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ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF VETERINARY MICROBIOLOGY, PARASITOLOGY AND POULTRY HEALTH

DETECTION OF *SALMONELLA FROM* DAIRY FARM, CHILDREN AND MILK, AND ITS ANTIMICROBIAL RESISTANCE PROFILE IN AND AROUND BISHOFTU TOWN: ONE HEALTH APPROACH

MSC THESIS IN ONE HEALTH

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Detection of *Salmonella* from Dairy farm, Children, Milk, and Its Antimicrobial Resistance Profile In and Around Bishoftu Town: One Health Approach

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Detection of *Salmonella* from Dairy farm, Children and Milk with Its Environment and Antimicrobial Resistance Profile In and Around Bishoftu Town**:** One Health approach

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by: **Milkesa Hailu Dibaba** Entitled: **Detection of *Salmonella* from Dairy farm, Children, Milk, and Its Antimicrobial Resistance Profile In and Around Bishoftu Town: One Health approach** And recommend that it be accepted as fulfilling the thesis requirement for the degree of: **Master of Science in One Health**

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Final approval and acceptance of the MSc thesis is contingent upon the submission of its final copy to the CGS/FGC through the departmental graduate committee (DGC) of the candidate's major department. I hereby certify that I have read the revised version of this thesis prepared under my direction and recommend that it be accepted as fulfilling the thesis requirement.

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Declaration

Milkesa Hailu Dibaba , declare that this thesis **Detection of *Salmonella* from Dairy farm, Children, Milk, and Its Antimicrobial Resistance Profile In and Around Bishoftu Town: One Health approach** and the work presented in it are my own. I confirm that: This work has been conducted independently and has not been submitted elsewhere for any academic award. All sources of information have been acknowledged and referenced appropriately. Any assistance received during the research process has been clearly stated. Ethical approval has been obtained where necessary, and all research has been conducted in accordance with the ethical standards of Addis Ababa University.

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ABBREVIATIONS and ACRONYMS

AMR	Antimicrobial Resistance
AST	antimicrobial susceptibility testing
BAM	Bacteriological Analytical Manual Method
CDC	Center of Disease Control
CVMA	College of Veterinary Medicine and Agriculture
DNA	Deoxyribonucleic Acid
GAP	Good Agricultural Practices
GMP	Good Manufacturing Practices
H2S	Hydrogen sulphide
LPS	Lipopolysaccharide
MDR	Multidrug-resistant
NTS	Non typhoidal Salmonella
iNTS	invasive Non typhoidal Salmonella
PI	Pathogenic Islands
RVS	Rappaport-Vassiliadis with soya
TSI	Triple Sugar Iron
USFDA	U.S. Food and Drug Administration
VP	Voges Proskauer
WHO	World Health Organization
XLD	Xylose lysine deoxycholate agar

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ABSTRACT

Salmonella enterica is a bacterial pathogen that is a significant risk to human, animal and environmental health. It is a major cause of food borne morbidity and mortality and is severe in under-five children. The presence of the pathogen in lactating cow, milk and milk environment, and related public health risks are not well studied through One Health lens. This research was aimed to investigate the prevalence and anti-microbial resistance profile of *salmonella enterica* in lactating cow, milk, milk environment (equipment swabs, milkier hand swabs, udder swabs, floor swabs) and under-five aged children in and around Bishoftu town central Ethiopia. A total of 336 samples were collected; 214 from lactating cows, milk, milk environment, and 122 from stool of under-five years aged children. Isolation was made by using standard microbiological methods which are culture-based detection, Omnilog, and antimicrobial susceptibility test by the Kirby–Bauer disk diffusion method against 10 antimicrobials. The overall prevalence of *Salmonella enterica* was found to be 4 (1.2 %). Stool sample collected from children under-five, 2/122 samples 1.6% (95% CI: 0.002-0.058) and sample collected from dairy farms; 2 (0.9 %), (1(2.3 %) milk sample and 1(2.2 %)) udder swab samples were confirmed to be Salmonella spp. positive. Antimicrobial resistance profile was observed Ampicillin, and Cefoxitin (3/4 (75%)); whereas no resistance was recorded in Amikacine, Gentamicin and Ciprofloxacin. On the other hand, a MDR profile of recorded in two isolates to wards more than three classes of drugs. This study revealed the occurrence of *Salmonella enterica* in lactating cows, milk, (and its environments) and children under-five. It is an alarming and further exploring towards the genetic relatedness among the isolates, quantifying the public risks associated with milk consumption, an integrated surveillance system that tracks Salmonella across human, animal, and environment are needed to reduce the public health treats in this.

Keywords: *Antimicrobial Resistance, Bishoftu, Dairy Farm, Milk, Salmonella*

1. INTRODUCTION

World Health Organization, report that over 2 billion people worldwide suffer from diarrheal diseases annually (Popa & Popa, 2021), One in every four cases of these diseases is attributed to Salmonella (Lertworapreecha *et al*., 2012). Salmonella is estimated to cause approximately 115 million human infections and 370,000 deaths annually. The WHO reports that around one in ten individuals suffer from foodborne Salmonella infections each year, leading to the loss of millions of healthy life years (Temesgen *et al*., 2025). It is also one of the most important bacterial zoonotic diseases, estimated to cause, 155,000 deaths yearly worldwide (Patients *et al*., 2012). *Salmonella speceis* is recognized as a major zoonotic foodborne pathogen of economic significance in animals and humans; it causes an estimated 90 million cases of gastroenteritis mostly reported as a food borne disease, it has been estimated that about 10% of the cases are due to direct contact with animals (Galán-Relaño *et al*., 2023).

Salmonella was first identified in 1884 by American bacteriologist D. E. Salmon, who isolated the organism from porcine intestinal germs. In 1888, Salmon and Smith successfully cultured the bacterium, and the first documented case and isolation in humans was reported the same year by Gartner(Oludairo *et al*., 2022). Salmonella is a bacterial genus comprising numerous closely related species and remains a leading cause of morbidity and mortality worldwide. It poses significant public health challenges and contributes to economic burdens in both developed and economically disadvantaged nations due to the costs associated with monitoring, surveillance, prevention, and treatment of salmonellosis (Buckner *et al*., 2016).

Salmonella belong to the family Enterobacteriaceae. The organisms are negative to Gram stain and oxidase test and they are motile (due to the presence of peritrichous flagella), rod shaped, non-spore producing and facultative anaerobes (Lertworapreecha *et al*., 2012). The bacterial cytoskeleton, composed of an actin-like protein, maintains the rod-shaped structure of Salmonella. This bacterium can be pathogenic to both humans and animals. The two species, *Salmonella enterica* and *Salmonella bongori*, are responsible for infectious diseases in humans and animals, which are clinically characterized by one or more of three major syndromes: septicemia, acute enteritis, and chronic enteritis (Kebede, 2023). Over 2600 serotypes have been reported (Yue *et al*., 2022). This bacterium causes a foodborne illness in humans and animals

and can readily contaminate meat, eggs, milk, and other food products. Its ability to spread easily presents significant challenges in prevention and control. (Lee *et al*., 2015) Salmonella is a genus of highly diverse bacteria that live in the digestive tract of humans and animals. They are widespread in the environment thanks to their ability to survive and adapt even under extreme conditions (Galán-Relaño *et al*., 2023). Numerous Salmonella serovars exhibit a broad host range, capable of infecting various animals, including mammals, birds, reptiles, amphibians, and insects. Additionally, Salmonella can proliferate in plants and persist in protozoa, soil, and water. Therefore, minimizing human infections necessitates reducing Salmonella presence in animals and limiting its transmission from environmental sources (Silva *et al*., 2014).

Salmonella enterica is categorized into two groups based on the clinical characteristics of human infections. Typhoidal Salmonella is exclusive to humans and causes typhoid fever, whereas Non-typhoidal Salmonella (NTS) has a broad host range and is responsible for various illnesses beyond typhoid fever. NTS serotypes are a major cause of bacterial diarrhea and invasive infections, presenting a significant health risk to young children, the elderly, and immune compromised individuals, particularly in developing countries. (Temesgen *et al*., 2025)

In sub-Saharan Africa Invasive non-typhoidal Salmonella (iNTS) disease manifesting as bloodstream infection with high mortality is responsible for a huge public health burden (Van Puyvelde *et al*., 2023), and one of the three most prevalent pathogens responsible for bacterial bloodstream infections in both adults and children in sub-Saharan Africa (Reddy *et al*., 2010). Invasive non-typhoidal Salmonella (NTS) disease is widespread in both rural and urban areas of sub-Saharan Africa. In some African communities, the mortality burden from childhood invasive bacterial infections may exceed that of childhood malaria (Berkley *et al.,* 2005). *Salmonella typhimurium* and *Salmonella enteritidis* are the most frequently identified NTS serotypes responsible for human infections in the region (Morpeth *et al*., 2009).

In Ethiopia, the incidence of foodborne salmonellosis remains unknown, and the risk factors contributing to the contamination of animal products have not been well documented. Additionally, no studies have been conducted to attribute sources to human infections. However, there is a significant presence of carrier food animals, which may contribute to the spread of the disease. (7.07% in cattle to 43.81% in pigs) and the wide spread raw animal product

consumption habit in a noteworthy segment of the population are suggestive of the risk of acquiring Salmonella from animal products (Tadesse & Gebremedhin, 2015).

Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century that threatens the effective prevention and treatment of an ever-increasing range of infections caused by microbes' no longer susceptible to the common medicines used to treat them (Prestinaci *et al*., 2015). Salmonella, a common foodborne pathogen, is among the bacteria that have developed resistance to various antibiotics (Nair *et al*., 2018). The severity and life-threatening nature of 11–20 million salmonellosis cases annually are influenced by host factors, Salmonella serotypes, and the presence of AMR genes. These infections result in approximately 161,000 deaths each year (Buckner *et al*., 2016).

The inappropriate use of antibiotics in livestock particularly, dairy farms have contributed to the rise of Salmonella which can increase human health risks. Some Multi drug resistant (MDR) Salmonella infections in humans have been connected to exposure to dairy farms or contaminated dairy products (Temesgen *et al*., 2025). Salmonellosis predominantly impacts developing countries, where key contributing factors include overcrowding, poverty, shifts in dietary habits, large-scale food services, complex and prolonged food supply chains with increased global movement, inadequate sanitation, and poor overall hygiene practices (Bekele & Lulu, 2017). Therefore, the current study was carried out to assess the prevalence and antimicrobial resistance of milk-borne *Salmonella* of children (below five ages) through dairy farms and its environment.

Research Question: What are the prevalence, distribution, and antimicrobial resistance profile of *Salmonella*, across dairy farm, children (below five ages) and milk and its environment?

Objectives of the study:

- To determine the prevalence and distribution of Salmonella in lactating cow, milk with its environment, and children.
- To analyze the prevalence and distribution between Salmonella isolates from animals, humans and environment.
- To assess the antimicrobial resistance profiles of isolated *Salmonella species* against commonly used antibiotics in study area.

2. LITERATURE REVIEW

2.1. History of Salmonellosis

Salmonellosis has existed for centuries. Advances in modern technology have revealed that typhoid fever played a role in a plague that devastated Athens around 430 B.C., causing the death of nearly one-third of the city's population (Patients *et al*., 2012). 1607-1624: Typhoid fever is thought to have caused the deaths of more than 6,000 settlers in Jamestown, Virginia. In 1879, German bacteriologist Dr. Karl Joseph Eberth identified bacilli in the abdominal lymph nodes and spleen of typhoid fever patients. His findings were later published and validated by other bacteriologists, including Robert Koch between 1880 and 1881 (Lee *et al*., 2015). This event marked the first visualization of Salmonella, though it had not yet been formally identified. In 1885, American veterinary scientist Daniel E. Salmon was credited with discovering the first strain of Salmonella. However, it was actually Theobald Smith, Salmon's research assistant, who isolated the first strain, *Salmonella cholera suis*. As Salmon was in charge of the research, he ultimately received recognition for the discovery. (Evans Chazireni, 2018). In 1888, Gartner isolated Salmonella bacteria as the causative agent of gastroenteritis from a fatal case involving a young man who had consumed raw meat from a diseased cow (Rahman *et al*., 2018).

Mary Mallon, a cook in the United States, was recognized as the first documented asymptomatic carrier of typhoid fever between 1906 and 1915. Unaware of her condition, she unintentionally transmitted the disease to many individuals through her work, leading to multiple outbreaks. Among the well-known figures suspected to have died from Salmonella infection are U.S. President William Henry Harrison and Wilbur Wright, one of the pioneering Wright brothers. *Salmonella Enteritidis* gained prominence as a major cause of human illness in the United States, accounting for just 5% of all Salmonella isolates in 1976 (Lee *et al*., 2015). Since the 2000s, Salmonella has remained a major public health concern, with the rise of antimicrobial resistance further complicating efforts to control and prevent salmonellosis (Alcaine *et al*., 2007).

2.2. Classification and Nomenclature

The classification and nomenclature of Salmonella are complex and have undergone revisions over time(Su & Chiu, 2005). The antigenic classification system for various Salmonella serotypes used today is the result of decades of research on antibody interactions with surface antigens, originally established by Kauffmann and White nearly a century ago. The World Health Organization Collaborating Centre for Reference and Research on Salmonella at the Pasteur Institute in Paris, France, is responsible for updating this classification. New serotypes are identified and reported annually in Research in Microbiology, and as of the latest report published in 2004, the genus Salmonella comprises a total of 2,541 serotypes (Popoff *et al*., 2004). The terms "serotype" and "serovar" are often used interchangeably; however, according to the Rules of the Bacteriological Code (1990 Revision) established by the Judicial Commission of the International Committee on the Systematics of Prokaryotes, "serovar" is the preferred term. Consequently, the Kauffmann-White classification scheme adopts "serovar." Salmonella nomenclature remains complex and continues to evolve. Currently, the Centers for Disease Control and Prevention (CDC) follows a nomenclature system based on recommendations from the WHO Collaborating Centre (Su & Chiu, 2005).

The genus Salmonella is part of the Enterobacteriaceae family, consisting of rod-shaped, Gram-negative bacteria. The type species of Salmonella is primarily responsible for most Salmonella-related infections in both humans and animals(OMSA, 2020). The genus Salmonella comprises only two species: *Salmonella enterica*, which is further classified into six subspecies *(S. enterica* subsp. *enterica, S. enterica* subsp. *salamae, S. enterica* subsp. *arizonae, S. enterica* subsp. *diarizonae, S. enterica* subsp**.** *houtenae,* and *S. enterica* subsp. *indica),* and *Salmonella bongori* (Le & Michel, 2002). *S. enterica* subsp. Enterica the most common subspecies, containing many important serovars causing human illness, *S. enterica* subsp. *Salamae* the Found in reptiles and occasionally in humans, *S. enterica* subsp. *Arizonae* Often associated with reptiles and occasionally in humans, (OMSA, 2020) . *enterica* subsp. *Diarizonae* loosely related to *S. enterica* subsp.*arizonae. S. enterica* subsp. *Houtenae* found in various animals, including reptiles, birds, and mammals, *S.nterica* subsp. *indica* Found in various animals, including reptiles, birds, and mammals, and *Salmonella bongori* is The second recognized species, less commonly associated with human infections, (Su & Chiu, 2005)

Salmonella serotypes are classified based on their somatic (O) and flagellar (H) antigens, a system established by Kauffman and White. Over 2,650 serotypes: The genus Salmonella contains over 2,650 serotypes, each with a unique antigenic profile. The full name of a serotype is given as, for example, "*Salmonella enterica* subsp. *enterica* serotype *Typhimurium*," but can be abbreviated to "*Salmonella Typhimurium* (Su & Chiu, 2005).

Previously, many Salmonella strains were considered separate species, but with the development of serotyping, they were reclassified as serotypes within *S. enterica*. In 1989, the serotype V was reclassified as a separate species, resurrecting the name *S. bongori* and the current nomenclature recognizes six subspecies under *S. enterica*, with serotypes assigned within each subspecies(Su & Chiu, 2005). Despite widespread familiarity among specialists, the conventional practice of using species names for Salmonella serotypes remains prevalent. Proper nomenclature is essential for the precise identification, monitoring, and management of Salmonella strains. A clear understanding of its classification and naming conventions aids in detecting outbreaks, tracing contamination sources, and implementing effective control strategies (OMSA, 2020).

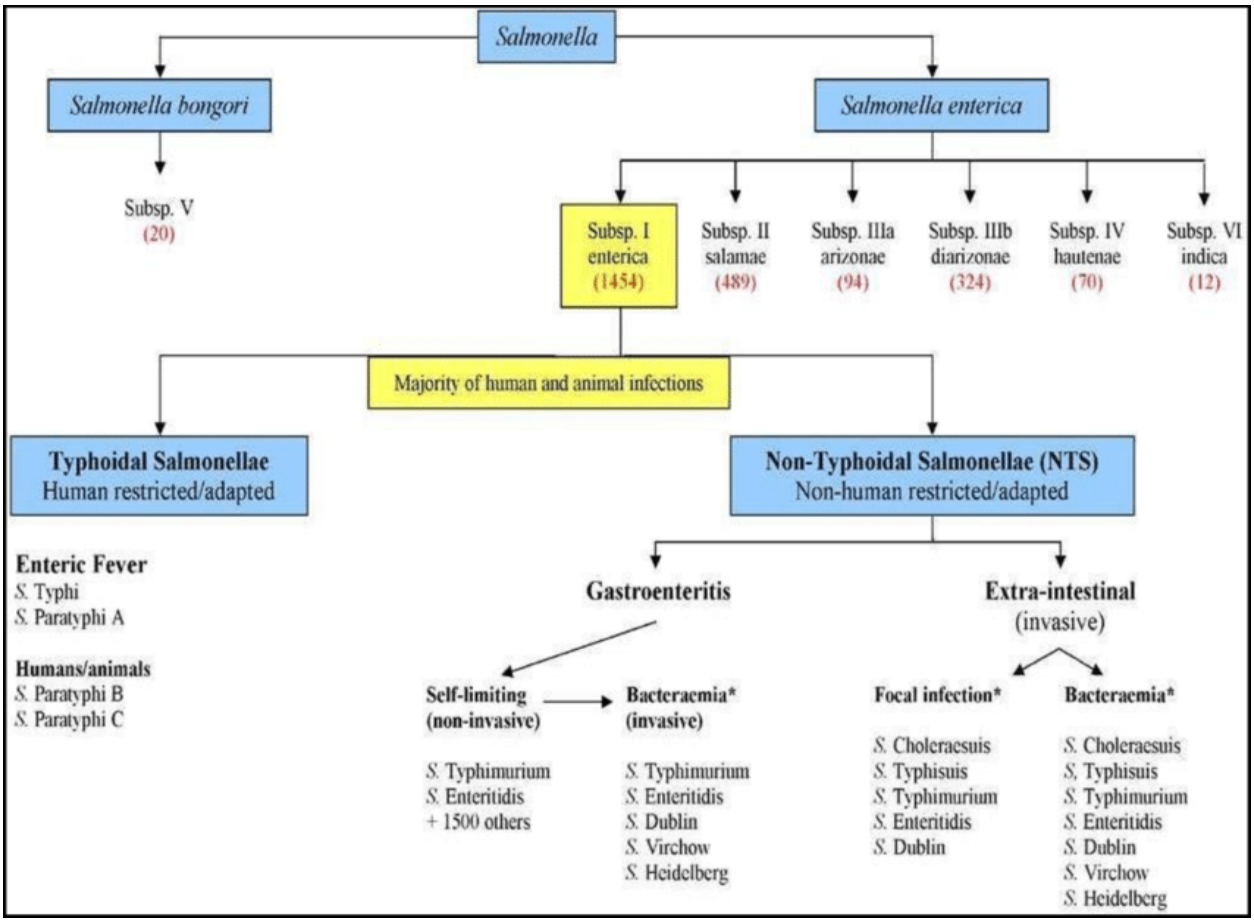

Figure 1: Salmonella classification **Source** (Hasan, (2021)

2.3. One Health Perspective of Salmonella Serovars

Salmonella is a diverse genus comprising over 2500 serovars which can cause a range of infections (Cuypers *et al*., 2023). Salmonella serotypes are classified into three categories: host-restricted, host-specific, and generalist serotypes. This classification has significant epidemiological and public health implications, influencing disease transmission, host susceptibility, and control strategies (Uzzau *et al*., 2000). Typhoidal Salmonella serovars *Typhi* and *Paratyphi* A exclusively infect humans and cause systemic disease. Non-typhoidal Salmonella (NTS) infections highlight the intricate relationship between humans, animals, and the environment, embodying the "One Health" approach. Many NTS serovars have zoonotic reservoirs, are predominantly linked to foodborne transmission, and can endure in the environment for prolonged periods (Cuypers *et al*., 2023).

From a One Health standpoint, certain Salmonella serovars hold considerable significance due to their profound effects on human, animal, and environmental health. Below is an overview of key serovars, based on the referenced sources.

2.3.1. *Salmonella Typhimurium*

Salmonella Typhimurium is a serovar of *Salmonella enterica*, recognized as one of the most extensively studied. *S. enterica* are differentiated based on their surface antigens O and H antigens which are utilized in serotyping to classify specific strains. Variations in these antigens contribute to differences in virulence, host range, and other biological characteristics (Sabbagh *et al*., 2010). This serovar is globally widespread and frequently implicated in foodborne outbreaks. Animal-to-human transmission is primarily associated with the consumption of contaminated animal products. The growing prevalence of multidrug resistance in this serovar is an increasing public health concern (Ramatla *et al*., 2022). In Ethiopia, Salmonella is highly prevalent among asymptomatic food handlers, diarrheal patients, and animals, with *Salmonella Typhimurium* being the most commonly detected serovar in human samples. Animal isolates have exhibited significant resistance to tetracycline, ampicillin, and streptomycin (Kahsay *et al*., 2023).

2.3.2. Salmonella Enteritidis

Salmonella enterica serovar Enteritidis (*S. Enteritidis*) is a significant cause of human salmonellosis globally (One of the most important Salmonella serovars responsible for human infection. One of its defining traits is its strong ability to form biofilms, with around 80% of infections attributed to this mode of growth. The biofilm lifestyle appears to resistance to antibiotics and immune responses response compared to the planktonic (free-floating) form of *Salmonella Enteritidis*. It is frequently linked to contaminated eggs, representing a significant public health risk (Ramatla *et al*., 2022).

A cross-sectional study examining Salmonella prevalence in poultry farms across central Ethiopia primarily focused on *Salmonella Typhimurium*. However, it also detected *Salmonella Enteritidis* in 23.3% (10 out of 43) of PCR-positive Salmonella isolates, providing direct evidence of *S. Enteritidis* presence in poultry within this specific region (Waktole *et al*., 2024). The virulence factors of Salmonella are primarily encoded within Salmonella Pathogenicity Islands (SPIs), enabling the bacteria to evade host immune defenses, establish infection,

replicate, and disseminate within complex host environments. SPI-1 plays a crucial role in facilitating Salmonella invasion into host cells and modulating immune responses (Xu *et al*., 2023)

2.3.3. Salmonella Hadar

Salmonella enterica serovar Hadar is a significant serovar associated with poultry, particularly turkeys, and is a cause of human salmonellosis. S. Hadar belongs to the O: 8 (C2-C3) serogroup and has the antigenic formula 6, 8:z10: e,n,x. It's prevalent in the U.S. and Europe, ranking among the top ten serovars isolated from non-clinical non-human sources in the U.S. and among the top four in human isolates in Europe (Rowe et al., 1980). It has also been reported in Asia (Thailand and China) and Africa. Emerging concern: Identified as a significant serovar in South Africa, isolated from animals, humans, and the environment (Ramatla *et al*., 2022). *Salmonella Hadar* is present across animal populations, food sources, and humans. There is also a concerning rise in multidrug-resistant strains. Ongoing surveillance and monitoring of *S. Hadar* particularly through phage typing and antimicrobial resistance profiling play a critical role in detecting outbreaks, pinpointing sources of infection, and guiding effective prevention and control strategies against salmonellosis (Pereira et al., 2007).

2.3.4. Salmonella Infantis

Salmonella enterica serovar Infantis (*S. Infantis*) is a globally distributed serovar affecting both humans and animals (Panzenhagen *et al*., 2018). A growing concern is the emergence of multidrug-resistant (MDR) strains of *Salmonella Infantis*, particularly those carrying plasmids such as pESI, which confer resistance to multiple antibiotics (Gymoese *et al*., 2019). The spread of these MDR strains presents a serious One Health challenge. A study conducted in Addis Ababa identified *Salmonella Infantis* as the most prevalent serotype, accounting for 36.4% of Salmonella isolates in minced meat samples from supermarkets. This underscores a considerable contamination concern within the city's meat supply chain. The study further revealed that 62.5% of *S. Infantis* isolates were derived from minced beef, whereas 18.8% originated from mutton and pork, indicating a potentially higher risk associated with beef products (Tadesse & Gebremedhin, 2015). The occurrence in raw milk and human stool samples demonstrates that the distribution and dissemination of virulence genes, although the S.infantis source of

infection not known due to lake of background information, but the milk are one of the most common sources known of human salmonellosis (Al-Rubaey *et al*., 2020).

2.3.5. Salmonella concord

*Salmonella enteric*a serovar Concord (*S. Concord*) is a non-typhoidal Salmonella (NTS) serovar that is rarely documented. The earliest reported isolates of S. Concord date back to 1944, with origins in both the United States and the United Kingdom. These initial detections involved samples from poultry in the U.S. and human stool specimens in the U.K (New & Man, 1944). Several studies have documented the occurrence of *Salmonella enterica serovar Concord (S. Concord)* in Ethiopia between 1974 and 1981, as well as in Saudi Arabia in 1982. From 2003 onward, a significant rise in *S. Concord* infections among humans was observed in both Europe and the United States (Sjölund-Karlsson *et al*., 2011). The spread of *Salmonella enterica serovar Concord (S. Concord*) has been associated with the international adoption of Ethiopian children. Infected adoptees were either asymptomatic or presented with mild gastrointestinal symptoms, such as abdominal discomfort, diarrhea, or bloody diarrhea, sometimes accompanied by fever. A study conducted in Ethiopia reported that 30.6% of *S. Concord* infections resulted in bloodstream involvement, and exhibits significant genetic diversity and a polyphyletic structure, with multiple distinct lineages. Four of these lineages were prevalent in Ethiopia, characterized by multidrug resistance (MDR) and ceftriaxone resistance. In contrast, other lineages have a broader global distribution, lack antimicrobial resistance (AMR) markers, and include isolates associated with both human infections and food sources. (Cuypers *et al*., 2023)

2.3.6. Salmonella enterica serovar Dublin (S. Dublin)

This serovar primarily impacts cattle, leading to enteritis and systemic infections. While human cases are relatively rare compared to its prevalence in cattle, transmission can occur through the consumption of contaminated beef or milk from infected animals (Tomaso & Methner, 2018). The bacterial invasome of Salmonella incorporates several virulence factors that enhance its pathogenic potential. Key contributors include the Gifsy-2 prophage and two distinct type 6 secretion systems (T6SSs), located within Salmonella pathogenicity islands SPI-6 and SPI-19. Additionally, virulence genes ggt and PagN play a role in bacterial invasion. While the Vi antigen and the virulence plasmid have (M. Mohammed *et al*., 2017)

2.3.7. Salmonella Gallinarum and Salmonella Pullorum

S. Gallinarum and *S. Pullorum* are highly pathogenic to poultry, capable of causing severe disease. These serovars are significant avian pathogens, leading to considerable economic losses in the poultry industry (Xiong *et al*., 2018). A thorough understanding of their unique traits, modes of transmission, and control strategies is vital for efficient disease management in poultry production. The advancement of rapid and precise diagnostic methods is key to early detection and effective outbreak prevention (Wales & Lawes, 2023).

2.4. Pathogenicity and Virulence Factors

Virulence among Salmonella serovars varies considerably. *S. Typhi*, for instance, is highly adapted to human hosts and leads to systemic infections, such as typhoid fever. In contrast, *S. Typhimurium* and *S. Enteritidis* primarily cause gastroenteritis. These differences stem from variations in the composition and expression of virulence factors, including the presence or absence of specific Salmonella pathogenicity islands (SPIs), plasmids, and other genetic elements that define each serovar's pathogenic profile (Xu *et al*., 2023).

Most virulence genes in Salmonella are concentrated in specific regions scattered throughout the chromosome, known as Salmonella pathogenicity islands (SPI) (Van Asten & Van Dijk, 2005). Salmonella pathogenicity islands (SPIs) are genomic regions acquired through horizontal gene transfer that contain clusters of virulence genes. These regions play a crucial role in the pathogen's ability to infect and survive within host organisms (Ramatla *et al*., 2024).

2.4.1. SPI-1 and SPI-2

The most prominent Salmonella pathogenicity islands (SPIs) encode the Type III Secretion System (T3SS), which functions like a molecular syringe. This system delivers effector proteins directly into host cells, altering their processes to promote bacterial invasion and intracellular persistence (Ibarra & Steele-Mortimer, 2009).

2.4.2. Type III Secretion System (T3SS)

The Type III Secretion System (T3SS) plays a critical role in Salmonella pathogenesis. SPI-1 T3SS facilitates the invasion of epithelial cells in the small and large intestine, triggering

inflammation and diarrhea, which are key features of early infection. Meanwhile, SPI-2 T3SS enables Salmonella to survive and multiply within macrophages; immune cells responsible for pathogen clearance allowing the bacteria to persist and spread to other organs (Nikiema *et al*., 2021).

2.4.3. Lipopolysaccharide (LPS)

The outer membrane of Salmonella contains lipopolysaccharide (LPS), which functions as an endotoxin. Upon release, LPS triggers a potent inflammatory response in the host, leading to key symptoms of salmonellosis such as fever, chills, and abdominal pain. In the intracellular Survival Mechanisms the Type III Secretion System (T3SS) encoded within SPI-2, along with additional virulence factors from Salmonella pathogenicity islands (SPIs) 2, 3, 5-8, 10-13, and 16, enables Salmonella to survive within macrophages. These factors allow the bacterium to endure the acidic conditions of the phagosome, facilitating intracellular replication and persistence within the host. Additionally, certain Salmonella strains produce toxins, including enterotoxins, which further exacerbate gastrointestinal symptoms, contributing to diarrhea and other clinical manifestations (Bahramianfard *et al*., 2021).

2.4.4. Fimbriae and Flagella

Fimbriae are slender, filamentous structures projecting from the surface of Salmonella that play a crucial role in adherence. They enable the bacterium to attach firmly to the epithelial cells lining the intestinal tract, which is a key step in establishing infection. This attachment facilitates colonization and assists the pathogen in invading host tissues. In contrast, flagella are long, whip-like appendages that provide Salmonella with motility. These structures allow the bacterium to navigate its environment, swim toward favorable conditions, and disseminate within the host. This movement aids in locating target tissues and evading the host's immune defenses, contributing to the progression and spread of infection (Dibb-Fuller *et al.*, 1999).

2.4.5. Virulence Plasmids

Extra chromosomal DNA molecules, such as plasmids, can encode various virulence factors, including the spv ABCD system. This system plays a crucial role in systemic virulence, enabling Salmonella to disseminate beyond the intestines and infect other organs (Bahramianfard *et al*., 2021). Typhoidal Salmonella strains, such as *Salmonella Typhi*, are responsible for typhoid

fever, a severe systemic infection. These pathogens possess key virulence factors, including typhoid toxin and the Vi antigen, which enhance immune evasion and facilitate disease progression (Silva *et al.*, 2017). In contrast, non-typhoidal Salmonella strains, like *Salmonella Enteritidis* and *Salmonella Typhimurium*, primarily cause gastroenteritis. Their pathogenicity is linked to various genes, including invA, fimA, stn, spvR, spvC, spiC, and pipD, which contribute to bacterial invasion, survival, and overall virulence (Ramatla *et al*., 2024).

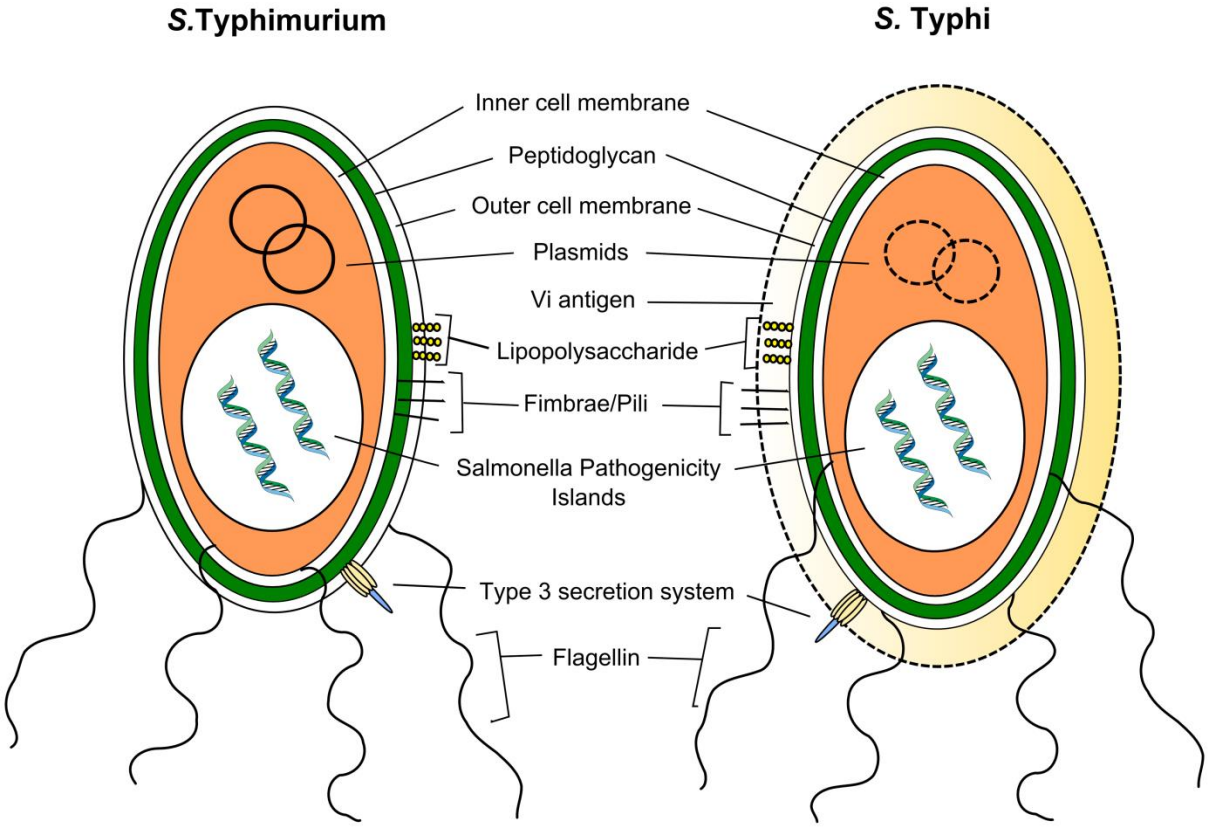

Figure 2: (Source: https://doi.org/10.1371/journal.ppat.1002933.g002)

2.5. Salmonella Occurrence as One Health Perspectives in Ethiopia

Salmonella infections pose a major public health challenge in Ethiopia, affecting both human populations and livestock, with implications for food safety and disease control (Tadesse, 2014). One Health approach highlights the interconnectedness of these aspects and emphasizes the need for multidisciplinary efforts to control the spread of this pathogen. The prevalence of human salmonellosis in Ethiopia varies considerably depending on the study and methodology used (Nyokabi et al., 2023). Several studies provide different prevalence rates (Table1)

Table1: Salmonella Occurrence in Ethiopia

category	Subcategory		Prevalence/Finding	Source
Human	human stools		4.8%, 10.8%	Abate & Assefa, 2021 , Lamboro *et al*., 2016
	diarrheic children		8.72%, 6.2%, 6.9%	Tadesse, 2014 **,** Beyene & Tasew, 2014 **,** Assefa & Girma, 2019
	diarrheic adults		5.68%	Tadesse, 2014 **(**humanSalmonellosis**)**
	in carriers		1.08%	Tadesse, 2014
Animal	Cattle (carcass)		7.07%	, Tadesse & Tessema, 2014 (salmonella in food animals)
	Sheep (carcass)		8.41%	Tadesse & Tessema, 2014
	Goats (carcass)		9.01%	Tadesse & Tessema, 2014
	Pigs (carcass)		43.81%	Tadesse & Tessema, 2014
	Poultry (farm-level)		50.6% farm, 14.4% sample	Waktole *et al*., 2024
	Goat meat (carcass)		3.86%	Tadesse & Gebremedhin, 2015 (Salmonella in raw animal products)
	Beef (carcass)		4.53%	Tadesse & Gebremedhin, 2015
	Minced beef		8.34%	Tadesse & Gebremedhin, 2015
	Raw Milk		10.76%, 7.1%	Tadesse & Gebremedhin, 2015, Temesgen *et al.*, 2025
Environment	Water	non-recycled surface water	0.31%.	Rocha *et al*., 2022
		groundwater	0.17%),	Rocha *et al*., 2022
	Plants	Vegetable, farms fertilized with animal manure	2.3%	Yalew, 2020

2.5.1. Human Salmonellosis

A systematic review and meta-analysis of studies conducted between 1974 and 2012 reported a pooled prevalence of Salmonella in human stool samples, with rates of 8.72% in diarrheic children, 5.68% in diarrheic adults, and 1.08% in asymptomatic carriers (Tadesse, 2014). Another meta-analysis covering the period from 2010 to 2020 found a pooled prevalence of 4.8% in human stools (Abate & Assefa, 2021) The most frequently identified serotypes among patients were *S. Concord, S. Typhi, S. Typhimurium*, and *S. Paratyphi*, collectively accounting for 82.1% of isolates. Notably, non-typhoidal Salmonella strains constituted 57.9% of patient isolates, underscoring their significant role in gastroenteritis cases (Tadesse, 2014); (Tadesse & Tessema, 2014).

2.5.2. Animal Salmonellosis

A meta-analysis of studies conducted in Ethiopia revealed a notable prevalence of Salmonella in slaughtered animals: 7.07% in cattle, 8.41% in sheep, 9.01% in goats, and 43.81% in pigs. In poultry farms, 57.9% of the identified isolates belonged to non-typhoidal Salmonella (Waktole *et al*., 2024). Specific serovars exhibited host-associated patterns, with *S. Mishmarhaemek* predominating in cattle, *S. Infantis* in small ruminants, *S. Gallinarum* and *S. Pullorum* in poultry, and *S. Hadar* in pigs. Importantly, *S. Typhimurium* was detected across all host species, underscoring its significance in zoonotic transmission (Tadesse & Tessema, 2014)

2.5.3. Environmental Salmonellosis

Studies in Addis Ababa have shown a prevalence of 2.3% Salmonella in vegetable farms fertilized with animal manure (Yalew, 2020). Manure has the potential to serve as an environmental contaminant, contributing to crop contamination. Research examining the ability of melon plants to absorb Salmonella from contaminated irrigation water revealed that while the pathogen could persist in the soil and rhizosphere, it was absent from the internal tissues of the melon after surface disinfection. These findings suggest that although soil contamination is possible, the likelihood of Salmonella penetrating and contaminating the edible portion of the melon remains minimal. (Jechalke *et al*., 2019); (Rocha *et al*., 2022).

Studies indicate that Salmonella can persist in soil for as long as six weeks, increasing the likelihood of contamination in tomato plants. This survival capability presents a potential risk for

the infection of future crops. (Lopez-Velasco *et al*., 2012); (Hailu *et al*., 2024). Proper soil management is crucial in preventing the spread of Salmonella. The Addis Ababa study identified animal manure used as fertilizer as a major contributor to soil contamination, emphasizing the need for effective control measures to mitigate the risk of pathogen transmission (Yalew, 2020). Irrigation water contaminated with Salmonella can serve as a pathway for soil contamination, increasing the risk of crop exposure to the pathogen (Jechalke *et al*., 2019). Previous crops can serve as a source of Salmonella contamination for subsequent crops, especially if crop debris is not properly managed (Hailu *et al*., 2024).

A meta-analysis estimated the prevalence of Salmonella in non-recycled surface water sources at 0.31%, underscoring the risk of waterborne contamination, particularly in regions with insufficient sanitation and water treatment. Although Salmonella was detected at a lower frequency in groundwater (0.17%), it still poses a potential threat to water quality and public health (Rocha *et al*., 2022). The use of contaminated irrigation water can lead to the contamination of soil and crops, posing a risk to human health (Jechalke *et al*., 2019).

2.6. Transmission Vehicles of Salmonella

Salmonella is extensively distributed in the environment and demonstrates a remarkable ability to persist in various food sources. Traditionally, poultry, eggs, and dairy products have been identified as the primary vectors of salmonellosis, posing significant public health risks. However, in recent years, fresh produce including fruits and vegetables has emerged as a growing concern in Salmonella transmission (Bouchrif *et al*., 2009). The primary route of transmission in both humans and animals is through the consumption of food or water that has been contaminated with fecal matter (Figure 3) (Oludairo *et al*., 2023). Contamination can occur at multiple stages throughout the food chain, from agricultural practices and irrigation with contaminated water to handling, processing, and distribution. This highlights the need for stringent food safety measures to mitigate the risk of Salmonella-related infections (Bouchrif *et al*., 2009).

Environmental factors including temperature, rainfall, pollution levels, nutrient availability, and other climate-related changes can significantly influence the growth and spread of Salmonella. These conditions may create environments that promote bacterial survival, reproduction, and

transmission (Billah & Rahman, 2024). The persistence of Salmonella in the environment serves as a primary infection source, as the bacterium can survive for extended periods. It is subsequently transmitted to vectors such as rats, flies, and birds, which can shed the pathogen for weeks or even months. Following direct transmission, livestock including swine, cattle, and poultry play a critical role in disease spread. These animals become infected primarily through oral exposure, often via contaminated surroundings or feed. Humans acquire Salmonella infections by consuming food or water contaminated through these animal reservoirs. However, *Salmonella Typhi* and *Salmonella Paratyphi*; in lack of animal reservoirs, with transmission occurring through the ingestion of improperly handled food contaminated by infected individuals (Newell *et al*., 2010).

Figure 3: Transmission cycle salmonellosis (Source: (Liu *et al*., 2023))

2.6.1. Foodborne Salmonella in Ethiopia

In recent years, the incidence of foodborne, Salmonella infections in Ethiopia has risen significantly. Research from various regions across the country has confirmed the presence of Salmonella in humans, food-producing animals, and a wide range of food products(Table2) (Ejo *et al.*, 2016).

Table2: summary of Salmonella prevalence from different food sources in Ethiopia based on published data from foodborne outbreak investigations:

Food Source	Salmonella Prevalence (%)	References
goat carcasses, beef carcasses, minced beef and milk	3.86%, 4.53%, 8.34% and 10.76% respectively.	Tadesse & Gebremedhin, 2015
Kitfo (minced raw meat)	45%	Kahsay & Hagos, 2020
Street vendor foods (kitfo, tibs, etc.)	30-50%	Reda *et al*., 2017
Processed meat (salami, sausage)	20-30%	Waktole *et al*., 2024
Raw milk	5-15%	Castañeda-Salazar *et al*., 2021
Eggs	10-20%	. Tadesse & Ali, 2024

2.7. Environmental Stresses Affecting Salmonella from One Health Perspective

These environmental shifts pose an increasing threat to public health, particularly for vulnerable groups such as young children, the elderly, and individuals with weakened immune systems. Changes in temperature, humidity, and extreme weather patterns create favorable conditions for Salmonella survival and proliferation. Rising global temperatures accelerate bacterial growth in food sources, while altered precipitation and flooding events facilitate the spread of Salmonella in water systems and agricultural environments. Consequently, fresh produce often consumed raw is at heightened risk of contamination through irrigation with contaminated water, soil exposure, and post-harvest handling (Awad *et al*., 2024), rising temperatures, a direct result of climate change, are enhancing Salmonella proliferation and extending its survival, increasing the

risk of contamination and foodborne illness (Morgado *et al*., 2021). A temperature rise of 1°C has been associated with an 8.8% increase in Salmonella-related illness cases, highlighting the significant impact of climate change on foodborne infections, (Dietrich *et al*., 2023). The combination of temperature, rainfall, and drought can affect Salmonella persistence and spread in the environment (Awad *et al*., 2024).

2.8. Salmonella and Its Antimicrobial Resistant Profile in Ethiopia

Some strains of Salmonella have developed resistance to certain antibiotics, which can make it more challenge to treat infections (Marchello *et al*., 2020). Antimicrobial resistance (AMR) in Salmonella is a significant public health concern globally. In Ethiopia Studies have consistently shown a high prevalence of antimicrobial resistance in Salmonella isolates (Table3) (Wabeto *et al*., 2017); (Dessale *et al*., 2023). Study at the Wolaita Sodo municipal abattoir indicates that Salmonella in 12.5% of sampled carcasses. These isolates exhibited resistance to 1 to 12 antimicrobial drugs, with high resistance rates to tetracycline (83.93%), nitrofurantoin (73.21%), and streptomycin (66%) (Wabeto *et al*., 2017); in Debre Markos town found a high prevalence of resistance to chloramphenicol (71.4%) among Salmonella isolates from under-five diarrheic children (Dessale *et al*., 2023). A cross-sectional study across central Ethiopia found Salmonella in 50.6% of poultry farms, with a 14.4% overall sample-level prevalence. Many isolates are found multidrug resistance to ten of the tested antibiotics (Ejo *et al*., 2016). A study in Bishoftu, Ethiopia, found that 95.5% of Salmonella isolates from cattle, beef, and diarrheic patients were resistant to at least one of the 14 antimicrobials tested (Gutema *et al*., 2021).

Salmonella acquires antibiotic resistance through multiple mechanisms. Mutations in target genes can reduce susceptibility by altering the binding sites of antibiotics. Some strains produce enzymes that inactivate antibiotics, neutralizing their effects. Additionally, modifications to the bacterial cell wall can limit antibiotic entry, further decreasing effectiveness. Salmonella may also develop efflux pumps, actively expelling antibiotics from the cell, preventing their therapeutic action (Moraes *et al*., 2024).

Table3: Trends in Salmonella Antimicrobial Resistance in Ethiopia

Antibiotic Class	Resistance (%)	Source(s)
Ampicillin	80.6%,93.3%, 100%	Waktole *et al*., 2024, Gebeyehu *et al*., 2022, Abebe *et al*., 2018, Muse et al., 2024
Tetracycline	63.5%, 83.93%, 50%)	Waktole *et al*., 2024, Wabeto *et al*., 2017, Bedassa *et al*., 2023
Sulfamethoxazole-Trimethoprim	46.7%, 25%, 40%	Waktole *et al*., 2024 , Temesgen *et al*., 2025, Mekonnen *et al*., 2022, Mohammed & Dubie, 2022
Kanamycin	100%, 82%, 73.3%	Abdi *et al*., 2017, Abunna *et al*., 2017, Mohammed & Dubie, 2022
Nalidixic Acid	97.8%, 75%, 67.7%	Abdi *et al*., 2017, Abunna *et al*., 2017, Mohammed & Dubie, 2022
Cefoxitin	97.8%, 63.6%, 53%	Abdi *et al*., 2017, Abunna *et al*., 2018, Mohammed & Dubie, 2022
Streptomycin	97.8%, 66%, 72.7 %, 67%	Abdi *et al*., 2017, Wabeto *et al*., 2017, Abunna *et al*., 2018, Ayichew *et al*., 2024
Chloramphenicol	91.3%, 33.3%, 50%	Abdi *et al*., 2017, Muse *et al*., 2024, Ayichew *et al*., 2024)
Ciprofloxacin	8.7%, 31.1%, 15.5%	Abate & Assefa, 2021, Sisay, 2021,
Ceftriaxone	12.2%, 16% , 58%	Abate & Assefa, 2021, Sisay, 2021, Ayichew *et al*., 2024

2.8.1. Contributing Factors to Antimicrobial Resistance in Ethiopia

In Ethiopia, the rise of antimicrobial resistance (AMR) presents a major public health threat that spans the food production system, healthcare facilities, and environmental settings. Epidemiological research has revealed concerningly high levels of AMR in food-producing animals, food handlers, and environmental samples. Notably, bacteria isolated from live animals show a 20% prevalence of resistance, with particularly high levels detected in milk and meat

products (Woldu, 2024). The overuse and misuse of antibiotics in human and animal healthcare contribute significantly to the development and spread of AMR. The widespread use of antibiotics in livestock, especially for growth enhancement and disease prevention, plays a significant role in the spread of antimicrobial resistance (AMR). Additionally, inadequate sanitation and hygiene measures facilitate the transmission of Salmonella and contribute to the emergence of resistant bacterial strains, exacerbating public health risks (Kemal *et al*., 2015).

2.8.2. Impact of Antimicrobial Resistance

The improper use of antibiotics in human medicine, animal health, and agriculture accelerates the spread of antimicrobial resistance (AMR), fueling what has been called a "Silent Pandemic." If left unaddressed, AMR could surpass all other causes of death by 2050, threatening the effectiveness of routine medical procedures and potentially resulting in millions of deaths each year. Economically, AMR is projected to cause trillions of dollars in losses and place immense strain on both healthcare systems and the agricultural sector (Ahmed *et al.,* 2024). Antimicrobial resistance (AMR) in Salmonella significantly complicates treatment efforts in both humans and animals, as conventional antibiotics become less effective against resistant strains. This resistance often results in prolonged illness, increased severity of symptoms, and a higher likelihood of complications. Consequently, healthcare costs rise due to extended hospitalization, additional diagnostic procedures, and the need for alternative, often more expensive, antimicrobial therapies (Kemal *et al*., 2015).

2.9. Salmonella Control Strategies with One Health approach

The One Health framework acknowledges the intricate connection between human, animal, and environmental health. Foodborne illnesses, such as salmonellosis, exemplify this interdependence, as pathogens can be transmitted from animals to humans via contaminated food sources (Silva *et al*., 2014). Effectively mitigating Salmonella infections necessitates a multidisciplinary approach, bringing together healthcare professionals, veterinarians, farmers, food producers, and public health authorities. Through coordinated efforts in surveillance, biosecurity, food safety practices, and antimicrobial stewardship, the One Health strategy enhances disease prevention and control, reducing Salmonella-related risk (Wang *et al*., 2021).

The most effective approach to controlling Salmonella is through prevention by Implementing strict food safety practices across the entire food chain, Good Agricultural Practices (GAPs) The practices aim to minimize contamination of food products during production, Good Manufacturing Practices (GMPs) The practices ensure safe food processing and handling, and Proper hygiene practices during food preparation and consumption (Silva *et al*., 2014).

Vaccination of poultry against Salmonella is a key strategy to reduce the prevalence of the pathogen in poultry flocks. Research into new antibiotic alternatives, such as bacteriophages and probiotics, is crucial, and Continuous surveillance and monitoring of Salmonella occurrence and antimicrobial resistance patterns and Whole Genome Sequencing (WGS) is a powerful instrument for controlling Salmonella outbreaks and understanding transmission routes (King *et al*., 2008). Effective Salmonella control depends entirely on coordinated efforts and transparent communication among key stakeholders. Farmers, food producers, healthcare professionals, veterinarians, and public health authorities must work together to implement preventive measures, enhance surveillance, and enforce food safety standards. This integrated approach strengthens disease management, reduces transmission risks, and safeguards both human and animal health (Silva *et al*., 2014).

Ethiopia has adopted a One Health approach to address zoonotic diseases, including Salmonella, which is listed as a second-tier priority disease in the country's National One Health Strategic Plan (2018-2022) (Fascendini, 2019). Ethiopia is enhancing its surveillance and reporting systems to track Salmonella outbreaks and trends more effectively. This effort involves comprehensive data collection from both human and animal populations, facilitating early detection and response. Additionally, the country is implementing a range of preventive and control measures to mitigate the spread of infection and safeguard public health (Gebreyohannes *et al*., 2018).

3. MATERIALS AND METHODS

3.1. Study Area

This study was conducted in and around Bishoftu (figure 4); a town situated 45 km southeast of Addis Ababa, within the East Shewa zone of Oromia, Ethiopia. Geographically, the town lies at 9° North latitude and 40° East longitude, with an altitude of 1850 meters above sea level in Ethiopia's central highlands. The region experiences an annual rainfall of 866 mm, predominantly occurring during the long rainy season (June to September), while the short rainy season extends from March to May. The dry season lasts from October to February. The area's mean annual temperatures range from a maximum of 26°C to a minimum of 14°C, accompanied by an average relative humidity of 61.3% (Biazen, 2014) (NMSA, 2010). The study area hosts a thriving smallholder urban dairy industry, which plays a vital role in local food production. Despite this, comprehensive data on the interconnected dynamics of human, animal, and environmental health particularly concerning milk-borne zoonotic pathogens like Salmonella remains limited. This gap in knowledge could pose significant public health risks and contribute to economic challenges, ultimately affecting the livelihoods of smallholder dairy producers. Currently, a large number of small holder urban dairy productions are operating in the present study area. However, information on humans, Animals, and environmental linkage of milk-borne zoonosis such as Salmonella remains scarce. Thus, lack of information could result in public health risks and economic losses affecting the livelihoods of smallholder dairy producers.

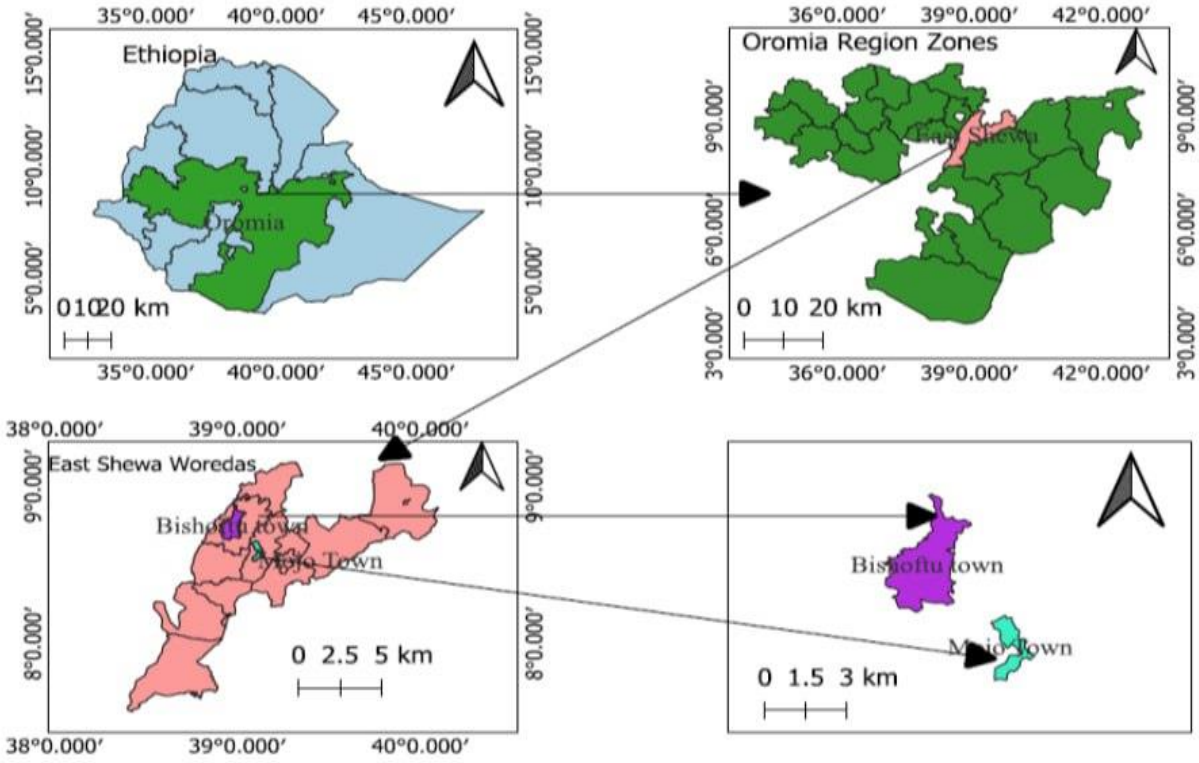

Fig.4: District Map of Ethiopia Showing Study Area

3.2. Study Design, Population, and Sampling

A cross-sectional study design was used to assess the prevalence and distribution of Salmonella in dairy farm, children and milk with its environment, using Omni Log Methods and assess the antimicrobial resistance profiles of Salmonella strains against commonly used antibiotics in the study area from November 2024 to May 2025.

A total of 336 samples were collected from 28 small and medium dairy farm; most of them (90%) were crossbreeds whereas a few were local (10%) Concerning management, of herds were semi-intensive kept indoors and received concentrate feeds in addition to hay and crop residues (such as corn stalks, wheat/barley straw and other leftovers from grain threshing) that rent milk for surround children, under-five children that use rented milk from small and medium dairy farm in the area and, milk; with its environment. Those samples were collected from Milk and rectal faces of 44 lactating cows; stool of 122 under-five children that use cow milk in the study area and, environmental samples from 26 milk container (instrument) swab, 26 milkier hand swab and 28 floor swab and 46 teat swab. Each sample was labeled to indicating code number

and other particulars of the sample and taken into transport medium (peptone water**)** by placed in ice box containing ice pack and bring to the laboratory for further processing and microbiological analysis.

In this study , raw milk and fecal samples of milking cows from small and medium dairy farm that rent milk for surround children , stool of under-five children that use cow milk in the study area, floor swap, udder swap, milkier hand swabs and swabs from milk containers were gathered from various sources (small and medium dairy farm in and around (modjo, dukdm) Bishoftu town.

A stratified random sampling method was used to collect samples from dairy farms. The farms were categorized based on their herd size into three strata; small-scale < 10 animals, medium-scale 10 to 50 animals, and large-scale >50 animals using the classification made by Megersa and his friends (Megersa *et al*., 2011)**.** A simple random sampling technique was employed to select dairy farms, and Lactating cows within the farm were selected by a simple random lottery technique from each dairy farm**.** Under-five children that use rented milk from selected dairy farms were selected purposively and a simple random lottery method for collected sample. All samples were clearly labeled with the date of sampling, the type of sample, and the name of the farm and children then held in an icebox with ice packs and transported to the Veterinary Public Health (VPH) laboratory of the College of Veterinary medicine and agriculture, Addis Ababa University (AAU-CVMA). In the laboratory, the samples were cultured in enrichment medium by 37°C for 24h and inoculated onto a standard bacteriological media. After the isolation of Salmonella, the positive isolates were transported to Animal Health Institutes (AHI), Sebeta by standard transporting medium for confirmation.

3.3. Sample Size Determination

The sample size was calculated for two independent populations using previous prevalence of Salmonellosis for diarrheic children 8.72% (Tadesse, 2014) , for lactating dairy cow 2.7% (Mekonnen et al., 2022), at of desired absolute precision and 95% confidence interval. Using the formula recommended by Thrustfield (2005);

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

$$d^2$$

Where, n = required sample size, Pexp = expected prevalence, d = desired absolute precision

Accordingly, 336 which are 122 samples are going to be collected from children, 44 from cow's faces, 44 from cow's milk, and from its environment 46, udder swab 28 floor swab, 26 milk material and 26 milkier hand swabs

3.4. Sample Collection and Transportation

Samples were obtained from various sources, such as milk, feces of milking cows from small and medium dairy farm, swap from udder, milkier hand, milk containers and floor and, stool of under-five children that use cow milk. 3-5g of fecal samples were collected directly from the rectum and placed in a 20 ml universal screw-capped bottle containing 10 ml of peptone water as transport media. During milking, the milk samples were collected from the teat and milking material. The swab samples were collected before milking using a sterile wooden cotton swab and placed in a sterile test tube containing 10 ml of buffered peptone water as transport media. One stool specimen per child was collected in a sterile container with transport media buffered peptone water (Rhiwadi-301019 India). The child's caretaker or parent together with research assistants were requested to collect the child's stool by using the spoon built into the cap of the container with 10ml of peptone water, approximately 1-2g (Microbiology, 2023) of the stool was collected and the cap was replaced and tightly closed. All samples were labeled and transported immediately to the Veterinary Public Health Laboratory of the AAU-CVMA for bacterial isolation. Finally , the suspected colony of Salmonella was confirmed at the Animal Health Institute (AHI), Sebata by using the OmniLog system and antimicrobial susceptibility testing (AST) was performed on the isolates.

3.5. Isolation and Identification

The detection, enumeration, and serotyping of Salmonella followed the microbiology of the food chain guidelines, specifically the horizontal method outlined in ISO-6579-1:2017 (ISO 6579-1, 2017). The procedure consisted of three key stages: pre-enrichment, selective enrichment, and selective plating to isolate Salmonella colonies. In the pre-enrichment phase, 1 ml of the collected milk sample was added to 9 ml of buffered peptone water (Rhiwadi-301019 India),

homogenized, and incubated at 37°C for 24 hours to facilitate the recovery of Salmonella. For the secondary enrichment, Rappaport Vassiliadis with soya broth (RVS) was equilibrated to room temperature following the manufacturer's instructions. After thorough mixing of the primary enrichment sample, a 0.1 ml aliquot was transferred to 10 ml of Rappaport-Vassiliadis with soya broth (RVS Goa-403 722 INDIA) and incubated at 41.5°C for 24 hours. The enriched samples were then streaked on to Xylose-Lysine Deoxycholate (XLD) agar (XL 297 INDIA) using a 10 µl loop and incubated at 37°C for 24 hours. Presumptive Salmonella colonies, identified by their characteristic pink coloration with or without black centers, were sub-cultured on XLD agar under identical conditions for further confirmation. Three to five typical Salmonella colonies were selected, streaked onto nutrient agar (A-902 A India), and incubated at 37°C for 24 hours for biochemical identification.

3.6. Biochemical Characterization of Salmonella Isolates

Biochemical tests were conducted to confirm the identity of potential Salmonella isolates, including the Triple Sugar Iron (TSI) test, Indole test, Citrate utilization test, motility test, and urease test. Salmonella isolates were identified based on specific metabolic characteristics: a red slant (alkaline) with a yellow butt (acid) in the TSI test, blackening due to hydrogen sulfide (HS) production, and gas formation in the butt. Additionally, the isolates exhibited negative Indole and urease tests (yellow coloration), while demonstrating positive citrate utilization (deep blue slant). Isolates fulfilling these criteria were subsequently transferred to Nutrient Agar (NA) for OmniLog-based identification and antimicrobial susceptibility testing.

3.7. Identification of Salmonella Using Omnilog

Suspected Salmonella isolates, identified through various biochemical tests, were inoculated on Nutrient Agar and transferred to the Sebeta Animal Health Institute (AHI) for confirmation using OmniLog technology. The isolates were cultured on Biolog Universal Growth (BUG) agar medium, where a single colony was suspended in a specialized gelling inoculation fluid (IFA), ensuring standardized cell density. A 100 µL aliquot of the cell suspension was then inoculated into a well of the GEN III MicroPlate, which was incubated under controlled conditions. Over a 22-hour incubation period at 33°C, bacterial metabolism interacted with the biochemical tests embedded within the Micro Plate. This metabolic activity generated a unique fingerprint,

enabling precise identification of Salmonella based on its distinctive biochemical profile. (Blvd, 2008) The Biolog Micro Station reader analyzed the metabolic fingerprint, and the data was imported into OmniLog Data Collection software. The software then searched an extensive database, rapidly identifying the Salmonella isolate within seconds.

3.8. Antimicrobial Susceptibility Test

Salmonella isolates confirmed through OmniLog were subjected to antimicrobial susceptibility testing (AST) using the Kirby–Bauer agar disc diffusion method, as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018). The isolates were tested against ten selected antibiotics commonly used in treatment: Ampicillin (AMP 10µg), Cefotaxime (CTX 10µg), Cefoxitin (FOX 10µg), Ceftazidime (CAZ 30µg), Ceftriaxone (CTX 30µg), Tetracycline (TET 30µg), Ciprofloxacin (CIP 10µg), Trimethoprim/Sulfamethoxazole (SXT 25µg), Amikacin (AMK 10µg), and Gentamicin (GEN 10µg). Pure colonies from nutrient agar plates were transferred into tubes containing 5 ml of sterile saline solution (0.85%). The bacterial suspension was then adjusted to a 0.5 McFarland turbidity standard using a densitometer. A sterile cotton swab was used to evenly inoculate the surface of Mueller-Hinton agar plates, which were left to dry for three minutes in a biosafety cabinet. Following this, antimicrobial discs with known concentrations were carefully placed onto the agar surface. The plates were incubated for 22 hours at 37°C, allowing the antibiotics to diffuse and interact with the bacterial isolates. After incubation, the diameters of the inhibition zones were measured to the nearest millimeter using a caliper. The results were then compared against established standards and classified as resistant, intermediate, or susceptible based on interpretive criteria (CLSI, 2018). (Table 4)

Table 4: List of antimicrobial discs used, their strength, and zone diameter
Interpretive breakpoints in mm

Antimicrobial classes	Antimicrobial agents	Abbreviations	Susceptible(S)	Intermediate(I)	Resistant (R)
Penicillin	Ampicillin	AMP	≥17	15-16	≤14
Cephalosporin	Cefotaxime	CTX	≥26	23–25	≤22
	Cefoxitin	FOX	≥18	15-17	≤14

	Ceftazidime	CAZ	≥21	18–20	≤17
	ceftriaxone	CTX.	≥23	20-22	≤19
Tetracyclines	Tetracycline	TET	≥15	12-14	≤11
Quinolones and fluoroquinolones	Ciprofloxacin	CIP	≥21	16-20	≤15
Folate pathway inhibitors	Trimethoprim/ Sulfamethoxazole	SXT	≥16	11-15	≤10
Aminoglycoside	Amikacine	AMK	≥17	15–16	≤14
	Gentamicin	GEN	≥15	13–14	≤12

3.9. Characteristics of the Study Participants

A total of 122 children that have directly related to the small and medium dairy farm from study area were enrolled in this study. The age of those children were under-five which 30 of them (0-11months), 78 of them (12-23months) and 14of them (24-59months) (Hugho *et al*., 2023). Forty five percent were males (n=56) 45.9% and (n=66) 54.1% were female children. 28 different small (<10 = 9) and medium<50 = 19) (Megersa *et al.*, 2011) dairy farm with milkier hand swab and swab from farm environment were included (Table 5).

Table 5: Characteristics of the study participants

Characteristic		Frequency	Percentage
Children sex	Female	66	54.1 %
	Male	56	45.9 %
		122	
Children Age	0–11 months	30	24.6 %
	12–23 months	78	63.9 %

	24–58 months	14	11.5 %
		122	
Farm type	Medium 10-50	9	32.1 %
	Small <10	19	67.9 %
		28	
Fecal samples		44	20.6 %
Milk samples		44	20.6 %
Floor swabs		28	13.1 %
Udder swabs		46	21.5 %
Material swabs		26	12.1 %
Hand swabs		26	12.1 %
		214	

3.10. Data Management and Analysis

The collected data was entered and managed using **Microsoft Excel**, while all statistical analyses were conducted using **SPSS software version 20**. Descriptive statistics, including percentages and frequency distributions, was applied to characterize the dataset and summarize key findings. The prevalence of *Salmonella* spp. was assessed using percentage calculations. Additionally, the association between various risk factors such as young children (*under five years old*), cows, milk, and environmental samples and their antimicrobial resistance profiles was evaluated using the **Chi-square (χ^2) test**. A **95% confidence interval** with **5% precision** used to determine statistical significance, ensuring robust and reliable results.

3.11. Ethical Considerations

College of Veterinary Medicine Animal Research Ethics Committee of Addis Ababa University and Aklilu Lamma Institute of Pathobiology Institutional Research Ethics Committee (ALIPB-IRERC) were granted this study with reference number **VM/ERC/04/82/17/2025** and **Ref. No.: ALIPB-IRERC/165/2017/25**. All procedures in this study were conducted by skilled professionals in strict accordance with the ethical guidelines and regulations set forth by the ethics committee. The welfare and well-being of the animals involved were carefully safeguarded throughout the research process. Prior to the study's commencement, verbal consent was obtained from farm owners for the collection of milk and fecal samples from their animals, along with milk-related materials, milker hand swabs, and floor swabs. Additionally, informed consent was secured from all human participants, ensuring they fully understood the study's objectives, methodologies, potential risks, and associated benefits.

3.12. Limitations of Study

Further exploring the genetic relatedness among the isolates from the different sources by DNA extraction; identification of antimicrobial resistance (AMR) genes *in Salmonella enterica*, due to Insufficient availability of materials and equipment, including DNA extraction kits, PCR primers, and DNA sequencing reagents, hindered the execution of necessary procedures.

4. RESULTS

4.1. Growth on Media and Biochemical Test

A total of 336 samples were subjected to bacteriological analysis using various media. Initially, 30 samples exhibited characteristics suggestive of Salmonella colonies on xylose-lysine-deoxycholate (XLD) agar (ANNEX 6). However, subsequent biochemical testing confirmed only 20 isolates (5.95%) as Salmonella positive. Biochemically confirmed Salmonella isolates displayed the following characteristics: Triple Sugar Iron (TSI) Test: Red slant (alkaline) with a yellow butt (acid), indicating fermentation patterns, Hydrogen Sulfide (HS) Production, Blackening of the medium, Gas Production: Observed in the butt. Indole Test Negative, Urease Test: Negative (yellow coloration), Citrate Utilization Test: Positive (deep blue slant). These biochemical profiles confirmed the presence of Salmonella isolates, which were further, analyzed (ANNEX7).

4.2. Omnilog Test

All twenty (20) biochemical test positive sample were processed by omnilog test (ANNEX 8) that only four (4) samples were salmonella positive. All four positive samples were identified as *Salmonella enterica* which were two (2) from children, one (1) from milk, and one (1) from udder swab that collected from small and medium dairy farm and under-five children that use milk from those dairy farms in and around Bishoftu town.

4.3. Prevalence of Salmonella

A presence or absence of Salmonella were bacteriologically tested for a total of 336 samples that collected from lactating cows feces, milk, udder swab, milk material swab, milkier hand swab, floor swab of small and medium dairy farm of study area and stool of under-five children that use milk from those farm (Table 6). Salmonella was isolated from 4 (1.2 %) of total sample tested. From 336 samples, 122 were stool samples from under-five children, of which 2 samples 1.6% (95% CI: 0.002-0.058) and 214 sample collected from feces, milk, udder swab, milk material swab, milkier hand swab, floor swab of 28 different small and medium dairy farm; 2 sample 0.9 % (95% CI: 0.001-0.033) which 1sample 2.3 % (95% CI: 0.154-0.267) of 44 milk

sample and 1; 2.2 %(95% CI: 0.158-0.272)of 46 udder swab sample were confirmed to be Salmonella spp. positive by conventional microbiology methods.

Table 6: Positive samples collected from children, lactating cow, and milk with its environmental (N = 336)

Sample Category	Total	Confirmed *Salmonella Enterica*	Prevalence
Children stool	122	2	1.6 %
Feces	44	0	0 %
Milk	44	1	2.3 %
Udder swab	46	1	2.2 %
Milkier hand swab	26	0	0 %
Milk material swab	26	0	0 %
Floor swab	28	0	0 %
Total	**336**	**4**	**1.2 %**

A total of 214 Samples from farms were collected 19 small farms (131 samples) and 9 medium dairy farms (83 samples) were collected. Salmonella isolates were obtained from medium size dairy farm in which 2/78 (2.6 %) that X^2 3.899 with a p-value of <0.05 (Table 7). This suggests a statistically significant association between farm type and the occurrence of the pathogen across dairy farms.

Farm management and hygienic practices such as, time of cleaning per day (*Salmonella* isolates were obtained from cleaning one time per day 2/50(4 %), No *Salmonella* isolates were obtained from cleaning two time per day and three time per X^2 6.290 and a p-value of <0.05). This shows a statistically significant association between hygienic practice of farm and the occurrence of the pathogen across dairy farms.

4.4. Prevalence of Salmonella in Children Under-Five Age

Salmonella isolates were detected in Under-five that have used milk from the medium size dairy farms which is 2/78 (2.6 %) X^2 3.899 with a p-value of <0.05 which suggests a statistically significant association between the occurrence of the pathogen in children and type of farm they use(Table 7). Moreover, Salmonella isolate were also detected in children who used raw milk 2/28 (7.1 %) with X^2 6.826 p-< 0.05) which suggests a statistically significant association between the consumption of raw milk and the occurrence of the pathogen For children. Salmonella isolate were also detected from children who ho have history of diarrhea 2/23 (8.7 %) which Pearson X^2 8.752 and a p< 0.05 which suggests a statistically significant association between the occurrence of the pathogen and diarrhea.

Table 7: Salmonella isolate and Characteristics of the study participants

Characteristic		Salmonella isolate	prevalence	PValue	X^2
Children sex	Female	1/66	1.5%	0.95	0.04
	Male	1/56	1.8%		
		2/122	1.6%		
Children Age	0–11 months	30		0.203	3.19
	12–23 months	78			
	24–58 months	14			
Children use raw milk		2/28	7.1%	0.009	6.826
Children with history of Diarrhea		2/23	8.7%	0.005	8.75
		122			

Farm type	Medium 10-50	9	2.6%	0.048	3.899
	Small <10	19			
		28			
Farm management	Clean one time/day	2/50	4%	0.043	6.290
	Hand wash/milking	2/100	2%	0.123	2.306
Fecal samples		0/44	0%		
Milk samples		1/44	2.3%		
Floor swabs		0/28	0%		
Udder swabs		1/46	2.2%		
Material swabs		0/26	0%		
Hand swabs		0/26	0%		
		2/214	0.9%		

4.5. Antimicrobial Resistance Profiles of Salmonella

The highest antimicrobial resistance detected in Ampicillin 3/4 (75%), and Cefoxitin 3/4 (75%); whereas no resistance was observed in Amikacine, Gentamicin and Ciprofloxacin. A MDR resistance profile was also detected in two of the isolate more than three classes of drugs and one of them was found to be susceptible to 8/10 antibiotics which means 80% susceptibility (Table 8).

Table 8: Antibiotic susceptibility test result of *Salmonella* isolates

Antibiotics used for test	Salmonella positive Isolate								**R and S rate (%) of each antibiotics for all isolate**	
	1 Milk Sample		1 Udder swab		children stool 1		children stool 2			
	zone diamete	Status	zone diamete	Status	zone diamete	Status	zone diamete	Status		
Ampicillin	13	R	11	R	14	R	17	S	75%R	25%S
Cefotaxime	28	S	21	R	20	R	27	S	50%R	25%S
Cefoxitin	R	R	16	R	18	R	18	S	75%R	25%S
Ceftazidime	20	I	23	S	16	R	21	S	25%R	50%S
ceftriaxone	28	S	26	S	19	R	28	S	25%R	75%S
Tetracycline	16	S	15	R	9	R	17	S	50%R	50%S
Ciprofloxacin	32	S	30	S	16	I	32	S	0%R	75%S
Trimethoprim/Sulfamethoxazole	20	S	21	S	10	R	16	R	50%R	50%S
Amikacine	19	S	18	S	17	S	19	S	0%R	75%S
Gentamicin	18	S	15	S	16	S	14	I	0%R	75%S
Resistant rate (%) of each isolate for total Antibiotics	20%R 70%S		40% MDR 50%S		70% MDR 20%S		10%R 80%S			

Abbreviations, R=resistant, I=Intermedium, S=sensitivity,/Susceptibility

5. DISCUSSION

In recent year, non-typhoidal Salmonella (NTS) infections have become a considerable threat to public health. This pathogen has increased in incidence and is a health concern mostly regarded as foodborne. It is highly important to appreciate the magnitude of the problem and implementing strategic, well-organized, and effective measures to reduce its incidence and impact (Kumar *et al*., 2025). The current study investigate non-typhoidal salmonella (NTS) prevalence along lactating Dairy cattle, under-five Children that directly and commonly used milk from those dairy cattle and Milk with its environment and Antimicrobial Resistance Profile In and Around Bishoftu Town.

In present study the 2.3 % prevalence of milk sample *Salmonella enterica* isolates were which aligns with previous study by (Temesgen *et al*., 2025) that was 2.44% prevalence , in Addis Ababa (Liyuwork *et al*., 2013) 2.1% prevalence, and in jigjiga city Ethiopia 3.3% (Reta *et al*., 2016). On other way the present prevalence of *Salmonella enterica* in milk sample is much lower than previous study 4.95% in Bedele and Nekemte Districts, Western Ethiopia (Eshetu *et al*., 2023), Northwest Ethiopia 10.3% (Beyene *et al*., 2024), Central Ethiopia 10% by (Geletu *et al*., 2022), 9.3%. Wolaita Zone, Southern Ethiopia(Asefa *et al*., 2023), Dairy Farms in and Around Meki Town, Oromia, Ethiopia 7.02% (Abunna, 2018), in Ethiopia 19.7% (Bedassa *et al*., 2023), in modjo twon (Abunna *et al*., 2017) in southern Ethiopia12.1%, Dale district (12.69%), Arsi Negele (10.54%), Hawassa (8.54%), (Gebeyehu *et al*., 2022); and study from outside Ethiopia the Tamale Metropolis of Ghana 6.0% (Adzitey *et al*., 2020), in Egypt (A. M. Ahmed & Shimamoto, 2014) 4.3%, in India 11.9% (Singh *et al*., 2018), and higher than other previous study sebeta twon 0.7% (Lakew, 2020), 1.5% walaita sodo (Ayichew *et al*., 2024), and Jordan 1.6 % (Obaidat & Stringer, 2019), 1.6% in Italy (Bonardi *et al*., 2017).

The prevalence of *salmonella enterica* isolated from Udder/ Teat swab in current study was 2.2% which is comparable with 1.96% in walaita sodo (Ayichew *et al*., 2024). The of prevalence of *salmonella* differences may be attributed to variations in sampling methods, microbiological testing protocols, farm biosecurity measures, milk hygiene practices, and the cleanliness of feed and water, all of which influence the level of Salmonella contamination in milk (Yirsa & Tigistu, 2025). No *salmonella species* was detected in lactating cows Faces , compared to bishoftu central Ethiopia 4.76% (Temesgen *et al*., 2025), 4%central Ethiopia (Geletu *et al*., 2022) , 3% in Adama

twon (Abunna *et al*., 2018) and 2.7% in Addis Ababa (Mekonnen *et al*., 2022) The differences may be due to the environmental condition, of study area, and the laboratory method used for the bacterial identification

Milkier Hand swab in dairy farm were 0.0% with the same report 0.0% in Central Ethiopia (Geletu *et al*., 2022), and Asella town (Beyene & Yibeltie, 2015)**,** 0.0 % central ethiopia (Geletu *et al*., 2022)**,** .0.0%Adama Ethiopia (Abunna *et al*., 2018), However there were prevalence report of 5.26% Dairy Farms in and Around Meki Town (Abunna, 2018), 1.7% in walaita sodo (Ayichew *et al*., 2024) and No salmonella enterica isolate from floor swab as the same report from wlaita sodo 0.0% and Swap of milk container were 0%; as the same (Temesgen *et al*., 2025) in bishoftu central Ethiopia. On the other hand there is 10.53% Dairy Farms in and Around Meki Town (Abunna, 2018) 1.82% walaita sodo the (Ayichew *et al*., 2024), and 2.44% floor swab isolate (Temesgen *et al*., 2025) this differences may be due to the environmental condition, study area, farm management and the laboratory method used for the bacterial identification

In this study, the prevalence of *Salmonella interica* from stool of under-five children was 1.6% that comparable with 1% south Ethiopia (Abebe *et al*., 2018) 2.6%), Bahir Dar Zuria (Balew & Kibret, 2023) , Debre Markos town 3.15% (Dessale *et al*., 2023), and study from abroad 2.4% in Canada (Faulder *et al*., 2017), in Nigeria 1.6% (Adagbada *et al*., 2014), 3.1%, in Tanzania (Hugho *et al*., 2024) and lower than previous study in Jigjiga, Eastern Ethiopia 3.8% (Muse *et al*., 2024), 6.2%) Jimma (G. Beyene & Tasew, 2014), 6.9% (Assefa & Girma, 2019) and study of outside Ethiopia in Bangladish 4.34% (Uddin *et al*., 2021), 8.63% in Nepal (Pokhrel *et al*., 2023), this might be due to variations in personal hygiene and environmental sanitation, and food handling practices of the community and due to climatic differences which affect the viability of pathogens.

Among global health problems, antimicrobial resistance (AMR) is the one that best illustrates the One Health approach. The One Health approach is defined as a joint effort of various disciplines that come together to provide solutions for human, animal, and environmental health. AMR is linked to each of these three components due to the irresponsible and excessive use of antimicrobials in various sectors (Velazquez-Meza *et al*., 2022). In the current study the investigated of (4/336) isolates of *salmonella entrica* from Animal, human and environmental

were evaluated for resistance and susceptibility of ten (10) frequently used antimicrobials using CLSI-2018 guidance. The testing revealed that, 25%, 25% , 25%, 50%, 75% , 50%, 75% , 50%, 75% 75% Susceptibility, and 75%, 50%, 75%, 25%, 25%, 50%, 0%, 50% , 0%, 0% resistant to Ampicillin, Cefotaxime, Cefoxitin, Ceftazidime, ceftriaxone, Tetracycline, Ciprofloxacin, Trimethoprim/Sulfamethoxazole, Amikacine , Gentamicin respectively. This drug resistance and susceptibility profile was comparable with previous study such as, study in walaita sodos (Ayichew *et al*., 2024) Ampicillin 75% Resistant, and 25% susceptible, Tetracycline 58% resistant, 42% susceptible, in Addis Ababa tetracycline 50% resistant (Mekonnen *et al*., 2022) 41.41% resistant of tetracycline. Study at bishoftu twon 75% ciprofloxacin Sensitivity. (Temesgen *et al*., 2025) and other study in central Ethiopia (Waktole *et al*., 2024) Gentamicin 73.3% Sensitive, Amikacin 89.5% Sensitive Southwest Ethiopia (Lamboro *et al*., 2016), Ceftriaxone 61.5%, 38.5%,Cipofloxacine susceptibility report in central Ethiopia, (Geletu *et al*., 2022). On the other hand resistant profile is incomparable with previous study 100% ampicillin resistance (Yirsa & Tigistu, 2025) and Southwest Ethiopia (Lamboro *et al*., 2016) , 94.4% in Bahir dar (Guesh, 2017) and 96.4% , 79.3%, 97.06%, 96.7%, 82.03% tetracycline resistant in modjo twon (Abunna *et al*., 2017), in Bale goba (Assefa & Girma, 2019), in Adama (Abunna, 2018) , (Yirsa & Tigistu, 2025) in gonder, in Italy (Di Taranto *et al*., 2025) respectively and ciprofloxacin 77.3% dairy farms in Uruguay (Casaux *et al*., 2023) .The study also revealed that from the total of four (4) salmonella isolate one of them were susceptible to 8/10 antibiotics which means 80% susceptibility, and two of them (50%) were resistant to more than three class of drugs which means multi drug resistant(MDR) which align with 46.4% Northwest Ethiopia (Beyene *et al*., 2024), in Senegal 50% (Park *et al*., 2021), in Uruguay 56.0% (Casaux *et al*., 2023) , but there are higher prevalence in previous study MDR prevalence of *Salmonella enterica* 78.35% pediatric populations in Ethiopia (Tilahun *et al*., 2025), Burkina Faso 83%; Ghana 66%,Kenya 100%(Park *et al*., 2021); in Jordan 82.1% (Obaidat & Stringer, 2019). But lower report Guinea-Bissau 22% (Park *et al*., 2021). The differences in the findings may be related to differences in the types of antibiotics frequently used in the study area and antibiotic usage.

6. CONCLUSIONS

Globally, Salmonella continues to pose a serious public health threat, and Ethiopia is no exception. Tackling this complex issue requires a One Health approach, which acknowledges the intricate connections between human, animal, and environmental health. Given the multifaceted nature of Salmonella transmission, effective control strategies must integrate coordinated efforts across sectors to mitigate risks and enhance disease prevention. The present study revealed the occurrence of *Salmonella enterica* in lactating cow, milk and its environment at farms and under-five children which was user of milk from those farm and very vulnerable to this pathogen. The study investigate 1.2% *salmonella interica* from total sample which were 2.3% from milk, 2.2% from udder swab, and 1.6% from under-five children which was more prominent for children with history of diarrhea. Since the prevalence is similar across animal-related sources (milk and udder swab) and children, there's potential cross-contamination or **zoonotic transmission** happening either directly (via contact) or indirectly (via food chain). In the current study the prevalence of antimicrobial-resistant in Ampicillin and Cefoxitin which 3/4 (75%), Trimethoprim/ Sulfamethoxazole and Tetracycline 50% with 50% MDR *Salmonella enterica* in both human and animal isolate. A 50% MDR prevalence suggests that half of the infections caused by *Salmonella enterica* might not respond to common antibiotics. That makes treating infections far more complicated, leading to: Longer illness durations, increased hospitalization rates, Greater risk of severe outcomes, especially in vulnerable populations such as under-five children, and similar resistance levels are seen in both human and animal isolates.

7. RECOMENDATIONS

- Further exploring the genetic relatedness among the isolates from animals, humans and environmental sources are needed
- Quantifying the public risks associated with milk consumption, an integrated surveillance system that tracks Salmonella across human, animal, and environment are needed to reduce children morbidity and mortality from *Salmonella enterica* .
- Conducting further studies on the environmental persistence of Salmonella in urban settings and developing targeted interventions are needed
- Since similar resistance levels are seen in human, animals and environmental isolates, establish a formal One Health task force involving human health, veterinary, and environmental agencies to coordinate Salmonella control efforts.
- Encourage routine testing of milk with milk products and farm environments for MDR pathogens.
- Launch campaigns on the risks of raw milk consumption and misuse of antibiotics.

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ANNEX 1: Sample Record sheet**e**

Sample ID	farm Number and site	Sample Type	Farm size small, medium	floor type	Age	milking place	cleaning pen	manure desposal	Result RPVS, XLD	

ANNEX 2: Biochemical test record sheet

Sample Id	TSI test	CITR test	Indole test	Motility test	Urea test
	+ve	+ve	-ve	+ve	-ve

ANNEX 3: Media for non-selective pre-enrichments and selective Enrichment, and Reagents used for Biochemical confirmation

ANNE 4: Procedures for preparation of different media

1. **Buffered Peptone Water (BPW) (B/4-6 M.I.D.C India)**

Preparation: 20 grams of dehydrated medium was added to 1 liter of distilled water. Mixed well and distributed into final containers. Then, it was sterilized by autoclaving at 121°C for 15 minutes.

2. **Rappaport Vassiliadis Soya Broth (Goa-403 722 INDIA)**

Preparation: 27.11 grams of dehydrated medium was suspended in 1000 ml of distilled water. It was heated to dissolve the medium completely. Then, it was sterilized by autoclaving at 115°C for 15 minutes and dispensed into tubes.

3. **Xylose-Lysine Deoxycholate Agar (XLD) Agar media (XL 297 INDIA)**

Preparation: 58.68 grams were suspended in 1000 ml of distilled water. Next, it was heated with frequent agitation until the medium boiled. Then it was transferred immediately to a water bath at 45-50 °C. Mixed well and poured in to sterile petri plates.

4. Nutrient agar (A-902 A India)

Preparation: 20.5gm of the dehydrated culture media was suspended in 1 liter of distilled water. Heated to boiled and mixed to be dissolved completely. Then, the medium was sterilized in the autoclave at 121°C for 15 minutes.

5. Triple Sugar Iron (TSI) M021-500G INDIA)

Preparation: 65.0 grams the medium was weighed and combined to 1 liter of deionized water. Mixed well thoroughly and boiled to dissolve completely. Sterilized by autoclaving at121°C for 15 minutes; After autoclaving, slant tubes and allowed to cool, and final pH 7.3 ± 0.2 at 25°.

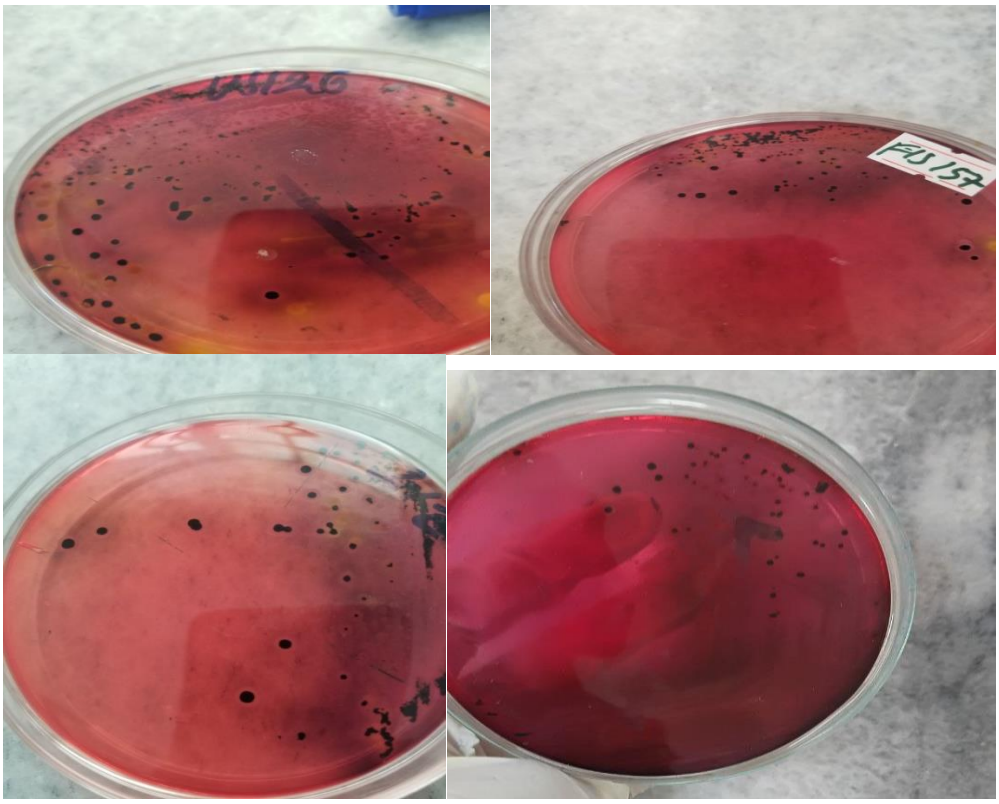
6. Simmons Citrate Agar (M099-500G INDIA)

Preparation: 23.2 grams was suspended in 1-liter cold distilled water. Then, it was heated to boil with frequent agitation to dissolve completely. Distributed into tubes and sterilized by autoclaving at 121 oC for 15 minutes and cooled in a slant position.

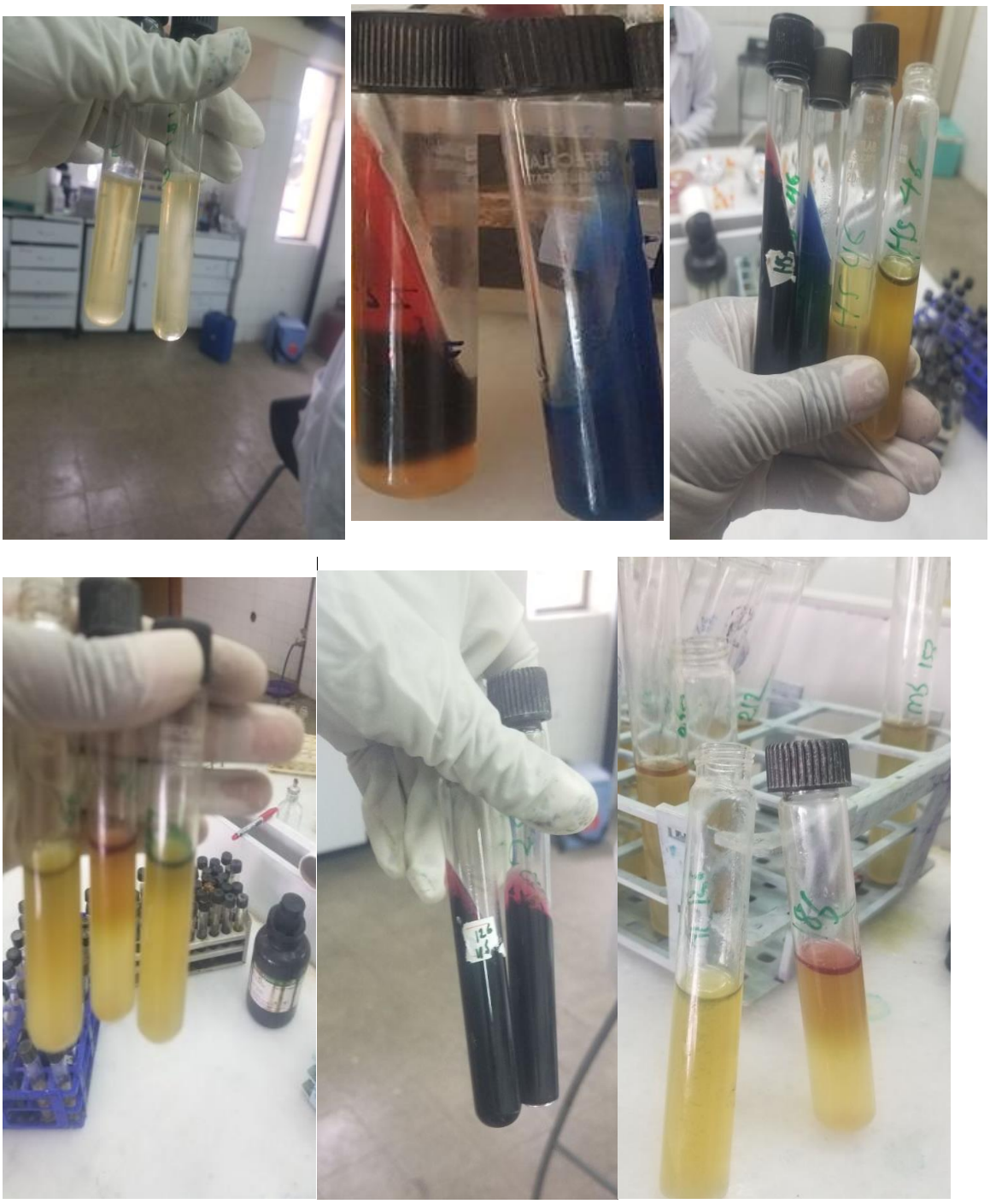
8. **Urea agar base (CM0053, UK)**

Preparation: **2**.4 grams was suspended in 95 ml of distilled water and dissolved completely by boiling. Then, it was sterilized by autoclaving at 115^{o} C for 20 minutes and cooled to 50°C and 5 ml of sterile 40% Urea Solution SR0020 was introduced aseptically. Mixed well and 10 ml amounts were distributed into sterile containers and allowed to set in the slope position.

ANNEX 5: Pictures taken during sample collection laboratory work

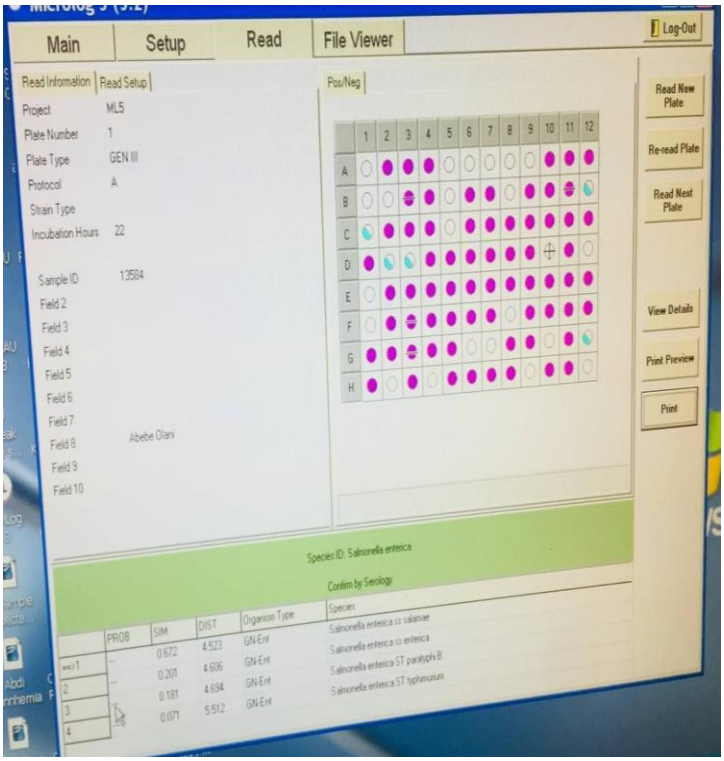


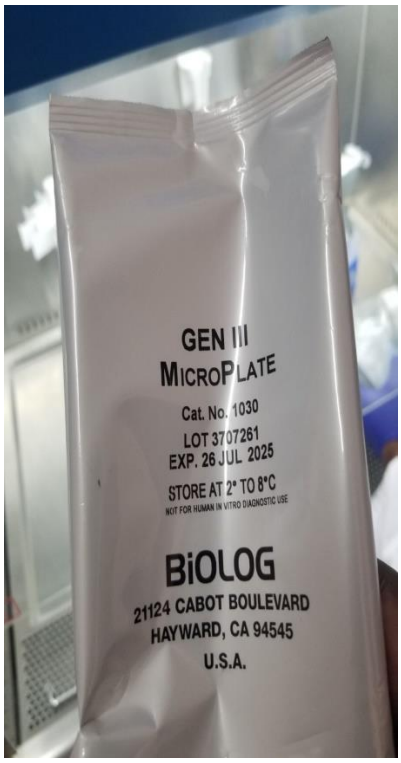
ANNEX 6: Growth of Salmonella enterica on XLD Agar

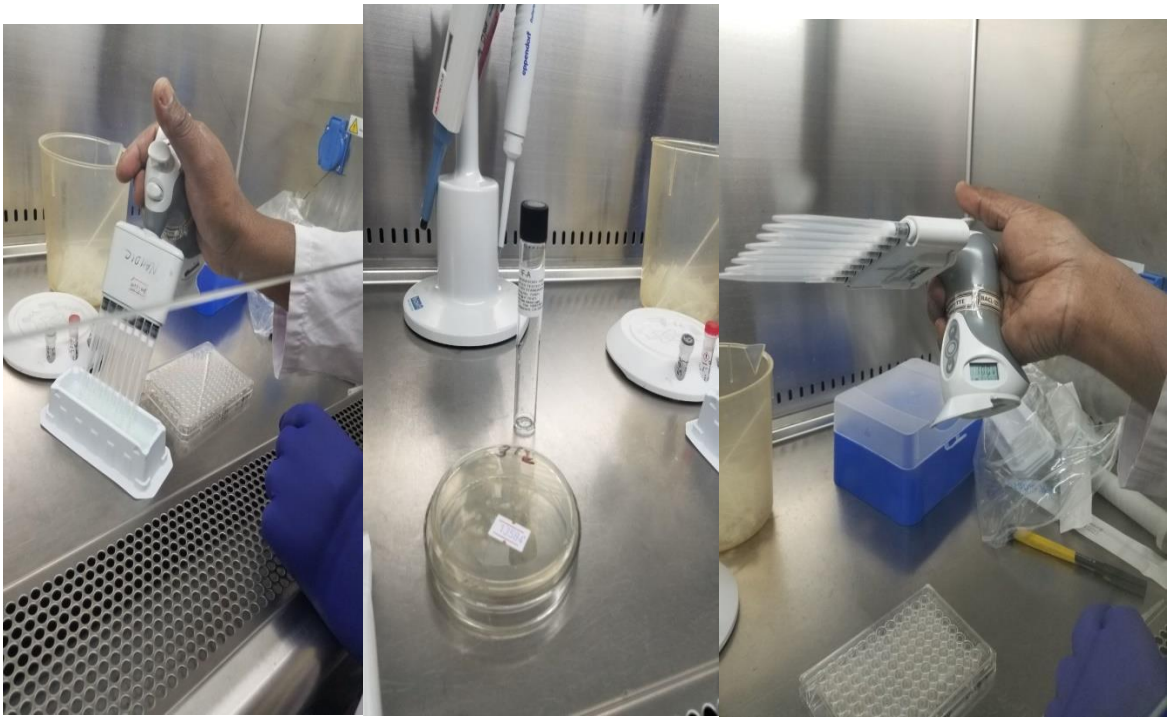


ANNEX 7: Biochemical test result of *Salmonella enterica*

ANNEX 7: Biochemical test result of *Salmonella enterica*







ANNEX 8: Picture showing Omnilog result and activity

ANNEX 9: Picture showing Measurements zone of inhibition of antibiotics

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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/04/82/17/2025

Name of Applicant: **Milkesa Hailu** (DVM, MSc student)

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

Title of the project: *detection of Salmonella species from dairy cattle, children, milk with its environment and antimicrobial resistance profile in and around Bishoftu town: One Health approach*

Date of application:	**December, 2024**
Nature of the project:	**field investigation**
Target animal species:	**Cattle**
Number of animals involved:	**60**
Study area:	**Bishoftu, Ethiopia**

Minutes No. and date of review: **VM/ERC/04/17/025, 25/02/2025**

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 **Milkesa Hailu**.

Additional clearance from concerned body is required for samples to be collected from human subjects

The Federal Democratic Republic of Ethiopia
Addis Ababa University
College of Veterinary Medicine and Agriculture

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature