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ASSESSMENT OF ANTIMICROBIAL USAGE PRACTICES AND ANTIMICROBIAL SUSCEPTIBILITY OF *LISTERIA MONOCYTOGENES* ALONG THE BEEF VALUE CHAIN IN ADAMA AND MODJO, ETHIOPIA: ONE HEALTH PERSPECTIVES

MSc THESIS

BY:

MESKEREM MULISA MISGANA

JUNE, 2026

BISHOTU, ETHIOPIA

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A Thesis Submitted to the Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture in Partial Fulfillment of the Requirements for Degree of Masters of Science in One Health

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Assessment of antimicrobial use practices and antimicrobial susceptibility of *Listeria monocytogenes* along the beef value chain in Adama and Modjo, Ethiopia: One Health perspectives.

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This is to certify that the thesis Prepared by Meskerem Mulisa Misgana entitled: Assessment of Antimicrobial Usage Practices and Antimicrobial Susceptibility of *Listeria monocytogenes* along the Beef Value Chain in Adama and Modjo, Ethiopia: One Health Perspectives and submitted in partial fulfillment of the requirement for the degree of Master of Science in One Health complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

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STATEMENT OF AUTHOR

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LIST OF ABREVIATIONS

AHI	Animal Health Institute
AMR	Antimicrobial resistance
AMU	Antimicrobial Use
AST	Antimicrobial susceptibility test
CAMP	Christie Atkins,Mumch-Petersen
CNS	Central Nervous System
DALYs	Disability Adjusted Live Years
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked immunosorbent assays
HCCA	α-Cyano-4-hydroxycinnamic acid
hlyA	Hemolysin A
IMS	Immunomagnetic separation
KAP	Knowledge attitude and practice
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization-Time of Flight
PCR	Polymerase Chain Reaction
PALCAM	Polymixin Acriflavin Lithium Chloride Ceftazidime Aesculin Mannitol
SPSS	Statistical package for social science

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ABSTRACT

Listeriosis, caused by *Listeria monocytogenes*, is a significant foodborne zoonotic disease with serious public health implications. This study assessed antimicrobial use (AMU) practices and antimicrobial susceptibility patterns of *L. monocytogenes* along the beef value chain in Adama and Modjo, Ethiopia, using a One Health approach. A cross-sectional study was conducted from November 2025 to May 2026. Data were collected through questionnaires (n = 60) and laboratory analysis of 460 samples, including carcass swabs, fecal samples, hand swabs, and environmental samples. Isolation and identification of *L. monocytogenes* were performed using standard culture methods, biochemical tests, and MALDI-TOF mass spectrometry. Antimicrobial susceptibility testing (AST) was carried out using the disk diffusion method, and detection of the virulence gene (*hlyA*) was conducted using PCR. The results showed that 74.8% of respondents had poor knowledge of AMU and antimicrobial resistance (AMR), while 88.7% demonstrated a favorable attitude toward the use of antibiotics for growth promotion. Out of 460 samples, 106 (23%) showed presumptive Listeria growth, of which 59 isolates were confirmed by biochemical tests. Of the 29 isolates further analyzed using MALDI-TOF, 6 were confirmed as *L. monocytogenes*, predominantly from carcass samples (n = 5) and one from a water sample. All confirmed isolates carried the *hlyA* virulence gene. Antimicrobial susceptibility testing showed that all isolates were fully susceptible to ampicillin, gentamicin, ciprofloxacin, cotrimoxazole, streptomycin, and chloramphenicol. However, high levels of resistance were observed to penicillin (100%), vancomycin (66.7%), and tetracycline (50%). In conclusion, poor antimicrobial use practices and the presence of resistant and virulent *L. monocytogenes* strains indicate a potential public health risk. Strengthening antimicrobial stewardship and improving hygiene practices along the beef value chain are essential.

Keywords*: Antimicrobial use; Antimicrobial resistance; Beef values chain; Listeria monocytogenes, One Health.*

1. INTRODUCTION

Antimicrobial resistance (AMR) is a major global public health and development threat. It happens when microbes stop responding to antimicrobial medications. As a result, infections become difficult to treat, raising the risk of disease transmission, prolonged illness, death, and significant economic losses. Globally, AMR was associated with 4.95 million fatalities in 2019 and directly caused 1.27 million deaths. AMR is complex issue that affects the health of humans, animals, and the environment, requiring a coordinated One Health approach (Murray *et al.*, 2022;WHO, 2023)

Antimicrobial use (AMU) in livestock production is essential for maintaining animal productivity and health. Antimicrobials are administered for therapeutic, growth promotion, prophylaxis, and metaphylaxis purposes (Economou and Gousia, 2015;Cameron and McAllister, 2016). However, their misuse contribute to the emergence and spread of antimicrobial resistant (AMR) bacteria , which can enter the environment and the food chain, thus posing a potential risk to human health (Aarestrup *et al*., 2008; Tsitsos *et al.*, 2021)

The food chain represents a critical interface linking humans, animals, and environmental health with one health framework (FAO, 2024). Livestock production can act as a reservoir for resistant bacteria that can be transmitted to humans through direct contact with animals, contaminated food products, or environmental pathways (Khairullah *et al*., 2025). The beef value chain includes several stages, including production, slaughter, processing, distribution, and consumption (Rekwot *et al.*, 2022). At each of these stages, there are potential points of contamination with antimicrobial resistance pathogens from the hide or intestines of the cattle or from the environment in which animals are reared, slaughtered, transported, and displayed for in the markets (Tassew *et al.*, 2010; Mshana *et al.*, 2021).

Among the develop antimicrobial resistance bacteria, *L. monocytogenes* causes severe foodborne disease, listeriosis (Wiktorczyk *et al.*, 2023). It poses a significant risk to the food industry, especially for ready-to-eat products, due to its ability to persist in adverse conditions and its association with severe health outcomes in vulnerable populations (Sharma *et al.*, 2017; Dos Reis *et al.*, 2022). *L. monocytogenes* is widely distributed in nature; it can be found in soil,

water, vegetation and the faeces of some animals and can contaminate food products (Bento and Tobin, 2026). The pathogen can be transmitted to human through direct animal contact, the consumption of contaminated food and through environmental pathways such as manure runoff and irrigation water (Pagliano *et al*., 2016; Tola, 2024)

In Ethiopia, livestock production is an integral part of the country's agriculture sector and plays an essential role in national economic development and food security (Wakaso, Mummed and Yesuf, 2025). Antimicrobials are widely used in Ethiopian livestock's production for enhancing the livestock productivity and profitability (Gemeda *et al.,* 2020). However, extensive use of antimicrobials in livestock production has contributed to the AMR (Takele *et al.,* 2023; Gizaw *et al.,* 2023). The presence of antimicrobial resistant *L. monocytogenes* in raw meat and abattoirs and butcher shops environment, has been reported (Borena *et al.*, 2022; Getaneh *et al.,* 2025); indicating significant health and food safety risks due to the traditional widespread consumption of raw meat like kitfo and dulet (Mereta *et al.,* 2020).

Listeria monocytogenes are mainly spread via contaminated food. Agricultural workers are at higher risk to contracting *L. monocytogenes* through direct contact with infected livestock such as cattle, sheep, and goats compared to other segments of the populations (Pagliano *et al.,* 2016;Tola, 2024). Although antibiotics such as ampicillin and gentamicin are commonly used for treating infections due to *L. monocytogenes*, the misuse of these drugs can lead to the emergence of resistant strains, affecting both public health and animal welfare (Dos Reis *et al.,* 2022; Beyene, 2016).

Furthermore several studies were conducted in Ethiopia (Jimma, Ambo, and Holeta town) municipal abattoir and butcher shops have revealed the presence of antimicrobial resistant *L, monocytogenes* in foods of animal origin, including beef and dairy products, which is 7% (Getaneh *et al.,* 2025), and 4.4% (Gebremedhin *et al.,* 2021) respectively. Despite increasing evidence of antimicrobial resistance in foodborne pathogens in Ethiopia, there is limited information on antimicrobial use practices and resistance patterns of *L. monocytogenes* along the beef value chain in Adama and Modjo towns.

OBJECTIVES:

General Objectives

The general objectives of this study were to assess antimicrobial use practices, the magnitude of *L. monocytogenes*, its antimicrobial susceptibility patterns, and virulence gene profiles along the beef value chain in Adama and Modjo, Ethiopia, using a One Health approach.

Specific objectives

- To assess the knowledge, attitudes, and practices of beef farm workers in Adama and Modjo regarding Antimicrobial use and Antimicrobial resistance.
- To determine the occurrence of *Listeria monocytogen*es along the beef value chain.
- To evaluate the antimicrobial susceptibility patterns of isolated *Listeria monocytogenes* isolates from the beef value chain in Adama and Modjo town.
- To assess virulence gene profile of isolated *Listeria monocytogenes* isolates from the beef value chain.

2. LITERATURE REVIEW

2.1.Overview of Antimicrobial Resistance in the Food Chain

Antimicrobial resistance in animal food is the rising human health concern that provokes when microorganisms develop the ability to survive exposure to drugs designed to destroy them. Since AMR can have detrimental effects on human health worldwide, it continues to be a key concern for many food stakeholders. AMR pathogen contaminates food at any stage, from the field to retail (Samtiya *et al.,* 2022). Food chain ecosystems harbor foodborne pathogens, commensals and bacteria that together with bacteriophages provide a pool of genomes that may include genes encoding resistance to antimicrobials (Economou and Gousia, 2015).

Bacteria found in the food chain can be intrinsically resistant strains and can be enriched or selected in a bacterial population following exposure to antimicrobials, particularly also sanitizers and biocides. Gene mutation or horizontal gene transfer from the large pool of resistance genes found throughout the entire food chain can cause resistance (Woolhouse *et al.*, 2015). Foodborne Pathogens are the leading cause of foodborne illness worldwide, and they are particularly severe in low-income nations such as Ethiopia. They contaminate food at all stages of the food chain, including agricultural production, animal sources, and preparation by food handlers (Asfaw *et al.*, 2022).

Antimicrobial resistant zoonotic pathogens on food pathogens on food provide a direct risk to public health because they expand the gene pool from which pathogenic bacteria might acquire resistance characteristics. Antimicrobial resistant bacteria such as Salmonella spp, Escherichia coli O157:H7, *L. monocytogenes*, and Campylobacter spp are regularly implicated in outbreaks, which are often associated to contaminated meat, dairy, and ready-to consume products (Verraes *et al.*, 2013; Asfaw *et al.*, 2022).

2.2.Antimicrobial Use Practices in Beef Production in Ethiopia

Beef fattening has gained increased acceptance in livestock husbandry due to its high turnover rate, youth employment opportunities, national cultural esteem for the meat, and ability to combat malnutrition (Agmas and Adugna, 2018). Numerous antimicrobial medications are used

to enhance health, promote growth, increase feed efficiency, and lower the prevalence of sickness. But when they are taken without a veterinarian's supervision, they are applied incorrectly because people don't know enough about their dosage, withdrawal period, and adverse effects. Antimicrobial residues are undesirable substances that can remain in the food chain and may be harmful to people (Beyene, 2016).

Antibiotics are broadly used across the current Ethiopia's beef production for several purposes such as therapeutic, metaphylaxis, prophylaxis, and growth promotion. The most commonly used antibiotics in Ethiopia by animal farm owners in different area are tetracycline, ampicillin, cotrimoxazole, gentamicin, and quinolone, penicillin, Penstrep (penicillin and streptomycin fixed combination), and sulfa drugs (Geta and Kibret, 2021; Ragassa and Berhanu, 2023; Takele *et al.*, 2023).

Due to a lack of veterinary oversight, antimicrobial use practices in Ethiopian beef farms are typified by a high rate of self-prescription and non-therapeutic use of antibiotics. Important behaviors include not adhering to recommended treatment plans, getting medications from pharmacies and open markets, and farmers generally not knowing about (AMR) and appropriate usage procedures (Mesafint *et al.*, 2024).

2.3.Overview of *Listeria Monocytogenes*

The fatal foodborne illness listeriosis is caused by the Gram-positive, facultative anaerobic, halotolerant rod *L. monocytogenes*. It is characterized by catalase positive, oxidase negative, esculin and sodium hypurate hydrolysis (Wiktorczyk *et al.,* 2023). *L. monocytogenes* can grow in a wide range of temperatures (0^0C-45^0C), pH (4.3-9.6), tolerates high salt concentrations (up to 10.0% NaCl) and low water activity (Aw to 0.90) (Allerberger and Wagner, 2010). The high incidence of the bacteria (gastroenteritis septicemia, meningoencephalitis, abortion, and miscarriages) is due to its ability to survive the acidic environment of the stomach, and adapt and maintain pH homeostasis (Dhama *et al.*, 2015). *L. monocytogenes* has been isolated worldwide from humans, animals, poultry, environmental sources like soil, drainage to stream, decaying plants and food sources such as meat and its products, seafood, and vegetables (Derra *et al.*, 2013; Dhama *et al.*, 2015).

Transmission occurs through the food chain, particularly from raw dairy, meat, and produce. In developing countries, factors such as poor hygiene and limited diagnostic capabilities contribute to the spread of listeriosis in both human and animal populations. Once in the intestines, it can invade epithelial cells, allowing it to bypass the host's immune defenses. *L. monocytogenes* can enter food processing and survive in food and processing environments due to having the potential to create biofilms. The pathogen is a major worry for the food industry and public health (Gandhi and Chikindas, 2007).

The survival and persistence of *L. monocytogenes* throughout the beef production from production to consumer pose important challenges to food safety and public health. On the farm, the bacterium can be found farm environments, and animal feed, where it can survive for extended periods due to its resilience to environmental stressors. Cattle can carry *L. monocytogenes* asymptomatically, leading to fecal contamination of their surroundings. During transport, the risk of cross-contamination increases, especially if temperature control measures are inadequate. The processing environment at the abattoir further complicates matters, as carcasses can become contaminated during slaughter and handling, with biofilms providing a persistent reservoir for the pathogen (Chowdhury and Anand, 2023).

In retail settings such as butcher shops, *L. monocytogenes* can thrive if proper sanitation protocols are not followed. The potential for cross-contamination during consumer handling emphasizes the importance of education on safe food practices. Environmental factors within food manufacturing plants such as moisture and organic matter can further enhance the pathogen's ability to persist despite sanitation efforts (Silva *et al.*, 2017).

2.3.1. Epidemiology of Listeria monocytogenes

The clinical *L. monocytogenes* in animals occur mainly in northern and southern latitudes and are much less common in tropical and subtropical than in temperate climates. Spread in nature, especially in the food chain, most cases occur sporadically but foodborne and nosocomial outbreaks have been recognized in unpasteurized milk, soft cheeses, processed meats, and contaminated vegetables (Kundul and Ame, 2022). In Ethiopia, the epidemiological distribution of *L. monocytogenes*, which affects both humans and animals, is alarming; the infections are common throughout the nation, with an average frequency of 21.6% for listeria species and 6.9%

for *L. monocytogenes* in particular foods of animal's origins The prevalence rates are higher in human individuals (6.4%) than in animals (4.7%) and foods of animal's origins (5.1%) (Tola, 2024). The overall prevalence of *L. monocytogenes* from various sources was 4.2% (Tadesse *et al.*, 2024).

Geographically, *L. monocytogenes* has been reported in various regions across Ethiopia, with significant findings emerging from urban centers like Addis Ababa, Ambo, Holeta, and Jimma, as well as rural areas in Tigray. A study carried out in the Tigray region revealed that the total incidence of *L. monocytogenes* among pregnant women was 8.5%, underscoring the serious threat this disease poses to the natio's public health (Wiktorczyk *et al* 2023). Furthermore, the high incidence of listeriosis in Ethiopia is a result of inadequate food handling procedures and the use of raw and unpasteurized dairy products, underscoring the need for better food safety regulations and public health initiatives (Getaneh *et al.*, 2025).

In Ethiopia, consuming contaminated food such as raw or undercooked animal items, uncooked dairy products, and ready to eat foods is the main way that *L. monocytogenes* is spread (Tadesse *et al.*, 2024). Humans, particularly agricultural workers, can infect with the pathogen by direct contact with ill and apparently health livestock, such as bovine, ovine and caprine. Likewise, the bacteria may be excreted in animal fluids and faeces, contaminating the environment and perhaps creating pathways for transmission (Kundul and Ame, 2022). Also, environmental contamination including soil and water can act as bacterial reservoirs, aiding in the bacteria's spread throughout the food chain (Getaneh *et al.*, 2025).

2.3.2. *Clinical signs*

Listeriosis, caused by *L. monocytogenes*, presents with a range of clinical signs that vary depending on the host's health status and the form of the infection. The clinical signs observed in affected animals and humans include meningitis, stillbirth, fever, watery diarrhea, nausea, headache, and joint and muscle pain (Sagni, 2020). The diagnosis of listeriosis is based on clinical symptoms and laboratory detection of the bacteria in blood, cerebrospinal fluid, or other relevant samples. Early detection and effective antibiotic therapy, often with ampicillin and an aminoglycoside, are critical for reducing the high fatality rate associated with invasive listeriosis (Schlech , 2019; Osek and Wieczorek, 2022).

Most commonly, sepsis, meningitis, and rhombencephalitis are among the clinical symptoms that Listeria monocytogenes can induce, especially in immunocompromised hosts. The latter symptom is similar to the ruminant veterinary infection known as "circling disease." Neonatal infection may happen from the passage of Listeria from the gastrointestinal system through a birth canal or from maternal chorioamnionitis, sometimes known as "early onset" sepsis, “late onset" meningitis) (Sagni, 2020; Tadesse *et al.*, 2024;Tola, 2024).

2.3.3. Antimicrobial Resistance Listeria monocytogenes

Antimicrobial resistance has been facilitated by the unchecked use of antibiotics in food-producing animals due to the rising demand for animal-sourced protein. *L. monocytogenes* show high resistance rate to clindamycin, vancomycin, and tetracycline in different point along the beef production chain included, abattoirs carcass, butchers, and slaughterhouse environment (Tsitsos *et al.*, 2025). Also *L. monocytogenes* isolated from the food animal sources resist to tetracycline amoxicillin nalidixic acid, penicillin and cephalothin (Tadesse *et al.*, 2024). Molecular subtyping of human and animal Listeria strains and current developments in antibiotic resistance is not well documented in Ethiopia (Tola, 2024).

L. monocytogenes has emerged as a significant foodborne pathogen, with increasing concerns regarding its antimicrobial resistance patterns. Studies have shown that this bacterium exhibits notable resistance to various antibiotics, particularly nalidixic acid, with resistance rates reported as high as 96.15% (Hailu *et al.*, 2024). This resistance poses a public health threat, especially in regions where Listeria contamination is prevalent in food products, such as raw milk and meat. There is still not much resistance to first-line medications like ampicillin and vancomycin, with susceptibility rates around 88.46% and 84.62%, respectively. However, the presence of multidrug-resistant strains, which account for 42.31% of isolates, underscores the urgent need for improved surveillance and management strategies in both veterinary and public health sectors to mitigate the risks associated with Listeria infections (Hailu *et al.*, 2024).

Additionally, a number of factors, such as improper food handling procedures and the abuse of antibiotics in agricultural activities, can be linked to the development of AMR in Listeria species. The abuse of antibiotics in livestock agriculture has created selective pressure that has led to the evolution of resistant strains that can infect people through tainted food(Tola, 2024). This

scenario highlights the critical intersection between animal health and public health, necessitating a One Health approach to address the challenges posed by antimicrobial resistance in *L. monocytogenes*. Continuous monitoring of resistance patterns and the implementation of effective food safety measures are essential to protect vulnerable populations and reduce the incidence of listeriosis (Tola, 2024).

2.3.4. Prevalence of Listeria monocytogenes in beef value chain in Ethiopia

Several studies show that the rates of contamination vary by geography and meat product type. The study conducted in Jimma town revealed that the overall prevalence of Listeria spp was 29.7%, with *L. monocytogenes* making up 23.1% of the isolates from meat sample (Abas *et al.*, 2025) In regions where meat eating practices like kitifo and dulet, which are frequently served with minimal cooking; this emphasizes the ongoing risk associated with raw and undercooked beef products (Kocho, 2007) The epidemiological prevalence of *Listeria species* is induced by several factors, such as cleanliness of meat handling and processing procedures. According to another studies, listeria innocua was the most common species, with. *L. monocytogenes* recovered from 4.4% of meat samples in a study done in Ambo and Holeta (Gebremedhin *et al.*, 2021).

Furthermore, significant rates of antibiotic resistance have been linked to *L. monocytogenes*, making treatment options more difficult for those who are infected. Concerns over the efficacy of current treatment regimens were raised by (Mereta *et al.*, 2020), observation that a considerable percentage of *L. monocytogenes* isolates showed resistance to conventional antibiotics such as tetracycline and penicillin (Hailu *et al.*, 2024).

2.3.5. Detection of Listeria monocytogenes

For the purpose of disease control and prevention, *L. monocytogenes* identification is crucial. Over time, the cold enrichment technique gave way to conventional and molecular technologies for the detection and isolation of *L. monocytogenes*. It takes a lot of time and effort to detect Listeria species using traditional morphological or biochemical methods (Law *et al*., 2015). According to (D'Urso and his colleague, 2009), molecular techniques such as PCR target specific target genes, while DNA sequencing is significantly faster, more precise, and hence more appropriate for detecting *L. monocytogenes*.

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, a number of traditional and quick techniques are currently available (Välimaa, *et.al.*, 2015). For the rapid identification of listeria in food samples, immune- capture techniques, culture- based methods, enzyme- linked immunosorbent assays, and antibody-based tests are increasingly used in the traditional way of isolating *L. monocytogenes* (Jadhav *et al*, 2012).

In order to isolate and identify *L. monocytogenes* using culture-based techniques, selection agents must be applied together with an enrichment process that suppresses completing microflora. This enrichment process makes it easier for *L. monocytogenes* to proliferate to detectable quantities and for damaged or stressed cells to recover (Chen *et al*., 2009). Listeria species can be identified using selective media such Oxford and PALCAM agar. A biochemical test was carried out for identification and validation after a pure culture of Listeria was isolated (Jadhav *et al*, 2012).

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2.3.6. Control and prevention

Treatment of listeriosis is usually with a combination of ampicillin and an aminoglycoside but other regimens have been used. The mortality rate is high, reflecting the combination of an immunocompromised host and an often delayed diagnosis (Sagni, 2020). Preventing *L. monocytogenes* contamination in livestock-origin foods involves implementing strict hygiene practices throughout the food production chain. This includes ensuring proper sanitation in livestock handling and slaughtering processes, as well as stringent hygiene measures during food processing and preparation. For instance, regular cleaning and disinfection of equipment, surfaces, and utensils used in meat and dairy production can significantly reduce the risk of cross-contamination (Tassew *et al.*, 2010)

Moreover, educating farmers and food handlers about the risks associated with Listeria and the importance of good agricultural and manufacturing practices is crucial for minimizing contamination (Abebe *et al*, 2020). In addition to improving hygiene practices, proper cooking and food storage techniques are vital in reducing the risk of listeriosis. *L. monocytogenes* can survive and multiply at refrigeration temperatures, making it essential to maintain proper cooling

and storage conditions for perishable foods (Alessandria *et al.*, 2010). Cooking foods to safe internal temperatures and avoiding the consumption of raw or undercooked animal products can effectively kill the bacteria. Additionally, promoting pasteurization of dairy products can significantly decrease the chance of listeria contamination (Asfaw *et al.*, 2022).

2.4.One Health Implication of Antimicrobial Resistance of *Listeria Monocytogenes*

Listeria monocytogenes is a zoonotic pathogen that offers considerable difficulties to human, animal, plant, and environmental health systems. The extensive distribution of *L. monocytogenes* in soil, water, plants, animals, industrial settings, and farms highlights the difficulty of preventing its spread. Changing agricultural and production practices, particularly organic farming and biodiversity promotion, could unintentionally lead to environmental contamination (Ryzhova *et al.*, 2025).

The prevalence of *L. monocytogenes* in Ethiopia's beef value chain raises serious public health issues. The presence of Listeria monocytogenes in Ethiopian livestock-origin goods poses major public health implications, particularly for vulnerable populations such as pregnant women, the elderly and immunocompromised persons. The integration of a One Health approach, which creating awareness to the farmers, and animals' product consumers, and implementation of food safety hygienic practices is essential for effective surveillance and control of Listeria infections (Abate, 2023).

In order to effectively combat *L. monocytogenes* AMR, human, animal, plant, an environmental health must all be taken into account at similar time. Plants can harbor *L. monocytogenes* on leaves and fruits following contamination from soil, manure, or irritation; humans are exposed through the consumption of contaminated animal and plant products; animals can be asymptomatic carriers shedding the pathogen in feces or milk, and environmental reservoirs like soil and water continue the cycle. These interrelated processes show that single-domain initiative, such better food processing, are insufficient in the absence of coordinated action throughout the whole food chain. So, it is necessary to develop surveillance, risk assessments, and mitigation strategies that incorporate data and treatments from all One Health components (Ryzhova *et al.*, 2025).

2.4.1. Transmission Pathways

According to (Gasanov *et al*, 2005), *L. monocytogenes* is widely distributed in the natural environment (soil, sea, river, and tap water, plants, seeds, silage pits, and even algae). A number of sources have been identified as potential food contamination routes and human, animal, and plant transmission of *L. monocytogenes* (Stea *et al*., 2015; Matle *et al.*, 2019). The main ways that *L. monocytogenes* is transmitted to humans are by consumption of contaminated unpasteurized dairy products and meat products with pathogens (Manjengwa, 2022).

There is a high danger of contamination with *L. monocytogenes* during the manufacturing operations of animal origin foods (Quereda *et al.*, 2021). With regard to beef farm, the fecal shedding of *L. monocytogenes* can lead to contamination scenarios with potential implications for food safety (Fig. 1). It contributes to a higher load of *L. monocytogenes* in the immediate barn environment; increase the risk of additional animals becoming infected (Schoder *et al.*, 2022). Risk factors for *L. monocytogenes* contamination of feed and crop include the use of manure from infected animals to fertilize fields and farm runoff contaminating water supplies (Lyautey *et al.*, 2007; Schoder *et al.* , 2022).

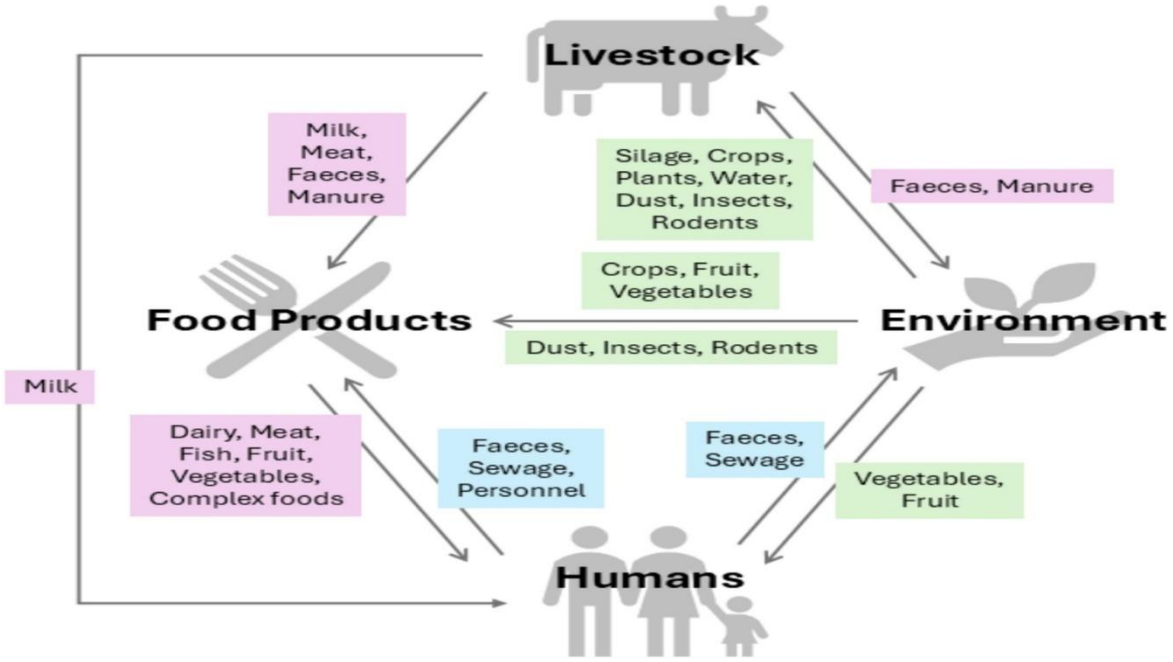

Figure 1: Transmission pathways of Listeria among three domains of one health.
Source: (Walland *et al.*, 2015).

2.4.2. Public health and food safety risk

Regarding *L. monocytogenes* public health concerns, listeriosis ranked fifth in terms of DALYs and fourth in terms of fatalities , and causes 23, 150 illnesses, 5463 deaths in 2010 (Noordhout *et al.*, 2014). *L. monocytogenes* is most commonly associated with pregnancy loss in healthy women, as well as septicaemia of CNS sickness (Distribution, 2025). Although HPV infects all human populations, pregnant women, newborns, the elderly and immunocompromised people are particularly vulnerable (Kundul and Ame, 2022). these are initiated vi direct and in direct contacts of infected animal tissues(Constable, 2021).

Food products may become contaminated by resistant bacteria released into the environment by infected animals, which could further spread resistant pathogens throughout the food chain. In addition to endangering human health, this may make livestock products less marketable, which would have a detrimental effect on food security and farmers' livelihoods (Woldemichael *et al*., 2025). Higher mortality rates and longer illness duration are caused by infections that are hard to treat when resistant bacteria are spread through direct contact or the food chain (Kallu *et al.*, 2024; Woldemichael *et al*., 2025).

2.4.3. Virulence gene of Listeria. monocytogenes

L. monocytogenes has several virulence genes such as *plcA, prfA, hlyA, mpl, actA, plcB, and iap,* that are important for its pathogenicity and completing the cell infection cycle (Swetha *et al.*, 2021). Significantly, *hlyA* and *prfA* were commonly reported in Ethiopian beef value chain (Getaneh *et al.*, 2025), and determined by using conventional PCR and were genomic DNA extracted by QIAamp DNA Mini Kit (Technologies, 2007), Gene sequencing techniques including PCR, pulsed-field gel electrophoresis, and randomly amplified polymorphic DNA are regularly employed to find the epidemiological and evolutionary relationships among *L. monocytogenes* isolates recovered from various sources (Getaneh *et al.*, 2025).

2.4.4. Economic and animal welfare impacts

The burden of Listeriosis and AMR along *L. monocytogenes* leads to increased healthcare costs, extended hospital stays, and higher mortality rates, thereby impacting the overall economy and healthcare resources (Woldu, 2024). Foodborne illnesses detrimentally impact human health and well-being while also imposing significant economic repercussions on individuals, families,

communities, corporations, and nations countries (WHO, 2017).The dynamic workforce of a nation, along with the expenses associated with prevention. treatment, product recalls, and investigations, imposes a significant financial strain on both governments and consumers, as well as the food industry (Mensah and Ofosu, 2020).

In addition to posing a threat to food safety and public health, *L. monocytogenes* can cause significant economic losses when it affects livestock production (Bagatella *et al,*, 2022). This may result in huge financial losses for animals sectors (Dhama *et al.,* 2015). In the food industry, *L. monocytogenes* cause a serious threat to food safety and quality (Centorame *et al.*, 2017) and has a major negative impact on trade, tourism, and health care system (WHO, 2017). Moreover, food traceability has become much more complicated, products have been taken from the consumer market, and sales of the implicated products have decreased (Lee and Yoon, 2021).

3. MATERIALS AND METHODS

3.1. Description of Study Area

The study was conducted from November 2025 to May 2026 at beef farms, slaughterhouses, and butcher shops found in Adama and Mojo towns, which are located in the East Shewa zone of Oromia, Ethiopia. Mojo is a town found at 8°39′N latitude and 39°5′E longitude, and at an altitude of 1,790 meters above sea level. It is situated at a distance of 66 km southeast of Addis Ababa, the capital city of Ethiopia. The average annual rainfall is 866 mm, with a bimodal rainfall pattern; main rainy season is from June to September, and the short rainy season is from March to May. Modjo town is the center of livestock industry and several national animal food chains such as beef and dairy chain, municipal and export abattoirs, and butchers.

The town of Adama is found at about 99 km southeast of Addis Ababa (39.17°E and 8.33°N) with an altitude of 1712 meters above sea level in the rift valley. Its annual rainfall ranges from 600 mm to 1150 mm and it has a temperature of 12.9°C to 33°C (CSA, 2020).

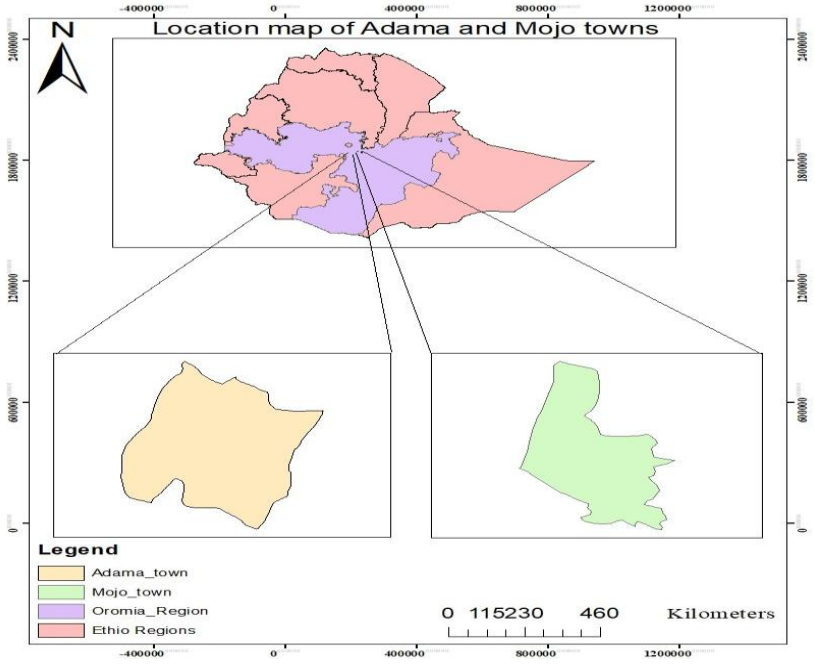

Figure 2: Map of Study Area

This Map is created by using ArcGIS pro

3.2. Study Population

The target study population was defined within a One Health framework, incorporating the animal, environmental, and human components of the beef value chain in Adama and Modjo, Ethiopia. The animal components comprised beef cattle that received fatting care in the animal fattening. The environmental component included farm environments and processing environments such as animal housing units, farm floor drainage water, carcass surfaces, abattoir equipment, and retail butcher shop environments. The human component consisted of individuals involved at various level of the beef value chain, including owners, workers, managers, and veterinarians, who were the source of information in this study.

3.3. Study Design

This study employed a cross-sectional design integrating both laboratory-based investigation and structured questionnaire survey to assess antimicrobial usage practice and antimicrobial susceptibility of listeria monocytogenes along beef value chain in Adama and Modjo, Ethiopia.

3.4.Sample Size Determination and Sampling

Since no published data are available on the *L. monocytogenes* prevalence along the beef value chain in the current study area, the sample size was determine by taking 4.2% as expected pooled prevalence of *L. monocytogenes* (Tadesse *et al.*, 2024) by using the formula recommended by (Thrusfield, 2018), with 95% confidence interval and 5% desired absolute precision.

$$\mathrm{N} = \frac{\mathrm{Z}^2 \times P(1-P)}{d^2} = \frac{(1.96)^2 \times 0.042(1-0.042)}{(0.05)^2} = 62$$

Where, Z = 1.96 (95% confidence level), P = 4.2% (expected prevalence, d=5% (standard deviation). A contingency allowance of 10% (6 samples) was added to account for potential sample loss or contamination, resulting in the required sample size being calculated to be 68 from each beef value stage with a total sample of 204. However, to increase the precision and accuracy of the study total samples of 460 were collected.

3.4.1. Sampling techniques

Systematic random sampling was applied to collect carcass swab samples from abattoirs and butcher shops. A sampling interval (k) was calculated (e.g., collecting a sample from every k-th carcass) to ensure that the samples were evenly distributed across the slaughter and processing periods. In contrast, simple random sampling was used to select individual cattle for fecal sampling within the selected farms, equipment for equipment swab sampling, and workers for hand swab sampling.

3.5.Data Collection Methods

3.5.1. Knowledge Attitude and Practice Survey

Structured questionnaires were administered through face-to-face interviews with selected participants (workers, owners, managers, and veterinarians) using the Kobo Toolbox app to evaluate AMU patterns (frequency and rationale for AMU, types of drugs used, and sources of drugs), waste management practices, and other related issues regarding the relationship between AMU and antimicrobial resistance (AMR). The questionnaire format was adopted from (Tadesse *et al.*, 2025), with modifications made and pretested at farm manager office to validate the questions for the collection of the needed information.

Informed consent was read to the respondents, and their written consent to participate in the survey was obtained prior to start (Annex 1). Data was collected from a total of 60 farms representing 27 from Adama and 33 from Modjo. The questionnaire includes respondent's socio-demographic characteristics and KAP on AMU and AMR. Finally, the collected information was exported to Excel for additional documentation.

3.5.2 Sample collection and transportation

Sample collection was strictly adhering to sterile procedures and the cold-chain principle to maintain sample integrity. Animal samples feces were collected directly from the rectum of selected cattle at farms. Carcass swabs where collected using sterile gauze by swabbing standardized anatomical sites (abdomen, thorax and breast) of selected carcass (ISO, 2015). Environmental swabs samples strictly was collected by sterile swabs taken from critical contact points in Farms (water, floors swab, and equipments swab) abattoirs (floors, equipment surfaces,

Vehicle) and butcher shops (cutting tables, knives, and refrigerators swab); Hand swabs collected from the hands of farm worker, selected abattoir workers and meat handlers after standardized work periods. The total amount of samples collected from respective group was indicated in table 1.

All samples were collected using appropriate sterile media Buffered Peptone Water for swabs. Each sample was clearly labeled with a unique ID code indicating the sample type, source, and date. Samples were immediately transported to the AAU, CVMA Microbiology laboratory, using an ice box containing an ice pack ensuring temperatures are maintained at +4 °C throughout transport.

Table 1: Summary of Collected Samples

Sample Source	Sample Types	Number
Animal	Fecal	98
	Carcass	112
	Total	210
	Vehicle	5
Environment	Equipments	99
	Floor	36
	Water	39
	Total	179
Human	Hand swab	71
Total		**460**

3.6. Laboratory Analysis

3.6.1. Isolation and identification of Listeria monocytogenes

Isolation and identification of *L. monocytogenes* were performed following the procedures recommended by the (ISO, 2022) that involve the primary enrichment and secondary selective enrichment, and plating. The primary enrichment of all collected samples was done in 10 ml of Half Fraser broth containing selective supplements that were incubated at 37 °C for 24 h. Then,

0.1ml of this primary enrichment broth were transferred into tubes containing 10ml full strength Fraser broth with selective supplements and incubated at 37°C for 48hr. After the enrichment, a loopful of inoculum from the selective broth were streaked onto Polymixin Acriflavin Lithium Chloride Ceftazidime Aesculin Mannitol (PALCAM) agar and incubated for 48 h at 37°C.

Suspected listeria colonies from PALCAM agar, characterized by typical green colonies surrounded by a black zone, were subcultured onto brain heart infusion agar and incubated at 37°C for 24h. Then purified suspected colonies were then subjected to Gram's staining and different biochemical tests, including motility, catalase test and Christie-Atkins-Munch-Petersen (CAMP) tests. Bacteria that looked coccoid to rod shaped and gram-positive, formed of gas bubbles in 3% hydrogen peroxide, and tumbling motility on semi-solid agar at 25°c were considered Listeria.

To confirm *L. monocytogenes* hemolytic activity and differentiate it from other Listeria species, the CAMP test was performed. On a 5% sheep blood agar plate, a single colony of a pure culture of the test isolate was streaked in a straight line. A *Staphylococcus aureus (*ATCC 25923) strain was streaked perpendicular to the presumptive colony, and then the plate was aerobically incubated at 37°C for 24 to 48 hours.

The result indicated by the formation of an arrowhead-shaped zone of increased β-hemolysis pointing toward the *Staphylococcus aureus* streak was considered *L. monocytogenes*. Presumptive *Listeria* colonies were picked and sub-cultured into Brain Heart Infusion Broth and incubated at 37°C for 24hr. Subsequently, 30% glycerol was added, and the cultures were preserved at -20°C for further identification and analysis.

3.6.2. MALDI-TOF based Identification of Listeria monocytogenes

MALDI-TOF was conducted at the Animal health institute (AHI) for identification of presumptive *L. monocytogenes* isolates based on a procedure recommended by the Bruker MALDI Biotyper manufacturer (Bruker, 2015). Presumptive culture from the brain heart infusion broth was subcultured on brain heart infusion agar and incubated for 24 hr. at 37°c. On a MALDI target plate, a single colony from brain heart infusion agar was selected by tips and applied as a thin layer. Each area was then treated with 1µl of 70% formic acid and dried at room

temperature. Then 1µl of α-Cyano-4-hydroxycinnamic acid (HCCA matrix solution) was added after drying, and the mixture was once more allowed to air dry. Finally, the MALDI target was inserted into the MALDI-TOF mass spectrometer and analysis was performed using Biotyper software.

3.6.3. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing of *L. monocytogenes* isolates was conducted by using disk-diffusion method, following the standardized protocols established by the Clinical and Laboratory Standards Institute (CLSI, 2023). A loopful of preserved *L. monocytogenes* was sub-cultured on brain heart infusion agar incubate at 37°C for 24hr. Around 2-3 pure fresh colonies of confirmed *L. monocytogenes* were transferred to 5ml of normal saline. Then the bacterial suspension was adjusted to 0.5 McFarland turbidity standards and transferred onto Mueller-Hinton agar plates by using a sterile cotton swab.

The inoculated plates were dried at room temperature, and antibiotic impregnated disks were applied to the surface of the inoculated plates. The plates were then incubated at 37°C for 24hr. The *L. monocytogenes* isolates were tested against a panel of 11 antimicrobial discs include, ampicillin (AMP,10µg), tetracycline (Tet,30µg), gentamicin (G,30µg), ciprofloxacin (CIP,5µg), Penicillin(P,5µg), Chloramphenicol (C,30µg), Streptomycin (S,25µg), Vancomycin(VAN,30µg), erythromycin (ERY,15µg), amoxicillin-clavulanate, (AC,30µg), and cotrimoxazole (STX,25µg). *L. monocytogenes* ATCC 19115 was used as positive control and diameter of the inhibition zone was measured using a caliper. Finally, the results were interpreted as Susceptible (S), Intermediate (I), and Resistant (R).

3.6.4. Detection of virulence genes in L. monocytogenes strains

Conventional PCR was performed to detect *hlyA* virulence gene of 6 *L. monocytogenes* isolates at AAU, CVMA Molecular biology laboratory. The genomic DNA of *L. monocytogenes* isolates was extracted by using a QIAamp DNA Mini Kit (Technologies, 2007)(Annex 4). The PCR amplification was performed using specific primers (Ashwani *et al*, *20*15; Paziak *et al*,1999) were used. More specifically, the reaction mixture was prepared in a final volume of 25 µl (Table 2).

The amplification of target gene protocol involved an initial denaturation step at 95°C for 2 min, 30 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, extension at 72 for 72° C for 90 seconds, final extension at 72°C for 10 min. The amplified product was detected by electrophoresis on 1.5% agarose gels stained with ethidium bromide, 100 base pair DNA ladder was used to identify the amplified product size. *L. monocytogenes* ATCC 19115 were used as positive control.

Table 2: Master mix and primer sequence used for amplification of the hlyA gene *of L. monocytogenes* by conventional PCR.

Reagents	**Volume**
Molecular Water	19.3µl
PCR reaction buffer	2.5µl
Primer-hlyA-Fov: GCAGTTGCAAGCGCTTGGAGTGAA	0.25µl
Primer-hlyA-Rev: GCAACGTATCCTCCAGAGTGATCG	0.25µl
DNA Template	2µl
Dntp	0.5µl
Taq Polymerase	0.2µl
Total	25µl

3.7.Data Management and Statistical Analysis

The survey data collected by Kobo Toolbox and laboratory results are entered to Microsoft Excel spreadsheet and then cleaned, coded, and exported to SPSS for analysis. Analysis was begun with descriptive statistics such as frequencies and percentages used to characterize the population KAP. Frequency and percentage were used to summarize the magnitude of *L. monocytogenes* and the antimicrobial susceptibility patterns Due to low detection of *L. monocytogenes* as positive isolation, regression analyses were not performed to identify statistically significant risk factors associated with the isolation of *L. monocytogenes* and the isolation of AMR *L. monocytogenes* strains.

3.8. Ethical Approval and Consideration

This study was conducted in compliance with national and international ethical guidelines. Before any data collection began, formal ethical clearance was obtained from the College of Veterinary Medicine and Agriculture (Ref.No: VM/ERC/08/118/8/2026 and Akililu Lemma Institute of Pathobiology (ALIHR IRERC-006/2018/2026) (Annex6), Addis Ababa University of Research and Review Committee. Prior to the KAP survey, all study participants received a clear explanation of the study purpose and mythology.

Informed consent was sought and documented for every participant, noting their freedom to leave at any time without consequence. All collected data was handled with strict confidentiality. Personal identifiers were removed, and code numbers were used instead of names. Data storage was secure, ensuring the anonymity of all human participants and study sites.

4. RESULTS

4.1.Beef farm Questionnaire Survey Results

4.1.1. Socio-demographic characteristics of the respondents.

A total of 60 respondents were included in the questionnaire survey, of whom 27 (45%) were from Adama town and 33 (55%) from Modjo town. The majority of participants were male (53; 88.3%), while females accounted for 7 (11.7%). Regarding educational status, 25 (41.7%) of respondents had primary-level education, whereas 5 (8.3%) had no formal education (Table 3).

Table 3: Socio-demographic characteristics of study participant

Study Variable	**Categories**	**Number (percent) of respondents**
Gender of respondent	Male	53(88.3%)
	Female	7(11.7%)
Age of respondent	<20	9(15%)
	20-30	25(41.6%)
	30-40	20(33.3%)
	>40	6(10%)
Education level	No formal Education	5(8.3%)
	Primary Education	25(41.7%)
	Secondary Education	11(18.3%)
	University/ college graduate	19(31.7%)
Role of study participants	Owners	25(41.7%)
	Managers	3(5%)
	Workers	23(38.3%)
	Veterinarian	9(15)
Experience in year	<5	53(88.3)
	5-10	4(6.7)
	11-20	3(5)

4.1.2. Knowledge of beef farm workers towards AMU and AMR

More than half of the respondents (51.7%) observed withdrawal periods after administering antibiotics before selling or slaughter animals, while 30% indicated that they did not know about withdrawal period. Most (81.6%) beef farm workers had no received any training and information related to AMU and AMR and only 18.3% of respondents had information about AMU and AMR. Among all respondents, 96.7 had knowledge to improper use of antimicrobial in animals could affect human health, and 65% of respondents report that misuse of antibiotics to AMR development. Only 15% of respondents could correctly identify which disease requires antimicrobial treatment (Table 4). Overall, about 24.6% of the respondents had good knowledge, while 74.8% had poor knowledge regarding AMU and AMR.

Table 4: Knowledge of beef farm workers regarding AMU and AMR (n=6)

Variables	**Number (%)**
Do you observe withdrawal periods after administering antibiotics before selling or slaughter animals?	
Yes	31(51.7)
No	11(18.3)
I don't know	18(30)
Have you received any training or information regarding AMU and AMR?	
Yes	11(18.3)
No	49(87.7)
Do you know improper use of antibiotics in animal can affect human health?	
Yes	58(96.7)
No	2(3.3)

I can correctly identify which disease requires an antimicrobial	
Strongly disagree	2(3.3)
Disagree	1(1.7)
Neutral	4(6.7)
Agree	5(8.3)
Strongly agree	9(15)
I don't know	39(65)
Do you know that misuse of antibiotics can contribute to antimicrobial resistance?	
Yes	39(65)
No	0(0)
I don't know	21(35)

4.1.3. Attitude of beef farm workers towards AMU and AMR

The majority of the respondents, 53 (88.7%) of the study area agreed with the use of antibiotics in animal feed to make cattle grow faster is acceptable, while only 7(11.7%) disagreed (Table 5).

Table 5: Attitude of beef farm workers towards AMU and AMR

Variables	Number (%)
Do you think that using antibiotics in animal feed to make cattle grow faster is acceptable?	
Agree	53(88.3)
Disagree	7(11.7)
The use of antimicrobials on my farm contributes to the global problem of AMR.	
Strongly disagree	1(1.7)
Disagree	2(3.4)
Neutral	4(6.7)
Agree	17(28.3)
Strongly agree	14(23.4)
I don't know	22(36.6)

4.1.4. Practice of beef farm workers related to AMU and AMR

Most respondents (88.3%) had regular access to veterinary services. All participants (100%) used antimicrobials, with administration decisions made by animal health professionals. Nearly all (96.7%) obtained antimicrobials from veterinary pharmacies. Common preventive practices included quarantining (70%) and vaccination (53.2%), while other measures were less frequently applied. Half of the respondents (50%) used antimicrobials 3–4 times in the past month, but only 18.3% kept usage records. The majority (80%) properly returned leftover antibiotics, although some retained or disposed of them inappropriately. Most respondents (58.4%) did not discontinue treatment early, and 66.7% reported using antimicrobials for growth promotion (Table 6).

Table 6: Practice Of beef farm workers Related to AMU and AMR

Variables	**Number (%)**
Do have access to veterinary services?	
Yes regular	53(88.3)
Yes Irregular	7(11.7)
Do you use antimicrobials in your farming?	
Yes	60(100)
No	0(0))
Which of the following preventative health measures do you use?	
Vaccination	32(53.2)
Quarantining	42(70)
Pest/vector control	7(11.7)
Strict cleanliness/disinfection protocols	4(6.7)
Who is the primary person responsible for deciding when to administer an antimicrobial to an individual sick animal?	
Animal health professional /Vet	60(100)
Trained farm manager	0
Farmer /owner	0
Where do you obtain antimicrobials drug?	
Veterinary clinics	16(26.7)

Agricultural supply stores	1(1.7)
Pharmacy	58(96.7)
Other Farmer	0
How many times were antimicrobials administered on your farm in the last month?	
1-2 times	20(33.3)
3-4 times	30(50)
5 or more times	10(16.7)
Do you keep records of antibiotic use?	
Yes	11(18.3)
No	49(81.7)
How do you dispose of unused and leftover antibiotics?	
Throw away anywhere	1(1.7)
Burn/bury	7(11.7)
Return to supplier	48(80)
Keep for later use	7(11.7)
I often discontinue treatment as soon as the animal looks better, even if the course is not complete.	
Strongly disagree	25(41.7)
Disagree	10(16.7)
Neutral	8(13.3)
Agree	6(10)
I don't know	11(18.3)
I use antimicrobials to promote growth or improve feed efficiency in my cattle.	
Strongly disagree	3(5)
Disagree	4(6.7)
Neutral	2(3.3)
Agree	11(18.3)
Strongly agree	40(66.7)

Multiple responses were allowed for preventive measures and sources of antimicrobials.

4.1.5. Type and Purpose of Antimicrobial use

Nearly half of the total respondents (48%) reported using antimicrobials for growth promotion (Figure 3).

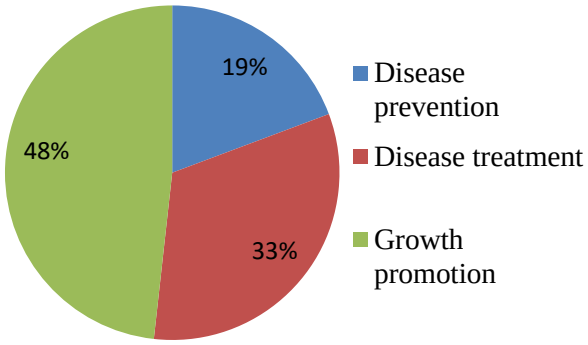

Figure 3: The primary purpose of antimicrobial administration on beef farming in Adama and Modjo (n =60)

The most commonly used veterinary drugs were ivermectins (81.7%), followed by oxytetracycline (55%), albendazole (35%), penstrep (21.7%), penicillin (20%), and sulfonamides (11.7%) (Figure 4).

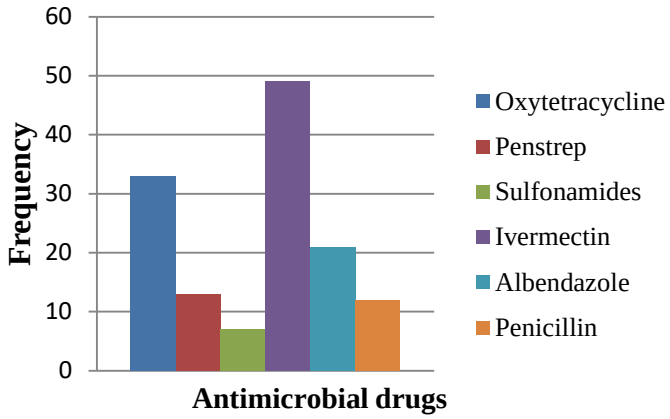

Figure 4: Common veterinary drugs used in beef farms in the study area.

Regarding waste management practices, the majority of participants (57%) disposed of manure and other waste through open dumping within the farm compound, while 25% disposed of it outside the farm and 17% practiced composting (Figure 5).

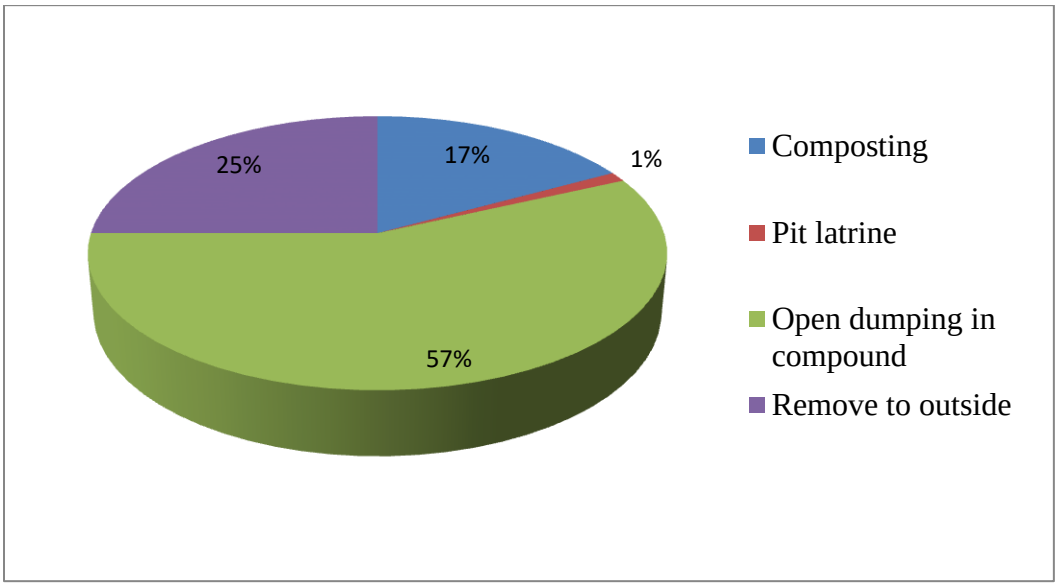

Figure 5: Manure and west management practices in beef farms.

4.2.Occurrence of *Listeria Monocytogenes* Isolates

A total of 460 samples were collected from beef farms (205; 44.6%), abattoirs (145; 31.5%), and butcher shops (110; 23.9%). Of these, 106 (23%) showed presumptive Listeria growth on PALCAM agar. The highest isolation rate was observed in beef farms (26.8%), followed by butcher shops (23.6%) and abattoirs (17.2%) (Table 7).

Table 7: Growth of Presumptive *Listeria* Isolates across sample source

	Growth PALCAM	
Sample Source	**Number of Sample tested**	**Number of Positive (%)**
Beef Farm	205	55(26.8)
Abattoir	145	25(17.2)
Butcher Shop	110	26(23.6)
Total	460	106(23)

The occurrence of presumptive Listeria isolates varied across One Health domains. The highest prevalence was recorded in animal samples (26.2%), followed by human hand swabs (23.9%)

and environmental samples (18.9%). Among individual sample types, fecal samples (33.7%) and equipment swabs (25.2%) showed relatively higher contamination levels (Table 8).

Table 8: Growth of Presumptive *Listeria* Isolates across One Health domain.

Sample source	Sample type	Number of tested sample	Number of Positive (%)
Animal	Fecal	98	33(33.7)
	Carcass swab	112	22(19.6)
	Total	**210**	**55(26.2)**
Human	Hand swab	71	17(23.9)
	Total	71	17(23.9)
Environment	Floor swab	36	2(5.6)
	Equipments swab	99	25(25.2)
	Water	39	7(17.9)
	Vehicles swab	5	0(0)
	Total	179	34(18.9)
Total		**460**	**106(23)**

Following biochemical confirmation (catalase test, motility, and CAMP test), 59 (55.7%) of the 106 presumptive isolates were identified as *Listeria monocytogenes*. The highest confirmation rate was observed in abattoir samples (68%), followed by butcher shops (57.6%) and farms (49%) (Table 9).

Out of the 59 biochemically confirmed isolates, 29 were further analyzed using MALDI-TOF mass spectrometry. Of these, 6 (20.6%) were confirmed as *Listeria monocytogenes*. The highest detection rate was observed in butcher shop samples (37.5%), followed by abattoir samples (20%) and farm samples (9%) (Table 10).

Table 9: Percentage of *Listeria monocytogenes* isolates confirmed by appropriate biochemical test (n =106)

Sample source	**Sample Type**	**Number of tested**	**Number of positive (%)**
Farm	Fecal	24	11(45.8)
	Hand swab	13	6(46.1)
	Equipment swab	10	5(50)
	Floor swab	2	1(50)
	Water	6	4(66.6)
	Total	55	27(49)
Abattoir	Carcass swab	12	10(83.3)
	Fecal	9	5(55.5)
	Equipment swab	2	1(50)
	Hand swab	1	0
	Water	1	1(100)
	Total	25	17(68)
Butcher shop	Carcass swab	10	4(40)
	Equipment swab	13	9(69.2)
	Hand swab	3	2(66.6)
	Total	26	15(57.6)
Total		**106**	**59(55.7)**

Table 10: Percentage of *Listeria monocytogenes* detected by MALD-TOF

Sample source	Sample Type	Number of tested (%)	Number of positive (%)
Farm	Fecal	5	0
	Hand swab	2	0
	Equipment swab	2	0
	Floor swab	1	0
	Water	1	1(100)
	Total	**11**	**1(9)**
Abattoir	Carcass swab	7	2(28.5)
	Fecal	3	0
	Total	**10**	**2(20)**
Butcher shop	Carcass swab	4	3(75)
	Equipment swab	3	0

	Hand swab	1	0
	Total	**8**	**3(37.5)**
Total		**29**	**6(20.6)**

4.3. Antimicrobial Susceptibility Test of *Listeria Monocytogenes*

The six confirmed isolates of *L. monocytogenes*, one from farm beef farm, two from abattoir, and three from butcher shop where subjected for antimicrobial susceptibility test with 11 different antibiotics. All isolates (100%) were susceptible to ampicillin, gentamicin, ciprofloxacin, chloramphenicol, streptomycin, amoxicillin-clavulanic acid, and cotrimoxazole. In contrast, all isolates (100%) showed resistance to penicillin. High levels of resistance were also observed for vancomycin (66.7%) and tetracycline (50%). Intermediate susceptibility was most commonly observed for erythromycin (66.7%), with the remaining isolates (33.3%) being fully susceptible (Table 11).

Table 11: Antimicrobial susceptibility patterns of *Listeria monocytogenes* isolates

Antibiotics	**Antimicrobial class**	**Number of isolates (%)**		
		Sensitive	**Resistant**	**Intermediate**
Ampicillin (10µg)	Beta-lactam	6(100)	0	0
Tetracycline (30µg)	Tetracycline	3(50)	3(50)	0
Gentamycin(30µg)	Aminoglycosides	6(100)	0	0
Ciprofloxacin(5µg)	Fluoroquinolones	6(100)	0	0
Penicillin (5µg)	Beta-lactam	0	6(100)	0
Chloramphenicol(30µg)	Amphenicols	6(100)	0	0
Vancomycin (30µg)	Glycopeptides	2(33.3)	4(66.7)	0
Erythromycin (15µg)	Macrolides	2(33.3)	0	4(66.7)
Amoxicillin-clavulanate (30µg)	Beta-lactam	6(100)	0	0
Cotrimoxazole (25µg)	Sulfonamide	6(100)	0	0
Streptomycin (25µg)	Aminoglycosides	6(100)	0	0

4.4. Detection of Selected Virulence Gene of *Listeria monocytogenes*

The isolates confirmed by MALDI-TOF were further analyzed for the presence of the virulence gene (hlyA) using PCR. All tested isolates (n = 6) were positive for the hlyA gene, producing the expected amplicon size of 456 base pairs, as indicated by comparison with the 100 bp DNA ladder. These isolates were obtained from water and carcass samples and exhibited resistance to tetracycline, penicillin, and vancomycin.

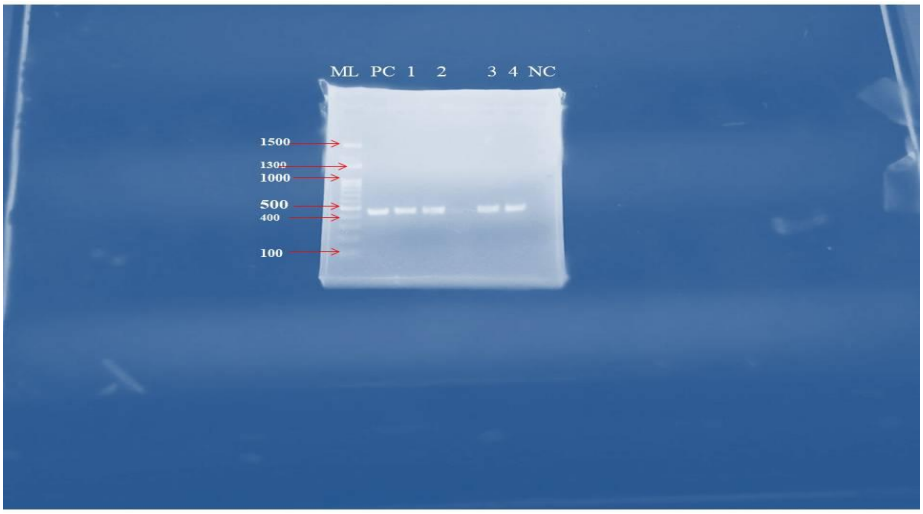

Figure 6: Agarose gel electrophoresis of PCR of hlyA (456) gene for detection of hemolysis virulence gene of *L. monocytogenes* isolates result.

Lane ML: 100bp DNA ladder; lane PC: positive control; lanes 1–4: positive Listeria monocytogenes isolates for hlyA virulence gene; Lane NC: negative control.

5. DISCUSSION

Antimicrobial resistance is a critical issue that threatens global food security, public health, and economic stability. Improper antimicrobial administration in beef production creates a powerful selective pressure on the microbial community contributing to antimicrobial resistant bacteria, which can spread through the environment and the food chain, thus posing a potential risk to human health (Economou and Gousia, 2015; Cameron and McAllister, 2016). *L. monocytogenes* is a foodborne pathogen that causes a serious foodborne disease. Furthermore antimicrobial resistant and virulent strains of *L. monocytogenes* can be transmitted from animal to humans through the consumption of contaminated food and environmental exposure (Tsitsos *et al.*, 2025).

Monitoring AMU practices is critical to minimizing AMR and limiting the incidence of *L. monocytogenes* in the food chain, as well as reducing environmental and public health risks (Majumder *et al.*, 2020; Sagar *et al.*, 2023). In this context, the present study was conducted to assess antimicrobial usage practices, antimicrobial susceptibility patterns, and virulence of *L. monocytogenes* along the beef value chain using a One Health approach.

The current study integrated laboratory analysis of *L. monocytogenes* with KAP assessment towards AMU and AMR. The result showed significant gaps in awareness among beef farm workers regarding AMU and AMR, alongside the presence of potentially pathogenic *L. monocytogenes*. The high proportion of participants with poor knowledge (74.8%) indicates a significant gap in antimicrobial stewardship awareness. Additionally, a large proportion of respondents expressed a positive attitude toward the use of antibiotics for growth promotion, reflecting inappropriate perceptions.

The decision on antibiotic use was made by animal health professionals, and antimicrobials were mainly obtained from veterinary pharmacies. *L. monocytogenes* isolates were recovered from fecal samples, hand swabs of farm workers and butchers, and carcass samples. All tested isolates were positive for the *hlyA* virulence gene, a key virulence determinant. The isolates showed resistance to penicillin, tetracycline, and vancomycin, while remaining susceptible to ampicillin.

Taken together, these findings indicate that *L. monocytogenes* in the study area poses a potential health threat to both animals and humans and requires prompt intervention.

The study findings revealed that respondents were predominantly male (88.3%), with most aged between 30–40 years. This is consistent with studies conducted in Ethiopia (Teshome *et al.*, 2021; Tufa *et al.*, 2023), which similarly reported male dominance and comparable age distributions among livestock workers. The majority of respondents had primary-level education. Educational status is an important factor influencing antimicrobial use practices. This finding aligns with Tufa et al. (2023), who reported that limited education contributes to inappropriate antimicrobial use and low AMR awareness (Tufa *et al.*, 2023).

The study also revealed that most respondents had poor attitudes toward prudent AMU. A high proportion (88.7%) agreed that antibiotics could be used for growth promotion, indicating misuse practices. Similar findings were reported in Turkey (Ozturk *et al.*, 2019; Alabi and Lin, 2025), where farmers believed antibiotics improve animal weight gain. This highlights the need for targeted training programs.

Regarding knowledge, about half of the respondents (51.7%) were aware of withdrawal periods, though this level remains insufficient. This was comparable to findings by Ragassa and Berhanu (2023). Additionally, 65% acknowledged that misuse contributes to AMR; however, knowledge about correct drug selection, dosing, and timing was very low (Ragassa and Berhanu, 2023). Only 18.3% of respondents had received training on AMU and AMR, which is lower than reports from Bangladesh Kalam *et al.*, (2022). This low level of training contributes to poor practices and highlights the urgent need for awareness programs. Almost all respondents didn't have the knowledge of the right drug, dose, and time to use drug to reduce the emergencies of AMR. Low proportions (15%) of respondents strongly agreed with the right uses of antimicrobials in the beef value chain. This revealed there is the knowledge gap in the study area and it is agreed with report of (Takele *et al.*, 2023), which reported only 2% of the respondents were know about the proper use of the drug as a livestock producers. The majority of the respondents (75.4%) were lack of knowledge regarding AMU and AMR. This findings were agreed with the previous research published by Takele *et al.*, (2023) (94%) and (Tufa *et al.*, 2018) (80%). The low knowledge level of the respondents might be associated with limited

educational level, inadequate training on prudent use of antimicrobial and low awareness of antimicrobial resistance, which influence the AMR, food safety, and public health.

The study indicated that small proportion of respondents (18.3%) had training or information regarding AMU and AMR which lower than the previous study report Kalam *et al.*, (2022) (50%) in Bangladesh. This study finding implies the emergency of AMR threat in beef value chain due to low level of information and training on AMU and AMR (Jahromi *et al.*, 2025). Moreover, in this study we find the highest respondents (88.7%) use antibiotics in animal feed for growth promotion which indicates inappropriate feeling towards prudent antimicrobial use in beef production system. The use of antibiotics for growth promotion has been widely recognized as a major driver of antimicrobial resistance (Alharbi *et al.*, 2025). This finding revealed the imminent need of associated training to increase knowledge and practice of the farm workers on antimicrobial usage patterns and its public health risk beyond animal health, as antibiotic and resistant organism shade to the surrounding community (Beyene, 2016).

The current finding on which is the improper uses of antibiotics was 96.7% is in agreement with national and international finding, The study done in UK by (Tang *et al*., 2023) has stated similar outcomes ('poor regulation control of antimicrobial drugs, and inappropriate use of antibiotics') with the current findings and another national scholars (Asfaw *et al.*, 2022; Endale *et al.*,2023), which is that improper use (growth promotion, self-administration, overdose, and underdose) of antibiotic like colistin poses a high risk of AMR (Tang *et al*., 2023). It indicate the major emergence of AMR worldwide, affecting humans, the environment, and animals (One health domains) (Beyene, 2016; Tufa *et al.*, 2023; Al-Khalaifah *et al.*, 2025) via improper use antibiotics, and insufficient monitoring of emergence of AMR in animal beef value chain.

The current finding indicated that most respondents of beef farm workers had moderate appropriate practices (had moderately good access to veterinary services (88.3%), and all respondents (100%) reported that antimicrobial use choice was made by animal health professionals). This finding is promising improvement related to various studies conducted in Ethiopia, which indicated that the use antimicrobial was commonly determined by farmers or drug sellers without the involvement of the veterinarians/professionals (Gemeda *et al.*, 2020; Gebeyehu *et al.*, 2021). However, the study conducted in intensive and semi-intensive beef

production in our country has revealed that limited access to veterinary services and self-prescribing of antimicrobials misuse, which in turn fosters the emergence of antimicrobial resistance (Reda *et al.*, 2025). This AMR can affect all One Health domains, since resistant bacteria can transfer from animal to human through food chains (direct contact, consumption of contaminated food, and environment pathways).

In the current study, almost all respondents (96.8%) gained antimicrobials from veterinary pharmacies, which indicate a relatively precise supply chain. however, this finding was disagreed with the previous study report in which, antimicrobials were frequently (67.8%) accessed from informal drug store without prescription, providing to inappropriate use and increased risk of antimicrobial resistance (Gebrekirstos *et al.*, 2017). Concerning AMU practices, almost 100% of the respondents reported using antimicrobials in their beef farms for different purposes. This practice is similar to the previous report's other part of Ethiopia, which revealed that antimicrobial are commonly used in livestock production by livestock keepers for disease prevention and control (Gemeda *et al.*, 2020). The extensive use of antimicrobials observed in the current study may cause the emergence of antimicrobial resistance.

The significant finding in this study is that 66.7% of the respondents strongly agreed, 18.3 agreed that antimicrobials are used to promote growth of beef animals. This finding indicates a high level of non-therapeutic use of antibiotics and it is agreed with the previous study reporting extensive use of antimicrobials as growth promoters in beef production, even though increasing awareness of AMR hazards (Gemeda *et al.*, 2020; Alabi, Chenia and Lin, 2025; Mekonen *et al.*, 2025). The current antimicrobial use practice has a significant one health concern; inducing the emergence and spread of resistant bacteria in beef production and potentially to humans via the food chain. In the current study, waste management related practices indicate potential environmental hazard, the respondents reported there is high (83.3%) open dumping of manure. the previous study also report high level of poor manure management, which is similar with the current finding (Odetokun *et al.*, 2026). While the current finding reveals better access veterinary services and professional decision-making in antimicrobial uses compared to various previous Ethiopian reports, critical practices gaps remain in recordkeeping, non-therapeutic antibiotic use, and waste management practices

The current study provides a crucial insight in to the isolation and identification of *L. monocytogenes* in beef value chain. The overall positive Listeria presumptive colony growth on PALCAM Listeria selective media was 106 (23%) across the total 460 tested samples. The high presumptive positive colony growth (26.8%) was identified from beef farm, followed by 23.6%, and 17.2% from butcher shop and slaughterhouses respectively. However, out of 106 positive listeria colony isolated by selective media, only 12.8% (n=59) samples was identified as *L. monocytogenes* by appropriate biochemical test. These results showed that the selective culture method is not enough to identify *L. monocytogenes* and emphasize the significance of confirmatory biochemical characterization. This finding was in align with the findings of Szabo *et al*., 1990) reported that the percentage of presumptive isolates obtained from selective media was reduced after biochemical tests due to the presence of non-pathogenic Listeria species or other related gram positive bacteria.

It was reported *L. monocytogenes*, can be transmitted from animal to humans through direct contact or the consumption of contaminated bovine raw meat (Heredia and García, 2018). Both animals and humans excrete the microorganism into the environment through faeces and sewage, propagating the contamination cycle (Barbuddhe *et al.*, 2008). *L. monocytogenes* is a significant human and animal pathogen that underlines the importance of the implication of the One Health concept (Tsitsos *et al.*, 2025). As part evaluation the chain of transmission this pathogen we were evaluated the magnitude of virulent *L. monocytogenes* and its antimicrobial susceptibility level from samples of carcass swabs of slaughter house and butcher shop and water sample of beef farm. Butcher shops had the greatest occurrence of *L. monocytogenes*, followed by abattoirs and beef farms. These findings could be because carcasses were exposed to pathogens during slaughter, processing, and retailing. The observation of contamination at this level indicates that the potential source of infection of meat was improperly cooked or untreated meat poses a potential source of infection for consumption. This finding highlight the critical requirement for better food safety measures in beef processing chain to combat the risk of *L monocytogenes* infections across the One Health approach.

The current study have been evaluated the antimicrobial susceptibility confirmed *L. monocytogenes.* Higher resistance were observed to penicillin, vancomycin, and tetracycline while all isolates were susceptible to ampicillin, gentamicin, ciprofloxacin, chloramphenicol,

Amoxicillin-clavulanate , and co-trimoxazole. The result is in agreed to the finding of (Conter *et al.*, 2009), in Parma, Italy in which they reported higher resistance for penicillin, vancomycin and tetracycline. The current isolate susceptible to gentamicin observed was contradicted to the finding of (Rippa *et al.*, 2024)reported in Italy, which revealed the resistance of gentamycin.

These current findings agreed with previous study that has reported changing levels of AMR in Listeria *monocytogenes* in our country(Getaneh *et al.*, 2025). The study reported (Luque-Sastre *et al.*, 2018) confirmed that though several isolates remained susceptible to ampicillin and gentamicin; there were growing trends of resistance to penicillin and tetracycline across various epidemiological zone, and indicates there potential shift in the susceptibility patterns of Listeria monocytogenes, which influenced by several factors such as antibiotic use, and selective pressure it exerts on bacterial population. In contrast, Iwu and Okoh (2020) found that isolates from food sources showed a lower resistance rate to penicillin (approximately 40%) but high resistance to tetracycline (up to 70%).(Iwu and Okoh, 2020) The increased resistance observed in our study might be a localized phenomenon or an emerging trend within specific settings, such as farms and abattoirs, where the use of antibiotics is widespread.

The high resistance to penicillin is particularly concerning because traditionally penicillin has been considered a first-line treatment for Listeria infections. The findings warrant a possible compromise in therapeutic choices for serious infections with this pathogen. Moreover, the intermediate susceptibility to erythromycin observed in our study may be an early stage of a resistance mechanism that needs further studies. This finding was similar to the study conducted in north part of Ethiopia that indicate 100% of the erythromycin was susceptible to listeria monocytogenes (Nasr *et al.*, 2026),Tigray region While there is some reassurance in susceptibility to other antibiotics such as ampicillin and gentamicin, the presence of multidrug-resistant strains is a significant public health risk. This is especially relevant considering the increasing global incidence of listeriosis, which can have serious consequences for vulnerable groups.

The virulence of *L. monocytogenes* isolates was detected by targeting *hlyA* (456pb); all tested isolates (n=6) were positive. The isolates were obtained from carcasses and water sample and showed resistance to tetracycline, penicillin, and vancomycin. The detection of the *hlyA* gene is

an important public health issue because the gene encodes listeriolysin O, a major virulence factor that enables *L. monocytogenes* to survive and multiply in host cells (Alharbi *et al.*, 2025). Similar studies have reported a high prevalence of the *hlyA* gene among pathogenic *L. monocytogenes* isolates obtained from animal samples (Katkar *et al.*, 2024).

The occurrence of hlyA gene of *L. monocytogenes* isolates from carcass and water samples indicated possible contamination of the beef processing environment and the risk of transmission of virulent *L. monocytogenes* through contaminated meat and water sources along the beef value chain. Furthermore, the existence of virulence gene and AMR among the isolates characterizes *L. monocytogenes* an important One Health concern, as resistant and pathogenic strains may persist in food chains and cause a serious risk to one health (Duma *et al.*, 2024).

Limitation of this study

The current finding has several limitations that may have influenced its overall findings. First, not all presumptive *Listeria* isolates were confirmed using MALDI-TOF analysis due to financial constraints and limited laboratory resources. Consequently, the overall prevalence of *L. monocytogenes* along the beef value chain could not be determined with complete accuracy. Second, the study did not assess the association between antimicrobial use patterns and antimicrobial resistance in *L. monocytogenes*, which limits the ability to establish a direct relationship between these variables. Third, only a single virulence gene (*hlyA*) of *L. monocytogenes* was investigated. Other important virulence associated genes were not examined due to a lack of specific primers and resource limitations, which may have restricted a widespread understanding of the pathogenic potential of the isolates. Finally, the molecular characterization performed in this study may not full represent the complete virulence profile of the detected strains.

6. CONCLUSION AND RECOMMENDATIONS

This study demonstrated the coexistence of poor AMU practices and antimicrobial resistant *L. monocytogenes* along the beef value chain in Adama and Modjo towns. The low level of knowledge among farm workers (74.8%) and the widespread non-therapeutic use of antimicrobials contribute to the emergence of resistance, despite antibiotic administration being primarily guided by veterinary professionals. All confirmed isolates harbored the *hly*A virulence gene, indicating pathogenic potential, and showed resistance to penicillin, tetracycline, and vancomycin, while remaining susceptible to commonly used antibiotics such as ampicillin and gentamicin. These findings highlight the presence of virulent and resistant strains, posing a potential public health risk to consumers. Overall, the study underscores the need for integrated One Health interventions to improve antimicrobial stewardship and food safety practices along the beef value chain.

Based on these findings, the following recommendations are forwarded:

- Beef farm workers in Adama and Modjo towns should receive targeted training on prudent AMU from relevant stakeholders.
- Training on hygienic meat handling practices should be provided to abattoir and butcher workers to prevent contamination by pathogenic organisms, including *L. monocytogenes*.
- Further molecular studies are needed to investigate additional virulence genes of *L. monocytogenes* to better inform treatment strategies and control measures within the One Health framework.

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8. APPENDEXS

Appendexs1: Informed Consent

My name is **Meskerem Mulisa**. I am working on my MSc research at Addis Ababa University College of Veterinary Medicine and Agriculture. I would like to interview you with a few questions about the antimicrobial use practice, sanitary conditions of your farm and taking fecal samples from randomly selected cattle and swab samples from your hand and farm environment with the objectives of assessing the knowledge, attitudes, and practices of beef farm workers towards antimicrobial uses and antimicrobial resistance, which is important to improve ecosystem health and food safety. 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 the interview.

Thank you for your participation

Appendex 2: Questionnaire survey

A. Basic information

Site: ☐Adama ☐Mojo

Beef farm ID: _____

GPS: _____

Date: ____/____/__2025___

B. Demographic Information

1 Gender: ☐Female ☐Male

2. Age: _____

3. Education level: ☐No formal ☐Primary ☐Secondary ☐college/University

4. Position/role: ☐Owner ☐Manger ☐Worker ☐Veterinarian ☐Other

5. How many years of experience do you have managing a beef farm?

☐< 5 years☐ 5-10 years ☐11-20 years ☐> 20 years

C. Knowledge, Attitudes, and Practices related question

1. Do have access to veterinary service? ☐Yes regular ☐Yes irregular ☐No
2. If yes to Q1, how would you rate the QUALITY of the veterinary service you access?

☐Very poor ☐Poor ☐Good ☐Excellent

1. If yes to Q1, how would you rate the price/affordability of the veterinary service you access?

☐Very expensive ☐Expensive ☐Reasonable ☐Affordable ☐Very affordable

2. Which of the following preventative health measures do you use?

☐Vaccination program (routine) ☐Quarantining new animals for a specific period

☐Routine pest/vector control (flies, ticks, etc.) ☐Strict cleanliness/disinfection protocols

☐None of the above

3. Do you use antimicrobials in your farming? ☐Yes ☐No
4. For what primary purpose were antimicrobials administered on your farm? (Select all that apply)

   ☐Disease prevention ☐Disease treatment ☐Growth promotion ☐Other

5. In the last 12 months, for what main purpose were the antimicrobials administered on your farm?

☐Treatment of respiratory diseases ☐Treatment of foot rot /lameness

☐Treatment of diarrhea ☐Treatment of bloat

☐Metaphylaxis ☐Prophylaxis

6. Who is the primary person responsible for deciding when to administer an antimicrobial to an individual sick animal? (select all applies)

☐Animal health professional /Vet (always) ☐Trained farm manager (following vet protocol)

☐Farmer /owner (based on personal experience) ☐ Expert in the drug shop ☐Other

7. Where do you obtain antimicrobials drug?

☐Veterinary clinics ☐Agricultural supply stores

☐Pharmacy /Drug store ☐Other Farmer (informal sources)

8. How often do you administer antimicrobials? ☐Never ☐ Rarely ☐Frequently
9. Please specify the time period in the last month ☐0 times ☐ 1-2 times ☐3-4 times

   ☐5 or more times

10. Do you keep records of antibiotic use? ☐Yes ☐No
11. When you treat an animal, do you record the following information? (Select all that apply)

    ☐Date of treatment ☐ Antimicrobial product ☐used Dosage and route

    ☐Withdrawal Period/Slaughter date ☐No, I do not keep formal records

12. List the antimicrobial and/or drugs used in the farm (select all possible

☐Oxytetracycline ☐ Penstrep ☐Sulfonamides ☐Ivermectin

☐Albendazole ☐Penicillin ☐Others

13. Do you observe withdrawal periods after administering antibiotics before selling or slaughter animals? ☐Yes ☐NO ☐I don't know

14. How do you dispose of unused and leftover antibiotics?

☐Throw away anywhere ☐Burn/bury ☐Return to supplier ☐Keep for later use

15. How do you manage manure or other wastes

☐Composting ☐Pit latrine ☐Open dumping in compound

☐Remove to outside ☐Other

16. Have you received any training or information regarding antimicrobial use and antimicrobial resistance? ☐Yes ☐NO

17. From which source did you receive this information? ☐Government extension services

☐ NGO services ☐Veterinary professionals ☐Other

18. Do you think that using antibiotics in animal feed to make cattle grow faster is acceptable?

☐Agree ☐ Disagree

19. Do you believe improper use of antibiotics in animal can affect human health? ☐Yes ☐No

20. The use of antimicrobials on my farm contributes to the global problem of AMR)

☐Strongly disagree ☐Disagree ☐Neutral ☐Agree ☐Strongly agree ☐I don't know

21. I often discontinue treatment as soon as the animal looks better, even if the course is not complete. ☐Strongly disagree ☐Disagree ☐Neutral ☐Agree

☐Strongly agree ☐I don't know

22. I use antimicrobials to promote growth or improve feed efficiency in my cattle.

☐Strongly disagree ☐Disagree ☐Neutral ☐Agree ☐Strongly agree

23. I am confident I can correctly identify which disease requires an antimicrobial vs. which does. ☐Strongly disagree ☐Disagree ☐Neutral ☐Agree

☐Strongly agree ☐ I don't know

24. Do you believe that misuse of antibiotics can contribute to antimicrobial resistance?

☐Yes ☐No ☐I don't know

Appendix 3: Sample collection procedures

1. **Procedures for fecal sample collection**

- ✓ Properly restrain the animal in a crush or with the help of an assistant to prevent injury.
- ✓ Wear protective gloves and ensure all materials are within reach.
- ✓ Clean the perianal area gently with a disposable towel to remove dirt or debris.
- ✓ Lubricate a gloved finger with sterile lubricant.
- ✓ Insert the lubricated finger gently into the rectum
- ✓ Collect fecal material directly from the rectal ampulla using gloved fingers.
- ✓ Withdraw the hand carefully and immediately transfer the feces into a sterile labeled container.
- ✓ Label the sample with the animal ID, location, date, and time of collection.
- ✓ Place the collected sample in a cool box with ice packs (4°C) immediately after collection.
- ✓ Transport the samples to the laboratory within 4–6 hours of collection whenever possible.

2. **Procedure for Carcass Swab Sample Collection**

- ✓ Identify standard standardized anatomical sites (abdomen, thorax and breast) each representing different contamination risks.
- ✓ Dip the sterile swab in sterile buffer or transport medium before swabbing.
- ✓ Rub the moistened swab vertically, then horizontally, and finally diagonally to ensure full coverage
- ✓ After swabbing, immediately insert the swab into sterile tube containing sterile buffered peptone water
- ✓ -Label the sample with ID, location, date, and time of collection
- ✓ Keep samples in a cool box at 4°C and transport to the laboratory within 4–6 hours of collection.

Appendix 4: Procedures of media preparation, isolation and identification

Buffer peptone water (OXOID CM1049)

Typical formula (g/l):

Enzymatic digest of casein10; sodium chloride 5; Anhydrous di-sodium hydrogen phosphate 3.5, potassium dihyrogen phosphate 1.5

Preparation:

Add 20g of the powder to 1 liter of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121°c for 15 minutes.

Fraser Broth Base (OXOID CM0895)

Typical formula (g/l):

Proteose peptone 5; Tryptone 5; Meat extraxt 5;Yeast extract 5; Sodium chloride 20;Di-sodium hydrogen phosphate 12; Potassium dihydrogen phosphate 1.35; Aescluin 1;Lithium chloride 3; PH 7.2± 0.2at 25°c

Preparation:

To make Half Fraser Broth: Add 12.9g to 225ml of distilled water. Mix well to dissolved and sterilize by autoclaving at 121°c for 15 minutes. Cool to below 50°c and 1vial of SR0166E (Half Fraser Selective Supplement) reconstituted.

To make Fraser Broth: Add 28.7g to 500ml of distilled water. Mix well to dissolved and sterilize by autoclaving at 121°c for 15 minutes. Cool to below 50°c and 1vial of SR0156E (Fraser Selective Supplement) reconstituted.

PALCAM AGAR BASE (OXOID CM0877)

Typical formula (g/l):

Colombia Blood Agar Base 39;Yeast extract 3;Glucose 0.5; Aesculin 0.8;Ferric ammonium citrate 0.5; Mannitol 10;Pienol red 0.08;Lithium chloride 15; Ph 7.2± 0.2 at 25°c

Preparation:

Suspend 34.5g in 500ml of distilled water. Bring gently to boiling to dissolve completely. Sterilize by autoclaving at 121°c for 15 minutes. Cool to below 50°c and aseptically add the contents of 1vial of PALCAM Selective Supplement (SR0150E) reconstituted as directed. Mix well and pour into sterile petridishes.

Brain Heart Infusion Broth (OXOID CM1135B)

Typical formula (g/l):

Brain infusion solids 12.5; Beef heart infusion solids 5; Proteose peptone 10; Glucose 2; Disodium phosphate 2.5; PH 7.4± 0.2 at 25°c

Preparation:

Suspend brain Heart infusion Broth 34.5g in 1000ml of distilled water, heat to dissolve completely. Cool and dispense into final containers. Sterilize by autoclaving at 121°c for 15 minutes.

Brain Heart Infusion Agar (OXOID CM1136)

Typical formula (g/l):

Brain infusion solids 12.5; Beef heart infusion solids 5; Proteose peptone 10; Glucose 2; Disodium phosphate 2.5; Agar 10; PH 7.4± 0.2 at 25°c

Preparation:

Suspend 47g in 1 liter of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121°c for 15 minutes, and pour into sterile petridishes.

Mueller Hinton Agar

Typical formula (g/l):

Casein Acid hydrolysate 17.5; Beef Extract powder 2; Starch 1.5; Agar 17; Ph 7.3±0.1 at 25°c

Preparation:

Suspend 38g of powder in 1000ml of distilled water. Boil with frequent agitation to dissolve the powder completely. Sterilize by autoclaving at 121°c for 15 minutes and pour into sterile petridishes.

1. Extraction of genomic DNA from bacterial suspension cultures

Pipet 1 ml of bacterial culture into a 1.5 ml microcentrifuge tube, and centrifuge for 5 min at 5000 x g (7500 rpm).

Add 180µl Buffer ATL and 20µl Proteinase K, mix by vortexing and incubate at 56°C until completely lysed (1–3 h).Vortex occasionally during incubation.

Add 200µl Buffer AL. Mix thoroughly by vortexing for 15 s and Incubate at 70°C for 10 min.

Add 200µl ethanol (96–100%). Vortex for 15 s, briefly centrifuge the tube to remove drops from the lid.

Pipet the mixture onto the QIAamp Mini spin column (in a 2 ml collection tube). Centrifuge at 6000 x *g* (8000 rpm) for 1 min. Discard the flow-through and collection tube.

Add 500 µl Buffer AW1. Centrifuge at 6000 x *g* (8000 rpm) for 1 min. Discard the flow-through and collection tube.

Place the QIAamp Mini spin column in a new 2 ml collection tube and add 500µl Buffer AW2. Centrifuge at full speed (20,000 x *g*; 14,000 rpm) for 3 min. Discard the flow-through and collection tube.

Place the QIAamp Mini spin column in a new 1.5 ml microcentrifuge tube (not provided), add 200µl Buffer AE or distilled water and incubate at room temperature for 1 min. Centrifuge at 6000 x *g* (8000 rpm) for 1 min to elute the DNA, and stored at −20 °C until used.

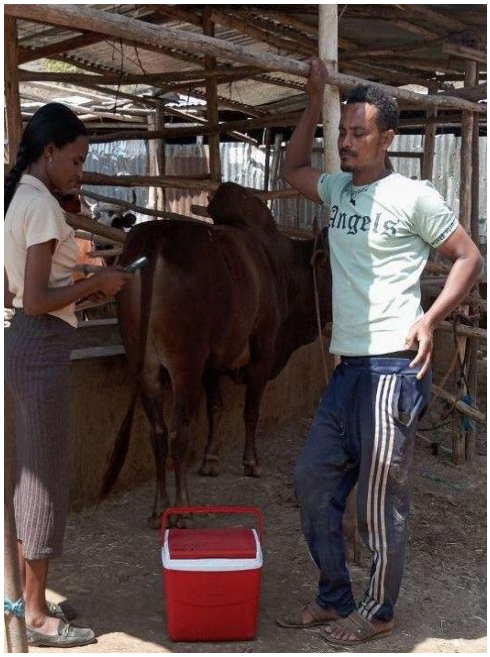
2. Polymerase chain reaction and Gel electrophoresis procedure

Prepare 20µl of PCR master mix and 5µl of DNA template (extracted DNA sample) in PCR tube. Place the PCR tube into the thermo cycler, cycling at 30 cycles of 95 °C for 2min during initial denaturation, 95 °C for 15 s during denaturation, 60 °C for 30 s during annealing and 72 °C for 90 seconds during extension, followed by a final extension at 72 °C for 10 min.

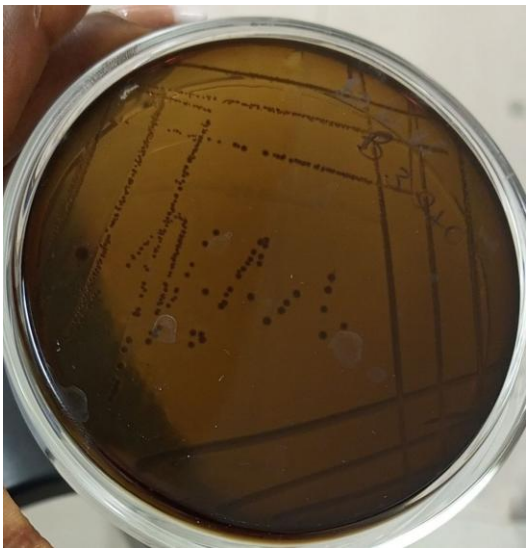
3. **Gel electrophoresis procedure**

- ✓ Weigh 1.5g of agarose powder and 100 mL TAE Buffer (1.5% agarose gel), mix well by helps of microwave.
- ✓ Cool to below 50°c and 2.5µl ethidium bromide mix well.
- ✓ Pour the gel in casting tray and then cooled for gel solidification. .
- ✓ Mix 5µl amplicon with 1µl loading dye
- ✓ Pipette sample and molecular ladder into gel wells.
- ✓ Gel electrophoresis power on, ensuring the DNA will migrate toward the positive electrode
- ✓ Visualized and captured on UV light.

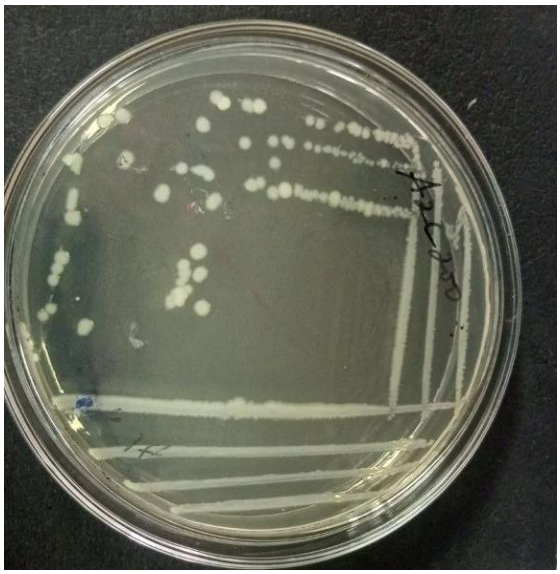
Appendex 5: Pictures taken during the study period



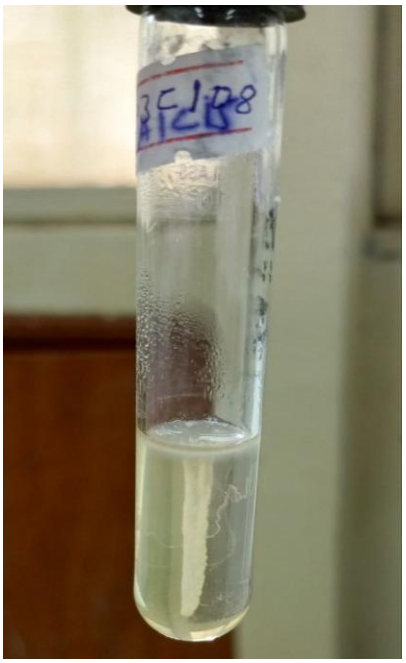
A) Interviewing an farm worker B) Primary selective enrichment of sample and PALCAM plate



C) Culturing under Biological safety cabinet **D)** Listeria on PALCAM Agar

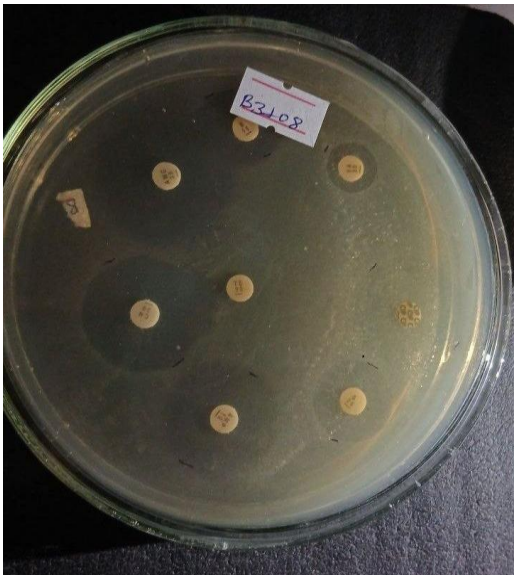


E) *Listeria monocytogenes* on BHI agar



F) Motility test of Listeria

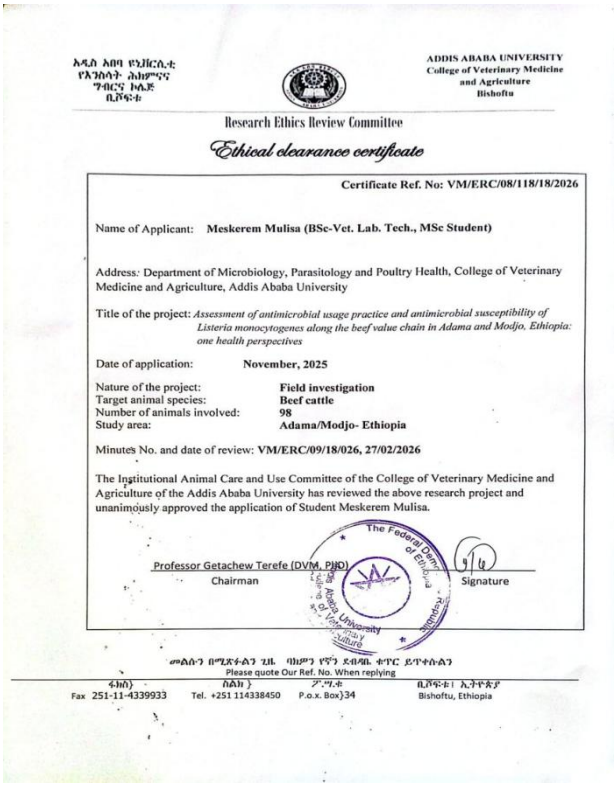
G) CAMP test result



H) Antimicrobial susceptibility test result

I. Conducting agarose Gelelecterophoresis at CVMA

Appendex 6: Ethical clearance certificate



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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/08/118/18/2026

Name of Applicant: **Meskerem Mulisa (BSc-Vet. Lab. Tech., MSc Student)**

Address: Department of Microbiology, Parasitology and Poultry Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Assessment of antimicrobial usage practice and antimicrobial susceptibility of Listeria monocytogenes along the beef value chain in Adama and Modjo, Ethiopia: one health perspectives*

Date of application: **November, 2025**

Nature of the project: **Field investigation**
Target animal species: **Beef cattle**
Number of animals involved: **98**
Study area: **Adama/Modjo- Ethiopia**

Minutes No. and date of review: **VM/ERC/09/18/026, 27/02/2026**

The Institutional Animal Care and Use Committee of the College of Veterinary Medicine and Agriculture of the Addis Ababa University has reviewed the above research project and unanimously approved the application of Student Meskerem Mulisa.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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Addis Ababa University
Aklilu Lemma Institute of Health Research

Ethical Clearance Certificate

Ref. No.: ALIHR IRERC-006/2018/2026

Date: June 9, 2026

Title: [Assessment of Antimicrobial Usage Practices and the Emergence of Antimicrobial Resistant Listeria Monocytogenes Along the Beef Value Chain in Adama and Modjo, Ethiopia: One Health Perspective]

Principal Investigator: Meskerem Mulisa,

Recommendation by the ALIHR -IRERC

Dear: Meskerem,

The ALIHR-IRERC has reviewed your above mentioned research proposal and noted its merit. The IRERC would like to remind you, as PI, to submit progress reports of the work every 6 months and the final report upon completion of the study. Furthermore, you are expected to notify the ALIHR-IRERC ahead of time for any amendment or modification in the protocol or premature suspension or termination of the study.

STATUS: Approved

National Ethics Review Board Clearance: not required

IRERC Chairperson: Nigatu Kebede, Prof.
Signature:

IRERC Secretary: Abebe Animut, PhD
Signature:

Approved by
Name: Prof. Wakgari Deressa, Director, ALIHR
Signature:
Date:

CC:
- IRERC Office
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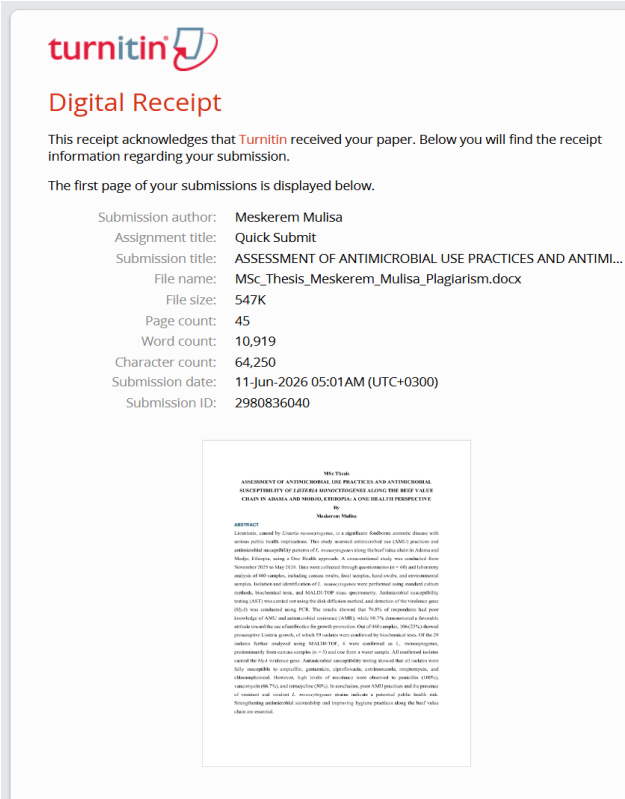
Appendix 7: Plagiarism Report



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MSc Thesis

ASSESSMENT OF ANTIMICROBIAL USE PRACTICES AND ANTIMICROBIAL SUSCEPTIBILITY OF LISTERIA MONOCYTOGENES ALONG THE BEEF VALUE CHAIN IN ADAMA AND MODJO, ETHIOPIA: A ONE HEALTH PERSPECTIVE

By

Meskerem Mulisa

ABSTRACT

ASSESSMENT OF ANTIMICROBIAL USE PRACTICES AND ANTIMICROBIAL SUSCEPTIBILITY OF LISTERIA MONOCYTOGENES ALONG THE BEEF VALUE CHAIN IN ADAMA AND MODJO, ETHIOPIA: A ONE HEALTH PERSPECTIVE

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