protracted in cattle (OIE, 2008).

2.10. Pathogenesis and clinical features

The pathogenesis of *L. monocytogenes*is one of the better understood amongst human food borne pathogens. Transmission of *L. monocytogenes* by food first requires penetration of the organism through the intestine. There are many virulence factors involved in the intracellular journey that *L. monocytogenes*must endure in order to survive the host's immune system. The initial step for the development of listeriosis typically involves the ingestion of the organism, followed by its survival against the non-specific immune system defenses of the gastrointestinal tract. There, it can multiply in the intestines and be eliminated in the feces. After food contaminated with *L. monocytogenes*has been ingested, the organism is believed to invade the intestinal epithelium and/or Peyer's patches. From here, the bacteria can enter the draining lymph nodes and disseminate via the bloodstream to the liver and spleen. In most cases infections by *L. moncytogenes*are limited, with clinical symptoms appearing in the immunocompromised, the elderly, pregnant women, and neonates. However, it is equally important to note that listeriosis has one of the highest case-fatality ratios of all food borne bacterial infections. Once inside the host cell, *L. monocytogenes*is able to circumvent the immune response. Once the bacterium has escaped the vacuole it enters the host cell cytoplasm, where growth and multiplication can occur (Riemann and Cliver, 2006).

Most *Listeria* species are destroyed by gastric acids. Use of antacids and H-blockers increases survival rate and are considered as risk factors for developing listeriosis. An alternative route of entry has been proposed for CNS infection through damaged oral, nasal or ocular mucosal surfaces via the neural sheath of peripheral nerve endings, particularly the trigeminal nerve. It is postulated that centripetal migration along cranial nerves leads to infection of the CNS. In the brain stem, there is unilateral bacterial localization with focal micro-abscess formation. *L. monocytogenes*has the ability to invade both phagocytic and non phagocytic cells, to survive and replicate intracellulary and transfer from cell to cell without exposure to humoral defense mechanisms. Virulent strains also possess a cytolytictoxin, listeriolysin, which destroys the membranes of phagocytic vacuoles allowing *Listeria* to escape into the cytoplasm. In the

cytoplasm, the organism utilizes cellular microfilaments to generate tail-like structures which confer motility (Fentahun and Fressebihat, 2012).

The clinical outcomes of listeriosis depends on the number of organisms ingested, pathogenic properties of the strains and the immune status of the host. There are three major clinical forms of listeriosis. These are septicemia, encephalitic and abortion forms (Fentahun and Fressebihat, 2012). Most and perhaps all forms of listeriosis in humans resulted from food borne transmission. There are two main forms of illness associated with *L. monocytogenes*infection. Non-invasive listeriosis is the mild form of disease, while invasive listeriosis is the severe form of disease and can be fatal (FDA 2012). In its most severe form, listeriosis is an invasive disease that affects immunocompromised patients and has the highest case-fatality rate of food borne illnesses. In immunocompetent persons, it can also cause severe disease attributed by some investigators to ingestion of high infective doses, as well as outbreaks of benign febrile gastroenteritis (Siegman-Igra*et al*., 2002). The affected individuals show flu like symptoms and may develop septicemia and meningitis. In pregnant women, uterine infection can occur resulting in abortion and fetal infection (Dhama*et al*., 2013).

Septicemic Form:

This form is known in the formation of septicemia. The septicemic form is marked by depression, in appetence, fever and death. The septicemic disease in sheep and goats usually occurs within 2 days of introduction to silage and abortions 6 - 13 days later. The syndrome being a general one comprising weakness, emaciation, diarrhea in some cases with hepatic necrosis and gastroenteritis at necropsy (Fentahun and Fressebihat, 2012).

Encephalitic Form:

The encephalitic form, sometimes called “circling disease”, is the most common form in ruminants. The course of the disease is more acute and frequently fatal in sheep and goats, but subacute to chronic in cattle (Fentahun and Fressebihat, 2012).

Abortion:

Abortion is common in ruminants usually late termafter 12 weeks in sheep. The fetus may be macerated or delivered weak and moribund. Retained placenta and metritis may be resulted. Outbreaks of abortion occur more commonly in sheep and goats, and there will be a blood stained vaginal discharge for several days (Fentahun and Fressebihat, 2012).

2.11. Disease status in Ethiopia

Listeriosis, a bacterial disease in humans and animals, is mostly caused by ingestion of *L. monocytogenes* via contaminated food and/or water, or by a zoonotic infection (Pal, 2007; Derra*et al*., 2013). In Ethiopia, the study has shown the presence and distribution of *L. monocytogenes*and other *Listeria* species in a variety of raw and ready-to-eat food products in Addis Ababa with a prevalence of 5.1% (Molla*et al*., 2004). Derra*et al*., (2013) described 4.1% of prevalence from raw meat and dairy products like raw milk, cottage cheese and cream cake collected from the capital and five neighboring towns in Ethiopia.

2.12. Diagnosis

Listeria species are closely related bacteria that share many morphological and biochemical characteristics. Apart from being catalase positive, and indole and oxidase negative, *Listeria* species can hydrolyseaesculin, but not urea. These common biochemical features have frequently been exploited for the differentiation of *Listeria* species from other bacteria. The application of molecular techniques has facilitated the identification and characterization of major virulence-associated genes and proteins in *L. monocytogenes*. The development of serological and nucleic-acid-based detection procedures has enhanced the laboratory detection and differentiation of *Listeria* species (Liu, 2006).

This bacterium has been found in numerous raw and processed foods. The presence of low numbers of *L. monocytogenes*in fresh produce or in products that are cooked before consumption is considered as safe (Markkula, 2013). Listeriosis can be tentatively diagnosed on the basis of clinical symptoms and demonstration of the organism in smear by Gram's staining. The organism can be isolated from clinical specimens such as blood, CSF and meconium of newborns or foetus in abortion cases, and faeces, vomitus, food stuffs/animal feed and vaginal secretions of infected individuals or animals (Pal, 2007). In view of high cost, more time and skills required by isolation procedures along with their impracticability to screen large numbers of samples, the serological, novel and pathogenicity marker based methods play an important role in its rapid and reliable diagnosis. Listeriosis should be differentiated from influenza, tuberculous meningitis in humans and rabies, brucellosis, pasteurellosis, toxoplasmosis in animals especially in abortion cases (Barbuddhe and Malik, 2009).

The patterns of hemolysis on sheep blood agar, CAMP test and acid production from a short range of sugars are useful differentiating laboratory method for *Listeria* species. *L. monocytogene*is CAMP positive when cross streaked with a beta-toxin producingSA on blood agar. In semisolid motility media incubated at room temperature (25°C), a characteristic umbrella pattern of motility develops 3 to 4 mm below the surface, due to the microaerophilic nature of *Listeria*. On sheep blood agar, most strains of *L. monocytogenes*produce a narrow zone of hemolysis (Fentahun and Fressebihat, 2012).

2.13. Treatment

Because foodborne listeriosis outbreaks are often associated with high case-fatality rates, it is imperative that effective antibiotics are used for treatment. As such, current treatments for listeriosis involve a combination of ampicillin and gentamicin. Two to three weeks of therapy appears to be sufficient to prevent relapses. Most β-lactam antibiotics can be used (Riemann and Cliver, 2007).

And Patients sensitive to penicillin are given trimethoprim (alone or in a combination with sulfamethoxazole), tetracycline, erythromycin or chloramphenicol(Navratilova *et al*., 2004). The reference treatment is currently based on a synergistic association of high doses of aminopenicillin (ampicillin or amoxicillin) and gentamicin. Although rifampin, vancomycin, linezolid, and carbapenems have been proposed as possible alternatives trimethoprim is generally used in case of intolerance of beta-lactams (Morvan*et al*., 2010).

Antibiotic therapy is the treatment of choice in most of the complications and the dose and duration of the treatment differ accordingly. For instance, bacteremia should be treated for 2 weeks, if the patient is immuno-competent. However, longer courses may be required in the immuno-compromised patient. Similarly, meningitis should be treated for 3 weeks; while endocarditis for 4-6 weeks; and brain abscess for a minimum of 6 weeks. Other effective agents like co-trimaxazole can be used for empirical antimicrobial treatment(Firehiwot, 2007).

2.14. Prevention and control

Listeria is present in many RTE or minimally processed foods, and therefore, prevention of illness from foodborne sources is difficult. The introduction of HACCP programs in the food industry has been identified as an important factor in diminishing the environmental contamination by *L. monocytogenes*in 'ready-to-eat' and minimally processed foods not subject to further processing. For immunocompromised patients, a number of recommendations have been made to decrease the risk of foodborne listeriosis (Reimann and Cliver, 2007).

Several studies have shown that the main strains contaminating the final products originate from the processing environments and are different from the strains present in raw material. To efficiently control *L. monocytogenes*and decrease the number of listeriosis cases, the contamination routes in the food chain need to be identified (Markkula, 2013).

Usually foodborne microbes are killed by cooking or chilling. Strict adherence to the food hygiene practices during production and storage and also during transportation and preparation of food is a mandate and appropriate trade restrictions should be implemented in the event of an outbreak of foodborne pathogen. Avoiding cross contaminations of cooked with other raw food materials, avoid of consumption of raw or uncooked products especially milk, adopting good husbandry practices including of proper biosecurity measures, cleaning, sanitation, hygiene, disinfection, quarantine, therapeutic regimens and appropriate vaccination programme are effective measures to mitigate listeriosis (Dhama*et al*., 2013).

There are strategies of effective disease surveillance programme and prompt investigation of reported listeriosis, health promotional initiatives to raise the awareness of the disease and the preventive measures, food safety training targeting to the food trade and food handlers, and enforcement of regulations designed to minimize *L. monocytogenes*in foods. Prevention and control measure are to be adopted to reduce infection burden in animals and in the processing steps of the food product obtained from the food animals till consumed (CHP, 2013).

As more information became available linking listeriosis with food consumption, food control agencies and private industry developed programmes to reduce the incidence of foodborne listeriosis. Industry initiated HACCP programmes and increased sanitation efforts to eliminate contamination. Food control agencies should expand programmes to prevent contaminated foods from entering commerce. The identification of CCPs has caused processors to find ways to eliminate the need for additional CCPs (Beverly,2004). There should be also consumer education campaigns that focus on food safety (WHO/FAO, 2004). Implementation of HACCP and rapid microbiological analysis will aid in controlling pathogens in food manufacturing facility (Jadhav and Pal, 2001; Bhunia, 2008).Proper motivation and training of employees and managers is vital to keep consumers safe. Thus, there is a crucial need to fill gaps in the knowledge base for designing effective training for newly hired and hard-to-reach employees in a retail food service environment (Crandall, 2011).

Surveillance of listeriosis and prompt investigation allow early identification of potential source of the infection and early intervention to eliminate the risk to the public (CHP, 2013). Using of novel techniques to control *L. monocytogenes*growth such as the incorporation of laboratory merit and further research is needed with the aim of protecting public health (Chitlapilly-Dass, 2011). Comprehensive education program for the consumer and food handler, both in commercial establishment, about the origin and personal and environmental hygiene is of paramount importance (Pal, 2007).

2.15. Public health importance

2.15.1. Economic and health impact

Listeriamonocytogenes is an opportunistic intracellular pathogen that has become an important cause of human foodborne infections worldwide. *Listeria* species are present in a variety of environments including the soil, water, effluents and foods. With manufactured RTE foods being consumed in increasing quantities, it is no surprise that *L. monocytogenes* has become recognized as an important opportunistic human foodborne pathogen. Although *L. monocytogenes* is infective to all human population groups, it has a propensity to cause especially severe problems in pregnant women, neonates, the elderly and immunosuppressed individuals (Liu, 2006).

During the early stages of infection, human listeriosis often displays non-specific flu-like symptoms like chills, fatigue, headache, and muscular and joint pain and gastroenteritis. However, without appropriate antibiotic treatment, it can develop into septicaemia, meningitis, encephalitis, abortion and, in some cases, death (Pal, 2007). Indeed, with mortality rates on average approaching 30 %, *L. monocytogenes* far exceeds other common food borne pathogens, such as *Salmonella enteritidis* with a mortality of 0.38 %, *Campylobacter* species 0.02-0.1% and *Vibrio* species 0.005-0.01 % in terms of disease severity (Liu, 2006).

It is estimated that microbial pathogens in foods are the cause for 6.5-33 million cases of human illness and up to 9,000 deaths in the United States each year. Over 40 different foodborne microbial pathogens, including fungi, viruses, parasites, and bacteria, are believed to cause human illnesses. For six bacterial pathogens, *Campylobacter* species, *Salmonella* species, *Yersinia enterocolitica*, *Escherichia coli* O157: H7, *Escherichia coli* O157 non-O157 STEC, and *L.monocytogenes*, the costs of human illnesses are estimated to be $9.3-$12.9 billion annually. To estimate medical costs and productivity losses, ERS uses four severity categories for acute illnesses: those who did not visit a physician, visited a physician, were hospitalized, or died prematurely. The lifetime consequences of chronic disease are included in the cost estimates for *E. coli* O157: H7 and fetal listeriosis. ERS estimates that each year in the United States, the costs of the acute illness from food borne *Listeria* is $2.3 billion (Beverly, 2004).

3. MATERIALS AND METHODS

3.1. Study area

The study was carried out in Addis Ababa Central Ethiopia. Addis Ababa is the capital city of Federal Democratic Republic of Ethiopia, and it has an area of 51,000 hectare in the central highlands with an average altitude of 2000-2560 meters above sea level. The area is characterized by bimodal rainfall with an average of 1100 mm, the highest percentage of rain falls during the long rainy season from June to September. The short rainy season is from February to April. Addis Ababa has an estimated human population of 3.15 million (CSA, 2007).

Figure 1: Map of Addis Ababa

3.2. Study abattoir and origin of samples

Addis Ababa Abattoir Enterprise was established before 65 years ago, and is located at the heart of Addis Ababa. The abattoir has different components such as slaughter hall, chilling room, detention meat room, condemned meat room, hide and skin room, veterinary office, water supply (cold and warm), electric generator, vehicles and incinerator. The abattoir has six separate slaughter halls for bovine (three), ovine and caprine (two), and swine (one).

The abattoir is a high output abattoir in the country providing 50% of the daily beef requirements of the city's residents. Most of the cattle slaughtered at the abattoir are adult males of local zebu, through lesser numbers of crossbred males, calves as well as culled dairy cows. Species of animals slaughtered include bovine, ovine, caprine and swine (CSA, 2005).

In the abattoir, regular meat inspection is being conducted by meat inspector as well as veterinarians from Ministry of Agriculture. The abattoir has both clean and dirty areas, so that after skinning and evisceration, carcass follows the clean lines until inspection and transporting while those offal, skins etc, to dirty areas as by product preparation, like pet animal feeds, hide and skin for sale as well as, those unfit ones and condemned organs to incinerators for burn (CSA, 2005).

The swabsample was collected from sheep meat from the Addis Ababa Abattoir Enterprise and butcher shops located in the city. Furthermore, swab samples were collected from equipments like knives, cutting tables and hooks.

3.3. Study population and Sample size determination

The study population represented sheep meat and equipments likeknives, cutting tables and hooks.

The approximate sample size required was determined, according to Thrusfield (2005), from expected prevalence of 50% with defined precision of 5% and level of confidence of 95%.

$$n = \frac{1.96^2 \, Pexp \, (1-Pexp)}{d^2}$$

Where, n = required sample size

Pexp = expected prevalence

d = desired absolute precision

Therefore, by using estimated prevalence of 50% in raw meat of sheep and taking a confidence interval of 95% and 5% absolute precision, the minimum sample size required for this study was 768 sheep meat swab samples. A total of 873 samples comprising of 384 sheep meat swab samples from the butcher shops, 384 sheep meat swab samples from the Addis Ababa Abattoir Enterprise were used for the study. In addition, 105 swab samples fromequipments like knives, cutting tables and hooks(Table 1).

Table 1: Distribution of the type and number of samples collected.

Type of sample	Number of samples
Sheep meat swab samples	768
Knives	40
Cutting tables	45
Hooks	20
Total	**873**

3.4. Study methodology

Study design

A cross-sectional study was conducted to determine the prevalence of *L. monocytogenes* and antibiotic susceptibility test from September 2013 to May 2014 in sheep meat slaughtered at Addis Ababa Abattoir Enterprise and meat presented for sale in different butcher houses in the city at the Food Microbiology Laboratory of Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. On each sampling day, all the required samples (sheep meat swab sample, knives, cutting tables and hooks) were taken.

3.5. Sampling techniques

In Addis Ababa Municipal Abattoir, the maximum numbers of animals are slaughtered on Wednesdays and Fridays and mainly during holidays.Carcasses were examined just after evisceration before washing. The meat was swabbed without distinction of race, sex or age at Addis Ababa Abattoir Enterprise and different butcher shops during several visits. The carcasses were chosen in a systematic random sampling method and examined just after the stage of evisceration. And for the butcher shops, convincing sampling type was performed and the samples were taken only from the sites where they were sold to the consumers.

All samples were collected aseptically using disposable gloves to avoid contamination, and the samples were labeled with necessary information including the date of sampling, sample code and sample type.The selected meat was swabbed aseptically using the method described in ISO11290-1 (1996) by placing sterile template (10 x 10 cm) on specific sites of a carcass. A sterile cotton tipped swab (2x3 cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England) rubbed first horizontally and then vertically several times on the carcasses. The abdomen (flank), thorax (lateral), crutch, breast (lateral), which were sites with the highest rate of contamination was chosen for sampling. On completion of the rubbing process, and leaving the cotton swab in the test tube. Finally, the carcass swabs taken was kept in a transport medium (buffered peptone water) and transported to the Food Microbiology of Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia for microbiological analysis. Up on arrival, the samples was stored in refrigerator at 4°C.

3.6. Isolation and identification of *Listeriamonocytogenes*

The techniques recommended by the International Standards Organization (ISO 11290-1, 1996) and the French Association for Standardization (AFNOR, 1993) were employed for the isolation and identification of *Listeria monocytogenes*.

- **Primary selective enrichment**

Each sample unit which are kept in buffered peptone water was mixed thoroughly to ensure the homogeneity of its contents and about 0.1 ml unit was obtained aseptically in to 10ml of prepared listeria enrichmentbroth (LEB) followed by mixing and the sample was kept inside incubator and incubated at 30°C for 48 hrs.

- **Secondary selective enrichment**

The secondary selective enrichment medium with full concentration of selective agents was employed. From the pre-enrichment culture (*Listeria* Enrichment Broth), after being well mixed 0.1 ml was transferred into 10 ml of Half Fraser broth and was incubated at 35°C for 24 hours.

- **Isolation and identification**

From Half Fraser Broth showing black color, a loopful of the culture was streaked onto PALCAM agar plates and OXA agar plates and incubated at 37°C for 24 to 48 hours.
Identification of *Listeria* species on PALCAM agar plates was based on aesculin hydrolysis and mannitol fermentation. All *Listeria* species hydrolysedaesculin as evidenced by a blackening of the medium. Mannitol fermentation was demonstrated by a color change in the colony and/or surrounding medium from red or gray to yellow due to the production of acidic end products.

The selectivity of the PALCAM medium is achieved through the presence of lithium chloride, polymixin B sulphate and acriflavine hydrochloride present in the medium base and Ceftazidime provided by PALCAM antimicrobial supplement. These agents effectively suppress growth of most commonly occurring non-*Listeria* species of bacteria present in food samples.

On PALCAM agar; typical colonies were grey-green with a black sunken center and a black halo, and on Oxford agar, colonies appeared brown black or greenish black with a depressed center and a surrounding black halo (Falana*et al*., 2003).

- **Confirmation**

Colonies suspected to be *Listeria* was transferred ontoTryptose yeast extract agar plate (TSYEA) and was incubated at 37°C for 18 to 24 hours. Those assumed *Listeria* colonies were characterized by using Gram's staining, characteristics of haemolysis, carbohydrate utilization and CAMP (Christie Atkins Munch Peterson) test following standard methods (ISO 11290-1, 1996; AFNOR, 1993).

The CAMP test was undertaken using *Staphylococcus aureus* (CIP: Collection of Institute of Pasteur, 5710). It was streaked vertically in a single line across a sheep blood agar plate and *Listeria* isolates horizontally to *S. aureus* streak (Lovett *et al*., 1989). The plates were incubated at 37°C for 18 to 24 hours. An enhanced zone of beta hemolysis between the test strain and culture of *S. aureus*was considered a positive reaction (ISO 11290-1, 1996). *L. monocytogenes* showed an enhanced zone of hemolysis, forming anarrow head towards the *S. aureus*culture.

For the carbohydrate utilization test, isolated colonies from TSYEA was transferred into test tubes containing xylose, rhamnose and mannitol and incubated at 37°C for up to 5 days. Positive reactions were indicated by yellow color (acid formation).

3.7.Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed for *L. monocytogenes*and other *Listeria* isolates by using Muller Hinton Agar. The common conventional antimicrobial drugs (Amoxyclav, Chloramphenicol, Ampicillin, Streptomycin, Tetracycline, Vancomycin, Ciprofluxcin, Gentamicin,Sulfamethtrimethoprin, penicillin, Co-trimexazole,oxacillin and Clindamycin) were tested.

The method applied for antimicrobial testing was agar plate antibiotic disk diffusion method, using Kirby-Bauer technique by 0.5 McFarland Standard (Mac Gowan*et al.,* 1990; Antunes*et al.*, 2002; Hansen *et al*., 2005; Quinn *et al*., 2004). About 2-3 pure colonies of the isolates was taken from the tryptone yeast extract agar and suspended in Muller Hinton Broth (MHB) and then, incubated at 37°C for 1-2hrs. The suspension was checked for the development of slight turbidity. It was innoculated, by dipping a sterile cotton swab into, it and wiping on the Muller Hinton agar, according to the standard procedure (NCCLS), and then the antimicrobial discs was firmly placed on it, and the plates were incubated at 37°C for 24 hrs.

The results were interpreted in accordance with the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2004). The resistance profile of the strains was reported according to the abbreviation for the antibiotics to which they showed resistance.

3.8. Questionnaire survey

Questionnaire survey was conducted to the meat value chains in the study sites and a detailed and organized questionnaire format was designed. A structured questionnaire were prepared and pre-tested and 50 butchers and 50 abattoir workers were surveyed. The questions and answers were written in English and entered.

3.9. Data management and analysis

The data collected through questionnaire survey and laboratory results of the collected samples were entered in to databases using Microsoft Excel computer program and analyzed using SPSS version 20 statistical computer software programs. Descriptive statistics were used to describe the nature and the characteristics of the data. Comparisons between prevalence of groups were analysed by using Chi-square (χ^2)test. For all tests, p-value less than 0.05 were considered to be significant.

4. RESULTS

4.1. Prevalence of *Listeria monocytogenes* in abattoir and butcher shops

From a total of 873 samples, the overall prevalence of *L. monocytogenes* was 36 (4.1%)(Figure 2).The prevalence of isolation of *L. monocytogenes* varied between sample sources. Out of each 384 samples collected from the abattoir and butcher shops, the prevalence of *L. monocytogenes* were 2.1% and 5.5% respectively. The result was higher in butcher shops than abattoir and there was significance difference in prevalence of *L. monocytogenes* from these sources of samples ($p<0.05$) (Table 3). Out of 105 equipment samples collected from both in abattoir and butcher shops, the prevalence of *L. monocytogenes* was 6.7%. There was no significance difference in prevalence of *L. monocytogenes* both in case of abattoir and butcher shops ($p>0.05$).

Table 2: Overall prevalence of *Listeria monocytogenes* from different source of samples.

Sample Type	No. examined	Prevalence (%)	95 % CI
Abattoir	384	8(2.1) a	0.1-4.1
Butcher	384	21(5.5) ab	3.5-7.5
Cutting table	45	4(8.9) ab	3.1-14.7
Hook	20	(0.0) b	
Knife	40	3(7.5) a	1.3-13.7
Total	873	36(4.1)	

*[ab]Proportions (%) with similar letters are not statistically significant (with p-value = 0.05). CI= confidence interval; %= percent of prevalence.

Table 2 in the above demonstrated the overall prevalence of *L. monocytogenes* in different sample sources when they were analyzed together which had the overall prevalence of 4.1. Cutting table was found to have the highest prevalence (8.9%) followed by knife (7.5%). Whereas the least prevalence was found to be hook (0.0%) that had statistically significant difference comparing with the others. Even though there was difference in prevalence among the others (abattoir, butcher, cutting table and knife), it was not statistically significant. This is also illustrated in Figure 2 below.

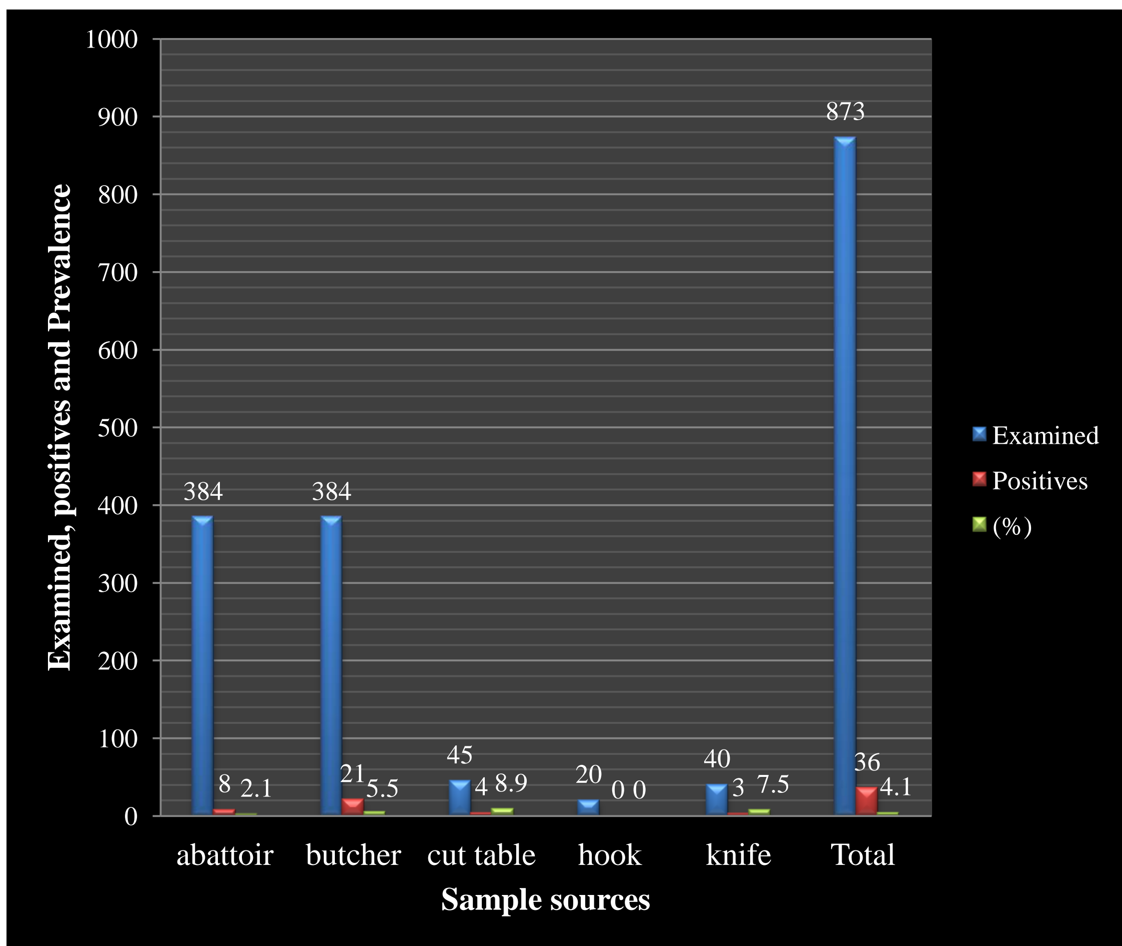

Figure 2: Overall prevalence of *L.monocytogenes*

4.2.Prevalence of*Listeria monocytogenes*in abattoir and butcher shops

Out of the total 768 swab samples examined during the study period8(2.1%) and 21(5.5%)were positive for *L. monocytogenes*.

Table 3: Prevalence of *Listeria monocytogenes* from different sources of samples

Source of sample	No. of examined	Prevalence (%)	OR	CI of OR	χ^2	p-value
Abattoir	384	8(2.1)	1			
Butcher	384	21(5.5)	2.7	1.2-6.2	6.1	0.02
Total	768	29(3.8)				

OR= odds ratio; CI= confidence interval;χ^2= Chi square

The total prevalence of the *L. monocytogenes* from abattoir and butcher shops was 3.8% (N=768). The prevalence in butcher has higher with statically significant difference (P=0.02). As the table 3 in the above indicated, the prevalence of the disease in butcher houses was almost three times (OR= 2.7, CI= 1.2-6.2) higher than the causative agent identified from abattoir. It is also indicated in the figure below (Figure 3).

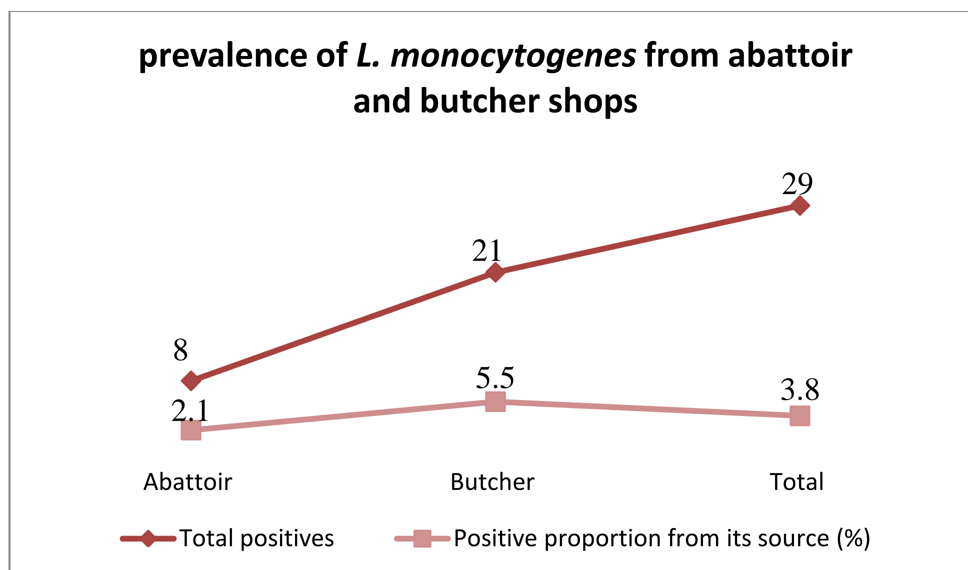

Fig 3: Proportion of positive prevalence in abattoir and butcher shops

4.3.Contamination rate of*Listeria monocytogenes* in equipments

Table 4: Prevalence of *listeria monocytogenes* in meat contact surface materials.

Source of sample	No. examined	Total positive	Prevalence (%)	χ^2	p-value
Hook	20	0	0.0		
Knife	40	3	3(7.5)	3.1	0.2
Cutting table	45	4	4(8.9)		
Total	105	7	7(6.7)		

Table 4 in the above indicated the contamination rate of *L.monocytogenes*in meat surface contact materials (hook, knife and cutting table). Although there was no even one sample positive for

Hooks, there is no statistically significant difference among hook, knife and cutting table (P= 0.2). It is illustrated in Figure 3 below.

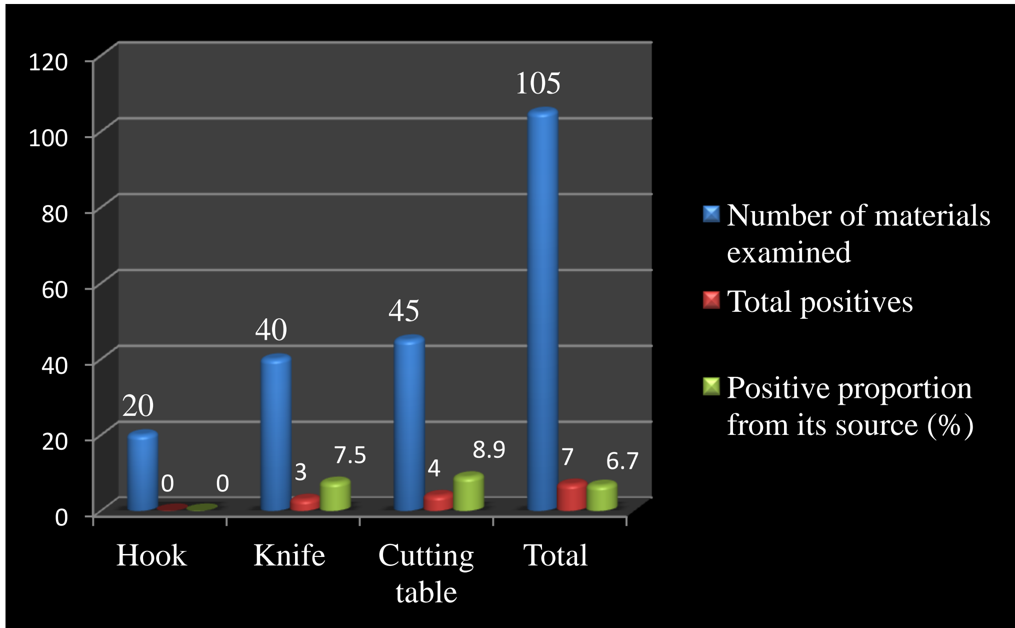

Figure 4: The positive proportion of the surface materials to *L. monocytogenes*

4.4. Antimicrobial susceptibility

A total of 36 isolates of*Listeria monocytogenes* were tested for antimicrobial susceptibility. Allisolates of *L. monocytogenes* were susceptible to Amoxyclav. Out of 36 isolates,28(77.8%) were resistant to tetracycline and 9 (25%) were resistant to penicillin. four (11.1%) were equally resistant to streptomycin and ampicillin, and 7 (19.4%) were equally resistant to sulfamethoprim and oxacillin; 2 (5.6%) were equally resistant to clindamycin and vancomycin. Interestingly, 34 (94.1%) isolates of *L. monocytogenes* were equally susceptible to vancomycin and co-trimexasole. The details of susceptibility pattern of the isolates are presented in Table 5.The study also revealed multi-drug resistance isolates in 10/36 (22.7%), 13/36 (36.1%) and 24/36 (66.7%) for one, two and two or more drug antimicrobials respectively.

Table 5: Susceptibility of *Listeria monocytogenes* isolates to different antimicrobials

Antimicrobial	*Listeria monocytogenes*		
	S	**R**	**I**
	N (%)	N (%)	N (%)
Ampicillin	32 (88.9)	4 (11.1)	0
Chloramphenicol	32 (88.9)	3 (8.3)	1 (2.8)
Ciprofloxacin	28 (77.8)	7 (19.4)	1 (2.8)
Penicillin	24 (66.7)	9 (25)	3 (8.3)
Tetracycline	4 (11.1)	28 (77.8)	4 (11.1)
Vancomycin	34 (94.4)	2 (5.6)	0
Co-trimexazole	34 (94.4)	2 (5.6)	0
Streptomycin	30 (83.3)	4 (11.1)	2 (5.6)
Gentamycin	35 (97.2)	1 (2.8)	0
Amoxyclav	36 (100)	0	0
Cindamycin	29 (80.5)	2 (5.6)	5 (13.9)
Sulfamethrimethoprim	24 (66.7)	7 (19.4)	5 (13.9)
Oxacillin	28 (77.8)	7 (19.4)	1 (2.8)

S= Susceptible; R= Resistant; I= Intermediate

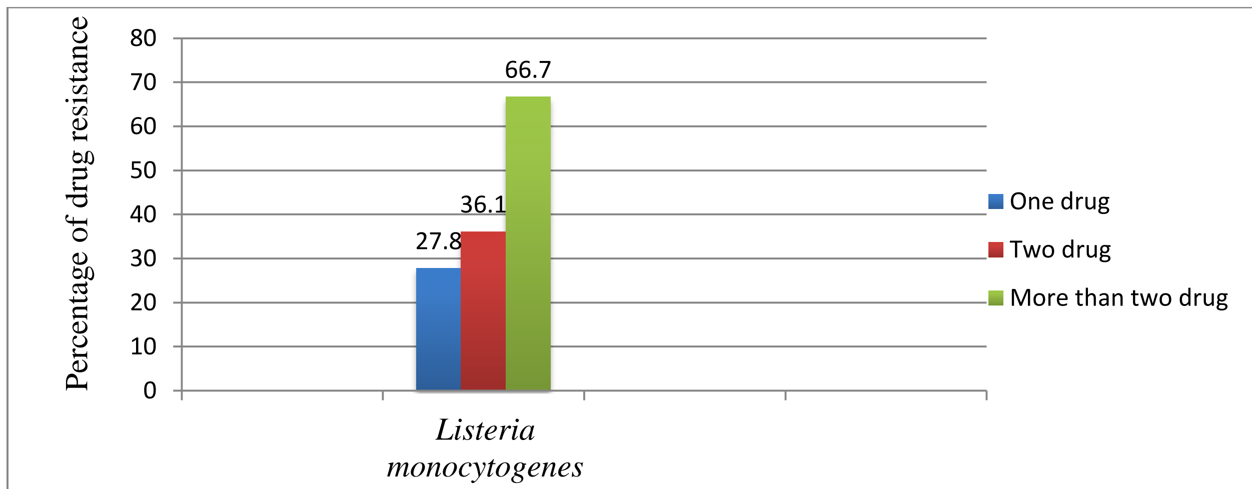

Fig 5: Multidrug resistance in *Listeria monocytogenes* for selected antimicrobial agents

4.5. Findings of questionnaire survey

4.5.1. Findings of questionnaire survey in abattoir

A total of 50 respondents were surveyed from the abattoir. About (60%) of the abattoir workers have completed high school level. Out of 50 respondents all (100%) had taken a lesson on personal hygiene. From the total of respondents (56%) and (36%) wash their hands once and twice per day during the course of working time respectively. And (74%) of the respondents reported to use detergent. Most of the respondents (92%) wash their hands after toilet.

All (100%) of the respondents cleaned the working surfaces between each process and after work. About (80%) of the respondents wash their working knives after the completion of the work and the rests wash several times during the course of working time.

As on observational assessment, 76% of the closets of butchers are dirty. And almost all of the workers in the working room wear aprons and wear a hair covering. 78% of them do not wear any jeweler materials. About the hygienic status of the Abattoir it is in a medium status.

Table 6: Summary of observational assessment and knowledge of workers on hygienic practices in abattoir

Abattoir activity	Performance	No. of respondents	Percent
Educational status	1-4	5	10
	5-8	15	30
	9-12	30	60
Lesson on personal hygiene	Yes	50	100
	No		
Time interval of washing hands	Once	28	56
	Twice	18	36
	Other	4	8
Washing of hands	With water only	13	26
	With detergent	37	74
Washing of hands after toilet	Yes	46	92
	No	4	8
Clean and disinfect working surfaces	Before work	0	0
	Betweeneach process	50	100
Washing of knives	After work	40	80
	Between process	10	20
View of closets	Neat	12	24
	Dirty	38	76
Wearing of aprons	Yes	50	100
	No	0	0
Hair	Covered	50	100
	Not covered	0	0
Wearing of jeweler materials	Worn	11	22
	Not worn	39	78

4.5.2.Findings of questionnaire survey in butcher shops

A total of 50 respondents were surveyed from butcher shops. About (48%) of the butchers in an educational level of elementary and (38%) have completed high school level. 50% of the respondents had taken a lesson on personal hygiene. About 46% and 36% of the respondents wash their hands twice and once per day during the course of working time respectively. And (86%) of the respondents reported to use a detergent.As observed during the current study, about (96%) of the respondents wash their hands after toilet.

Although, about (38%) of the respondents reported the cashier is handling the money. The majority (62%) of the respondents handle the money by themselves. Most of the butchers (72%) cleaned the working surfaces and similarly washing of knives about (7.8%) performed after work.

As on observational assessment, (60%) of the closet of the butchers is dirty. And most of them (74%) didn't wear a hair covering. Wearing of jeweler materials were observed in (38%) of the butchers. About the hygienic status of the butcher shops (50%), (30%) and (20%) had poor, moderate and good status respectively.

Table 7: Summary of observational assessment and knowledge of workers on hygienic practice in butcher shops.

Questionnaire and observation type	Performance	No. of respondents	Percent
Educational status	1-4	7	14
	5-8	24	48
	9-12	19	38
Lesson on personal hygiene	Yes	25	50
	No	25	50
Time interval of washing hands	Once	18	36
	Twice	23	46
	Other	9	18
Washing of hands	With water only	7	14
	Water and Detergent	43	86
Washing of hands after toilet	Yes	48	94
	No	2	6
Handling money	Cashier	19	38
	Butcher	31	62
Cleaning working surfaces	Before work	14	28
	After work	36	72
Washing of knives	After work	39	78
	Between each selling	11	22
View of closets	Neat	20	40
	Dirty	30	60
Hair covering	Covered	13	26
	Not covered	37	74
Wearing of jeweler materials	Worn	19	38
	Not worn	31	62
Hygienic status of the butcher house	Good	10	20
	Moderate	15	30
	Poor	25	50

5. DISCUSSION

Production of safe food has important economic implications in an increasingly competitive global market (Addis *et al*., 2011).*Listeria* species are ubiquitous in nature and has been isolated from wide environmental sources (Liu, 2008). The organism possesses ability to survive in harsh conditions and therefore, can persist in environment. Because of such persistence *Listeria* species can easily enter in the food chain. Of the known *Listeria* species, *L. monocytogenes* is pathogenic to humans and animals (Pal, 2007; Raorane *et al*., 2014).

Raw meat and other raw food products commonlyfound in the retail environment may be contaminated withpathogens, including *L. monocytogenes*.Retail environments are much more open with many people coming and going. These open retail environments may allow for the introduction of *L. monocytogenes* at various points and times of the day, potentiallymaking control of *L. monocytogenes* in the retailenvironment more difficult (Cutter *et al*., 2006).The detection and identification of *Listeria* species have attracted the attention of many authors. This specific interest is related to the presence of *L. monocytogenes*, one of the most important food-borne pathogens, in the genus. It is often found in various uncooked foods, such as meat, cheese, and vegetables. It is widely diffused in the environment and this fact can cause the contamination of food during production and distribution. However, *L. monocytogenes* has been the main representative of the genus to be studied (Cocolin *et al*., 2002).

5.1. Prevalence of *L. monocytogenes* in raw sheep meat

The specific prevalence of *L. monocytogenes* based on sample source was found to be statisticallysignificant. In this study, the prevalence of *L. monocytogenes* in sheep meat was 2.1% in abattoir. This is in agreement with(Pociecha *et al.*, 1991) who noted a prevalence of 3.2% from ovine carcassin Island and 5% from ovine carcass in Brazil slaughter house (Ankpolat *et*

al., 2004). In the current study, the overall prevalence of *L. monocytogenes* was4.1. The prevalence was still higher in other country likeAustralia with 16% (Ibrahim and Mac Rae, 1991), and 40% (Mac Gowan *et al*., 1994) in lamb.

Listeria monocytogenes has been found in different kinds of raw meat; there has been a relatively high frequency of positive findings amounting to by 20.8% by Sramova, *et al*. (2000) and 12.5% by Karpiskova (1998).The prevalence of the pathogen (2.1%) was found to be in agreement with findings of Ankpolat *et al*. (2004) who recorded 5%. On the contrary, there was no detection of *L. monocytogenes* at abattoir from sheep carcass in Germany (Cohen *et al*., 2006). And a prevalence of 50% by Abay *et al*. (2012) from sheep minced beef which is very high from the current study.

The study also revealed the prevalence of *L. monocytogenes* in sheep meat was 3.8% both in abattoir and butcher shops. This is lower than Kwiatek *et al*. (1992) who observed a prevalence of 9.3% in sheep meat.This could be attributed to the high microbial loads on raw meat entering the process and thus increase the potential for contamination of the processing environment and if separation is not adequately maintained the finished product(Gilbert *et al*., 2009).

In New Zealand, a prevalence of 30% *L. monocytogenes* was recorded by Gilbert *et al*.(2009) which was very high than the current study reported 2.1% prevalence of *L. monocytogenes*in abattoir.The reason for this attributed to the differences in hygienic conditions of slaughter houses, storage and processing in different countries. In Ethiopia only few researches done on this, by Molla *et al*. (2004) who observed a prevalence of 5.1% in raw and ready to eat food products and one previous study revealed that a prevalence of 5.4% by (Firehiwot, 2007) from raw meat, milk and milk products.

Other relative studies done on a prevalence of 4.0% by Al Ali, *et al*. (2012) of *L. monocytogenes*from gall bladder of sheep in slaughter houses.And several studies confirmed that a prevalence of 4% by Ndahi *et al*. (2013) in ready to eat foods, 2.4% by Ennaja *et al*. (2008) from meat and meat products in Morocco and 4.7% by Yucel *et al*, (2005) from meat products in turkey.

The specific prevalence of *L. monocytogenes* from equipments was found to be statistically not significant. Dirty or contaminated equipments can contaminate the safe food. Improperly cleaned equipment can be a source of *L. monocytogenes* contamination. Based on FDA reports and foodborne outbreak reports provided to the CDC, three risk factors have been identified most frequently as contributing to the contamination, spread and growth of foodborne pathogens, including *L. monocytogenes*, in processing or retail environments. They are cross-contamination; improper cleaning and sanitation; or improper time and temperature control (Cutter *et al*., 2006).

In the present study, the equipments were potential source of contamination with a prevalence of 6.7% which was lower than Lowry and Tiong (1988)and Dunja (2011) who reported 13% and 11.4% prevalence of *L. monocytogenes* in food contact surfaces, respectively.Therefore, Control measures to reduce the carriage of these pathogens in ruminants prior to slaughter are reviewed with reference to the current regulations and guidelines relating to the primary production.This study result suggests that a prevalence of 6.7% of *L. monocytogenes*. The prevalence was higher in other country a prevalence of 25.64% which is reported by Jankuloski *et al*, (2007).

The variation of prevalence in the two study sites may be because of environmental contaminations and poor sanitary conditions while handling of the meat before reach to the consumer. This indicates that the meat was free from *L. monocytogenes* wring distribution while slaughtering and the contamination occurs in an increasing level along the food value chain starting from slaughtering at the abattoir level, during distribution of the meat and improper handling of the meat handlers who sold it.

5.2. Antimicrobial susceptibility

Antibiotic resistant bacteria pose a growing problem of concern, worldwide since the bacteria can be easily circulated in the environment.Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous. A relatively high number of strains are resistance to the antimicrobials commonly used in the therapeutic protocols of many humansand animal infections (Normanno*et al*., 2007).In the current study, a resistance for tetracycline was 77.8%. In the contrary 0% was reported Conter *et al*. (2013). Findings of resistant as well as poly-resistant strains of *L. monocytogenes* to antimicrobial drugs are rather sporadic. It is pertinent to mention that such strains have been isolated from foodswere frequently found resistance to tetracycline and penicillin (Navratilova *et al*., 2004).

In this study the results regarding tetracycline resistance for *L. monocytogenes*which is higher than the previous study 25% and in case of amoxyclav (100%) were sensitive which is lower than a study 75% reported by Gupta and Sharm (2013) respectively.Thus, the resistance figures from different countries can considerably vary from very low to very high, probably reflecting the use of antimicrobials in those countries.Excepting tetracycline and penicillin, most antimicrobial drugs tested with in this study effectively prevented the growth of *L. monocytogenes*.

The current study revealed that,Chloramphenicol resistance was found to be 8.3% which is lower than a study by Goulet and Sharm (2013) who reported 50%.It is worth mentioning that *L. monocytogenes*isolates examined in this study from all types of samples were resistant to 19.4% and 77.8%. In the contrary, percentages of resistance to ciprofloxacin and tetracycline were 1.8%, and 9%, respectively reported by Zhang (2005).In the current study, resistance for ampicillinis 11.1%which is higher than and ciprofloxacin resistance 19.4% which is lower than a study, who reported 7.4% and 22.2%of resistance Nayak *et al*. (2012) respectively.

The frequency of multi drug resistant *L. monocytogenes* in the current study was as high as previously reported in other countries. In Iran a study by Ebrahim *et al*. (2012) revealed that 73.8% and 17.0% were resistant to one or more antimicrobials and three or more antimicrobials respectively.

A larger sample size is needed to determine if there are differences between antimicrobial resistance patterns of the isolates among different sample sources. Considering that *L. monocytogenes* is slowly becoming antibiotic resistant, a continued surveillance of emerging antimicrobial resistance of this pathogen is important for effective treatment (Conter *et al*., 2009).In conclusion, because of the increase of antimicrobial resistance showed by *L. monocytogenes*, a continuous surveillance of emerging antimicrobial resistance among this pathogen is important to ensure effective treatment of listeriosis.

5.3. Questionnaire survey

Employees can contaminate food with *L. monocytogenes* if proper personal hygiene policies are not followed or if employeesdo not take the proper steps to safely receive, store,prepare and serve food.They also may be a source for *L. monocytogenes* since some humans are known to carry the pathogen in theirgastrointestinal tracts. Poor personal hygiene practices, suchas improper hand washing or dirty uniforms, can lead to thecontamination of food and equipment with *L. monocytogenes* (Cutter, *et al*., 2006).

Proper motivation and training of employees and managers is vital to keep consumers safe (Bricher, 2007). In this study, 100% of the respondents from the abattoir had taken a lesson on personal hygiene but from the butcher shops only 50% of the respondents had taken a lesson on personal hygiene. Therefore, all currently available educational approaches need to be critically evaluated and adopting of employees training to minimize *L. monocytogenes* cross contamination (Crandal, 2011).Furthermore, 92% of the abattoir workers and 96% of the

butchers washed their hands after the use of toilet. In contrary, few workers did not wash their hands. This may contribute to a low level of contamination of meat.One previous study revealed that, a prevalence of 16.7% and 27.8% from smoked and cooked meat products and from fermented dry meat products respectively (Navratilova, 2004).

The results of this study showed that most of the respondents 74% of abattoir workers and 86% of butchers used a detergent for washing of hands. Findings of *L. monocytogenes*in swabs from tools and working surfaceswitness the fact that contamination of meatand meat products is due to secondary soiling fromthe environment or equipment of meat-processingplants. Contamination generally increasedduring cutting, probably as a result of crosscontamination.Also, in the retail and food service environment, contamination may be transferred between ready-to-eat products (Lianou and Sofos, 2007).

The type of handling that ready-to-eat meat receives may also influence the level of *L. monocytogenes* contamination. In a survey of retail packaged meats there was a significantly higher prevalence of *L. monocytogenes* reported in products cut into cubes 61.5% out of 13 compared with sliced products 4.6% out of 196 (Angelidis and Koutsoumanis, 2006).

6. CONCLUSION AND RECOMMENDATIONS

The consumption ofimproper meat is not safe from consumer point of view, as it may lead to the transmission of various diseases. In this study, the results of bacteriological assessment showed that raw meat from market and slaughter houses are a source of *L. monocytogenes*. In addition, the presence of this bacteria may be attributed to the unclean working environment,poor sanitary conditions of persons who are contacting with the meat and their equipment materials used.This may result in low meat quality and might potentially cause food poisoning especially in susceptiblegroups which includespregnant womens,young, elderly and immunocompromised individuals.Due to high risk andpublic health concern, it may cause a high case fatality rate. The detection of this bacteria in ready to eat processed food makes it unfit for human consumption.

Listeria monocytogenes may not be seen as potential clinical threat in Ethiopia today, with the increasing trend oftransnational spread and emerging diseases. The probable risk that it might pose in the years to comecannot be ignored.The present study demonstrated the possible risk of *L. monocytogenes* after consuming meat and RTE food stuffs available in the markets, and also highlighted the need for an effective and efficient storage process to keep such food safe, till they reached the consumers.Numerous risk factors are associated with thecontamination and growth of *L. monocytogenes* in abattoir and market places. These factors need to be addressed and considered a serious hazard to identify control measures for an effectiveprevention and control program of the organism. Further, sources of infection and modes of transmission should be ascertained. And addressing communication, risk perception and consumer practices to the public are mandatory.

Furthermore, the present study detected widespread resistance by *L. monocytogenes* to commonly used antimicrobials. In addition, the prevalence of multi-drug resistance of the bacterium is also phenomenon, which gives cause for serious concern.

Therefore, based on the above conclusive remarks, the following recommendations are forwarded:

Understanding the sources of the pathogen and factors that contribute to the risk of contamination, growth and spread of the pathogen are important building blocks to an effective control program.

The best approach for preventing listeriosis is reducing the exposure of susceptible populations to contaminated food.The production of microbiologically safe food is fundamentally based on the implementation and application of general preventative measures, good hygienic practices and good manufacturing practices.And food contamination needs to be controlled and information provided to the people who are at a greater risk.

Creating public awareness by disseminating the information is necessary and an extensive survey of the prevalence of *L. monocytogenes* in whole of Ethiopia must be undertaken.

In order to detect early changes in bacteria susceptibilities before a high prevalence of resistance is developed, regular monitoring of antimicrobial resistance topathogenic bacteria should be practiced. The genetic mechanisms, which mediate antimicrobial resistance in this bacteria, would also need further studies.

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8. APENDICES

Appendix I: Questionnaire

Date of collection ______

Code No. ______

Observational assessment and knowledge of butchers on hygienic practicesin butcher shops.

1. Worker's name ___________________ Sub-city _____________ Educational status __________
2. Have you ever received any lessons in personal hygiene? Yes _____ No ____
3. How often do you wash your hands? Once _____ Twice _____ Other ____
4. Washing hands With water only _____With water and soap ____
5. Do you wash your hands after toilet? Yes ____ No ____
6. Who is handling the money? Butcher _____Cashier_____
7. How often do you clean and disinfect working surfaces? Before work ____ After work _____
8. Washing knives After work _____ Between each process_____
9. The view of the closets of the butchers? Neat ____ Dirty ____
10. Aprons Used _____Not used _____
11. Hair Covered _____ Not covered _____
12. Jewellery materials Worn _____ Not worn _____
13. Hygienic status of the butcher shops Good _______ Moderate ______ Poor _______

Date of collection ______

Code No. ______

Observational assessment and knowledge of worker's on hygienic practicesin Abatttoir.

1. Worker's name ______________________ Sub-city __________ Educational status _________
2. Have you ever received any lessons in personal hygiene? Yes _____ No ____
3. How often do you wash your hands? Once _____ Twice _____ Other ____
4. Washing hands With water only _____With water and soap ____
5. Do you wash your hands after toilet? Yes ____ No ____
6. How often do you clean and disinfect working surfaces? Before work ____ After work _____
7. Washing knives After work _____ Between each process_____
8. The view of the closets of the butchers? Neat ____ Dirty ____
9. Wearing of aprons Yes _____ No _____
10. Hair Covered _____ Not covered _____
11. Jeweller materials Worn _____ Not worn _____

Appendix II: Laboratory Data Collecting Sheet

		Isolation and primary identification	Confirmation

S.N	Sample code	Gram stain	Motility test	Catalase	Oxidase	Hemolysis	CAMP test	Carbohydrate utilization tests		
								D-mannitol	Rhamnose	Xylose

Appendix III: Procedure of Gram's staining

1. Prepare the smear and heat fix.
2. Stain with crystal violet for 60 seconds and rinse with tap water and drain.
3. Flood the slides with iodine and allow remaining 60 seconds and rinsing with tap water and drain.
4. Decolorize with 95% ethanol until 15 seconds and rinse with tap water and drain.
5. Counter stain with safranin for 60 seconds and rinse with tap water and drain.
6. Examine microscopically under oil immersion.

AppendixIV: Procedure for catalase test

1. Place a drop of 3% H_2O_2 on a glass slide.

2. Touch a sterile loop to a culture of the organism to be tested and pick up a visible mass of cells (colony).
3. Mix the organism in the drop of hydrogen peroxide.
4. Observe for immediate and vigorous bubbling.
   Interpretation: Bubbling indicates a positive test and no bubbling indicates a negative test.

Appendix V: Procedure for oxidase test

1. Prepare a solution of 1% tetramethyl-p-phenylenediaminedihydrochloride.
2. Piece of filter paper is moistened in a petridish with fresh reagent.
3. The test bacterium is streaked firmly across the filter paper with a glass rod.
4. A dark purple color along the streak line with in 10 seconds indicates a positive reaction.

Appendix VI: Procedure for haemolysis test

1. Isolates colony was taken with an inoculating needle from a typical colony on TSYEA.
2. Streak the sample in to 7% Sheep Blood Agar Base.
3. It was incubated at 37°C for 24 hours.
4. After incubation positive test cultures show narrow, clear and light zones (β-haemolysis).

Appendix VII: Procedure for CAMP test

1. Take a colony culture with an inoculating needle from a typical colony on TSYEA
2. *Staphylococcus aureus* was taken (CIP: Collection of Institute of Pasteur, 5710).
3. It was streaked vertically in a single line across a sheep blood agar plate and *Listeria* isolates horizontally to *S. aureus* streak and
4. The plates were incubated at 37°C for 18 to 24 hours.

5. An enhanced zone of beta hemolysis between the test strain and culture of *S. aureus*was considered a positive reaction.*L.monocytogenes*showed an enhanced zone of hemolysis, forming anarrow head towards the *S. aureus*culture.

Appendix IX: Procedure for carbohydrate utilization test

1. Isolated colonies from TSYEA was transferred into test tubes containing xylose, rhamnose and mannitol and
2. It was incubated at 37°C for up to 5 days.
3. Positive reactions were indicated by yellowcolor (acid formation).

Appendix X: Composition and preparation of culture media used for the study.

❖ **Pre-enrichment - *Listeria* enrichment broth**

Specifications; KM 10505

<u>Composition(gm/l)</u>

Peptone mixture …………………….. 20

Yeast extracts ………………………...6.0

Sodium chloride ……………………...5.0

Potassium dihydrogen phosphate …....2.5

Glucose ………………………………2.5

Nalidixic Acid ……………………….0.04

AcriflavinHCl ……………………..…0.015

Cyclohexamide……………………....0.05

pH 7.3+/- 0.2

Preparation;

✓ 36.1 gm of powder was weighed and added to 1lt of deionized water (conductivity <10ms).

- ✓ Then warmed until complete dissolution
- ✓ It was mixed well and 225 ml wasdistributed into each of 250ml erlenymer flasks and sterilized by autoclaving at 121°C for 15 minutes.

❖ **Secondary selective enrichment media (*Listeria* Fraser broth)**

Specification KM 10335

Use: For isolation and enumeration of *Listeria* species.

Composition (gm/l)

Peptone mixture ………..10

Yeast extract ……………5

Sodium chloride ………..20

KH_2PO ……………...….1.35

Na_2HPO …………..……9.5

Beef extract …………….5.0

Nalidixic Acid …………..0.010

AcriflavinHCl ….….……0.0125

Aesculine …………...….1.0

Lithium chloride …….....3.0

PH= 7.2+/- 0.2

Preparation

- ✓ 27.4 gm of the powder was weighed and added to 500ml of the deionized water(conductivity<10ms),
- ✓ Then it was well mixed and sterilized by autoclaving at 121° C for 15 minutes.
- ✓ After sterilization, it was allowed to cool to around 47°C and previously preparedand filtered (sterilized), 5 ml of 5% Ferric ammonium citrate* supplement was added tothis broth.
- ✓ Then it was well mixed and 10ml of the broth was aseptically dispensed intosterile tubes.

*Ferric ammonium citrate (17% Fe)-MERCK.

❖ ***Listeria* isolation agar**; two selective media were used for this purpose

A. Oxford Agar

Specification: KM1049

Use: A selective medium for the isolation of *Listeria monocytogenes*from food and clinical materials.

Composition (gm/l)

Coloumbia agar ……………..42.0

Aesculine…………………… 1.0

Ferric Ammonium citrate ….. 0.5

Lithium chloride …………....15.0

pH= 7.2 +/- 0.2

Preparation

- ✓ 5 g of the powder was weighed and added to 1lt of deionized water and then it was allowed to soak for 10 minute and
- ✓ Swirled to mix and sterilized by autoclaving at 121°C for 15 minutes.
- ✓ It was kept at room temperature until it cooled to around 47° C and 2 vials of dry powderof the selective supplement KM ‘SO49 was suspended with 1 ml distilled water andadded into this medium.
- ✓ At last this prepared medium was thoroughly mixed by agitatingand it was pour plated into sterile petridish.

KM SO49 supplement contains;

CCNAF selective supplement (MICRO TRADE)

Formula;

CEFOTITAN ……………………… 1 mg

COLISTIN ………………………….10 mg

FOSOMYCIN ………………………5 mg

ACRIFLAVINE……………………. 2.5 mg

NATAMYCIN ……………………..12.5 mg

B. PALCAM (Polymixinacriflavin lithium chloride ceftazidime, aesculin and mannitol) agar base

Specification; KM S079

Use: An important selective differential medium for the isolation of *Listeria monocytogenes* from food, clinical and environmental specimens.

<u>Composition (gm/l)</u>

Columbia peptone mix …...….23.0

Aesculine ………………...….0.8

Ferric ammonium citrate …..... 0.5

Lithium chloride …………… 15.0

Corn starch ………………….1.0

Yeast extract ………………. 3.0

Mannitol …………………....10.0

Sodium chloride …………….5.0

Glucose ……………………..0.5

Phenol red …………………. 0.08

Agar ………………………. 12.0

pH=7.2 +/- 0.2

Preparation

- ✓ 1liter of PALCAM medium was prepared by weighing and adding of 70.8 g of thepowder into one liter of deionized water.
- ✓ Followed by mixing and sterilizing of themedium at 121^{0}C for 15 minutes.
- ✓ Then, it was allowed to cool to around 47 ^{0}C and 2 vials of theselective supplement KMSO79 was added (as in OXA case), mixed and pour plated. Atthis level the PALCAM medium was used after keeping for some time to allow drying ofthe medium.

KMSO79 selective supplement- PAC (MICRO TRADE)

Formula; Polymixin B ……. 6.25 mg

Cetrazidine ……………… 10 mg

Acriflavine……………….. 2.5 mg

D. Tryptose yeast extract agar

Specification; KM 1116

Use: an agar for performing total viable count by the pour plate method.

Composition(gm/l)

Tryptone ……………5.0

Yeast extract ………. 2.5

Glucose ……………. 1.0

Agar ……………….. 15.0

pH 7.0+/-0.2

Preparation

- ✓ 23.5 g of the ingredients was suspended in1 lit of the deionized water andboiled with frequent stirring.
- ✓ Then, it was dispensed into screw-capped tubes andautoclaved at 121^{0}C for 15 minutes and the rack was kept in slant position in order to prepare slants.

E. Tryptone Soya Yeast Extract Agar (TSYEA)

Composition(gm/l)

Tryptone...............................17.0

Soya peptone……………… 3

Sodium Chloride …………. 5.0

Di potassium phosphate ….. 2.5

Yeast extract ……………... 6

Glucose …………………… 2.5

Agar ………………………. 15.0

Preparation

- ✓ 1liter of deionised water dissolved in 40gm powder and mix well
- ✓ Heat with frequent agitation and boil for one measure to completely dissolve the powder

- ✓ Autocalve at 121° C for15minutes
- ✓ Dispense in to petridishes

F. Blood Agar Base

<u>Composition (gm/l)</u>

Heart infusion from (solids) ……..…2.0

Pancreatic digest of casein………....13.0

Yeast extract ……………………..…5

Agar……………………………...15.0

Sodium chloride ……………………5.0

Preparation

- ✓ 1liter of deionised water dissolved in 40gm powder and mix well.
- ✓ Heat with frequent agitation and boil for one measure to completely dissolve the powder.
- ✓ Autocalve at 121° C for15minutes.
- ✓ Cool the base to 45 to 50°C and add 5% sterile, defibrinated sheep blood
- ✓ Dispense in to petridishes.

G. Carbohydrate utilization broths (rhamanose, xylose and mannitol)

i. Purple broth base

<u>Composition (gm/l)</u>

Peptone from meat ………….. 5

Peptone from casein …………5

Purple base ………………….. 0.018

Sodium chloride

Preparation

- ✓ Dissolve 15gm of powder in 1lit of purified water
- ✓ Autoclave at 121° C for 15 minutes and cool to about 60°C

ii. Carbohydrate solution

Rhamanose

Xylose

Mannitol

Preparation

- ✓ Dissolve 5gm of each carbohydrate in 100ml of water separately
- ✓ Sterilization by filtration

iii. Complete medium

Preparation

- ✓ For each carbohydrate, add aseptically 0.5 ml of filter sterilized carbohydrate solution to 4.5 ml of Phenol red solution prepared.

H:Buffered peptone water (Oxoid, England)

Composition (g/l)

Peptone ………….10.0

Sodium chloride …5.0

Final pH 7.5 ± 0.2 (at 25 ^{o}C)

Preparation

15 g of the powder was dissolved in 1 liter of distilled water. Stirred and dissolved completely. Then, sterilized by autoclaving at 121°C for 15 minutes after dispensing into the test tubes.