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ASSESSMENT OF THE HYGIENIC STATUS OF MUNICIPAL ABATTOIRS IN CENTRAL ETHIOPIA TOWARD DESIGNING STRATEGIES FOR IMPROVED MEAT SAFETY MANAGEMENT SYSTEMS

PHD DISSERTATION

BY

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I, the undersigned, hereby affirm that the work in this doctoral dissertation is wholly original and properly cited all pertinent sources. By signing this document, I genuinely affirm that I have not submitted this doctoral dissertation to any other institution, anywhere, in order to obtain any academic degree, or certificate.

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Endalu Mulatu, Ahmad Hamedy, Kebede Amenu, Nebyou Moje, Biruhtesfa Asrade, Hika Waktole, Bekele Megersa.2026. Assessment of Infrastructures, Operational Facilities and Food Safety Practices in Municipal Abattoirs in Central Ethiopia. *Discover food journal*: **6** (157). https://doi.org/10.1007/s44187-026-00931-y

Endalu Mulatu, Ahmad Hamedy. Nebyou Moje, Biruhtesfa Asrade, Hika Waktole, Bekele Megersa. Microbial Quality of Beef Carcasses and Hygiene Practices at Municipal Abattoirs of Central Ethiopia: A Cross-Sectional Study. *Environmental Health Insights*. 2026;**20:**1-14. https://doi.org/10.1177/11786302261470215

Endalu Mulatu, Christian Berens, Christian Menge, Kebede Amenu, Nebyou Moje Hawas, Biruhtesfa Asrade, Hika Waktole, Bekele Megersa, Ahmad Hamedy. Molecular Characteristics and Antimicrobial Susceptibility of Shiga Toxin-Encoding Escherichia Coli O157:H7 On Beef Carcasses From Municipal Abattoirs in Central Ethiopia. BMC Microbiology. 2026. https://doi.org/10.1186/s12866-026-05667-4

LIST OF ACRONYMS

AD	Allele differences
AMR	Anti-microbial resistance
APC	Aerobic Plate Count
BPW	Buffered Peptone Water
CAC	Codex Alimentarius Commission
CCP	Critical Control Points
CDC	Center for Disease Control
cgMLST	Core genome Multilocus sequence types
CLSI	Clinical and Laboratory Standards Institute
CT-SMAC	Cefixime and potassium tellurite supplement Sorbitol MacCkonkey
DNA	Deoxyribonucleic Acid
EMB	Eosin methylene blue
ESS	Ethiopian Statistical Service
EUC	European Union Commission
FAO	Food and Agriculture Organization of United Nations
USFDA	United State Federal Food and Drug Authority
FBD	Foodborne diseases
FC	Fecal Coliform
GIT	Gastro Intestinal Tract
HUS	Hemolytic uremic syndrome
IMS	Immuno magnetic separation
ISO	International Standards Organization
LMICs	Low- and middle-income countries
MDR	Multi-drug resistance
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence types
MOA	Ministry of Agriculture
MST	Minimum spanning tree

mTSB+n	Modified tryptone soya broth+ novobiocin
NRCSDWC	National Research Council Safe Drinking Water Committee
PBST	Phosphate buffered saline containing+ tween
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
STEC	Shiga toxin producing *E.coli*
WGS	Whole Genome Sequencing

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ABSTRACT

Ensuring the safety of meat products is a significant public health concern in many developing countries, where inadequate slaughterhouse infrastructure and poor hygienic practices result in microbial contamination of meat. A cross-sectional study was conducted to investigate infrastructure, operational facilities, and hygienic practices across 30 municipal abattoirs in central Ethiopia, together with the identification of pathogenic *Escherichia coli.* A structured questionnaire, checklist, and direct observation were used to collect information related to infrastructural conditions, operational facilities, and hygienic practices among 30 municipal abattoirs in central Ethiopia. Microbiological sampling was conducted in some selected abattoirs, including carcass swabs, workers' hand swabs, knife swabs, water samples, fecal samples, and hide samples. Standard microbiological plating method were employed to determine the aerobic plate count and fecal coliform count, while whole-genome sequencing was performed to investigate the genetic relatedness of *E. coli* O157:H7 from carcasses with the other source *E. coli* O157:H7 as well as occurrence of antimicrobial resistance and virulence gene investigated. The results indicated significant deficiencies in abattoir infrastructure and operational facilities. More than 50 % of the municipal abattoirs faced deficiencies in terms of required facilities such as hot water sources, equipment sterilizers, chilling rooms, and proper personal hygiene facilities, designated and well-designed hand-washing stations, showers, and restrooms, skin and bone storage rooms. In 70 % of municipal abattoirs, pest control facility, wildlife and stray dog control, and deep freezers were lacking, provision of personal protective equipment, separate room for skinning, evisceration, and viscera cleaning. Deficiencies in hygiene and food safety practices were also observed. In all municipal (100%) abattoirs, equipment sterilization between uses was not practiced, refrigerated transport for carcasses was unavailable and routine water quality testing was not conducted. Approximately, in three-fourths of the municipal abattoirs carcass processing took place on floor, and demonstrated inadequate enforcement of personal protective equipment use and lacked separate green offal, viscera and red meat transportation. Significant gaps in workers' hygiene and safety practices were identified. The use of aprons (44.37%) and head covers (45.77%) was reported by less than half of the workers, although 63.38% reported consistently wearing the clean personal

protective equipment available to them. Task alternation and equipment sharing were common, with 50% of workers engaging in these practices. Sterilization of knives between each use was not practiced in all abattoirs workers, and only 44.37% of workers performed regular medical checkups. About 35.21% workers practiced hand washing with soap while only 9.15% of respondents use gloves during the slaughtering process. Among the respondents, 52.11% of the workers had received on job food safety training. The study showed that the overall mean aerobic plate count of 6.12 $\log_{10}$ CFU/cm^2 and fecal coliform count of 4.58 $\log_{10}$ CFU/cm^2 on beef carcasses. Carcass swabs had a significantly higher mean aerobic plate count (6.12 $\log_{10}$ CFU/cm^2) compared to knife (5.54 $\log_{10}$ CFU/cm^2; p=0.006) and hand swabs (5.19 $\log_{10}$ CFU/cm^2, p=0.000) while knife swabs showed a higher mean fecal coliform count (5.05 $\log_{10}$ CFU/cm^2) than carcass swabs (p = 0.005). Water samples from municipal abattoir from Holeta, Batu and Gelan were contaminated with *E.coli.* In addition, prevalence of *E. coli* O157:H7 was 1.4% and 1.1% in feces and on carcasses, respectively Most importantly, *E. coli* O157:H7 was found to be negative in water, hide, hand and knife samples. Genomic analysis indicated clonal relationships among isolates from the same abattoirs and genetic diversity among isolates from different abattoirs. All isolates had multiple virulence factors such as adherence factors (*eae, iha, and tir*), type III translocated proteins (*espA, espB, espJ, espP, etpD, nleA, nleB,* and *nleC*), outer membrane proteins (*chuA, ompT*, and *traT*), toxins (*astA, ehxA*, and *toxB*), and other pathogenicity-related factors (*gad, iss, katP*, and *terC*). All strains carried the *stx2* subtype *stx2c* and were completely susceptible to tested antimicrobial agents no antimicrobial resistance genes were detected. In conclusion, this study revealed substantial deficiencies in abattoir infrastructure and hygiene practices. The microbiological analysis indicated that the microbial load on beef carcasses exceeded acceptable limits both in aerobic plate count and fecal coliform count. The microbial quality of workers hand and knife were also beyond acceptable limit. The findings also indicated that contact with fecal material during evisceration is a potential source of carcass contamination with *E. coli* O157:H7. Therefore, hygienic practices, abattoir infrastructure, and food safety regulations must be the future focus areas in the action plan to combat carcass contamination.

Keywords: *Abattoir, Hygienic Practices, Meat Safety , Public Health, Ethiopia*

1. CHAPTER I: GENERAL INTRODUCTION

More than 60% of disease-causing pathogens are zoonotic which shared between humans and animals, contaminated foods of animal origin play a paramount role in the transmission of these pathogens to humans (Chitnis *et al.*, 2020). Food safety has been a fundamental public health concern to human development since ancient times (Yun Li and Shi, 2022) and remains a subject of global discussion. Prioritizing food safety involves enhancing food safety systems in exporting countries, contributing to consumer health and protection and advancing food regulations and standards (Rahmat *et al.*, 2016). These efforts primarily focus on activities to reduce the risk of contamination within the food supply chain, whether arising from natural causes, accidental, negligence, or violations of food safety principles due to technical ignorance (Mitenius and van de Ligt, 2023).

Food safety and food security are closely interconnected concepts, so that improving food safety is essential for achieving sustainable food security (FAO, 2019). Unsafe food contaminated with pathogens, toxins, or chemical residues causes foodborne illnesses that undermine food security in two ways (WHO, 2015). First, illnesses such as diarrhea impair nutrient absorption and weaken immunity, leading to malnutrition even when sufficient foods are consumed. Secondly, foodborne disease reduces productivity and increases medical expenses, limiting household access to adequate food. Recognizing this, the World Health Organization (WHO), Food and Agriculture Organization of United Nations (FAO), and World Organization for Animal Health have reached consensus that a risk-based, farm-to-table approach is the most effective way to ensure food safety (Unnevehr, 2015). Such an integrated approach protects public health, reduces economic losses, enhances market access, and strengthens overall food security.

Globally, unsafe food contributes to approximately 600 million cases of foodborne illness and 420, 000 deaths annually, with children under five years of age accounting for nearly 30% of these deaths. World health organization (WHO) estimated that approximately 33 million years of healthy lives are lost worldwide each year due to consumption of unsafe

food, a number that is likely underestimated. The burden of foodborne diseases (FBD) is disproportionally distributed across the globe and inversely correlated with the socio-economic development of countries (WHO, 2026d). Over 90% of foodborne illnesses occur in low- and middle-income countries (LMICs). This creates massive health and economic strains, including an estimated more than one hundred billion US dollars annual loss due to reduced productivity and medical expenses (Grace, 2023). It also poses a significant barrier to international trade in food commodities (Tibebu *et al.*, 2024) due difficulties in complying with food safety standard requirements (Rahmat *et al*., 2016).

Food contamination by pathogenic microorganisms such as bacteria, viruses, parasites and prions is a worldwide public health concern. Many countries have reported increasing incidences of foodborne illnesses in recent years (WHO, 2026b). Human infection occurs through the consumption of contaminated animal-origin food or products (milk, meat, egg, and fish) (Chang *et al.*, 2015; Thapa *et al.*, 2020).

Meat and meat products are among food types most commonly contaminated with pathogens associated with FBD. Microbial contamination of meat and meat products is unavoidable as microorganisms are present on and in animals and their immediate surroundings. Meat producers and processors face many challenges to keep contamination levels as low as possible to ensure the microbial safety and product quality throughout the entire meat chain (Terrell and Hernandez-Jover, 2023).

Unhygienic meat handling and processing practices by meat handlers during processing are considered a major contributing factor to meat contamination (Das *et al.*, 2019) Personal hygiene and maintaining the health of the employees in the meat and meat processing facilities are critical for preventing food-borne illnesses and safeguarding public health. Furthermore, inadequate slaughterhouse operational facilities can lead to unhygienic handling of carcass resulting in cross-contamination, spreading foodborne pathogens from hides/feces to beef carcasses (WHO, 2026c). *Salmonella*, *E. coli* O157:H7, and *Staphylococcus aureus* are among the major bacterial contaminants of carcasses in slaughterhouses (Ahmad *et al.*, 2013). These pathogens can cause a wide

spectrum of human illnesses ranging from asymptomatic infection to severe diarrhea (WHO, 2022).

Among these pathogens, *E. coli O157:H7* is considered one of the most significant foodborne pathogens because of its ability to cause severe illnesses, including hemorrhagic colitis and hemolytic uremic syndrome (Ayenew *et al*., 2021). The presence of antimicrobial-resistant microbes in this setting exacerbates the burden of FBD (Founou *et al.*, 2016) and can result in human infections that can be tough or impossible to treat (WHO, 2011). These conditions are associated with unhygienic meat handling and processing practices and render slaughterhouses focal points for the spread of potential food borne pathogens to the public and work places where the occupational disease acquired.

Therefore, reducing foodborne health risks to the public requires regular abattoir sanitary-hygienic evaluation and various-intervention approaches aimed at reducing contamination of meat and meat products within slaughterhouse environments and ensuring food safety (Atsbha *et al.*, 2018). In Ethiopia, the practice of consuming raw or undercooked meat is increasingly common including in major urban centers, which may create favorable conditions for the transmission of foodborne pathogens to consumers (Gebeyehu *et al.*, 2022). According to researchers report there is an evidence of beef carcass contamination with potential pathogens like *E. coli* O157: H7 (Atnafie *et al.*, 2017; Mengistu *et al.*, 2017; Ayenew *et al.*, 2021; Tadese *et al.*, 2021), *Staphylococcus aureus* (Beyene *et al.*, 2017; Adugna *et al.*, 2018; Gizaw *et al.*, 2020) and *Salmonella* spp (Sibhat *et al.*, 2011; Alemu and Zewde, 2012; Tadesse and Gebremedhin, 2015; Wabeto *et al.*, 2017; Atsbha *et al*., 2018; Zelalem *et al.*, 2019) at municipal abattoirs in different part of the country. These studies further identified unhygienic carcass handling during slaughter processes as a major source of contamination and strongly recommended the implementation of improved food safety interventions (Zelalem *et al.,* 2019).

1.1.Background

1.1.1. Economic importance of the livestock and beef sector

Mixed crop-livestock farming is the dominant agricultural system in Ethiopia, supporting majority of agricultural activity and livelihoods (Assefa *et al.*, 2024). Demands for animal products are rising globally, and Ethiopia possesses significant potential to meet this demand through meat and meat product production for both domestic and international markets (Muleta and Dida, 2025). Ethiopia has 55 million cattle population; 78% out of total livestock population and contributing billions of dollars to the economy through the provision of meat, milk, hides and draft power. The total combined output value (e.g., beef, milk and draft power) of cattle production was 11.9 billion United states dollar (USD), which was 11.2% of the GDP in Ethiopia in 2021 (Li *et al.*, 2023). The beef cattle production sector plays a crucial role in the livelihoods of pastoral farmers in Ethiopia, and it is the main source of beef meat for domestic and foreign markets. As compared to other African countries, Ethiopia has a huge beef cattle population (Abebe *et al.*, 2022).

In 15 years Ethiopia showed 82.01% growth in total meat production, reaching 123,670 tons by the end of 2019. Cattle meat emerged as the dominant contributor, accounting for 67,488 tons or 54.6% of the total meat production, followed by sheep at 26,969 tons (21.8%), goats at 18,806 tons (15.2%), poultry at 8,285 tons (6.7%), and camels at 2,122 tons (1.7%) (Begna, 2025). This rapid expansion highlights the increasing economic and nutritional importance of the meat sector within Ethiopia's agricultural economy.

In Ethiopia meat consumption is embedded in special occasions and cultural practices rather than being everyday dietary staple. On the other hand, cultural taboos and religious beliefs limit the species of animals used for meat. Among the meat being consumed, beef was the most widely consumed meat type followed by chevon and mutton (Biru *et al.*, 2014). Population growth, rising income and urbanization have fueled a significant

increase in demand for meat and meat products in developing countries (Latino *et al.*, 2020).

1.1.2. Meat safety and public health significance

Meat is a high-quality, complete protein source containing all essential amino acids required for human tissue repair, growth, and immune function. It is a nutrient-dense, easily digestible food providing critical micronutrients like iron, zinc, and vitamin B12, which are often limited in plant-based diets (Klurfeld, 2018; Sheffield *et al.*, 2024; Barr *et al.*, 2025;). Consequently, meat contributes substantially to nutritional security and human health, particularly in populations vulnerable to protein and micronutrient deficiencies.

Despite its nutritional importance, meat also represents one of the major vehicles for transmission of foodborne pathogens to humans globally, food-borne microorganisms are major pathogens affecting food safety and cause human illness most commonly associated with consumption of animal products contaminated with vegetative pathogens or their toxins (Abebe *et al.*, 2020). Approximately one in ten people worldwide fall ill after consuming contaminated food (WHO, 2026d). Among the various food sources, contaminated meat represents a major contributor to the global burden of FBD, accounting for approximately 41% of the total FBD burden and contributing to 33% of all food-related fatalities (Pires *et al.,* 2021).

Ensuring meat safety is critical for protecting public health from zoonotic foodborne illnesses and antimicrobial resistance pathogens. This requires strict hygiene protocol from primary production to slaughter, processing, transportation, retail, and final consumption (Oluwafemi *et al.*, 2013). Failures in hygienic meat handling and slaughterhouse sanitation can facilitate contamination of carcasses with pathogenic microorganisms originating from animal hides, feces, contaminated equipment, water sources, or food handlers. In Ethiopia, consumption of raw or partially cooked meat is a widely practiced tradition and may be considered a significant contributor to zoonotic

diseases (Assefa, 2019; Deneke *et al.*, 2022). Information on the public risk perceptions and protective behaviors are essential for prevention and control of these diseases (Tsegaye *et al.*, 2022). More than 200 zoonotic diseases are known to affect humans, representing a significant share of both emerging and existing infectious diseases worldwide. These diseases pose a major global public health challenge, particularly in LMICs (Pantha *et al.*, 2025). Unsafe meat poses several public health risks, including salmonellosis, *E. coli,* campylobacteriosis accompanied by mild to severe diarrhea, vomiting, dehydration, and potential mortality (Adesola *et al.*, 2024; Ali and Alsayeqh, 2022).

1.1.3. Abattoirs as critical control points in meat safety

Abattoirs play an important role in surveillance, control, and prevention of diseases of animal and public health importance (Nastasijevic *et al.*, 2023). An abattoir, or slaughterhouse, is a specialized, licensed facility designed for the hygienic slaughtering, dressing, and processing of livestock into meat products for human consumption. Its primary functions include humane animal slaughter; ante-mortem and post-mortem inspection; disease surveillance; carcass processing; and by-product rendering, all of which involve critical slaughter processes and potential points of contamination (FAO, 1985). According to Food and Agriculture Organization of United Nations (FAO) guidelines abattoir slaughter practices a requires structured process (as shown in Figure 1 below) that focus on humane handling, stunning, and hygienic processing to ensure food safety (FAO, 2026b). Abattoirs are essential components of the meat supply chain and key for meat processing and food safety management functions integral to urban food security (Nigussie *et al.*, 2025).

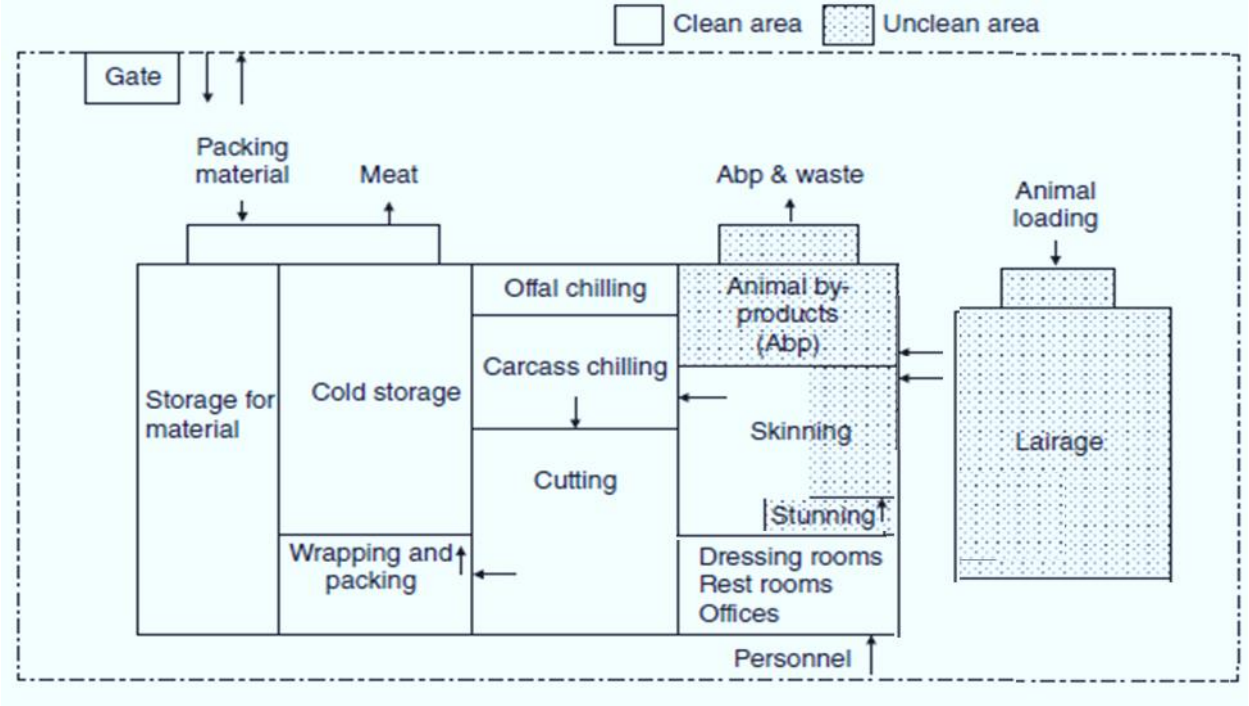


Figure 1: Schematic layout of a slaughterhouse adopted from Ninios *et al.* (2014).

Abattoirs in Ethiopia are classified as export abattoirs which designed to high standard and supply meat for global market and domestic abattoirs are abattoir or slaughterhouse that is approved and registered by the regional regulatory authority and engaged in slaughtering of animals for human consumption within the country (MOA, 2021). Abattoirs in Ethiopia are primarily public-owned facilities that provide slaughter services for local community typically handling cattle, sheep, and goats (Zaba *et al.*, 2025). Regulatory oversight of these municipal abattoirs faces severe challenges due to fragmented legal frameworks, weak enforcement, and poor infrastructure (Wolde *et al.*, 2026). Key infrastructure and operational facilities for a standard abattoir includes a functional lairage, slaughter hall with designated rooms for offal, skin, carcass dressing/splitting areas, bones, cold storage, waste disposal systems, potable water supply, hot water supply, equipment sterilizers, carcass processing equipment's (knife, hooks and carcass hanging rail system), personal protective equipment's (PPE), veterinary offices and laboratories, adequate light and sanitation infrastructure as well as staff amenities like rest room, bathroom and break areas (FAO, 1988; Mann *et al.*, 1983; Skaarup, 1985). Abattoir operational facilities in many developing countries, including

Ethiopia, are frequently substandard and are characterized by poor design, dilapidated infrastructure, and a lack of essential equipment (Ovuru *et al.,* 2024; Yimana and Hassen, 2024; Nigussie *et al.,* 2025; Zaba *et al.,* 2025)

1.1.4. Hygienic requirements and standards for abattoirs

The abattoirs' adherence to the enforcement of hygienic practices is a determining factor for the safety of meat, which includes facility, personal, and process sanitation and safety, all of which are intended for preventing pathogens from contaminating the meat (FAO, 1991). Hygienic failures at any stage of slaughter and meat processing may facilitate the introduction and spread of biological, chemical, and physical hazards within the meat supply chain. According to Codex Alimentarius Commission (CAC) good hygiene practices in abattoirs are a fundamental part of meat safety systems that are considered crucial for preventing contamination and providing safety for meat for human consumption during, slaughter, carcass dressing, processing, storage, and transportation. These practices are a set of measures that include the design of the facility, sanitation, personal hygiene of workers, hygienic handling of carcasses, temperature, and waste disposal, all of which are intended for reducing contamination and ensure the safety of meat intended for human consumption during the slaughtering and processing of meat (CAC, 2005). Failure to implement effective pre-operational sanitation measures may increase the risk of carcass contamination during slaughter operations.

Pre-operational activities include ante-mortem inspection of animals to identify for signs of illness and stress, cleaning and disinfection of the slaughter halls, equipment, floor, walls, and drains, verification of potable water supply, and inspection of sanitation facilities such as hand-washing stations and toilets (CAC, 2005). Personnel-related hygiene activities: include the washing of hands with soap and disinfectants before the start of work and during operations, wearing clean hygienic attire such as aprons, gloves, boots, and hair cover, prohibition of eating, smoking, and wearing of jewelry during work, routine medical examination, and training of workers in hygiene and food safety principles (FAO, 2004b). During slaughter and carcass processing, hygienic operational

procedures are necessary to minimize cross-contamination. These activities also include hygienic methods of stunning and bleeding to prevent contamination of carcasses, hygienic removal of hides or skins without any contact between the outer surface and the exposed meat surfaces, hygienic evisceration to prevent puncture of gastrointestinal organs, immediate trimming or washing of visibly contaminated areas on carcasses, and separation of edible and inedible offal using special equipment and containers (FAO, 1991). The post-operational and storage activities include prompt chilling of carcasses at appropriate temperatures, storage and transport under cold conditions, avoiding cross-contamination between raw meats and wastes, and post-mortem inspection of meats (CAC, 2005; CAC., 2022).

Equipment and utensil hygiene includes frequent cleaning and sterilization of knives, hooks, saws, and tables, often in hot water or food grade chemical sanitizers, between carcass operations, and checking the efficacy of cleaning (CAC, 2005; FAO, 2004b). The environmental hygiene includes implementing efficient pest control measures, ventilation that limits condensation and dust, systematic removal of blood and solid wastes, and hygienic handling of effluents. So, poor hygiene practices in such places are a major cause of public health hazards. Despite the existence of these internationally recognized hygienic standards, implementation in many developing countries remains inadequate. The lack of regulation and poor hygiene conditions may result in the transmission of zoonotic pathogens from animals to humans, mainly affecting workers at slaughterhouses who are in close proximity to animals as well as animal products (Hossain *et al.*, 2025).

The knowledge gap of food safety and improper hygienic practices of food handlers are the key factors for the transmission of foodborne diseases (Islam *et al.,* 2024). Workers are a major contributor to meat contamination at slaughterhouses, as they are a potential causes of contamination. Inadequate hygiene practices, such as contaminated hands, clothes, and equipment like knives, directly cause contamination of meat products. The major contamination hazards are failure to sanitize equipment, poor personal hygiene practices, inadequate handling of hides/viscera, and lack of training (Tarekegn *et al.*, 2023; Okoli *et al.*, 2025; Kurniawan *et al.*, 2026). Hygienic safety of food products is a

major health duty for meat product handlers, as inadequate hygiene practices may cause health hazards to consumers (Ahad *et al.*, 2025).

In Ethiopia the prevalence of good hygienic practices in municipal abattoirs is low (Zewude *et al.*, 2025). Common hygienic challenges in municipal abattoirs include inadequate sanitation of slaughter floors and equipment, poor waste disposal systems, irregular water supply, and overcrowded or poorly designed facilities that make effective cleaning difficult (Gutema *et al.*, 2021; Desisa and Mitiku, 2025; Zewude *et al.,* 2025). These problems are often compounded by limited staff training in hygienic practices, lack of protective clothing use (Gutema *et al.,* 2021; Zelalem *et al.*, 2021), absence of regular health checkups and poor personal hygiene (Zemachu *et al.*, 2023; Ahad *et al.,* 2025), as well as the presence of pests such as flies and rodents (Zewude *et al.,* 2025). Inadequate separation of clean and dirty operations, improper handling of carcasses, and insufficient meat inspection further increase the risk of contamination and compromise the overall safety and quality of meat produced which may ultimately lead to serious public health issue (Yimana and Hassen, 2024).

1.1.5. Microbial quality of carcass

Microbial contamination of meat carcasses during slaughtering and processing is an inevitable. The microbial counts that exceed the maximum limit show that the hygiene of meat production at the abattoir is inadequate and that measures are required to ensure food safety (Tsitsos *et al.*, 2022). Beef carcasses can be easily contaminated with various pathogenic bacteria during dressing, evisceration, processing, and storage. Most commonly the sources of the microbial contamination can be the skin, feet, stomach, intestinal contents, hands, clothes of employees, equipment, and water used for washing carcasses, as well as dust and aerosols (McEvoy *et al.*, 2000; Koutsoumanis and Sofos, 2004). The contamination of carcasses and various meat cuts in terms of numbers and kinds of various spoilage bacteria, yeast, molds, pathogens, and emerging pathogens has been studied and reported extensively (Cole *et al.*, 2011). The microbial quality of carcasses is commonly assessed using indicator microorganisms such as aerobic plate

counts (APC), Enterobacteriaceae counts, and fecal coliform counts. These microbiological indicators are widely used to evaluate the overall hygienic performance of slaughterhouses and the effectiveness of sanitation procedures during meat processing. Elevated APC values generally reflect poor overall hygiene and increased microbial contamination, whereas high Enterobacteriaceae or fecal coliform counts indicate fecal contamination and inadequate sanitary practices during slaughter and carcass handling. International microbial standards for beef carcasses recommend maintaining relatively low microbial loads to ensure meat safety and quality.

Acceptable microbial quality for a beef carcass requires low levels of contamination, generally maintaining APC from 3.5 log cfu/cm^2 daily mean log to 5.0 log cfu/cm^2 daily mean log, with Enterobacteriaceae from 1.5 log cfu/cm^2 daily mean log to 2.5 log cfu/cm^2 daily mean log. Beef carcasses in municipal abattoirs of Ethiopia often exhibit poor microbial quality, frequently exceeding maximum limit for total aerobic counts and fecal coliform counts. High bacterial loads are caused by poor hygienic conditions during slaughter, transportation, and display in butcheries, compounded by high environmental temperatures (Bersisa *et al.*, 2019; Berhanu *et al.*, 2025; Kemal and Tabit, 2025).

The critical control points (CCPs) in municipal abattoirs involve stages that prevent biological, chemical, and physical contaminants, with an emphasis on hygiene from slaughter to dispatch. These includes pre-slaughter animal inspection, hygienic skinning, evisceration, carcass chilling, and final dispatch. These steps prevent contamination and ensure food safety (FAO, 2004a).Among these stages, hide removal and eviscerations are particularly critical because improper handling may result in direct transfer of fecal pathogens onto carcass surfaces. Effective monitoring and control of these critical points are therefore essential for reducing microbial contamination and ensuring meat safety. In addition to indicator microorganisms, the presence of specific foodborne pathogens such as *Escherichia coli* O157:H7*, Salmonella spp., and Staphylococcus aureus* on carcasses represents a significant public health concern.

1.1.6. E. coli O157:H7 as a foodborne pathogen

E. coli O157:H7 has emerged as a significant foodborne pathogen(Gambushe *et al.*, 2022), causing illnesses ranging from bloody diarrhea to severe hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura Center for disease control (CDC) (CDC, 2026). It is estimated that up to 10% of patients with Shiga toxin producing *E.coli* (STEC) infection may develop HUS, with a case-fatality rate ranging from 3 to 5%. Overall, HUS is the most common cause of acute renal failure in young children. It can cause neurological complications (such as seizure, stroke and coma) in 25% of HUS patients and chronic renal sequelae, usually mild, in around 50% of survivors (WHO, 2026c). Although precise global incidence figures are challenging to estimate due to underreporting and limited surveillance in many regions, systematic analyses suggest that *E. coli* O157:H7 cause approximately 2.8 million acute illnesses annually worldwide, including thousands of cases of the severe complication HUS and hundreds of deaths (Majowicz *et al.*, 2014). *E. coli* O157:H7 is also one of the causes of gastrointestinal diseases in developed countries causing millions of illnesses annually. The condition is primarily transmitted through contaminated food and water from cattle and ruminant reservoirs, this pathogen is difficult to control in non-heated foods due to its acid tolerance and often treated by supportive care (Gambushe *et al.,* 2022).The prevention of infection requires control measures at all stages of the food chain, from agricultural production on the farm to processing, manufacturing and preparation of foods in both commercial establishments and household kitchens (WHO, 2026c).

The prevalence estimate of *E. coli* O157:H7 in cattle at the global level was 5.68% . Reported pooled prevalence estimates were highest in Africa (31.2%), followed by North America (7.35%), Oceania (6.85%), Europe (5.15%), Asia (4.69%), and Latin America and the Caribbean (1.65%) (Islam *et al.*, 2014). The global prevalence of *E. coli O157:H7* in carcasses ranges between 1 and 20%, depending on hygiene practices and regional factors (Thomas *et al.*, 2025). The occurrence of *Escherichia coli* in foods of animal origin in Ethiopia remains a concern, largely due to practices such as illegal

slaughtering of animals in open fields and inadequate hygienic conditions in abattoirs (Assefa, 2019). In addition, the pooled prevalence of *E. coli O157:H7* in beef carcasses at Ethiopian abattoirs has been reported to be 5% (Zelalem *et al .,*2019). However, despite growing evidence of carcass contamination, comprehensive investigations integrating slaughterhouse hygienic conditions, microbial quality assessment, and molecular characterization of *E. coli* O157:H7 isolates remain limited in Ethiopia.

1.1.7. Antimicrobial resistance in E. coli O157:H7

Antimicrobials are natural or synthetic substances used to inhibit or eliminate the development of microorganisms (Sankar, 2023). Antimicrobial Resistance (AMR) occurs when microorganisms such as bacteria, viruses, fungi and parasites develop the ability to survive drugs that that were previously effective for treatment. Antimicrobial Resistance is a serious global health threat. Every year, over one million people die from antimicrobial-resistant infections. When harmful bacteria in contaminated food are resistant to antimicrobials, they can cause infections that don't respond to medications, such as antibiotics, making treatment more challenging and sometimes even resulting in life-threatening conditions (FAO, 2026a). The appearance of antimicrobial resistance can be ascribed to anthropological, animal, and environmental factors. Human-related causes include antimicrobial overuse and misuse in medicine, antibiotic-containing cosmetics and biocides utilization, and inadequate sanitation and hygiene in public settings. Environmental factors including naturally existing resistance genes, improper disposal of unused antimicrobials, contamination from waste in public settings, animal farms, and pharmaceutical industries, and the use of agricultural and sanitation chemicals facilitate its emergence and spread (Endale *et al.*, 2023).

Food production industries in the developing countries use antimicrobials as growth promoters in food animals to satisfy the market demand and to increase their profit margin (Pokharel *et al.*, 2020). However, guidelines regarding antimicrobials use are either totally absent or poorly implemented (Thakur and Panda, 2017). The issue of antimicrobial residues in foods of animal origin has not given due attention in developing

countries. As a result, the prevalence of veterinary drug residues in foods of animal origin in some African countries has been reported to be as high as 94%, compared to less than 1% in Europe (Mensah, 2014).

In developing countries, antimicrobial resistant pathogens are commonly found in food animals (Grace, 2015). AMR is a growing health crisis, particularly in low-resource settings like Ethiopia (Woldu, 2024). The study conducted in central Ethiopia and Amhara region revealed that there is evidence of lack of awareness among food animal owners about the use of antibiotics and presence of widespread misuse and improper drug dispensing and handling practices (Tufa *et al.*, 2018; Ture *et al.*, 2019; Geta and Kibret, 2021). The most likely reason for drug residues might be due to improper drug usage and failure to keep the withdrawal period (Ture et al., 2019; Tadele *et al.*, 2022;). In Ethiopia a meta-analysis report revealed that comparable high antimicrobial resistant pathogens prevalence estimates were found from milk than eggs and meat. The pooled prevalence of anti-microbial resistant in bacteria from food producing live animals was 20%. High estimates of antimicrobial resistant pooled prevalence were found in bacteria identified from milk, food handlers and the environmental samples with 29%, and 28% in meat. In foods of non-animal origin, the prevalence was lower with 13%. In milk, the highest antimicrobial resistant estimate was found for penicillin (69%) followed by amoxicillin (51%). Regarding multi-drug resistance, the overall pooled prevalence was 74% among antimicrobial resistant positive samples. Microbes reported having a higher multi-drug resistances (MDR) pattern were *Staphylococcus* spp (96%), *Salmonella* spp (81%) and *Escherichia coli* (77%) (Gemeda *et al.*, 2021). Recent disease surveillance findings from more than 4000 health centers across Ethiopia have shown a rising trend in cases of antimicrobial resistance, which occurs when pathogens change over time and no longer respond to medicines, i.e: strains of infection-causing E. coli bacteria are showing increasing resistance to stronger antibiotics (WHO, 2026a).

Meta-analysis report showed that a pooled analysis of twenty antibiotics for food from animal sources, clindamycin resistance in *E. coli* isolates reached highest estimated pooled prevalence of 100.0%, followed by cefuroxime at 93.5%, neomycin at 74.1%, and

amoxicillin at 73.4%. Conversely, the lowest resistance estimates were for Norfloxacin at 1.7%, Meropenem at 4.8%, and Cefotaxime at 8.6%. The pooled prevalence of multi drug resistance for E. coli isolates was found to be 49.9%. The overall pooled prevalence of AMR for *E. coli* was 40.3%. The pooled prevalence of piperacillin resistance in *E. coli* isolates from human sources is 92.3%, followed by ampicillin at 86.4% and cephazolin at 74.3%. In contrast, the lowest resistance estimates were for imipenem at 11.9%, followed by chloramphenicol at 12.5% and azithromycin at 15.8%. The pooled prevalence of MDR was 80.7% (95% CI: 70.7–90.7). The overall estimated pooled prevalence for AMR was 55.5% (Zegene *et al.*, 2025).

1.2. Research Gaps and Rationale for the Study

Despite municipal abattoirs are very important in ensuring meat safety and protecting public health (Nastasijevic *et al.,* 2023), there is limited systematic evidence on the infrastructure and operational standards of abattoirs in Ethiopia. Most of the available studies has emphasized on microbial contamination outcomes (Assefa, 2019; Zelalem *et al.*, 2019; Gazu *et al.*, 2023), while little emphasis has been placed on physical infrastructure, availability of essential facilities, and compliance with recommended hygienic standards. The relative importance of infrastructural and operational facility deficiency issues influencing hygienic performance remains poorly characterized. Furthermore, although *E. coli* O157:H7 has been reported in beef carcasses and abattoir environments in various regions of Ethiopia *(*Zelalem *et al*., 2019), there is a scarcity of detailed information on the specific sources and pathways of carcass contamination within municipal abattoirs. This is important in ensuring the identification of critical control points necessary for effective intervention. Therefore, it is needed for an integrated assessment that simultaneously evaluates abattoir infrastructure and operational facilities, hygienic practices, and carcass microbiological outcomes, including the occurrence and source of contamination of *E. coli* O157:H7 and its antimicrobial susceptibility patterns. Such an approach is critical to produce context-specific evidence that explains the existence of contamination and its associated factors. Such a study is

justified to fill critical knowledge gaps and to inform the design of tailored interventions to improve meat safety in municipal abattoirs of Ethiopia.

1.2.1. General objective

The study targets an integrated assessment of municipal abattoirs in central Ethiopia by evaluating their physical infrastructure, availability and functionality of operational facilities, and compliance with hygienic standards, alongside determining the occurrence, sources, and contamination pathways of *Escherichia coli* O157:H7 in beef carcasses and abattoir environments, including its antimicrobial susceptibility patterns, in order to identify critical control points and generate evidence-based interventions for improving meat safety and public health.

1.2.2. Specific objectives

- To assess infrastructures, operational facilities and food safety practices in municipal abattoirs in central Ethiopia
- To investigate microbial quality of beef carcasses and associated hygienic practices at municipal abattoirs of central Ethiopia
- To investigate the occurrence of antimicrobial susceptibility, resistance and virulence gene of *Escherichia coli* O157:H7 isolated from beef carcasses and abattoir environments in central Ethiopia.
- To investigate the genetic relatedness of *Escherichia coli* O157:H7 isolated from beef carcasses and abattoir environments in central Ethiopia.

1.3. Relevance of the Study to Policy and Public Health

The findings of this study have important implications for food safety policy, meat inspection systems, slaughterhouse management, and public health protection in Ethiopia. By generating empirical evidence on infrastructural gaps, hygienic deficiencies, and contamination risks, the study can inform regulatory authorities on priority areas for investment, renovation, and enforcement of minimum abattoir standards. This is

particularly relevant for municipal abattoirs, which supply meat for local consumption yet often operate under weak regulatory oversight. From a public health perspective, the study contributes to food safety policy development by providing data on carcass contamination source with *E. coli O157:H7* and associated antimicrobial resistance patterns**.** Such evidence is critical for strengthening national food safety strategies, improving routine surveillance of foodborne pathogens, and integrating AMR monitoring within the meat production chain. Given the widespread consumption of raw and undercooked meat in Ethiopia, the public health relevance of these findings is especially significant. Moreover, the study supports national and international efforts to combat antimicrobial resistance by highlighting abattoirs as potential hotspots for the emergence and dissemination of resistant foodborne pathogens. The results can inform Ethiopia's engagement with One Health initiatives and AMR action plans by linking animal health, food safety, and human health outcomes. In addition, the study may serve as a baseline reference for future research, surveillance programs, and policy evaluations related to meat safety and slaughterhouse hygiene in Ethiopia. The integrated methodological approach combining infrastructural assessment, hygienic evaluation, microbiological analysis, and molecular characterization may also provide a useful framework for similar investigations in other low-resource settings.

1.4. Description of the Study Area, Sample Size determination and Methods

This study was conducted in 30 municipal abattoirs in the central parts of Ethiopia, within a 150 km radius of the capital, Addis Ababa. The study areas encompassing Shagar city, towns in West, East, Southwest and North Shewa zones of Oromia Regional State, North Shewa zone of the Amhara Regional State, and the Gurage zone and East Gurage zone of the Central Ethiopia Regional State (Figure 2). According EthiopianStatisticalService (ESS) the study area population was approximately 14,379,971 at zonal and city level (ESS, 2024). For human consumption on average 1000 animals were slaughtered daily in this area's at municipal abattoirs.

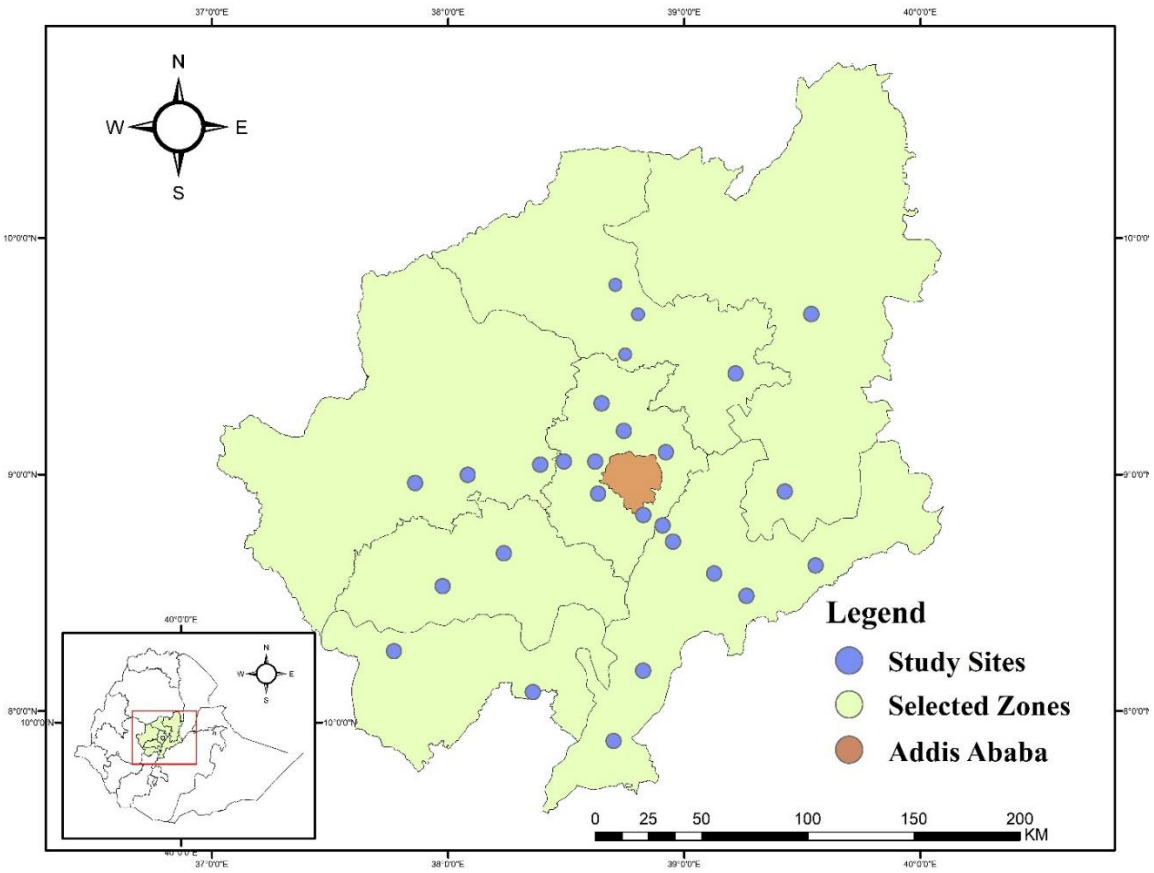


Figure 2: Map of Ethiopia showing the study Area (ArcGIS 10.8. 2).

A cross-sectional study was conducted on municipal abattoirs of towns and city at the central part of Ethiopia within a 150 km radius from Addis Ababa from January 2024 to February 2025. The municipal abattoirs were selected based on accessibility alongside the main road network. Therefore, all available and accessible municipal abattoirs within this area were included in the study, resulting in a total of 30 municipal abattoirs. They operate under different ownership modalities including private enterprises, cooperatives and the public sector. They supply only the domestic/local market's demand for meat and governed under regulatory authority of their respective municipalities. A pre-tested checklists and questionaries were used to assess infrastructure, operational facility and abattoir hygiene and food safety practices of municipal abattoirs and workers. The infrastructure and operational facility within the abattoirs were evaluated using a structured checklist based on guidelines of the FAO and the WHO for developing countries (FAO, 1988; Mann *et al*., 1983). Additionally, a semi-structured questionnaire

was designed to assess abattoirs' hygiene and food safety practices based on the FAO (FAO, 1991, 2004b; Skaarup, 1985) and CAC (CAC, 2005). The questionaries and checklists were reviewed by subject-matter experts to ensure content validity. Data were collected through interview with the abattoir managers and responses were triangulated with on-site observations of municipal abattoirs' infrastructure, operational facilities and food safety practices. The interviews were carried out through face-to-face in a private setting to encourage honest responses and participants were assured of the confidentiality of the information they provided. Sample size was determined using Yamane's (1967) formula for a finite population for workers meat safety practice assessment (Yamane, 1967).

$$n = \frac{N}{1 + NE^2}$$

Where: - n=number of required sample size; N=total population of abattoir workers (219); E = 0.05 absolute precision. So the required sample size is 142 and they were selected using a simple random sampling method. Since the number of workers varies across abattoirs, the sample size for each location was determined proportionally

Sample size determination for carcass swab sampling for microbial quality assessment from five selected municipal abattoirs (Adama, Holeta, Gelan, Batu and Sebeta) were determined by formula given below by Charan and Biswas, 2013.

$$N = \frac{(Z_{1-\alpha/2})\text{^}2 * SD^2}{d^2}$$

Where:- N = Sample size

$Z_{1-\alpha/2}$ = Is standard normal variate (at 5% type 1 error (P<0.05)). It is 1.96

SD = Standard deviation of variable. Value of standard deviation can be taken from previously done study or through pilot study.

d = Absolute error or precision has to be decided by researcher (0.205). Based on the above formula the estimated sample size was 50.06 for each abattoir considering SD= 0.74 (Bersisa *et al.,* 2019) and absolute error = ±0.205$\log_{10}$CFU/cm^2. However the sample size was increased to be 51 carcass swab samples that were collected from each

abattoir. Overall 255 carcass swab samples, 30 hand swabs from workers, 30 knife swabs, and 30 water samples were collected.

The sample size the for detection of carcass contamination *Escherichia coli O157: H7* was determined using the formula for simple random sampling given below by Thrusfield, 2005(Thrusfield, 2005).

$$n = \frac{Z^2 * p(1-p)}{d^2}$$

Where: - n=number of required sample size; Z=normal distribution (95%); P= expected prevalence; d=precision absolute desired. For the sample size calculations, 95% confidence interval and 5% precision were used. Accordingly, the sample size of apparently healthy to be slaughtered cattle and beef carcass swab at abattoir was determined based on a previous study report on prevalence of *E. coli O157: H7 (*Zelalem *et al.,* 2019). Based on the above formula a total of 72 animals were sampled with 5% expected prevalence from each of three (Adama, Batu and Holeta) selected municipal abattoirs. The abattoirs were selected based on their accessibility. Isolation of conducted *E. coli O157: H7* according to protocol of International Standards Organization (ISO) (ISO16654:2001, 2001). Antimicrobial resistance test was conducted on isolates of *E. coli* O157:H7 following the standard protocol described by Clinical and Laboratory Standards Institute (CLSI), 2024.

1.5. Ethical approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki, and Ethical approval for the study was obtained from the Institutional Review Board (Research Ethics Committee) of the College of Veterinary Medicine and Agriculture Addis Ababa University (Certificate Ref. No: VM/ERC/03/11/16/2024). Before conducting the interviews, the purpose of the study was clearly explained to the participants and their informed consent to participate was obtained.

1.6. Outline of the Thesis

This thesis is organized into six chapters. Chapter one gives general overview of the study, which include general introduction and background, research gap and rationale of the study, objectives, relevance of the study and overview and outline of the study. Chapters two elaborate the extent of infrastructures, operational facilities and food safety practices in municipal abattoirs in central Ethiopia which was assessed by semi-structured questionnarries and direct observation The study results highlight critical gaps in the adequacy of operational facilities and poor compliance with food safety standards and hygiene practices in the surveyed municipal abattoirs. Chapter three addresses carcass microbial quality and meat safety practices of workers at municipal abattoirs in central Ethiopia. It is assessed by standard microbiological plating out method and semi-structured questionnarries. The findings of this study revealed that microbial quality of the beef carcasses exceeded acceptable limits, indicating a critical gap in basic hygiene compliance. Chapter four gives information occurrence, antimicrobial resistance and sources of contamination of Carcasses with *E. coli O157:H7* at Municipal Abattoirs in central Ethiopia. Whole genome sequencing was performed to assessed genetic relatedness of *E.coli* O157:H7 from carcass with other sources and detected the occurrence of anti-microbial resistance and virulence gene. The study findings suggest that contact with fecal material during evisceration is a possible source contaminating the carcasses with *E. coli* O157:H7 and no anti-microbial resistance gene detected in all study isolates. Chapter five consolidates the findings of the three papers described from chapter 2 to 4. The study highlights poor infrastructural and operational factors in abattoirs created favorable environment for the development and transmission of microbial contaminants due to unhygienic handling of carcasses. Finally, Chapter Six presents the general conclusions and recommendations of the study. The chapter summarizes the major findings and provides recommendations for improving abattoir infrastructure, hygienic slaughter practices, food safety monitoring systems, and public health interventions aimed at reducing carcass contamination and enhancing meat safety in Ethiopia.

2. CHAPTER II: ASSESSMENT OF INFRASTRUCTURES, OPERATIONAL FACILITIES AND FOOD SAFETY PRACTICES IN MUNICIPAL ABATTOIRS IN CENTRAL ETHIOPIA

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ABSTRACT

Suboptimal infrastructures, inadequate facilities, poor hygienic practices in carcass processing represent significant challenges to meat safety and public health. A cross-sectional study was conducted to assess infrastructures, operational facilities, and hygienic meat handling and food safety practices at 30 municipal abattoirs selected from the central part of Ethiopia. Data were collected through semi-structured checklists, questionnaires and direct observations. The study showed that 83.3 % of the municipal abattoirs are in unacceptable proximity to residential areas. About 63.3 % exhibited pest harborage, breeding places, or signs of pest activity. Furthermore, significant defects were noted in municipal abattoirs, with 60 % of the walls in poor condition and inadequate floor sloping (53.3 %) toward the drains. Hot water sources, quality control laboratories, and equipment sterilizers were lacking in all surveyed municipal abattoirs. Other operational facilities such as stunning pens and chilling rooms were missing in 96.7 % of municipal abattoirs while, isolation pens for disease suspected animals were absent in 63.3 % of municipal abattoirs. On the other hand personal hygiene facilities were similarly insufficient or failed to meet acceptable standards in many surveyed municipal abattoirs. For instance, designated and well-designed hand-washing stations (76.7 %), showers (60 %), and restrooms (53.3 %) were absent. Additionally, the provision of sufficient cleaning and disinfection chemicals (83.3 %) and personal protective equipment (76.7 %) were also lacking. In 70 % of municipal abattoirs, pest control, wildlife and stray dog control, and deep freezers were lacking. Skin and bone storage rooms were lacking in 60 % of the municipal abattoirs. In about two-third of the municipal abattoirs, separate room for skinning, evisceration, and viscera cleaning were lacking. In terms of hygiene and food safety practices, none of the municipal abattoirs implemented equipment sterilization between uses, and employed refrigerated transport for carcasses. The issue related to hygiene and food safety continues to be compromised as most municipal abattoirs (83.3 %); workers and utensils were not restricted to designated working areas and water quality testing was not practiced. About three-fourths of the municipal abattoirs did not process carcasses in a vertical position, and the majority (83.3 %) demonstrated inadequate enforcement of personal protective

equipment use and lacked separate green offal, viscera and red meat transportation. The study results highlight critical gaps in the adequacy of operational facilities and poor compliance with food safety standards and hygiene practices in the surveyed municipal abattoirs. Taking these into considerations, the finding offer policymakers practical evidence to guide regulatory improvements, resource allocation, and capacity building efforts to improve municipal abattoirs.

Keywords: *Abattoir hygiene, Infrastructure, Facility, Meat safety, Public health, Ethiopia*

2.1. Introduction

Food safety is a growing global public health concern, affecting the health and well-being of millions of people worldwide. Each year, about 600 million individuals become ill from consuming contaminated food resulting in 420,000 deaths and losses of 33 million healthy life years lost in terms of Disability Adjusted Life Years (DALYs) (WHO, 2025). In 2010, the global burden attributed to animal-sourced food was estimated at 168 DALYs per 100,000, accounting for about 35 % of the estimated total global burden of FBD (Li *et al.*, 2019). Still ensuring the production of safe animal-origin food remains a major challenge in Low and Middle-income Countries (Seyoum *et al.*, 2024). Meat-borne pathogens significantly contribute to this burden. Meat is a highly perishable food product which needs serious emphasis about its quality, stability and safety (Khaled *et al.*, 2021).

Abattoir is a premise where animals are slaughtered and semi-processed for human consumption with a crucial role in the safe meat supply chain (Rodarte *et al.*, 2023). Its primary role is to create standard and suitable conditions for the safe handling and processing of meat by avoiding contamination and further protecting consumer health (García-Díez *et al.*, 2023). In order to play such roles, abattoirs need to be properly equipped with the required operating facilities and good infrastructures (Mann *et al.*, 1983; Skaarup, 1985). Additionally, maintaining good hygienic practices in abattoirs and

adherence of personnel to strict hygiene standards during carcass processing stages like; bleeding, skinning, evisceration, storage, transportation chilling, etc are crucial for preventing contamination and ensuring meat Safety (CAC, 2005). Food processing establishment auditing and inspection play a vital role in preventing foodborne illnesses by identifying risks, ensuring adherence to internationally recognized food safety standards, and encourages in culture of continuous food safety improvement (FAO, 2003; Griffith *et al.*, 2010; Powell *et al.*, 2011).

A standard abattoir consists components such as lairage; where animals are held, rested, and supplied with water before slaughter and ante-mortem inspection performed. stunning area; where they are rendered unconscious, followed immediately by the bleeding area and the slaughter or dressing area; where effective blood removal. carcass skinning or dehairing, evisceration, and splitting takes place respectively. In addition, it possess meat inspection area; where post-mortem examination carried out, chilling rooms; to rapidly reduce temperature. Abattoirs also include offal processing rooms, cutting and deboning sections, and condemned meat rooms to safely handle edible and inedible products, supported by waste disposal systems, effluent treatment plants, potable water supply, staff hygiene facilities, and veterinary offices (Mann *et al.,* 1983;FAO, 1988).

Several studies from various regions of Ethiopia showed beef carcass contamination at abattoirs has been a major concern, resulting in significant public health problems, primarily due to the presence of pathogenic microorganisms such as *S. aureus* (Bersisa *et al.*, 2019; Kebede and Getu, 2023), *Salmonella* (Tadesse *et al.*, 2024) and *Escherichia coli* O157:H7 (Tarekegn *et al.*, 2023). These issues are often attributed to limitations in food safety knowledge, attitudes (Kebede and Getu, 2023) and practices (Abunna *et al.*, 2022) at municipal abattoirs. Despite the fact that abattoirs are central to ensuring meat safety, in many developing countries like Ethiopia, they operate with inadequate infrastructures, poor facilities, and limited compliance with food safety standard practices (Ovuru *et al.*, 2024). However, the status of abattoirs is not well-documented, creating gaps in evidence which necessarily needs to guide for potential interventions for

improvement (Ovuru *et al*., 2024; Nigussie *et al.*, 2025). Taking these and limited comprehensive studies focusing on their operational and food safety conditions, this study was designed to assess the infrastructures, operational facilities and hygienic food safety practices at municipal abattoirs in the central part of Ethiopia.

2.2. Materials and Methods

2.2.1. Study area

The study was conducted in the central parts of Ethiopia encompassing Shagar city, towns in West, East, Southwest and North Shewa zones of Oromia Regional State, North Shewa zone of the Amhara Regional State, and the Gurage zone and East Gurage zone of the Central Ethiopia Regional State as indicated in figure 3 within a 150 km radius of the capital, Addis Ababa, alongside the main road. The ESS projected the 2024 population of the area to be approximately 14,379,971 at zonal and city level (ESS, 2024). Agriculture is the primary source of livelihood for the rural population, and livestock production, both intensive and extensive farming systems, is a common activity across the area. On average 1000 animals were slaughtered daily in this area's municipal abattoirs for human consumption. The area is characterized by rapid population growth and urbanization, which has contributed to a potential increase in the demand for meat consumption and production. The abattoirs were initially established on the outskirts of towns or cities and are now located within the urban centers due to the continuous expansion of urban areas.

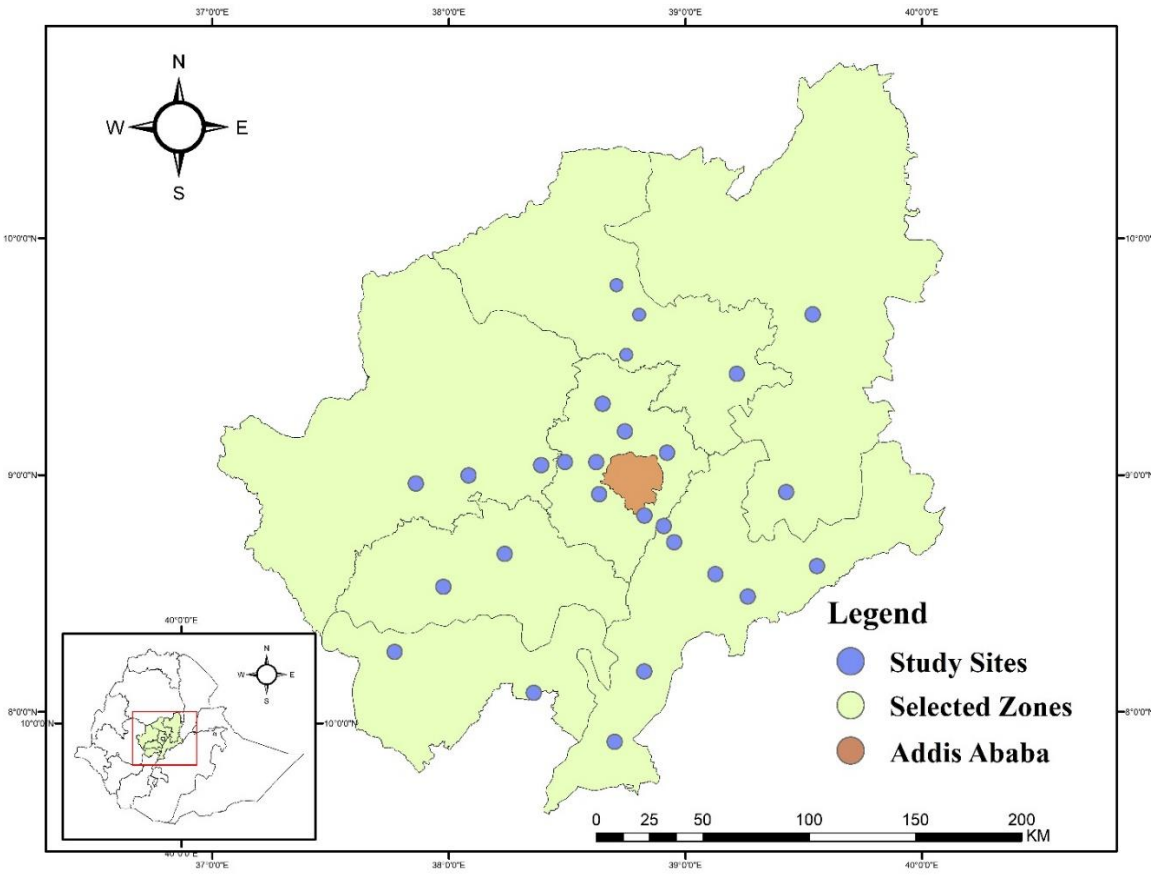


Figure 3: Map of Ethiopia showing the study Area (ArcGIS 10.8. 2).

2.2.2. Study design and sampling

A cross-sectional study was conducted on municipal abattoirs of towns and city at the central part of Ethiopia within a 150 km radius from Addis Ababa from January 2024 to February 2025. The municipal abattoirs were selected based on accessibility alongside the main road network. Therefore, all available and accessible municipal abattoirs within this area were included in the study, resulting a total of 30 municipal abattoirs. They operates under different ownership modalities including private enterprises, cooperatives and the public sector. They supply only the domestic/local market's demand for meat and governed under regulatory authority of their respective municipalities.

2.2.3. Data collection

A pre-tested checklists and questionaries were used to assess infrastructure, operational facility and abattoir hygiene and food safety practices of municipal abattoirs. The infrastructure and operational facility within the abattoirs were evaluated using a structured checklist which incorporated the minimum prerequisites of abattoir facilities and equipment, which are mandatory for a small-scale abattoir as set by the FAO and the WHO for developing countries (Mann *et al*., 1983; FAO, 1988). The checklist comprises four parts such as abattoir general information, location, layout and operational facility questions. Additionally, a semi-structured questionnaire was designed to assess abattoirs' hygiene and food safety practices based on the FAO (Skaarup, 1985; FAO, 1991, 2004) and CAC (CAC, 2005) guidelines comprising two parts; abattoir general information and food safety practice questions. The questionaries and checklists were reviewed by subject-matter experts to ensure content validity. Data were collected through interview with the abattoir managers and responses were triangulated with on-site observations of municipal abattoirs' infrastructure, operational facilities and food safety practices. The interviews were carried out through face-to-face in a private setting to encourage honest responses and participants were assured of the confidentiality of the information they provided.

2.2.4. Data analyses

Data were coded and analyzed using Stata Statistical Software, release 14.2 (Stata Corp LP, College Station, TX). Descriptive statistics were utilized to report overall scores in percentages for food safety practice questions, layout, location and operational facility information. To summarize the overall operational facility assessment and food safety practices of all municipal abattoirs; each correct response was assigned a score of one. The scores were added with maximum of 28 for operational facility assessment and 15 for food safety practice questions. The total scores obtained for operational facility assessment was divided by 28 and multiplied by 100. For food safety practices the total score obtained was divided by 15 and multiplied by 100. Municipal abattoirs with score

of ≥75% were categorized as “Good” (with overall best performance), 74-60% as “Fair” (was tolerable, but there's room for improvement), <60% as “Unsatisfactory” (is lacking key areas and need focused improvement).

2.3. Results

2.3.1. The municipal abattoirs location

All municipal abattoirs site were accessible for transporting livestock and finished products using vehicle. Half of the surveyed sites were free from trees, while less than half allow for future expansion, and had no signs of pest activity or breeding at the location. Proximity of the majority of municipal abattoirs to residential areas was not acceptable (Figure 4).

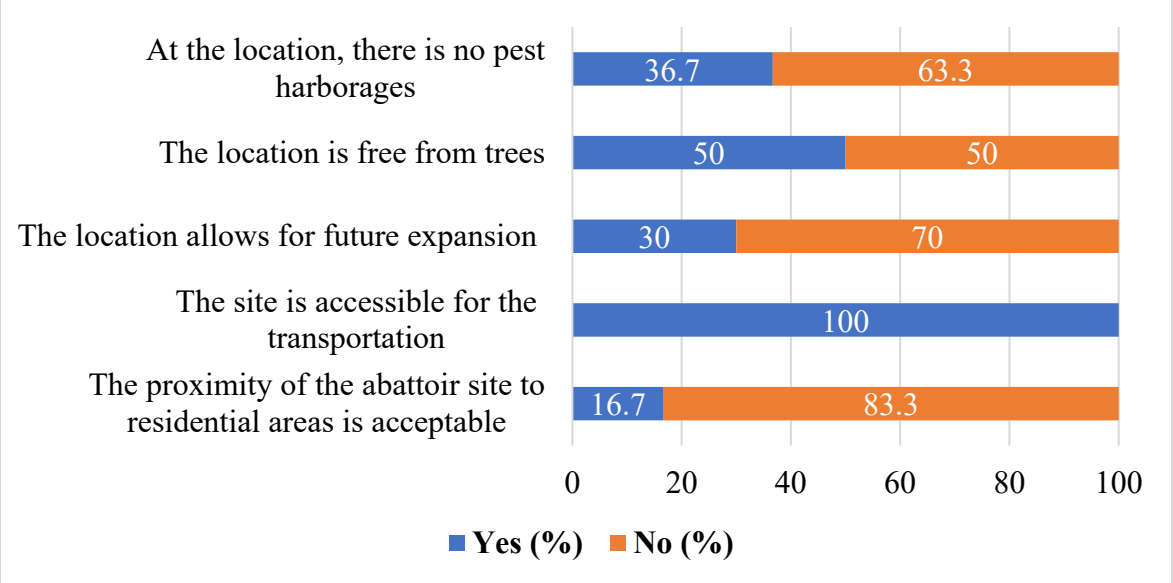


Figure 4: Location preferences of Municipal abattoirs from the Central part of Ethiopia

2.3.2. The municipal abattoirs layout

Figure 5 provides a summary of the municipal abattoir's layout. Most of the municipal abattoirs have roofing materials (galvanized non corrosive) that comply with hygiene

standards and layout effective for managing the flow of livestock during the slaughtering process. However, less than half of abattoirs flooring (sloping sufficiently towards a drain) and wall designs meet hygiene standards and were in good conditions.

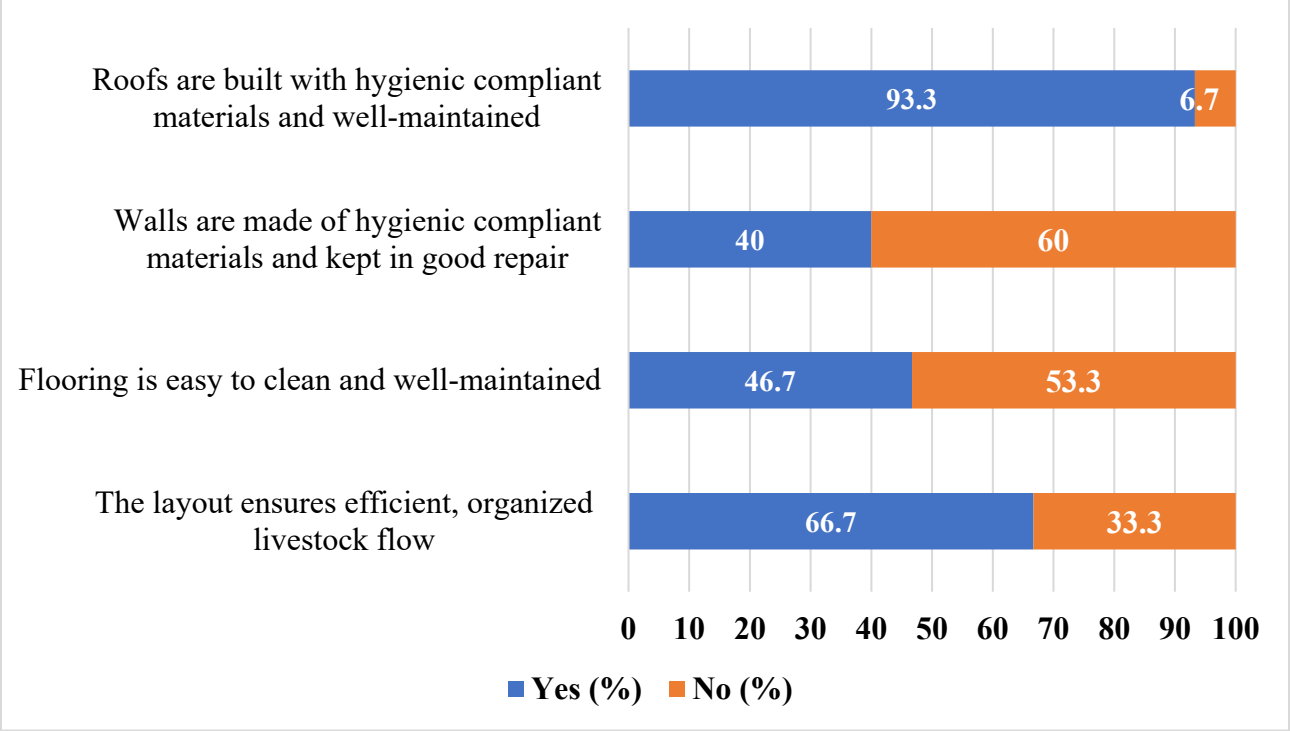


Figure 5: Layout of Municipal abattoirs from the Central part of Ethiopia

2.3.3. The municipal abattoirs operational facilities

The assessment of the municipal abattoir facilities showed that majority (80-100 %) possess livestock reception area, carcass bleeding/dressing facilities, water supply, meat inspector office provision, and security measures. However, they had critical deficiencies (0-3.3 % compliance) in essential areas including stunning pens, hot water supply, quality control laboratories, chilling rooms, and equipment sterilizers. Significant deficiencies existed in the majority of assessed facilities such as wildlife/stray dog barriers, foot baths, hand-washing areas, showers, and restrooms. Additionally, key operational areas which include isolation pens, pest control, cleaning chemical supply, personal protective equipment (PPE) provision, and designated waste disposal were lacking in majority of municipal abattoirs (>50 %) (Table 1).

Table 1: Summary of operational facilities' assessment results of different Municipal abattoirs from the Central part of Ethiopia

Operational Facility Assessment Criteria	Frequency	%
Reception and examination area for the livestock	27	90.0
The facility has well-designed holding pens	20	66.7
Isolation pen for suspected animals	11	36.7
It has a stunning pen	1	3.3
Adequate facilities for bleeding and dressing of carcasses	30	100.0
The facility is supplied with a hot water source	0	0.0
The slaughterhouse provided with store for hides and hooves, and horns	12	40.0
The facility provided a deep freezer (-18/-20 ^{0}C)	9	30.0
Slaughterhouse has a sufficient supply of water	26	86.7
The facility has designated areas for washing hands	7	23.3
The facility has well-designed designated areas for showers	12	40.0
Adequate and well-designed restroom facilities	14	46.7
The slaughterhouse has sufficient electricity in all work areas	23	76.7
The slaughterhouse has efficient facilities for the control of pests	9	30.0
The facility has a quality control laboratory	0	0.0
The facility has well-designed and maintained employee break areas	6	20.0
Adequate space is provided for employee locker rooms	9	30.0
Meat Inspector Office provision	24	80.0
Sufficient facilities for the disposal of condemned meat and organs	19	63.3
The facility has designated areas for the proper disposal of solid waste	17	56.7
Security measures are in place to prevent unauthorized access into the facility	27	90.0
No entry for wildlife and stray dogs	9	30.0
Provided with a foot bath	8	26.7
Provided a chilling room	1	3.3
Provided sufficient food grade Cleaning and disinfection chemicals	5	16.7
Provided sufficient PPE	7	23.3
Provided with sterilizers for the equipment used in the abattoirs	0	0
Separate room for skinning, evisceration and offal cleaning	10	33.3

In majority of municipal abattoirs (70%) there was no wildlife and stray dog control. In those areas open air disposal of offal and bones were observed. Additionally, stray dogs and vultures were commonly observed in those premises area (Figure 6 A, B and C).



Figure 6: Municipal abattoirs environment in the Central part of Ethiopia: A) Stray dogs in the compound of abattoir, B) Disposed offal and bones next to the abattoir fences and C) Vultures in the abattoir compound

Nearly two third (60 %) of the municipal abattoirs have no sufficient storage for skin, bone, horns and piled in the compound of abattoirs (Figure 7).



Figure 7: Improper storage of skin, horn and bone in some Municipal abattoirs in the Central part of Ethiopia.

2.3.4. The municipal abattoirs hygiene and food safety practices

In all municipal abattoirs, application of humane killing and ante-mortem examination are routinely practiced. Post-mortem examinations were practiced in majority of municipal abattoirs. Additionally, above three-fourth of municipal abattoirs practiced cleaning of meat transport vans between batches, effective communication of inspection results, and maintained regular cleaning of slaughtering halls, which can be regarded as good practices that contribute positively to overall hygiene and food safety (Table 2).

Despite some positive practices, several essential food safety measures were critically absent in many municipal abattoirs. None of the facilities reported the utilization of equipment sterilization between batches or using refrigerated vehicles for transporting carcasses. Only less than one-fourth of municipal abattoirs implemented important protocols such as separating viscera, green offal and carcasses during transport, enforcing the use of personal protective equipment, testing water safety, and restriction of personnel and equipment to designated areas. Furthermore, majority of municipal abattoirs practiced carcass processing on the floor (Table 2 and Figure 8).

Table 2: Summary of abattoir hygiene and food safety indicators of Municipal abattoirs located in the Central part of Ethiopia

Abattoir hygiene and food safety indicators	Frequency	%
Animals were stunned before being slaughtered	30	100.0
Personnel and utensils stay/work in designated areas only	5	16.7
Slaughtering halls are always cleaned between batches	23	76.7
Processing equipment and utensils are always sterilized between batches	0	0.0
The use of personal protective equipment is consistently enforced	5	16.7
Wastewater disposal practices comply with environmental regulations	18	60.0
Meat Inspection areas are cleaned between every activity	18	60.0
There are mechanisms in place to communicate inspection results	26	86.7
The abattoir water supply is regularly tested for safety (pathogen and heavy metals)	5	16.7
Vertical positioning of the carcass during skinning and evisceration to prevent contamination	8	26.7
Conduct a thorough ante-mortem examination	30	100.0
Conduct a thorough post-mortem examination	26	86.7
Meat transporting vans are always cleaned between batches	29	96.3
Carcass is transported with refrigerated vehicles	0	0.0
Visceral organs, green offal and carcasses were transported separately	5	16.7



Figure 8: Carcass processing on the floor (evisceration and de-hiding on the floor) in some Municipal abattoirs the Central part of Ethiopia

About 40 % of municipal abattoirs waste water management does not obey environmental protection regulations. The observed wastes were seen in both abattoir compounds and surrounding of the abattoirs which potentially have a negative impact on the environment (Figure 9).



Figure 9: Improper wastewater disposal in some Municipal abattoirs in the Central part of Ethiopia

2.3.5. The overall operational facility and food safety practice performance of the municipal abattoirs

The overall operational facility assessment and food safety practice performance were unsatisfactory in nearly three fourth of the assessed municipal abattoirs (Figure 10).



Figure 10: Overall operational facility and food safety practice performance of Municipal abattoirs in the Central part of Ethiopia.

2.4. Discussion

The role of abattoirs in ensuring meat safety is fundamental in safeguarding the public health. However, inadequate infrastructure and operational facilities leads to poor hygiene practices compromising meat safety with increasing the risk of foodborne illness to the public (Ibrahim *et al.*, 2021). This study assessed infrastructures, food safety practices and operational facilities in municipal abattoirs within approximately 150-km radius of Addis Ababa, in the central part of Ethiopia, alongside the main road due to its accessibility. According to FAO (2005) abattoirs should not be located close to dwellings, schools, churches and other public or commercial buildings due to possible nuisance from noise, smell, congestion, etc. In contrary, most of the municipal abattoirs in the present study were located within the close range of less than 500 meter from residential areas. Furthermore, spaces for future expansion were not available in most of the studied

municipal abattoirs as well as there was pest harborage, breeding place, or sign of pest activity. This finding is in agreement with the previous findings of abattoirs' proximity to residential areas (Gali *et al.*, 2020; Odekanle *et al.*, 2020; Agu *et al.*, 2021) but disagree with Skaarup recommendation for abattoirs to be free of shrubbery or vegetation close to the structure and the land acquired should be sufficient to permit future expansion, as overcrowding of facilities may give sanitation problems (Skaarup, 1985).

Defects were observed in operational facilities including in wall, floor designs are consistent with previous research conducted in developing regions, where inadequate abattoir infrastructure has been identified as a major contributor to meat contamination risks like studies in other African countries reported that poor facility design causes microbial contamination risks (Rodarte *et al*., 2023; Ovuru *et al*., 2024). Furthermore, the facility issues such as poorly maintained surfaces, ill-designed workflows and equipment compromise operational hygiene and facilitate cross-contamination (EFSABIOHAZPanel *et al.*, 2024).

The availability of hot water and steam is a critical infrastructural requirement for effective cleaning and sterilization in abattoirs, which is vital for preventing cross-contamination. These essential components were completely absent across all surveyed municipal abattoirs. Similarly, other study findings have documented lack hot water sources and equipment sterilizer from municipal abattoirs in Ethiopia (Gutema *et al.*, 2021; Yimana and Hassen, 2024), both of which are crucial facilities during slaughtering process (FAO, 2025). Similar to our findings, quality control laboratories, and isolation pens, deep freezer (Yimana and Hassen, 2024), stunning pens, chilling rooms (Gali *et al*., 2020; Zaba *et al.*, 2025)were reported to be lacking in different abattoirs investigated by other authors. The abattoir should possess an isolation pen for diseased animals and must be held in isolation pens and examined separately. The chilling or refrigerating room is necessary in abattoirs to ensure **a** critical control point for meat safety, quality, and compliance (Mann *et al*., 1983). It is essential to prevent and reduce the deterioration process of meat and microorganism development by chilling which needs to be carried out quickly after carcass wash at the end of the slaughter process (Cano-Muñoz, 1991).

In most cases, essential personal hygiene facilities including well-designed equipment for washing hands, showers, restrooms, provision of cleaning and disinfection chemicals and provision of sufficient personal protective equipment were lacking in most of the municipal abattoirs. These identified gaps were also reported from Southern Ethiopia, Kenya, and Nigeria where the absence of disinfection facilities and hand washing facilities (Lawan *et al.*, 2013; Cook *et al.*, 2017) and restrooms in abattoirs was reported (Agu *et al*., 2021; Zaba *et al*., 2025). As meat handlers hand is identified as the source of pathogens for food contamination; sanitation and hygiene facilities are critical in minimizing contamination. Thorough and proper hand washing remains the most effective method for removing pathogens and preventing foodborne infections. Hand washing should be with potable water for rinsing and soaps to loosen microbes from the skin. It has to be practiced after any activity that soils hands and certainly before handling and processing of food (Todd *et al.*, 2010). Lack of operational facilities can result in an increased risk of zoonotic and parasitic diseases transmission due to the inadequate support for proper slaughter hygiene within the abattoir (Zaba *et al*., 2025).

Most municipal abattoirs lacked other facilities including pest, wildlife and stray dog control systems, and dedicated storage for skins and bones and separate room for evisceration and viscera cleaning similar to reports elsewhere (Agu *et al.,* 2021; Abunna *et al.,*2022; Yimana and Hassen, 2024). Additionally, observations of the municipal abattoirs environment also revealed the open-air collection and storage of bones and hooves, which attracts pests and scavengers and heightens contamination risks (Figure 7). These findings are consistent with previous studies conducted in Kenya, Ethiopia and other developing countries where absence of wildlife, stray dog and pest control (Ovuru et al., 2024; Zaba *et al*., 2025), skin and bone storage room and separate rooms for skinning and evisceration was reported (Agu *et al*., 2021; Yimana and Hassen, 2024). Effective pest control systems should be in place to prevent access, harborage and infestation in food handling areas (CAC, 2005). Pests can contaminate food products and act as vectors for various foodborne pathogens (Rossi and Andreucci, 2014). To prevent access of unauthorized persons, the public, dogs and other animals, fencing must be

erected around the slaughterhouse area. The fencing should have contact with the ground at the lower edge and should be high enough to prevent access to the grounds (Skaarup, 1985). Stray dogs serve as reservoirs for diverse pathogens, including parasites, bacteria and arthropods (Cordero *et al.*, 2023). The consumption of infected meat (e.g. condemned carcasses or offal containing parasitic cysts) by dogs creates a critical pathway for zoonotic disease transmission of tapeworm (Rodarte *et al*., 2023).

The wastes from abattoirs are a hub of pathogens that can be cause for infection of humans and animals (Mozhiarasi and Natarajan, 2025). These wastes, particularly blood, fecal matter and offal, are rich in microorganisms capable of contaminating the environment and water body. Improper or unhygienic disposal of wastewater from abattoirs contributes to serious environmental pollution, including soil and water contamination, and poses substantial risks to public health (Kothari *et al.*, 2024). Hence, effective waste management is needed to prevent environmental, water source contamination as well as domestic and wild animals and human health (Skaarup, 1985). This study revealed that less than half municipal abattoirs waste water management was not compliant with environmental protection regulations (Figure 9). Another study from Ethiopia also reported ineffective abattoir wastewater management (Yimana and Hassen, 2024).

The assessment of hygiene and food safety practices in the surveyed municipal abattoirs of Central Ethiopia revealed significant non-compliance with key standards. These critical gaps such as absence of equipment sterilization, refrigerated transport, and water quality testing directly increase the risk of serious foodborne contamination. Comparable deficiencies have been reported in abattoirs across sub-Saharan Africa, including those in Ethiopia and Ghana, where a lack of sterilization equipment (Gutema *et al*., 2021; Asati *et al.*, 2024)and carcass transportation at room temperature were reported (Gutema *et al*., 2021; Kebede and Getu, 2023). As the microbial quality of water is a significant predictor of the microbial quality of meat (Berhanu *et al.*, 2025) water used for the processing of carcasses should not compromise the safety of meat, and needs regular testing for safety (CAC., 2022). Sterilization of equipment in slaughterhouses is mandatory to minimize

microbial loads, prevent cross-contamination and ensure the safety of meat products. The presence of blood, tissue and feces on equipment and abattoir environments creates favorable conditions for the growth of pathogenic bacteria (CAC, 2005). Maintaining proper temperature during meat transportation is a fundamental element that directly impacts the safety and shelf life of food (James and James, 2010).

Another concern observed was, in most municipal abattoirs workers and utensils have been moving freely without being restricted to designated clean or dirty zones. This increases the risk of cross-contamination, compromising meat safety. Strict separation of operational areas is essential to maintain hygienic processing and prevent pathogen spread. This likely requires comprehensive structural redesign, and rigorous regulatory enforcement for municipal abattoirs in central Ethiopia, supported by a full environmental impact assessment. Our observation is in agreement with Yimana and Hassen, 2024, who reported that workers were involved in the whole slaughtering process irrespective of the clean and dirty areas in the abattoirs. The majority of municipal abattoirs do not process carcasses in a vertical position which is carcasses were processed horizontally on the floor (Figure 8). This finding aligns with Zaba *et al*, 2025 where the lack of a functional rail system for elevating carcass and horizontal handling of carcasses was observed in the majority of surveyed municipal abattoirs (Zaba *et al*., 2025). Similarly, Yimana and Hassen reported, carcass processing took place horizontally on the floor (Yimana and Hassen, 2024). The present study is also in consistent with the study from Nigeria, where dressing carcasses are reported to took place directly on slaughterhouse floors (Njoga *et al.*, 2023). Processing of carcasses vertically during slaughter operations is important in maintaining hygiene and critical in minimizing contamination (FAO, 1991). Necessary separate facilities should be provided for personnel’s working on different carcass production stages (CAC, 2005). The separation of clean and unclean operations is crucial in abattoirs to ensure the safety of meat. This involves a well-planned plant layout, where the purpose of any structure should be to prevent the products' contamination by pathogenic microorganisms (Skaarup, 1985).
According to the CAC, the person who handles meat should wear appropriate personal protective equipment and different meat products should be transported separately to

prevent cross-contamination (CAC, 2005). Thus, adhering to these standards is essential for ensuring food safety throughout the meat processing and distribution chain. However, the current study showed low enforcement of personal protective equipment usage and lack of separate transportation of green offal, viscera and red meat were observed in the majority of municipal abattoirs . This report showed similarity with that of Kassaw *et al*, 2025 where adherence to personal protective equipment use gaps was reported. Poor food hygiene practices mainly worsen food safety and increase foodborne illness transmission (Zenbaba *et al.*, 2022). The recent study revealed that majority of municipal abattoirs overall operational facility assessment and food safety practice performance was unsatisfactory. This aligns with the report that showed poor infrastructure could not support the production of safe and wholesome meat (Lawan *et al.,* 2013). Substandard facility results in poor food safety practices comprising meat safety and posing in public health risk (Ovuru *et al*., 2024; Nigussie *et al.,* 2025).

2.5. Conclusions

This study revealed that the locations of the majority of the municipal abattoirs in central Ethiopia are in close proximity with residential areas, which is not acceptable due to its potential public health risks. Additionally, serious deficiencies were also observed in wall and flooring design and adherence to hygienic standards and good maintenance. Most notably, in majority of municipal abattoirs essential operational facilities were missing and food safety practices were not satisfactory. To enhance public health and meat safety, it is recommended that abattoirs located close to dwelling areas need to be relocated and restructured. Sufficient operational facilities should be provided and established, along with the sustainable enforcement of food safety standards practices. The environmental effects should be comprehensively assessed. The study finding can used by policymakers as practical evidence to guide regulatory improvements, resource allocation, and capacity building efforts in municipal abattoirs.

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Authors' contribution

Endalu Mulatu: Writing review and editing, Writing original manuscript, Fund acquisition Visualization, Validation, Research design, Data collection and Investigation, Data curation and analysis. Nebyou Moje: Fund acquisition, Validation, Data curation, Conceptualization, Writing review and editing, Resources, Research design. Biruhtesfa Asrade and Kebede Amenu: Writing review and editing, research design, Fund acquisition. Bekele Megresa, Ahmad Hamedy and Hika Waktole: Writing review and editing, Resources, Research design, Supervision, Data analysis, Fund acquisition, Conceptualization.

Availability of data and materials

The data used are available from the corresponding author on reasonable request.

Declarations

Ethical Approval

The study was conducted according to the guidelines of the Declaration of Helsinki, and Ethical approval for the study was obtained from the Institutional Review Board (research ethics committee) of the College of Veterinary Medicine and Agriculture Addis Ababa University (Certificate Ref. No: VM/ERC/03/11/16/2024).

Consent to participate

Before conducting the interviews, the purpose of the study was clearly explained to the participants and their informed consent to participate was obtained.

Consent to publish

Consent for publication was obtained from all participants.

Competing interests

The authors declare no competing interests.

2.6. References

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3. CHAPTER III: MICROBIAL QUALITY OF BEEF CARCASSES AND HYGIENE PRACTICES AT MUNICIPAL ABATTOIRS OF CENTRAL ETHIOPIA: A CROSS-SECTIONAL STUDY.

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ABSTRACT

Microbial contamination of beef carcasses occurs during slaughtering process. Cross-sectional study was conducted to assess microbial quality of carcasses and workers' meat safety practices at five municipal abattoirs in the central part of Ethiopia. Overall, 255 carcass swab samples, along with 30 samples each from hands and knife swabs, and water were collected for microbial quality assessment using standard plating method. Additionally, semi structured questionnaires were administered to 142 abattoir workers to assess their meat safety practices. The study showed that the overall mean aerobic plate count of 6.12 $\log_{10}$ CFU/cm^2 and fecal coliform count of 4.58 $\log_{10}$ CFU/cm^2 on beef carcasses. Carcass swabs had a significantly higher mean aerobic plate count (6.12 $\log_{10}$ CFU/cm^2) compared to knife (5.54 $\log_{10}$ CFU/cm^2; p=0.006) and hand swabs (5.19 $\log_{10}$ CFU/cm^2, p=0.000) while knife swabs showed a higher mean fecal coliform count (5.05 $\log_{10}$ CFU/cm^2) than carcass swabs (p = 0.005). Overall 60% of water samples were positive for *E. coli* and fecal coliforms. Furthermore, the use of personal protective equipment including overall coats and boots was 99.30%. All abattoir workers reported abstaining from eating, drinking and smoking at work, and 73.94% did not wear jewelry during slaughter processing. The use of aprons (44.37%) and head covers (45.77%) was practiced by less than half of the respondents, yet 63.38% of workers consistently wore clean personal protective equipment. Task alternation and equipment sharing were common, with 50% of workers engaging in these practices. Sterilization of knives between each use was not practiced in all abattoirs workers, and only 44.37% of workers performed regular medical checkups. About 35.21% of workers practiced hand washing with soap while only 9.15% used gloves during the slaughtering process. Among the respondents, 52.11% of them had received on job food safety training. In conclusion, the study showed high microbial loads on beef carcasses and a low meat safety practice rate (39.44%), highlighting the need for strict and effective multi-stakeholder food safety measures.

Keywords: *Aerobic plate count, Fecal coliform, Hygienic practices, Meat safety, Public health, Ethiopia*

3.1. Introduction

Adequate quality and quantities of safe and nutritious food is important to sustain quality life and promote good health (WHO, 2025). Meat from healthy animal is nearly free from pathogenic microorganisms (Gill, 1979) and highly nutritious and contributes numerous essential nutrients that are tough to obtain in adequate quantity from other food sources (Geiker *et al.*, 2021). However, its nature provides ideal conditions for microbes to grow. Some microorganisms cause spoilage, while others pose a threat to human health (Saucier, 2016). Provision of beef meat that meets maximum microbial quality limit requirements is expected to ensure safety, healthy, and wholesome meat (Diyantoro and Wardhana, 2019).

The safety of meat has been at the forefront of societal concerns in recent years, and indications exist that challenges to meat safety will continue in the future (Sofos, 2008). The consumption of contaminated food is a cause for more than 200 various diseases, ranging from diarrhea to cancer (WHO, 2025). Major and highly reported outbreaks of foodborne disease have been related to the consumption of contaminated meat and have led to increased interest in meat safety among stakeholders (Azad and Rahman, 2022).

Determining the microbial quality and safety of meat is crucial because of its high potential to harbor pathogens (Al-Mazrouei *et al.*, 2024). Sources of microbial contamination of a carcass include the animal, workers, the clothing of workers, equipment and water (FAO, 1985). Microorganisms associated with the live animal are primarily located on the surface of the animal's hide, hair, hooves and in the gastrointestinal tract (Gill, 1979). Additionally, beef carcass processing procedures, like skinning, evisceration, and chilling, often lead to meat contamination by foodborne pathogens (Ncoko *et al.*, 2020) due to poor hygienic and sanitation practices at the abattoir (Bersisa *et al.*, 2019).

Food handler’s action and practices in a food processing facility are crucial for producing safe food (Griffith, 2013). Improper handling during processing can transfer pathogens,

including human-specific foodborne pathogens or contaminants from animals and utensils to the carcass (Havelaar *et al.*, 2013). These non-compliance with food safety protocols contributes to the carcass contamination through several pathways. Furthermore, the key risk factors include negligence of washing hands properly after handling contaminated material before handling beef carcass, working while ill or with open wounds, using the same equipment for dirty and clean areas, improper evisceration and skinning techniques, inadequate equipment cleaning and sanitation, lack of use of proper personal protective equipment (PPE) and equipment sterilization and improper chilling or storage conditions (FAO, 2004b; CAC, 2005).

Inadequate hygienic practices are among the factors that compromises the microbial quality of carcasses in developing countries (Tanganyika *et al.*, 2017; Adjei *et al.*, 2022; Kenaw *et al.*, 2024; Koech *et al.*, 2024). In Africa alone, foodborne diseases are responsible for approximately 91 million illnesses and 137,000 deaths annually (WHO, 2019). The microbial quality of carcass is a serious public health concern in Ethiopia (Asfaw *et al.*, 2022) where consumption of raw and undercooked meat was common (Azage and Kibret, 2017). While there has been a report on abattoir workers' hygienic practices at Adama Municipal Abattoir (Yimana and Hassen, 2024). Microbial quality of carcass, meat contact surfaces and water quality have not been comprehensively studied at the Adama, Batu, Gelan, Holeta and Sebeta municipal abattoirs. Therefore, this study aims to determine the microbial quality of beef carcasses, selected contact surfaces, and process water, as well as to assess meat safety practices among workers in five municipal abattoirs in Central Ethiopia. The study has significant implications for public health and food safety regulations and policies by identifying microbial contamination along the beef production chain and evaluating worker hygiene practices, findings that can inform targeted interventions.

3.2.Materials and Methods

3.2.1. Study Area

The study abattoirs were located in towns of Adama, Batu, Gelan, Holeta and Sebeta. The towns are situated within a radius of 150 km from Addis Ababa, Ethiopia (Figure 11). They are regulated by the respective town municipalities. The abattoirs serve more than 500 local butcher shops which found in the study area and provide meat inspections and animal slaughtering services. Meat processed in the abattoirs is used for local consumption. According to the Ethiopian Statistical Service 2024 population projection, the population of the area is more than 700,000 (Ethiopian Statistical Service, 2024). The area is characterized by rapid growth in urbanization and population growth.

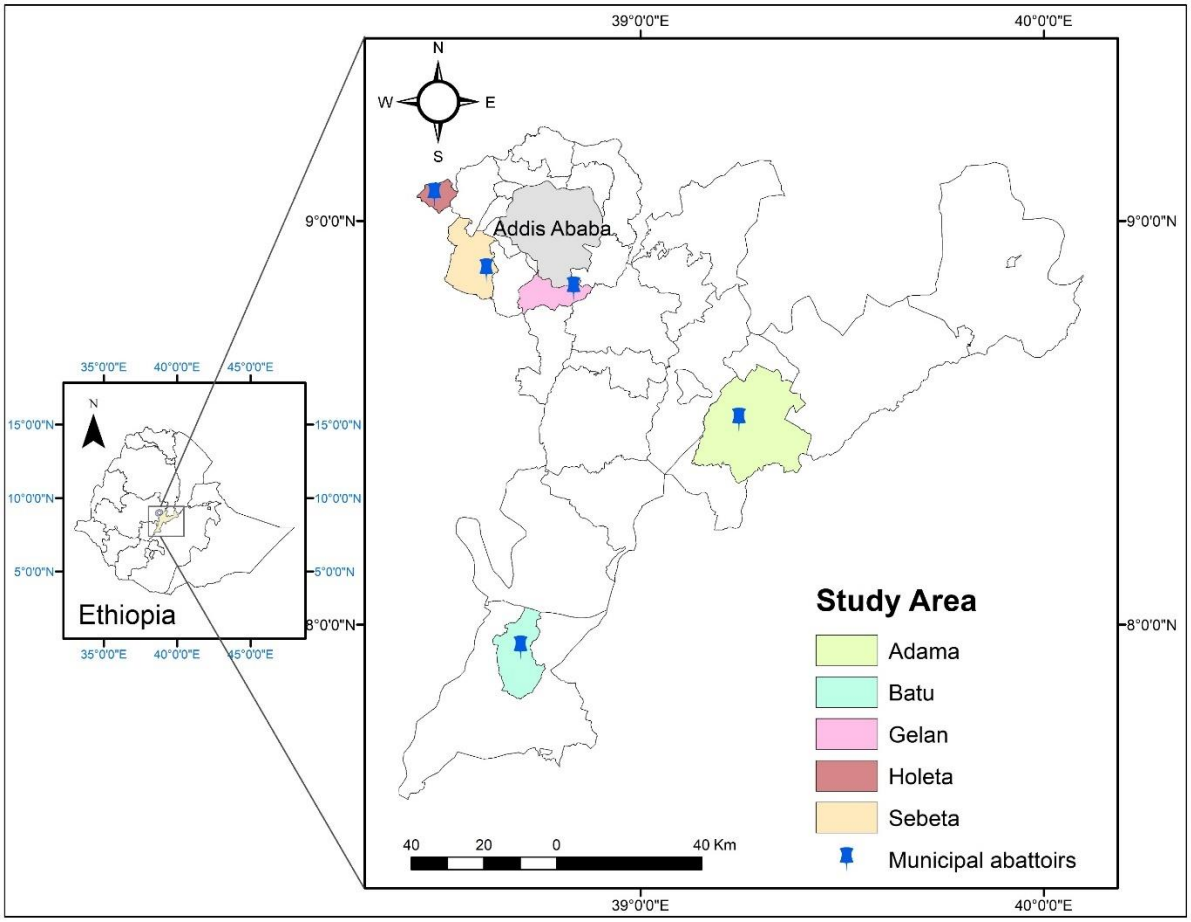


Figure 11: Map of Ethiopia showing study abattoirs (ArcGIS 10.8. 2).

3.2.2. Beef Carcass Processing Procedures at Abattoirs

Apparently healthy animals, ready-to-be-slaughtered were presented to the abattoirs before mid-day and thorough antemortem examinations were performed and animals deemed fit for slaughtering were passed. The slaughter service started at midnight with mechanical stunning of animals by stabbing at the atlanto-occipital region using a sharp edge of the knife, immediately followed by bleeding, and then removal of the head and the feet with the carcass in a horizontal position on the floor. At Adama municipal abattoir, carcass processing steps were performed in a vertical position after hanging the carcass by hooks and sliding it over the rail system. However, in the remaining abattoirs, all carcass processing steps took place on the floor in a horizontal position. Then carcasses were transported at room temperature. Finally, the slaughter hall and the transporting vans were regularly cleaned.

3.2.3. Study Design, Sample Size and Data Collection

A cross-sectional study design was conducted from January, 2024 to February, 2025 on five municipal abattoirs located in Adama, Batu, Gelan, Holeta and Sebeta towns to assess abattoir workers' meat safety practices and microbial quality of beef carcasses. Sample size was determined using Yamane's (1967) formula for a finite population for workers meat safety practice assessment (Yamane, 1967).

$$n = \frac{N}{1 + NE^2}$$

Where: - n=number of required sample size; N=total population of abattoir workers (219); E = 0.05 absolute precision. So the required sample size is 142 and they were selected using a simple random sampling method. Since the number of workers varies across abattoirs, the sample size for each location was determined proportionally (Figure 12). Semi-structured questionnaires were designed to assess abattoir workers' meat safety practices, based on the Food and Agriculture Organization of the United Nations guidelines and Codex Alimentarius Commission (FAO, 2004b; CAC, 2005). The

questionnaires were reviewed by subject-matter experts to ensure content validity. The questionnaire was pre-tested on 15 municipal abattoir workers representing 10 % of the sample size to evaluate clarity, consistency, and applicability of the questions (Bujang *et al.*, 2024). Prior to the pilot test, all participants were informed the purpose and objectives of the study. Informed consent was obtained from each participant before data collection commenced. To ensure privacy and confidentiality, all data were collected in a private setting. Internal consistency reliability of the meat safety practice items was evaluated using Cronbach's alpha, which showed acceptable reliability (Cronbach's alpha = 0.78) (NH *et al.*, 2015). Data collection was carried out through face-to-face interviews and onsite observation of meat safety practice was conducted. Before conducting the interviews, the purpose of the study was clearly explained to the participants and their informed consent to participate was obtained. To obtain honest responses, participants were assured of the confidentiality of the information they provided. The data were collected by trained expert in the field of specialization. Inter-rater reliability was not assessed because all observational and questionnaire data were collected by a single trained expert.

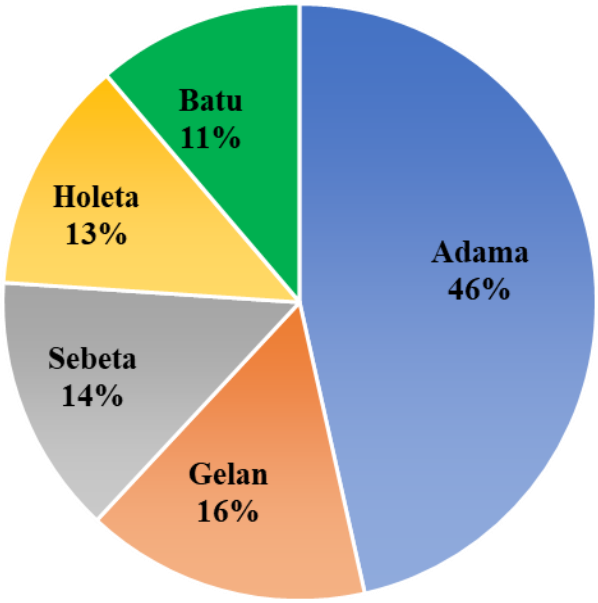


Figure 12: Proportion of sample size of abattoir workers from different study abattoirs.

Sample size determination for carcass swab sampling was determined by formula given below (Charan and Biswas, 2013)

$$N = \frac{(Z_{1-\alpha/2})\text{^}2 * SD^2}{d^2}$$

Where:- N = Sample size

$Z_{1-\alpha/2}$ = Is standard normal variate (at 5% type 1 error (P<0.05)). It is 1.96

SD = Standard deviation of variable. Value of standard deviation can be taken from previously done study or through pilot study.

d = Absolute error or precision has to be decided by researcher (0.205). Based on the above formula the estimated sample size was 50.06 for each abattoir considering SD = 0.74 (Bersisa *et al.*, 2019) and absolute error = ± 0.205 $\log_{10}$ CFU/cm^2. However the sample size was increased to be 51 carcass swab samples that were collected from each abattoir.

3.3.4. Microbial Quality Assessment

Sample collection

A total of 255 beef carcass swab samples were collected from five abattoirs (51 beef carcass swab samples per abattoir) using systematic random sampling method. An equal distribution of samples carcasses samples was purposefully employed to allow for balanced representation in the five locations. At each sampling day five carcass swab samples were collected on weekly basis for each abattoir. A total of 30 hand swabs from workers, 30 knife swabs, and 30 water samples were collected during each visit to the abattoirs before the starting carcass processing. Water samples were collected several times repeatedly within the duration of sampling period to account for variation in water quality whereas different individuals were sampled for hand and knife swab. Carcass swabs, were collected using sterile non-absorbent cotton wool swab moistened with 0.1% peptone water by the swabbing technique on an area of 100 cm^2 marked with a sterile plastic frame (10 cm × 10 cm) from anatomical carcass sites, the rump, flank, brisket and hind the fore leg through wiping sampling site for a total of approximately 10 times in the vertical and 10 times in the horizontal direction (ISO17604:2015, 2015). Swab samples

collected from different anatomical sites of each carcass were pooled together and analyzed.

Abattoir workers' hands, those who come into direct contact with carcass, and knife swabs were collected before the slaughtering operation began. All samples are transported to the Microbiology laboratory, College of Veterinary Medicine and Agriculture, Addis Ababa University using an icebox. At the laboratory, the serial dilutions were made using sterile diluents, from 10^{-1}to10^{-9} in sterile 10mL sterile test tubes and 0.1 mL of homogenate was plated out on agar media of interest at an appropriate time and temperature. For aerobic plate count (APC), 0.1 mL of homogenate was plated onto the surface of plate count agar (Oxoid, Basingstoke, Hampshire, UK). Plates were incubated at 35°C for 48±3 hours. A violet red bile agar (Oxoid, Basingstoke, Hampshire, UK); was used to enumerate fecal coliform count (FCC); 0.1 mL of the homogenate was evenly spread onto agar plates and incubated at 35°C for 24 hours, typical and atypical colonies were enumerated. Plates containing between 15 and 300 colonies were counted (TheUSFoodandDrugAdministration, 2025). Water samples used for carcass wash sources in the abattoirs were collected to determine the presence of fecal coliforms and *E. coli* in 100 mL of water using the qualitative method (positive/ negative). The qualitative method is sufficient because, as a standard, drinking water contains no fecal coliforms and *E. coli* per 100 mL (NRCSDWC, 1977; WHO, 2017). Water used for food processing should be of potable(CAC., 2022). Counted colonies were calculated as follows:

$$N = \frac{\sum C}{[(1xn_1) + (0.1xn_2)xd]}$$

where N = Number of colonies per ml or g of product, Σ c = Sum of all colonies on all plates counted, n_1 = Number of plates in first dilution counted, n_2 = Number of plates in second dilution counted, d = Dilution from which the first counts were obtained (US Food and Drug Administration (USFDA), 2025) and expressed in CFU/cm^2. The results of total microbial counts obtained in CFU/cm^2 were converted into $\log_{10}$ by multiplying $\log_{10}$ with (N(CFU/cm^2).Where N = Number of colonies per ml or g.

Occurrence of fecal coliforms and E. coli in water

For the enumeration of microbial quality of water, fecal coliforms were obtained by pouring 100 ml of the sampled water onto a membrane filter paper 47 mm in diameter, 0.45 μm pore size, to trap as well as isolate bacteria. Following filtration, the filter paper was then placed in a Petri dish, holding the prepared violet red bile agar (Oxoid Basingstoke, UK), cooled and incubated at 35°C for 24 hours. Purple-red colonies that are 0.5 mm or larger in diameter and surrounded by a zone of precipitated bile acids transferred to eosin methylene blue (EMB) agar (Oxoid, Basingstoke, UK). Further isolation of *E. coli* was performed using EMB. The Petri dishes were incubated for 24 hours at 35°C. Greenish metallic blue colonies in EMB agar were regarded as presumptive for *E. coli*. Gram stain and biochemical tests (IMViC tests) were utilized to confirm the *E. coli* isolates (USFDA, 2025).

3.3.5. Data Analyses

The data were coded and analyzed using Stata Statistical Software, release 14.2 (Stata Corp LP, College Station, TX). Descriptive statistics were utilized to report overall scores for workers' food safety practice questions using percentages and frequencies. The questionnaire comprises three parts general information of abattoirs (12 questions), general demographic characteristics of abattoir workers (5 questions) and 14 meat safety practice questions. For meat safety practice questions, each positive response was assigned a score of 1 or otherwise 0, and the scores were summed out of 14 questions. According to Abunna *et al.,* 2022 total score obtained was divided by 14 and multiplied by 100 to obtain a percentage. Accordingly, abattoir workers with ≥70% scores were classified as having acceptable level of meat safety practice, while those with <70% scores were classified as having unacceptable level of meat safety practices This cut-off point was used to create a binary dataset to determine the overall meat safety practice (Abunna *et al.*, 2022). Microbial quality of water is determined using the qualitative method (positive/ negative) as it should be free from fecal coliform and *E. coli* bacteria

can be demonstrated in 100 ml of water (National Research Council Safe Drinking Water Committee (NRCSDWC), 1977; WHO, 2017). The results of total microbial counts (CFU/cm^2) were converted into log_{10} and descriptive statistics was used to calculate mean, standard error, minimum and maximum values, considering the type of sample and origin. Carcass microbial limit was determined as unacceptable if APC>5.0 log_{10} CFU/cm^2 and FCC>3.0 log_{10} CFU/cm^2 (Stone *et al.*, 2025). Microbial counts (microbial quality) were compared using one way ANOVA and Z-test for mean comparison. Linear regression analysis was performed to assess the association between abattoir and microbial load parameters (APC and FCC) and effect size and Confidence interval (CI) were reported. The comparison of acceptable meat safety practices with socio-demographics was performed with Chi-square test. P-value < 0.05 was considered statistically significant with a 95% level of confidence.

3.3. Results

3.3.1. Socio-demographic Characteristics of Abattoir Workers

The majority of workers were male, age group above 30, less than five years work experience and primary/secondary educational status. Acceptable meat safety practice varies between educational status, experience and food safety training groups (P<0.05) (Table 3).

Table 3: Socio-demographic characteristics and acceptable practices of abattoir workers.

Variable	**Category (levels)**	**Frequency (N)**	**Acceptable Practice frequency (N)**	**Percent (95%CI))**	***P-value**
Age	18-30	41	22	53.7 (37.4-69.3)	ref
	>30	101	34	33.7 (20.1 - 50.6)	0.283
Gender	Male	130	50	38.5 (24.2 - 55.5)	ref
	Female	12	6	50.0 (35.1 - 67.1)	0.434

Educational Status	Primary/secondary	111	40	36 (22.1 - 53.1)	ref
	Above secondary	31	16	51.6 (35.01 - 67.1)	0.000
Experience	<5	83	37	44.6 (28.5 - 60.3)	ref
	≥5	59	19	32.2 (18.1 - 48.1	0.019
Food Safety Training	Yes	74	39	52.7 (37.4 - 69.3)	ref
	No	68	17	25.0 (12.4 - 40.3)	0.001

*: P<0.05 indicates statistically significant values

3.3.2. Food Safety Practices of the Abattoir Workers

While nearly all workers used overall coat and boots, less than fifty percent of them washed their hands with soap before handling meat, used gloves, aprons, and head covers and regular health check-ups. There is no practice of sterilization of knives between each activity (Table 4).

Table 4: Meat safety practices of the abattoir workers.

Food safety practice statements	**Frequency**	**%**
Regular health check-up	63	44.37
Use gloves	13	9.15
Use apron	63	44.37
Use boots	141	99.30
Use Overall coat	141	99.30
Use head covers	65	45.77
Do not wear jewelry	105	73.94
Do not eat or drink at the workplace	142	100
Hand wash with soap before handling meat	50	35.21
Always use clean PPE	90	63.38
Do not smoke while on work	142	100

Do not handle meat when ill	109	76.76
No exchanging/crisscrossing of personnel or equipment/ stay in designated areas	71	50
Knives were properly sterilized between each activity.	0	0
Over all workers acceptable level meat safety practice	**56**	**39.44**

3.3.3. Risky Meat Safety Practices of Workers at each Abattoir

Figure 13 provides comparison of abattoirs with regard to meat safety practices and showing Adama abattoir workers showed good meat safety practices.

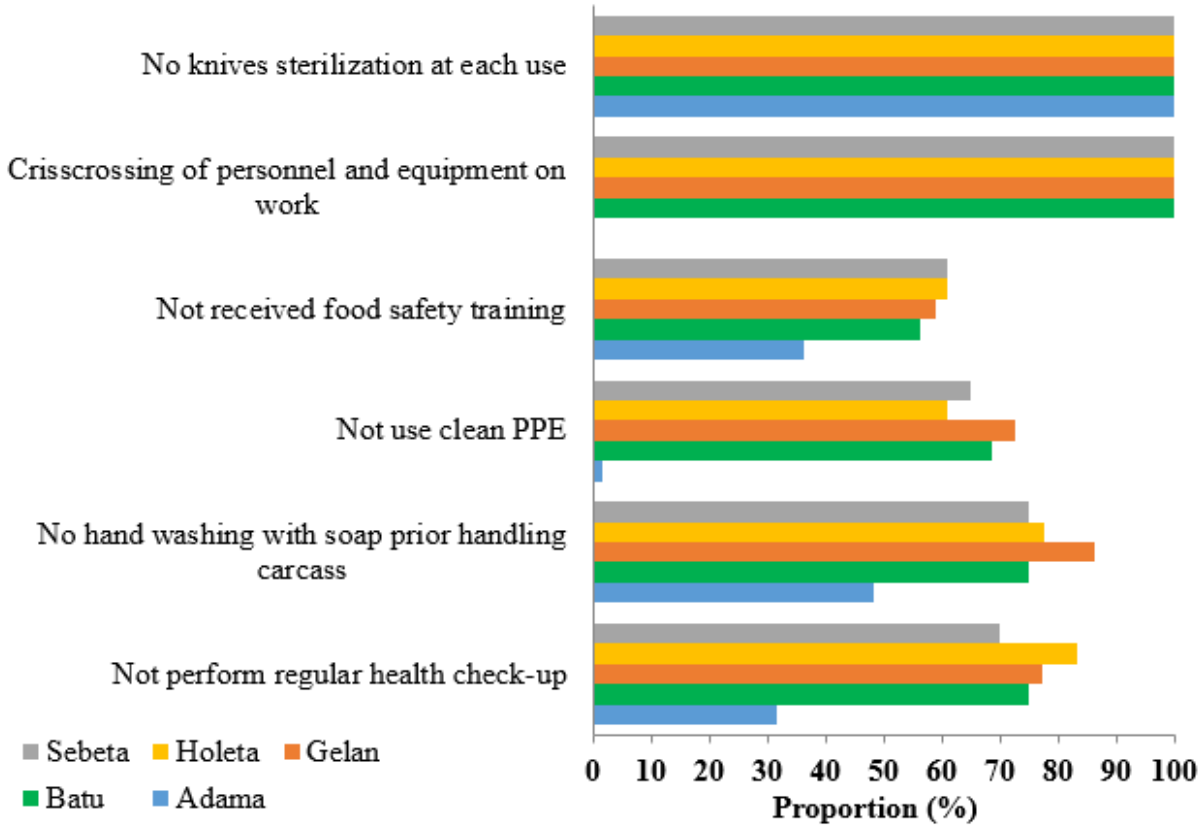


Figure 13: Workers' risky meat safety practices at each abattoir.

3.3.4. Microbial Quality of the Beef Carcass

The overall beef carcass aerobic plate count and coliform count are 6.12 $\log_{10}$ CFU/cm^2 and 4.58 $\log_{10}$ CFU/cm^2, respectively. A significant difference was observed between the Adama municipal abattoir and the remaining abattoirs in both aerobic plate and fecal coliform counts (Table 5).

Table 5**:** Microbial quality of carcass.

Abattoirs	**APC log_{10} CFU/cm^2**							**FCC (log_{10} CFU/cm^2)**						
	Mean	**SD**	**min**	**Max**	**P-value**	**Coef**	**95% CI**	**mean**	**SD**	**min**	**Max**	**P-value**	**Coef**	**95% CI**
Adama	5.67	0.83	4.18	7.93	ref	Ref	ref	4.13	0.89	2.75	6.04	Ref	ref	ref
Batu	6.27	0.84	4.72	8.19	*0.006	0.60	0.26-0.94	4.79	0.79	3.39	6.73	*0.001	0.65	0.32-0.99
Gelan	6.22	0.89	4.05	7.84	*0.015	0.55	0.21-0.89	4.68	0.95	2.71	6.99	*0.014	0.55	0.21-0.88
Holeta	6.24	0.83	4.76	8.54	*0.010	0.58	0.24-0.92	4.66	0.83	3.11	6.58	*0.021	0.53	0.19-0.86
Sebeta	6.20	0.94	4.09	8.69	*0.021	0.53	0.19-0.87	4.65	0.80	2.60	6.11	*0.027	0.51	0.18-0.85
Total	**6.12**	**0.89**	**4.05**	**8.69**				**4.58**	**0.88**	**2.60**	**6.99**			

*: Statistically significant values

3.3.5. Microbial Quality of Workers' Hands and Knives

The mean APC and FCC for knives and workers' hands exceeded the maximum limit allowed as depicted herein Table 6.

Table 6**:** Microbial quality of knife and hand (n=60).

Abattoir	**Hand**				**Knife**			
	APC (log_{10} CFU/cm^2)		**FCC (log_{10} CFU/cm^2)**		**APC (log_{10} CFU/cm^2)**		**FCC (log_{10} CFU/cm^2)**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adama	4.98	0.71	4.94	0.45	5.33	0.56	4.76	0.54
Batu	5.08	0.98	4.73	0.34	5.61	0.48	5.39	0.77
Gelan	5.43	0.67	4.72	0.47	5.78	0.73	5.12	0.54
Holeta	5.22	0.94	4.92	0.64	5.46	0.73	4.96	0.54
Sebeta	5.25	0.53	4.99	0.41	5.50	0.39	5.01	0.46
Total	**5.19**	**0.75**	**4.86**	**0.45**	**5.54**	**0.58**	**5.05**	**0.67**

3.3.6. Occurrence of fecal coliform and E. coli in water samples

Water samples from Batu, Gelan and Holeta municipal abattoirs were 100 % positive for fecal coliforms and *E. coli* while water samples from Adama and Sebeta municipal abattoirs were 100% negative. Overall 60% of water sample collected from five abattoirs were positive for fecal coliforms and *E. coli.*

3.3.7. Microbial loads across the three swab samples

The mean total aerobic count was highest in carcass swabs (6.12 log_{10} CFU/cm^2), followed by knife swabs (5.54 log_{10} CFU/cm^2), and lowest in hand swabs (5.19 log_{10} CFU/cm^2). Significant differences were observed between carcass and knife swabs ($p = 0.006$) and between carcass and hand swabs ($p < 0.001$). For Fecal coliform counts, knife swabs showed a higher mean (5.05 log_{10} CFU/cm^2) than carcass swabs (4.58 log_{10}

CFU/cm²), with a significant difference (p = 0.005). However, there is no significance difference between carcass and hand swabs samples (Table 7).

Table 7: Comparison of the microbial loads across the three swab samples

Sample type	**N**	**Mean** (log_{10} CFU/cm²)	**SD**	**Z-value**	**P-value**
Aerobic plate count					
Carcass swab	255	6.12	0.89	ref	ref
Knife swab	30	5.54	0.58	3.48	*0.006
Hand swab	30	5.19	0.75	5.50	*0.000
Fecal Coliform count					
Carcass swab	255	4.58	0.88	ref	ref
Knife swab	30	5.05	0.67	-2.83	*0.005
Hand swab	30	4.86	0.45	-1.71	0.087

* Statistically significant values

3.4. Discussion

Microbiological investigation is a critical quality management tool in the food business (Camargo *et al.*, 2019). Contaminated meat has been cause for numerous cases of foodborne illness and poses serious challenges in developing countries (Kebede and Getu, 2023). The study showed the overall mean beef carcass APC was 6.12 $\log_{10}$ CFU/cm^2 and FCC 4.58 $\log_{10}$ CFU/cm^{2,} were high. This finding showed resemblance with a study from Haramaya, Ethiopia where 7.50 $\log_{10}$ CFU/cm^2 FCC was reported (Edget *et al.*, 2017). Similarly 3.61 $\log_{10}$ CFU/cm^2 FCC was reported from Dessie, Ethiopia (Berhanu *et al.*, 2025) which was high. Fecal coliforms exist in the intestines and feces of animals (Jiang *et al.*, 2020). The elevated fecal coliform count is an indicator of possible fecal contamination (Martin *et al.*, 2016). A poor hygienic and sanitation practice during carcass processing procedures at the abattoir deteriorates microbial quality of carcasses (Bersisa *et al.*, 2019).

An APC of the this study is comparable to the report from Dire Dawa abattoir, which is 5.63 $\log_{10}$ CFU/cm^2, though the highest APC 7.11 $\log_{10}$ CFU/cm^2 was reported from Haramaya municipal abattoir (Mengistu *et al.*, 2017). On the other hand the lowest APC 4.53 $\log_{10}$ CFU/cm^2 reported from the carcass at Bishoftu municipal abattoir (Bersisa *et al.*, 2019). The microbial quality of carcass of our study was beyond acceptable limit both in APC and FCC (Stone *et al.*, 2025). The observed differences between studies might be due to methodological variations, including differences in sampling sites, microbiological techniques, incubation conditions, and the hygiene indicators evaluated. In our finding, a significant difference was observed in the microbial quality of carcasses between Adama municipal abattoir and the remaining abattoirs. This disparity might be associated with better hygienic practices of abattoir workers at Adama compared to others, where workers demonstrated better exposure to food safety trainings and medical check-up in addition to good hygienic practices. Good hygienic practices reduce carcass contamination by limiting the transfer of pathogens from hides, feces, or equipment to meat during carcass processing (FAO, 2004a). Additionally, at the Adama municipal abattoir, the carcass processing steps were performed in a vertical position, with carcasses

hanged by hooks and slid over the rail system. However, in the remaining abattoirs, all carcass processing steps took place on the floor. Vertical processing of the carcass is imperative in maintaining hygiene and critical in minimizing contamination (FAO, 1991).

The microbial profile of food contact surfaces is a suitable indicator of a food safety management system (Illés *et al.*, 2018). The ability of microorganisms to adhere to surfaces has significant implications for food safety. Microorganisms present on animal tissues can contribute to food spoilage and pose risk to food safety (Frank, 2001). Based on the standards used in the food processing industry, a standard of less than 1.3 $\log_{10}$ CFU/cm^2 was used for APC and less than 1.0 $\log_{10}$ CFU/cm^2 for *E. coli* count (Sneed *et al.*, 2004). According to our study APC for knives and workers' hand was 5.54 $\log_{10}$ CFU/cm^2 and 5.19 $\log_{10}$ CFU/cm^2,respectively. The FCC was 5.05 $\log_{10}$ CFU/cm^2 for the knife and 4.86 $\log_{10}$ CFU/cm^2 for the hands of meat handler's. Other studies have reported higher loads including an APC 6.96 $\log_{10}$ CFU/cm^2 for hand and 7.84 for knives $\log_{10}$ CFU/cm^2 (Birhanu *et al.*, 2017) as well as FCC of 6.0 $\log_{10}$ CFU/cm ^{2} (Ayalew *et al.*, 2015)for knives , but lower FCC 3.39 $\log_{10}$ CFU/cm^2 for hand (Berhanu *et al.*, 2025). Comparisons of the swab samples reveal significant differences in microbial contamination across sample types, with carcasses showing the highest aerobic counts, followed by knives and hand, suggesting that carcasses might be contaminated by multiple sources in the processing chain. Additionally, knives can be contaminated through various means during evisceration, trimming, and surface contact, while hands become contaminated by touching contaminated surfaces. Consequently, swabs from knife and hands had higher coliform levels than carcasses, indicating poor hygiene practices among workers that likely pose risk of carcass contamination by coliforms (Birhanu *et al.*, 2017; Berhanu *et al.*, 2025).

Water used for food handling and processing must be of potable quality (CAC, 2022) with no detectable fecal coliform and no *E. coli* bacteria present in 100 ml of water (National Research Council Safe Drinking Water Committee, 1977; WHO, 2017). However, except for Adama and Sebeta, all water samples from Batu, Gelan and Holeta municipal abattoirs were positive for fecal coliforms and *E. coli*. This might be due to

abattoir water tanks were not cleaned regularly or covered properly with lid. Absence of cleaning of water tank/vessel regularly deteriorates the microbiological and physicochemical quality of stored water (Manga *et al.*, 2021), thereby posing a risk of food contamination. Furthermore borehole water sources were utilized without treatment in some abattoirs, which is a likely source of contamination as untreated borehole or ground water may harbor pathogenic microorganisms (Lutterodt *et al.*, 2018; Olalemi *et al.*, 2021). Reports from abattoirs in Eastern Cape Province of South Africa, Lilongwe, Malawi, Dessie of Ethiopia, inconsistent with this study where abattoir water samples were reported to be positive for fecal coliforms and *E. coli* (Tanganyika *et al.*, 2017; Ncoko *et al.*, 2020; Berhanu *et al.*, 2025). On the other hand study from Bishoftu municipal abattoir reported abattoir water to have unacceptable microbial limit (Bersisa *et al.*, 2019). There is an evidence of lack of water quality testing in the municipal abattoirs of central Ethiopia (Mulatu *et al.*, 2026).

The questionnaire survey in our study showed that 73.94% of workers did not wear jewelry during carcass processing. This was in alignment with the report that indicated the majority of workers removed their stuff, such as rings, necklaces and watches, before processing meat (Zelalem *et al.*, 2021). These findings cannot fulfill the criteria of not a single person working with meat handling should wear any jewelry, badges or buttons that may come loose and be accidentally included in the product (FAO, 1991).

This study showed that workers who performed regular medical checkups and did not work while ill were 44.37% and 76.76%, respectively. Yimana and Hassen, 2024 also reported that the majority of workers did not execute regular health checkups and refrained from handling carcasses during illness (Yimana and Hassen, 2024). It is expected that those who come into direct or indirect contact with the carcass during their work should: undergo a medical examination before and throughout their employment; refrain from working while clinically affected by, or suspected of carrying, communicable agents that could be transmitted through meat (CAC, 2005).

Maintaining an appropriate standard of personal hygiene, wearing protective clothing, and ensuring that non-disposable protective clothing is cleaned before and during work is a mandatory practice for those who come into direct or indirect contact with carcasses (CAC, 2005). Our study showed that hand washing with soap was practiced among 35.21% of workers. This infers the study of Yimana and Hassen, 2024 who reported that three-fourths of workers do not wash their hands before handling meat. The use of gloves at work was very low with only 9.15% of workers used it. This was in alignment with the report from Jigjiga (Tegegne and Phyo, 2017), where above three-quarters of workers do not use gloves at the time of processing carcasses. Very low glove usage and inadequate hand washing by food handlers, particularly when handling food, significantly increase the risk of foodborne illnesses (Green *et al.*, 2007). Furthermore, the use of overall coats and boots by workers was 99.30% which is supported by the finding from Mekelle abattoir, Ethiopia (Haileselassie *et al.*, 2013).

On the other hand, the use of an apron and a head cover of workers were 44.37% and 45.77% respectively. About 63.38%, of workers always use clean protective equipment they had. This corresponds with a report from Gopalganj, Bangladesh, which shows that the majority of workers did not use aprons and hair covers (Hossain *et al.*, 2025) and Ambo, Ethiopia, where cleaning of personal protective equipment was reported mainly twice a week and weekly (Tadese *et al.*, 2021). The current finding shows that, all abattoir workers obey to the rule of not smoking, eating and drinking at work, while they did not strictly follow other food safety rules. Similar findings has been reported by a previous study where none of workers from an abattoir in Kajiado, Kenya consume food, drink and smoking upon processing carcasses (Kimindu *et al.*, 2024). When engaged in food handling activities, personnel should refrain from behavior which could result in contamination of food, for example: smoking or vaping; spitting; chewing, eating, or drinking (CAC, 2022).

The study showed that 50% of workers alternate tasks and exchange equipment with one another. The knife, sterilization between each activity, was not carried out by anyone. The finding aligns with a study conducted in Debrebrhan abattoir, where equipment

sanitation and sterility practice are reported to be poor (Asfaw *et al.*, 2023). Likewise, the exchanging/crisscrossing of personnel and equipment between dirty and clean areas was also reported (Yimana and Hassen, 2024). This practice increases the risk of cross-contamination, compromising the hygienic status of the processing environment and potentially leading to the spread of foodborne pathogens, and needs enforcement of rules (FAO, 2003). Equipment used for processing should be sterilized after each use and designated between dirty and clean areas (FAO, 1991).

The most important finding with regards to public health in this study was the absence of knife sterilization in all five abattoirs, signaling a breach of basic sanitation standards. The use of knives is constant throughout the process of slaughtering, including activities like skinning and evisceration. Without any sterilization, knives serve as a means of transmission for microorganisms. Knife contamination poses a high risk of transmitting pathogens from animal hides, intestinal material, or even other contaminated carcasses to the surface of fresh meat, thereby increasing chances of cross-contamination in the production process (Asfaw *et al.*, 2023). Poor hand hygiene exacerbates this problem because hands serve as another means of transmission of microbes. High numbers of microbes found in both knives and hands prove this point.

This study showed that, 52.11% of workers received on job food safety training. This finding is in concordance with the study conducted at the Bishoftu municipal abattoir, where more than half of the workers received on-job food safety training (Gutema *et al.*, 2021). Carcass processing should be carried out by personnel with the appropriate training (CAC, 2005). The overall acceptable levels of meat safety practice of abattoir workers were 39.44%. This study is in line with report from Bishoftu (Abunna *et al.*, 2022), Jigjiga (Ahad *et al.*, 2025), Dessie and Kombolcha (Gebeyehu and Tsegaye, 2022) Ethiopia where most of meat handlers had poor food safety practices. Poor meat handling practices can significantly increase the risk of meat contamination, leading to foodborne illnesses (Hasan *et al.*, 2024). The overall meat hygiene practice was unacceptable while more than half of workers received on job food safety training. This might be due to inadequacy operational facility which had critical role in comprising of food safety

practices (Ovuru *et al.*, 2024; Nigussie *et al.*, 2025). Moreover, low enforcement of food safety rules in food establishment leads in deterioration of microbial quality of food (Subedi *et al.*, 2025). From a public health perspective, high microbial load of carcass resulted from unhygienic practices (Mulatu *et al.*, 2026). Similar deficiencies in meat safety practices and microbial quality reported in Bishoftu, Jigjiga, Dessie, and Kombolcha, suggesting that inadequate hygiene compliance and weak enforcement of food safety measures may represent systemic challenges across Ethiopian municipal abattoirs. These recurring findings collectively suggest the presence of systemic hygiene deficiencies, infrastructure limitations, and regulatory enforcement gaps across Ethiopian municipal abattoirs.

3.5. Conclusion

The findings of the study showed high carcass APC and FCC, poor knife sterilization, limited handwashing with soap, low glove/apron/head-cover use, and fecal contamination of water in three abattoirs with overall acceptable meat safety practice rate of 39.44%. Based on the findings, this study recommends sustainable hygiene training with enforcement of mandatory knife sterilization, infrastructure upgrades to vertical carcass processing, and regular water quality testing with borehole treatment. Stakeholder collaboration is also vital for improving the microbial quality of meat.

Limitations of the study

It is important to consider the limitation of the study when interpreting its findings. A possible limitation of the study is its research design, which relied on cross-sectional study approach which cannot establish causal relationship and indicator-based microbiological assessment rather than specific pathogen detection. The absence of evaluating risks associated with specific foodborne pathogens underscores the need for further investigation of pathogens of food safety and the public health significance**.** Moreover, the reliance on self-reports regarding the behavior of workers could lead to social desirability bias, where individuals may report their adherence to the recommended

hygienic behavior more often than it actually occurs. The study design does not allow for direct linkage between individual worker practices and specific carcass contamination levels, limiting the ability to attribute contamination sources to particular behaviors or risky practices. Clustering or nested sampling effects within abattoirs were not statistically addressed. The present study assessed carcass hygiene using FCC as an indicator of fecal contamination and hygienic practices during carcass processing. However, the use of FCC alone has some limitations. FCC may not capture the broader spectrum of microorganisms included in the Enterobacteriaceae family.

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Availability of data and materials

The data used are available from the corresponding author on reasonable request.

Statements and declarations

Ethical Approval

The study was conducted according to the guidelines of the Declaration of Helsinki, and Ethical approval for the study was obtained from the Institutional Review Board (research ethics committee) of the College of Veterinary Medicine and Agriculture Addis Ababa University (Certificate Ref. No: VM/ERC/03/11/16/2024).

Consent to participate

Before conducting the study, the purpose of the study was clearly explained to the participants and their informed consent was obtained.

Consent to publish

Consent was obtained from all participants

Declaration of conflicting interest

The authors declare no competing interests.

Author contributions

Endalu Mulatu: Writing review and editing, Writing original manuscript, Visualization, Validation, Research design, Data collection and Laboratory Investigation, Data curation and analysis, Fund acquisition, and Conceptualization. Nebyou Moje: Fund acquisition, Validation, Conceptualization, Writing review and editing, Research design. Biruhtesfa Asrade : Writing review and editing, research design, Fund acquisition. Ahmad Hamedy, Bekele Megresa and Hika Waktole: Writing review and editing, Resources, Research design, Supervision, Data analysis, Fund acquisition, Conceptualization.

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4. CHAPTER IV: MOLECULAR CHARACTERISTICS AND ANTIMICROBIAL SUSCEPTIBILITY OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* O157:H7 FROM BEEF CATTLE FECES AND CARCASSES IN MUNICIPAL ABATTOIRS IN CENTRAL ETHIOPIA

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ABSTRACT

Pathogenic *Escherichia coli* O157:H7 has been reported to occur on beef carcasses in Ethiopia which can pose a significant public health risk. This study was conducted to assess the occurrence, potential source of contamination and the antimicrobial susceptibility of *E. coli* O157:H7 on bovine carcasses from three municipal abattoirs from Central Ethiopia. A total of 216 fecal and hide samples each, 540 carcass swabs, 18 water samples, and 18 swabs each from workers' hands and knives were collected. The study revealed a 1.4% and 1.1% prevalence of *E. coli* O157:H7 in feces and on carcasses, respectively. *E. coli* O157:H7 was not detected (0%) in the remaining samples including water, hides, workers' hands and knives. A whole-genome-sequencing-based core genome multilocus sequence typing (cgMLST) analysis of the *E. coli* O157:H7 isolates from the same municipal abattoir showed clonal relatedness, i.e. clustering together with no allele differences (AD) in the cgMLST scheme. However, genetic diversity was observed between isolates originating from different abattoirs, with allelic differences ranging from 140 to 226 AD. All strains carried virulence genes encoding adherence factors (*eae*, *iha*, and *tir*), type III secretion system translocated proteins (*espA*, *espB*, *espJ*, *espP*, *etpD*, *nleA*, *nleB*, and *nleC*), outer membrane proteins (*chuA*, *ompT*, and *traT*), toxins (*astA*, *ehxA*, and *toxB*), and other pathogenicity-related factors (*gad*, *iss*, *katP*, and *terC*). All strains carried the *stx2* gene subtype *stx2c*. All isolates were susceptible to the antimicrobials for which valid clinical breakpoints were available and displayed wild-type MIC distributions for most antimicrobials evaluated using available EUCAST ECOFFs. Neither acquired antimicrobial resistance genes nor known resistance-associated chromosomal mutations were detected. The study findings suggest that contact with fecal material during evisceration is a possible source for contaminating the carcasses with *E. coli* O157:H7. Therefore, strict hygienic practice should be followed during carcass processing.

Keywords: *Antimicrobial susceptibility, E. coli O157:H7, Genetic relatedness, Food safety, Meat processing, Public health, Ethiopia*

4.1. Introduction

In both developing and developed countries, foodborne disease is a major cause of morbidity and mortality (Singha *et al.*, 2023). *Escherichia coli* O157:H7 is a major pathogenic *E. coli* serotype which has become a critical food-borne pathogen since the early 1980s, as strains of this serotype frequently possess genes encoding for Shiga toxins (*stx*) (Rani *et al.*, 2021). They are characterized by the presence of one or both Shiga toxin genes (*stx1* and/or *stx2*) (Shan *et al.*, 2021). Stx-producing *E. coli* (STEC) are typically found on food products mostly due to cross-contamination with fecal matter. STEC O157:H7 are of major public health concern due to the severity of illness they can cause (Oluwarinde *et al.*, 2023). This includes diarrhea, hemorrhagic colitis, and the hemolytic-uremic syndrome (HUS) in humans (Gambushe *et al.*, 2022). STEC O157:H7 are one of the most frequently reported foodborne pathogens to have emerged in the past four decades (Singha *et al.*, 2023).

Virulence genes play an essential role for high-level colonization of the human gut, adherence to host cells, and systemic damage (Kolodziejek *et al.*, 2022). Shiga toxin genes are the principal virulence determinants associated with disease severity (Wang *et al.*, 2024). Antimicrobial susceptibility testing is important in the detection of resistant strains of microbes, for choosing the right therapeutic approach in combating bacterial infections, and to assess the effectiveness of new antimicrobial agents (Kadeřábková *et al.*, 2024). Apart from the clinical importance, antimicrobial resistance could contribute to bacterial fitness, survival, and propagation, hence enabling the establishment and spread of resistant strains in the food production system (Nasrollahian *et al.*, 2024). Furthermore, several virulence genes present in *E. coli* O157:H7 are associated with plasmids that can even harbor antimicrobial resistance genes, leading to the possibility of co-selection of both resistance and virulence properties (Naidoo and Zishiri, 2023; Castañeda-Barba *et al.*, 2024). Resistant strains of microbes can make disease treatment challenging, leading to prolonged illness, increased complications, and potentially higher mortality rates, especially in vulnerable populations such as children, the elderly, and immuno-compromised individuals (WHO, 2023). It has been estimated that bacterial

antimicrobial resistance was directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths (Murray *et al.*, 2022). Because of its public health significance, the increasingly reported antimicrobial-resistance of *E. coli* O157:H7 in the food supply chain poses an additional major threat to food safety (Abebe *et al.*, 2023). Although antibiotic treatment for patients suffering from severe clinical manifestations has been much debated, since exposure of STEC to antimicrobial compounds may induce toxin expression, potentially worsening clinical outcome (Ramstad *et al.*, 2021). Antimicrobial susceptibility testing should prioritize the surveillance of resistance trends, the identification of public health risks, and monitoring of resistance strains in the food chain. Thus, antibiotics are not considered part of the standard treatment for patients infected with STEC, as they may possibly increase the risk of subsequent HUS (WHO, 2026).

Contamination of beef carcasses with *E. coli* O157:H7 in abattoirs largely originates from the animals themselves and can occur during the slaughtering process (Abdissa *et al.*, 2017). Furthermore, cross-contamination can arise during distribution, further processing, and storage (Gutema, Abdi*, et al.*, 2021). Identifying the causal source of *E. coli* O157:H7 contamination during the slaughtering process is critical for establishing targeted interventions that help minimize pathogen transfer and safeguard the public (Wilson *et al.*, 2018; Gutema, Abdi*, et al.*, 2021). According to previous studies, contamination of carcasses with *E. coli* O157:H7 at the slaughterhouse is of public health concern in Ethiopia, where unhygienic food safety practices are common (Zelalem *et al.*, 2019). However, limited information exists regarding the genetic relatedness of *E. coli* O157:H7 from different sources in municipal abattoirs and its association with carcass contamination as well as its virulence and antimicrobial resistance genes. Therefore, this study aimed to address occurrence, antimicrobial susceptibility along with the presence of resistance and virulence genes and the genetic relatedness of *E. coli* O157:H7 isolates from carcass samples and other associated sources in different municipal abattoirs in the Central part of Ethiopia.

4.2. Material and Methods

4.2.1. Study Area

The abattoirs, from which the *E. coli* O157:H7 strains were isolated, were located in the Central part of Ethiopia, specifically in the towns of Adama, Batu and Holeta. These are situated within a radius of 150 km from Addis Ababa. The abattoirs are regulated by the respective town municipalities. Butcher shop owners buy healthy ready to be slaughtered cattle from various open markets near the towns and transport them to the municipal abattoir. The municipal abattoirs at Adama, Batu, and Holeta provide animal slaughtering services, meat inspection, and meat processing for an average of 90, 15, and 25 animals per day, respectively, thereby supplying more than 400 butcher shops across the towns. Meat processed in these abattoirs is used for local consumption in the respective towns. According to the 2024 population projection of the Ethiopian Central Statistical Service, the estimated populations of Adama, Batu, and Holeta were 504,345, 99,928, and 53,354 inhabitants, respectively (EthiopianStatisticalService, 2024) (Figure 14).

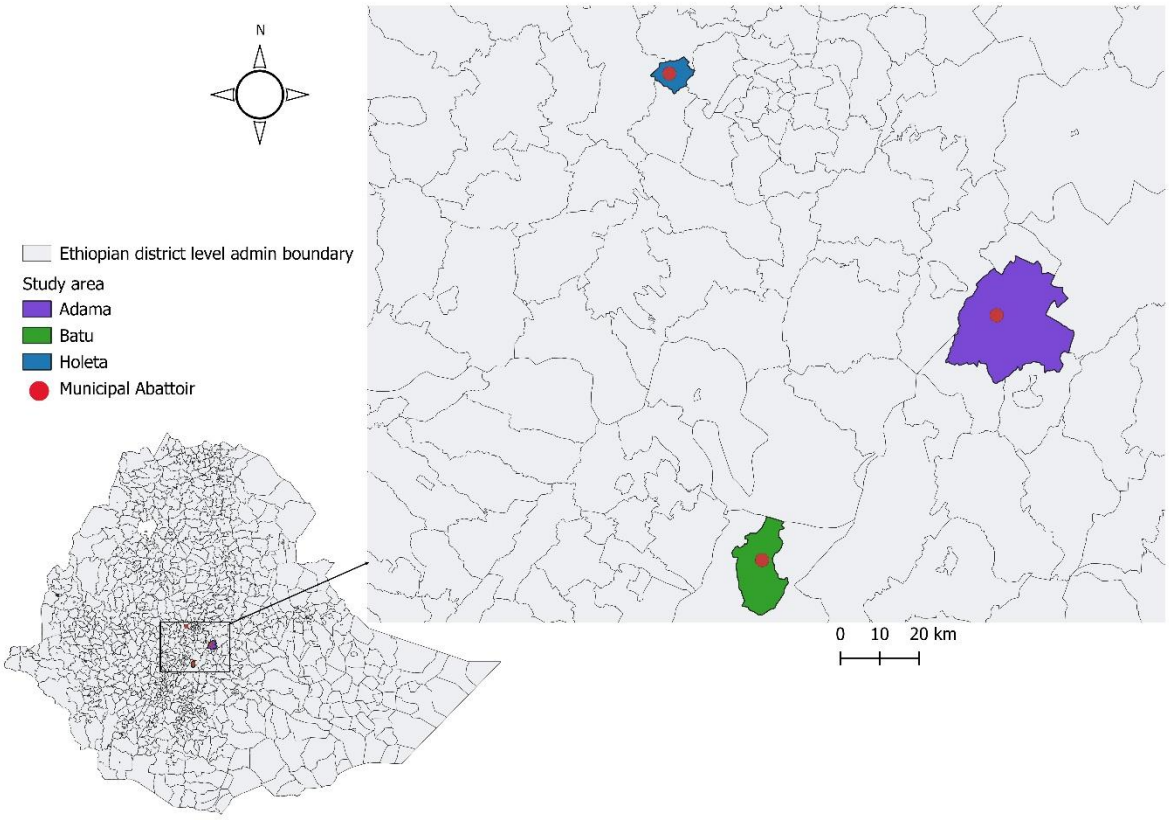


Figure 14**:** Map of Ethiopia showing the study area (QGIS Version 3.44.8)

4.2.2. Study design and sample size determination

A cross-sectional study was conducted from January 2024 to February 2025 at the three municipal abattoirs. Systematic random sampling was used to select apparently healthy and ready to be slaughtered cattle and subsequent sample collection. The sample size required was determined using the formula given below by Thrusfield, 2005 (Thrusfield, 2005) using an expected prevalence of 5% for *E. coli* O157:H7 (Zelalem *et al.*, 2019).

$$n = \frac{Z^2 * p(1-p)}{d^2}$$

Where: n=number of samples required; Z=normal distribution (95%); p=expected prevalence; d=absolute precision desired. For the sample size calculations, a 95% confidence interval and 5% precision were used. Accordingly, the sample size of apparently healthy to be slaughtered cattle and beef carcass swabs at each abattoir was determined. Based on the expected prevalence and other parameters used in the formula,

the sample size was estimated to be 72 cattle per abattoir. Accordingly,72 hide swabs and 72 fecal samples were collected from each municipal abattoir. This resulted in a total of 216 hide and 216 fecal samples collected from the three abattoirs from the same animals. Although the calculated minimum carcass sample size was 72 per abattoir, the number of carcass swab samples was increased to 180 from each abattoir to improve the likelihood of pathogen isolation, so that a total of 540 carcass swab samples were collected from the three municipal abattoirs. Among the 540 carcass swab samples collected, 216 were obtained from the same animals from which fecal and hide samples had also been collected.

The three abattoirs for this study were selected from the 30 surveyed municipal abattoirs in Central Ethiopia based on their accessibility (Endalu *et al.*, 2026). At each visit to an abattoir, one pooled sample of workers' hand swabs, one pooled sample of knife swabs, and one sample of water which was used for cleaning equipment, workers' hands, and carcasses were collected before carcass processing began. Subsequently, a total of 18 pooled samples deemed sufficient for each item were collected from the three abattoirs. Water samples were collected repeatedly within the duration of the sampling period to account for variations in water quality whereas different individuals were sampled for hand and knife swabs. On each sampling day, six feces and hide swab samples and fifteen carcass swab samples were collected from each abattoir. The number of carcass swab samples collected was increased purposefully to evaluate potential cross-contamination occurring between carcasses during processing on the same sampling day. A total of 36 visits (12 visits per abattoir) were performed to collect the samples. Sampling was conducted during repeated visits between January 2024 and February 2025.

4.2.3. Sample collection

The procedure of slaughtering started by stunning the animals through a precise incision at the atlanto-occipital region by a sharp knife. Then, there was immediate bleeding and removal of the head, feet, and the carcass in a horizontal position on the floor (Gutema, Agga*, et al.*, 2021). Approximately 25 g of fecal material was collected using sterile

rectal gloves. Hide swab samples were collected by using 2.5 × 2.5 mm sterile cotton tipped swabs in polypropylene tubes (ThermoFisher Scientific, CA, USA) soaked in approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, UK). Hides were swabbed from the neck of the animals over the line of bleeding before slaughtering near the bleeding area over an area of approximately 10 × 10 cm. The hide of the ventral midline part of the animal was also swabbed similarly at the midline to determine its contact to the carcass during de-hiding. Similarly, the external parts of the carcasses were swabbed in the rump, midline, and brisket areas. Sterile cotton swabs in polypropylene tubes were soaked in approximately 10 ml of buffered peptone water and rubbed against the carcass, first horizontally, then vertically. For the internal part of the carcass, where contact with other carcasses is not possible, the thoracic and the pelvic parts of both sides were sampled through the evisceration opening. For every sampling, a sterile latex glove was used to avoid cross contamination and every procedure was done as aseptic as possible. Swab samples collected from different anatomical sites of each carcass were pooled together and analyzed. All samples collected were transported in an ice box to the microbiology laboratory of the College of Veterinary medicine and Agriculture, Addis Ababa University, in accordance with the guidelines of the international standards organization (ISO17604:2015, 2015; ISO/TS17728:2015, 2015; ISO18593:2018, 2018).

4.2.4. Isolation of E. coli O157:H7

Fecal samples of 25 g were weighed for accuracy and placed in a sterile stomacher bag. A modified tryptone soy broth containing 20 mg/l novobiocin (Oxoid Ltd., Basingstoke, Hampshire, UK) (mTSB+n) was added at a ratio of 1:9 followed by agitation at low speed for 30 s. In all other swab samples, 90 ml of mTSB+n were added to a sterile 100 ml test tube and homogenized using a vortex mixer. All enriched samples with mTSB+n were incubated at 41.5 °C for 24 h and processed by an immuno magnetic separation (IMS) kit using anti-*E. coli* O157 dynabeads (Dynabeads® MAX *E. coli* O157 Kit, ThermoFisher Scientific, CA, USA) according to manufacturer's instruction. Roughly 50 µl of the IMS bead and bacteria complex were streaked onto Sorbitol MacConkey agar (Oxoid Ltd., Basingstoke, Hampshire, UK) containing 5 mg/100 ml cefixime and 0.25

g/100 ml potassium tellurite supplement (SMAC Media CT supplement, Applied Biosystems by ThermoFisher Scientific, UK) (CT-SMAC) and incubated at 37 °C for 20–24 h. The CT-SMAC agar plates were examined for the presence of non-sorbitol fermenting colonies and such colonies were sub-cultured on nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, UK). Bacterial cultures from the nutrient agar were transferred to tryptophan medium (Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 37 °C for 20–24 h. After incubation, indole production was tested by adding two drops of Kovac's reagent (HiMedia Laboratories Pvt. Limited, India) to the cultures. Indole-positive isolates were inoculated onto nutrient agar plates (Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 37 °C for 24 h for further strain identification by latex agglutination using a specific latex kit (*Escherichia coli* O157 Latex Test, Oxoid Ltd., Basingstoke, Hampshire, UK). The tests were performed according to the manufacturer's instructions. A drop of test latex and 0.085% sterile saline water were separately dispensed into the reaction card. A few presumptive colonies (on average 2 colonies) of *E. coli* O157:H7 were taken and emulsified into the saline water on the latex card, then slowly mixed with the test latex and checked for agglutination within one minute (ISO16654:2001, 2001).

4.2.5. Molecular characterization of E. coli O157:H7

Isolates were pre-screened for the presence of *E. coli* via the *uidA* gene, for the Stx-encoding genes *stx1* and *stx2*, the *eae* gene, and for an O157 GenoSeroType by conventional end-point PCR. Two to five colonies of each isolate were resuspended in 200 µL PBS, heat-denatured (95 °C; 10 min), briefly centrifuged and stored at -20 °C. The target sequences of the heat-denatured samples were amplified in three different PCRs with primers described previously (Table 8) at a concentration of 10 nmol/mL each, either in a single gene (*eae*, O157) or a multiplex PCR (*stx1*, *stx2*, *uidA*) using OneTaq 2X Master Mix with Standard Buffer (New England Biolabs; Frankfurt/Main, Germany) and 2 µL of the heat-denatured crude lysate. Thermocycling conditions were as follows: 94 °C for 30 s, 30 cycles of 94 °C for 30 s, 55 °C (*eae*) or 58 °C (O157) or 63 °C (*stx1*, *stx2*, *uidA*) for 40 s, and 68 °C for 40 s, and a final extension at 68 °C for 5 min. The

PCR-amplified fragments (in a volume of 10 µL) were separated by electrophoresis for 55 min at 100 V in a 1.5% agarose gel stained with Gel red (Merck, Darmstadt, Germany) and visualized under UV light.

Table 8**:** Primers used for target gene amplification

Primer name	**Target gene**	**Primer sequence [5' → 3']**	**Fragment length [bp]**	**Reference**
uidA-F	*uidA*	TGGTGATTACCGACGAAAACGGC	162	(Shimpoh *et al.*, 2017)
uidA-R		ACGCGTGGTTACAGTCTTGCG		
MP4-stx1A-F	*stx1*	CGATGTTACGGTTTGTTACTGTGACAGC	244	(Müller *et al.*, 2007)
MP4-stx1A-R		AATGCCACGCTTCCCAGAATTG		
MP3-stx2A-F	*stx2*	GTTTTGACCATCTTCGTCTGATTATTGAG	324	
MP3-stx2A-R		AGCGTAAGGCTTCTGCTGTGAC		
EAE-F	*eae*	ATTACTGAGATTAAGGCTGAT	682	(Blanco *et al.*, 2004)
EAE-R		ATTTATTTGCAGCCCCCCAT		
5'O157	*wzx*	GCTGCTTATGCAGATGCTC	133	(Monday *et al.*, 2007)
3'O157		CGACTTCACTACCGAACACTA		

4.2.6. DNA isolation and whole genome sequencing

PCR-positive isolates were grown overnight at 37 °C in 5 ml Luria-Bertani broth (Carl Roth GmbH, Karlsruhe, Germany). Genomic DNA was purified with the DNeasy® UltraClean® Microbial Kit (Qiagen GmbH, Hilden, Germany). DNA concentrations were determined with a NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer (Thermofisher, Dreieich, Germany). DNA sequencing was performed by Novogene (Illumina NovaSeq 6000 or NovaSeq X Plus, 2 x 150 bp; Martinsried, Germany). General data and assembly statistics of the isolates sequenced are shown in Table 9

Table 9: Assembly statistics of the *E. coli* O157:H7 isolates

Isolate ID	N50 [bp]	Total number of contigs (number of contigs ≥50 kb)	Largest contig [bp]	Total [bp]	Sequencing depth estimate [x-fold][a]
25E0148	244,781	174 (25)	615,028	5,373,064	294
25E0149	244,781	170 (26)	617,809	5,372,523	313
25E0150	244,781	168 (25)	617,809	5,375,181	334
25E0151	209,225	182 (26)	481,153	5,350,667	264
25E0152	209,803	181 (26)	481,244	5,352,925	267
25E0153	209,225	187 (26)	520,301	5,349,346	248
25E0163	157,822	188 (26)	503,810	5,350,116	231
25E0164	157,823	191 (25)	503,743	5,351,865	207
25E0165	157,823	191 (25)	503,810	5,351,192	210

[a] based on the total number of sequenced bases divided by the length of the *E. coli* K-12 MG1655 (GenBank accession number: U00096.3) reference genome in bp (Blattner *et al.*, 1997).

4.2.7. Identification and processing of strains from Enterobase

The online resource Enterobase (v1.2.0) contains 430,090 *Escherichia*/*Shigella* entries (accessed on 12.02.2026) (Enterobase, 2026). Despite this large number, it is still a convenience dataset with a non-representative and geographically skewed collection of whole-genome sequences. Fifty-five entries from Africa with an O157 GenoSeroType were identified using the search parameters "Serotype = O157" and "Country = Name of an individual African State". Their annotated assembled contigs were downloaded from the EnteroBase website, uploaded in Geneious Prime (Dotmatics, Boston, USA; v2025.1.3) and exported in .fasta format for further bioinformatic analysis (Zhou *et al.*, 2020). All strain assemblies were checked for an O157:H7 GenoSeroType with SerotypeFinder (v2.0; Center for Genomic Epidemiology website) as well as the presence/absence of the *eae* and *stx* genes, including the latter's subtype.

4.2.8. Genetic relatedness, linkage, antimicrobial resistance genes and virulence factors of E. coli O157:H7

Bioinformatic analysis of the *E. coli* O157:H7 isolates was conducted using the SnakeColi pipeline (https://gitlab.com/micweber/snake_coli). All programs and tools used were run with their default settings. Raw Illumina sequencing data were quality-checked with FASTQC (v0.11.9). Contamination of the samples was assessed using Kraken2 (v2.1.3) (Wood *et al.*, 2019) and MetaPhlan (v4.2.2) (Blanco-Míguez *et al.*, 2023). Samples with more than 10% contamination with other species and/or fewer than 500,000 reads assigned to *E. coli* were excluded. De novo assemblies were generated with Shovill (https://github.com/tseemann/shovill) in the version 1.1.0 with default settings using SPADes (Bankevich *et al.*, 2012) (v4.1.0) as the assembler. Assembly quality was evaluated with QUAST (v5.3.0) (Mikheenko *et al.*, 2018). Contig sequences were annotated with Bakta (v1.9.4) (Schwengers *et al.*, 2021).

Multilocus sequence types (MLST) were assigned using the mlst tool applying the *E. coli* Achtman scheme from PubMLST (Jolley *et al.*, 2018). Virulence factor genes were

detected using ABRicate (v1.0.1; 80% for minimum DNA identity and 80% for minimum sequence coverage) with the VFDB (v2025-01-14) or the VirulenceFinder (v2022-12-02) databases (Scheutz *et al.*, 2024; Zhou *et al.*, 2025). The .fastq files of the isolates 25E0148, 25E0149 and 25E0150, each with a truncated and non-typable *stx2* gene, were imported in Geneious v2025.1.3, mapped to the *stx2c* gene (from 25E0151 from this manuscript) using the Geneious mapper with medium sensitivity and no fine-tuning. Screening for intestinal pathotype markers and genoserotypes was performed with ECTyper (v.2.0.0; ≥95% identity and ≥90% coverage for O-antigen alleles, and ≥90% identity and ≥50% coverage for H-antigen alleles) (Bessonov *et al.*, 2021). Antimicrobial resistance genes and resistance-associated chromosomal mutations were detected with AMRFinderPlus (v4.0.23) (Feldgarden *et al.*, 2021) or ResFinder (v4.7.2). Phylogroups of all strains were identified using EZClermont (v0.7) (Waters *et al.*, 2020). The core genome multilocus sequence type (cgMLST) of an isolate served to indicate genetic relatedness of the isolates and was determined using the Enterobase scheme as implemented in MBioSEQ Ridom Typer, v11 (Bruker, Karlsruhe, Germany). Alleles from the cgMLST, that were not present in a pairwise comparison of two isolates were not counted in the analysis of these two isolates. The number of alleles not successfully called varied for the individual isolates between 24-30 alleles and accumulated to 59 alleles for all isolates together. The threshold for a cluster was set to 10 allelic differences (AD) (Zhou *et al.*, 2020).

4.2.9. Antimicrobial susceptibility testing of E. coli O157:H7

Antimicrobial resistance testing via disk diffusion was conducted for the *E. coli* O157:H7 isolates following standard protocols described by the CLSI 2024. The disk diffusion method was employed for antimicrobial susceptibility testing using eleven antibiotic disks (Oxoid Ltd., Basingstoke, Hampshire, UK), namely: Amikacin (30 µg), Meropenem (10 µg), Trimethoprim/Sulfamethoxazole (25 µg), Streptomycin (10 µg), Ceftriaxone (30 µg), Gentamicin (10 µg), Ampicillin (10 µg), Ciprofloxacin (5 µg), Ceftazidime (30 µg), Cefoxitin (30 µg), Tetracycline (30 µg). The *E. coli* O157:H7 isolates were inoculated onto nutrient agar plates (Oxoid Ltd., Basingstoke, Hampshire,

UK) and incubated at 37 °C for 24 h. Colonies were suspended in sterile 0.85% saline and adjusted to a turbidity equivalent to 0.5 McFarland using a densitometer (DEN-1 McFarland Densitometer, Grant Instruments Ltd, England). A sterile cotton swab was dipped into the adjusted bacterial suspension and spread evenly via repeated rubbing on a Mueller-Hinton agar plate (Oxoid Ltd., Basingstoke, Hampshire, UK). The selected antibiotic-impregnated disks were placed at a minimum distance of 24 mm on the surface of the inoculated plate using a disk dispenser (Oxoid Ltd., Basingstoke, Hampshire, UK) and incubated at 37 °C for 24 h. After incubation, the diameters of inhibition zones were measured using a digital caliper.

The *E. coli* O157:H7 strains were additionally analyzed in Germany for their antibiotic susceptibility via broth microdilution using the "Grosstierplatte" microtiter plate (E1-150-100 for large livestock species such as cattle) containing 14 antimicrobials (Ampicillin, Ceftiofur, Cephalothin, Colistin, Enrofloxacin, Erythromycin, Florfenicol, Gentamicin, Penicillin G, Spectinomycin, Tetracycline, Tiamulin, Tilmicosin, and Tulathromycin), one combination of two antimicrobials (Trimethoprim/Sulfamethoxazole), and one antimicrobial/inhibitor combination (Amoxicillin/Clavulanic acid), based on the MICRONAUT AST system (MERLIN Diagnostika, Bornheim-Hersel, Germany) following CLSI 2018 protocols. The isolates were streaked out onto blood agar (sifin diagnostics, Berlin, Germany), single colonies picked to prepare suspensions corresponding to 0.5 McFarland units with a 0.9% NaCl solution (Carl Roth, Karlsruhe, Germany) and transferred to cation-adjusted Mueller-Hinton-Bouillon (Sigma-Aldrich Chemie B.V., The Netherlands). The microtiter plates were then inoculated with this bacterial suspension following the manufacturer's instructions (MERLIN Diagnostika, Bornheim-Hersel, Germany) and incubated for 18-20h at 37 °C. After incubation, automated turbidity reading was carried out using a microplate reader (Tecan, Crailsheim, Germany). The *E. coli* reference strain ATCC 25922 served as quality control strain (Richter *et al.*, 2025). To obtain minimum inhibitory concentration (MIC) values for all strain/drug combinations, these rules were implemented (i) if the lowest test concentration of the antibiotic tested already inhibited a strain or (ii) if a strain still showed growth at the highest antibiotic concentration in the

panel: for (i) the lowest antibiotic concentration served as MIC value and for (ii) an antibiotic concentration twice higher than the highest concentration in the panel was set as MIC value (Ruddat *et al.*, 2012; Richter *et al.*, 2025). The EUCAST (https://mic.eucast.org/search/) ECOFF values for *E. coli* were used to differentiate the isolates into non-wild type strains and wild type strains based on their respective MIC value. The tentative ECOFF was applied for the antibiotic combination amoxicillin/clavulanic acid. For tulathromycin, there is no ECOFF for *E. coli* or other Enterobacterales. The ECOFF values for *Mannheimia haemolytica* and *Pasteurella multocida* for tulathromycin and the (T)ECOFF value of azithromycin for *E. coli* served as a rough orientation.

4.2.10. Data Management and Analyses

The collected data were entered into Microsoft Excel and checked before analysis. The data were analyzed using R version 4.1.2. Descriptive statistics were used to summarize the results as frequency distributions and prevalence with 95% confidence interval. MBioSEQ Ridom Typer, v11, was used to generate or analyze the genetic relatedness and linkage of *E. coli* O157:H7 sequence data. For antimicrobial susceptibility analysis, the inhibition zone results around individual antibiotic disks were interpreted and the isolates classified as Sensitive, Intermediate and Resistant.

4.3. Results

4.3.1. Occurrence of Escherichia coli O157:H7

All swab samples taken from the worker's hands, their knives and the water used for cleaning tested negative for *E. coli* O157:H7. The strains were only isolated from animal materials (feces and carcasses), but with low prevalence of 1.4% (95%CI: 0.3 – 4.0) for the fecal material and 1.1% (95%CI: 0.4 – 2.4%) for the carcass swabs (Table 10).

Table 10**:** Occurrence of E. coli O157:H7 from different samples

Sample type	Sample size	Positive	
		No.	% (95% CL)
Hand swab	18	0	0
Knife swab	18	0	0
Water	18	0	0
Feces	216	3	1.4 (0.3 – 4.0)
Hide swab	216	0	0
Carcass swab	540	6	1.1 (0.4 – 2.4)

4.3.2. Genetic relatedness and linkage of E. coli O157:H7 from the study

As shown in figure 15, the isolates from the animals at the same abattoir were clonal, showing no allelic differences in their cgMLST profiles. However, isolates from different abattoirs exhibited larger allelic differences (AD) from 140-226 AD indicating low to no genetic relatedness.

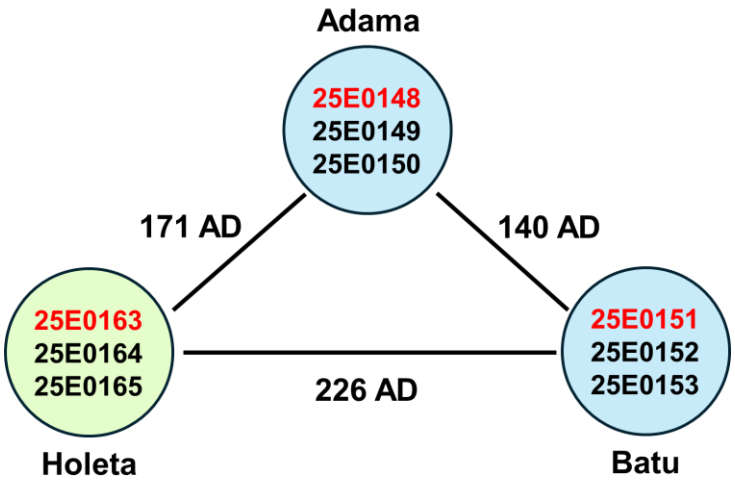


Figure 15: Minimum spanning tree of E. coli O157:H7 isolates from municipal abattoirs in Central Ethiopia. The isolates are color-coded according to their Warwick MLST (ST11: blue; ST1183: green). Circles enclose isolate clusters with no allelic differences (AD) in the Enterobase cgMLST scheme. They are from the same abattoir, as indicated above or below the cluster, representing samples from fecal material (red) and animal

carcasses (black). The numbers of allelic differences (AD) between the isolate clusters are indicated.

4.3.3. Genetic relatedness and linkage of the isolates with E. coli O157:H7 in comparison with selected African isolates currently available in Enterobase

A search of the EnteroBase collection of *E. coli* strains yielded 48 entries featuring isolates from Ethiopia, one from Morocco and six from South Africa with an O157:H7 GenoSeroType and whole-genome-sequence data. Four isolates from Ethiopia and two from South Africa were eliminated from further analysis due to low coverage (< 100-fold). The remaining isolates were analyzed for their genetic relatedness by determining their cgMLSTs. All but one of the aEPEC isolates, lacking any *stx* genes, were located in cluster #1, interspersed with three STEC isolates. The study isolates were located in the clusters #7-9 of the minimum spanning tree (MST). The MST clusters #7 and #8 are closely related to the MST clusters #3 and #2, containing strains from Enterobase, being separated by only 22 or 13 allelic differences, respectively. The MST Cluster #9, representing isolates with ST1183, obtained in the Holeta abattoir, is not closely related to any of the other MST clusters or any individual isolates. All Ethiopian isolates, except for MB5R, are present in clusters, indicating group-specific close relatedness.

The isolates from this study formed three clusters (#7-9; see also Figure 15) and the other isolates from Ethiopia formed six clusters (#1-6).The remaining strains from Morocco and South Africa were not part of any cluster and are of intermediate (< 50 AD) to distant (> 100 AD) relatedness (Figure 16) to their closest neighbor in the MST.

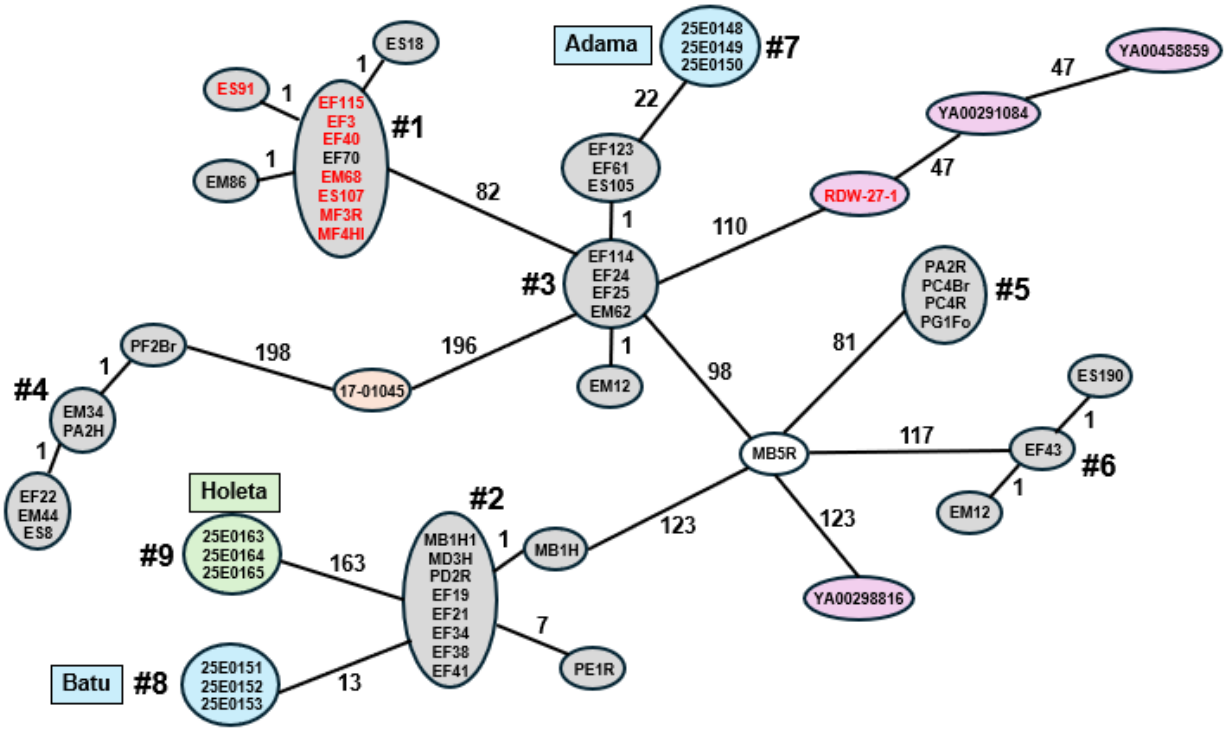


Figure 16**:** Minimum spanning tree of E. coli O157:H7 STEC (in black) and aEPEC (in red) isolates from Africa [Ethiopia (n = 53, grey, green, blue), Morocco (n = 1; orange), South Africa (n = 4; lilac)]. Distances are not to scale, but have been adjusted for better visibility. Numbers indicate allelic differences between isolates or isolate clusters (grey or colored and numbered from #1 to #9). Clusters contain all isolates with less than ten allelic differences. The study isolates are colored as in Figure 15 with their isolation site added and are located in the clusters #7, #8, and #9.

4.3.4.Antimicrobial resistance phenotypic and genotypic profiles of E. coli O157:H7 in comparison with selected African isolates.

All isolates obtained were susceptible to all antimicrobials tested by disk diffusion method. The MIC values of all but two isolates from this study demonstrated a wild type phenotype to nearly all antibiotics in the broth microdilution assay. There is no ECOFF value for tulathromycin for *E. coli*. ECOFFs for the Gram-negative species *Mannheimia haemolytica* (16 µg/ml) and *Pasteurella multocida* (8 µg/ml) and a (T)ECOFF for

azithromycin and *E. coli* (16 µg/ml) may provide contextual support for an estimated wild-type-like MIC distribution. Only spectinomycin displayed an MIC value corresponding to a non-wild type ECOFF phenotype in the isolates 25E0149 and 25E0150. Since no clinical breakpoints exist for spectinomycin for *Enterobacterales* in humans and animals, no assumption on a susceptible, intermediate or resistant phenotype can be made. No acquired antimicrobial resistance genes or chromosomal mutations were detected in any of the isolates.

Analysis of the whole genome assemblies of *E. coli* O157:H7 isolates sampled in Africa and currently available in the EnteroBase dataset revealed that none of the Ethiopian STEC or aEPEC strains contained any acquired AMR genes. In contrast, the Moroccan and two of the South African isolates were multi-resistant (Table 11), indicating resistance to three or more of the following antibiotics: Streptomycin, beta-Lactams (Penicillins and/or Cephalosporins), Macrolides, Florfenicol, Tetracyclines, Sulfonamides, and Trimethoprim.

Table 11**:** Antimicrobial resistance genes identified in WGS assemblies of African *E. coli* O157:H7 isolates uploaded to EnteroBase.

Strain ID	**EnteroBase ID**	**Isolated in**	**AMR genes**
17-01045	ESC_NA8248AA	Morocco	*aph*(3")-Ib/*aph*(6)-Id, *bla*$_{\text{TEM-1B}}$, s*ul2*
YA00298816	ESC_YA9014AA	South Africa	*aph*(3")-Ib/*aph*(6)-Id, *bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{TEM-1B}}$, *floR*, *tet*(A), *sul2*/*dfrA*14
YA00458859	ESC_FB8383AA	South Africa	*aph*(3")-Ib/*aph*(6)-Id, *bla*$_{\text{CMY-2}}$, *floR*, *mef*(C)/*mph*(G), *tet*(A), *sul2*

4.3.5. Virulence factors identified in the E. coli O157:H7 isolates

All strains isolated here carried the same repertoire of virulence genes encoding adherence factors (*eae*, *iha*, and *tir*), type III secretion system translocated proteins (*espA*, *espB*, *espJ*, *espP*, *etpD*, *nleA*, *nleB*, and *nleC*), outer membrane proteins (*chuA*, *ompT*, and *traT*), toxins (*astA*, *ehxA*, and *toxB*), and other pathogenicity-related factors (*gad*, *iss*, *katP*, and *terC*). Only the strain 25E0165, isolated from the Holeta abattoir, carried additionally a *tccP* gene. None of the strains carried an *stx*1 gene. All strains carried the *stx*2 gene subtype *stx*2c. The enterohemolysin gene *ehxA* was identified by sequence analysis as belonging to subtype B (Cookson *et al.*, 2007).

4.4. Discussion

Slaughtering animals for human consumption demands a hygienic procedure in which protocols leading to acceptable hygienic levels are implemented meticulously. However, several steps, such as evisceration and de-hiding, can lead to carcass contamination by *E. coli* O157:H7 during the slaughtering process (Gutema *et al.*, 2021). The prevalence of *E. coli* O157:H7 (1.89%) in fecal matter reported by Abdissa *et al.,* 2017. agrees well with the current study (1.4%). The non-occurrence of *E. coli* O157:H7 on workers' hands, knives, and hide swabs as well as in water samples was similar to previous reports showing no (0%) detection of O157:H7 on hands (Chala *et al.*, 2025), knives (Atnafie *et al.*, 2017) and water (Abunna *et al.*, 2023). The overall occurrence of *E. coli* O157:H7 on carcasses was 1.1%, which is comparable to findings reported in earlier investigations conducted at Addis Ababa and Debre Berhan with 0.54% and 0.8% prevalence on carcass swabs at abattoirs and retail shops, respectively (Abdissa *et al.,* 2017). In Ethiopia, the practice of unhygienic handling procedures in municipal abattoirs leads to carcass contamination with pathogenic microorganisms (Bersisa *et al.*, 2019). This is a significant public health issue in the country where the consumption of uncooked beef is a common tradition (Seleshe *et al.*, 2014).

The cgMLST analysis of the nine *E. coli* O157:H7 isolates obtained in this study revealed a clonal relatedness among isolates obtained from feces and carcasses processed within the same municipal abattoir. This suggests that cross-contamination occurred during slaughter and carcass handling. In line with the recent findings, fecal material released during the evisceration stage has been identified as one of the major sources of carcass contamination by *E. coli* O157:H7 in the abattoir environment (Atnafie *et al.*, 2017; Gutema *et al.*, 2023). The evisceration process involves the removal of the entire gastrointestinal tract including the intestines whereby any accidental rupture of the intestines may result in the release of fecal content containing pathogenic or zoonotic microorganisms contaminating the surface of the carcass (Alvseike *et al.*, 2020).

Such contamination may be worsened by inadequate hygienic practices prevailing during carcass processing, including improper evisceration techniques, insufficient hand washing by slaughterhouse personnel, and lack of effective knife sterilization between carcasses (Sebsibe and Asfaw, 2020). The most widely mentioned procedure for knife sterilization is hot-water decontamination at 82 °C in a water bath (CAC, 2005; CAC, 2022). Adequate cleaning or disinfection is essential to prevent cross-contamination between carcasses and, thus, reduce pathogen transfer (Raschle *et al.*, 2019). Repeated use of contaminated knives and equipment without proper sanitation would facilitate the transfer of pathogens from colonized intestinal content to previously uncontaminated carcasses (Geresu and Desta, 2021). Moreover, the absence of routine hand hygiene practices among abattoir workers can further contribute to the dissemination of pathogenic microorganisms within the processing environment (Okoli *et al.*, 2025). Therefore, poor adherence to sanitary measures during slaughter operations significantly increases the risk of carcass contamination and may ultimately compromise meat safety and public health (Hossain *et al.*, 2025). In fact, at the abattoirs in the study area there is no practice of knife sterilization due to the absence of a hot water knife sterilizer, and no proper hand washing between each activity.

The cgMLST analysis of *E. coli* O157:H7 isolates from the Adama and Batu municipal abattoirs showed a close relationship with isolates reported from Bishoftu (Gutema *et al.*,

2023). A number of factors can facilitate the circulation and spread of closely related pathogenic organisms by increasing contact between sources of contamination and susceptible hosts, influencing disease transmission (Wesolowski *et al.*, 2014; Stein and Chirilã, 2017; Lessani *et al.*, 2024). The townships Adama, Batu and Bishoftu are all located in the East Shewa administrative zone. The animal movements within this zone can facilitate the spread of genetically closely related strains of the pathogens (Widgren, 2016; O'Shaughnessy *et al.*, 2025). Therefore, close relationship between isolates from Adama, Batu and Bishoftu municipal abattoirs might be due to the municipal abattoirs sharing the same beef supply market. In contrast, isolates from the Holeta municipal abattoir were not closely related to the Bishoftu isolates.

All study isolates were susceptible to nearly all antimicrobials tested in this study. Solely two isolates (25E0149, 25E0150) displayed a non-wild type phenotype towards Spectinomycin, without encoding a gene mediating resistance to Spectinomycin. Strain status as resistant/intermediate/susceptible cannot be assessed due to the lack of a defined clinical breakpoint for Spectinomycin (CLSI, 2018, 2020). Similarly, 100% susceptibility to Chloramphenicol, Ciprofloxacin (5 µg), Ceftazidime (30 µg), Tetracycline (30 µg), and Gentamicin (10 µg) has been reported previously (Chala *et al.*, 2025). Likewise, 100% susceptibility rate to Ampicillin (10 µg) and Trimethoprim/Sulfamethoxazole (25 µg) has also been reported (Abebe *et al.*, 2023). The antimicrobial susceptibility testing performed here showed similarity to a study with *E. coli* strains isolated from raw beef samples from Addis Ababa, which reported a susceptibility of 100% of the strains to Amikacin (30 µg) and Ceftriaxone (30 µg) (Haile *et al.*, 2022). Other studies have reported a 97.3% susceptibility rate to Meropenem (10 µg) (Abera *et al.*, 2025), complete susceptibility to Streptomycin (10 µg) (Ayenew *et al.*, 2021) and also to Cefoxitin (30µg) (Beyi *et al.*, 2017). In contrast to recent findings, previous studies conducted in Ethiopia have reported the occurrence of antimicrobial resistant *E. coli* O157:H7 in Ethiopia (Ayenew *et al.*, 2021; Abebe *et al.*, 2023) . All study isolates did not encode antimicrobial resistance genes to any of the antimicrobials tested, which is consistent with the report by Gutema *et al.* who also did not identify any antimicrobial resistance genes in their isolates (Gutema *et al.*, 2023).

The closer relatedness to isolates from the study by Gutema *et al.,* is also supported by the very similar virulence factor profiles of the isolates in the two studies. Compared to the isolates from Gutema *et al*. (Gutema *et al.*, 2023), the isolates in this study all featured an *stx2c* gene, but lacked genes encoding *espF* and *tccP* (except for the strain 25E0165 isolated from the Holeta abattoir encoding *tccP*), carrying *espP* instead. Similar virulence gene profiles have been reported in previous studies of *E. coli* O157:H7 isolates, where adhesion (*eae*, *tir*), T3SS-associated (*espA*, *espB*, *espJ*), non-LEE effector (*nleA*, *nleB*, *nleC*), stress-response (*gad*, *iss*, *katP*, *terC*), and plasmid-encoded toxin genes (*ehxA*, *toxB*, *etpD*) were consistently detected (Bumunang *et al.*, 2022; Naidoo and Zishiri, 2023). Similar findings have been reported in Ethiopia and other African countries, where *E. coli* O157:H7 isolates from animal and human sources were shown to harbor key adhesion (*eae*), toxin (*stx*2, *ehx*A), plasmid-encoded (*katP*, *etpD*), and non-LEE effector genes (*nleB*, *nleC*, *terC*), with several studies documenting the occurrence of the *stx2c* subtype among STEC strains associated with severe disease (Abreham *et al.*, 2019; Karama *et al.*, 2019; Ayenew *et al.*, 2021; Olawole *et al.*, 2025). All isolates from this study carried *stx2c* and lacked *stx1* is clinically relevant, as *stx2c* alone can be associated with an increased risk of severe disease, including hemolytic uremic syndrome (Eklund *et al.*, 2002). Additionally, the presence of *eae, tir, ehxA, toxB, esp*, and *nle* genes, along with plasmid-associated virulence determinants shows pathogenic potential of these strains. The *ehxA* subtype B is strongly associated with O157:H7 STEC, the presence of an *eae* gene and an *stx2c* subtype and also bloody diarrhea and HUS (Hua *et al.*, 2021). These genomic profiles indicate pathogenic potential and warrant public-health attention, although toxin expression and pathogenicity were not experimentally assessed.

4.5. Conclusion

The findings of this study indicated that carcass contamination with *E. coli* O157:H7 at the visited abattoirs is consistent with a common fecal source or cross-contamination during slaughter and carcass processing. All isolates carried *stx2c*, a marker associated with increased risk of severe disease including hemolytic uremic syndrome. The presence

of *eae*, *tir*, *ehxA*, *toxB*, *esp*, *nle*, and plasmid-associated virulence genes supports their pathogenic potential and public health relevance. They were susceptible to the antimicrobials tested and no resistance genes were detected. Therefore, strict hygienic practice should be followed during carcass processing to prevent public health risks.

Limitations

Comparative genomic data on human clinical STEC O157:H7 from Ethiopia or neighboring regions are too limited to make a strong public health interpretation. Since this study targeted the isolation and characterization of O157 using IMS and CT-SMAC, we could not detect non-O157 STEC (including serogroups O26, O45, O103, O111, O121, and O145) or LEE-negative strains, which is a limitation of the study.

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Availability of data and materials

The DNA sequence datasets generated during the current study are available in the National Center for Biotechnology Information https://www.ncbi.nlm.nih.gov/ with the SRA accession numbers: SRR37129132, SRR37129129, SRR37129126, SRR37129134, SRR37129131, SRR37129128, SRR37129133, SRR37129130, SRR37129127.

Declarations

Ethical Approval

All animal related procedures were performed in accordance with institutional and national/international guidelines for animal welfare and ethical standards relevant to abattoir based studies. For the hand swab sampling, the study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Informed consent was obtained prior to participation. Ethical approval for the study was obtained from the Institutional Review Board (research ethics committee) of the College of Veterinary Medicine and Agriculture Addis Ababa University (Certificate Ref. No: VM/ERC/03/11/16/2024).

Consent to participate

Before conducting the study, the purpose of the study was clearly explained to the individual participants and their informed consent was obtained. Furthermore, before the sampling process, the animal owners were informed about the aim and purpose of the research, and their consent to take the samples was obtained.

Consent to publish

Not applicable

Competing interests

The authors declare no competing interests.

Author contributions

Endalu Mulatu: Writing review and editing, Writing original manuscript, Validation, Research design, Data collection and Investigation, Data curation and analysis and Conceptualization. Nebyou Moje Hawas, Kebede Amenu and Biruhtesfa Asrade: Review editing and research design. Christian Berens: Methodology, Review writing and editing, Bioinformatic data analysis, Whole Genome Sequencing. Christian Menge: Review editing. Bekele Megresa, Ahmad Hamedy and Hika Waktole: Writing review and editing, Research design, Supervision, Data analysis, Conceptualization, Validation.

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5. CHAPTER V: GENERAL DISCUSSION

Meat safety and quality are an essential aspect in the protection of public health, especially in developing countries where municipal abattoirs represent major sources of meat supply for urban populations. Meat safety is critical to public health where contamination with pathogens such as *Salmonella*, *E. coli*, and *Staphylococcus aureus* likely occur under inadequate infrastructure (deficient drainage systems, inadequate potable water supply, and insufficient lighting), weak inspection systems, insufficient handler training, and no routine microbial monitoring (Buhari *et al.,* 2020; Adesola *et al.,* 2024; Adesiyan *et al.,* 2025). These conditions increase carcass contamination during slaughter and processing, exposing consumers to foodborne infections, antimicrobial resistance, and economic burdens. Therefore, improving meat safety at municipal abattoirs at developing countries such as Ethiopia represents a major public health priority affecting millions who depend on these facilities for red meat supply. In the present study, the municipal abattoirs in central Ethiopia were comprehensively evaluated in terms of three important aspects: (1) status of the infrastructure of abattoirs and operational facilities, (2) hygiene and food safety practices among abattoir workers, and (3) microbiological quality of carcasses along with molecular characterization and antimicrobial susceptibility of *E. coli* O157:H7.

One of the major findings in this research is that there is an wide spread inadequacy in terms of infrastructural and operational facilities across the municipal abattoirs investigated. This study is in agreement with report with Ovuru *et al., 2023* and Nigussie *et al.,* 2025 who reports insufficiency of abattoir operational facilities in developing countries. Presence of appropriate infrastructure and operational facilities of abattoirs is fundamental prerequisite for maintaining hygienic slaughter practices in slaughtering activities (Hossain *et al*., 2025). However, in the present investigation, it was observed that 83.3% of the municipal abattoirs were situated in close proximity to residential areas. According to international recommendations and guidelines in the establishment of slaughterhouses, it is important that abattoirs should not be established in close proximity to residential areas, schools, and other public places in order to prevent environmental

pollution (FAO, 1985). Establishing abattoirs in close proximity to residential areas is likely to cause environmental contamination by improperly disposing of slaughter wastes, contributing to environmental pollution and nuisance conditions, and even exposing the public to zoonotic diseases. In addition, 70% of the abattoirs were found to be lacking sufficient land for expansion. It is important to note that sufficient allocation of land is an important prerequisite in planning the layout of abattoirs, expansion of operational activities, and the installation of improved waste management practices (Buncic, 2006). Similar findings have been reported indicating close proximity of slaughterhouses to residential areas (Ibimode *et al.*, 2024; Olawole *et al.*, 2025). Structural defects in the abattoir buildings were also observed, especially in the design of the walls and floors. Cracks in the floor surfaces and the porous nature of the surfaces make the abattoir structures difficult to clean, disinfect and favor microbial growth (Dallagi *et al.*, 2023). Previous research reported that poorly maintained and designed slaughterhouse structures where meat and meat byproducts coming from such conditions microbial quality was deteriorated through by bacterial contamination (Alam *et al.*, 2022).

Another major infrastructural deficiency identified in this study was the lack of essential operational facilities that are required for hygienic practices in the slaughterhouses. The absence of hot water supply systems, steam sterilizers and chilling rooms was evident in more than 50% of the municipal abattoirs that were assessed in the study. Availability of continuous hot water supply systems is essential for the sterilization of knives and equipment between the processes of handling the animal carcasses (Yang *et al.*, 2024). The international guidelines on food safety emphasize the need for the sterilization of knives using hot water at about 82°C for the purpose of eliminating the possibility of the spread of disease-causing organisms during the handling of animal carcasses (EUC, 2004). Similarly, chilling rooms were not found to be available in over 50 % of the abattoirs under survey. The rapid chilling of carcasses immediately after slaughter is widely accepted as a critical control point in the processing of meat in which chilling of carcasses slows microbial growth, extends shelf life, and preserves meat quality, and maintains the quality of the final product (Nethra *et al.*, 2023).Temperature of carcasses is one of the main factors in bacterial growth, and inadequate refrigeration can allow

pathogens to grow and multiply (Hazards, 2014). Absence of chilling room and hot water availability was reported previously (Gutema *et al*., 2021; Yimana and Hassen, 2024).

This study found that hand washing stations; showers, toilets, and availability of cleaning agents were inadequate in more than 60% of abattoirs. Personal hygiene is an important aspect of food safety. Workers in meat processing operations need to have adequate facilities that enable them to practice personal hygiene during slaughter operations (Zanin *et al.*, 2017). Previous studies have shown that hands of meat handlers can play an important role in the transmission of pathogens to meat products and to ensure safety of meat adequate hand washing stations with potable water, soap, and disinfectants are available (Asati *et al.*, 2024).

Pest control measures were also inadequate in 70% of the abattoirs studied. The presence of pests, stray dogs, and wildlife within the slaughterhouse compounds shows a low level of biosecurity and inadequate fencing of the abattoir compounds. The presence of pests and stray animals within slaughterhouse premises is inconsistent with recommended hygienic standards (FAO, 1991). The observed waste management practices within the studied abattoirs were also found to be insufficient. If these wastes are not well managed and disposed of after treatment, they can cause serious environmental and health problems (Franke-Whittle and Insam, 2013). Aerobic plate counts 6.12 log10 CFU/cm^2 and FCC 4.58 log10 CFU/cm^2 on beef carcasses exceeded internationally accepted microbial count limits. Similarly, previous studies have reported elevated microbial loads on carcasses (Kemal and Tabit, 2025). Hygienic carcass processing reduce microbial contamination (Nakamura *et al.*, 2023). High microbial contamination was also found on meat contact surfaces, including knives and workers' hands in studied abattoirs. These findings indicate deficiencies in cleaning and sanitation procedures during slaughter operations such as knives and workers' hands are used during the handling of carcasses, thus playing a crucial role in the transfer of microorganisms from one carcass to another (Durmuşoğlu *et al.*, 2020). The high level of microbes on the knives and workers' hands shows that adequate sanitation procedures are not being used in the abattoirs under study.

The recent finding agrees with report from Bishoftu municipal abattoir where knife and worker's hand microbial quality was high (Bersisa *et al.,* 2019).

Water quality represents a critical determinant of microbial contamination during meat processing (Alegbeleye and Mateo-Sagasta, 2026). Water used in washing carcasses and equipment must be of potable quality so that it does not become a source of contamination in the process (Rahman, 2020).However, in the present study, it was found that 60% of the water samples used in abattoirs were contaminated with fecal coliform. Previous reports showed that water used for carcass wash at slaughterhouse are positive for *E. coli* and fecal coliform (Kimindu *et al.*, 2025). Sources of water be continuously investigated to eliminate or reduce the risk of contamination of meat processed in the abattoirs (Ncoko *et al.*, 2020). The behavioral practice of food handlers determines food safety outcomes (Fayemi *et al.*, 2026). From the results of the questionnaire survey, it was found that the compliance rate for a few food safety practices was very low in the abattoir workers' practices. Though the majority (over 60%) of the abattoir workers claimed to have removed their jewelry before handling the meat, other important practices such as washing their hands regularly with soap and using gloves were not well implemented in the abattoir. The limited use of protective equipment such as gloves, aprons, and head covers increases the risk of contamination during carcass processing. This study agrees with previous report where hand washing with soap and use of glove was not practiced (Zewude *et al.,* 2025).

Another key observation from the study was that 55.63% of abattoir workers were not subjected to routine medical examination. Food handlers who are suffering from communicable diseases may transfer pathogens to food products if proper precautions are not taken and regular medical examination of workers is an essential to prevent disease transmission and to ensure public health safety (Tappes *et al.*, 2019). The low percentage of workers who underwent regular medical examination implies that occupational health monitoring systems in these abattoirs were poor. Previous report agreed with this finding where meat handlers were reported not to check their health status regularly (Ashuro *et al.*, 2023). Regular medical check-ups for food handlers significantly improve food safety

by reducing foodborne illness outbreaks. Studies indicating that staffs with routine screenings are over eight times more likely to follow safe food practices (Azanaw *et al.,* 2019). It was found that despite the fact that more than half of the workers were trained in food safety during their jobs, the overall level of hygienic practice was still found to be unsatisfactory. This finding suggests that food safety compliance is influenced not only by worker knowledge but also by infrastructural capacity, institutional supervision, and availability of hygienic resources. It is possible that workers are aware of the food handling and storage practices but are not in a position to implement the practices because they do not have the necessary equipment and facilities.

More importantly, the detection of *E. coli O157:H7* in the samples taken from the carcasses serves to reinforce the importance of improving hygiene practices in the slaughterhouses. Although the prevalence in the study was relatively low (1.1%), the public health implications of the detection of this pathogen on the carcasses are considerable, as it has the potential to cause severe human illness even at low infectious doses (Doyle *et al.*, 2019). The whole genomic sequence analysis revealed that there was a close genetic link between the isolates obtained from the fecal samples and those obtained from the carcasses that were slaughtered in the same abattoir. This finding strongly suggests that cross-contamination occurred during slaughter operations.

Evisceration has been identified as a critical step in the slaughtering process where carcass contamination is likely to occur. Accidental rupture of the gastrointestinal tract (GIT) may occur during evisceration which may result in the discharge of the content of the GIT, including pathogenic microorganisms, onto the carcass surface. In addition, if knives are not sterilized between carcasses, there are possibilities of the spread of pathogenic microorganisms from contaminated carcasses to uncontaminated carcasses (Leps *et al.*, 2013). In this study, the absence of hot water knife sterilization systems was likely to contribute to the risk of cross-contamination during carcass processing. Interestingly, all isolates identified in the present study were found to be susceptible to antimicrobial agents and did not exhibit any antimicrobial resistance genes. This may imply that antimicrobial-resistant strains of *E. coli* O157:H7 may currently have limited

circulation within the study area. Moreover monitoring antimicrobial resistance is very important because these pathogens can develop resistance genes through the food chain, resulting in treatment failure, increased severity of human disease, and increased mortality rates. This is because antibiotics are used in food animals, and these pathogens can develop resistance to these drugs, and the resistance is transferred to humans through the food chain (Salam *et al.*, 2023; Ho *et al.*, 2025).

6. CHAPTER VI: GENERAL CONCLUSSION AND RECOMMENDATIONS

In conclusion, the findings of this study demonstrate that meat safety in municipal abattoirs is influenced by the complex interaction between infrastructural deficiencies, inadequate operational facilities, and poor hygienic practices, which collectively contribute to microbial contamination. Inadequate infrastructural conditions and operational deficiencies create conditions favorable for cross-contamination and persistence of foodborne pathogens during slaughter operations. These deficiencies compromise microbial meat quality and may increase the risk of foodborne disease transmission within the community.

From the results of the current study, the following practical recommendations are made to enhance the safety of meat products, improve slaughterhouse operational practices , and ensure public safety:

- The municipal authorities' needs to upgrade abattoir infrastructure and operational facilities based on international regulations
- Enforce strict hygiene and sanitation practices through routine cleaning and disinfection of equipment, surfaces, and workplaces as well as standard operating practices for safe slaughtering, particularly during critical contamination points such as evisceration.
- Offer continuous training for slaughterhouse employees that focuses on aspects of hygiene, food safety, and prevention of contamination.
- Enhance compliance with national meat safety standards by implementing integrated policies targeting infrastructure, hygiene, and microbiological safety simultaneously.
- Encourage cooperation between public health agencies, veterinary offices, and municipals on improvement of meat safety.

In short, an integrated strategy considering infrastructure, facilities, hygiene, and microbiological safety is necessary. Without coordinated improvements across these domains, sustainable enhancement of meat safety systems is unlikely to be achieved.

Implementation of such integrated strategies may substantially reduce carcass contamination and improve meat safety outcomes in Ethiopia.

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8. APPENDICES

Appendix 1: Ethical approval certificate



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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu

Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/03/11/16/2024

Name of Applicant: **Endalu Mulatu Lemi (MSc, PhD student)**

Address: Department of Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Assessment of hygienic status of municipal abattoirs in Ethiopia towards designing strategies for improved meat safety management system*

Date of application:	**May, 2024**
Nature of the project:	**Abattoir investigation and questionnaire survey**
Target animal species:	**Cattle**
Number of animals involved:	**216**
Study area:	**Central Ethiopia, Sidama and Southern Regional States**

Minutes No. and date of review: **VM/ERC/03/16/024, 16/05/2024**

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 **Endalu Mulatu Lemi.**

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

መልሱን በሚጽፉልን ጊዜ አባክዎን የኛን ደብዳቤ ቁጥር ይጥቀሱልን
Please quote Our Ref. No. When replying

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

Appendix 2: Survey data collection



External view of some municipal abattoirs



Internal view of some municipal abattoirs (cracked wall and floor)



Carcass processing on the floor in some abattoirs.



Vertical carcass processing in few abattoirs



Solid waste management in some abattoirs





Improper solid and liquid waste management in some abattoirs

Appendix 3: Microbiological investigation of *E.coli O157:H7*

Sample Collection

↓

Sample Preparation / Homogenization

↓

Selective Enrichment in Modified tryptone soya broth+ novobiocin

↓

Immunomagnetic Separation IMS

↓

Selective Plating on Cefixime and potassium tellurite supplement Sorbitol MacCkonkey

↓

Selection of Presumptive Non-sorbitol Fermenting Colonies

↓

Biochemical Confirmation

↓

Serological Confirmation (O157 and H-antigen)

↓

Molecular Confirmation of virulence genes

↓

Confirmed *E. coli* O157:H7 isolate



Sample collection



Sample culturing

Appendix 4: Biochemical test of *E.coli O157:H7*

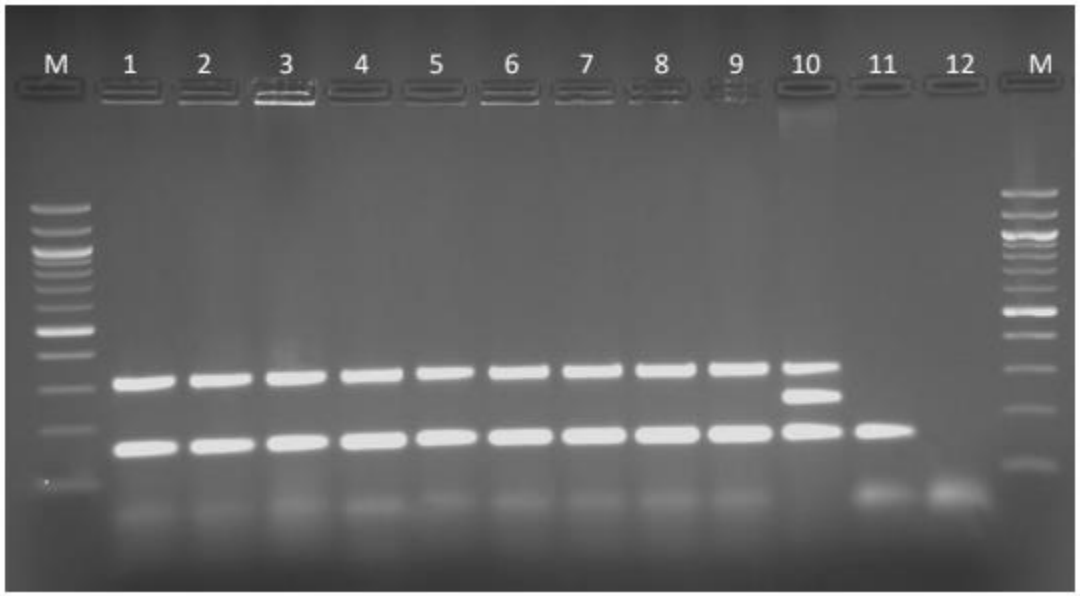


Biochemical tests

Appendix 5: Molecular investigation of *E.coli O157:H7*



DNA Extraction



Supplemental figure PCR for detection of the uidA gene (162 bp) to identify Escherichia coli and the stx1 (244 bp) and stx2 (324 bp) genes to identify Shiga toxins2: 25E0149 (stx2-positive, uidA-positive)

1: 25E0148 (stx2-positive, uidA-positive)

2: 25E0149 (stx2-positive, uidA-positive)

3: 25E0150 (stx2-positive, uidA-positive)

4: 25E0151 (stx2-positive, uidA-positive)

5: 25E0152 (stx2-positive, uidA-positive)

6: 25E0153 (stx2-positive, uidA-positive)

7: 25E0163 (stx2-positive, uidA-positive)

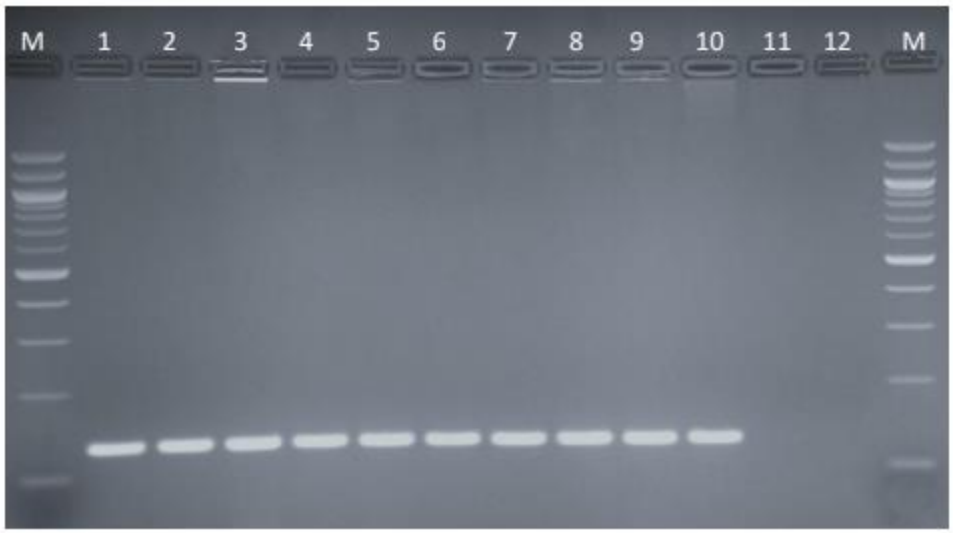
8: 25E0164 (stx2-positive, uidA-positive)

9: 25E0165 (stx2-positive, uidA-positive)

10: 12E0108 (EDL933; stx1-positive, stx2-positive, uidA-positive), positive control

11: 26E0039 (MG1655; uidA-positive), negative control

12: no template negative control



Supplemental figure PCR for detection of the O157-specific wzy gene (133 bp)1: 25E0148 (wzy-positive)

2: 25E0149 (wzy-positive)

3: 25E0150 (wzy-positive)

4: 25E0151 (wzy-positive)

5: 25E0152 (wzy-positive)

6: 25E0153 (wzy-positive)

7: 25E0163 (wzy-positive)

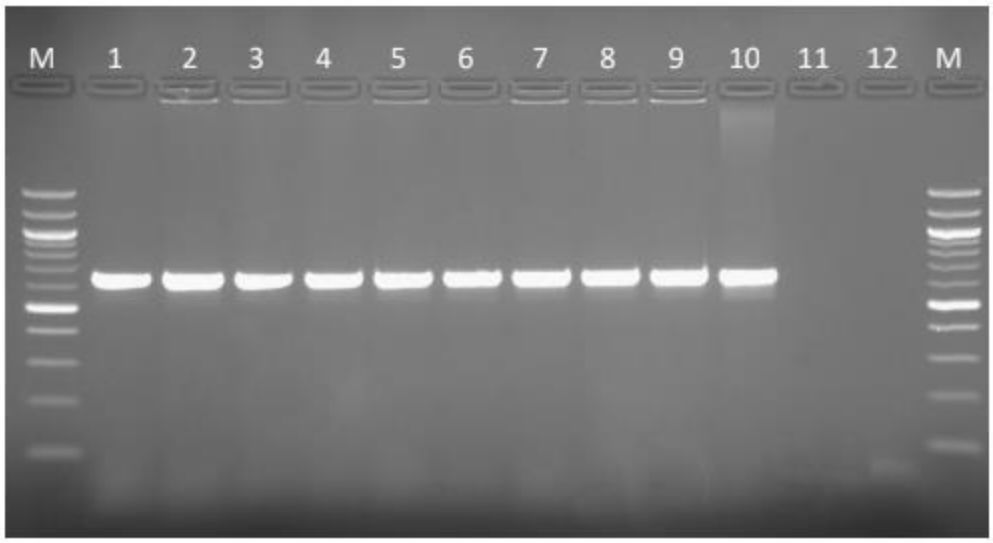
8: 25E0164 (wzy-positive)

9: 25E0165 (wzy-positive)

10: 12E108 (EDL933, O157; wzy-positive), positive control

11: 22E0584 (O26; wzy-negative), negative control

12: no template negative control



Supplemental figure PCR for detection of the eaeA gene (682 bp)

1: 25E0148 (eaeA-positive)

2: 25E0149 (eaeA-positive)

3: 25E0150 (eaeA-positive)

4: 25E0151 (eaeA-positive)

5: 25E0152 (eaeA-positive)

6: 25E0153 (eaeA-positive)

7: 25E0163 (eaeA-positive)

8: 25E0164 (eaeA-positive)

9: 25E0165 (eaeA-positive)

10: 12E0108 (EDL933, eaeA-positive), positive control

11: 26E0039 (MG1655; eaeA-negative), negative control

12: no template negative control

Appendix 6: Anti-microbial susceptibility test of *E.coli O157:H7*



Antimicrobial susceptibility test

Appendix 7: Microbiological media preparation

Buffered Peptone Water (CM0509,Oxoid, Hamsphire, UK.)

Ingredients	**g/ L**
Peptone	10
Sodium chloride	5
Di-sodium phosphate,	3.5
Potassium dihydrogen phosphate	1.5

Preparation

Suspend Buffered Peptone Water (20 grams / litre) in de-ionised water. Heat to dissolve. Cool and dispense 10ml into final containers, universal bottles. Sterilize at 121°C for 15 minutes. When cool label each bottle and pack in units of 100 into labelled boxes.

Plate count agar (CM0325,Oxoid, Hamsphire, UK.)

Ingredients	**g/ L**
Enzymatic digest of casein	5.0
Yeast extract	2.5
Glucose	1.0
Agar	9.0

Preparation

Suspend 17.5g in 1 liter of distilled water. Dissolve by bringing to the boil with frequent stirring,
mix and distribute into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

Violet red bile glucose agar (CM0485, Oxoid, Hamsphire, UK.)

Ingredients	**g/ L**
Yeast extract grams per	3.0
Peptone	7.0
Sodium chloride	5.0
Bile salts No.	3 1.5
Glucose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0

Preparation

Suspend 38.5g in 1 litre of distilled water. With frequent agitation, bring to the boil to dissolve completely. Cool to 50°C. Mix well and pour into sterile Petri dishes or hold at 45°C when using the pour plate technique. DO NOT AUTOCLAVE.

Eosin methylene blue agar (CM0485, Oxoid, Hamsphire, UK.)

Ingredients	**g/ L**
Peptone grams	10.0
Lactose	10.0
Di-potassium hydrogen phosphate	2.0
Eosin Y	0.4
Methylene blue	0.06
Agar	15.0

Preparations

Suspend 37.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C. Mix well to ensure even dispersion of the medium in order to oxidize the methylene blue, which is an essential part of this medium, and pour into sterile Petri dishes.

Modified tryptone soya broth (CM 989, Oxoid, Hamsphire, UK.)

Ingredients	**g/ L**
Pancreatic digest of casein	17.0
Papaic digest of soybean meal	3.0
Sodium chloride	5.0
Di-potassium hydrogen phosphate	4.0
Glucose	2.5
Bile salts	1.5

Preparation

Add 16.5 grams to 500ml of distilled water, mix well and sterilize by autoclaving at 121°C for 15 minutes. Cool to approximately 50°C and add aseptically add one vial of Novobiocin supplement (SR181E) reconstituted as directed. Mix well and aseptically distribute into sterile containers.

MacConkey agar (CM0813/ CB0813, Oxoid, Hamsphire, UK.)

Ingredients	**g/ L**
Peptone	20.0

Sorbitol	10.0
Bile salts No	3 1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0

Preparation

Suspend 51.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C. Mix well and pour into sterile Petri dishes. Alternatively, cool to 50°C and aseptically add the contents of 2 vials of Cefixime-Tellurite Supplement (SR0172E) reconstituted as directed. Mix well and pour into sterile Petri dishes.

MacConkey Agar (CM0007Oxoid, Hamsphire, UK.)

Ingredients	**g/ L**
Peptone grams	20.0
Lactose	10.0
Bile salts	5.0
Sodium chloride	5.0
Neutral red	0.075
Agar	12.0

Preparation

Suspend 52g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C. Mix well and pour into sterile Petri dishes.

Lysine iron agar (CM0381, Oxoid, Hamsphire, UK.)

Ingredients	**g/ L**
Peptone	5.0
Yeast extract	3.0
Glucose	1.0
L-lysine	10.0
Ferric ammonium citrate	0.5
Sodium thiosulphate	0.04
Bromocresol purple	0.02
Agar	14.5

Preparation

Suspend 34g in 1 litre of distilled water. Boil to dissolve the medium completely. Dispense into tubes and sterilize by autoclaving at 121°C for 15 minutes. Cool the tubes in an inclined position to form slants with deep butts.

Mueller-Hinton agar (CM0337, Oxoid, Hamsphire, UK.)

Ingredients	**g/ L**
Beef, dehydrated infusion	300.0
Casein hydrolysate	17.5
Soluble starch	1.5
Agar	17.0

Preparation

Suspend 38g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C. Mix well and pour into sterile Petri dishes.

Nutrient agar (CM0003, Oxoid, Hamsphire, UK.)

Ingredients	**g/ L**
Lab-Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0

Preparation

Suspend 28g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool to 50°C. Mix well and pour into sterile containers.

Appendix 8: Chemical and reagents

Kovac's reagent (R008, HiMedia Laboratories Pvt. Limited, India)

Ingredients

p-dimethyl amino benzaldehyde 5.0 gm
Amyl alcohol 75.0 ml
Hydrochloric Acid 25.0 ml

Directions

1. Take 5 ml of a 24 - 48 hours old culture of the organism under investigation.
2. Add 0.2 - 0.3 ml of Kovac's reagent.
3. Observed for a red coloured ring which indicates positive indole test.

Cefixime-Tellurite supplement (SMAC Media CT supplement, Applied Biosystems by ThermoFisher Scientific, UK)

Ingredients

Cefixime	1mg
Potassium tellurite	50mg

Directions

Aseptically add 10ml of sterile distilled water to 1 vial and invert gently to dissolve. Aseptically add the vial contents to 2 litres of sterile Sorbitol MacConkey Agar (CM0813) prepared as directed and cooled to 50°C. Mix well and pour into sterile Petri dishes.

Appendix 9: Informed consent

Assessment of The Hygienic Status of Municipal Abattoirs In Ethiopia Toward Designing Strategies For Improved Meat Safety Management Systems

Informed consent

Dear respondent

Greeting!

I am working on a research project, "ASSESSMENT OF THE HYGIENIC STATUS OF MUNICIPAL ABATTOIRS IN ETHIOPIA TOWARD DESIGNING STRATEGIES FOR IMPROVED MEAT SAFETY MANAGEMENT SYSTEMS". The study aims to assess the hygienic status of municipal abattoirs in Ethiopia toward designing strategies for improved meat safety management systems.

The interview will take about 30 minutes to finish. Apart from the importance of your answers to all of my questions, you may decline to answer any of the questions and also you can withdraw from the study at any time if you don't feel comfortable.

There is no immediate benefit to you now but as part of the research, the output will be useful prevention of the pathogen. On the other hand what I want to note you is that I respect your privacy and confidentiality. This means I will not disclose your name and other personal identifiable information to anybody outside of the research.

Please do not indicate your name and other respondents on the questionnaires rather use numbers instead of name chronologically.

Based on the above information do you agree to participate in the study? Encircle on your decision from the following choose.

A) Yes B) No

If you have any questions or require further information about the study, please do not hesitate to contact me at: E-mail: endalu.mulatu@aau.edu.et or mobile telephone: +251940760547

Thank you for considering this request,

Best regards!

በኢትዮጵያ የማዘጋጃ ቤት አርድ ቤቶች የንጽህና ሁኔታ ግምገማ ለተሻሻለ የስጋ ደህንነት አስተዳደር ስርዓት ስልቶች ንድፍ

የተሳትፎ ፈቃድ መግለጫ

ክቡር/ክብርት ተሳታፊ

ሰላምታ!

እኔ "በኢትዮጵያ የማዘጋጃ ቤት አርድ ቤቶች የንጽህና ሁኔታ ግምገማ ለተሻሻለ የስጋ ደህንነት አስተዳደር ስርዓት ስልቶች ንድፍ" በሚል ርዕስ የምርምር ፕሮጀክት ላይ እየሰራሁ ነው። ጥናቱ በኢትዮጵያ የማዘጋጃ ቤት አርድ ቤቶች የንጽህና ሁኔታን ለመገምገም እና የተሻሻለ የስጋ ደህንነት አስተዳደር ስርዓት ስልቶችን ለመንደፍ ይደረጋል።

ቃለ መጠይቁ ለመጨረስ 30 ደቂቃ ያህል ይወስዳል። ለሁሉም ጥያቄዎች መልስዎ አስፈላጊ ቢሆንም፣ የማትፈልጉትን ጥያቄ መልስ ማስቀረት ይችላሉ፣ እንዲሁም በማንኛውም ጊዜ ካልተስማማዎት ከጥናቱ መውጣት ይችላሉ።

ለእርስዎ በአሁኑ ጊዜ ቀጥተኛ ጥቅም የለም፣ ነገር ግን የምርምሩ ውጤት በተዛማጅ በሽታ መከላከል ውስጥ ጠቃሚ ይሆናል። እንዲሁም የግል መረጃዎን እና ሚስጥራዊነትዎን እንደምከብር ልገልጽልዎ እፈልጋለሁ፤ ስምዎን እና ሌሎች የሚለዩ መረጃዎችን ከምርምሩ ውጭ ለማንም አልገልጽም።

እባክዎ በመጠይቁ ላይ ስምዎን አትጻፉ፤ በስም ፋንታ በተከታታይ ቁጥር ይጠቀሙ።

ከላይ በተጠቀሰው መረጃ መሰረት በጥናቱ ለመሳተፍ ይስማማሉ? ከሚከተሉት በማክበብ ውሳኔዎን አንዱን ይምረጡ።

ሀ) አዎ ለ) አይ

ስለ ጥናቱ ጥያቄ ካለዎት ወይም ተጨማሪ መረጃ ከፈለጉ፣ በኢሜይል endalu.mulatu@aau.edu.et ወይም በስልክ +251940760547 ያነጋግሩኝ።

ለተሳትፎዎ እናመሰግናለን፣

ከአክብሮት ጋር!

Haala qulqullina mana qalmaa bulchiinsa magaalaan hogganaman keessatti qorannoo tooftaalee sirna nageenya foonii fooyya'aa qopheessuu

Ibsa Hayyamaa Hirmaannaa

Kabajamo Obboo/Aaddee

Akkam jirtu!

Ani hojii qorannoo mata-duree "Haala qulqullina mana qalmaa bulchiinsa magaalaan hogganaman keessatti qorannoo tooftaalee sirna nageenya foonii fooyya'aa qopheessuu" jedhu irratti hojjechaa jira. Qorannoon kun haala qulqullina manneen qalmaa mootummaa Itoophiyaa kanneen bulchiinsa magaalaan hogganaman keessatti qorachuu fi tooftaalee sirna nageenya foonii fooyya'aa qopheessuuf kaayyeffata.

Gaaffiin kun yeroo daqiiqaa 30 keessaa ni xumurama. Deebiin keessan gaaffilee hundaaf barbaachisaa ta'us, gaaffii hin barbaadne deebisuu dhiisuu ni dandeessu; akkasumas yeroo kamiyyuu yoo isiniif hin mijanne qorannicha keessaa bahuu ni dandeessu.

Ammaaf bu'aan kallattiin isin irraa argattan hinjiru; garuu bu'aan qorannichaa ittisa dhukkubaaf ni fayyada. Akkasumas iccitii fi yaadi dhuunfaa keessan nan kabaja; jechuun maqaa keessan fi odeeffannoo eenyummaa keessan ibsu kamiyyuu nama qorannicha alaa jiruuf hin ibsu.

Maqaa keessan gaaffilee irratti hin barreessinaa; maqaa bakka buusuuf lakkoofsa tartiibaan fayyadamaa.

Odeeffannoo armaan olii irratti hundaa'uun qorannicha keessatti hirmaachuuf itti waliigaltu? Murtee keessaan kanneen armaan gadii keessaa filachuun ibsa.

A) Eeyyee B) Lakki

Gaaffii yoo qabaattan yookaan odeeffannoo dabalataa yoo barbaaddan: endalu.mulatu@aau.edu.et ykn bilbila +251940760547 irratti na qunnamaa.

Hirmaanna keessaniif galatoomaa,

Nagaa wajjiin!

Appendix 10: Questionaries

Questionnaire

Part 1: General Information

1. Name of enumerator: _____
2. Date of interview: _____
3. Region: _____
4. Zone: _____
5. District: _____
6. Name of Town: _____
7. Name/Code of respondent: _____

Part 2: Slaughter Facility Characteristics

1. Facility name: _____
2. Address (Kebele): _____
3. Year established: _____
4. Number of butcher shops served: _____
5. Daily slaughter capacity: _____
6. Actual daily slaughter number: _____
7. Services provided: _____

□ A) Slaughtering of sheep and goats

□ B) Slaughtering of cattle

□ C) Slaughtering of sheep, goats, and cattle

□ D) Slaughtering of pigs

□ E) Slaughtering of camels

□ Other: _____

8. Ownership: □ Government □ Private □ Cooperatives
9. Total premise area: _____
10. Slaughter hall area: _____
11. Distance from residential area: _____

12. How many kilometers is the slaughterhouse from the main road? _____ km

Part III: Location Assessment

1. Slaughterhouse name: _____

2. The proximity of the slaughterhouse site to residential areas is acceptable. ☐ Yes ☐ No

3. Environmental impact considerations (environment, water, air pollution) have been adequately addressed. ☐ Yes ☐ No

4. The accessibility of the site for transportation of livestock and finished products is suitable. ☐ Yes ☐ No

5. Adequate measures are in place to manage and minimize odors associated with slaughterhouse operations. ☐ Yes ☐ No

6. The location allows for future expansion of the slaughterhouse facility. ☐ Yes ☐ No

7. The location is free from trees. ☐ Yes ☐ No

8. There is no harborage or breeding place for pests at the location. ☐ Yes ☐ No

Part IV: Layout Assessment

9. The slaughterhouse layout allows efficient and organized livestock flow (entrance and exit). ☐ Yes ☐ No

10. The slaughterhouse infrastructure allows separation between clean and dirty areas. ☐ Yes ☐ No

11. There is enough and separate space for carcass processing activities. ☐ Yes ☐ No

12. The layout has safety features to ensure workers' well-being. ☐ Yes ☐ No

13. Flooring materials are easy to clean and sanitize and are in good repair. ☐ Yes ☐ No

14. Walls are constructed with hygienic materials and are in good repair. ☐ Yes ☐ No

15. Roofs are constructed with hygienic materials and are in good repair. ☐ Yes ☐ No

PartV: Facility Assessment

16. Reception and examination area for livestock is available. ☐ Yes ☐ No

17. The facility has a well-designed holding pen. ☐ Yes ☐ No

18. Isolation pen for suspected animals is available. ☐ Yes ☐ No

19. Stunning pen is available. ☐ Yes ☐ No

20. Adequate facilities for proper bleeding and dressing of carcasses are available. ☐ Yes ☐ No

21. The facility is supplied with a hot water source. ☐ Yes ☐ No

22. Store for hides, hooves, and horns is available. ☐ Yes ☐ No

23. The facility provides a deep freezer-18/-20 oC. ☐ Yes ☐ No

24. Slaughterhouse has sufficient water supply. ☐ Yes ☐ No

25. Adequate ventilation systems are available. ☐ Yes ☐ No

26. Designated areas for washing hands are available. ☐ Yes ☐ No

27. Designated shower areas are available. ☐ Yes ☐ No

28. Adequate restroom facilities are available. ☐ Yes ☐ No

29. Electricity supply is available in all work areas. ☐ Yes ☐ No

30. Efficient pest control facilities are available. ☐ Yes ☐ No

31. A quality control laboratory is available. ☐ Yes ☐ No

32. Employee break areas are available and maintained. ☐ Yes ☐ No

33. Adequate employee locker rooms are available. ☐ Yes ☐ No

34. Meat inspector office is provided. ☐ Yes ☐ No

35. Facilities exist for disposal of condemned meat and organs. ☐ Yes ☐ No

36. Areas are designated for solid waste disposal. ☐ Yes ☐ No

37. Security measures prevent unauthorized access. ☐ Yes ☐ No

38. Entry of wildlife and stray dogs is prevented. ☐ Yes ☐ No

39. Foot bath is provided. ☐ Yes ☐ No

40. Vehicle bath is provided. ☐ Yes ☐ No

41. Cold room is available. ☐ Yes ☐ No

42. Sufficient cleaning and disinfection chemicals are provided. ☐ Yes ☐ No

43. Personal protective equipment is sufficiently provided. ☐ Yes ☐ No

44. Equipment sterilizers are provided. ☐ Yes ☐ No

Part 6: Abattoir Hygiene and Food Safety Indicators Questionnaire

Instruction: Please tick the appropriate box.

1. Animals are stunned before being slaughtered.☐ Yes ☐ No

2. Personnel and utensils stay/work only in designated areas.☐ Yes ☐ No

3. Slaughtering halls are cleaned between batches.☐ Yes ☐ No

4. Processing equipment and utensils are sterilized between batches.☐ Yes ☐ No

5. The use of personal protective equipment is consistently enforced.☐ Yes ☐ No

6. Wastewater disposal practices comply with environmental regulations.☐ Yes ☐ No

7. Meat inspection areas are cleaned between every activity.☐ Yes ☐ No

8. There are mechanisms to communicate meat inspection results.☐ Yes ☐ No

9. The abattoir water supply is regularly tested for safety (pathogens and heavy metals).☐ Yes ☐ No

10. The carcass is kept vertical during skinning and evisceration to prevent contamination.☐ Yes ☐ No

11. A thorough ante-mortem examination is conducted.☐ Yes ☐ No

12. A thorough post-mortem examination is conducted.☐ Yes ☐ No

13. Meat transporting vans are cleaned between batches.☐ Yes ☐ No

14. Carcasses are transported using refrigerated vehicles.☐ Yes ☐ No

15. Visceral organs, green offal, and carcasses are transported separately.☐ Yes ☐ No

መጠይቅ

ክፍል 1፡ አጠቃላይ መረጃ

1. የመረጃ ሰብሳቢ ስም: _____

2. የቃለ መጠይቅ ቀን: _____

3. ክልል: _____

4. ዞን: _____

5. ወረዳ: _____

6. የከተማ ስም: _____

7. የተጠያቂ ስም/ኮድ: _____

ክፍል 2፡ የቀራው ባህሪያት

1. የቀራ ቤት ስም: _____

2. አድራሻ (ቀበሌ): _____

3. የተቋቋመበት ዓመት: _____

4. የሚያገለግላቸው የስጋ ቤቶች ብዛት: _____

5. የቀን የማረድ አቅም: _____

6. በቀን የሚታረዱ እንስሳት ብዛት: _____

7. የሚሰጠው አገልግሎት: _____

□ A) በግና ፍየል ማረድ

□ B) ከብት ማረድ

□ C) በግ፣ ፍየል እና ከብት ማረድ

□ D) አሳማ ማረድ

□ E) ግመል ማረድ

□ ሌላ: _____

8. ባለቤትነት: □ መንግስት □ ግል □ ህብረት ስራ

9. አጠቃላይ የቦታ መጠን: _____

10. የማረጃ አዳራሽ ስፋት (በካሬ ሜትር): _____ $ሜ^2$

11. ከመኖሪያ አካባቢ ያለው ርቀት (በኪሎ ሜትር): _____ ኪ.ሜ

12. ቀራው ከዋናው መንገድ ስንት ኪሎ ሜትር ይርቃል? _____ ኪ.ሜ

ክፍል 3: የቀራ ቦታ ግምገማ

1. የቀራ ስም: _____

2. የቀራ ቦታ ከ መኖሪያ አካባቢ ቅርብ መሆኑ ተቀባይነት አለው። ☐ አዎ ☐ አይ

3. የአካባቢ ተፅዕኖ (አካባቢ፣ ውሃ፣ አየር) በበቂ ሁኔታ ታስበዋል። ☐ አዎ ☐ አይ

4. ቦታው ለእንስሳት እና ምርቶች መጓጓዣ ተስማሚ ነው። ☐ አዎ ☐ አይ

5. ከቀራ ስራ የሚመጡ ሽታዎችን ለመቀነስ በቂ እርምጃ አለ። ☐ አዎ ☐ አይ

6. ቦታው ለወደፊት መስፋፋት ያስችላል። ☐ አዎ ☐ አይ

7. ቦታው ከዛፎች ነፃ ነው። ☐ አዎ ☐ አይ

8. የተባዮች መኖሪያ ወይም መራቢያ ቦታ የለም። ☐ አዎ ☐ አይ

ክፍል 4: የቀራ ዲዛይን አቀራረብ ግምገማ

9. የቀራ አቀራረብ የእንስሳት መግቢያና መውጫ በተደራጀ ሁኔታ ያስችላል። ☐ አዎ ☐ አይ

10. ንጹህ እና ቆሻሻ ክፍሎች ተለይተው አሉ። ☐ አዎ ☐ አይ

11. ለሥጋ ማቀነባበር በቂ እና የተለየ ቦታ አለ። ☐ አዎ ☐ አይ

12. ለሰራተኞች ደህንነት የሚያስጠብቁ የደህንነት መዋቅሮች አሉ። ☐ አዎ ☐ አይ

13. የመሬት ወለል ቀላል ለማጽዳት እና ለማሳነጽ የሚቻል ነው። ☐ አዎ ☐ አይ

14. ግድግዳዎች ንጽህናን የሚያሟሉ ናቸው። ☐ አዎ ☐ አይ

15. ጣሪያዎች ንጽህናን የሚያሟሉ ናቸው። ☐ አዎ ☐ አይ

ክፍል 5፡ የቀራ ኢቃ እና ፋሲሊቲ አቅርቦት ግምገማ

16. የእንስሳት መቀበያ እና ምርመራ ቦታ አለ። ☐ አዎ ☐ አይ

17. የእንስሳት መጠበቂያ ቦታ አለ። ☐ አዎ ☐ አይ

18. የተጠረጠሩ እንስሳት ለማስለያ ቦታ አለ። ☐ አዎ ☐ አይ

19. የማደንዘዣ ቦታ አለ። ☐ አዎ ☐ አይ

20. ለመደም እና ለመልበስ በቂ መሳሪያዎች አሉ። ☐ አዎ ☐ አይ

21. ሙቅ ውሃ ይገኛል። ☐ አዎ ☐ አይ

22. ለቆዳ እና ቀንድ ማከማቻ አለ። ☐ አዎ ☐ አይ

23. ከፍተኛ ማቀዝቀዣ -18/-20 oC አለ። ☐ አዎ ☐ አይ

24. በቂ የውሃ አቅርቦት አለ። ☐ አዎ ☐ አይ

25. የአየር ማውጫ ስርዓት አለ። ☐ አዎ ☐ አይ

26. የእጅ መታጠቢያ ቦታ አለ። ☐ አዎ ☐ አይ

27. የመታጠቢያ ሻወር ቦታ አለ። ☐ አዎ ☐ አይ

28. በቂ የመጸዳጃ ቤት አለ። ☐ አዎ ☐ አይ

29. በሁሉም የስራ ቦታዎች ኤሌክትሪክ አለ። ☐ አዎ ☐ አይ

30. የተባይ መቆጣጠሪያ ዘዴ አለ። ☐ አዎ ☐ አይ

31. የጥራት መቆጣጠሪያ ላቦራቶሪ አለ። ☐ አዎ ☐ አይ

32. የሰራተኞች ዕረፍት ቦታ አለ። ☐ አዎ ☐ አይ

33. የሰራተኞች ልብስ መቀየሪያ ክፍል አለ። ☐ አዎ ☐ አይ

34. የሥጋ መመርመሪያ ቢሮ አለ። ☐ አዎ ☐ አይ

35. የተከለከለ ሥጋ ለማስወገድ መስሪያ አለ። ☐ አዎ ☐ አይ

36. የደረቅ ቆሻሻ መጣያ ቦታ አለ። ☐ አዎ ☐ አይ

37. የደህንነት ጥበቃ አለ። ☐ አዎ ☐ አይ

38. የዱር እንስሳት እና ውሾች መግባት አይችሉም። ☐ አዎ ☐ አይ

39. የእግር ማጠቢያ አለ። ☐ አዎ ☐ አይ

40. የተሽከርካሪ ማጠቢያ አለ። ☐ አዎ ☐ አይ

41. ቀዝቃዛ ክፍል አለ። ☐ አዎ ☐ አይ

42. በቂ የማጽጃ ኬሚካሎች አሉ። ☐ አዎ ☐ አይ

43. የግል መከላከያ መሳሪያዎች አሉ። ☐ አዎ ☐ አይ

44. የመሳሪያ/ ቢላ ማሳነጫ አለ። ☐ አዎ ☐ አይ

ክፍል 6፡ የቀራ ንጽህና እና የምግብ ደህንነት መጠን መለኪያዎች መጠይቅ

1. እንስሳት ከመታረድ በፊት ይደንዝዛሉ? (stunning ይደረጋል). ☐ አዎ ☐ አይ

2. ሰራተኞች እና መሳሪያዎች በተመደቡ ቦታዎች ብቻ ይሰራሉ? ☐ አዎ ☐ አይ

3. የማረጃ አዳራሾች በእያንዳንዱ ዙር መካከል ይጸዳሉ? ☐ አዎ ☐ አይ

4. እቃዎች በእያንዳንዱ ዙር መካከል ይስቴራላይዝ ይደረጋሉ ☐ አዎ ☐ አይ

5. የግል መከላከያ መሳሪያ (PPE) መጠቀም ክትትል ይደረጋል? ☐ አዎ ☐ አይ

6. የቆሻሻ ውሃ አወጣጥ ሂደቶች የአካባቢ ደንቦችን ይከተላሉ?☐ አዎ ☐ አይ

7. የስጋ ምርመራ ቦታዎች ከእያንዳንዱ ስራ በኋላ ይጸዳሉ? ☐ አዎ ☐ አይ

8. የምርመራ ውጤቶችን ለማስተላለፍ ስርዓቶች አሉ? ☐ አዎ ☐ አይ

9. ውሃ አቅርቦት ለደህንነት በመደበኛነት ይመረመራል? ☐ አዎ ☐ አይ

10. በስኪኒንግ እና ኢቪሰሬሽን ጊዜ ካርካስ በቀጥታ አቀማመጥ ይይዛል እንዲሁም ብክለትን ለመከላከል.☐ አዎ ☐ አይ

11. ሙሉ የየእንስሳት እርድ በፊት የጠና ምርመራ ይደረጋል? ☐ አዎ ☐ አይ

12. ሙሉ የሥጋ ምርመራ ይደረጋል?☐ አዎ ☐ አይ

13. የስጋ መጓጓዣ መኪናዎች በእያንዳንዱ ዙር መካከል ይጸዳሉ? ☐ አዎ ☐ አይ

14. ሥጋ በቀዝቃዛ መኪናዎች ይጓጓዛሉ? ☐ አዎ ☐ አይ

15. የሆድ እቃ እና ስጋ በተለያዩ ይጓጓዛሉ. ☐ አዎ ☐ አይ

Gaaffannoo

Kutaa 1: Odeeffannoo Waliigalaa

1. Maqaa odeeffannoo walitti qabaa: _____

2. Guyyaa gaafannoo: _____

3. Naannoo: _____

4. Godina: _____

5. Aanaa: _____

6. Maqaa magaalaa: _____

7. Maqaa/Koodii deebisaa: _____

Kutaa 2: Amaloota Mana Qalmaa

1. Maqaa mana qalmaa: _____

2. Teessoo (Kebele): _____

3. Bara hundeeffamaa: _____

4. Baay'ina manneen foonii tajaajilaman: _____

5. Dandeettii qalmaa guyyaa tokkoo: _____

6. Baay'ina qalmaa guyyaa tokkoo: _____

7. Tajaajiloota kennaman: _____

□ A) Hoolaa fi re'ee qaluu

□ B) Loon qaluu

□ C) Hoolaa, re'ee fi loon qaluu

□ D) Booyyee qaluu

□ E) Gaala qaluu

□ Kan biraa: _____

8. Abbummaa: □ Mootummaa □ Dhuunfaa □ Waldaa hojii gamtaa

9. Bal'ina waliigalaa bakka hojii: _____

10. Bal'ina mana qalmaa keessaa (iskuweer meetiraan): _____ m^2

11. Fageenya mana jireenyaa irraa (kiiloo meetiraan): _____ km

12. Manni qalmaa kun karaa guddaa irraa kiiloo meetira meeqa fagoo dha? _____ km

Kutaa 3: Madaallii Bakka Mana Qalmaa

1. Maqaa mana qalmaa: _____

2. Fageenyi mana qalmaa fi naannoo jireenyaa fudhatama qaba. ☐ Eeyyee ☐ Lakkii

3. Miidhaan naannoo (bishaan, qilleensa) sirnaan ilaalameera. ☐ Eeyyee ☐ Lakkii

4. Iddoon kun geejjiba horii fi oomishaaf mijataadha. ☐ Eeyyee ☐ Lakkii

5. Foolii hojii mana qalmaa hir'isuuf tarkaanfiin gahaan jira. ☐ Eeyyee ☐ Lakkii

6. Iddoon kun babal'ina gara fuulduraaf mijataadha. ☐ Eeyyee ☐ Lakkii

7. Iddoon sun muka irraa bilisa dha. ☐ Eeyyee ☐ Lakkii

8. Bakki qubannaa ilbiisotaa hin jiru. ☐ Eeyyee ☐ Lakkii

Kutaa 4: Madaallii Qindaa'ina Mana Qalmaa

9. Qindaa'inni mana qalmaa seensa fi ba'iinsa horii ni dandeessisa. ☐ Eeyyee ☐ Lakkii

10. Bakki qulqulluu fi bakki xuraa'aa addaan baafameera. ☐ Eeyyee ☐ Lakkii

11. Hojii qopheessuu fooniif bakka gahaan jira. ☐ Eeyyee ☐ Lakkii

12. Nageenya hojjettootaaf sirni eegumsa jira. ☐ Eeyyee ☐ Lakkii

13. Lafti galma qalmaa salphaa dha qulqulleessuuf. ☐ Eeyyee ☐ Lakkii

14. Keenyan mana hojii qulqullinaaf mijataa dha. ☐ Eeyyee ☐ Lakkii

15. Baaxii mana hojii qulqullinaaf mijataa dha. ☐ Eeyyee ☐ Lakkii

Kutaa 5: Madaallii dhiyeessii meeshaalee fi Tajaajila Mana Qalmaa

16. Bakki simannaa fi qorannaa bineensotaa jira. ☐ Eeyyee ☐ Lakkii

17. Bakki tursiisa bineensotaa jira. ☐ Eeyyee ☐ Lakkii

18. Bakki horii dhukkubaan shakkamanii tursiisan jira. ☐ Eeyyee ☐ Lakkii

19. Bakki itti hojii of wallalchisan gochuu jira. ☐ Eeyyee ☐ Lakkii

20. Meeshaaleen dhiiga baasuu fi qopheessuu gahaa jiru. ☐ Eeyyee ☐ Lakkii

21. Bishaan ho'aa jira. ☐ Eeyyee ☐ Lakkii

22. Bakki kuusaa gogaa, gaafa fi miila jira. ☐ Eeyyee ☐ Lakkii

23. Freezer guddaan jira -18/-20 oC. ☐ Eeyyee ☐ Lakkii

24. Bishaan gahaan jira. ☐ Eeyyee ☐ Lakkii

25. Sirni qilleensa gaahaatti kutaa qalmaa keessa marsiisu jira. ☐ Eeyyee ☐ Lakkii

26. Bakki harka dhiqannaa jira. ☐ Eeyyee ☐ Lakkii

27. Bakki dhiqannaa qaamaa jira. ☐ Eeyyee ☐ Lakkii

28. Mana fincaanii gahaa jira. ☐ Eeyyee ☐ Lakkii

29. Humni ibsaa (electricity) jira. ☐ Eeyyee ☐ Lakkii

30. Sirni to'annoo ilbiisotaa jira. ☐ Eeyyee ☐ Lakkii

31. Laaboraatooriin to'annoo qulqullinaa jira. ☐ Eeyyee ☐ Lakkii

32. Bakki boqonnaa hojjettootaa jira. ☐ Eeyyee ☐ Lakkii

33. Kutaan uffata jijjiiruu hojjettootaa jira. ☐ Eeyyee ☐ Lakkii

34. Waajjirri qorataa foonii jira. ☐ Eeyyee ☐ Lakkii

35. Bakki tursiisu foon dhorkamaa jira. ☐ Eeyyee ☐ Lakkii

36. Bakki gatinsa balfaa jabaataa jira. ☐ Eeyyee ☐ Lakkii

37. Eegumsi nageenyaa jira. ☐ Eeyyee ☐ Lakkii

38. Seensa bineensota bosonaa fi saree daangeffameera. ☐ Eeyyee ☐ Lakkii

39. Bakki miila dhiqannaa jira. ☐ Eeyyee ☐ Lakkii

40. Bakki konkolaataa dhiqannaa jira. ☐ Eeyyee ☐ Lakkii

41. Kuta qabbanaa'aa jira. ☐ Eeyyee ☐ Lakkii

42. Keemikaalli qulqulleessuu gahaan jira. ☐ Eeyyee ☐ Lakkii

43. Meeshaan of eegu hojjettootaa gahaan jira. ☐ Eeyyee ☐ Lakkii

44. Meeshaan sterilizer jira. ☐ Eeyyee ☐ Lakkii

Kutaa 6: Gaaffii Qorannoo Agarsiistota Qulqullinaa Mana Qalamaa fi Nageenya Foonii

1. Beeyladoonni qalamuun dura of wallalchisamuu? ☐ Eeyyee ☐ Lakkii

2. Hojjettoonni fi meeshaaleen iddoo ramadame keessatti qofa ni hojjetu? ☐ Eeyyee ☐ Lakkii

3. Kutaan qalmaa yeroo yerootti ni qulqullaa'a? ☐ Eeyyee ☐ Lakkii

4. Meeshaaleen hojii fi mi'aawwaan yeroo yerootti ni qulqulla'uu?. ☐ Eeyyee ☐ Lakkii

5. Itti fayyadamiinsi meeshaalee ittisa dhuunfaa (PPE) yeroo hunda hojiirra olmaan isaa ni mirkanaa'a? ☐ Eeyyee ☐ Lakkii

6. Akkaataa dhangala'aa xuraa'aa dhabamsiisuu seerota naannoo ni kabaja? ☐ Eeyyee ☐ Lakkii

7. Iddoon qorannoo foonii yeroo hunda hojii booda ni qulqullaa'a? ☐ Eeyyee ☐ Lakkii

8. Bu'aan qorannoo foonii sirni ittin maamltoota beeksisan jira?. ☐ Eeyyee ☐ Lakkii

9. Bishaan mana qalmaa yeroo yerootti nageenyaaf ni qoratamaa? ☐ Eeyyee ☐ Lakkii

10. Gogaa baasuu fi qaama keessaa/ mi'a garaa baasuu haala dhaabbata/ foon rarrafafee dalagama?. ☐ Eeyyee ☐ Lakkii

11. Qorannoon fayyumma horii qalma dura guutuu ni gaggeeffamaa? ☐ Eeyyee ☐ Lakkii

12. Qorannoon fayyummaa foonii guutuu ni gaggeeffamaa? ☐ Eeyyee ☐ Lakkii

13. Konkolaattonni fe'umsaa foonii marsaa marsaan yeroo hunda ni qulqullaa'u? ☐ Eeyyee ☐ Lakkii

14. Foon konkolaattoota qabbanaa'oo irratti ni geeffamu? ☐ Eeyyee ☐ Lakkii

15. Qaamoleen mi'I garaafi foon addaan addaan ni geeffamu. ☐ Eeyyee ☐ Lakkii

Questionnaire: Assessment of Hygienic Status of Abattoir Workers Toward Meat Safety Practices

Section 1: General Information

1. Name of the abattoir: _____

2. Age: ☐ Above 30 ☐ Below 30

3. Education: ☐ Above secondary ☐ Below secondary

4. Experience: ☐ Above 5 years ☐ Below 5 years

5. Position: ☐ Management ☐ Meat inspector ☐ Cleaner ☐ Evisceration ☐ Offal room ☐ Skinning ☐ Carcass loading/unloading

6. Gender: ☐ Male ☐ Female

Section 2: Food Safety Practices

7. Regular health check-up: ☐ Yes ☐ No

8. Use gloves: ☐ Yes ☐ No

9. Use apron: ☐ Yes ☐ No

10. Use boots: ☐ Yes ☐ 0 No

11. Use overall coat: ☐ Yes ☐ No

12. Use head covers: ☐ Yes ☐ No

13. Do not wear jewelry: ☐ Yes ☐ No

14. Do not eat or drink at the workplace: ☐ Yes ☐ No

15. Hand wash with soap before handling meat: ☐ Yes ☐ No

16. Always use clean personal protective equipment: ☐ Yes ☐ No

17. Received on-job food safety training: ☐ Yes ☐ No

18. Do not handle meat when you are ill: ☐ Yes ☐ No

የቀራ ሰራተኞች የንጽህና ሁኔታ እና ወደ የስጋ ደህንነት አሰራሮች ያላቸው ተግባር ግምገማ

ክፍል 1: አጠቃላይ መረጃ

1. የማረጃ ቤቱ ስም: _____

2. ዕድሜ: ☐ ከ30 በላይ ☐ ከ30 በታች

3. የትምህርት ደረጃ: ☐ ከሁለተኛ ደረጃ በላይ ☐ ከሁለተኛ ደረጃ በታች

4. የስራ ልምድ: ☐ ከ5 ዓመት በላይ ☐ ከ5 ዓመት በታች

5. የስራ ኃላፊነት: ☐ አስተዳደር ☐ የስጋ መርመራ ባለሙያ ☐ ንፁህ ሰራተኛ ☐ ኢቪሰሬሽን ☐ አፋል ክፍል ☐ ስኪኒንግ ☐ ካርካስ መጫን/መውረድ

6. ፆታ: ☐ ወንድ ☐ ሴት

ክፍል 2: የምግብ ደህንነት ልምዶች

7. መደበኛ የጤና ምርመራ: ☐ አዎ ☐ አይ

8. ጓንት መጠቀም: ☐ አዎ ☐ አይ

9. አፕሮን መጠቀም: ☐ አዎ ☐ አይ

10. ቡት መጠቀም: ☐ አዎ ☐ አይ

11. አቨርአል ኮት መጠቀም: ☐ አዎ ☐ አይ

12. የራስ መሸፈኛ መጠቀም: ☐ አዎ ☐ አይ

13. ጌጣጌጥ አለመልበስ: ☐ አዎ ☐ አይ

14. በስራ ቦታ መብላት ወይም መጠጣት አለመፍቀድ: ☐ አዎ ☐ አይ

15. ስጋ ከመንካት በፊት እጅ በሳሙና መታጠብ: ☐ አዎ ☐ አይ

16. ንፁህ የግል መከላከያ መሳሪያ መጠቀም: ☐ አዎ ☐ አይ

17. በስራ ላይ የምግብ ደህንነት ስልጠና መቀበል: ☐ አዎ ☐ አይ

18. በህመም ጊዜ ስጋ አለመንካት: ☐ አዎ ☐ አይ

Qorannoo Haala Qulqullinaa Hojjettoota Mana Qalamaa fi Itti Fayyadama Gochaalee Nageenya Foonii

Kutaa 1: Odeeffannoo Waliigalaa

1. Maqaa mana qalamuu (abattoir): _____

2. Umurii: ☐ 30 ol ☐ 30 gadi

3. Sadarkaa barnootaa: ☐ Sekondarii ol ☐ Sekondarii gadi

4. Muuxannoo hojii: ☐ Waggaa 5 ol ☐ Waggaa 5 gadi

5. Iddoo hojii: ☐ Bulchiinsa ☐ Qorataa foonii ☐ Qulqulleessaa ☐ Evisereshinii ☐ Kutaa Offal ☐ Skinning ☐ Fe'uu/baasuu karkaasii

6. Saala: ☐ Dhiira ☐ Dubartii

Kutaa 2: Gocha Nageenya Nyaataa

7. Qorannoo fayyaa yeroo yerootti godhachuu: ☐ Eeyyee ☐ Lakkii

8. Gloozii fayyadamuu: ☐ Eeyyee ☐ Lakkii

9. Apeeroon fayyadamuu: ☐ Eeyyee ☐ Lakkii

10. Bootii fayyadamuu: ☐ Eeyyee ☐ Lakkii

11. Kootii hojii (overall) fayyadamuu: ☐ Eeyyee ☐ Lakkii

12. Uffata mataa fayyadamuu: ☐ Eeyyee ☐ Lakkii

13. Faaya uffachuu dhiisuu: ☐ Eeyyee ☐ Lakkii

14. Bakka hojii keessatti nyaachuu ykn dhuguu dhiisuu: ☐ Eeyyee ☐ Lakkii

15. Harka saaphanaa waliin dhiqachuu dura foon tuquu: ☐ Eeyyee ☐ Lakkii

16. Meeshaa ittisa dhuunfaa qulqulluu fayyadamuu: ☐ Eeyyee ☐ Lakkii

17. Leenjii nageenya nyaataa hojii irratti argachuu: ☐ Eeyyee ☐ Lakkii

18. Yeroo dhukkubsatan foon tuquu dhiisuu: ☐ Eeyyee ☐ Lakkii

Appendix 11: Publications

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Discover Food

RESEARCH Open Access

Assessment of infrastructures, operational facilities and food safety practices in municipal abattoirs in central Ethiopia

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Abstract

Suboptimal infrastructures, inadequate facilities, poor hygienic practices in carcass processing represent significant challenges to meat safety and public health. A cross-sectional study was conducted to assess infrastructures, operational facilities, and hygienic meat handling and food safety practices at 30 municipal abattoirs selected from the central part of Ethiopia. Data were collected through semi-structured checklists, questionnaires and direct observations. The study showed that 83.3% of the municipal abattoirs are in unacceptable proximity to residential areas. About 63.3% exhibited pest harborage, breeding places, or signs of pest activity. Furthermore, significant defects were noted in municipal abattoirs, with 60% of the walls in poor condition and inadequate floor sloping (53.3%) toward the drains. Hot water sources, quality control laboratories, and equipment sterilizers were lacking in all surveyed municipal abattoirs. Other operational facilities such as stunning pens and chilling rooms were missing in 96.7% of municipal abattoirs while, isolation pens for disease suspected animals were absent in 63.3% of municipal abattoirs. On the other hand personal hygiene facilities were similarly insufficient or failed to meet acceptable standards in many surveyed municipal abattoirs. For instance, designated and well-designed hand-washing stations (76.7%), showers (60%), and restrooms (53.3%) were absent. Additionally, the provision of sufficient cleaning and disinfection chemicals (83.3%) and personal protective equipment (76.7%) were also lacking. In 70% of municipal abattoirs, pest control, wildlife and stray dog control, and deep freezers were lacking. Skin and bone storage rooms were lacking in 60% of the municipal abattoirs. In about two-third of the municipal abattoirs, separate room for skinning, evisceration, and viscera cleaning were lacking. In terms of hygiene and food safety practices, none of the municipal abattoirs implemented equipment sterilization between uses, and employed refrigerated transport for carcasses. The issue related to hygiene and food safety continue to be compromised as most municipal abattoirs (83.3%); workers and utensils were not restricted to designated working areas and water quality testing was not practiced. About three-fourths of the municipal abattoirs did not process carcasses in a vertical position, and the majority (83.3%) demonstrated inadequate enforcement of personal protective

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Research Article

Microbial Quality of Beef Carcasses and Hygiene Practices at Municipal Abattoirs of Central Ethiopia: A Cross-Sectional Study

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Sage

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Abstract

Microbial contamination of beef carcasses occurs during slaughtering process. Cross-sectional study was conducted to assess microbial quality of carcasses and workers' meat safety practices at five municipal abattoirs in the central part of Ethiopia. Overall, 255 carcass swab samples, along with 30 samples each from hands and knife swabs, and water were collected for microbial quality assessment using standard plating method. Additionally, semi structured questionnaires were administered to 142 abattoir workers to assess their meat safety practices. The study showed that the overall mean aerobic plate count of 6.12 $\log_{10}$ CFU/cm^2 and fecal coliform count of 4.58 $\log_{10}$ CFU/cm^2 on beef carcasses. Carcass swabs had a significantly higher mean aerobic plate count (6.12 $\log_{10}$ CFU/cm^2) compared to knife (5.54 $\log_{10}$ CFU/cm^2; $p=0.006$) and hand swabs (5.19 $\log_{10}$ CFU/cm^2, $p=0.000$) while knife swabs showed a higher mean fecal coliform count (5.05 $\log_{10}$ CFU/cm^2) than carcass swabs ($p=0.005$). Overall 60% of water samples were positive for *E. coli* and fecal coliforms. Furthermore, the use of personal protective equipment including overall coats and boots was 99.30%. All abattoir workers reported abstaining from eating, drinking and smoking at work, and 73.94% did not wear jewelry during slaughter processing. The use of aprons (44.37%) and head covers (45.77%) was practiced by less than half of the respondents, yet 63.38% of workers consistently wore clean personal protective equipment. Task alternation and equipment sharing were common, with 50% of workers engaging in these practices. Sterilization of knives between each use was not practiced by all abattoirs workers, and only 44.37% of workers performed regular medical checkups. About 35.21% of workers practiced hand washing with soap while only 9.15% used gloves during the slaughtering process. Among the respondents, 52.11% of them had received on job food safety training. In conclusion, the study showed high microbial loads on beef carcasses and a low meat safety practice rate (39.44%), highlighting the need for strict and effective multi-stakeholder food safety measures.

Keywords

aerobic plate count, fecal coliform, hygienic practices, meat safety, public health, Ethiopia

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BMC Microbiology

RESEARCH | Article in Press

Molecular characteristics and antimicrobial susceptibility of Shiga toxin-producing *Escherichia coli* O157:H7 from beef cattle feces and carcasses in municipal abattoirs in Central Ethiopia

Endalu Mulatu · Christian Berens · Christian Menge · Kebede Amenu · Nebyou Moje Hawas · Biruhtesfa Asrade · Hika Waktole · Bekele Megersa · Ahmad Hamedy

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Abstract

Pathogenic *Escherichia coli* O157:H7 has been reported to occur on beef carcasses in Ethiopia which can pose a significant public health risk. This study was conducted to assess the occurrence, potential source of contamination and the antimicrobial susceptibility of *E. coli* O157:H7 on bovine carcasses from three municipal abattoirs from Central Ethiopia. A total of 216 fecal and hide samples each, 540 carcass swabs, 18 water samples, and 18 swabs each from workers' hands and knives were collected. The study revealed a 1.4% and 1.1% prevalence of *E. coli* O157:H7 in feces and on carcasses, respectively. *E. coli* O157:H7 was not detected (0%) in the remaining samples including water, hides, workers' hands and knives. A whole-genome-sequencing-based core genome multilocus sequence typing (cgMLST) analysis of the *E. coli* O157:H7 isolates from the same municipal abattoir showed clonal relatedness, i.e. clustering together with no allelic differences (AD) in the cgMLST scheme. However, genetic diversity was observed between isolates originating from different abattoirs, with allelic differences ranging from 140 to 226 AD. All strains carried virulence genes encoding adherence factors (*eae*, *iha*, and *tir*), type III secretion system translocated proteins (*espA*, *espB*, *espJ*, *espF*, *espD*, *nleA*, *nleB*, and *nleC*), outer membrane proteins (*ehaA*, *ompT*, and *traT*), toxins (*astA*, *ehaA*, and *tcpB*), and other pathogenicity-related factors (*gad*, *tsx*, *katP*, and *terC*). All strains carried the *stx2* gene subtype *stx2c*. All isolates were susceptible to the antimicrobials for which valid clinical breakpoints were available and displayed wild-type MIC distributions for most antimicrobials evaluated using available EUCAST ECOFFs. Neither acquired antimicrobial resistance genes nor known resistance-associated chromosomal mutations were detected. The study findings suggest that contact with fecal material during evisceration is a possible source for contaminating the carcasses with *E. coli* O157:H7. Therefore, strict hygienic practice should be followed during carcass processing.

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