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COLLEGE OF VETERINARY MEDICINE AND AGRICLUTURE

OCCURRENCE AND ANTIMICROBIAL SUSCEPITIBILITY OF SALMONELLA AT HUMAN ANIMAL ENVIRONMENT INTERFACE IN HOUSEHOLD SETTING BISHOFTU, ETHIOPIA

BY

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JUNE, 2026

BISHOFTU, ETHIOPIA

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A THESIS SUBMITTED TO THE COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH

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Occurrence and Antimicrobial Susceptibility of Salmonella at Human Animal Environment Interface in Household Setting Bishoftu, Ethiopia

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This is to certify that the thesis prepared by Fozia Hussien entitled: Occurrence and Antimicrobial susceptibility of Salmonella at Human Animal Environment Interface in Household Setting Bishoftu, Ethiopia and submitted in partial fulfilment of the requirement for the degree of Master of Science in One Health complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

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STATEMENT OF THE AUTHOR

I hereby affirm that this thesis represents my original work, and I acknowledge all sources of material used in its creation. This thesis is being submitted as part of the requirements for a postgraduate (MSc) degree at Addis Ababa University College of Veterinary Medicine and Agriculture. It will be archived in the University/College Library for borrowing under library regulations. I solemnly declare that this thesis has not been submitted to any other academic institution to obtain any degree, diploma, or certificate. Brief quotations from this thesis are permissible without special authorization, provided proper acknowledgment of the sources is included.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
DALY	Disability-Adjusted Life Year
GBD	Global Burden of Disease
iNTS	invasive Non-Typhoidal Salmonella
LMICs	Low and Middle-Income Countries
MDR	Multi-Drug Resistance
WHO	World Health Organization
NTS	Non-Typhoidal Salmonella
SPI	Salmonella plasmid virulence
LPS	Lipopolysaccharide
ELISA	Enzyme linked immunosorbent assay
Bfw	Buffered peptone water
XLD	Xylose Lysine Deoxycholate
HE	Hextone Enetric
PCR	Polymerise Chain Reaction
WGS	Whole Genomic Sequence
RV	Rapaport Vassilidas
CLSI	Clinical and laboratory standard institution
PFGE	Pulse-Field Gel Electrophoresis
MLST	Multi Locus Sequence Typing
TSB	Tryptone Soya Broth

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ABSTRACT

Salmonella enterica is a leading cause of zoonotic illness, posing a significant public health concern in household settings, particularly where humans and animals share space and access to clean water, hygiene, and sanitation facilities is limited. The emergence of antimicrobial resistance complicates control efforts. From December 2025 to April 2026, a cross-sectional study was conducted to assess the occurrence and antimicrobial susceptibility of *Salmonella* at the human animal environment interface in household settings in Bishoftu, Ethiopia. A total of 266 households were included, and 1463 samples were collected from infant stool, soil, water, hand rinse, food, and animal feces. A household survey was conducted on hygiene, sanitation practices, and animal waste management, and antimicrobial use. Salmonella was confirmed using Matrix- Assisted Laser Desorption/Ionization time- of- flight (MALDI- TOF) mass spectrometry. Antimicrobial susceptibility testing was performed using the Kirby- Bauer disk diffusion method for nine antibiotics. Critical sanitation gaps included infrequent water treatment (93.98%) and (73.68%) shared latrines, most without handwashing facility (78.5%). The animal waste management system was poor; 73.7% of households either disposed of waste in a waste bin or rubbish pile or did nothing at all, indicating that nearly three-quarters of households lacked proper waste management practices. 80.1% caregivers were unaware of AMR transmission between animals and humans. Over all Salmonella prevalence was 2.12% and the highest prevalence was recorded in animal feces (6.8%). Of 31 82.8% were susceptible, 5.4% showed intermediate resistance, and 11.8% of them exhibit resistance and the highest resistance was tetracycline (32.26%). MDR occurred in 6.5% isolates and all recorded from soil. In conclusion, the study demonstrates critical gaps in sanitation and animal waste management. These finding confirms that poor household practice and animal waste contribute to *Salmonella* transmission and antimicrobial resistance, underscoring the urgent need for integrated one health interventions.

***Keywords**: Salmonella, One health, Antimicrobial resistance, Child care, Household*

1. INTRODUCTION

1.1 Background

Salmonella enterica is a gram-negative, rod shaped bacterium. It the most abundant organism, with more than 2500 serovars having been identified. These serovars are further subdivided, based on their clinical and epidemiological profiles, into typhoidal and non-typhoidal *Salmonella* (NTS) (Eng *et al.,* 2015). Furthermore, typhoidal serovars are subdivided into *Salmonella Typhi* and *Salmonella Paratyphi*. Typhoid serotypes Typhi and Paratyphi both cause human-restricted diseases, including typhoid fever and paratyphoid. On the contrary, NTS causes zoonotic diseases that are widely distributed across human animals and the environment. The zoonotic importance of NTS makes it specifically concerning as it can cross-contaminate species and ecological boundaries through water, food, livestock, and wildlife reservoirs (Majowicz *et al.,* 2010). This can infect many different animals (livestock, wildlife) and spread across ecological settings like farms, water systems, and forests thereby emerging as foodborne threats.

Foodborne infections pose a significant risk to public health, safety, and the global economy worldwide. Foodborne illness causes around 600 million infections and 420,000 mortalities annually. *Salmonella,* which is one of the major foodborne infections, is the third leading cause of death from diarrheal illness worldwide. It accounts for an estimated 115 million human illness and 370,000 deaths annually (Qin *et al.,* 2022). The World Health Organization (WHO) estimates that around one in ten individuals contract foodborne salmonella infection annually, leading to the loss of millions of healthy life years(Lee *et al.,* 2021).

In Ethiopia, diarrheal disease remains one of the leading causes of mortality and morbidity among children under five years of age, with Salmonella being the major causative agent. The burden the disease in Ethiopia is high, with an annual incidence rate of 186.7 thousand and a mortality rate of 150.7 per 100000 children. Several studies have shown the widespread occurrence of NTS in humans, animals, and the environment. In the report, there is a significant level of *Salmonella* carriage in asymptomatic diarrheal patients, food handlers, and livestock populations (Tweldemedhin *et al.,* 2022).

Studying *Salmonella* at the human-animal-environment interface matters given the close contact between people, livestock, pets and with animal and fecal contamination that drive household-level transmission as key one health concern. Poor domestic animal husbandry close proximity to children and inadequate waste management increase the likelihood of zoonotic transmission within household environment where close human-livestock cohabitation is common (Paulos *et al.,* 2025). Children who commonly play in areas shared with animals are highly vulnerable to this environmental contamination due to their developmental stage, characterized by crawling and extensive hand to mouth explanatory behavior.

In recent years, 75% of emerging and re-emerging infectious diseases worldwide have been zoonotic that originate from and more commonly found to affect the poorer and marginalized population, particularly in low-income countries (Schiller *et al.,* 2026). The one health concept recognizes that the health of human, domestic and wild animals, and the wider environment, including the ecosystem, is closely linked and interdependent. Over the last decade, changes to the ecosystem, climate, demographics, socio-cultural, and economic factors in LMICs have continued to reinforce the importance of one health in these settings (Giacomini *et al.,* 2025).

1.2 Statement of the Problem

Salmonella infection continues to pose a significant public health threat globally, imposing an economic burden in both high and low-income countries due to expenses related to surveillance, prevention, and treatment of the disease. The burden is significantly high in low and middle-income countries, where poor hygiene practices, limited access to safe water, and a weak food safety system (Prüss-Ustün *et al.,* 2019).In Ethiopia's urban, and peri-urban areas, raising animals in the family's compound is an alternate method of covering daily expenses and offering a ready supply of animal protein (Ali and Neka 2012).

Even though there has been a lot of progress in increasing access to WASH services, unsanitary waste disposal and poor domestic animal husbandry continue to undermine these efforts, raising major public health concerns. The interaction between humans and animals in the home may encourage the transfer of infectious pathogens from poorly managed excrement to humans (Odo and Mekonnen 2021). In this condition zoonotic pathogens can easily spread between human and animals through direct and indirect contact with animal and contaminated environment which makes it important to address the problem in human animal environment interface (Penakalapati *et al.,* 2017).

The current health system is in danger due to extensive availability and abuse of antibiotics as well as the rise in AMR. In addition, budgetary constraints and difficulties with regulatory standards have resulted in a lack of new pharmaceutical products. Livestock productions and ecosystems have a major impact on altering the resistance profile of microorganisms because antimicrobials used to treat human infection are frequently used in animals, and exposed organisms have been passed from animal to human through food and released into the environment through human and animal excretion (Tweldemedhin *et al.,* 2022).

1.3 Significance of the Study

This study is undertaken to provide data and analysis on the occurrence and antimicrobial resistance pattern of Salmonella species isolated from human, animal and environmental samples in household settings in Bishoftu, Ethiopia. By implementing one health approach, the study examines *Salmonella* prevalence and susceptibility profile across human animal and environmental matrices. The result will also contribute in understanding the local burden of Salmonella and the role of animals and environment in its spread and its resistant trends, supporting the development of targeted and evidence-based interventions. Additionally, the study will be helpful for future research and source of reference data, surveillance and public health planning. It will also helpful in identifying crucial control points in household interface and advise the implementation of one health approach which is essential in mitigating the problem.

General objective

The overall objective of the study was to assess the occurrence and antimicrobial susceptibility pattern of Salmonella at human, animal, environment interface in household setting in Bishoftu, Ethiopia.

Specific objectives

- To assess the clean water, hygiene practice and animal waste management practice in household.
- To determine the occurrence and prevalence of Salmonella at human, animals, and environment interface in household settings in Bishoftu, Ethiopia.
- To determine the antimicrobial susceptibility patterns of the identified Salmonella

2. LITERATURE REVIEW

3.1. General Overview of *Salmonella*

Salmonella is the leading cause of foodborne illness worldwide, and its zoonotic potential puts the health of humans and animals at risk. Theobald Smith first discovered the bacteria in 1885 in the lab of Dr. Daniel E. Salmon at the Bureau of Animal Industry in the United States (Jajere *et al.,* 2019). *Salmonella* is a gram-negative, rod-shaped, facultative bacterium belonging to the Enterobacteriaceae family. The genus has two major species. *Salmonella enterica* and *Salmonella bongori*, of which *Salmonella enterica* is the most significant in terms of one health importance (Eng *et al.,* 2015).

Based on the pathogenicity and host adaptation, *Salmonella* serovars are divided into two major clinical and epidemiological groups: typhoidal and non-typhoidal *Salmonella*. Typhoidal *Salmonella* is further divided into typhoidal and paratyphoid fever, also known as enteric fever. is caused by systemic infections with *Salmonella enterica* subspecies serovars typhi and paratyphi A, B, and C. Typhoid and paratyphoid infections primarily cause bacteremia, febrile illnesses, with prolonged high fever, headache, and malaise being characteristic symptoms. If treatment is not received, typhoid and paratyphoid fever can cause ileus, gastrointestinal bleeding, intestinal perforation, septic shock, and death. (Stanaway *et al.,* 2019).

The non-typhoidal serovars are more prevalent, have broader host ranges, are difficult to control, and cause a sizable amount of salmonellosis in both humans and animals. Although non-typhoidal Salmonella serovars usually induce gastroenteritis that resolves on its own, invasive non-typhoidal Salmonella (iNTS) have emerged that can cause serious systemic illness (Hagedoorn *et al.,* 2023). Non-typhoidal *Salmonella* is of great global economic and public health concern, being one of the most commonly reported bacteria transmitted via animal and animal products and mainly implicated by ingestion of food of animal origin that is contaminated (Antunes *et al.,* 2016)

3.2. Antigenic Structure

The Kauffmann-White, a classification system, is often resorted to for the determination of the antigenic structure of *Salmonella* serotypes, which are divided into three major groups determined by their antigens: somatic (O), flagellar (H), and capsular (k) antigen (Brenner *et al.*, 2000). The somatic (O) antigen, which is heat-stable and produces the oligosaccharide component of bacterial cells' lipopolysaccharide (LPS), is found near the outer membrane of the cell. A particular *Salmonella* serotype may exhibit many O antigens. The H antigens, which are heat-labile and found in the bacterial flagella, play a huge role in triggering host immunological response. Many *Salmonella* species have two flagellar genes, which allow them to express one of the phases I or phase II H antigens. Many of the phase I antigens are serotype specific, with the role of determining immunological identity, while the phase II antigens are regarded as the most globally recognized antigens among all serotypes (McQuiston *et al.*, 2008).

The Capsular (K) antigens, that are polysaccharide, heat-sensitive, is present in the bacterial capsule. The virulence-associated Vi antigen is present in certain serotypes, like S. typhi, S. paratyphi C, and S. Dublin (Brenner *et al.*, 2000). So far, more than 2500 serotypes have been characterized, and more than 1500 of them are included in *Salmonella enterica* subspecies enterica. The majority of human infections are due to these serotypes, thus knowledge of their antigenic structure composition is of great importance for epidemiologic diagnosis as well as vaccine development (Guibourdenche *et al.*, 2010).

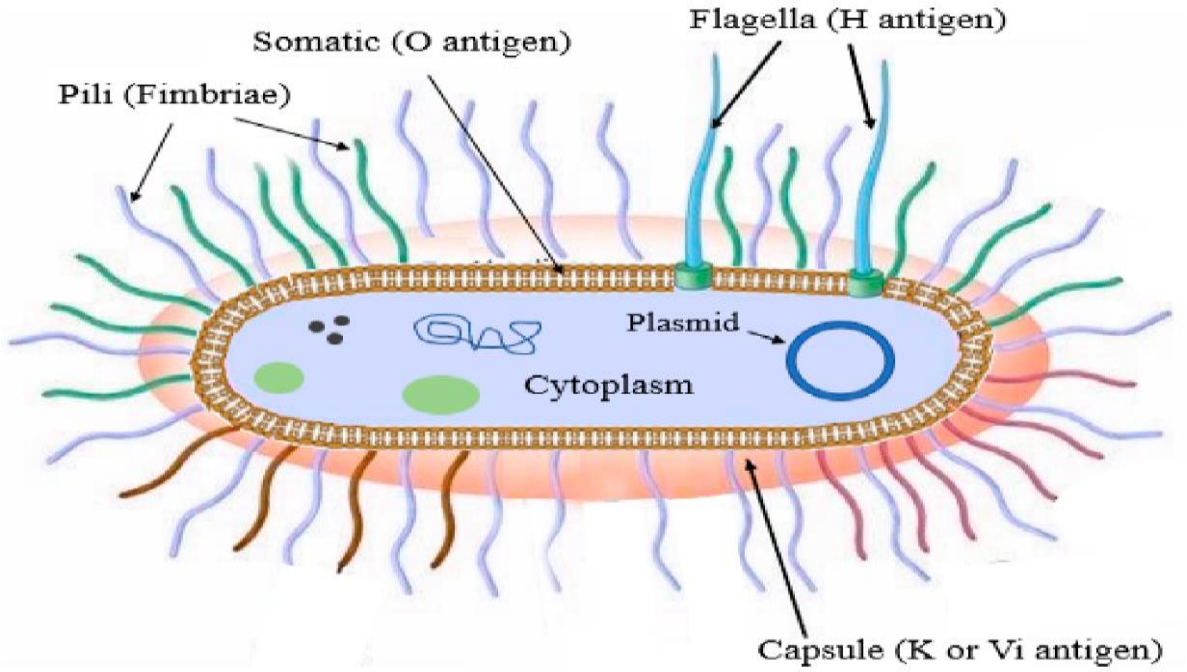

Figure 1: Schematic presentation of *Salmonella* surface antigens
Source:https://www.cram.com/flashcards/enterobacteriaceae-5178261

3.3. Virulence Factor and Pathogenicity

The pathogen *Salmonella enterica* has a wide range of virulence characteristics, which enable it to enter, persist, and proliferate within host cells. The elements are divided into various divisions and include the bacteria's capacity to cling to surfaces, form fimbriae, move with a tail, form a protective coating, and have secretion systems. Together, these virulence factors enable the bacterium to adhere to host cells, enter the cells, evade the immune system, and travel through the bloodstream to cause infections (Jennings *et.al.*, 2017).

The *Salmonella* pathogenicity islands (SPIs) encode the type III secretion system (T3SS), which is essential to this process. More precisely, SPI-2 is involved in intracellular persistence and dissemination throughout the body, whereas SPI-1 is responsible for epithelial cell invasion and induction of macrophage death. Other SPIs, SPI-3 to SPI-6, increase *Salmonella's* adaptability and virulence in various habitats by secreting toxins, influencing the host's defence, and assisting in coping with environmental stress (Ebert *et al.*, 2004).

Aside from the chromosomal SPIs, the spv (Salmonella plasmid virulence) locus, which enhances bacterial growth in the liver and spleen during systemic infections, is found in virulence plasmids, which are typically 50-100 kb in size. Furthermore, *Salmonella* can produce a variety of toxins, including haemolysin HyIE, endotoxins, enterotoxins, and cytotoxins (Barth and Bauerfeind 2005). All these toxins increase the severity of illness. In contrast, enterotoxins and cytotoxins cause diarrhoea and tissue damage by harming intestinal and epithelial cells, whereas endotoxins (lipopolysaccharide, LPS) trigger inflammatory reactions and septic shock. A shift in the pathological result from moderate gastroenteritis to severe systemic sickness has been linked to variation in toxin production corresponding to serovars such as S. typhi (Gal-Moret *et.al*., 2014).

Furthermore, the bacterial surface elements, such as flagella and fimbriae, are crucial for both immune system evasion and host adaptability. Fimbriae are in charge of bacteria's ability to adhere to intestinal mucosa, create biofilms, and survive both inside and outside the host. On the other hand, the mobility and antigenic variety provided by flagella enable the bacteria to evade detection. The bacterial detection is less vulnerable to the host antimicrobial peptides and acidic conditions because of lipopolysaccharide (LPS) coating and other polysaccharides on their surface. Together, these virulence characteristics enable *Salmonella enterica* to inhabit diverse ecological niches, colonize a variety of hosts, and cause a broad spectrum of disease (Ilyas *et al*., 2017).

2.4. Salmonella at Human Animal Environmental Interface

In Ethiopia salmonella poses huge public health concern as zoonotic and foodborne. *Salmonella* is widely spread not only in the countryside of the country but also urban and peri-urban areas. The main source of the contamination related with close contact with animal which is common in our country where we live in proximity with animal and marginalized household settings, poor food handling and personal environmental hygiene practices which leads to recurrent of the infection (Tilahun *et al.*, 2025).

The system of livestock farming in Ethiopia is so tightly integrated with family living the risk of salmonella passing along the human animal environment interface is greatly raised. Backyard or neighbourhood rearing of poultry, cattle, small ruminant is common and thus leads to direct and indirect human contact through manure handling, feeding, or cleaning practice. The most recent cross-sectional study conducted which was carried out in Amhara regional state across reported that humans, animals were infected with *Salmonella* duty poor hygiene, shared household setting and improper waste management (Mengistu *et al.,* 2025).

Aside from livestock, domestic pets in Ethiopian households are also significant carriers of Salmonella. The feces of animals, lack of hygiene in the kitchen, and common living areas are the source of environmental contamination lead to indirect exposure. Kiflu *et al* (2017) isolated salmonella from 11.7 % 0f healthy looking dogs in Addis Abeba, which pointed out the possibility of zoonotic infection through close pet-human contact and lack of hygiene. In another recent showed that vegetable farm around Addis Abeba, which is treated animal manure, had the presence of multidrug- resistance of *Salmonella enterica*, thus demonstrating how contaminated waste from household livestock or pets could spread to the larger domestic environment and highlights the close relation of Salmonella infection at human animal environment interface (Waktole *et al.,* 2024)

2.4.1. Transmission of Salmonella

Salmonellosis in humans and animal is serious public health issue with considerable health social economic issue in both developed and developing countries. The infection is mostly devastating in low- and middle-income countries (LMICs) (Majowicz *et al.,* 2010) where the environment plays huge role which is favourable condition for the bacteria. Many animal species especially chickens, pigeons, and reptiles as potential reservoirs for *Salmonella enterica* causative agent for Salmonellosis (Djeffal *et al.,* 2017).
In household settings, particularly in low- and middle-income countries, the close cohabitation of humans and livestock creates a significant hotspot for the transmission of *Salmonella.* The occurrence of Salmonella infections at this interface is facilitated by the contamination of the immediate environment, such as courtyards and water sources, with animal feces. Poultry and livestock, often asymptomatic carriers, serve as primary reservoirs, and transmission to humans occurs through direct contact or via contaminated food and

water (Wilson *et al.*, 2025). Studies from regions like Ethiopia have demonstrated a high prevalence of *Salmonella* in backyard poultry and in the household environment, which correlates with a high incidence of diarrheal diseases, especially among children who have the closest contact with animals and the environment (Beyene *et al.*, 2024).

The severity of infection depends on the host's health and the strain of *Salmonella*. Population at risk mainly the elders, children under five and immunocompromised patient due to weak immune system. HIV/AIDS individuals especially those with low CD4 counts are susceptible to non-typhoidal infection (NTDs) (Marchello *et al.*, 2022).

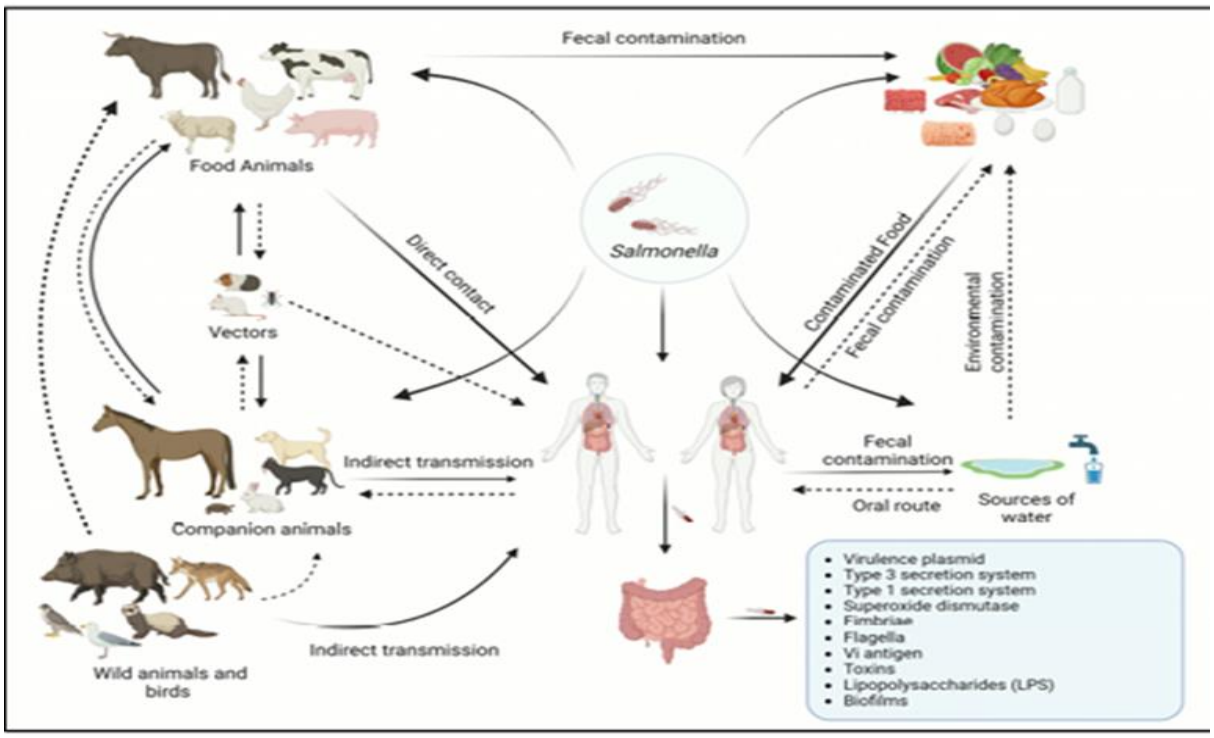

Figure *2*: Schematic representation of the transmission pathways of salmonella at human animal environmental interface

Source*:* (Lamichhane *et al.*, 2024)

2.4.2. Clinical aspects of Salmonella

Typhoidal *Salmonella* infection which is human restricted infection consists 2 serotypes. These serotypes are *Salmonella typhi* and *Salmonella Paratyphi* and they further divided into *S. Typhi* and para typhi A, B, C and cause typhoidal or enteric fever. The enteric fever causes a serious illness and endemic in our country Ethiopia. They are widespread in place with poor sanitation and limited to access to clean water (Asnake *et al.,* 2025). The incubation period is two weeks where they invade the intestinal mucosa during this period and spread to the blood leading to bacteraemia and the symptom includes prolonged fever, malaise, abdominal tenderness, and enlargement of the liver and spleen the degree of the symptoms varies based on the individual immune statues of host and virulence of the infecting strains (Wain *et al.*,1998).

In contrary non typhoidal *Salmonella* (NTS) cause broad-spectrum disease both in humans and animals. They play a major role in causing gastroenteritis which is self-limiting and characterized by diarrhoea, abdominal cramps, nausea, vomiting, and fever; however, invasive infections sometimes occur in individuals with suppressed immunity, new-borns, and old people. They route of transmission through consuming contaminated animal products and enters the body and attaches and penetrate intestinal epithelial cell where it produces toxins and produce where it disrupters the mucosal barrier and activate inflammatory response(Guiney *et al*., 2011).

2.4.3. Growth and environmental survival

Salmonella is a non-fastidious bacterium because it can grow in various environmental conditions outside a living host. Most serovars can grow at temperatures between 5°C and 47°C, with an optimal range of 32°C-35°C. However, the same serotypes can grow at a much wider temperature range, as low as 2-4°C or as high as 54°C, and become heat-sensitive, being killed at temperatures of 70°C and above. The pH range conducive to the growth of *Salmonella* is 4 to 9, with an optimal range between 6.5 and 7.5. *Salmonella* can endure dry conditions but can also survive in water for varying durations, from days to several months, making it extremely persistent (Alegbeleye *et al.*, 2023)

These traits have a considerable impact on environmental preservation and climate change effects. The combination of humidity, warmer temperatures, and shifted rains might lead to the microbial presence and spreading of *Salmonella* in all parts of the ecosystem, such as soil, water, and food. Besides, the bacterium's ability to survive under various conditions allows it to thrive even when climate changes cause a rise in temperature and humidity in a particular area, hence it can be in more locations and during more months which would then affect the degree of infection in both humans and animals. Thus, environmental factors, made worse by climate change, are the main determinants in the understanding of the ecology and transmission dynamics of *Salmonella* (Morgado *et al.*, 2021).

2.5. Detection of *Salmonella*

2.51. Serological method of detection

Serological testing is an important tool, especially in the diagnosis of diseases such as salmonellosis. These tests are designed to accurately determine specific antibodies or antigens in a patient's blood serum, providing a means of confirming the presence of pathogens or monitoring the body's immune response(Avolio *et al.*, 2025) . The enzyme-linked immunosorbent assay (ELISA) is a commonly used serological technique in the diagnostic toolbox. By enabling quick and precise identification of Salmonella infections, the use of serological testing in the diagnosis of salmonellosis greatly improves patient care and public health management. Early diagnosis and treatment are made possible by tests like the Widal test and the enzyme-linked immunosorbent assay (ELISA), which assist in identifying antibodies or antigens unique to Salmonella serovars. In endemic areas, the Widal test is frequently used to diagnose typhoid fever; when paired with clinical signs, it has a sensitivity of 70–80% and a specificity of 90% (Nazir *et al.*, 2025).

2.5.2. Cultural bacterial isolation

Salmonella bacteriological detection commences with cultural isolation on media that are both selective and differential. After the pre-enrichment step, in buffered peptone water (Bfw), the samples are placed on the selective agar xylose lysine Deoxycholate agar (XLD), hektoen enteric (HE), or MacConkey agar, which not only kill the competing bacteria but also allow differentiating based on the production of either lactose or hydrogen sulfate. *Salmonella* usually exhibits a growth pattern of colonies on XLD or HE agar that do not ferment lactose and are thus coloured black due to the H_2S production. Then these colonies are sub-cultured onto nutrient agar for the isolation of pure cultures that will be subjected to biochemical identification tests (John *et al.*, 2008).

2.5.3. Molecular characterization

Molecular profiling of *Salmonella* is a necessary method for the comprehension of its genetic variety, distribution, and virulence mechanisms. Molecular methods provide a precise identification of the microorganism's species, serovar, and even strain, which is not the case with the traditional culture-based techniques (Li *et al.*, 2021). The most common molecular methods are PCR, PFGE, MLST, and WGS. While the latter reacts to and confirms the presence of specific virulence genes, such as invA, hilA, and stn, in a matter of hours, it also provides highly accurate detection of pathogenic strains in the sample footprints, whether they are of clinical, animal, or environmental origin. On the other hand, PFGE and MLST are the most incisive tools in the hands of epidemiology since the former allows the tracing of DNA patterns and the latter is the tracing of the variation in sequences (Yan *et al.*, 2021).

Whole-genome sequencing (WGS) has completely changed the way *Salmonella* is molecularly characterized and by this provided a complete picture of AMR genes, virulence factors, and phylogenetic relationships. Apart from this, WGS has enabled the tracking of outbreaks at a very high resolution as well as the identification of transmission routes among humans, animals, and the environment, which is in accordance with the One Health approach(Pornsukarom *et al.*, 2018).

Furthermore, through the molecular characterization, the monitoring of the emergence of MDR strains which are now being reported more often worldwide and that represent a major public health concern, is easier The combination of molecular data with traditional epidemiological information not only strengthens outbreak response but also informs targeted interventions, hence being an integral part of modern *Salmonella* surveillance (Chen *et al.*, 2025).

2.6. Antimicrobial Resistance of *Salmonella*

The emergence of antimicrobial-resistant *Salmonella* species is another global challenge, especially low and middle-income countries(LMICs), where there is increased misuse of antimicrobial agents in humans as well as in animals is associated with different biological, pharmacological societal factors that worsen the problem in low and middle-income countries(Sulis *et.al.*, 2022). Antimicrobial-resistant *Salmonella* is a global problem in today's healthcare procedures, and a strain on the verge of pan-resistance was recently discovered (Le Hello *et al.*, 2013). The prevalence of multidrug-resistant *Salmonella* has grown, and outbreaks caused by MDR strains have been observed in Sub-Saharan Africa. Infections with MDR pathogens are associated with increased morbidity and death, most likely due to the co-selection of drug resistance and virulence (Mølbak *et.al*., 2005).

In developing countries, including Ethiopia, a high frequency of resistant *Salmonella* spp. against different antimicrobial agents has been reported. The therapeutic management of the disease is difficult because isolation of *Salmonella* species (Belachew *et al.*, 2021). In most laboratories remains a challenge due to inadequate laboratory facilities required for the accurate detection of bacteria and to perform antimicrobial susceptibility testing. Hence, drug sensitivity tests are not routinely carried out, and treatment alternatives are not available in most health care facilities (Tadesse *et.al.*, 2014).

The household environment also acts as a crucial amplifier for antimicrobial resistance (AMR). The unrestricted use of antibiotics in animal husbandry for prophylaxis or growth promotion exerts a selective pressure that drives the emergence of resistant *Salmonella* strains. These resistant bacteria are then shed into the environment and can be transmitted to humans. Consequently, multidrug-resistant *Salmonella* isolates, showing resistance to first-line antibiotics like ampicillin, tetracycline, and co-trimoxazole, are frequently reported in household studies (Abate *et al.*, 2021).

Table 1*:* Reported prevalence and antimicrobial resistant of Salmonella in Ethiopia

Study area	Study unit	Sample type	Prevalence	AMR	Reference
Bahirdar, Desse, Deberemarkos Gondor	Humans Animals Chicken	stool Cloacal swab, rectal swab	12.1% 4.1% 3.2%	Human resistant 79.6% amp,tet 71.1% Animal amp, tet 100%	(G. Mengistu *et al.,* 2025a)
Bahirdar Gondor	Human Animal Environment	Stool& hand swab Feces& milk Swab& farm sewage	4.3%&1.7% 11.9% &10.3% 5.2% & 10.5%	Resistant Tet 42.9% Amp57.1% Clp 35.7%	(Beyene et.al., 2024)
Adama Modjo	Human Animal Environment	Stool Cloacal swab, fecal droppings &feed samples floor swab,	2.8% 0.3% &5.5% 3.4%	High resistant showed in Strepto 80% Tet 76%	(Belina *et al.,* 2021)

2.7. Prevention and Control of *Salmonella*

To achieve effective control and prevention of *Salmonella*, we must apply a one health approach, which is a holistic and transdisciplinary approach that enables us to apply prevention and control across the human, animal, and environmental sectors. Timely identification of *Salmonella* depends on the coordinated surveillance system of the country, which mainly monitors in humans, animals, and the environment (Belina *et al.,* 2021).

For instance, a Study conducted by Beyene *et al* (2024) highlights that the combination of clinical samples from humans, animal feces, and environmental swabs strengthens the detection of circulating *Salmonella* strains and identifies a possible source of zoonotic transmission. Similar to this, environmental monitoring, which includes testing food, soil, and water, is essential for identifying hotspots for contamination and stopping outbreaks before they start (Lulie *et al.,* 2024).

Intervention at several levels is required for control and preventive strategies. Biosecurity measures such as restricting access to animal housing and isolating from household settings,

routine cleaning, and effective waste management that reduces cross-contamination with that specific household setting (Mkangara *et.al.*, 2023). Additionally, keeping up proper animal husbandry lowers pathogen shedding and enhances animal health. Food safety intervention is also crucial; proper safety handling, proper cooking and hygienic storage prevent contamination of meat, eggs, and diary product at every stage of food chain. Combining both hygienic practice and biosecurity are essential in the prevention of Salmonella in all sector (Nazir *et al.*, 2025).

4. MATERIALS AND METHODS

3.1. Study Area Description

The study was conducted in Bishoftu town, located in East Shewa Zone of Oromia regional state in Ethiopia. The town is 47 km southeast of Addis Ababa, the capital city of Ethiopia. It lies at 8°04′5″N latitude and 38°05′9″E longitude, with a maximum altitude of 1850 meters above sea level (NMSA, 2010). The total human population of the town is estimated at around 207,383 (NMSA, 2022). Bishoftu is considered as urban and peri-urban livestock corridor area in Ethiopia. Smallholder farming in particular often occurs within the household compounds or confined areas, resulting in frequent human-animal interaction and environment contamination. The area is known for its dense population and expanding animal husbandry practices, which increase the close interaction between animals and people. The town is divided into three sub-cities and total of eleven Kebeles.

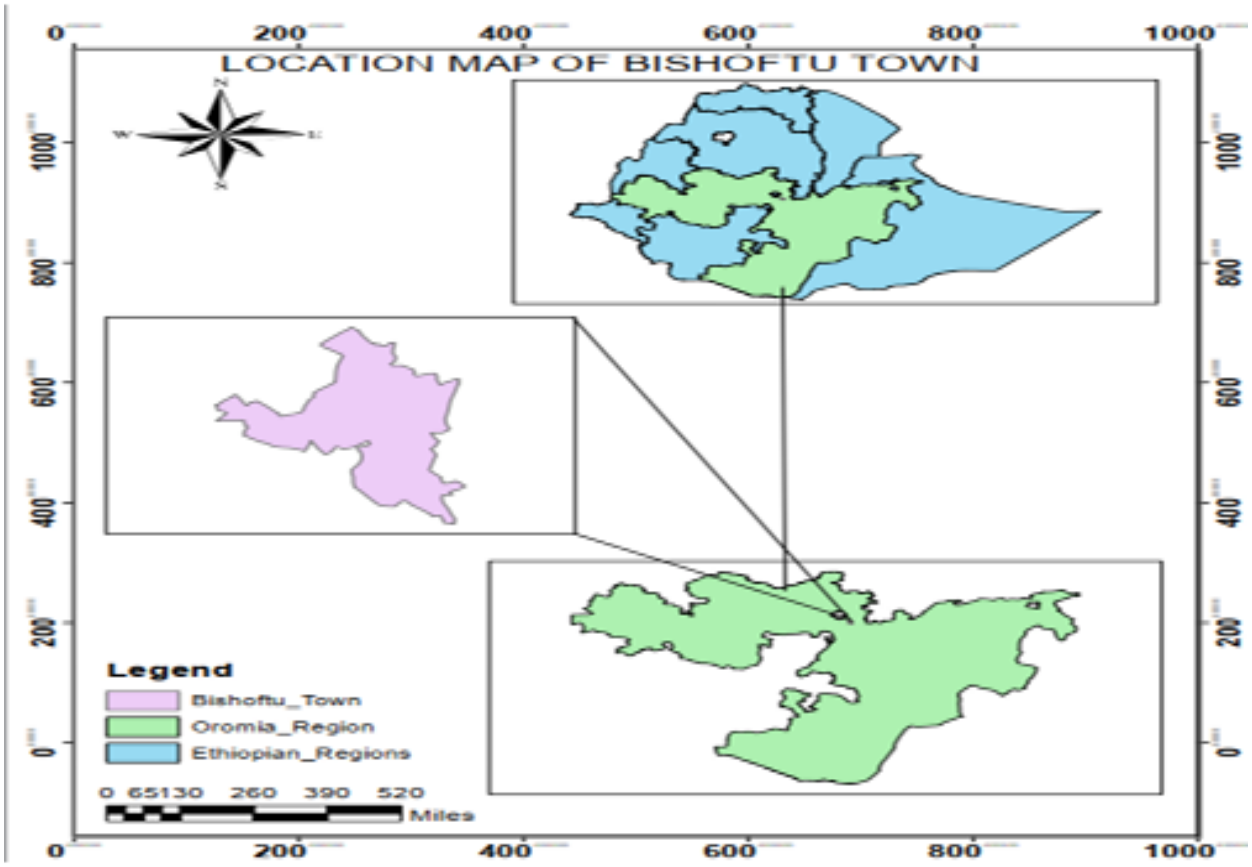

Figure 3: Map of the study area

This map is developed using ArcGIS from Ethiopian shape files.

3.2. Study Design and Population

A cross-sectional study was conducted from December 15, 2025-April 15, 2026. Based on information that was obtained from Bishoftu health office and the pilot research conducted prior to the actual an area formerly known as Kebeles two found in Dhibayu sub city were purposely selected based on comparatively high infant population and wide spread practice of animal husbandry practice in resident compounds. Households having an infant of six to twelve months old and at least one domestic animal were recruited rolling basis into the study.

3.3. Sample Size Determination and Sampling

The sample size was calculated based on expected prevalence of 22.5% of diarrhoea in infants aged between six to twelve months' prevalence of diarrhoea in infants into account (EDHS, 2017), 5% of margin of error and a 95% confidence level using the following formula, 266 households will be selected, and the calculation will be carried out based on the following formula.

$$n = \frac{Z^2 \times Pexp\ (1-Pexp)}{d^2}$$

Z= 1.96(95% Confidence level)

D= 0.05 (desired precision)

Pexp= proportion (22.5%)

n= 266

3.4. Data Collection Tools and Methods

3.4.1. Household survey

A structured questionnaire was administered on rolling bases used to collect data on infants' potential exposure to *Salmonella* and the antimicrobial resistance of *Salmonella*. The questionnaire format was designed to base on previous similar tests. The questionnaire was first developed in English language and then was translated to the local dialects (Amharic or Afan Oromo) by a qualified language expert. The draft questionnaire was pretested with three caregivers who were excluded from the final study. Finally, the questionnaire was uploaded into Research Electronic Data Capture (REDCap), an online data collection application (REDCap, https://projectredcap.org) that allows offline data collection, and a face-to-face interview with the infant's primary caregiver will be performed using a smart tablet. The structure of the questionnaire consists of four themes or categories:

i) Social-demographic characteristics of infant caregivers,
ii) Access to clean water, sanitation and hygiene services
iii) Animal ownership and hygienic waste management practices, and
iv) Childcare practices in the household
v) Knowledge of caregivers on antimicrobial usage.

Health extension worker, who serve as facilitator in identifying the households who are eligible for the study after explaining the objective and providing written study information about the study.

3.4.2. Sample collection

Parallel to questionnaire survey, a total of 266 participating households were visited in the morning, and samples such as stool samples from infants, soil from household yard, hand rinse of infant caregiver and fresh animal fecal samples, drinking water food samples of the infants were collected. All the samples were collected once a week on the same sampling day.

Infant stool samples were collected, following prior notification and provision of a disposable diaper on the preceding evening. On each sample collection date, approximately 2 grams of stool was transferred into a pre-labelled sterile collection container. Soil samples

were collected from three to five mapped sites within the household compound. A sterilized spatula was used to collect the 18 soil at a 45^0 angle to a depth of 5 cm. For cemented surfaces, sterile dry gauze was used to rub the surface horizontally and vertically from three to five sites. The soil or swab samples were then placed in labelled sterile whirl-pack bags to ensure aseptic conditions.

The caregiver hand rinse sample was collected by slowly inserting the caregiver's hands into a whirl-pack bag containing 225 ml Buffer Peptone Water (BPW) until the broth covered the hand up to the wrist and rubbing the hands gently to release any contamination. Animal fecal samples were collected from the ground of one animal species in a selected household. In case the household owns different animal species, the sample was collected from the one most likely to have contact with infants such as poultry and pet animals. Up to 25 grams of freshly voided fecal material was collected and placed in a pre-labelled sterile whirl-pack bag.

Drinking water samples was collected from the source used from infant consumption 25ml of water was transferred into pre-labelled whirl-pack bag. Infant food also was collected by transferring approximately 25 grams of prepared food into a pre-labelled whirl-pack bag. Finally, all collected samples were transported within 6 hours to the Enteric-pathogens laboratory found in Addis Ababa University, College of Veterinary Medicine and Agriculture using an ice box containing an ice pack and stored at +4 °C until processing within 24 hours.

3.4.3. Isolation of Salmonella species

Identification of *Salmonella* will be performed based on the recommended bacteriological technique which includes a series of procedures, i.e. primary enrichment, selective enrichment and molecular confirmation method (Antunes *et al.*, 2016).

The primary enrichment will be done by measuring 2 g infant stool sample will be inoculated 8 ml BPW (Oxoid, UK) and 25 g of animal feces, food sample and soil samples into 225 ml BPW. The bag containing soil samples were homogenized by hands before incubation. For drinking water, 25 ml water of water will be passed through sterile filter paper with 45 nanometre pore size to trap the particle, after which the filter paper was aseptically transferred into 90 ml of BPW for pre-enrichment sample

All the pre-enriched samples, including the hand rinse samples will be incubated at 37^0c for 24 hr. Then, from the pre-enrichment culture 0.1 aliquot will be transferred into a tube containing 10 ml Rapaport-vassilidas (RV) broth (Oxoid, UK), selective secondary enrichment, and incubated for 24 hr at 41^0c). A loop full of aliquot from the selective enrichment will be streaked on Xylose Lysine Deoxycholate (XLD) (Oxoid, UK) agar plates and incubated at 37^0c for 24 hr. The plates will be screened for the growth of *Salmonella*, which appeared to be small black colonies. A typical one colony from each plate will be sub-cultured into Tryptic Soya Broth (Oxoid, UK) and incubated at 37°C for 24 h and then preserved at -20°C as glycerol stocks at 1:2 cultures and 80% glycerol ratio for future isolation and analysis.

3.4.4. Matrix-assisted laser desorption and ionization technology (MALDI-TOF)

The Extended Direct Transfer (eDT) procedure of MALDI-TOF was conducted at Animal health institute (AHI) for confirming presumptive *Salmonella* colonies based on a protocol suggested by Bruker MALDI Biotyper Manufacturer (Bruker *et.al,* 2015). A presumptive salmonella isolate from the glycerol stock was sub-cultured on nutrient agar and incubated at 37°C for 24 h. A single colony from nutrient agar was then be picked by stick and smeared as a thin layer on a spot on a MALDI target plate. One µl of 70% formic acid was added to each spot and allowed to dry at room temperature. After drying, 1µl of α-Cyano-4-hydroxycinnamic acid (HCCA matrix solution) was then added and again left to air dry. We inserted the prepared target plate into MALDI-TOF Biotyper instrument for spectral acquisition and identification according to the manufacturer's instructions.

3.4.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility of the *Salmonella* isolates was determined using the disk diffusion method (Kirby-Bauer) according to the Clinical and Laboratory Standards Institute (CLSI) Supplement M100 (CLSI, 2023). The following nine antimicrobial drugs were used: ampicillin (10 µg) and amoxicillin-clavulanic acid (30 µg) from the beta-lactamase group; gentamicin (10 µg) and streptomycin (10 µg) from the aminoglycoside group; tetracycline (30 µg) from the tetracycline group; ciprofloxacin (5 µg) and Norfloxacine (10 µg) from the

fluoroquinolone group; co-trimoxazole (25 µg) from the folate pathway inhibitors; and azithromycin (15 µg) from the macrolide group.

A loopful of the preserved Salmonella culture was sub-cultured on nutrient agar and incubated at 37°C for 12 h to obtain a pure, fresh culture for antimicrobial susceptibility testing. The test broth was adjusted to McFarland 0.5 turbidity to obtain the appropriate bacterial population. Mueller-Hinton agar plates (Oxoid, UK) were prepared according to the manufacturer's instructions. A sterile cotton swab was soaked in the inoculum suspension, then swabbed evenly across the surface of the Mueller-Hinton agar plates. Once the plates dried, antibiotic disks were positioned on the inoculated plates using an antibiotic disk dispenser. Plates were then incubated at 37°C for 18 hours. After incubation, the diameter of the inhibition zone around each disk was measured using a transparent ruler. Results were categorized as susceptible, intermediate or resistant based on the standardized table provided by CLSI (APPENDICES 2). Isolates that exhibited resistance to at least one antimicrobial from three antimicrobial classes were considered MDR Salmonella (Magiorakos *et al.*, 2012).

3.5. Data Management

The survey data was collected using REDCap, and the laboratory results were entered to Kobo Toolbox, web-based applications used to manage research data. Both datasets were downloaded and exported to a Microsoft Excel for cleaning and preparation prior for analysis. Statistical analyses were performed using STATA statistical software, version 14. Descriptive statistics such as frequency and percentages was used to summarize the occurrence of Salmonella and the antimicrobial susceptibility profiles across the sample sources.

3.6. Ethical Clearance

Community health extension worker facilitated the enrolment process, survey data and samples are collected after obtaining written informed consent from the child's primary caregiver. A copy of the consent form was provided to the participant for their permanent record. Respondents were informed that their responses would be kept confidential, and they are free to withdraw from the study at any time. The study was conducted only after obtaining informed written consent from all voluntary participants. If a household reuses the next eligible household was approached following the same protocol. Ethical clearance for the study was obtained from the scientific and ethical review committees of Addis Ababa University, Aklilu Lemma Institute of Pathobiology (Ref. No: ALIPB IRERC/97/2015/23) and the University of Iowa, USA (IRB ID No: 202302098).

4. RESULT

4.1. Household Survey

4.1.1. Demography and socio-economic characteristics of surveyed households

A total of 266 households were included in the survey. Among the infants in analysis, 137(51.5%) were females, and 129 (48.5%) were males. The majority of primary caregivers were mothers (88.35%), followed by fathers (3.01%) and grandmothers (2.63%). More than two-thirds (77.07%) of primary caregivers were housewives, and only 29.32% had attended higher education. Approximately 10.9% of the caregivers shared a living yard with multiple private family households, while 75.94% shared with multiple unrelated households. Additionally, 78.2% of the homes in the household had cement floors, whereas around 19.92% had dirt floors covered with carpet or vinyl. Most (66.17%) households had grass or dirt in the compound. The detailed socio-economic data is summarized in the table.

Table 2: Demographic and socioeconomic characteristics of caregivers and infants

Variables	Category	Frequency (n)	Percent (%)
Infant sex	Female	137	51.50
	Male	129	48.50
Relationship with the infant	Mother	233	88.35
	Father	8	3.01
	Grandmother	7	2.63
	Other Guardian	16	6.02
Marital status of caregiver	Married	236	88.72
	Single	17	6.39
	Divorced	13	4.89
Educational status	Secondary	103	38.72
	Primary	78	29.32
	Higher	78	29.32
	None	7	2.63
Occupation	Housewife	205	77.07
	Professional	28	10.53
	Vendor	11	4.14
	Janitor	11	4.14
	Secretarial	5	1.88
	None	3	1.13
	Childcare	2	0.75
	Hired help	1	0.38

Type of residence	Compound shared with other families	202	75.94
	Compound with multiple private family households	29	10.90
	Separate house, private yard	28	10.53
	Apartment or room in multi-unit building, no yard	6	2.63
	Free standing house, gated community	1	0.38
Floor type	Wood, parquet, cement or ceramic	208	78.20
	Dirt floor covered with carpet or vinyl	53	19.92
	Uncovered dirt	5	1.88
Material of ground in compound	Grass or dirt	176	66.17
	Cement, brick or tile	90	33.83
Flood history	No	195	73.31
	Yes, from rain flooding	63	23.68
	Yes, from community flood water	7	2.63
	Yes, from waste water drains	1	0.38

4.1.2. Household water, sanitation and Hygiene (WASH) characteristic

Out of 266 participants, the majority of households (63.91%) had access to tap water located within their compound, followed by those used bottled or packaged water (24.81%), while only 2.36% had a tap inside their private house (Table 3). Nearly all households (93.98%) reported using no water treatment method, with only 4.89% boiling water and 1.13% using Wuha agar (a local water treatment product. Regarding sanitation, latrine access was nearly universal (98.12%), though most latrines (73.7%) were located in the compound and shared with other households, while only 7.1% had a latrine inside their own household. Hand hygiene infrastructure near latrines was notably deficient, with only 21.1% having water available and just 14.7% having soap present for hand-washing, implying critical gaps in hygiene practices despite relatively good water access and latrine coverage.

Table 3: Household Water, Sanitation, and Hygiene (WASH) Characteristics (N=266)

Characteristic	Category	Frequency (n)	Percentage (%)
Water Source	Tap inside the house	7	2.36
	Tap in the compound	170	63.91
	Public tap	17	6.39
	Bottled or packed water	66	24.81
	Other	6	2.26
Water Treatment Option	Wuha agar	3	1.13
	Boiling	13	4.89
	None	250	93.98
Access to Latrine	Yes	261	98.12
	No	5	1.88
Location of Latrine	In the household	19	7.14
	In the compound, not shared	49	18.42
	In the compound, shared with other household	196	73.68
	Community/public latrine	2	0.75
Water for Hand Washing Near Latrine	Yes	56	21.05
	No	210	78.95
Soap Near Latrine	Yes	39	14.66
	No	227	85.34

4.1.3. *Animal ownership and waste management*

Table 3 shows a summary of 133 households that owned animals. Animal ownership by households (n=133) varies considerably with dogs (54.9%) and chickens (42.9%) were the most frequently owned animal species, followed by cats (36.1%), while ruminants including cattle and sheep (6% each), goats (3%) were owned by few households. Equine (0.8%) and other species (5.3%) were less commonly owned. Animal manure management showed that the majority of households either disposed of waste in a waste bin or rubbish pile (37.6%) or did nothing at all (36.1%), indicating that nearly three-quarters of households lack proper waste management practices. A smaller proportion of households used a drain or rubbish pile outside the house (12.8%) or deposited waste into a latrine pit (11.3%), while only 3% reported using animal feces as fertilizer.

Table 4: Animal Ownership and Waste Management Characteristics

Characteristic	Category	Frequency (n)	Percentage (%)
Animal Species at households	Dog	73	54.9
	Chicken	57	42.9
	Cat	48	36.1
	Cattle	8	6.0
	Sheep	8	6.0
	Goat	4	3.0
	Equine	1	0.8
	Others	7	5.3
Animal feces M anagement by households	Dispose in bin or rubbish pile	50	37.6
	Nothing is done	48	36.1
	Drain or pile outside of the house	17	12.8
	Latrine pit	15	11.3
	Used for crop fertilizer	4	3.0

4.1.4. *Infant Care Practice and Health History*

As presented in Table 5 majority of infants (90.2%) were reportedly breastfed, while only 9.8% were not. Regarding complementary feeding practices, nearly all (97.7%) of caregivers reported not following appropriate feeding practices. In terms of recent illness history, the majority of infants (70.93%) had no symptoms of illness in the last seven days. Among those showed illness, fever was the most commonly reported symptom (13.1%), followed by diarrhoea (6. 6%).Most of ill infants (83.8%) received no treatment at all, while only 15.0% received drugs or antimicrobials, and very few received zinc treatment (1%) or oral rehydration solution (0.4%).

Table 5: Infant Care Practice and Health History Characteristics (N=266)

Variables	Category	Frequency (n)	Percentage (%)
Breastfeeding	Yes	240	90.23
	No	26	9.77
Feeding Practice	Yes	6	2.26
	No	260	97.74
Signs and Symptoms in the Last 7 Days	No symptom at all	205	70.93
	Fever	38	13.12
	Diarrhoea	19	6.57
	Vomiting	14	4.84
	No appetite	10	3.46
	Fatigue	3	1.04
Treatment Received	No treatment	223	83.83
	Drugs/Antimicrobials	40	15.04
	Zinc treatment	2	0.75
	Oral rehydration solution	1	0.38

4.1.5. Antimicrobial uses and knowledge caregivers

The questionnaire survey on knowledge and practices of caregivers related to antimicrobial use showed that majority (92.5%) of them agree that drugs should be prescribed by professionals. Most caregivers (89.1%) reported using antimicrobials to treat infection, with smaller proportions using them for prevention (4.5%). Nearly half (48.1%) of caregivers did not know the cause for resistance. When they were asked about the effects of AMR, most caregivers (58.3%) admitted they did not know. While 35.7% correctly recognized non-response to microbial treatment. Surprisingly, 80.1% of caregivers did not know whether AMR can be transmitted between animals and humans.

Table 6: Antimicrobial uses and knowledge caregivers on drug use

Variables	Category	Frequency (n)	Percentage (%)
Drug must be administered by professionals	Yes	246	92.48
	No	20	7.52
Use of antimicrobials	Treat infection	237	89.10
	Prevent infection	12	4.51
	Both (treat and prevent)	7	2.63
	I don't know	10	3.76
Cause of resistance	Overdose	93	35.00
	I don't know	128	48.10
	Underdose	36	13.50
	Both (under-and overdose)	9	3.40
Effect of AMR	I do not know	155	58.30
	Non-response to microbial treatment	95	35.70
	Extra cost treatment	16	6.00
AMR can be passed to animals and vice versa	I do not know	213	80.08
	Yes	30	11.28
	No	23	8.65

4.2. Occurrence of *Salmonella* in Household Setting

Based on culture based detection, presumptive *Salmonella* was detected in 52 samples out of 1463 samples collected from 266 households. Among the 266 households, only 133 owned animals from which fecal samples were collected. The samples included six items: infant stool, soil, water, hand rinse, food, and animal feces, encompassing all 3 domains of One Health. *Salmonella* presumptively identified by culture based detection. Following confirmatory testing using MALDI-TOF where, 31 out of 52 presumptive were confirmed positive.

Table 7: Detection of *Salmonella* in various samples from households (n=266 and (animal) =133).

Sample type	Number of culture-positive (n=52)	Proportions of positives (%)	Overall prevalence
Infant stool	9	17.31	3.38
Soil	22	42.31	8.27
Water	2	3.85	0.75
Hand rinse	3	5.77	0.75
Food	2	3.85	0.75
Animal feces	14	26.92	10.53
Total	52	100%	3.6%

4.2.1. MALDI-TOF confirmation of Salmonella

All 52 presumptive *Salmonella* isolates were subjected to further confirmation using MALDI-TOF, and 31 were tested positive as *Salmonella* with the overall prevalence 2.12% across all 266 households. Among the sample types, animal faeces samples showed the highest prevalence (6.77%) followed by soil (4.5%) and stool samples (3%). Water and hand rinse samples each had one positive with overall prevalence 0.4% each whereas no *Salmonella* was confirmed in food samples despite initial culture positivity. Furthermore, although it was not statistically significant, households that owned animals had higher odds of *Salmonella* positivity compared to those that did not own animals.

Table 8: Detection of *Salmonella* in various samples using MALDI-TOF tests (n=266 & n (animal)=133).

Sample type	Number of sample tested (n=52)	Number of positives n (%)	Overall Prevalence (%)
Infant stool	9	8(88.9)	3.0
Soil	22	12(54.6)	4.5
Water	3	1(50)	0.4
Food	2	0	0.0
Hand rinse	2	1(50)	0.4
Animal feces	14	9(64.3)	6.77
Overall	52	31 (59.6)	2.12

4.3. Antimicrobial Susceptibility of *Salmonella*

A total of 31 *Salmonella* isolates were subjected to antimicrobial susceptibility testing. The isolates were obtained from the following sources: infant stool (8), soil (12), water (1), hand rinse (1), and animal (9). Overall, among the 31 *Salmonella* isolates, 82.8% were susceptible, 5.4% showed intermediate resistance, and 11.8% exhibited resistance. All isolates were completely susceptible to ciprofloxacin, co-trimoxazole, and norfloxacine. The highest resistance was recorded against tetracycline (32.26%), followed by azithromycin (22.6%). When evaluated by sample source, isolates from soil showed the highest level of resistance (15.3%), followed by animal stool (11.1%).

Table 9: Antimicrobial Susceptibility profile of Salmonella isolates (n=31)

Antimicrobial tested	**Antimicrobial class**	**Resistance pattern n (%)**		
		S	I	R
Ciprofloxacin	Fluoroquinolone	31(100%)	0	0
Co-Trimoxazole	Folate pathway	31(100%)	0	0
Norfloxacine	Fluoroquinolone	31(100%)	0	0
AMC	Beta lactamase	25(80.6%)	2(6.5%)	4(12.9%)
Ampicillin	Beta lactamase	27(87.1%)	0	4(12.9%)
Gentamicin	Aminoglycoside	28(90.3%)	0	3(9.7%)
Azithromycin	Macrolide	18(58.1%)	6(19.6%)	7(22.6%)
Tetracycline	Tetracycline group	14(45.2%)	7(22.6%)	10(32.3%)
Streptomycin	Aminoglycoside	26(83.9%)	0	5 (16.1%)

4.3.1. Multidrug resistance of salmonella

Among the Salmonella isolates, only 2 (6.5%) were multidrug-resistant, resistant to three or more classes of antimicrobials. Among the isolates, only those from soil exhibit a multidrug-resistant profile, specifically to beta lactams, tetracycline, aminoglycosides, and macrolide

5. DISCUSSION

The household setup, particularly living arrangements and population density, plays a key role in the transmission dynamics of enteric pathogens such as Salmonella (Pardhan-Ali *et al.,* 2013). Consistent with the main objective of the study, this was to assess the occurrence and antimicrobial pattern of Salmonella at human, animal, environment interface in household setting in Bishoftu, Ethiopia. The results of the household survey showed that the majority of households (75.94%) lived in shared compounds with multiple families, and 10.9% shared with multiple households. This high-density living setting creates a situation where contaminated environmental surface such as communal yards, shared latrine, and common walkways, become fomites which could latter facilitate the transmission of the infectious pathogen in the household (Penakalapati *et al.,* 2017).

Animal husbandry in household settings increases human-animal interaction and may facilitate the spread of pathogens between humans and animals via contaminated environmental surfaces. Children are particularly at high risk due to their tendency to ingest animal faeces or come into contact with contaminated fomites, which may carry pathogenic bacteria at high concentrations, causing disease (Pintar *et al.,* 2015). In this study, 50 of households own animals, of which 42.1% keep two or more species. The presence of domestic animals in densely populated compounds has been documented as a significant risk factor for diarrheal disease, as animals shed enteric pathogens into the soil (Conan *et al.,* 2017).

Access to safe drinking water is a key component in reducing feco-oral transmission. The study found majority of households (72.66%) used tap water for drinking purposes, while the small portion (24.1%) used bottled water. However, among the respondents who used tap water the overwhelming majority (93.98%) do not use any form of additional water treatment. Although the tap water considered as improved water source, the lack of household water treatment is concerning because water from improved source can still be contaminated during distribution and storage, particularly in low income setting where pipes may have leaks and not cleaned constantly (Felföldi *et al.,* 2010). For instant recent epidemiological studies have showed that household that do not use water treatment had 3.3 higher odd ratio of experiencing waterborne disease compared to those that treated their water (Daba *et al.,* 2025).

The study found that almost all households (98.12%) had access to a latrine, a figure significantly higher than the reported national sanitation coverage of 20 and above the reported urban sanitation coverage of 42 for Ethiopia (Desye *et al.,* 2023). The high latrine access in the study is comparable to a study conducted in Addis Ababa, where latrine coverage was documented at 99, reflecting the urban nature of the setting. However, the critical concern lies not in access to latrines or coverage, but in the type and management of these facilities, as the majority of households (73.68%) used latrines shared with other households in the same compounds, a portion comparable to 79.8% communal latrine usages reported in Addis Abeba. The increasing trend in urban settlement may be a contributing factor to the observed use of shared latrines (Kefeni and Yallew, 2018).

The high proportion of shared latrines can be attributed to infectious disease transmission, where relying on shared sanitation facilities carries a higher risk of adverse health outcomes, particularly for diarrheal diseases, due to lack of maintenance, lack of regular cleaning, and inconsistent hygiene practices among multiple users (Just *et al.,* 2018). Furthermore, the presence of shared latrines poses a particular risk to infants and children through their caregivers, who may serve as vectors for pathogen transmission and through the contaminated environment surrounding the latrine, where infants tend to touch and play anything on the ground and increases the vulnerability of infants to enteric pathogens such as salmonellosis in the household setting (Wolf *et al.,* 2019).

The absence of handwashing facilities near the latrine is a major hygienic gap, despite the high access of latrine observed in the household survey. In the survey more than three quarters of the households (78.95%) lacked water and soap near the latrine, representing significant WASH gap. The finding was comparable with hosanna town were 84.2% lack such facilities (Aydamo *et al.,* 2023) and slightly higher than the national urban estimate of 71 by EPHI and ICF (2021). The absence of handwashing station can be explained by different reasons like lack of awareness regarding the importance of personal hygiene and sanitation and limited space in shared compound.

According to EDHS household, household with soap available at handwashing station were 5.6 times more likely to have access to basic hygiene service compared to those without, reinforcing the presence of soap is important factor in enabling proper hand hygiene (Wolf *et al.,* 2019) .This finding is particularly important given among the very few households (21.05%) that did hand washing station near the latrine only 69. 6% of the used soap, with remainder washing hand with water alone is a practice that has been shown to be ineffective at removing enteric pathogen like Salmonella. Moreover, since majority of the caregivers were mothers (88.35%) who are responsible for feeding the infants and cleaning, the lack of handwashing station significantly increases the risk of contracting enteric pathogens (Wang *et al.,* 2024).

In addition to hand washing facility and practice animal waste management is a huge concern in household settings. The study found that among the 133 households that owned animal, improper disposal of animal faeces is widespread practice, were 48 (36.1%). Some households reported that nothing is done to manage the waste, and 17(12.8%) disposed of the waste in a rubbish pile outside of the compound, which can contribute to preventable disease burden. In 48.9% of the households, there is no formal and safe animal faeces disposal practice, which was significantly higher than 12.5% of households reported to dispose of animal faeces in the open environment in previous studies (Alemu and Mezgebu, 2023). The high prevalence of improper animal disposal can be due to many reasons, including the absence of designated waste disposal areas, a lack of awareness regarding the health risks, and cultural norms where animal faeces are repurposed for various domestic uses. Such practice increases the risk of environmental contamination with faecal pathogens, which may subsequently expose children and other vulnerable individuals to enteric pathogen infections (Kaur *et al*., 2017).

The other thing addressed in the study is the floor type of the house as well as compound where 78.2% of the household had cement floor and about 19.92% still had dirt floor cover with carpet. Regarding ground nature of the compound, 66.17% of the household had grass or dirt in the compound, creating a favourable environment where pathogen can persist and multiply. Different studies have demonstrated that *Salmonella* can survive in soil for weeks to months, especially in moist and shaded environment common in household compounds (Wang *et al.,* 2018). These findings highlight the importance of flooring materials and

compound ground cover as part of comprehensive WASH intervention to reduce enteric pathogen exposure in household setting.

According to the survey, the vast majority of caregivers (92.48%) knew drug must be administered only by health professionals, while 89.1% thought drugs are used to treat infection. However, despite the high percentage of knowledge regarding drugs use there is still a huge gap, as only the half of the caregivers cites any knowledge about AMR. Among those, only 35% identified over-dose as the main cause for AMR, while 13.5% cited as underdoes and 48.1% answered “I do not know”.

The caregivers identified overdose as the main reason for AMR more frequently than under dose, suggesting a misunderstanding of the AMR mechanism, where both are equally important drivers of AMR (WHO, 2021). Additionally, 80.8% of the respondents did not know that AMR can be passed from animals to humans, representing a critical One Health knowledge gap that undermines efforts to control AMR and zoonotic transmission (Ercumen *et al.,* 2017).The study found a confirmed Salmonella prevalence rate of 2.12% across all sample types after MALDI-TOF confirmation. The prevalence was lower than the 7.7% reported in a similar One Health study in north west Ethiopia (Beyene *et al.,* 2024). Although the prevalence is low, it is still clinically significant, as these isolates were recovered from infant stool, soil, water, hand rinse, food, and animal faeces, demonstrating the widespread presence of Salmonella across all three One Health domains. Soil accounted for the highest portion of confirmed positive (38.71%), followed by animal feces (29.03%) and infant stool (25.81%). This finding is consistent with the studies from similar low-income settings, where soil serves a major reservoir for enteric pathogen (Navab-Daneshmand *et al.,* 2018).

The high level of soil positivity in the study can be attributed to several reasons. First, majority of the household had grass and dirt in their compound, proving a favourable environment for salmonella survival, combined almost half of the household lacks proper animal waste management system, leading the contaminate of the soil and enabling transmission of the pathogen via fomites and environmental contamination, salmonella can persist in the environment contaminated from weeks to months underscoring the importance of waste management in preventing zoonotic transmission within the household settings (Perez *et al.,* 2025).

Antimicrobial resistance in bacteria has grown to be an urgent public health threat worldwide. Among the confirmed 31 *Salmonella* isolates more than half of them were susceptible, with very few showing resistance. The resistance prevalence reported in the study was lower than reports from Addis Abeba and the national systematic review (Kahsay *et al.,* 2023). The study found that all confirmed *Salmonella* isolates were susceptible to co-trimoxazole, ciprofloxacin, and Norfloxacine. The finding aligns with recent reports, where ciprofloxacin resistance in salmonella remains low, more likely due to restricted use of the drug in both human and veterinary medicine (Beyene *et al.,* 2024). The complete susceptibility of co-trimoxazole was higher compared to other studies, suggesting that it may remain an effective treatment option. However, these findings should be interpreted with caution, as the sample size analysed was small and continued monitoring is essential to detect and track the resistance pattern. The only MDR registered in this study was found soil with 6.5%. Which may indicate that household soil could be potential reservoir of MDR, possibly due to disposal of animal feces in the household. However, it should be taken into consideration the study only addresses the magnitude and distribution of antimicrobial resistant (AMR) in Salmonella isolates, rather than the transmission dynamics

Tetracycline exhibits the highest resistance level among the drugs tested, with resistance observed in soil, infant stool, and animal feces. The relatively high resistance can be due to many reasons, where there is widespread use of tetracycline in veterinary medicine, commonly used to treat infection in livestock. The presence of tetracycline in animals, soil, and infants provides strong circumstantial evidence of one health (Swarthout *et al.,* 2022). However, the current study did not investigate the genetic relatedness among the different sources, so further study using whole-genome sequencing, would elucidate the genetic relatedness among the isolates and determine the epidemiological links to better inform targeted interventions.

Aside from tetracycline various resistances was observed to ampicillin, streptomycin, and gentamycin across the sample sources. Resistance to ampicillin was reported in animal and soil but not in infant stool. Ampicillin have been one the oldest beta lactamase drug used among children over decades, and recent studies have documented high resistance rates ranging from 55.7- 100% across clinical sample (Yirsa and Tigistu, 2025). Therefore, the absence ampicillin resistance in infant isolate should not be interpreted as evidence that ampicillin remains as effective treatment and this finding is likely due to small sample size.

The high streptomycin resistance was observed in animal faeces reflects the historical use of this drug in Ethiopia livestock production as treatment for bacterial infection as well as growth promotion (Tufa *et al.,* 2023). Ampicillin and streptomycin are old antibiotics and out of routine clinical use in developed countries they are still in use in Ethiopian veterinary and community settings(Tufa *et al.,* 2024).

The pattern of occurrence and distribution of the pathogen in the study indicates the possibility of both horizontal zoonotic and reverse zoonotic transmission of enteric pathogens between animals and humans being mediated by environmental contamination. Other studies conducted in similar settings have reported the occurrence of salmonella in humans, animals, and the environment within household environments, highlighting the potential public health risk associated with close human–animal interactions and shared living spaces (Mengistu *et al.,* 2025).

This finding aligns with recent systematic review and meta-analysis in Ethiopia which reported salmonella prevalence among environmental samples being the highest with prevalence rate of 8.43% followed by animals (7.8%) and humans (4.79%) (Assefa *et al.,* 2026). The confirmation of salmonella in animal feces, soil, infants stool in the current study reflecting this national pattern that suggests contaminated soil and caregivers' hands serve as a critical transmission bridge. These findings emphasize the importance of a one-health approach in understanding the transmission dynamics in the household. However, as mentioned previously, the current study did not investigate the genetic relatedness of Salmonella isolates obtained from different sources (infants, animals, soil, and caregivers), which limits the ability to determine the direction or pathways of transmission of pathogenic salmonella from animals to humans.

Limitation

The study has several limitations that need to be mentioned. First all the presumptive Salmonella isolates were confirmed solely using MALDI-TOF which identifies bacteria only at the species level. Serological and molecular identification would have been preferable, as these methods could have provided deeper insight into whether the isolates recovered from infants were typhoidal or non-typhoidal, thereby facilitating comparison with other isolates from other sources such as soil and animal. Second, the study did not investigate the genetic relatedness of isolates or the epidemiological connections among Salmonella isolates recovered from household sources, including infant stool, animal feces, soil, water, and hand rinse. This absence of molecular epidemiological data limits the ability to infer transmission dynamics, including the directionality of transmission and the potential mediating role of environmental contamination and caregivers in the dissemination of Salmonella at the household level. Third, antimicrobial resistance profiling in this study was based exclusively on phenotypic susceptibility testing. The study did not include genotypic characterization of resistance, such as the detection of specific resistance genes or mobile genetic elements, which would have provided a more comprehensive understanding of the mechanisms underlying resistance to the tested antimicrobial agents. Finally, the overall study was confined to single Kebele, including multiple Kebeles within the town would have allowed for better population representation.

6. CONCLUSION AND RECOMMENDATIONS

The study demonstrated the occurrence of Salmonella at the human-animal environmental interface in the household setting in Bishoftu, Ethiopia. In the study, a total of 2.12 of samples tested positive for Salmonella across all 3 domains. Among the sample types, soil had the highest prevalence (4.5%), followed by animal feces (3.4%) and infant stool (3.0%), indicating that environmental contamination and domestic animals serve as potential infectious disease transmission in household setups. The household survey showed a critical gap in water, sanitation and hygiene practice, animal waste management system all of which essential components in preventing and controlling infectious diseases like salmonella. The study also addresses the AMR profiles among the isolates. Among the drug tested tetracycline showed the highest resistance level detected in soil, infant stool, and animal feces. The distribution and occurrence pattern of the pathogen suggest that both horizontal zoonotic and reverse zoonotic transmission of enteric pathogens between animals and humans may occur, mediated by environmental contamination. The study emphasizes the critical importance of one health approach in mitigating these challenges.

Based on the above conclusion, the following recommendations are forwarded:

- Enhancing childcare practice and household hygiene practice through health education by health extension workers particularly infant care givers on WASH practice and personal hygiene and environmental sanitation.
- Better management of animal waste is required to minimize and overlap sharing of space between human animal to environmental contamination and cross species transmission of enteric pathogen.
- Launch public campaigns to promote prudent antibiotic use, safe food hygiene, and Salmonella risk awareness to reduce transmission and combat antimicrobial resistance.
- Establish an integrated One Health surveillance system for real-time data sharing across human, animal, and environmental health sectors for effective control and prevention

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8. APPENDICES

Appendices 1: Principles and procedures of media preparation, isolation and identification tests

1. Pre-Enrichment and Transportation Media

1.1. Buffered Peptone Water (BPW) (Oxoid, Thermo Scientific, UK)

Preparation

Add 20 gram to 1 litre of distilled water

Mix well and gently heat

Distribute into final containers

Sterilize by autoclaving at 121°c for 15 minutes.

1.2. Tryptone Soya broth (TSB) (Oxoid, Thermo Scientific, UK)

Preparation

Add 30 gram to 1 litre of distilled water

Mix well and distribute into the final container

Sterilize by autoclaving at 1210c for 15 minutes

2. Media preparation for Isolation of Salmonella

A. Rapaport-vassilidas (RV) (Oxoid, UK)-for selective enrichment

Dissolve 26.75 grams in 1 litre distilled water.

Gently heat to completely dissolve the medium.

Dispense into final tubes

Sterilize by autoclaving at 1210c for 15 minutes

B. Xylose Lysine Deoxycholate (XLD) (Oxoid, UK)-for selective plating

Suspend 53 gram in 1 litre of distilled water

Gently heat to a boil, stirring frequently, until the medium is completely dissolved.

For XLD agar, no autoclave is needed after boiling place the media in water bath until the optimum temperature is reached, then dispense.

C. Nutrient Agar Preparation (Oxoid, Basingstoke, England)

Suspended 28 grams in 1000ml distilled water.

Heated to boil to dissolve the medium completely.

Sterilized by autoclaving at 121℃ for 15 minutes.

Mixed well and poured in to sterile Petri dishes.

D. Muller Hinton agar preparations for antimicrobial susceptibility test

38 gm of the medium was suspended in one litter of distilled water.

Mix well and gently heat to boiling with frequent stirring until the medium is completely dissolved.

Autoclaved at 121°C for 15 minutes then cooled to room temperature.

Pour into sterile Petri dishes under aseptic conditions.

Store the plates at 2-8 ºC.

Appendices 2: Antibiotic discs used during the antimicrobial susceptibility testing.

Antimicrobial Discs	Potency	Interpretation Criteria			Remark
		Susceptible	Intermediate	Resistance	
Ampicillin	10 μg	≥17mm	14-16mm	≤13mm	
Ciprofloxacin	5 mcg	≥26mm	22-25mm	≤21mm	
Tetracycline	30 μg	≥15mm	12-14mm	≤11mm	
Gentamicin	30 μg	≥15mm	13-14mm	≤12mm	
Streptomycin	25 mcg	≥15mm	12-14mm	≤11mm	

Co-trimoxazole	25 μg	≥16mm	11-15mm	≤10mm
Amoxicillin-clavulanate	20/10 μg	≥18mm	14-17mm	≤13mm
Azithromycin	15 μg	≥18mm	14-17mm	≤13mm
Norfloxacine	10 mcg	≥17mm	13-16mm	≤12mm

AMR result

No	Sample id	Dr.t	Cip	Cot	Nor	Amioxclav	Amp	Gen	Azt	Tet	Ste
1	31		35mm	27mm	28mm	22mm	23mm	26mm	14mm	12mm	19mm
2	32		34mm	29mm	30mm	30mm	25mm	24mm	13mm	10mm	20mm
3	36		39mm	29mm	30mm	27mm	24mm	20mm	28mm	0	20mm
4	56		33mm	25mm	21mm	17mm	20mm	10mm	17mm	0	18mm
5	91		35mm	28mm	30mm	24mm	22mm	28mm	20mm	10mm	27mm
6	92		36mm	18mm	30mm	28mm	24mm	29mm	19mm	12mm	20mm
7	101		40mm	25mm	29mm	29mm	25mm	25mm	10mm	8mm	20mm
8	104		36mm	25mm	30mm	23mm	24mm	27mm	15mm	8mm	21mm
9	113		31mm	26mm	24mm	27mm	25mm	25mm	16mm	15mm	22mm
10	116		38mm	25mm	31mm	26mm	24mm	27mm	18mm	14mm	18mm
11	121		37mm	28mm	22mm	32mm	25mm	30mm	18mm	16mm	20mm
12	146		38mm	31mm	36mm	30mm	25mm	31mm	16mm	0	20mm
13	171		30mm	22mm	26mm	30mm	24mm	27mm	22mm	10mm	25mm
14	222		36mm	25mm	32mm	32mm	26mm	31mm	26mm	17mm	20mm
15	232		32mm	0	22mm	30mm	20mm	27mm	0	17mm	20mm
16	242		32mm	30mm	24mm	28mm	24mm	30mm	22mm	21mm	0
17	246		29mm	32mm	23mm	28mm	22mm	25mm	14mm	16mm	22mm

18	261	34mm	28mm	26mm	31mm	23mm	30mm	20mm	16mm	20mm	
19	276	33mm	30mm	24mm	31mm	26mm	27mm	22mm	12mm	27mm	
20	352	38mm	27mm	32mm	15mm	20mm	30mm	30mm	13mm	21mm	
21	356	30mm	30mm	20mm	29mm	23mm	20mm	20mm	15mm	18mm	
22	441	37mm	25mm	30mm	24mm	24mm	30mm	18mm	16mm	24mm	
23	452	36mm	27mm	32mm	30mm	25mm	26mm	26mm	20mm	23mm	
24	456	34mm	31mm	32mm	28mm	20mm	26mm	26mm	20mm	20mm	
25	521	36mm	27mm	33mm	20mm	26mm	30mm	20mm	20mm	23mm	
26	672	30mm	25mm	25mm	20mm	24mm	25mm	10mm	13mm	17mm	
27	2342	32mm	25mm	18mm	31mm	0	29mm	18mm	15mm	0	
28	2382	33mm	26mm	17mm	20mm	22mm	12mm	0	14mm	0	
29	2632	33mm	29mm	20mm	17mm	0	10mm	0	0	20mm	
30	2626	36mm	22mm	22mm	17mm	0	27mm	22mm	14mm	0	
31	2642	33mm	29mm	31mm	25mm	0	19mm	0	0	17mm	

Appendices 3: Antimicrobial Susceptibility profile of Salmonella isolates based on sample source (n=31).

Antimicrobial tested	Infant stool n(%)			Soil n(%)			Water n(%)			Hand rinse n(%)			Animal n(%)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ciprofloxacin	8(100)	0	0	12(100)	0	0	1(100)	0	0	1(100)	0	0	1(100)		
Co-Trimoxazole	8(100)	0	0	12(100)	0	0	1(100)	0	0	1(100)	0	0	1(100)		
Norfloxacine	8(100)	0	0	12(100)	0	0	1(100)	0	0	1(100)	0	0	1(100)		

AMC	8(100)	0	0	8(66.71)	1(8.3)	3(25)	1(100)	0	0	1(100)	0	0	7(77.8)	1(11.1)	1(11.1)
Ampicillin	8(100)	0	0	9(75)	0	3(25)	1(100)	0	0	1(100)	0	0	8(88.9)	0	1(11.1)
Gentamicin	8(100)	0	0	10(83.3)	0	2(16.7)	1(100)	0	0	1(100)	0	0	8(88.9)	0	1(11.1)
Azithromycin	6(75)	1(12.5)	1(12.5)	6(50)	0	6(50)	0	1(100)	0	0	1(100)	0	6(66.7)	3(33.3)	0
Tetracycline	4(57.1)	1(12.5)	3(37.5)	6(50)	3(2)	3(25)	1(100)	0	0	0	0	1(100)	3(33.3)	3(33.3)	3(33.3)
Streptomycin	7(87.5)	0	1(12.5)	10(83.3	0	2(16.7)	1(100)	0	0	1(100)	0	0	6(66.7)	0	3(33.3

Appendices 4: Questionnaire

Enrollment and Questionnaire Survey

Enumerator ID: _____

Household ID: _____

Date of survey: DD/MM/YYYY

Time of survey: HH:MM

Part I: Infants' demography and Socio-demographic characteristics of caregivers

(Check all details against baseline questionnaire entry before starting)

Before I begin the interview, could you please bring the infant's Birth Certificate, and Health

Card? We will need to refer to those documents. I am going to ask you questions on different

topics and afterwards I will ask for a stool sample from the infant and will provide you with

instructions on how to collect this. The whole process should not take more than 45 minutes of your time.

1. In what month and year was the infant born? (Verify with infant's health card, if possible):

MM/YYYY_____

2. Sex of infant

    a. Male

b. Female

3. What is your relationship to the infant?

a. Mother

b. Father

c. Grandmother

d. Grandfather

e. Other guardian (record any further detail/explanation) _____

4. What is your marital status?

a. Single

b. Married or in partnership.

c. Divorced/widowed.

5. Age of the caregiver (years) _____

6. How many adults live in the household? _____

7. How many children between five and 18 years live in the household? _____

8. How many children under five live in the household? _____

9. How long have you been living continuously in this house? _____ Months

10. What is the highest level of school you attended?

a. None

b. Primary

c. Secondary

d. Higher

e. Prefer not to answer.

f. Don’t know.

11. What is your occupation?

a. Professional

b. Secretarial

c. Janitorial

d. Childcare

e. Market vendor

f. Other

12. Access to media (TV or radio)

a. yes

b. No

13. Household average monthly income (ETB)_____

14. What type of residence is this?

    a. Apartment or room in multi-unit building, no yard

    b. Free standing house, no compound yard

    c. Free standing house, private yard

    d. Free standing house, gated community

    e. Compound with multiple private family households

    f. Compound shared with multiple unrelated families.

15. Does your family own or rent this residence?

    a. Own

    b. Rent

    c. Live here for free

16. How many rooms does the house have? _____

17. What is the main material of the floor in the residence? (observe)

    a. Uncovered dirt

    b. Dirt floor covered with carpet or vinyl.

    c. Wood/parquet, marble/granite, cement, mosaic/tile

    d. Other: _____

18. What is the main material of the ground in the compound?

    a. Grass or dirt

    b Cement, brick, or tile

    c Other: _____

19. Is the compound enclosed by a lockable gate?

    a. Yes

    b. No

20. Does the compound ever flood?

    a. Yes, from rain on the ground inside the home.

    b. Yes, from community floodwater.

    c. Yes, from wastewater drains.

    d. No

21. Is there playground for children in the compound?

    a. Yes

b. No

Part III. Access to clean Water, Sanitation and Hygiene facilities

22. Where does your household normally collect water to drink?

a. Tap inside the house.

b. Tap in the compound.

c. Public tap or fountain

d. Protected spring

e. Unprotected spring

f.collected rainwater.

g. Borehole

h. Bottled or packed water

i.Other: _____

23. How long does it take to go there, get water, and come back?

a. Water source in the yard

b. Less than 30 minutes

c. Greater than 30 minutes

24. Household drinking water treatment options

a. Chlorine

b. Wuha Agar

c. Boiling

d. No usage of treatment

25. Do you have access to a toilet or latrine?

a. No, rely on open defecation/bucket/bag

b. Yes

26. Where is the toilet or latrine? (observe)

a. In the household

b. In the compound, not shared.

c. In the compound, shared with other households.

d. Community latrine

e. Public latrine

27. What type of latrine or sanitation system is it?

a Flush/pour flush toilet to piped sewer system.

b. Flush/pour flush toilet to underground pit/tank.

c. Flush/pour flush toilet to onsite, above ground, open pit.

d. Pit latrine with concrete slab (not pour flush)

e Pit latrine without slab (not pour flush)

f Bucket

g Bag

h Other: _____

28. Is the latrine serves single household?

a. Yes

b. No

29. Is there water for hand washing near the latrine?

a. Yes

b. No

30. Is there soap or other detergent for hand washing near the latrine?

a. Yes

b. No

Part IV. Animal ownership and waste management

31. Does your household own any animals?

a. Yes

b. No

32. How many animals of different species do you have?

Animal species	Number
Chicken	
Duck	
Pig	
Cattle	
Goat	
Sheep	
Equines	
Dog	
Cat	
Others	

33. What do you do with animal feces/where does it go (check all that apply)?

a. Nothing is done with animal feces.

b. Latrine pit

c. Waste bin or rubbish pile in compound

d. Used for crop fertilizer.

e. Drain or rubbish pile outside the compound.

34. Presence of animal feces in the compound(observe)

a. Yes

b. No

c. Animal species_____

35. Does the child ever touch or play with any of the household animals?

a. Yes

b. No

Part V. Infant care practices and health history .

36. Is the infant currently breastfed?

a. Yes

b. No

c. Don't know

37. Is the infant exclusively breastfed?

a. Yes

b. No

c. Don't know.

38 Who takes care of the infant when you are away? Select all that apply.

a. Father

b. Mother-in-law, grandmother, aunt

c. Youth < 18 years

d. Hired help.

e. Neighbor or friend

f. No other caregivers

39 Has the infant had any of the following symptoms in the past 7 days? Check all that apply.

a. Diarrhea (three or more loose stools per day at any point).

b. Fatigue

c. Vomiting

d. Unusual lack of appetite or willingness to take liquids.

e. Fever

f. Runny nose

g. Cough

h. Rash

i. Others (Specify)_____

40. Has anyone else in the household had the same symptoms in the past month?

a. Yes

b. No

41. Did you seek any health advice or treatment for the infant's symptoms?

a. None

b. Public hospital/clinic

c. Public health post /Community Health extension

d. Private hospital/clinic

e. Private pharmacy

f. Relative/friend

g. Others

41. During the time the infant had these symptoms, did he/she receive?

a. Hospitalization

b. Fluid made with powdered Oral Rehydration Solution (name brands)

c. Pre-packaged liquid Oral Rehydration Solution (name brands)

d. Homemade Oral Rehydration Solution

e. Zinc. Yes → Tablet, syrup?

f. Drugs/antimicrobials. Yes → Tablet, syrup, injection?

g. Antimotility (explain). Yes → Tablet, syrup?

h. Intravenous (explain)

i. Local/herbal medicine. Yes → Name?

Part VI. Knowledge of infant cargivers on rational use of antimicrobials in humans

42. Know drugs/antimicriobials to be used to

a.Treat infection in human

b. Prevent infections in humans

c. I do not know

43. Know/heard that antimicriobial should be prescribed and administered by health professionals.

a. yes

b No

44. Knowledge about antimicriobial resistance

a. Yes

b. No

45. Knowledge about the cause for antimicrobial resistance.

a. Administered under-dose

b.Administered over-dose

c. I do not know

d. other(specify)

46. Effect of antimicrobial resistance in humans

a. Non-response to micriobial infection treatment

b. Extra cost on the treatment of micriobial infection

c. I do not know

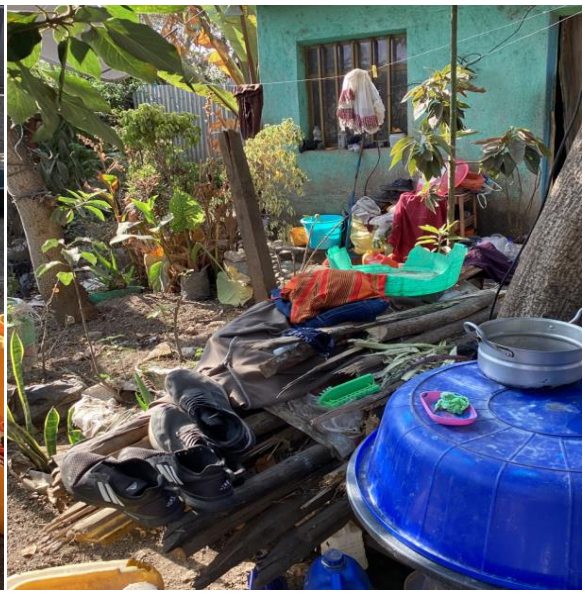
47. Antimicrobial resistant pathogens in humans can be passed to animals

a. Yes

b No

c. I do not know

Appendices 5: Pictures from sample collection and laboratory work

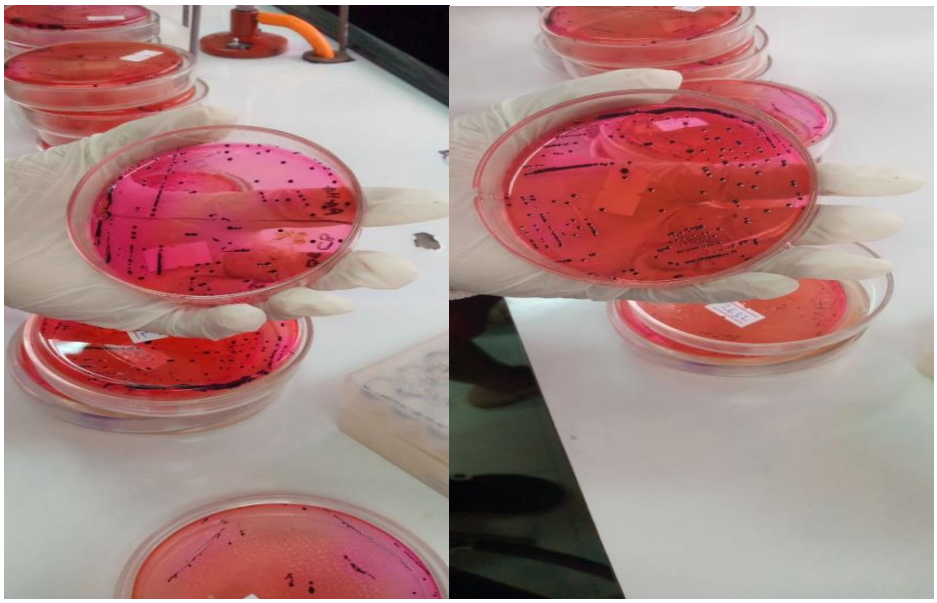


A. Pictures from households during sample collection time

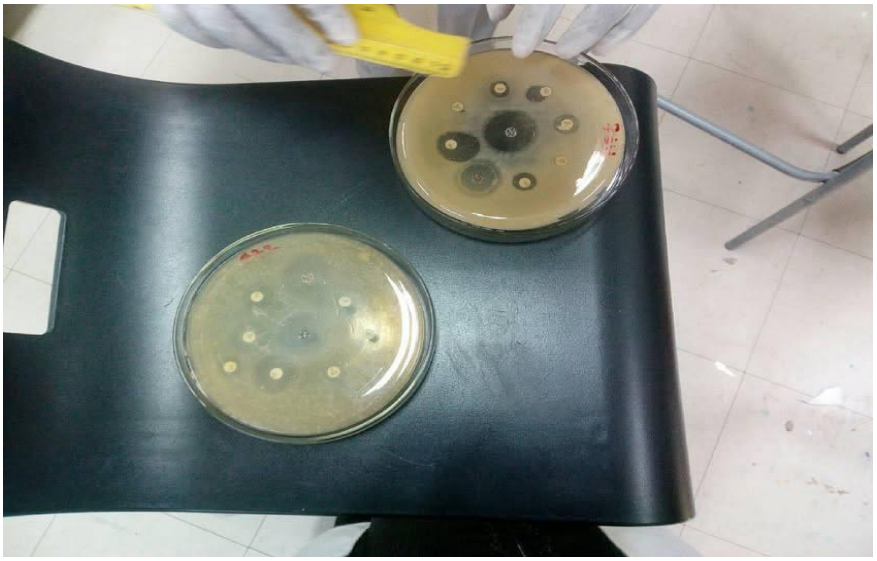
B Positive *Salmonella* in Rapaport-Vassilidis (RV) broth

.

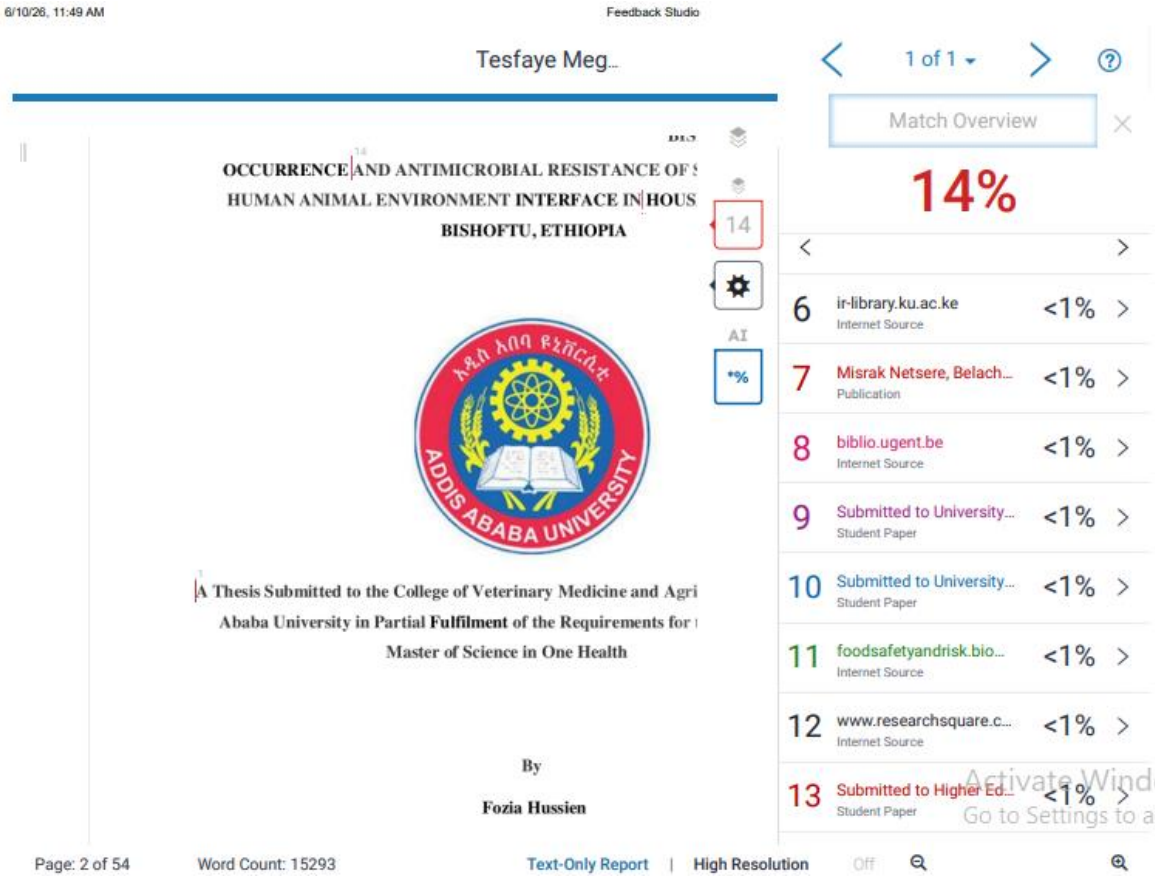
D. Inoculating the samples on XLD



D Positive Salmonella in XLD agar



E Antimicrobial susceptibility test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ADDIS ABABA UNIVERSITY
A Thesis Submitted to the College of Veterinary Medicine and Agri
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