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ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF LISTERIA MONOCYTOGENES IN MUNICIPAL ABATTOIR AND BUTCHER SHOPS IN ADDIS ABABA

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BY

AGARI FEYISA TUFA

COLLEGE OF VETERINARY MEDICNE AND AGRICULTURE

DEPARTMENT OF VETERINARY MICROBIOLOGY, IMMUNOLOGY AND VETERINARY PUBLIC HEALTH

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

BY

AGARI FEYISA (DVM)

JUNE, 2023

BISHOFTU, ETHIOPIA

Addis Ababa University

College Of Veterinary Medicine and Agriculture

Department Of Veterinary Microbiology, Immunology and Veterinary Public Health

As member of the Examining Board of the final MSc open Défense, we certify that we have read and evaluate the thesis prepared by **Agari Feyisa Tufa** entitled: **''Isolation and Antimicrobial Susceptibility Profile of *Listeria Monocytogenes* in Municipal Abattoir and Butcher Shops in Addis Ababa.''** And recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Veterinary Public Health

Dr. Dechassa Obsi	______________	26/06/2023
Chairman	Signature	Date
Dr. Gezahegne Alemayehu	______________	26/06/2023
External Evaluator	Signature	Date
Prof. Bekele Megersa	______________	26/06/2023
Internal Examiner	Signature	Date
Bedaso Mammo (PhD, Associate Professor)	______________	______________
Major Advisor	Signature	Date
Zerihun Beyene (DVM, MSc)	______________	______________
Co-Advisor	Signature	Date
Prof. Bekele Megersa	______________	______________
Department Chairman	Signature	Date

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my novel work and that all sources of material used for this thesis have been exactly acknowledged. This thesis has been submitted in partial fulfilment of the requirements for MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University or College library to be made available to borrowers under rules of the library. I uniquely 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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Name: Agari Feyisa Signature: _______________ Date_______________

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LIST OF ABBREVIATIONS

µL	Micro Litre
µm	Micro Meter
APIQTC	Animal Products and Inputs Quality Testing Center
ASF	Animal Source Food
BUG	Biological Universal Growth Agar
CAC	Codex Alimentarius Commission
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standard Institute
CNS	Central Nervous System
DALYs	Disability Adjusted Life Years
DNA	Deoxyribonucleic Acid
ELFA	Enzyme Linked immuno Fluorescent Assay
ELISA	Enzyme Linked Immuno Sorbent Assay
EU	European Union
FBD	Food Borne Diseases
HACCP	Hazard Analysis Critical Control Point
LAMP	Loop Mediated Isothermal Amplification
LS	Lysis Tube
MDA	Molecular Detection Assay
MDR	Multidrug Resistance
mL	millilitre
NC	Negative Control
PALCAM	Polymyxin Acriflavine Lithium Chloride Ceftazidime Aesculin Mannitol
PCR	Polymerase Chain Reaction
Ph	Hydrogen ion concentration
RC	Reagent Control
RNA	Ribonucleic Acid
TASF	Terrestrial Animal Source Food
USA	United State of America
USDA	United States Department of Agriculture
VIDAS	VITEK Immunodiagnostic Assay System

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ABSTRACT

Listeriosis, which is caused by *Listeria monocytogenes*, is one of the most common foods borne zoonotic infection having significant public health concerns. The persistence of *L. monocytogenes* in the food production environments and consumption of contaminated food of animal origin such as beef has great public health significance in Ethiopia where raw meat has always been considered as traditional delicacy. Therefore, a cross-sectional study was conducted from December 2022 to May, 2023 to isolate *L. monocytogenes* from raw beef meat and environmental samples at Addis Ababa abattoirs enterprise and butcher shops in Addis Ababa and assess its antimicrobial susceptibility pattern. A total of 280 samples consisting of carcass and environmental samples were collected from abattoir (n=150) and butcher shops (n=130) in the study area for microbiological analysis of this bacteria. After primary and secondary enrichments with demi Fraser and Fraser broth, all collected samples were subjected to 3M molecular detection assay for detection and then presumptive positive was transferred to Biolog identification system (GEN III MicroPlate) for confirmation according to manufacturer's protocol. The overall prevalence of *L. monocytogenes* was found to be 4.64% (13/280) of which 4.67% (7/150) were from abattoirs and 4.62% (6/130) were from butchers' shops. Out of 120 beef carcass samples collected 5.83% (7/120) were positive for *L. monocytogenes.* In this study the occurrence of *L. monocytogenes* was found to be 6% (3/50), 3.64% (2/55) and 4% (1/25) from personnel hands, knifes and cutting boards respectively. All *L. monocytogenes* isolates were subjected to antimicrobial susceptibility testing using eight available antibiotic disks. Out of 13 isolates, 92.3% (12/13) were equally resistant to tetracycline and amoxicillin whereas 84.6 % (11/13) and 76.9% (10/13) were resistant to streptomycin and ampicillin respectively. However, all isolates were found to be susceptible to Trimethoprim-sulpha methazole. Eleven (84.6%) isolates were found to be multi-drug resistant. This study showed that there was occurrence of antibiotics resistance developed against *L. monocytogenes* in Addis Ababa beef value chains, which requires improved good manufacturing and hygienic practices during meat production, HACCP implementation, prudent use of antimicrobial drugs and regular surveillance system.

Keywords: *3M molecular detection assay, Addis Ababa, antimicrobial susceptibility, beef meat, L. monocytogenes*

1. INTRODUCTION

More than 60% of human pathogens are zoonotic in origin which includes a wide variety of bacteria, viruses, fungi, protozoa, parasites, and other pathogens (Rahman *et al.,* 2020). Zoonotic diseases can spread to humans through direct contact or through food, water or the environment. Foodborne diseases (FBD) pose a serious public health and huge economic burden on both developing and developed countries. Over the years, the incidence and emergence of FBD has been overwhelmingly high globally (Mensah and Ofosu, 2020; Mansour *et al.,* 2021). Unsafe food consumption results in 600 million cases of FBD and 420,000 deaths every year from 56 million people die each year. This data shows that 7.69% (600 million cases) individuals of world populations from 7.8 billion suffer from FBD every year and 7.5% (420,000 death) of all deaths (56 million) annually are due to foodborne infections. More common foodborne illnesses were caused by bacteria (226,526,634 cases) followed by viruses (138,513,782) and parasites (10,284,561) globally (WHO, 2015).

Bacteria are the causative agent of two-thirds of foodborne disease outbreaks though there have been around 250 different food-borne diseases (Abebe *et al.,* 2020), with most of them being zoonotic in nature and having reservoirs in healthy food animals (Raheem, 2016; Kinyua, 2020). Vertebrate animal species are natural reservoirs for many pathogens of animal origin that cause human infections (zoonoses) and zoonotic pathogens can be transmitted through animal source food (ASF), and secondarily via non-ASF that become contaminated during their production process, which puts consumers of ASF at increased risk (Abebe *et al.,* 2020). One-third of the world's food-borne disease burden is associated with the consumption of contaminated terrestrial animal source food (TASF) (FAO, 2023). The burden associated with consumption of contaminated TASFs is estimated at 168 DALYs per 100 000 population (about 35 percent of the global burden of FBDs) (Li *et al.,* 2019).

Although there are 31 pathogens that have been identified as causing food-borne diseases (Zhao *et al.,* 2014), C*ampylobacter* species, *Salmonella* species, *Listeria* species, and *Escherichia coli O157:H7* have been generally found to be responsible for majority of food-borne outbreaks (Kidanu *et al.,* 2021). Although the number of *Listeria monocytogenes* (*L. monocytogenes*) related cases was lower than that caused by other bacteria, the fatality rate (22.41%) was much higher than that caused by other bacteria (WHO, 2015; Lee and Yoon,

2021). *L. monocytogenes* is reported to be highly implicated in food contamination leading to frequent food borne outbreaks in diverse regions of the world (Kinyua, 2020). World Health Organization classified *L. monocytogenes* as a major notable foodborne pathogen associated with high mortality and hospitalization (Abdeen *et al.,* 2021) and estimates the global burden of listeriosis to be 172,823 disability adjusted life years (DALYs) from 23,150 illnesses (De Noordhout *et al.,* 2015; Dufailu *et al.,* 2021).

Listeriosis, also called silage disease, circling diseases, and meningoencephalitis, is caused by *L. monocytogenes*. It is an infectious and fatal disease of animals, birds, fish, crustaceans, and humans (OIE, 2014). Listeriosis is one of the most serious and severe food-borne diseases (Beyza and Chirkena, 2019) and the most important emerging bacterial zoonotic diseases worldwide. Among the *Listeria* species, *L. monocytogenes* causes listeriosis in humans and animals and has the highest case fatality rate among foodborne diseases (Moti *et al.,* 2022). This causes severe and life-threatening infection mainly in high-risk groups such as pregnant women, neonates, elderly and immunocompromised patients with fatality rate in high-risk groups can be up to 30% (Ramaswamy *et al.,* 2007). *L. monocytogenes* is one of the most important pathogens responsible for foodborne illness which may result in meningitis, septicaemia, spontaneous abortion, perinatal infections and gastroenteritis (Liu, 2006; OIE, 2014; Şanlibaba *et al.,* 2018).

Listeriosis which is one of the most important foodborne bacterial zoonotic diseases of human being acquired through consumption of contaminated food of animal origin (Garedew *et al.,* 2015; Fisseha, 2017)**.** Meat and meat products may serve as vectors for pathogenic organisms and have frequently been contaminated with *L. monocytogenes* (Setiani *et al.,* 2019) and can become contaminated with bacteria during food processing or slaughtering (Abebe *et al.,* 2020). *L. monocytogenes* has also been reported in different meat-processing facilities such as abattoirs, meat-processing plants and butcheries (Carpentier and Cerf, 2011)**.** Cross contamination of carcasses and meat products occur during subsequent handling, processing, preparation and distribution (Dave and Ghaly, 2011). Hygienic and sanitary measures practiced across the production chain determine the level of *Listeria* contamination especially during slaughtering, evisceration and meat processing. *Listeria* could also be carried as environmental contaminants over the carcass meat through fomites, personnel, utensils and the water used for meat processing (Kumar *et al.,* 2016).

Even though listeriosis caused by *L. monocytogenes* is a global major public health issues, in developing countries especially in Africa there is limited research on *L. monocytogenes* (Odetokun and Adetunji, 2016). In Ethiopia, presence of Listeria monocytogenes in meats has not been widely surveyed (Zelalem *et al.,* 2019). Only few studies have been done on *L. monocytogenes* in a variety of raw and ready-to-eat food products of animal origin collected from different parts of the country. Gebretsadik *et al.* (2011) reported 2.6% of *L. monocytogenes* from raw beef in Addis Ababa. The systematic review and meta-analysis conducted on meat samples showed the pooled prevalence of 4% *L. monocytogenes* (Zelalem *et al.,* 2019). The study conducted in Jimma town municipal abattoir and butcher shops samples also revealed 7% positive finding for *L. monocytogenes* (Tiku *et al.,* 2020). Polymerase chain reaction (PCR) based detection of *L. monocytogenes* in retail meat in the area of Addis Ababa revealed that 6.8% of samples from raw beef were found to contain *L. monocytogenes* (Derra *et al.,* 2013). However, there is limited information regarding occurrences and contamination rate of *L. monocytogenes* in the municipal abattoirs and butcher shops in Addis Ababa. Therefore, this study was performed with objectives of;

- Isolating the *L. monocytogenes* from raw meat samples taken from Addis Ababa abattoirs enterprise and butcher shops found in Addis Ababa.
- Determining the antibiotic susceptibility profile of isolated *L. monocytogenes.*

2. LITERATURE REVIEW

2.1 History

Gustav Hulphers, a veterinarian at the Veterinary Institute of Stockholm, Sweden was first identified the bacterium *L. monocytogenes* in laboratory rabbits in 1910 (Valladares, 2019), from the necrotic liver of a rabbit in Sweden and named at that time Bacillus hepatis (Carvalho *et al.,* 2014; Matle *et al.,* 2020; Osek *et al.,* 2022). Hulphers described carefully the morphology, the cultural characteristics, the biochemical properties, the growth limits of the bacterium and the pathological-anatomical changes in rabbits (Valladares, 2019). In 1926, Murray, Webb and Swann discovered *Listeria* while investigating an epidemic in laboratory animals, rabbits and guinea pigs (BrugerePicoux, 2008; Mateus *et al.,* 2013) in research laboratories of Cambridge, United Kingdom, and named *Bacterium monocytogenes* (Matle *et al.,* 2020; Osek *et al.,* 2022).

A year later in 1927, Pirie (researcher from South Africa) also isolated a bacterium corresponding to the description given by Hulphers and Murray in gerbils (Iatera lobenquiae), known as desert or African jumping mouse, and called it *Listerella hepatolytica* in honour of Lord Lister, the father of antisepsis (Matle *et al.,* 2020). Thereafter, Pirie called the organism *Listerella monocytogenes* (Valladares, 2019; Matle *et al.,* 2020). Pirie changed the name of the bacterium to *L. monocytogenes* in 1940, as the name *Listerella* was used for other species (Lamont and Sobel, 2011; Valladares, 2019).

In 1929, the first human case of listeriosis in which the microorganism was obtained from blood was reported by Nyfeldt in Denmark (Valladares, 2019), who claimed that these bacteria were the cause of infectious mononucleosis (Osek *et al.,* 2022). In the same year Gill also described the illness in sheep called circling diseases caused by *L. monocytogenes* (Matle *et al.,* 2020). In 1919, an unknown bacterium of diphtheroid type was isolated from cerebrospinal fluid (CSF) of a soldier in France and after 20 years, the organism was identified as *L. monocytogenes* (Valladares, 2019).

Even though *L. monocytogenes* has been recognized as a human pathogen since 1929, the route of transmission only became clear since the 1980s when a series of outbreaks were found to be related to food (CHP, 2010; Hellström, 2011). The first confirmed food-borne

outbreak of listeriosis occurred in 1981 in Nova Scotia, Canada, and involved 41 patients due to sheep manure cultivated cabbage (Derra, 2007). After several outbreaks in the 1980s, the interest for the pathogen among food manufacturers were started to emerge (Klumpp and Loessner, 2013). Since then, *L. monocytogenes* outbreaks have been linked to consumption of contaminated foods, which include dairy products, meat products, seafood products and vegetables (Zuber *et al.*, 2019).

2.2 General Characteristics of *Listeria Monocytogenes*

Listeriosis is caused by members of the genus Listeria, in the family Listeriaceae (Spickler, 2019), listeria species belong to the phylum Firmicutes, class Bacilli, order Bacillales (Jadhav 2015), of about 0.5 µm in width and 1 µm – 1.5 µm in length (Wieczorek *et al.,* 2012). Listeria species are Gram positive and facultative anaerobic organisms. They are also non-spore forming, and short rod-shaped bacteria (Odetokun and Adetunji, 2016; Abebe *et al.,* 2020). The genus *Listeria* currently consists of 26 species (FAO and WHO, 2022). Among these different species, only *L. monocytogenes* is a human pathogen and *Listeria ivanovii* is an animal pathogen, which is rarely pathogenic in human (Zeinali *et al.,* 2017; Heredia and García, 2018; FAO and WHO, 2022). Based on the relatedness to *L. monocytogenes*, the species in the genera of *Listeria* are categorized in two groups of '*Listeria sensu strictu'*, which includes *L. monocytogenes, Listeria seeligeri, Listeria marthii, Listeria ivanovii, Listeria welshimeri,* and *Listeria innocua* and '*Listeria sensu lato'*, a group that includes the other *Listeria species* (Heredia and García, 2018).

L. monocytogenes can grow at the low temperature of the refrigerator, a range of pH values, a high salt concentration (WHO, 2017; FSS, 2019) and that requires minimum water activity of 0.92 (Rodrigues and Melo, 2017). It has an unusual characteristic of surviving in temperatures from -7°C to 45°C and can multiply in properly refrigerated food after contamination (CHP, 2010). *L. monocytogenes* can grow at pH range between 4.4 and 9.4 and can tolerate sodium chloride at concentration of less than 11.5% (Setiani *et al.,* 2019). In addition, *L. monocytogenes* has the ability to form biofilms which can contribute to its ability to colonize food processing facilities (Yada and Anato, 2019). These characteristics enable the pathogen to survive in food-processing environments and in foods, and make it a great concern for the food industry and a threat for public health (Gandhi and Chikindas, 2007). This versatile growth and survive under extreme environmental conditions of *L.*

monocytogenes posed at food-processing facilities are become a serious problem for food industry (Matle *et al.,* 2020)**.** *L. monocytogenes* has been divided into 13 serotypes (½a, ½b, ½c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7) based on somatic and flagellar antigens (Dhama *et al.,* 2015; Quereda *et al.,* 2021), with only three (1/2a, 1/2b, and 4b) causing more than 90% of invasive human infections (Burall *et al.,* 2017).

2.3 Sources, Routes and Mode of Transmission

L. monocytogenes is widely dispersed in the natural environment (CHP, 2010) and several sources have been indicated as possible contamination routes for food and transmission of *L. monocytogenes* to human beings (Matle *et al.,* 2020). It has been found in soil, vegetation, silage, sewage, water and faeces of healthy animals and humans (FSS, 2019), food-processing facilities and retail outlets (Matle *et al.,* 2020). Various food types such as raw and processed foods also can become contaminated with Listeria species. These foods are raw milk and dairy products, various meats and meat products such as beef, fermented sausages, fish products, ready-to-eat foods, vegetables (Saludes *et al.,* 2015), juices and related products, such as mixed salads (Heredia and García, 2018)

The route of transmission of *L. monocytogenes* to humans was unclear until the 1980s when several outbreaks indicated that the source of bacteria was food (Osek *et al.,* 2022). Since it is ubiquitous in the soil, vegetation, water, waste droppings of some animals and can easily contaminate foods at any point in the farm to fork production chain (Manjengwa, 2022), and therefore can easily contaminate food products of both animal and plant origin (Lambertz *et al.,* 2012). Consumption of implicated contaminated foods with high counts of this pathogen is the main route of transmission of infections (Manjengwa, 2022). Food-borne transmission constitutes the main acquisition route of Listeriosis (Churchill *et al.,* 2006).

Consumption of food of animal origin contaminated by *L. monocytogenes* is most common route of infection in humans (Abebe *et al.,* 2020), through ingestion of contaminated unpasteurized dairy products, raw meat, and meat products, soft cheese, fish and fish products (Moti *et al.,* 2022). It was reported that, approximately 99% of human listeriosis appear to be food-borne (Derra, 2007) and high risk of contamination with *L. monocytogenes* occurs during food manufacturing processes (Quereda *et al.,* 2021). The carcasses and their

products may be contaminated during slaughtering and meat processing (Babiker *et al.,* 2020).

Other possible route of infections with Listeriosis in humans are direct contact with infected animals and contaminated environments (Hilliard *et al.,* 2018), involving cross-contamination by employees, equipment, environment surfaces, animal skin, food additives, packing material (Babiker *et al.,* 2020) and footwear, gloves and aprons of the personnel involved in manufacturing (Gelbicova and Karpiskova, 2012). Rarely, *L. monocytogenes* can spread from infected animals directly to humans in addition to person to person spread from pregnant women to the foetus through the placenta (FSS, 2019) and can also enter the body by inhalation or inoculation into broken skin or the eye (Spickler, 2019).

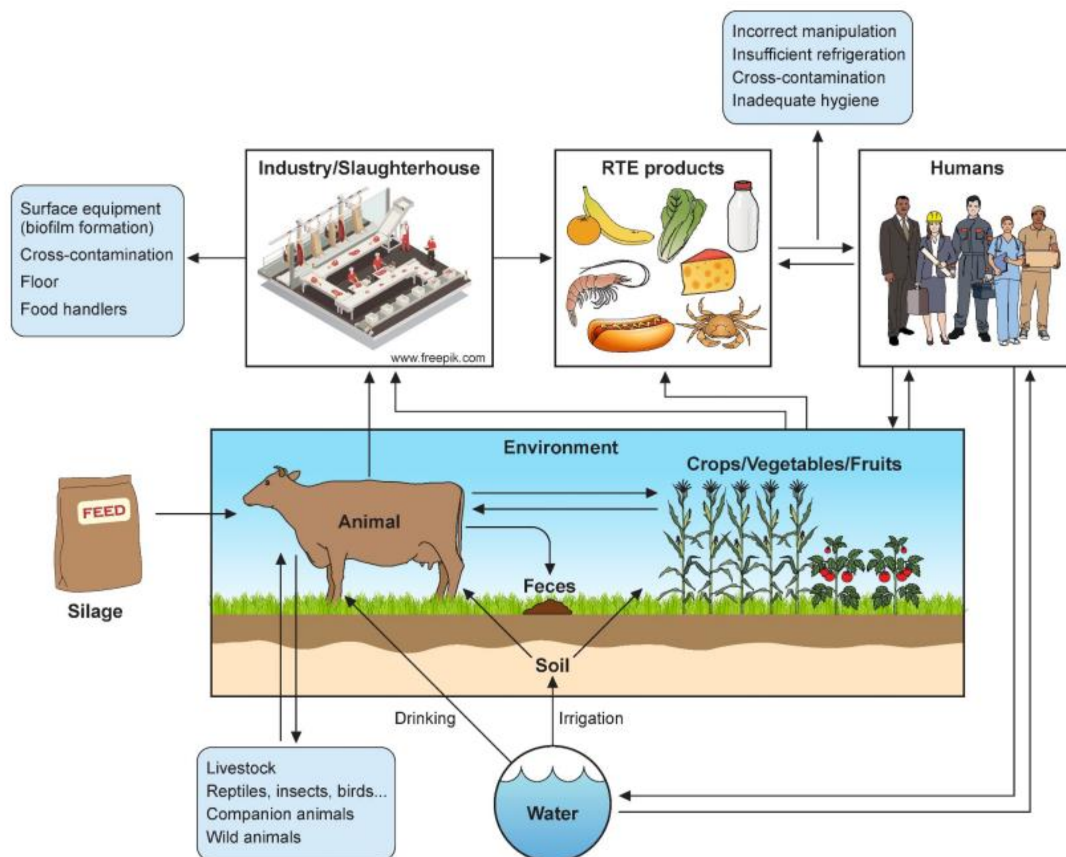

Figure 1: Summary of contamination sources of *L. monocytogenes*

Source: (Quereda *et al.,* 2021)

2.4 Pathogenesis of *Listeria Monocytogenes*

Before reaching the intestine, the ingested *Listeria* organisms must withstand the adverse environment of the stomach (Vazquez-Boland *et al.,* 2001). *L. monocytogenes* survives exposure to high acidity, bile salts, non-specific inflammatory attacks and proteolytic enzymes from the host system (Matle *et al.,* 2020). Invasion of host epithelial cells is governed by various surface proteins including InlA and InlB, which bind to receptors on the host cell. InlA binds to the host E-cadherin whereas InlB interacts with the hepatocyte growth factor (HGF) receptor (Titu, 2014) and then disseminate into circulatory system to cause systemic diseases (Vazquez-Boland *et al.,* 2001). Several different stages of adhesion and invasion of host cells including internalisation by host cells, intracellular multiplication, lysis of vacuole and intercellular spread to the adjacent cells are involved in infection process of host cell by Listeria monocytogenes (Vazquez-Boland *et al.,* 2001).

From the intestine, bacteria are then carried in macrophage cells to the liver and spleen, where most of them are destroyed by neutrophils acting in concert with Küpàer cells and some of them can escape into the cytosol by employing the pore forming protein Listeriolysin O (Kundul and Ame, 2022). This breaks down the vacuole wall and enables the bacteria to escape into the cytoplasm since any bacteria remaining in the vacuole are destroyed by the host cell (Yada and Anato, 2019). Once in the cytosol, ActA assembles asymmetrically on the bacterial surface and recruits host cell actin to polymerize into an actin tail. This machinery propels *L. monocytogenes* through the cell and enables spread into adjacent cells (Titu, 2014). *L. monocytogenes* has the capacity to pass three important barriers in the human host, namely the blood brain barrier, the placenta and intestinal epithelium and then subsequently disseminate to other organs (Chen *et al.,* 2009).

L. monocytogenes penetrate the endothelial layer of the placenta and thereby infect the foetus (Derra, 2007). Colonization of the trophoblast layer followed by translocation across the endothelial barrier would enable the bacteria to reach the foetal bloodstream, leading to generalized infection and subsequent death of the foetus in utero or to premature birth of a severely infected neonate with miliary pyogranulomatous lesions (Vazquez-Boland *et al.,* 2001). It can also access the Central Nervous system (CNS) by mechanisms including retrograde axonal transport that occurs through two different routes that utilizes the cranial nerves primarily the trigeminal nerve upon crossing of the oral epithelium and which

exploits the olfactory epithelium and crossing of the blood-brain barrier (Disson and Lecuit, 2012). In humans, CNS infection by *Listeria* species presents primarily in the form of meningitis, which is often associated with the presence of infectious foci in the brain parenchyma, especially in the brain stem (Vazquez-Boland *et al.,* 2001).

2.5 Clinical Signs

L. monocytogenes is widely distributed in nature and causes listeriosis in both animals and humans (Dhama *et al.,* 2015; FAO, 2023), with wide variety of clinical syndromes (Quereda *et al.,* 2021). The symptoms of listeriosis usually last 7 to 10 days. Although the incidence of listeriosis is lower than other foodborne pathogens, it results in approximately 30% rates of death (Cadirci *et al.,* 2018) and 95% hospitalization rate (Osek *et al.,* 2022). It can cause severe life-threatening infections such as septicaemia, meningitis, meningoencephalitis, spontaneous abortion, still birth, or foetal infection in high-risk groups (Abebe *et al.,* 2020). The clinical signs of listeriosis often appear after a long incubation time of 1 to 70 days (Buchanan *et al.,* 2018).

L. monocytogenes infection can be non-invasive or invasive (Orsi and Wiedmann, 2016), which occurs in humans and animals mainly due to ingestion of contaminated foods (Negi *et al.,* 2015). The invasive illness is characterized by severe symptoms such as meningitis, septicaemia, primary bacteraemia, endocarditis, non-meningitic central nervous system infection, conjunctivitis, and flu-like illness (Heredia and García, 2018). The invasive form of listeriosis mostly occurs in immunocompromised individuals (Osek *et al.,* 2022). The non-invasive form of listeriosis is characterized by febrile gastroenteritis (Buchanan *et al.,* 2017), can manifest in immune competent adults and it is usually self-resolving (Hellström, 2011).

2.5.1 Clinical signs in humans

Human beings' contract listeriosis infection by consumption or handling of food, contact with food contact surfaces, equipment and animal products contaminated with *L. monocyto genes* (Manjengwa, 2022). Expression of infections in humans can be invasive or non-invasive (Allerberger and Wagner, 2010; Desai *et al.,* 2019), depending on dose and mode

of infection, age and immune status of the individual, physiological stage and virulence of strain ingested. (Poimenidou *et al.,* 2018).

Invasive listeriosis is mainly a disease affecting susceptible individuals with underlying predisposing conditions including elderly, pregnant women and their unborn or new born infants, and patients with severe underlying diseases such as cancer, Acquired Immune Deficiency Syndrome (AIDS), or organ transplant (Hellström. 2011). It manifests itself as sepsis, meningitis, endocarditis, encephalitis, meningoencephalitis, septicaemia and brain infection (Hellström. 2011; Matle *et al.,* 2020). Invasive listeriosis is responsible for over 90% of hospitalisation and between 20% and 30% case fatality rate (Leong *et al.,* 2014). In pregnant women, infection may result in abortion, stillbirth or premature birth and may be preceded by influenza-like signs including fever (OIE, 2018). Pregnant woman has 17-fold increased risk of contracting invasive listeriosis, and this infection mostly occurs in the third trimester (Mateus *et al.,* 2013).

Non-invasive febrile gastroenteritis can manifest in immunocompetent adults and causes a self-limited acute febrile gastroenteritis among healthy people without severe complications in healthy individuals. Primary symptoms include fever, diarrhoea, arthromyalgia, and headache (Matle *et al.,* 2020; Quereda *et al.,* 2021). This form of Listeriosis in human beings has also been reported on the form of hepatitis, endocarditis, myocarditis, pneumonia, conjunctivitis, ophthalmitis, sinusitis, and joint infections (Amato *et al.,* 2017). Rarely occurred occupational hazardous cutaneous listeriosis is associated with exposure to animal products of conception removed from the birth canal. Typically, it manifests as nonpainful, nonpruritic, self-limited, localized, papulopustular, or vesiculopustular eruptions from which the organism can be isolated (Zelenik *et al.,* 2014).

2.5.2 Clinical signs in animals

Listeriosis is of major veterinary importance (Molla *et al.,* 2004), of mainly a disease of ruminants (Ivanek *et al.,* 2006). Asymptomatic carriage of *Listeria* is much more common than disease and clinical cases occur most often in cattle, sheep and goats, and the most complete descriptions of clinical signs are in these species (Spickler, 2019). Similarly, to humans, the clinical features of listeriosis in animals include septicaemia, rhombencephalitis, and abortion, especially in domestic ruminants (sheep, goats, and cattle)

(Quereda *et al.,* 2021). *L. monocytogenes* has been found in all syndromes, while *Listeria ivanovii* has been associated most often with reproductive losses in ruminants (Spickler, 2019).

The main clinical manifestations of animal listeriosis are encephalitis, abortion and septicaemia, and the disease is often associated with stored forages, usually silage, and contaminated farm environments (OIE, 2018; OIE, 2021). In ruminants, encephalitis is the most commonly recognized form of listeriosis and affects all ages and both sexes, sometimes as an epidemic in feedlot cattle or sheep (Hoelzer *et al.,* 2012; Constable, 2023). Generally, ruminants affected by encephalitis show marked neurological symptoms including circling, ataxia, opisthotonos, paralysis of cranial nerves combined with anorexia, hyperthermia, depression (Hoelzer *et al.,* 2012) and head pressing or turning of the head to one side (OIE, 2018).

Rarely, subclinical mastitis of ruminants has been associated with *L. monocytogenes* infection (Hellström. 2011; Quereda *et al.,* 2021). Gastrointestinal infections can occasionally occur in sheep (OIE, 2018). Iritis, uveitis, and keratoconjunctivitis have been reported in cattle associated with contact with contaminated silage (Laven and Lawrence, 2006).

2.6 Isolation and Identification Methods of *Listeria Monocytogenes*

Identification of *L. monocytogenes* is extremely important for prevention and disease control. The method used for detection and isolation of *L. monocytogenes* has evolved over the years from cold enrichment technique to conventional and molecular methods (OIE, 2014). A variety of conventional and rapid methods are currently available for the detection and identification of *L. monocytogenes* in samples from the food chain (primary production samples, feed, food samples, and environmental samples) and specimens from animal listeriosis (OIE, 2018; OIE, 2021). To be able to distinguish *L. monocytogenes* from other Listeria species, a rapid, specific and sensitive test is essential in which quick and accurate detection will lead to the control of the spread of this organism (Shearer *et al.,* 2001). There are two types of methods that can be used to isolate Listeria, which are conventional and rapid molecular test methods (Setiani *et al.,* 2019).

*2.6.1 **Conventional Method of Identification***

Currently conventional methods are widely used especially in developing nations for food safety tests (Manjengwa, 2022) and remain the gold standard against which other methods are validated. These methods use selective agents and enrichment procedures to reduce the number of contaminating microorganisms and allow multiplication of *L. monocytogenes* (OIE, 2018). Conventional bacteriological methods are important because their use results in obtaining a pure culture of the organism (FAO and WHO, 2022), which is useful for regulatory, epidemiological surveillance and outbreak management purposes. These methods are usually very sensitive and they do not require sophisticated and expensive equipment, allowing widespread use (OIE, 2018; OIE, 2021).

Various conventional methods are known for the isolation of *L. monocytogenes* from food and clinical samples that have gained acceptance for international regulatory purposes. All the methods differ from each other by their criteria of selecting primary, secondary and also selective agars (inhibitory compounds) in regard to the food types (Derra, 2007). Therefore, several methods have been applied for their detection and identification based on selective preenrichment, enrichment, and plating on agar plates, followed by the *Listeria* isolates cha racterization using conventional microbiological methods like sugar fermentation, colony morphology and hemolytic properties (Gasanov *et al.,* 2005). The conventional method of i solation of *L. monocytogenes* includes antibody-based tests, enzyme linked immunosorbent assay, culture-based methods and immune-capture techniques (Välimaa *et al.,* 2015).

Conventional methods for the isolation of *L. monocytogenes* from the food chain that have gained acceptance for international regulatory purposes include the European Committee for standardization (CEN, EN) and the International Organization for Standardization (ISO 11290) method and the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) method (OIE, 2018; OIE, 2021; Moti *et al.,* 2022).

Culture-Based Detection Methods

The isolation and identification of *L. monocytogenes* using culture-based methods involve the use of selective agents and enrichment procedure with purpose of inhibiting other competing microflora whilst the enrichment procedure allows the increase of *L. monocytogenes* to detectable levels and the recovery of injured or stressed cells (Chen *et al.,* 2017). Many cultural methods use selective enrichment followed by plating methods to test for the detection or not detection of *L. monocytogenes* in a food sample (usually 25g), with the typical detection limit being 1–5 CFU/test sample size (FAO and WHO, 2022). Culture-based tests are usually preferred as being sensitive, cheap and pure colonies of the targeted organisms obtained by culture-based assays are useful for epidemiological surveillance and outbreak management purposes (OIE, 2014).

There are three commonly used culture-based methods for isolation of *L. monocytogenes* in foods because of international regulations and requirements. These methods include the International Standard (ISO), USDA and One-Broth Listeria (Association Française de Normalization, AFNOR) method (Gómez *et al.,* 2014; FAO and WHO, 2022). The International Standard ISO-11290 and USDA methods" use a two-step enrichment in Fraser broth, but the Association Française de Normalization (AFNOR) uses the single-step broth method (Moti *et al.,* 2022).

The ISO 11290 method utilises Half Fraser Broth and Fraser Broth for primary and secondary enrichment, respectively (Matle *et al.,* 2020). While most *L. monocytogenes* are detected following primary enrichment in half-Fraser broth, secondary enrichment in Fraser broth often results in more *L. monocytogenes* positive samples, especially when the original food sample contains low levels of *L. monocytogenes* or has a high level of competing microflora (FAO and WHO, 2022)**.** The ISO 11290 recommends the use of Oxford and Polymyxin Acriflavine Lithium Chloride Ceftazidime Aesculin Mannitol (PALCAM) agar for detection and isolation (Leong *et al.,* 2016).

Both PALCAM and Oxford media are useful for the isolation of *Listeria species* from food which is rich in competitive microflora, and processed products where *Listeria* cells are often stressed or damaged (Law *et al.,* 2015). However, one of the main limitations of PALCAM and Oxford media is their inability to distinguish between pathogenic *L. monocytogenes* fr

om non pathogenic *Listeria* of other species (Zunabovic *et al.,* 2011). Presumptive *L. mono cytogenes* or *Listeria species* are sub cultured on a non-selective medium and confirm by means of appropriate morphological, physiological and biochemical tests described in the standard (OIE, 2021; FAO and WHO, 2022).

Some of the disadvantages of this group of methods include the relatively long period of time that the protocols require for completion, several hands-on manipulations, the requirement for many different chemicals, reagents, media and the relative subjectivity involved when interpreting typicality of colony on selective and differential agar plates (Jadhav *et al.,* 2012; OIE, 2018; FAO and WHO, 2022). Moreover, not all microbes will grow in a lab environment, and some damaged or stressed organisms may be viable but not cultivable (Manjengwa, 2022).

Biochemical Tests

After isolation of a pure culture of *Listeria*, a biochemical test was performed for identification and confirmation (Benetti *et al.,* 2013). Presumptive *Listeria* colonies on selective agar are confirmed by rapid tests and on biochemical properties such as Gram stain, catalase test, motility test, ability to produce haemolysis on blood agar plates, Christie–Atkins–Munch-Peterson test with *Rhodococcus equi* and *Staphylococcus aureus* and carbohydrate utilisation tests (Matle *et al.,* 2020). The common media for the biochemical tests include carbohydrate fermentation tests like rhamnose, D-xylose, mannitol; and haemolysis on blood agar (Benetti *et al.,* 2013).

L. monocytogenes is unreactive for oxidase and indole tests, ferments glucose and lactose with acid production, but no gas produced. It has a positive reaction to catalase, Voges Proskauer, and methyl red, and on blood agar Beta, haemolysis forms as a clear zone. The Christe Atkins Munch Peterson (CAMP) test for *Staphylococcus aureus* is positive for *Listeria monocytogenes* but negative for *Rhodoccocus equi* (Vazquez- Boland et al., 2001; Derra, 2007; Benetti *et al.,* 2014). Alternative to traditional biochemical testing which is time consuming and takes a week for differentiation of species using sugar utilization tests, currently commercial identification kits are widely used (Gasanov *et al.,* 2005).

Table 1: Biochemical properties of *L. monocytogenes*

Biochemical Characteristics	Reactions (Positive, +); (Negative, -)
Gram stain	+
Oxidase	-
Catalase	+
Indole	-
Urease	-
Mannitol	-
Xylose	-
Phosphatidylinositol phospholipase	+
Nitrate	-
α-Methyl-d-mannoside	+
Voges Proskauer	+
Arylamidase	-
D-Arabitol	+
Methyl red	+
Rhamnose	+

Sources: (Gasanov *et al.* (2005); Nayak *et al. (*2015); OIE (2018); Valladares (2019))

Among commercially available kits, Biolog OmniLog identification system (GENIII MicroPlate test panel) provides automated biochemical identification using a standardized micro method using 94 biochemical tests (71 carbon source utilization assays and 23 chemical sensitivity assays) to profile and identify a broad range of Gram-negative and Gram-positive bacteria. Biolog OmniLog identification system software is used to identify the bacterium from its phenotypic pattern in the GENIII MicroPlate (Biolog system user guide, Hayward, CA).

Immune Assay-Based Tests

Immunological detection based on antigen-antibody bindings is widely used for determining foodborne pathogens and rely mainly on the specific binding of an antibody to an antigen

(Zhao *et al.,* 2014). Antibodies specific to *Listeria* have been applied in food testing for many years and they are popular because of their simplicity, sensitivity, accuracy and also because testing can be carried out directly from enrichment media without tedious sample preparation (Gasanov *et al.,* 2005) and gave faster result and were less expensive (Priyanka *et al.,* 2016).

One of the most widely used to date immunological assays for foodborne pathogens detection is Enzyme-linked Immunosorbent Assay (ELISA), which is a very accurate and sensitive method for detecting antigens (Zhao *et al.,* 2014; Priyanka *et al.,* 2016). It is one of the most common antibody tests used for *L. monocytogenes* detection, especially in foods (Osek *et al.,* 2022). ELISA uses an antibody immobilized to a micro titre well for antigen capture in combination with a secondary antibody coupled to an enzyme (or another label) to detect the captured antigen (Gasanov *et al.,* 2005). The ELISA, including its variant enzyme-linked immunofluorescent assay (ELFA), is one of the most common antibody tests used for *L. monocytogenes* detection, especially in foods (Osek *et al.,* 2022). They are the most widely applied methods because they combine ease of use with the generation of rapid test results. Many of immunoassays are available as commercial kits and are approved by regulatory authorities (Gasanov *et al.,* 2005). Currently, high-throughput and automated ELISA systems such as Assurance EIA (BioControl) and VIDAS (BioMerieux) are commercially available for the detection of foodborne pathogens (Glynn *et al.,* 2006).

VITEK immunodiagnostic assay system (VIDAS) is system that performs entire ELISA procedure automatically (Law *et al.,* 2015b). It is an automated microbiology system utilizing growth-based technology for fast, accurate microbial identification, and antibiotic susceptibility testing (Osek *et al.,* 2022). VIDAS utilizes ELFA which is similar to ELISA, but it is a more sensitive fluorescent immunoassay for reporting the results. The VIDAS system involves the use of reagent strip and a plastic tube known as solid phase receptacle (SPR) (Law *et al.,* 2015b)

Although ELISA has been widely used in many laboratories, this method still requires various specialized equipment's and trained personnel (Muldoon *et al.,* 2007). However, Lateral flow immunoassays is other types of immunological detection methods which are rapid, cheap, simple and reliable. Dipstick and immunochromatographic strips for lateral

flow immunoassays have been developed for rapid on-site detection of foodborne pathogens such as *Listeria* species and *Salmonella* (Shukla *et al.,* 2011).

2.6.2 Nucleic acid-based methods of detection of Listeria monocytogenes

Morphological and biochemical approaches used for identification of *L. monocytogenes* are simple, sensitive, and inexpensive but laborious (Dwivedi and Jaykus, 2011; Law *et al.,* 2015) and time consuming as they require more than a week for detection and pathogen confirmation (Law *et al.,* 2015). Moreover, culture methods for detecting *L. monocytogene s* in edibles are slow, they may not be convenient for raw meat and foods that have short shelf lives (Jandaghi *et al.,* 2020). Therefore, due to a continued need to develop improved rapid methods for detection of foodborne pathogens (Fortes *et al.,* 2013), the trends for food testing in the food industry is towards the use of molecular methods (Gasanov *et al.,* 2005).

Nucleic acid-based methods operate by detecting specific Deoxyribo nucleic acid (DNA) or Ribo nucleic acid (RNA) sequences in the target pathogen by the target nucleic acid sequence hybridization to a synthetic oligonucleotide (probes/primers) complementary to the target sequence (Zhao *et al.,* 2014). Molecular methods detect differences at the DNA level and can differentiate between micro-organisms at the genus, species and even sub-species level (Liu *et al.,* 2003). Identification of *Listeria species* and *L. monocytogenes* using molecular methods is becoming increasingly popular. There is a wide range of molecular methods available for the identification and characterization of *Listeria* (Gasanov *et al.,* 2005). The recent nucleic acid-based methods include simple polymerase chain reaction (PCR), multiplex polymerase chain reaction (mPCR), real-time or quantitative polymerase chain reaction (qPCR), nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP) and microarray technology (Matle *et al.,* 2020; Wang *et al.,* 2020).

Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is the most well-known and established nucleic acid amplification technique for detecting pathogenic microorganisms (Janzten *et al.,* 2006; Zhao *et al.,* 2014). It is a technique whereby segments of DNA are amplified using a heat stable DNA polymerase and two primers (short DNA sequences specific to a particular gene) and

the amplified fragments are then detected, usually using agarose gel electrophoresis (Gasanov *et al.,* 2005). In this method, double-stranded DNA is denatured into single strands, and specific primers or single-stranded oligonucleotides anneal to these DNA strands, followed by extension of the primers complementary to the single stranded DNA, with a thermostable DNA polymerase. The amplification products by PCR are then visualized on electrophoresis by staining with ethidium bromide as bands (Zhao *et al.,* 2014).

PCR is now established as a reliable and reproducible technique for identification of *Listeria species* and more importantly for the differentiation of *Listeria monocytogenes* from other *Listeria species* using primers targeting genes of virulence factors or RNA sub-unit genes (Gasanov *et al.,* 2005). This detection method is highly specific, sensitive and inexpensive, but is susceptible to inhibitors. Moreover, the results of the amplified products can be interpreted by agarose gel electrophoresis, which takes a long time and has high technical requirements for the detection personnel (Wang, 2021).

DNA Microarrays

DNA microarrays are an existing new technology, which is based on DNA or RNA hybridization (Gasanov *et al.,* 2005). Microarrays which were originally used for the study of gene expression, but now has been widely used in the field of foodborne pathogen detection (Law *et al.,* 2015b). It is a collection of immobilized hybridization probes specific for multiple pathogens which on reaction with dye-labelled sample DNA bind and emit fluorescence. From the location of the fluorescence and the corresponding identity of the immobilized probe the suspect pathogen is identified (Ghatak, 2020). DNA microarrays are made up of chips or glass slides coated with hundreds of specific oligonucleotide probes which are chemically synthesized short sequences range from 25 to 80 bp (OIE, 2018).

DNA microarray is gaining importance currently and has become a useful tool due to its rapidness, sensitivity and specificity and it allows high throughput analysis (Priyanka *et al.,* 2016) and are commercially available from many different suppliers and can be custom made for specific purposes (Gasanov *et al.,* 2005).

DNA Hybridisation

DNA hybridization is the simplest molecular method used for the detection of *Listeria* and *L. monocytogenes* in foods using an oligonucleotide probe or primers commentary sequenc e to the target DNA containing a label for detection of presence of a target sequence (Gasanov *et al.,* 2005). DNA hybridisation tests have been extensively used for the differentiation of *L. monocytogenes* from other *Listeria species* by means of probes directed against specific genes. There are different commercially available kits for the testing of pure cultures or foods and environmental samples (Janzten *et al.,* 2006) like GeneTrak DNA hybridization kit (Neogen Corporation, Lansing MI) (Stephan *et al.,* 2003).

Quantitative Polymerase Chain Reaction

Quantitative PCR (qPCR) which is also called real-time PCR is an approach capable of continuously monitoring the PCR product formation throughout the reaction. It offers rapid, simultaneous amplification and sequence-specific-based detection of target genes (Zhao *et al.,* 2014). Real-time PCR had been a significant improvement towards the quantification of foodborne pathogens in samples (Ghatak, 2020), able to monitor the PCR information continuously during the entire reaction by measuring the fluorescent signal produced by specific dual labelled probes or intercalating dyes. The fluorescence intensity is proportional to the amount of PCR amplicons (Zhao *et al.,* 2014). The assay has been employed for the detection of many pathogens including those associated with foodborne illnesses, however it is a complex process to perform requiring expertise and instrumentation is costly and thus largely remains out of bounds for most laboratories (Ghatak, 2020).

Loop-Mediated Isothermal Amplification (LAMP)

Although PCR has been widely used in foodborne pathogens, it requires thermocycling to separate the double strands of DNA (Zhao *et al.,* 2014). To overcome the variable temperature amplification constraint of PCR requiring a thermocycler, LAMP was develop ed (Ghatak, 2020). LAMP is a technique to realize exponential amplification of nucleic acids at isothermal condition which has been used for rapid detection of animal pathogens (viruses, bacteria, parasites) (Wang, 2021). This was a major breakthrough as the need for precise temperature control was no longer needed (Ghatak, 2020) and has simpler hardware

requirements than PCR, as it may even work with a simple water bath setup (Zhao *et al.,* 2014).

It is based on the auto-cycling of DNA synthesis strand displacement carried out by large fragment of Bst DNA polymerase under isothermal conditions between 59°C and 65°C for 60 minutes. In LAMP, four primers comprising two inner primers and two outer primers are used to target six specific regions of target DNA (Law *et al.,* 2015b). The amplification is performed under isothermal conditions and the amplicons are mixtures of many different sizes of stem loop DNAs with several inverted repeats of the target sequence and cauliflower-like structures with multiple loops (Zhao *et al.,* 2014). The amplicons can be detected by SYBR Green I dye or agarose gel electrophoresis (Wang *et al.,* 2008; Zhao *et al.,* 2014).

3M Molecular Detection System

3M Molecular Detection System is systems which uses isothermal amplification methods for detection of foodborne pathogens (Fortes *et al.,* 2013). It is a new technology being extensively used in food analysis for the detection of *Listeria spp*., *Salmonella spp*. and *E. coli 0157:H7* (Loff *et al.,* 2014), and provides rapid and reliable results for the detection of *Listeria monocytogenes* at low levels on both clean and contaminated food processing surfaces (Miks-Krajnik *et al.,* 2016).

The 3M™ Molecular Detection Assay (MDA) - *Listeria* is used for the detection of *Listeria species* in foods, food related and environmental samples after enrichment (Bird *et al.,* 2015). Furthermore, the 3M™ Molecular Detection Assay (MDA) 2 – *L. monocytogenes* method is used for the rapid detection of *L. monocytogenes* in selected foods and environmental samples. After enrichment, *L. monocytogenes* is screened in the samples by 3M™ system following the manufacturer’s recommended protocol (ADAWR, 2019). P resumptive positive results are reported in real-time, while negative results are displayed after completion of the assay of 75 minutes (Bird *et al.,* 2015).

2.7 Treatment, Prevention and Control

An ideal antibiotic must have the ability to penetrate the host cell, bind tightly to an intracellular target and have the capacity to create depots therefore ensuring a long lasting optimal antibiotic concentration (Temple and Nahata, 2000; Kinyua, 2020). The intracellular nature of *L. monocytogenes* and invading virtually all cell types makes its effective antimicrobial treatment difficult (Dhama *et al.,* 2015; Pagliano *et al.,* 2017). Even though, majority of the people with *Listeria* infections can spontaneously clear the infection with in about seven days, patients at increased risk especially pregnant women, usually require immediate intravenous antibiotic treatment to prevent, halt, or slow the development of more severe diseases (Kundul and Ame, 2022).

Ampicillin or penicillin G in combination with an aminoglycoside like gentamicin are antibiotic of choice for treating human listeriosis whereas trimethoprim in combination with a sulphonamide such as sulfamethoxazole and co-trimoxazole is considered as second choice of therapy (Kovacevic *et al.,* 2013). High doses and early treatment are required for animal s with rhombencephalitis, and supportive treatment may also be required (Spickler, 2019). Nevertheless, *L. monocytogenes* growth towards resistance has been accelerating (Moti *et al.,* 2022), judicious use of these drugs in medical and veterinary practices along with monitoring of antibiotic susceptibility is very important (Barbosa *et al.,* 2013; Dhama *et al.,* 2015).

As *L. monocytogenes* is a ubiquitous organism, its complete elimination is an unrealistic aim (Yada and Anato, 2019). Thus, it is impossible to eradicate *L. monocytogenes* permanently from food environments (Buchanan *et al.,* 2017). Therefore, control is a more practical approach which can be achieved by attention to detail in hygiene strategies, monitoring occurrence of the organism or using novel control methods such as bacteriocins and bacteriophage (Leong *et al.,* 2016). As most cases of the human listeriosis are foodborne, various measures targeting food safety should be implemented to reduce the incidence of listeriosis (CHP, 2010)

Contamination of foods by *L. monocytogenes* can occur in all the steps from farm to table, which brings about the need to put forward control measures at every step (Hellström, 2011). The control of *L. monocytogenes* is required at all stages in the food chain and an integrated

approach is needed to prevent the multiplication of these bacteria in the final food product (Manjengwa, 2022). In food processing, a hazard analysis and critical control points (HACCP) program and good hygiene practices have to be followed and closely monitored since it can markedly contribute to the microbiological safety of foods and to less frequent findings of *L. monocytogenes* in food products (CHP, 2010; Kundul and Ame, 2022; Moti *et al.,* 2022). The establishment of HACCP is also important to ensure systematic control of meat slaughter processes regarding microbiological safety, spoilage, and storage stability (Wu *et al.,* 2020; Mpundu *et al.,* 2022).

Proper control of *L. monocytogenes* requires appropriate monitoring and surveillance, at the food plant as well as at governmental level (Hellström. 2011). Surveillance of foodborne disease is an essential component of a modern food safety system, and the data are important for risk assessment, which can guide risk management, including priority-setting and decision making (WHO, 2017). Increasing consumer awareness by providing continuous food safety education through various channels like social media on the need for safer food handling practices such as hand washing and safe storage of RTE food is highly important (Lin *et al.,* 2005).

2.8 Antimicrobial Resistance Profile of *Listeria Monocytogenes*

The discovery of antibiotics as effective therapeutic agents of bacteria has contributed to the protection of humans and animals over the past century. However, the accelerating development of antimicrobial resistant strains of bacteria has risen to the top of the list of global public health priorities (Asfaw *et al.,* 2022). Antimicrobial resistance is caused when pathogenic organisms evolve their mechanism to resist standard antibiotics (Mansour *et al.,* 2021), persist or grow in the presence of drugs designed to inhibit or kill them (FAO, 2023). Antimicrobial resistance is an important public health issue and one of the highest priorities of the World Health Organization, has led to higher patient morbidity and mortality rates and increased healthcare expenditure over the last decades (Friedman *et al.,* 2016; Dufailu *et al.,* 2021).

Antimicrobial resistance is also a food safety issue, as food animals are given antimicrobial agents to prevent disease or, in some places, to promote growth, so that resistant bacteria and resistance genes can pass through the food chain from food animals to humans (WHO,

2017). Previously susceptible *L. monocytogenes* become resistant to antimicrobial drugs currently in use for both human and veterinary medicine (Garedew *et al.,* 2015) and resistant *L. monocytogenes* strains have been reported against first-line antibiotics (Walsh *et al.,* 2001). Moreover, various studies have reported *L. monocytogenes* strains that showed multiple drug resistance to different antimicrobial substances (Altuntas *et al.,* 2012; Kidanu *et al.,* 2021).

In Ethiopia, antimicrobial susceptibility pattern of *L. monocytogenes* is lacking both in the veterinary and public health sectors (Kidanu *et al.,* 2021), except some fragmented study done in different parts of the countries. Isolated *L. monocytogenes* from RTE animal origin in Gondar town shows 66.7%, 50%, 37.5% and 16.6% resistance to penicillin, nalidixic acid, tetracycline and chloramphenicol respectively. Furthermore, 16.6% were found to be multi-drug resistant (Garedew *et al.,* 2015). Mulu (2014) also reported isolates of *L. monocytogenes* from sheep meat in municipal abattoir and butcher shops in Addis Ababa were 77.8% resistance to Tetracycline. *L. monocytogenes* isolated from pregnant women in Tigray region by Welekidan *et al.* (2020) were resistant to clindamycin (66.7%), penicillin G (66.7%), vancomycin (50%) and amoxicillin (50%).

2.9 Epidemiology of *Listeria Monocytogenes*

2.9.1 Global Epidemiology

Listeriosis is an emerging major public health concern infection worldwide due the occurrence of associated foodborne outbreak and significant risk of mortality and morbidity (CHP, 2010; Moti *et al.,* 2022). *L. monocytogenes* had caused episodes of human listeriosis throughout the world (Orsi and Wiedmann, 2016) and globally distributed with varying prevalence rates in the different regions (Dufailu *et al.,* 2021). The Center for Disease Control and Prevention (CDC) estimates approximately 1,600 cases of listeriosis, 1,500 hospitalizations with rate of 94%, and 260 deaths occur annually in the USA (Hammons and Oliver, 2014; Osek *et al.,* 2022). In Europe, the disease caused an estimated 1781 cases of illness, 399 deaths and almost 15000 DALYs in the WHO European Region in 2010 (WHO, 2017).

Approximately 99% of listeriosis cases result from contaminated food (Hammons and Oliver, 2014). *L. monocytogenes* is frequently isolated from food of animal origin such as ready-to-eat meat products, ground beef, meat and meat products (sausages), fish and fish products, milk, and pasteurized dairy products like soft cheese and ice cream (Abebe *et al.,* 2020). EFSA and ECDC. (2019) points out that RTE food represents one of the most important routes of *Listeria* transmission, with an occurrence of 2.7% for fish and fishery products, 1.4% for meat and meat products, 1.8% for RTE fruit and vegetables. In 2020, 14 Member States in European Union reported over all *L. monocytogenes* detection of 7.4% from RTE bovine meat products of 856 units tested in EU (EFSA and ECDC, 2021). In a survey of the 2000 – 2007 journals in China, the prevalence of *L. monocytogenes* in raw meat was reported at about 25% (Chen *et al.,* 2009).

In developing countries like Africa, most of the times there have been few or no reports on *L. monocytogenes* (Molla *et al.,* 2004). Thus, the epidemiology of listeriosis in Africa is less understood, and can lead to underestimation of the disease burden on the continent (Dufailu *et al.,* 2021). There has been no appropriate consideration of its incidence in Africa, and there have been few reports (Molla *et al.,* 2004). In Africa, *L. monocytogenes* has been isolated from various food and environmental sources in countries such as South Africa, Nigeria, Ethiopia, Ghana, Egypt and Botswana (Dufailu *et al.,* 2021). In South Africa, an outbreak of listeriosis between January 2017 and July 2018 caused 1,060 confirmed cases and 216 recorded deaths This outbreak awakened the world to the possible wide-spread of the disease in Africa (Tchatchouang *et al.,* 2020). The overall prevalence of *L. monocytogenes* in meat and meat products from 2014 to 2016 in South Africa was reported to be 14.7%, with meats from local markets and ports of entry recording prevalence rates of 15.0% and 12.4% respectively (Matle *et al.,* 2019).

Epidemiological investigations indicated that ready to eat processed meat products from a f ood production facility contaminated with *L. monocytogenes* were responsible for the outbr eak (Tchatchouang *et al.,* 2020). In Nigeria, the prevalence rate of 88% were reported by Lennox *et al.* (2017) in food of animal origin of which prevalence rate was 64.7% and 33.7% in chicken and fish respectively.

2.9.2 Occurrence of Listeria Monocytogenes in Ethiopia

Although foods of animal origin such as milk, cheese, meat, and poultry are consumed well in Ethiopia, published information on the status of food-borne Listeriosis caused by *L. monocytogenes* is very limited and incomplete in both the veterinary and public health sectors (Mulu, 2014). There were also no well-organized epidemiological surveillance systems at the national level (Moti *et al.,* 2022). However, there are some published information on the prevalence of *L. monocytogenes* in food of animal origin in different regions of Ethiopia in different samples (Abebe *et al.,* 2020; Kundul and Ame, 2022).

The overall occurrence of 4% *L. Monocytogenes* from raw meat samples of cattle collected from abattoirs, butchers, and restaurants of Ambo and Holeta Towns was reported by Gebremedhin *et al.* (2021). Tiku *et al.* (2020) also revealed 7% prevalence of *L. monocytogenes* along meat production chain in southwest Ethiopia. Isolates of 18.3% *L. monocytogenes* were also obtained by Derra (2007) from retail meat products in Addis Ababa and its surrounding towns. The study conducted on meat samples in Mekelle city were revealed 4.17% occurrence of *L. monocytogenes* (Kidanu *et al.,* 2021. Similarly, Zelalem *et al.* (2019) conducted meta-analysis on meat samples revealed pooled prevalence of 4% *L. monocytogenes* isolates.

Study conducted on human Listeriosis in Tigray Regional states, Northern Ethiopia among pregnant women shows prevalence rate of 8.5% (Welekidan *et al.,* 2019). Similarly, in women attending Jimma University centre, *L. monocytogenes* was isolated with prevalence rate of 5.56% (Girma *et al.,* 2021).

Table 2: Prevalence of *Listeria monocytogenes* from beef meat in Ethiopia

Study area	Sample unit	Prevalence	Author
Addis Ababa city	Butcher shops	2.6%	Gebretsadik *et al.* (2011)
Mekelle city	Butcher shops	8.33%	Kidanu *et al.* (2021)
	Restaurants	8.33%	
	Abattoir	1.67%	
Gondar Town	Public dinning places	6.6%	Garedew *et al.* (2015)
Bishoftu and Dukem Towns	Butcher shops, hotels and supermarkets	11.2%	Fisseha, (2017)
Addis Ababa and its surrounding towns	Retail shops	18.3%	Derra, (2007)
Jimma town	Municipal abattoirs and butcher shops	7%	Tiku *et al.* (2020)
Addis Ababa and its surrounding towns	Retail shops	6.8%	Derra *et al.* (2013)
Ambo and Holeta towns	Abattoir, Butcher shops and restaurants	4.4%	Gebremedhin *et al.* (2021)

2.10 Regulations Relating to *Listeria Monocytogenes*

Regulations relating to *L. monocytogenes* in food remain a serious threat to public health and a significant impediment to socio-economic development worldwide (Grace, 2015). Most food legislation requires the microbial criteria for food-borne bacteria such as *L. monocytogenes* or their toxins and metabolites in specific foods (Tabit, 2018). There are several regulations concerning the acceptable microbiological level of *L. monocytogenes* in RTE foods (Sanlibaba *et al.,* 2018). These criteria often prescribe the acceptable levels of these bacteria or their toxins in food products available on the market (Luber, 2011).

In responses to the threat posed by food-borne pathogens, many countries responded through implementation of strict regulations for microbiological standards or criteria in relation to contamination of food products (Matle *et al.,* 2020). In the United States, it is 0 CFU/g of *L. monocytogenes* per 25g of the food sample (Sanlibaba *et al.,* 2018). The United States Food and Drug Administration (FDA) imposes a zero-tolerance regulation to *L. mon*

ocytogenes in ready-to-eat and processed foods (Setiani *et al.,* 2019) and failure to comply in food processing facilities is considered as serious offence (Piet *et al.,* 2016).

The European union microbiological standard for *L. monocytogenes* in RTE food products is in accordance with Codex Alimentarius Commission (CAC) recommendations (Matle *et al.,* 2020). According to the European Commission (EC) regulations, the number of *L. mon ocytogenes* should be less than 100 CFU/g in RTE products at the time of consumption and throughout shelf life. RTE foods supporting the growth of *L. monocytogenes* must not pres ent in 25g of the sample before the food has left the immediate control of the food industry (Sanlibaba *et al.,* 2018). Australia, Canada and New Zealand implemented microbiological standards similar to those of Europe as recommended by CAC (Jadhav, 2015). In Turkey, the Turkish Food Codex has stipulated that *L. monocytogenes* must not be detected at all in the RTE foods (Sanlibaba *et al.,* 2018).

In Africa, in general there is little awareness or regulation relating to *L. monocytogenes*. One South African voluntary standard (South African National Standard, SANS: 885:2011), specifically refers to the prevalence of *L. monocytogenes* in processed meat products, allows a maximum of 100 CFU/g at the end of shelf-life (Leong *et al.,* 2016; Matle *et al.,* 2020).

In Ethiopia the main government organs responsible for food safety are the Ministry of Health, Ministry of Agriculture and the Quality and Standardization Authority of Ethiopia (Now named as Institute of Ethiopian standards). Institute of Ethiopian standards is empow ered to set food standards and has developed a number standards related to quality assurance and the safety of food of animal origin, which are supposed to be followed by all stakeholders implementing authorities and compliers like producers, market agents, industr ies (Jabbar and Grace, 2012). However, there was no recorded and ratified Ethiopian stand ard legislation which set safe limits for *L. monocytogenes* in food of animal origins including beef and its products.

2.11 Public Health and Economic Importance

Listeriosis was the fourth leading foodborne hazard in terms of deaths and the fifth in terms of DALYs. In 2010, listeriosis was responsible for 23,150 illnesses, 5463 deaths and 172,823 DALYs globally and an estimated 1781 cases of illness, 399 deaths and almost 15 000

DALYs in European Region (De Noordhout *et al.,* 2014). In developed countries like the USA, England and Wales, approximately 20% - 25% of infections have been reported to lead to abortion and still birth (McLauchlin *et al.,* 2004).

L. monocytogenes is most important as a cause of pregnancy losses in healthy women, and septicaemia or CNS disease (Spickler, 2019). Although it 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 (Kundul and Ame, 2022). Humans have developed fatal meningitis, sepsis, and papular exanthema on the arms after handling aborted material (Constable, 2023).

Foodborne disease not only adversely affects people's health and well-being but also has negative economic consequences for individuals, families, communities, businesses and countries (WHO, 2017). A country's vibrant workforce, cost involved in prevention, treatment, product recall and investigations put an enormous financial burden not only on governments but also on consumers and food industry (Mensah and Ofosu, 2020). In USA, the annual cost of *L. monocytogenes* was estimated to be US$ 2.322 billion, and the annual benefit of Listeria food safety measures was $0.012.4 billion (Ivanek *et al.,* 2004).

L. monocytogenes is the major concern for the food quality and safety in food industries (Centorame *et al.,* 2017) and place a substantial burden on health-care systems, trade and tourism (WHO, 2017). Furthermore, the traceability of foods has become vastly more complex (WHO, 2017), withdrawal of products from the consumer marketplace and a decrease in sales of the incriminated products (Lee *et al.,* 2021).

As in humans, reproductive losses, CNS disease and septicaemia are the most serious syndromes in ruminants. Animals may abort, typically late in gestation, or give birth to stillborn offspring, and some live neonates may develop septicaemia (Spickler, 2019). As an animal pathogen, listeria monocytogenes is a cause of concern not only in terms of public health and food safety but also as a significant cause of economic losses when livestock and their offspring are affected (Bagatella *et al.,* 2022) and may lead to huge economic losses in livestock industries (Dhama *et al.,* 2015).

3. MATERIAL AND METHODS

3.1 Study Area

The study was conducted from December, 2022 to May, 2023 at Addis Ababa abattoirs enterprise and butcher shops found in Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia and the residence of African Union. It is located at 9.1.1.48N and 38.44,.24E with 8°30'and 40°27' N latitude and 34°21' and 39°1' E longitude. Addis Ababa city has an area of 54,000 hectares with an average elevation of 2,400 meter above sea level and average minimum and maximum temperature of 11.10c and 23.80c respectively and an average annual rainfall of 1175.8. On July 1, 2022 Addis Ababa has estimated human population of 3,859,638 of which 1,821,690 are males and 2,037,948 are females (CSA,2013).

Addis Ababa Abattoirs Enterprise is high output abattoir in the country which providing 50% of the daily beef requirements of the city's residents (Mulu, 2014) in which higher number of cattle are often slaughtered and low number of shoats and swine are slaughtered on daily basis. Even though, it was difficult to know the exact origin of the animals, the slaughtered animals were mainly brought from highland areas of Jimma, Wollo, Wollega, Wolaita, Debreberhan, Hararghe as well as around Addis Ababa and from lowland areas of Awash, Borena and Afar. Regular meat inspection is being conducted by meat inspectors assigned from the Addis Ababa Agricultural Commission.

3.2 Sample Size Determination

The required sample size was determined by using the formula recommended by Thrusfield (2005) by considering the prevalence of 6.8% (Derra *et al.,* 2013) of *L. monocytogenes* previously done at Addis Ababa for raw beef meat samples and 6.7% (Mulu, 2014) for environmental samples (knife, cutting tables and personnel's hand) samples. This sample size determined using 95% confidence interval and 5% desired absolute precision.

$$n = \frac{Z2\ P\ exp\ (1-Pexp)}{sd2}$$

Where, n is required sample size; Pexp is the expected prevalence of 6.8% and 6.7% for carcass and environmental samples respectively, sd is the standard deviation (5%), and Z = 1.96.

Thus, the required sample size was calculated to be 98 and 96 for carcass and environmental samples respectively with total samples of 194. However, to increase the precision of the study total samples of 280 were taken. Accordingly, 150 samples consisting of 60 carcass swabs, 30 workers hands, 30 knifes and 30 from vehicles were taken from abattoir whereas 130 from butcher's shops (60 from beef carcass, 25 from cutting boards and 25 from knifes and 20 butcher workers hand) were allocated and sampled for microbiological analysis of *L. monocytogenes*.

3.3 Study Design

A cross-sectional study was conducted from December, 2022 to May, 2023 at Addis Ababa abattoirs enterprise and butcher shops in Addis Ababa. Beef carcasses swabs of slaughtered cattle at abattoir were sampled by using systematic random sampling depending on the number of animals slaughtered on sampling day. The beef carcasses presented for sales in different butcher shops and environmental samples (swab samples from knife, workers hands, vehicles and cutting boards) were also sampled using simple random sampling methods.

The samples were then transported to Animal Products and Inputs Quality Testing Centre (APIQTC), Addis Ababa. This Quality Assessment Centre was established by formerly named office Veterinary Drug and Animal Feed Administration and Control Authority (now changed to Ethiopian Agricultural Authority). This laboratory serves and mandated in analysing of different samples from animal origin like meat and meat products, milk and milk products and other animal source foods) for their microbiological quality.

3.4 Study Samples

Study samples were collected from beef carcasses and environmental swabs from Addis Ababa abattoirs enterprise and fresh beef meat samples as well as environmental swabs from several butcher shops found in Addis Ababa for microbiological analysis of *L.*

monocytogenes. On each sampling day, all the required samples including bovine meat swab sample, knives, cutting tables, workers hands and vehicles) were taken. Swab samples were collected aseptically using systematic random sampling technique from the carcasses of daily slaughtered cattle immediately after hide removal and evisceration at the abattoir. Fresh beef meat samples and environmental swabs of butcher shops were collected from legally registered butcher shops using simple random sampling techniques after obtaining a list of active and legally registered butcher shops in Addis Ababa and their distribution lines from Addis Ababa Abattoir Enterprise.

A total of 280 samples of beef carcasses and related environmental samples, comprised of 60 carcass swabs and 90 environmental swabs (Knifes, Worker's hand and vehicles) from Addis Ababa abattoirs enterprise and 60 beef carcasses and 70 environmental swabs (knifes, cutting boards and personnel hands) from butcher shops were collected for detection of *L. monocytogenes*. Beef carcass from butcher shops were purchased from butcher shops found in Addis Ababa.

3.5 Sample Collection and Transportation

The selected carcasses at Addis Ababa abattoirs enterprise were swabbed using the method described in ISO 17604 (2015) by placing sterile template (10 x 10 cm) to outline on specific sites of a carcass marked for swabbing just after the stage of evisceration. The abdomen (flank), thorax (lateral) and breast (lateral) which are sites with the highest rate of contamination (ISO 17604, 2015); were chosen for pooled sampling using the same swab over all the sites. A sterile cotton tipped gauze pad (10 x 10cm) fitted with shaft, was first soaked and rehydrated in an approximately 10 ml of buffered peptone water (3M Health care, USA) and rubbed first horizontally and then vertically several times on the beef carcasses without distinction of breed, sex or age. On completion of the rubbing process, carcass swabs were placed in screw-cap tubes with transport medium (buffered peptone water).

At each abattoir visit, environmental samples (abattoirs workers hand, knifes and vehicles) were also collected by swabbing the abattoirs workers hand, knifes and vehicles used for transporting carcass to butcher shops. All samples were collected aseptically using disposable gloves to avoid cross contamination and inserted into sterile tubes that contain

10 ml of buffered peptone water (3M Health care, USA). Samples of beef carcasses (250 grams) were purchased from each of the randomly selected butcher shops. All samples were collected aseptically and labelled with necessary information including the sample identification number, type of samples (carcass swabs, carcass and environmental swabs), sampling place and date of collection.

Finally, all collected samples were immediately transported to APIQTC, Addis Ababa, Ethiopia using ice box with ice packs in cold chain for microbiological analysis and samples were processed within 2 hrs of arrival at the laboratory.

3.6 Isolation and Identification of *Listeria monocytogenes*

3.6.1 Detection of Listeria Monocytogenes

The samples collected from abattoir (beef carcass swabs and environmental swabs) and carcass samples from butcher shops were tested for the presence of *L. monocytogenes* following the procedures recommended by the International Standards Organization (ISO 11290-1, 2017) with inclusion of 3M^{TM} Molecular detection system. Twenty-five (25) grams of carcass samples were weighted and chopped aseptically into small pieces using sterile knife. The carcass samples and swab samples were transferred in to sterile plastic bag containing 225 mL and 90 mL demi Fraser broth (3M Health Care, USA) respectively, then homogenized using a laboratory blender (homogenizer) at speed of 260rpm for 2 minutes and incubated at 37 °C for 24 - 48 hours. After the completion of the incubation period, 0.1 mL of this primary enrichment broth was transferred into tubes containing 10 mL of Fraser broth and incubated at 37 ◦C for 24 - 48 hours.

After sub cultured samples in Fraser broth were incubated for 48 h at 37°C, enrichments were gently agitated and 20 µL of each enrichment was aliquoted into a separate 3M lysis tube as manufacturer's protocol (3M Health care, USA https://www.3m.com/3M/en_US/food-safety-us/). After all samples were transferred to lysis tube, 20 µL negative control (NC)) and One reagent tube inserted and transferred into lysis tube. The rack of lysis tube (LS) was covered with the rack lid and firmly invert three to five times to mix, and LS tubes were placed in the heat block for 15 minutes at 100 °C. The rack of LS tubes was removed from heating block and allowed to cool for 10 minutes. Then, 20 µL of each sample lysate from

the upper portion of the fluid in the LS tube were transferred into corresponding reagent tube at angle to avoid disturbing the pellets and mixed by gently pipetting up and down five times and reagent tubes were covered with the provided cap. For quality control of the test method, 20 µL NC and Reagent control without sample lysate and Matric control containing sample lysate were used according to the 3M molecular detection assay protocol.

Capped tubes were loaded into clean and decontaminated 3M Molecular Detection Speed Loader Tray, then closed and latched tray lid. Launched 3M Molecular Detection Software were log in and samples code of each sample were coded in appropriate place complied in detection kits. Then 3M molecular detection instrument was turned on to run. Presumptive positive results were reported in real time, while negative results were displayed at the end of the 75 minutes run. All samples detected and identified as presumptive positive for *L. monocytogenes* using 3M™ molecular detection assay were further tested for isolation a nd identification.

3.6.2 Isolation and Identification

Presumptive positive results by 3M™ Molecular Detection Assay 2 – *L. monocytogenes* were confirmed by using ISO 11290 -1 (2017) methods followed by transferring positive isolates from secondary enrichment and direct plating onto selective media and GEN III MicroPlate automated confirmation (Biolog, Hayward, CA 94545, USA, https://www.biol og.com/wp-content/uploads/2020/04/00P_185_GEN_III_MicroPlate_IFU.pdf.). A loopful of full Fraser enrichment broth cultures (10 µL) detected to be positive were streaked onto Listeria selective agar and incubated at 37°C for 24 - 48 hours. Characteristic colonies presumed to be *Listeria* were transferred to Biolog Universal Growth (BUG) agar which is Biolog recommended agar media and incubated at 37°C for 24 hours.

Pure colonies from BUG Agar were inoculated into inoculating fluid - A (IF -A) using cotton tipped Inoculator swab and checked for its turbidity to standardize the bacterial conc entration. The inoculum was prepared at desired turbidity of 95% which is target cell density. The cell suspension in inoculating fluid was transferred to all wells of GEN III MicroPlate using multichannel pipet (8-Channel). The MicroPlate was covered with its lid and incubated at 33°C for 22 hours. Then, MicroPlate was read using Biolog microbial identification Systems software of Biolog MicroStation™ to confirm the *L. monocytogenes.*

3.7 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of *L. monocytogenes* isolates was examined against nine commercially available antimicrobial discs (Condalab, Spain) as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018) on Mueller-Hinton agar by using disk diffusion methods. The eight antimicrobial discs used include amoxicillin (2μg), ampi cillin (10μg), gentamicin (10μg), vancomycin (30μg), streptomycin (10μg), penicillin G (10μg), tetracycline (30μg), and trimethoprim-sulfamethoxazole (25 μg).

From each isolated colonies on BUG agar, two to three pure fresh colonies of the isolates of *L. monocytogenes* were taken and suspended in brain heart infusion broth and incubated at 37°C for 24 hours. Then, bacterial suspension was adjusted to 0.5 McFarland turbidity standard and bacterial suspension was spread on the Muller Hinton agar. After the plates were dried (about 10 min), with the aid of automatic disc dispenser, antibiotic impregnated discs were placed to the surface of the inoculated plates and incubated at 37°C for 24 hrs. Finally, the diameter of growth inhibition zone around each disc was measured, with the help of a calliper and the results were classified as Susceptible (S), Intermediate (I), and Resistant (R) categories in accordance with the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2018).

3.8 Questionnaire and Observational Survey.

A semi-structured and pretested questionnaire survey and observational checklist were used to assess the abattoirs and butcher shops workers knowledge and hygienic practice in processing and handling beef along the meat value chain. Questionnaire survey was conducted by translating questions to local languages (Afaan Oromo or Amharic) known by respondents and recorded on designed questionnaire format in which the questions and answers were written in English. The questionnaire was administered by using face-to-face method to collect their responses to a total of 120 respondents (60 from abattoir and 60 from butcheries shops) who have direct contact with beef carcasses. The questions were administered with the key elements including level of education, exposure to training on meat handling, hygiene and safety and personal hygienic practices. The demographic characteristics of respondents including sex, marital status, educational status time on work per day were also investigated.

3.9 Data Management and Analysis

The collected and generated data from laboratory tests and questionnaire surveys were entered into Microsoft Excel spreadsheets (Microsoft Corporation), transferred and analysed using STATA version 14.2 software (Stata Crop. College Station, USA). Descriptive statistics was used to summarize the occurrence or prevalence, socio demographic characteristics of the respondents and antimicrobial susceptibility data of *L. monocytogenes* using percentages.

3.10 Ethical Clearance

For this research, the ethical clearance was approved and obtained from Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture, Addis Ababa University (Certificate Ref. No: VM/ERC/21/04/15/2023).

On the other hand, personnel-based samples were collected after proper explanation of the objectives of this study to the abattoir and butcher shops workers and samples were taken based on their consent.

4. RESULTS

4.1 Overall prevalence of *Listeria Monocytogenes*

Among 280 samples collected both from abattoirs (n=150) and butcher shops (n=130) in Addis Ababa, the overall prevalence of *L. monocytogenes* was found to be 4.64 % (13/280). The occurrence of *L. monocytogenes* was 4.67 % (7/150) in abattoirs and 4.62 % (6/130) in butchers' shops. The occurrence was varied between sample sources which is slightly higher in abattoirs than butcher shops with no statistically significant difference (P > 0.05) (Table 3).

Table 3: Overall prevalence of *Listeria monocytogenes* by source of samples

Sample Source	Number of samples collected	*Listeria monocytogenes* (%)	
		Negative	**Positive**
Abattoir	150	143	4.67 % (7/150)
Butcher Shops	130	124	4.62 % (6/130)
Total	**280**	**167**	4.64 % (13/280)

From 120 meat samples (60 from abattoirs and 60 from butcher shops), 7(5.83%) were positive for *L. monocytogenes*, of which 4 (3.33%) were isolated from abattoir samples and 3(2.5%) were from the butcher shops. Out of 150 samples examined from Addis Ababa abattoirs enterprise, *L. monocytogenes* was isolated from one abattoir's knife swab sample (0.67%) and two (1.33 %) swab samples from abattoir's worker's hand. From 130 samples collected from butcher shops, one swab sample collected from butcher's hand and one swab sample from butcher's knife were tested positive for *L. monocytogenes*. The proportion of isolates were found to be 6% (3/50) and 3.64% (2/55) from personnel hand (abattoirs and butcher shops) and knives (from abattoirs and butcher shops) respectively.

From 30 swabs samples collected from abattoirs vehicles no *L. monocytogenes* were recovered. The distribution of *L. monocytogenes* isolates in various abattoir's samples were analysed. Accordingly, the proportion of isolates were carcass swab (6.67 %), workers' hand (6.67 %) and knife (3.33%). Six (4.61%) of 130 butcher shops samples were positive for

Listeria monocytogenes with occurrence rate of 5 %, 4 %, 5 % and 4 % from carcass, cutting boards, butcher's hand and knifes respectively (Table 4).

Table 4: Occurrence of *Listeria monocytogenes* by samples sources and sample types

Sample source	Sample types	No. Examined	Occurrence (%)
Abattoirs	Carcass swabs	60	4 (6.67%)
	Personnel hand	30	2 (6.67%)
	Knifes	30	1 (3.33%)
	Vehicles	30	0 (0%)
	Sub Total	**150**	**7(4.67%)**
Butcher shops	Carcass	60	3 (5%)
	Cutting boards	25	1 (4%)
	Personnel hand	20	1 (5%)
	Knifes	25	1 (4%)
	Sub Total	**130**	**6 (4.61)**
Total		**280**	**13(4.64%)**

4.2 Antimicrobial Susceptibility of *Listeria monocytogenes*

Antimicrobial susceptibility test was performed for *L. monocytogenes* isolates by using disc diffusion method on Muller Hinton Agar. The results of antibiotic resistance tests were considered as resistant, intermediate and susceptible according to CLSI (2018) criteria and the diameter of zone of inhibition as measured in millimetre by calliper. A total of thirteen confirmed isolates were assessed for antimicrobial susceptibility pattern with eight comme rcially available antibiotics disks. Out of 13 isolates, 12 (92.3%) were resistant to tetracycline and amoxicillin, 11(84.6) % were resistant to streptomycin, 10 (76.9%) were resistant to ampicillin and 8(61.5%) were resistant to vancomycin. However, all isolates were found susceptible to Trimethoprim-sulpha methazole, 10 (76.9%) were susceptible to penicillin G. Twelve (92.3%) isolates showed intermediate resistance to gentamycin. Furth ermore, eleven (84.6%) isolates were found to be MDR. One (7.7%) isolate was resistant to two antibiotics whereas ten (76.9%) were resistance to three and more antibiotics. The most

common MDR pattern was observed against amoxicillin, ampicillin, tetracycline and streptomycin (Figure 2).

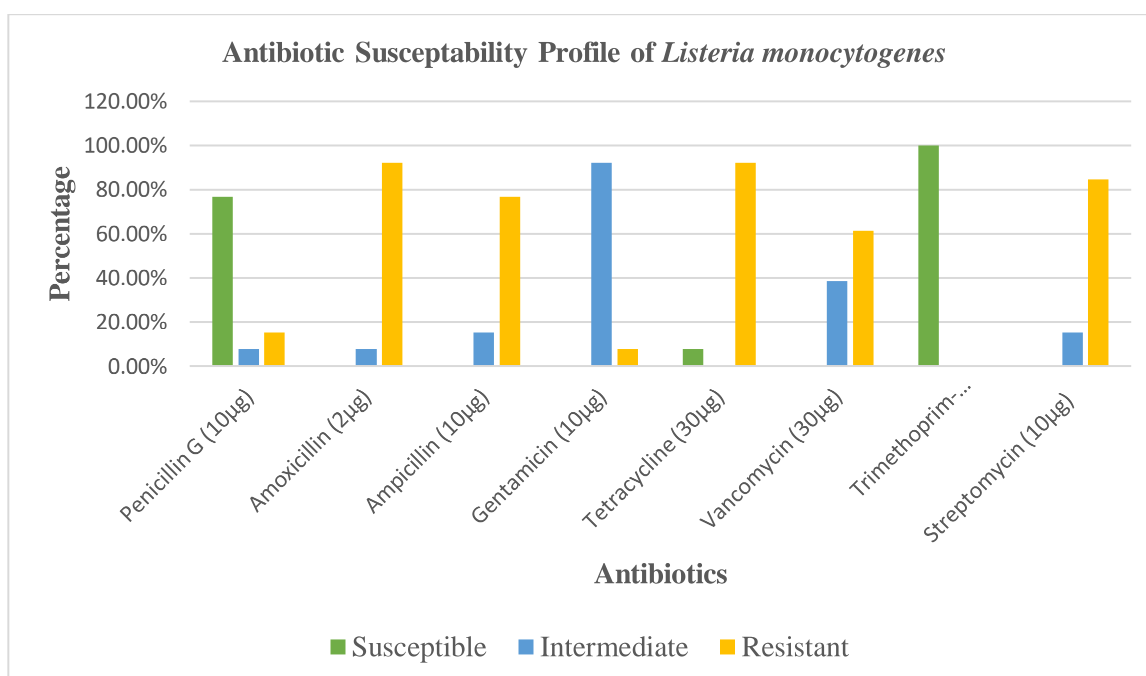

Figure 2: Antimicrobial Susceptibility Profiles of *Listeria Monocytogenes*

4.3 Socio-demographic characteristics of abattoir and butcher shops workers

All respondents from both abattoir and butcher shops were male only. Most respondents from abattoirs (46.67%) were in age of ≥ 35 years and 40% were in age between 18 – 24 years. Twenty-six (43.33%), 18 (30%) and 16 (26.67%) of butcher shops respondents were found in age ≥ 35, 25 -34 and 18 – 24 years respectively. Half (50%) of abattoir workers respondents attended primary education whereas as 45% of butcher shops respondents attended primary and secondary education each. The majority of the respondents from abattoirs had ≥ 4 years of work experience and worked ≤ 8 hr per day. Butcher shop respondents (65%) were working ≥ 9 hours per day (Table 5).

Table 5: Socio-demographic characteristics of respondents (abattoir and butcher shops)

Variables	Categories	Abattoirs personnel		Butcher shops personnel	
		Frequency	**%**	**Frequency**	**%**
Sex	Male	60	100 %	60	100 %
	Female	-	-	-	-
Age in Years	18-24	8	13.33%	16	26.67 %
	25-34	24	40 %	18	30 %
	≥ 35	28	46.67%	26	43.33 %
Marital Status	Single	14	23.33 %	22	36.67%
	Married	46	76.67%	38	63.33%
Education Level	Primary	30	50 %	27	45%
	Secondary	17	28.33%	27	45%
	Tertiary	13	21.67%	6	10%
Work experience in years	≤ 1	5	8.33 %	5	8.33%
	1-3	9	15 %	10	16.67
	≥ 4	46	76.67%	45	75 %
Working times per day	≤ 8 hours	60	100%	15	25 %
	≥ 9 hours	0	-	45	75 %

The majority of respondents from abattoirs had information (86.67%) and knowledge (73.33%) on meat hygiene and safety. However, the majority of respondents from butcher shops had no information (90%) on meat hygiene and safety. Majority of of respondents (91.67%) from abattoirs had training on meat hygiene whereas all respondents from butcher shops had no any training. Most of responents from both abattoir and butcher shops were washing their hands even though most of them did not wash their hands with soap or deteregents. From the observational assessment, 81.67 % of butcher shops respondents did not wear head cover while 26.67 % and 25% of abattoir workers respondents had no aprons and head cover respectively. Most butcher shops (61.67 %) respondents wore jewellery and 93.33% were handling money by themselves without having cashier (Table 6).

Table 6: Knowledge and Practices on Meat hygiene and safety of abattoirs and Butcher shops workers

Knowledge and practices	Categories	Abattoirs workers		Butcher Shops	
Knowledges		**Freq.**	**%**	**Freq.**	**%**
Information on Meat Safety and Hygiene	Yes	52	86.67 %	6	10 %
	No	8	13.33 %	54	90 %
Training on Meat Safety	Yes	55	91.67 %	0	-
	No	5	8.33%	60	100%
Knowledge on Meat Safety and Hygiene	Yes	44	73.33 %	5	8.33 %
	No	16	26.67 %	55	91.67 %
Practices					
Washing hands	Yes	60	100 %	60	100%
	No	0	-	0	-
Washing hands with soap	Yes	15	25 %	14	23.33 %
	No	45	75 %	46	76.67 %
Washing knifes	Yes	54	90 %	29	48.33 %
	No	6	10 %	31	51.67 %
Wearing aprons	Yes	44	73.33 %	-	-
	No	16	26.67 %	-	-
Wearing boots	Yes	60	100 %	-	-
	No	-	-	-	-
Wearing head cover	Yes	45	75 %	11	18.33 %
	No	15	25 %	49	81.67 %
Wearing jewellery	Worn	0		37	61.67 %
	Not worn	60	100%	23	38.33 %
Cash Handling	Cashier	-	-	4	6.67 %
	By Themselves	-	-	56	93.33 %

5. DISCUSSION

Isolation of *L. monocytogenes* from raw meat should be considered as a microbiological hazard detection for people (Gebremedhin *et al.,* 2021), since there was practicing of eating raw meat in Ethiopia where high prevalence of *L. monocytogenes* was recorded from cattle products (Derra, 2007). It is widely diffused in the environment and can cause the contamination of food during production and distribution which can easily enter in the food chain (Raorane *et al.,* 2014).

The present study revealed an overall prevalence of 4.64% for samples collected from abattoir and butcher shops collectively with no statistically significant difference based on sample sources. This finding was in agreement with previous study in Ethiopia in the same study area by Mulu (2014) who reported 4.1% at municipal abattoir and butcher shops and the findings of Babiker *et al.* (2020) who reported a prevalence of 5.5% at Tambool town slaughter point and butcher shops in Central Sudan. However, the present study was slightly lower when compared to 7% prevalence by Tiku *et al.* (2020) in abattoirs and butcher shops of Jimma town. In this study the prevalence of *L. monocytogenes* was 4.67% and 4.62% from abattoir and butcher shops respectively. This finding of 4.67% from abattoir samples were in agreement with Babiker *et al.* (2020) in slaughter house from Sudan with isolation rate of 6.6% and was slightly in consistence with 7% prevalence by Tiku *et al.* (2020) in abattoir samples of Jimma town. The current finding of 4.67% of *L. monocytogenes* from abattoir samples were higher when compared to 1.66% prevalence by Çadirci *et al.* (2018) from a slaughterhouse in Samsun, Turkey, 2.1% reported by Mulu (2014) from Addis Ababa abattoir enterprise, and1.67% by Kidanu *et al.* (2021) from abattoir in Mekelle city.

On contrary, the 33% prevalence recorded by Gebremedhin *et al.* (2021) in Beef at Abattoirs in Ambo and Holeta town, and 26.3% by Mpundu *et al. (*2022) in beef processing abattoirs in Namwala district, Zambia were much higher than the present finding. The difference in hygienic conditions of slaughter houses, storage and processing in different countries could be attributed for these different findings (Babiker *et al.,* 2020). Moreover, contamination differences were due to environmental dissimilarities such as slaughter throughputs schedules of individual abattoirs and abattoir designs (Fasanmi *et al.,* 2018).

In the current study, the isolation rate of *L. monocytogenes* from butcher shops was comparable to prevalence report of 5.5% by Mulu (2014) in butcher shops in Addis Ababa and pooled prevalence of 6% reported by a systematic review and meta-analysis by Zelalem *et al.,* (2019) from meat samples collected in butcher shops in Ethiopia. However, this prevalence was higher when compared to the findings of Gebremedhin *et al.* (2021) from Ambo and Holeta Towns (1.6%) and 2.6% by Babiker *et al.* (2020) from butcher shops of Aljazeera state, central Sudan. Slightly higher occurrence of *L. monocytogenes* of 7% was found by Tiku *et al.* (2020) in Jimma town butcher shops. The difference in the prevalence at different butcher shops are probably as a result of cross contamination (Fisseha, 2017), due to poor hygienic handling and processing, the suitability of the meat pH, water activity, and nutrient content (Alsheikh *et al.,* 2012). Furthermore, the temperature fluctuation during distribution, meat contamination by handlers, lack of hygiene and unsafe loading and unloading practices might have contributed for slight increment of meat contamination in retail outlets (Zelalem *et al.,* 2019).

In the present study, carcass was contaminated with *L. monocytogenes* both in abattoir and butcher shops. This finding was in agreement with reports of Mulu (2014); Babiker *et al.* (2020); Tiku *et al.* (2020); Gebremedhin *et al.* (2021) and Kidanu *et al.* (2021). This might be due to raw bovine carcasses were exposed to contaminants during slaughtering, processing and retailing (Kidanu *et al.,* 2021), during distribution of the meat and improper handling of the meat handlers who sold it (Mulu, 2014).

In this study, *L. monocytogenes* was revealed in 5.83% (7/120) of beef carcasses in Addis Ababa abattoirs enterprise and butcher shops in Addis Ababa. The practice of consuming raw beef in Ethiopia could therefore pose a public health risk as a result of potential contamination of beef with *L. monocytogenes* (Diriba *et al.,* 2021). Moreover, studies have shown that it has been known to contaminate raw meat slices, ground meat and meat processing environments which could aggravates the public health risk (Fisseha, 2017; Moti *et al.,* 2022).

The revealed isolation level of 5.83% from beef carcass is similar to finding of 6.8% by Derra *et al.* (2013) from Addis Ababa, 4.17% by Kidanu *et al.* (2021) from Mekelle city, 6.66% by Garedew *et al.* (2015) from Gondar town and 3.92% by Ndahi *et al.* (2014) from Nigeria. Furthermore, 6.6% prevalence of *L. monocytogenes* by Babiker *et al.* (2020) and

5% by Ankpolat *et al.* (2004) in beef carcass were in compliance with the current finding. The pooled prevalence of 4% reported by Systematic review and meta-analysis of Zelalem *et al.* (2019) from beef meat in Ethiopia, 4.44% isolation rate by Gebremedhin *et al.* (2021) from raw beef in Ambo and Holeta Towns and 4.2% estimate in meat products in Ireland by Leong *et al. (*2014) were also found in compliance with the present finding.

In contrary, the present finding of 5.83% *L. monocytogenes* from beef carcass was higher when compared to no detection from raw beef by Bingol *et al.* (2013) and 2.77% by Çadirci *et al.* (2018) from cattle carcass surface in Turkey, 0.77% by Vanderline *et al.* (1998) in Australia and 2.6% by Gebretsadik *et al.* (2011) in Addis Ababa. Furthermore, the current finding of 5.83% from beef carcass was also higher when compared to the 2.5% by Wieczorek *et al.,* (2012) and 3.4% by Ayaz *et al.,* (2018) from bovine carcass in Poland and Turkey respectively.

On the other hand, other researchers were reported much higher prevalence rate of 88.24% by Lennox *et al.* (2017) in Nigeria and 18.33% by Derra (2007) in Addis Ababa from beef carcass. The recent occurrence of *L. monocytogenes* in raw beef was also lower when compared to pooled prevalence of 8.7% in China and 9% in European union by Zhang *et al.* (2023), 10% by Zena and Khalil (2017) in Baghdad city, 34.8% by Wu *et al.* (2015) in China and 11% pooled prevalence by Diriba *et al.* (2021) in Ethiopia. Furthermore, higher prevalence level of 12% by Larrayane *et al.,* (2020), 23% by Saludes *et al,* (2015), and 18.7% by Kramarenko *et al.* (2013) were also recorded in Brazil, Chile and Estonia from beef carcass respectively when compared to the current finding.

The findings of different studies vary a lot which could be attributed to differences in the degree of contamination that occurs during the slaughtering, processing, and handling of the carcass meat (Gebremedhin *et al.,* 2021), the degree of sanitary measures applied during food processing and manufacture (Abdeen *et al.,* 2021) and different sampling techniques and method of analysis (Çadirci *et al.,* 2018). The difference in the study season, geographi c conditions, and sample size (Maktabi *et al.,* 2016) and live animals, environment and dirty transport crates may also contribute to contamination of *L. monocytogenes* of the abattoir and meat production chain at different level (Zelalem *et al.,* 2019).

Out of 160 environmental samples (knifes, cutting boards, workers hands and vehicles) collected both from abattoir and butcher shops, isolation rate of *L. monocytogenes* was 3.7 5% (6/160) of environmental swabs. The specific prevalence from equipment's (110 sampl es) excluding worker's hand was found to be 2.73% (3/110). Thus, dirty or contaminated equipment's can contaminate the safe food and can be a source of *L. monocytogenes* contamination (Cutter *et al.,* 2006) and be focal points for the microorganism (Çadirci *et al.,* 2018).

The present occurrence of 2.73% *L. monocytogenes* isolates from equipment's was higher when compared to no detection by Mpundu *et al.* (2022) from the environmental samples collected in Zambia. However, the current finding was lower when compared to 6.7% by Mulu (2014), 12.8% by Kahraman *et al.* (2010) and 8.8% by Alişarli *et al.* (2003) from equipment's. Furthermore, higher prevalence level of 25.64% by Jankuloski *et al.* (2007) in Republic of Macedonia, 7.6% by Babiker *et al.* (2020) in Central Sudan and 8.33% by Çadirci *et al.* (2018) in Turkey were also revealed from equipment's. As per different scholars explained the variances in isolation can probably by differences in the sampled environment and from the hygienic conditions of specific facilities (Mpundu *et al.,* 2022). The finding of this study indicates that there might be risks associated with the persistence of *L. monocytogenes* on equipment's used along beef meat value chain.

Overall prevalence of *L. monocytogenes* from 50 personnel hand swabs (30 from abattoir and 20 from butcher shops personnel) was found to be 6% (3/50). The present finding was higher than Kahraman *et al.* (2010) who revealed no detection from personnel hands in Finland, 1.4% report by Gudbjörnsdóttir *et al.* (2004) from personnel hand in Nordic countries at meat processing plant and 2.78% detection by Çadirci, *et al. (*2018) from hands of the workers in cattle slaughterhouse in Turkey. Therefore, personnel can be carriers of *L. monocytogenes* and can contaminate the carcass while handling the raw meat at abattoirs and butcher's shops while selling the consumer when they didn't comply with personnel hygiene rules.

L. monocytogenes was not detected from swabs samples taken from abattoir vehicles in this study. This finding was in agreement with the report of Akkaya *et al.* (2008) who revealed no detection from the floor of meat transport vehicle. On contrary, the recent finding was not in compliance with the 5% detection of Çadirci *et al.* (2018) for samples obtained from

the sides of the meat section of the transport vehicle which is the same sides of present study sampling. This might be linked to the short storage of carcasses in the studied vehicles after slaughter and dispatched to the butcher shops shortly.

In the current study, the prevalence of *L. monocytogenes* from knives and cutting boards were found to be 3.64% (2/55) both from abattoir and butcher shops, and 4% (1/25) from butcher shops respectively. The present finding of 3.66% isolation from knifes were higher when compared to 0.71% detection by Legnani *et al.* (2004) and 2.7% report of Çadirci *et al.* (2018). On the other hand, the current isolation rate revealed from knifes were lower when compared to 8.33% by Çadirci *et al. (*2018) from Turkey, 7.5% report by Mulu (2014) from Addis Ababa and 10% by Babiker *et al.* (2020) from Sudan. Moreover, the recent revealed 3.64% was lower than 8.57% by Akkaya *et al.* (2008) and 12.5% recorded prevalence from knifes by Zhao *et al.* (2021).

In the current study, *L. monocytogenes* was found in 4% of cutting boards which is in agreement with 3.1% detection of Zhao *et al.* (2021) from chopping board. However, it was lower than the 8.33% reports of Çadirci *et al.* (2018), 7.5% by Babiker *et al.* (2020) and 8.9% by Mulu (2014). Gebremedhin *et al.* (2021) stated that the types or quality of cutting boards, absence of frequent sanitation of the cutting boards and use of contaminated water for cutting boards washing might be also linked to increased chance of cutting boards contamination with *Listeria* species.

In the present study, *L. monocytogenes* was detected from bovine carcass both at abattoir and butcher shops as well as from environmental samples including knifes, cutting boards and personnel hand. This suggests that there were defects in hygienic and sanitary measures along bovine carcass meat value chain at study area.

Treating human and animal disease is becoming a huge problem because of evolvement of antibiotic resistant bacteria, posing 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 since high numbers of isolates are resistance to the antimicrobials commonly used for humans and animal infections (Mulu, 2014; Fisseha, 2017). In the present study, higher

percentage of resistant isolates were found for tetracycline and amoxicillin equally (92.3%) followed by streptomycin (84.6%), ampicillin (76.9%) and vancomycin (61.5%).

In this study, 92.3% resistance for tetracycline was in line with the 82.6% resistant report by Obaidat and Stringer (2019) and 77.8% by Mulu (2014), but higher than 37.5% and 55% resistance reported by Garedew *et al.* (2015) and Gebremedhin *et al.* (2021) respectively. In contrary to recent finding, Kinyua (2020) was reported 100% susceptibility of *L. monocytogenes* isolates to tetracycline. Amoxicillin also showed high resistance level (92.3%), which is in contrary with Gebremedhin *et al.* (2021) and Tiku *et al.* (2020) reports of 95% and 100% susceptibility of *L. monocytogenes* isolates to amoxicillin respectively. On other hand, Welekidan *et al.* (2020) reported 50% of *L. monocytogenes* isolated from pregnant women in Tigray region were resistant to amoxicillin.

Combination of ampicillin with an aminoglycoside such as gentamicin are the antibiotic of choice for treating human listeriosis (Kovacevic *et al.,* 2013), but in the present study ampicillin was 76.9% resistant to *L. monocytogenes* isolates which was in contrary with 88.46% and 97% susceptibility report by Kidanu *et al.* (2021) and Khen *et al.* (2015) respectively. All isolates were showed intermediate resistance to gentamycin which was in contrary with finding of Kinyua (2020); Tiku *et al.* (2020) and Mulu (2014) report of 100% and 97.2% susceptibility report respectively. Eleven (84.6%) isolates of *L. monocytogenes* had developed resistance to two and more different antimicrobial drugs and were developed MDR which was comparable with 95% MDR by Gebremedhin *et al.* (2021) and higher when compared to 66.7% by Mulu (2014).

In this study, higher percentage of resistant isolates were found against tetracycline and amoxicillin followed by streptomycin, ampicillin and vancomycin. High Intermediate resistances were also observed for Gentamycin. This resistance level may be attributed to the indiscriminate use of these antimicrobial agents in food producing animals at subtherap eutic levels or prophylactic doses (Tiku *et al.,* 2020). Hence, the use of antimicrobials in veterinary medicine is the main cause of the development of antimicrobial resistance foodborne bacterial pathogens including *L. monocytogenes* and can easily be transported from animal to human via food of animal origin consumptions (Escolar et al., 2017). Therefore, judicious use of these drugs in medical and veterinary practices along with

monitoring of antibiotic susceptibility is very important (Barbosa *et al.,* 2013; Dhama *et al.,* 2015).

From questionarries survey conducted, 91.67% of respondents from abattoirs had training on meat hygiene. However, in the present finding no butcher shops personnel were taken training on meat hygiene. Thus, untrained personnel pay no attention to the hygienic standards and as result contribute immensely to bacterial contamination of *L. monocytogen es*. Therefore, adopting of employees training to minimize Listeria monocytogenes cross contamination is highly important (Mulu, 2014), further more training the meat handlers on the meat safety and hygiene is highly important to avoid contamination of raw meat.

In the present assessment, even though all of respondents wash their hands while handling the carcass, 75 % from abattoirs and 76.67% respondents from butcher shops were not wash their hands with soaps. On contrary, Mulu (2014) showed that most of the respondents (74%) of abattoir workers and 86% of butcher's shops personnel were used a detergent for washing their hands. Poor personal hygiene practices like improper hand washing can lead to the contamination of raw meat and equipment's with Listeria monocytogenes which can pose high public health risk.

Even though all respondents from abattoirs were working ≤ 8 hours per day, most of butcher shops workers (75%) had ≥ 9 hours working times per day. As stated by Gebremedhin *et al.* (2021), this might be related to the change in the hygienic behaviour of works as they get exhausted or tired while working ≥ 9 hours per day. Most of butcher shops respondents (93.33%) were handling money by themselves without having cashier. However, the current finding was not in compliance with Mulu (2014) who reported 62% of the respondents handle the money by themselves. Moreover, most of butcher shops (61.67 %) respondents were worn jewellery which disagrees with 38% report of wearing jewellery materials by butchers' personnel by Mulu, (2014).

6. CONCLUSION AND RECOMMENDATIONS

L. monocytogenes which is caused listeriosis both in humans and animals have worldwide public health and economic impacts. It is most commonly foodborne emerging zoonotic pathogen, in which animal source food like raw beef meat play a great role in its transmission via consumption of contaminated meat. In the present study, *L. monocytogenes* was isolated from meat values chain of Addis Ababa, Ethiopia and environmental samples associated within these chains with overall proportion of 4.64% *L. monocytogenes* positive. The present findings revealed that meat and meat contact surfaces (personnel, knifes and cutting boards) are potential source of contamination of meat at abattoirs and butcher shops during slaughtering, processing and selling. The current isolation of *L. monocytogenes* along all chain of beef carcass production reflects that there was a failure in the application of good manufacturing and hygienic practices. Moreover, isolated *listeria monocytogenes* has developed resistance to most commonly used both in veterinary and human's antimicrobial agents in the country which has great public health importance.

Therefore, based on the above conclusion and findings the following recommendations are forwarded;

- Implementing of appropriate good manufacturing practices (GMPs) and hazard analysis and critical control point (HACCP) systems at abattoirs
- Implementing strict safety and standard hygienic practices along meat supply chain to minimize the level of contamination of raw beef meat before reaching the consumer.
- Providing periodic and regular training on safe and hygienic meat handling for all personnel at each stage of meat production chain.
- Frequent researches on isolation and antimicrobial susceptibility pattern of *L. mono cytogenes* and factors contributing to the contamination along meat value chains across the country and routine tests should be under taken.
- Performing periodic antimicrobial susceptibility test for *L. monocytogenes* isolates and follow-up on the prudent use of antimicrobial drugs.
- Formulating and implementing strict regulation on meat safety and hygiene along meat production chain and administration of antibiotics

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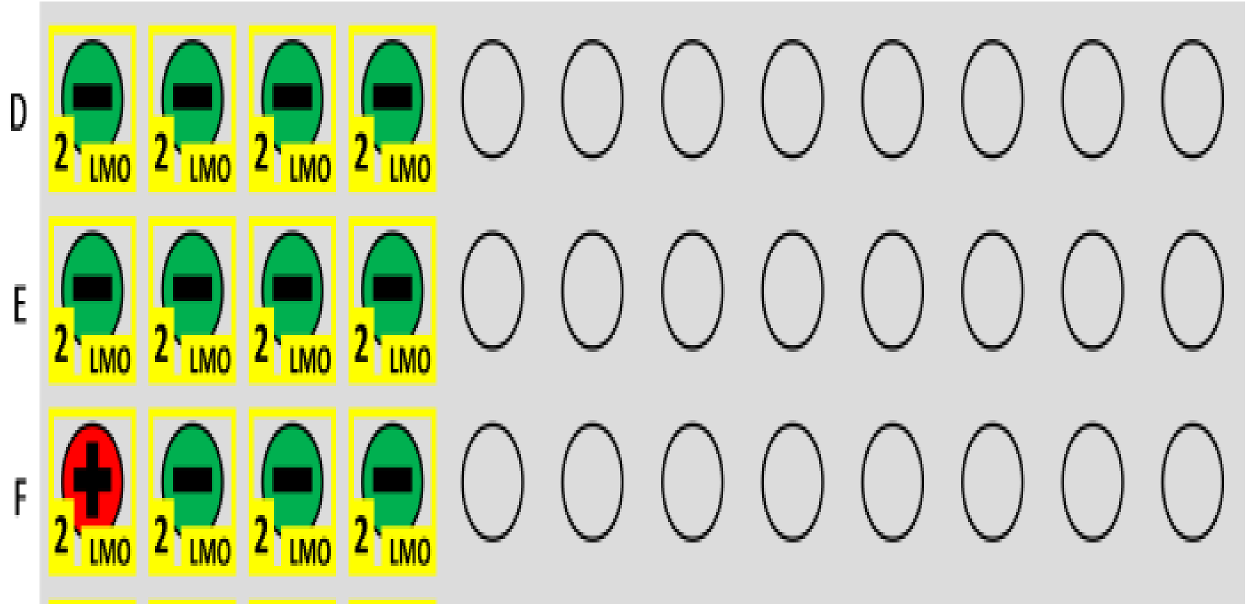
8. APPENDICES

Appendix 1: Pictures showing isolation and identification of *Listeria monocytogenes*



Detection of *L. monocytogenes* by 3M Molecular Detection System Assay

MicroLog Secure 6.2

Main | Setup | Read | File Viewer | (Admin) | Log-Out

New Batch

Read Plate | Open | Import | Save | Save As | Close | Delete | Print

Plate Information

Project * RESEARCH

Plate Type * GEN III

Protocol * A

Incubation Hours * 22

Temperature * 33

Title	Value
sample type	CARCASS
Sample ID Numbe	MTS-1947
IF Lot #	A
Plate Lot #	
Media used	BUG
OD/Transmittance	98
Technician	SINKE ARARSO AND AGARI FEYISA
analysis date	30/05/2023
purpose of the test	RESEARCH
supervisor	ZERIHUN BEYENE

Clear Information After Save

Add to List

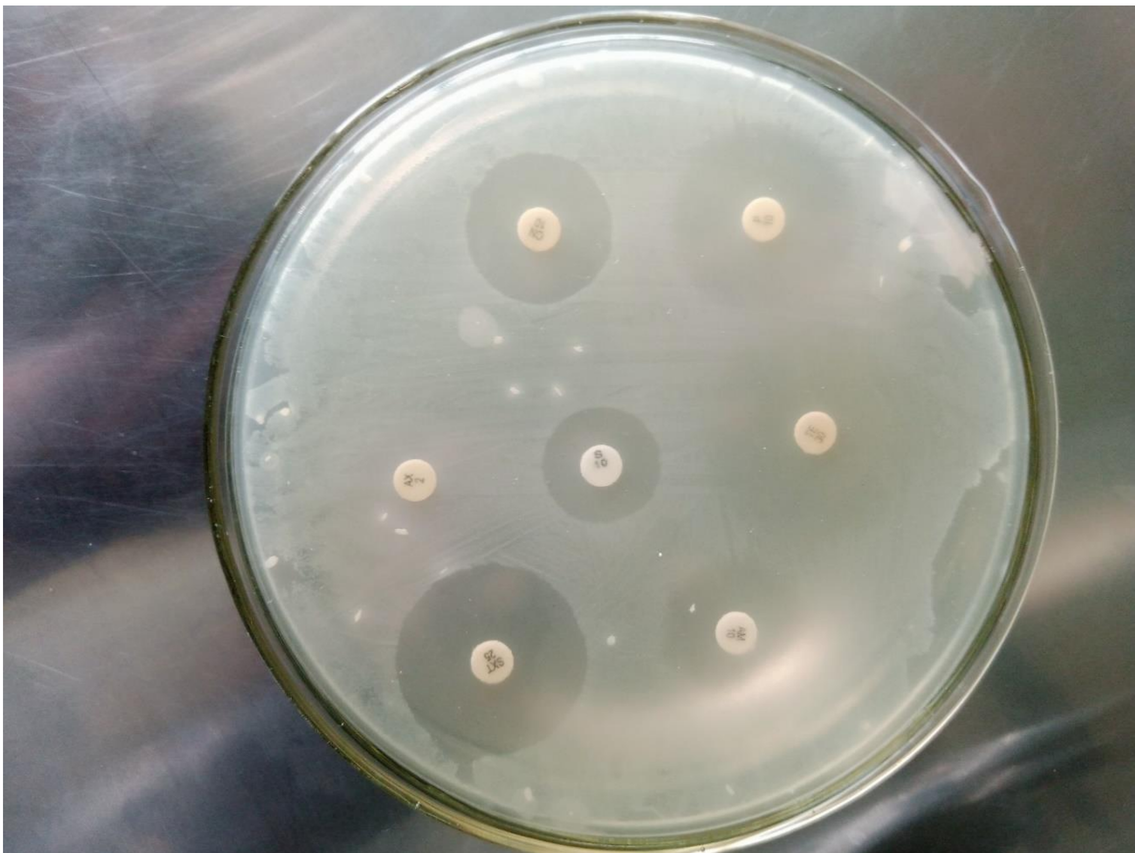
(* Required Fields)

Index	Results	Project	Plate ...	Pr...	Incub	Temp	sample type	Sample I...	I...	P	Media...	OD/Tr...	Technician	analysis d...	purpose ...	supervisor
1	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	CARCASS SWAB	MTS-1800	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
2	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	CARCASS SWAB	MTS-1812	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
3	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	CARCASS SWAB	MTS-1834	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
4	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	CARCASS SWAB	MTS-1867	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
5	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	WORKER HAND S	MTS-1816	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
6	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	WORKER HAND S	MTS-1888	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
7	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	ABATTOIR KNIFE	MTS-1904	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
8	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	CARCASS	MTS-1939	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
9	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	CARCASS	MTS-1947	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
10	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	CARCASS	MTS-1991	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
11	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	WORKER HAND (	MTS-2050	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
12	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	CUTTING BOARD	MTS-2005	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
13	Species ID: Listeria monocytogenes	RESEARCH	GEN III	A	22 Hrs	33 C	BUTCHER KNIFE	MTS-2026	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE
New		RESEARCH	GEN III	A	22 Hrs	33 C	CARCASS	MTS-1947	A		BUG	98	SINKE ARARSO AND AGARI F	30/05/2023	RESEARCH	ZERIHUN BEYENE

Type here to search

All confirmed isolates of *L. monocytogenes* by **GEN III MicroPlate**

Confirmed Carcass swabs samples from abattoir



Resistance of *L. monocytogenes* to Amoxicillin (AX2)

Appendix 2: Media and Equipment's used

Buffered peptone water

Specifications: (3M BPW 500, 3M Health Care, USA)

Formula	*grams per litre*
Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Potassium dihydrogen phosphate	1.5 g
Disodium hydrogen phosphate dodecahydrate	9.0 g
Water	1L

pH 7.0 ± 0.2 at 25°C

Directions:

- Suspend 25.5g of the powder in 1.0 litre of purified water. Mix thoroughly. If necessary, heat completely dissolve powder. Dispense. Autoclave 121°C for 15 minutes.

Demi or Half Fraser broth

Specifications: (BP0118005, 3M Health Care, USA)

Formula	*gram/Litre*
Proteose peptone	5.0 g
Tryptone	5.0 g
Meat extract	5.0 g
Yeast extract	5.0 g
Sodium chloride	20.0g
di-Sodium hydrogen phosphate	12 g
Potassium di-hydrogen phosphate	1.35 g
Aesculin	1.0 g
Lithium chloride	3.1 g
Ferric ammonium citrate	0.5 g
Nalidixic acid	10mg
Acriflavine hydrochloride	12.5 mg
Water	1L

pH 7.2 ± 0.2 at 25°C

Directions:

- Suspend 55g of the powder in 1.0 litre of purified water. Mix thoroughly. Autoclave 121°C for 15 minutes. Cool to room temperature.

Fraser broth base

Specifications: (BP0210500, 3M Health Care, USA)

Formula	***gram/Litre***
Proteose peptone	5.0 g
Tryptone	5.0 g
Meat extract	5.0 g
Yeast extract	5.0 g
Sodium chloride	20.0g
di-Sodium hydrogen phosphate	12 g
Potassium di-hydrogen phosphate	1.35 g
Aesculin	1.0 g
Lithium chloride	3.1 g
Ferric ammonium citrate	0.5 g
Nalidixic acid	20mg
Acriflavine hydrochloride	25 mg
Water	1L

pH 7.2 ± 0.2 at 25°C

Directions:

- Suspend 55g of the powder in 1.0 litre of purified water. Mix thoroughly. Autoclave 121°C for 15 minutes. Cool to room temperature.

Listeria Selective Agar

Part (I)

Specifications: (TM612, TITAN BIOTECH LTD, India)

Formula	***gram/Litre***
Agar	13.00

Peptide digest of animal tissue	10.00
Casein enzymic hydrolysate	10.00
Sodium chloride	5.0 g
Dextrose	1.00
Nalidixic acid	0.040
Acriflavine hydrochloride (Trypaflavine)	0.010
Thiamine dichloride	0.005

pH 7.4 ± 0.2 at 25°C

Directions:

- Suspend 39 g of part - I and 37.5g of part -II in 1000ml of distilled water. Gently heat to boiling with gentle swirling to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes.

Listeria Selective Agar

Part (II)

Specifications: (TM612, TITAN BIOTECH LTD, India)

Formula	***gram/Litre***
Potassium thiocyanate	37.500

pH 7.4 ± 0.2 at 25°C

Directions:

- Suspend 39 g of part - I and 37.5g of part -II in 1000ml of distilled water. Gently heat to boiling with gentle swirling to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes.

Muller Hinton Agar

Specifications: (MM019, Sisco research laboratories PVt. Ltd, India)

Ingredients	**Grams/Litre**
Beef infusion from	300.00
acid hydrolysate of casein	17.50
Starch	1.5

Agar	17.0

Final pH 7.4 +/- 0.2 at 25°C

Directions:

- Add 39.00g powder to distilled/ deionized water. Bring volume to 1.0 liter and mix thoroughly. Gently heat and bring to boiling. Autoclave at 15 psi at 121°C for 15 minutes.

Biolog universal Growth Agar

Specifications: (70101, Biolog, Inc, Hayward, CA94545, USA)

Final pH 3 +/- 0.1 at 25°C

Directions:

- Mix 57 grams of BUG^{TM} agar into one liter of purified water. Boil gently to dissolve components. Sterilize by autoclaving at 15 ibs pressure and 121°C for 15 minutes and dispense as desired.

Equipment's used

- Autoclave, Balance, Biosafety cabinet
- Incubator
- Pipettes or automatic pipettes
- Ice box
- Measuring cylinder
- Petri dishes
- Refrigerator
- Screw capped bottle
- Sterile bags, gauge, loop or sterile swabs inoculators
- Calliper, disk dispenser

Appendix 3: 3M Molecular Detection Assay 2 – *L. monocytogenes* (Media and Instruments)

- Demi Fraser broth
- Fraser broth
- Buffered Peptone Water

- Molecular Detection Instrument
- *L. monocytogenes* reagent tubes
- Lysis solution, reagent control and matric control tubes
- Speed Loader Tray, Heat block and chill block tray
- Cap/Decap Tool
- Empty lysis and reagent tube rack
- Pipettes capable of 20 µL
- Sterile filter tip pipet tips capable of 20 µL
- Incubator
- Refrigerator
- Computer with detection system software

Appendix 4: GEN III MicroPlate Material, Media and Reagents

- MicroPlates (Biolog GENIII MicroPlates)
- Biological Growth Agar (BUG Agar).
- Inoculating Fluid (Catalog No.72401- IF-A)
- Sterile disposable inoculator swabs
- Sterile disposable wooden agar plate streakers
- Sterile disposable 9-inch transfer pipets.
- Sterile disposable reservoirs.
- Multichannel Pipettes (8 channel electronic pipettor).
- Pipet Tips (Sterile racked pipet tips for Ovation multichannel pipettor)
- Turbidimeter
- Turbidity Standards (85% T; 65% T)
- OmniLog incubator/Reader
- Biolog's Microbial Identification Systems software
- Biolog MicroStation™ Machine

Appendix 5: Samples collection and Result Record Format

Sample Code	Sample Source	Sample Types						Result
		Carcass swab	Carcass	Knifes	Personnel hand	Cutting boards	Vehicles	

Appendix 6: Respondents Knowledge and Practices questionaries survey format

Informed consent

My name is Agari Feyisa. I am working my MSc research at Addis Ababa University College of Veterinary Medicine and Agriculture (AAU_CVMA). I would like to interview you a few questions about the sanitary condition of your slaughter houses and some of the questions require physical observation and taking swab samples from your hands with objectives of assessing knowledge and practice concerning slaughter safety and hygiene, which is important to improve the sanitary status so as to safeguard the safety of carcass reaching consumer from slaughter houses. Your willingness and cooperation are helpful in identifying knowledge and practice related problems related to the Addis Ababa abattoirs enterprise. All information that you give will be kept strictly confidential, your participation is voluntary and you can drop it any time you want.

If it is your permission to continue, please continue to the next section of interview.

Questionnaire Code: ______________________ (For Researchers only)

I: Sociodemographic characteristics

1. **Sex: ________________** Age in years: _________________
2. **Marital status**: _______________

   Single ________ Married__________

3. **Educational status**:

   Illiterate: _____ Informal Education: ____ Primary Education: _____ Secondary Education: ________ Other (Specify): _______

4. **Role in Abattoir**:

   Veterinarian/Meat inspector: _____ butchers: ______ Others: _______

5. **Employment status**:

   Permanent ___________ Temporary staff __________________

6. **Work experiences** in Abattoirs ______________

   Working times per day: ________

7. Knowledge and information on FBD, Meat hygiene and Safety

   Yes _____________ No __________

8. Receive job **related Training on** Meat Hygiene:

   Yes _______ No_________

9. **Hand washing**: Yes__________ No____________

   If yes, washing hands with: water only ______ With water and soap ____________

   Washing knives: Before work: ______ After work: ______Between each process_____

10. Using of following protective materials while working in the abattoir (**Observe**)

    Apron: ________ white coat: ______ Head Cover: ______ Gloves: _______ Boots: ____

11. Cash handling (**For butcher shops only**): By cashier ________ By them selves______

Appendix 7: Antimicrobial Susceptibility Test Result Record Format

Type of Drugs	Sensitive	Intermediate	Resistant
Amoxicillin			
Ampicillin			
Tetracycline			
Penicillin G			
Streptomycin			
Trimethoprim-sulfamethoxazole			
Vancomycin			
Gentamycin			

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/21/04/15/2023

Name and affiliation of applicant: **Agari Feyisa (DVM, MSc student)**
Department of Microbiology, immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Isolation and antimicrobial susceptibility profile of Listeria monocytogenes at Municipal abattoirs and butcher shops in Addis Ababa*

Date of application:	**December, 2022**
Nature of the project:	**Field investigation**
Target animal species:	**Cattle**
Number of animals involved:	**No live animal use**
Study area:	**Addis Ababa, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/15/022, 15/02/2023**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of Agari Feyisa.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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Please quote Our Ref. No. When replying

ፋክስ} Fax 251-11-4339933
ስልክ } Tel. +251 114338450
ፖ.ሣ.ቁ P.o.x. Box}34
ቢሾፍቱ፤ ኢትዮጵያ Bishoftu, Ethiopia

Ethical clearance certificate