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ISOLATION, MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE PATTERN OF *SALMONELLA* FROM POULTRY AND POULTRY PRODUCTS IN SELECTED TOWN OF CENTRAL ETHIOPIA.

BY

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JUNE 2022
BISHOFTU ETHIOPIA

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Research thesis submitted to the college of veterinary medicine, Addis Ababa University in partial fulfillment of the requirements for the degree of master of veterinary Science in Veterinary Public Health

BY

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APPROVAL SHEET

Adugna Emane Deressa has submitted a thesis proposal entitled "**ISOLATION, MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE PATTERNS OF SALMONELLA FROM POULTRY AND POULTRY PRODUCTS IN CENTRAL ETHIOPIA**" for presentation with my approval as a college advisor.

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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: Adugna Emane Deressa, entitled: **Isolation, molecular characterization and antimicrobial resistance patterns of *Salmonella* from poultry and poultry products in central Ethiopia** and recommended that the thesis is accepted as fulfilling the thesis requirement for the Degree of Master of Science in Veterinary Public Health.

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LIST OF ABBREVIATIONS

AMR Antimicrobial Resistance
BGA Brilliant green Agar
BPW Buffer Peptone Water
CDC Center for Disease Prevention and Control
CLSI Committee for Clinical Laboratory Standards
EFSA European Food Standard Authority
FAO Food and Agricultural Organization
ISO International Organization for Standardization
KAP Knowledge, Attitude and Practice
LDC Lysine Decarboxylation
MDR Multidrug Resistance
MRI Multi drug Resistance Index
NMSA National meteorological and statistical agency
NTS Non-Typhoidal *Salmonella*
OIE Organization for International Epizooties
PCR Polymerase Chain Reaction
PFGE Pulse Film Gel Electrophoresis
RVS Rappaport-Vassiliadis soy peptone
SPI *Salmonella* Pathogenicity Island
TSI Triple Sugar Iron
TT Tetrathione
UK United Kingdom
USA United States of America
USDA United State Development Agency
VP Voges-Proskauer
WHO World Health Organization
XLD Xylose Lysine Desoxycholate

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ABSTRACT

Salmonella is the leading cause of foodborne infection in food of animal origin including poultry derived food. Even though studies on occurrence of salmonella in poultry have been conducted in central Ethiopia, comprehensive study on salmonella contamination in poultry and poultry product is scarce. Therefore, the aim of this study is to isolate, molecularly detect and assess antimicrobial resistance patterns of salmonella in poultry and poultry products. A cross sectional study was conducted from November 2021 to June 2022 in central Ethiopia. A total of 560 samples were collected from different establishments and analyzed for presence of salmonella. Frequency and prevalence were calculated for descriptive analysis whereas uni and multivariate logistic regression were used to measure the association of risk factors with occurrence of salmonella. $p>0.05$ was considered significant. 73.2% of 18 farms were positive for salmonella with sample level prevalence 11.3% (63/560). Salmonella was more isolated from meat samples (11.6%), Bishoftu (11.9%), large flock size (12.5%) and Eggs from market (13.3%). Serotype distribution showed that S.Typhimurium was dominant among serovar isolated. Salmonella occurrence was significantly associated to sources of samples (P=0 .04). In addition, all salmonella positive samples from backyard chicken were found to be serovar S.Typhimurium, which suggests less hygiene status and biosecurity in backyard environment. Antibiogram profiles revealed that all isolate were resistant to minimum three and maximum to 14 of 15 tested drugs. The maximum and minimum resistance index recorded was 0.93 and 0.2 respectively. In conclusion, high prevalence of salmonella recorded in chicken meat that indicates less hygiene status of workers and the working environment. MDR feature of the strain also alerts risk for the public health of consumer. This suggests the need for further epidemiological study and implementation of strong regulation on poultry food chain through cross-sectoral collaboration to reduce the risk of drug resistant foodborne infection.

Key terms*: Antimicrobial resistance, Molecular techniques, Foodborne infection, Poultry, Salmonella.*

1. INTRODUCTION

Poultry plays an important role in the livelihood of poor rural households, peri-urban and urban areas of many developing countries including Ethiopia (Foley *et al*., 2011). In the last few years, small-scale semi-intensive poultry farm is being practiced in urban and peri-urban areas of Ethiopia. Because they are located near human residential areas and some poultry producers keep chickens in the same compound where they live, there is possibility of transmission of potential pathogens to humans, risking the family members to zoonotic infection (Ketema *et al*., 2018).

Foodborne diseases became the leading cause of morbidity and mortality in both in developing and developed countries of the world (WHO, 2020). These pathogenic microorganisms in the food chain are transmitted to humans through utilization of a variety of foods such as beef, dairy products, poultry products (Bell *et al*., 2016, Ximenes *et al*., 2019). Bacterial food contamination can occur at all stages of food production from farm to table and affects the quality of products such as meat and egg (Botteldoorn *et al*., 2003).

Salmonella is one of the bacterial pathogens circulated in farm animals including poultry. There are two spp of *Salmonella*, namely S.Enterica and S.Bongori. S. Bongori commonly found in cold-blooded creatures such as fish and reptiles while S.Enterica causes disease in warm-blooded animals including human beings. Some S.enterica serotype cause infection in different host while some are host specific i.e. they cause disease in single host only. for example, *Salmonella* enterica s.spp. gallinaruum cause infection in poultry, S.dublin in cattle, S.typhi and P.typhi in human, S,cholerasuis in pigs and so on (Bäumler, 1998).

Salmonellosis infection in poultry can be transmitted by two ways; vertical or horizontal transmissions. Horizontal transmission takes place through aerogens and/or fecooral transmission. It can also transmitted through fomite, polluted drinking water, polluted feed, dirty cages, asymptomatic infected animals, and feces of infected livestock (Zamora-Sanabria and Alvarado, 2017). Vertically it can be transmitted from infected mother to egg (Yanestria *et al.*, 2019).

Among foodborne infectious diseases in humans, *Salmonellosis* is one of the potential zoonotic diseases that cause heavy losses of lives and economy. *Salmonella* can cause disease in both human and animal (Hardy, 2004; Gut *et al*., 2018). In terms of public health, salmonellosis is regarded as the leading zoonotic disease (Shinohara *et al*., 2008). Salmonellosis is an enteric disease typically characterized by inducing acid, chronic diarrhea, and even death in humans and animals (Anamaria *et al.*, 2018).

According to a study conducted by Bhunia (2008), *Salmonella* causes about 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis, and 3 million deaths worldwide. Infections due to *Salmonella* have been associated with a wide variety of foods especially those of animal origins such as meat, chicken, and egg (Hernandez *et al*., 2005).

In some of the investigations of Salmonellosis outbreaks, apart from those related to food animals, there is also indirect contact with infected or contaminated food vendors (Loharikar *et al*., 2013). An example is a foodborne disease outbreak that originates from workers who were infected by direct contact with chickens from the farm (Hedican *et al*., 2010). Animals are the main reservoir for transmission of NTS infection. Transmission of NTS infection to humans can occur through the consumption of food or water contaminated with infected animals, as well as by direct contact with infected animals or consuming infected animals. The incidence of NTS around the world is high as bacteria can be found in the environment. This diversity of reservoirs creates significant difficulties for public health authorities in infection control (Dione *et al*., 2011).

Integrated surveillance of the common serovars of *Salmonella* circulating in poultry farms and in-contact humans, as well as their antimicrobial susceptibility status, is useful to envisage possible intervention strategies to control and prevent its impact on public health. A study conducted in central Ethiopia reported that 14.6% of poultry farms in central Ethiopia and 4.7% of the pooled fecal droppings of birds were positive for *Salmonella*. Another study in Southern Ethiopia conducted on three farms reported the detection of *Salmonella* from different samples at a rate of 16.7% (Abdi *et al*., 2017).

The emergence and spread of antimicrobial-resistant *Salmonella* strains have become a serious health hazard worldwide (Jones *et al*., 2008). Inappropriate use of antimicrobials in food-

producing animals during rearing has been reported to be a factor for the occurrence of AMR in *Salmonella* (Goldman, 2004; Angulo *et al*., 2004). Resistant *Salmonella* has been reported in several studies of samples from human, food animals and their products in different parts of Ethiopia (Ejo *et al.*, 2016; Beyene *et al*., 2011; Eguale *et al*., 2016, 2018; Ketema *et al.*, 2018). Multidrug resistance (MDR), to more than three antimicrobials, has been also reported among *Salmonella* isolates from humans and food animals in Ethiopia, including resistance to fluoroquinolones and third-generation cephalosporins, drugs of choice for invasive salmonellosis (Beyene *et al*., 2011; Eguale *et al*., 2016; Sarba *et al*., 2020).

Currently, there are several reports of resistant *Salmonella* isolate from different samples of food animals and their products in Ethiopia (Eguale, 2018, Kemal *et al*., 2016). Despite a number of published studies of *Salmonella* in poultry in central Ethiopia, there is no published data on the prevalence, serovars involved, and antimicrobial susceptibility of *Salmonella* from poultry and poultry products across the poultry food chain in Bishoftu, Modjo L/Tafo and AA. Therefore, the aim of this study is to determine the prevalence, serotype distribution, and investigate the antimicrobial resistance pattern of *Salmonella* isolates from poultry and poultry products in the study towns, Central Ethiopia.

1.1. Objectives

1.1.1. General objective

To isolate and characterize *Salmonella* isolates from poultry food chains

1.1.2. Specific objectives

- To isolate and characterize *Salmonella* from poultry and poultry products
- To assess the prevalence and risk factors of *Salmonella*
- To assess the antibiotic resistance pattern of *Salmonella* isolates

2. LITERATURE REVIEW

Salmonella comprises more than 2,500 recognized serotypes (Musa *et al*., 2017) which are divided into two species: *Salmonella* enterica (S. enterica) and *Salmonella* bongori (S. bongori). S. enterica is further classiffied into six subspecies (Dhama *et al*., 2013) and could be a major pathogen in humans as well as in animals (Tegegne, 2019; Musa *et al*., 2017). Those bacteria are Gram-negative, moderately anaerobic, non-spore-forming, and straight rods belonging to the Enterobacteriaceae family (Chlebicz and Sli, 2018) that are indistinguishable from E. coli under the microscope or conventional nutrient media (Bäumler, 1998).

As *Salmonella* is ubiquitous, its presence in the environment means there is an increased risk for humans and animals if exposed to this organism due to its zoonotic potential (Taylor *et al*., 2001). In addition, many animals are reservoirs of *Salmonella* as they harbor the bacteria in the intestines asymptomatically and can shed the organism into the environment (Preena, 2013).

The analysis of the genetic material of *Salmonella* serotypes reveals that there was a common ancestor existed 25 to 40 million years ago. The ability of this bacterium to adapt to the host environment for successful pathogenicity has led to the evolution of different *Salmonella* strains from this common ancestor (Bäumler, 1998).

Studies show a strong association between genetic changes and bacterial host adaptation (Toft & Andersson, 2010). Host adaption in *Salmonella* is demonstrated at a species level, for example, *Salmonella enteric*a has adapted to cause infection typically in warm-blooded hosts whereas *Salmonella bongori* has evolved to infect cold-blooded hosts such as snakes and lizards. Strains that have a high association of isolation with a particular host causing a systemic infection are said to be host adaptive or restrictive (Preena, 2013).

Two forms of *Salmonella* typhoid, S.Typhi, and S.paratyphi are stored in humans. These bacteria are spread by food or water intake. They cause enteric fever that is typically characterized by a period of incubation of a week or more accompanied by symptoms such as headache, stomach pain, and diarrhea (Eng *et al*., 2015; Bhan *et al*., 2005). Additionally, myalgia, bradycardia, hepatomegaly, splenomegaly, and chest and stomach rashes can be encountered in infected men (Eng *et al*., 2015; Kuvandik *et al*., 2009).

Salmonella strains other than S. Typhi and S. paratyphi are often referred to as Non-Typhoid *Salmonella* (NTS) and are mainly found in animals. NTS is zoonotic and causes an infection that is characterized by gastroenteritis, an inflammatory condition of the digestive tract, accompanied by symptoms such as myalgia, headache, nausea, vomiting, stomach cramps, and bloodless diarrhea. Rare symptoms such as hepatomegaly and splenomegaly can also be encountered in infected patients (Eng *et al*., 2015; Hohmann, 2001).

The *Salmonella* enterica serotype Dublin is typically associated with an infection in cattle and is considered host adaptive to this host. *Salmonella enteric* serotype choleraesuis are typically isolated from pigs and from their slaughterhouses, which is significant in the zoonotic transmission route (Paulin *et al.,* 2002). Studies have reported pigeons as the carriage of *S.* Typhimurium variant Copenhagen phage type 99 and are host restricted (Pasmans *et al*., 2003).

2.1. Salmonella in Poultry

Salmonella is common in galliform birds and has been isolated at high rates from commercially reared chicken, turkeys, and other poultry. Apart from the associated foodborne risk, farms may represent a direct risk to public health, even though relevant studies are so far missing and high biosecurity standards in most commercial poultry productions probably minimize the risk (Eguale *et al*., 2018).

The clinical symptoms associated with *Salmonella* infection vary considerably by age group and serotype. Infections with generalist serotypes rarely cause clinical disease in galliform birds and most animals become asymptomatic carriers, particularly during infections of young birds. Infections with the host adapted serotype Gallinarum biovars Gallinarum and Pullorum, however, cause severe disease with high mortality and immense economic losses on chicken and turkey farms (Shivaprasad, 2000).

Salmonella Gallinarum biovar Pullorum causes “Pullorum disease” in young animals, which is associated with septicemia and high mortality that can exceed 85% (Padron, 1990). *Salmonella* Gallinarum biovar Pullorum infections of adult birds are generally mild or asymptomatic, even though decreases in fertility and egg production, as well as increased mortality, have been observed in some instances. Adult animals can develop a carrier state, and transovarial

transmission is thought to be the primary route of transmission to young birds, even though rodents and other vectors are also thought to play an important epidemiologic role (Anderson *et al*., 2006). Clinical symptoms include anorexia, diarrhea, dehydration, decreased hatching, and high mortality. *Salmonella* Gallinarum biovar Gallinarum causes “fowl typhoid” in young and particularly adult birds (Shivaprasad, 2000).

Salmonella's mode of transmission can be horizontal or vertical. The transmission of bacteria from the parent to the newborn child is the vertical transmission. In *Salmonella* infection in poultry, which is caused by serovar enteritidis with a particular preference for the chicken reproductive system, vertical transmission is of great concern. In this case, it happens by transovarial infection when the mother bird has a systemic disease that comes about in ovary infection and egg production within the oviduct. Bacteria moving from cloacae into reproductive organs contribute to serovar enteritidis gaining access to eggs (Anamaria *et al*., 2018).

Horizontal transmission takes place through aerogens and/or feco-oral transmission. *Salmonella* is transmitted horizontally by fomites, contaminated drinking water, polluted feed, dirty cages, no signs of tainted animals, and dung of infected animals (Zamora-Sanabria and Alvarado, 2017; Yanestria *et al*., 2019**)**.

Clinical symptoms are very similar to those observed during infections with biovar Pullorum, and economic losses during outbreaks can be very high. Both Gallinarum biovars Gallinarum and Pullorum are host restricted and therefore pose a negligible risk to human health. In contrast, infections with *Salmonella* Enteritidis are typically asymptomatic in adult birds but can cause systemic disease in young birds, and transovarial transmission of serotype Enteritidis has also been described (Guard-Petter, 2001).

Infections with *Salmonella* Enteritidis pose a considerable risk to human health and have been estimated to inflict costs of approx. 1 billion US dollars per year on the USA economy (Roberts and Sockett, 1994). *Salmonella* prevalence varies considerably by poultry type, differs between serotypes and biovars, and intestinal carriage often appears to be lower than isolation rates from egg shells, dead birds, and environmental samples (Chao *et al*., 2007; Bailey *et al*.,1994).

Salmonella prevalence in hatcheries is estimated between 0 and 17% for chickens, compared to approx. 25% for geese, and 20-60% for ducks (Chao *et al*., 2007). *Salmonella* Gallinarum biovars Pullorum and Gallinarum have been eradicated in commercial poultry productions in the developed world, but are still important in backyard flocks as well as in the developing world. It is conceivable that serotype Enteritidis filled the ecologic niche left by the eradication of serotype Gallinarum biovar Gallinarum since a considerable increase in Enteritidis prevalence coincided with the eradication of biovar Gallinarum in the 1960s (Foley *et al*., 2008).

Modeling results have suggested a potential role of competitive exclusion between serotypes Enteritidis and Gallinarum biovar Gallinarum in poultry (Rabsch *et al*., 2000). *Salmonella* Enteritidis, as well as serotypes Typhimurium, Kentucky, and Heidelberg, are commonly detected among clinically healthy as well as sick chickens and turkeys, indicating a potentially important risk for human health as many people raise poultry in their backyards for meat, egg production, or as pets (CDC, 2000).

In addition to household exposure, human cases have been linked to poultry contact on farms, in agricultural feed stores, and at country fairs (CDC, 2009). Young hatchlings pose a particularly high risk for humans, and remarkably often infect children. The number of human outbreaks increases strikingly around Easter when chicken or duck hatchlings are especially popular pets. Such outbreaks have been documented every few years since the 1950s. To reduce the risk associated with hobby farming, the sale of poultry for meat or egg production at feed stores is prohibited in all USA states (CDC, 2007).

2.2. *Salmonella* in Poultry Food Products

Salmonellosis is one of the most frequently reported foodborne disease outbreaks worldwide but is mainly common in developing countries such as India, Asia, and Africa (Winter *et al*., 2015; Smith *et al*., 2016, Olobatoke, 2017). According to the WHO, *Salmonella* is among pathogens that caused the greatest impact on the human population and has been associated with outbreaks and sporadic cases of human foodborne diseases worldwide.

Recently, technological advancements in traveling, globalization, and growth in international trade between many countries in the world have led to the rapid dissemination of foodborne

pathogens, contaminants in foodstuffs, and other pathogens of potential threat to humans. Consequently, this leads to an increased perception of the need for the adoption of surveillance systems to ensure food safety, and identification of foods involved in foodborne outbreaks due to its economic importance. This is because the identification of only one contaminated food product may lead to the discarding of tones of foods resulting in economic losses to the production sector and international trade restrictions (Tauxe *et al*., 2010).

Poultry and poultry products such as eggs have been frequently reported to be associated with salmonellosis outbreaks and therefore, are generally recognized as primary sources of the disease (Saravanan *et al*., 2015). Typically, humans become infected through ingestion of foods contaminated with animal feces or cross-contaminated by other sources. Enteric fever, which is caused by the typhoidal strains *S.* Typhi and *S.* Paratyphi, has been reported endemic in Southeast and Central Asia, where it causes 200,000 deaths and 22 million illnesses per year (Crump *et al*., 2004).

Serovars of NTS are widespread and are commonly associated with specific animals. In the human hosts, they typically cause a self–limiting gastroenteritis with symptoms such as fever, diarrhea, vomiting, and stomach cramps (Langridge *et al*., 2012). These symptoms could be accompanied by prolonged fecal shedding of the bacteria for more than a month. Globally, gastroenteritis, the most common form of NTS infection, accounts for about 93.8 million cases and 155,000 deaths per year (Majowicz *et al*., 2010). Based on surveillance data for 2001-2005, the frequently isolated serovar responsible for NTS infection worldwide was SE (65%), followed by ST and SN, which accounted for 12% and 4% of the clinical isolates recovered, respectively (Galanis *et al*., 2006). Similarly, in Asia, Latin America, and Europe, SE was the common serotype identified accounting for 38%, 31%, and 87% of the clinical isolates respectively. Whereas, in Africa, both SE and ST were reportedly identified as the common serotypes occurring in 26% and 25% of the recovered clinical isolates (Galanis *et al*., 2006). In 2010 alone, the annual costs associated with salmonellosis were estimated at US$2.71 billion for 1.4 million cases (USDA, 2013). Similarly, in the US, the estimated costs of medical expenses, sick leaves and loss of productivity related to the high incidence of salmonellosis ranged from US$1.3 to US$4.0 billion a year (Taitt *et al*., 2004).

2.3. Salmonellosis as poultry foodborne infections

The World Health Organization (WHO) defined Zoonosis as: "any disease or infection that is naturally transmissible from vertebrate animals to humans". Some pathogens retain the ability to infect more than one mammalian host, including humans, and are regarded as zoonotic strains. Zoonotic infections thrive in individuals that are immunocompromised, which include the elderly, pregnant women, neonates, and adults with a predisposing disease. Nonetheless, they can also cause infection in healthy hosts. These zoonotic diseases are transmitted to humans through the consumption of food of animal origin. Food-borne illnesses happen from the utilization of foodstuffs particularly food of animal origins contaminated with pathogenic microbes, their poison, and chemicals (Assefa and Bihon, 2018; Haile *et al*., 2017; Faris, 2015). Bacteria (66%), chemicals (26%), and parasites (4%) are the most causes of food-borne infections (Addis and Sisay, 2015).

From the biological dangers, bacterial pathogens are the foremost genuine concern for the issues of food safety to consumers accounting for two-thirds of foodborne infection outbreaks (Zelalem *et al*., 2019; Addis and Sisay, 2015) where vertebrate animals appear to be common reservoirs that cause human diseases through the food chain (Carrique-Mas and Bryant, 2013). These pathogens are exposed to food during collection, slaughtering, preparing, storing, cooking, and bundling. However, environmental factors and the vulnerability of the human population to illness caused food-borne bacterial pathogens to advance (Hemalata *et al*., 2016).

Food of animal origin especially meat (beef, sheep, chicken, and pork), dairy products (milk, cheese, yogurt, and ice cream), and eggs are the three ways by which individuals are exposed to pathogenic microbes (Abunna *et al*., 2016). Due to their dietary esteem, primarily high protein and lipid content, dairy products are an appropriate growth environment for several microorganisms (Laslo and Gyorgy, 2019).

Salmonella is the most common zoonotic food-borne infection in the World (Addis *et al*., 2011). It is one of the major public health concerns as the leading cause of death, especially in the LMICs (Smith *et al*., 2016). The noticeable epidemiological figure is the common carrier status in animals (Kassaye *et al*., 2015). The incidence of *Salmonella* in developing countries, where ready-to-eat foods are prepared under less hygienic environments and fruits and vegetables are grown on

farms with poor management practices is much higher. In many of these countries, people who have less knowledge about the risks posed by foodborne pathogens sell these foods (Andoh *et al*., 2017).

Salmonella can be found naturally within the environment, domestic and wild animals (Heredia and Garcia, 2018). The essential living space of *Salmonella* species is within the intestinal tract of the animals such as farm animals, humans, fowls, reptiles, and insects. This implies that animals are the reservoir of foodborne infections of *Salmonella* (Addis and Sisay, 2015). Zoonotic pathogens exhibit a phenotype that allows them to maintain the ability to colonize and potentially cause infections in more than one host species. Conversely, some strains of pathogens, as observed within the *Salmonella*e, are significantly host restricted, or adapted, and are generally only able to cause disease in one host (Preena, 2013).

Nontyphoidal *Salmonella* species are zoonotic agents mostly isolated from foods of animal origin (Tegegne, 2019). Poultry (Balakrishnan et al., 2018), pigs and cattle (Aragaw *et al*., 2007) and their products like meat, eggs (Sanchez et al., 2002), and drain (Taddese *et al*., 2019) are the most commonly recognized food sources capable for outbreaks of human salmonellosis even though the microorganism has also been found in other foodstuffs (Heredia and Garcia, 2018).

Chicken products including eggs are widely recognized to be a critical reservoir for *Salmonella* and are recognized in sporadic cases and the outbreak of human salmonellosis (Kemal *et al*., 2015). According to a study conducted by Ejo *et al*. (2016), consumption of raw food, cross-contamination, inappropriate food storage, poor personal hygiene practices, insufficient warming of food items, and a delayed time-lapse between preparing and consuming food items was specified as risk factors to salmonellosis in humans. The bacteria enter the food chain at any point in animal feed, food manufacturing, processing, retailing, catering, preparation, and survive normal catering refrigeration temperatures and increase in number under conditions of thermal abuse (Tadesse and Gebremedhin, 2015).

Nontyphoidal *Salmonella* is one of the critical zoonotic bacterial of food-borne pathogens in humans. They are widely dispersed in nature (Kemal *et al*., 2015), and they are the main pathogenic bacteria in humans as well as in animals (Addis *et al*., 2011). They are the most commonly isolated bacterial agents of food-borne infection outbreaks (Balakrishnan *et al*.,

2018), and they account for about 93.8 million foodborne diseases and 155,000 deaths per year around the world (Heredia and Garcia, 2018).

Out of the 94 million NTS cases reported, 80.3 million cases were estimated as a foodborne origin (Majowicz *et al*., 2010). Among the NTS, *Salmonella* Typhimurium (ST), *Salmonella* Enteritidis (SE), *Salmonella* Heidelberg (SH), and *Salmonella* Newport (SN) are the epidemiologically important NTS serotypes – with poultry and poultry derived products as important reservoir sources – and have been associated with the majority of human salmonellosis worldwide (Eguale, 2018, Tarabees *et al*., 2017). *S. enterica* is widely distributed in the environment and has been associated with a variety of infections in cattle, pigs, and birds including poultry and free-living wild birds (Fagbamila *et al*., 2017). More than 150 serotypes can cause foodborne salmonellosis (Dhama *et al*., 2013); however, S. Typhimurium and S. enteritidis are more common (Tegegne, 2019; Tadesse and Tessema, 2014; Dhama *et al*., 2013).

Some of the investigations of Salmonellosis outbreaks, apart from those related to contact with live birds, can also be from indirect contact with contaminated food which may originate from workers who were infected by direct contact with chickens from the farm (Hedican *et al*., 2010). Transmission of NTS infection to humans can occur through the consumption of food or water contaminated with infected animals, as well as by direct contact with infected animals or consuming infected animals**.**

2.4. Antimicrobial Resistance of Salmonella

Antibiotics are used for the prevention, treatment, and control of infectious diseases in human and animals. The selectivity of antibiotics against bacterial interference can reduce harm to patients by ensuring the eradication of target bacteria. Excessive use of antibiotics, however, can cause antibiotic resistance. The role of livestock in emerging and propagating antimicrobial-resistant bacteria is poorly understood among humans (Fall-Niang *et al*., 2019). In the veterinary sector, inaccurate antibiotic use can promote antibiotic resistance in different bacteria types (Witaningrum *et al*., 2020) including Gram-positive bacteria (Tyasningsih *et al*., 2019; Effendi *et al*., 2019) and Gram-negative bacteria (Wibisono *et al*., *2020*; Putra *et al*., 2020).

NTS infection does not normally need antibiotic therapy, but complications including meningitis and septicemia need antibiotic treatments such as ciprofloxacin, ceftriaxone, and ampicillin. S.paratyphi usually has complications and needs serious treatment with antibiotics such as cefixime, chloramphenicol, amoxicillin, trimethoprim, azithromycin, aztreonam, cefotaxime, or ceftriaxone to avoid related death (Medalla *et al*., 2017). Over the years, cattle have been considered as an important storage facility for antimicrobial resistance with public health impacts; however, some evidence-based study has shown that resistant bacteria also found in poultry farms (Aarestrup, 2015).

According to the CDC report, more than 3,000 people in USA die every year due to antimicrobial resistance and more than 48 million cases of foodborne disease occur in the US every year where at least 70% of the pathogens are resistant to at least one antimicrobial (Cosby *et al*., 2015; Scharff, 2010).

The development of antibiotic-resistant *Salmonella* primarily results from the use of antibiotics as feed additives in animals to facilitate growth and antibiotics in veterinary medicine for the treatment of bacterial infections. This can establish an increased risk of transmission, through direct contact with animals or ingestion of food of infected animals with MDR strain *Salmonella* or indirectly via contamination of food and water from animal feces (Eng *et al*., 2015; Boerlin, 2008). The antibiotic resistance profile of *Salmonella* isolated from contaminated chicken meat showed that 3.7% were resistant to ampicillin, 11.1% were resistant to ofloxacin and 44.4% were resistant to nalidixic acid (Aprillian *et al*., 2015). Addressing this challenge will require efforts from various sectors to successfully control the spread and emergence of AMR in the poultry farm.

Salmonella enterica serotype Typhi and Paratyphi are host-adapted to humans causing a perfuse infection, typhoid fever. These serotypes show low levels of genetic variation has been investigated and revealed that evolution in this population seems to be characterized by the ongoing loss of gene function. A strong adaptive selection for mutations conferring antibiotic resistance in S. Typhi has been reported (Crump and Mintz, 2010). For example mutations in the gene gyrA is known to adaptively select for fluoroquinolone resistance in this serotypes. These revelations in evolutionary dynamics are important for understanding and applying implications for the control of typhoid (Holt *et al*., 2008).

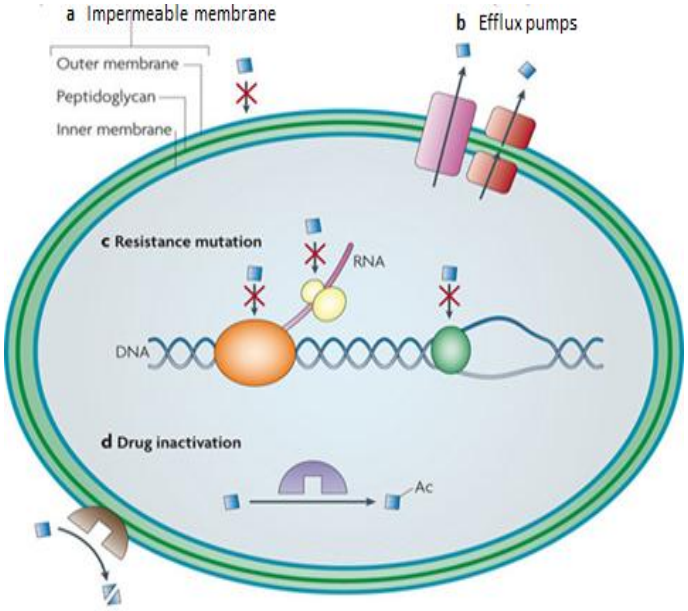


Figure 1: Mechanisms of drug resistance in a Gram-negative bacterium (Allen *et al.*, 2010)

2.5. Control and prevention of Salmonellosis

Salmonellosis is a major concern in food safety. The European Union has enacted regulations since January 2006 covering control measures implemented throughout the poultry production chain, including production levels, biosecurity measures, and prohibiting the use of antibiotics as growth promoters (OIE, 2019).

Poultry and poultry products are frequently associated with outbreaks of Salmonellosis and therefore, are generally recognized as a major source of disease spread (Saravanan *et al.*, 2015). *Salmonella* prevention and control can be achieved by adopting the principles of HACCP (Hazard Analysis Critical Control Point) (OIE, 2019). Hygiene and biosecurity should be part of the overall management of the farm. These steps are very important in infection control. Incoming poultry must have a high health status and must be purchased from reliable suppliers that have quality-assured breeding and hatchery facilities. Furthermore, *Salmonella* can be transmitted in chicken farms through vehicles, workers, clothing, footwear, equipment, water,

food, garbage, insects, rodents, wild birds, pets, equipment, and many other factors (Effendi *et al*., 2020).

Preventing the entry of *Salmonella* into the farm can be done by limiting people who enter the farm, wearing protective clothing and boots that have been disinfected. In addition, workers must know basic hygienic principles, such as keeping hands and feet clean. Farm environment must be clean and disinfected regularly. To do so it needs to test by taking samples on floors, walls, drinking water, eating places, and the environment. Additionally public awareness of the dangers of antimicrobial-resistant must be ensured about antibiotic use approach (Van-Immerseel *et al*., 2009; Wibisono *et al*., 2020).

Integrated surveillance and collaboration between human health, food safety, and animal health is important to mitigate the risk of AMR. One Health approaches and contamination strategies including livestock, retail, catering, and consumers are vital to minimize contamination and reduce transmission of *Salmonella*. In addition, continuous monitoring of the prevalence of *Salmonella* resistance globally priority for clinicians to support the best treatment options for Salmonellosis, especially for patients receiving antibiotic therapy (Aarestrup, 2015; Wibisono *et al*., 2020).

Actions should include active surveillance to monitor the emergence and spread of AMR. Infection prevention and control must also be taken into consideration to limit further spread. Creating awareness about inappropriate use of antibiotics, and antibiotic stewardship programs in hospitals, outpatients, and community pharmacies is critical to limit and regulate the ongoing use of antimicrobials (Van Immerseel *et al*., 2009).

2.6. Status of Salmonella prevalence in Poultry value chains in Ethiopia

Table 1: Status of Salmonella prevalence in Ethiopia

Area	Sources of samples	Prevalence	Reference
Adama and Modjo	Poultry	2.9%	(Dagnew *et al*., 2020)
Jimma	Poultry (eggs)	2.65%	(Taddese *et al*., 2019)
Haramaya	Poultry (eggs)	2.9%	(Tessema *et al.*, 2017)
Arsi Zone	Food items	9.4%	(Geresu *et al.*, 2021)
Bishoftu and AA	Poultry meat	23.6%	(Molla *et al*., 2003)
Haramaya	Poultry	2.7%	(Kemal *et al.*, 2016)
Southern Ethiopia	Poultry	16.67%	(Abdi *et al.*, 2017)
In and around AA	Poultry	14.6%	(Eguale, 2018)
Bishoftu and Modjo	Poultry	14.6%	(Asfaw *et al*., 2020)

3. MATERIALS AND METHODS

3.1. Study area

This study was conducted under project title **"**Biosecurity practices in Poultry Farms: isolation, identification and molecular characterization of major bacterial pathogens, investigation of major bacterial zoonosis and biosecurity based interventions towards enhancing production efficiency and profitability in poultry farms central Ethiopia (BPF-BZDI). Among central Ethiopia specifically Bishoftu, Modjo, Laga Tafo-Laga Dadi towns, and Addis Ababa City were selected for the study. Addis Ababa is the capital city and administrative center for the Federal Democratic Republic of Ethiopia. It is located at a latitude of 9 ^{0}C 3′ North and 38 ^{0}C 43′ East. Other study towns namely Bishoftu, Modjo, Laga Tafo-Laga Dadi are located within 21 to 60 km from Addis Ababa. Laga Tafo-Laga Dadi town is located in Oromia Regional State, Berek District, along the road to Debre Berhan at a distance of 21 km North-East of Addis Ababa. It is located between 9º 01'29" - 9º 06' N Latitude and between 38º 53'42" E - 38º 55'30" E longitude. Farmers in this study area mostly practiced a mixed crop-livestock farming system (CSA, 2012).

Bishoftu is located 50 km to the east of Addis Ababa on the road to the Adama. Bishoftu has more than hundreds of poultry farms under different establishments. Currently, most of the poultry and poultry products being supplied to Addis Ababa are from Bishoftu. Modjo town is located at 73 km South East of Addis Ababa. It is located at 8.36°N and 39.7°E. Small-scale urban and peri-urban poultry production is commonly practiced in these towns (CSA, 2012). A list of poultry farms in the towns was obtained from Woreda Agricultural Offices, of which representative farms from different localities in the towns were selected for inclusion in the study.

Map of the study are

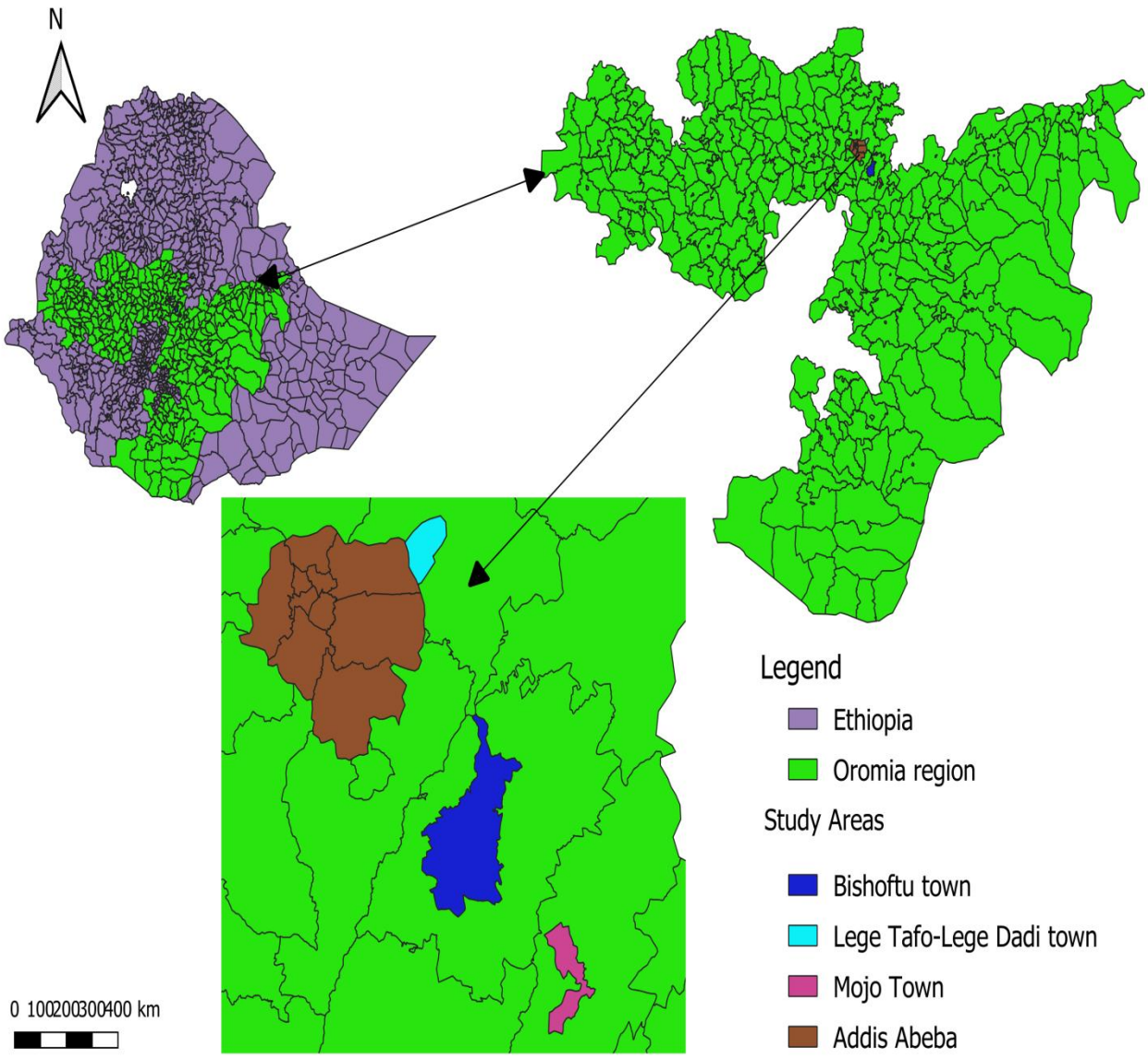


Figure 2: Geographical map of the study area

3.2. Study setting and sample type

The study comprises samples from live chickens kept at small-scale (50-1000 birds per farm) and medium-scale (>1000 birds per farm) (FAO, 2019) poultry farms found in this study towns. The study population consisted of healthy live chickens, apparently healthy slaughtered chicken, frozen chicken meat (FCM) from supermarkets, and eggs. All of the selected farms involved in the current study were under intensive management systems. The type of samples was Cloacal swab from the chicken, meat swab from apparently slaughtered chicken, chicken organ (liver and spleen), FCM carcass (any portion), and FCM swab from supermarkets, and egg swab from farm and shop. All of the farms involved in the current study kept their birds inside and uses the floor system. A total of 18 poultry farms included in the study i.e. for meat samples (n = 5), for cloacal swab (n=8) and for egg sample (n= 5) have farm size ranging from 200- 2000 poultry population. Additionally, backyard chicken (n=20) were included in the study unit.

3.3. Study Design and sample size estimation

A cross-sectional study was conducted from November 2021 to June 2022 to assess the prevalence, distribution, and antibiotic resistance patterns of *Salmonella* species from poultry and poultry products (eggs and chicken meat). The number of samples (from poultry food chain) was calculated using the Thrusfield (2007) formula with 0.5% expected prevalence and 5% desired absolute precision at a 95% confidence interval.

$$n = \frac{1.96^2 P_{exp}(1 - P_{exp})}{d^2},$$

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

Accordingly, the calculated sample size was 384. However, in this study 560 samples (200 fresh meat swabs, 100 giblets, 60 FCM swab and 20 FCM carcass, 100 Cloacal swabs, and 80-egg swabs) were collected from poultry farms and supermarkets.

3.4. Sampling technique and sample collection

The inclusion of farms in the study was based on the availability of establishment (farms and chicken slaughtering) in the study area, representation of the poultry farms and chicken meat sellers in the study area, and the willingness of the owners where random sampling technique was used to sample the unit from the study population. A total of 560 samples were collected from live chickens, carcasses, giblets and eggs for *Salmonella* detection. Eggs from farms and shops were collected randomly. Chicken meat swabs and giblets (Liver and spleen) were collected from the slaughtering site on the farms immediately during processing. The FCM swab and carcass were collected randomly from supermarkets. The number of samples from one farm was based on the size of the target population. Specifically, if the number of chickens being slaughtered is >= 300, 5% swab (i.e. 20 Breast and 20 leg/neck swabs) and 20 giblets (10 liver and 10 spleens) were sampled. The sample was collected by moistened sterile cotton swab with 10ml buffered peptone water (BPW). All samples were properly labeled by type, date of collection, and sources and immediately transported to the AAU CVMA microbiology laboratory in the icebox for tests.

3.5. Salmonella isolation and molecular characterization

3.5.1. Isolation of Salmonella

The technique recommended by the International Organization for Standardization ISO 6579 (2012) was employed to isolate and identify *Salmonella* organisms. Upon arrival at the laboratory, the meat samples and giblets were weighed and crushed into pieces. 25gm sample was added to 225ml of BPW, manually homogenized in a stomacher bag by gentle agitation for approximately 30 seconds, and was incubated at 37 º C for 24hr. Following incubation, 0.1 ml of the pre-enrichment was transferred by micropipette to 10 ml Rappaport-Vassiliadis soy peptone (RVS) (HiMedia, India) broth for selective enrichments. Then the inoculated Rappaport-Vassiliadis broth was incubated at 41.5^0c for 24 hours.

Following selective enrichment, a loop full of enrichment was streaked onto the surface of two selective agars: xylose lysine desoxycholate (XLD) and/or brilliant green agar (BGA), and incubated at 37 ^{0}C for 24 hours. Then the plates were observed for colony morphology of typical

Salmonella, a pink colony with a black center for XLD and red color of the agar for BGA. Then pure colony from this selective agar was streaked on nutrient agar until biochemical tests.

3.5.2. Biochemical tests

All suspected *Salmonella* colonies were streaked on nutrient agar and incubated for 24 hrs. Then a loop full of *Salmonella* colonies from nutrient agar was picked and inoculated into the following biochemical test tubes: triple sugar iron (TSI) agar, Simmons's citrate agar, Lysine decarboxylate broth, Tryptone soya broth (Indole), MR-VP broth, and incubated for 24 or 48 hours at 37^{0} C. Colonies producing an alkaline slant (red color) with acid (yellow color) but with the presence of H2S gas (black color of the medium) and gas production for TSI, positive for lysine (purple color), negative for indole test (yellow-brown ring), negative for Voges-Proskauer (no color change), positive for Methyl red (red color) and positive for citrate utilization (blue color) was considered to be *Salmonella*-positive. The overall procedure is available in **annex**.

3.5.3. Molecular characterization

Biochemically isolated *Salmonella* was preserved in BHI (HiMedia, India) broth by a 1.5ml cryovial tube and submitted to NVI (National Veterinary Institute), Bishoftu Ethiopia. The submitted sample was coded and transferred to the microbiology lab where the extraction was conducted.

Extraction of DNA- Extraction of DNA was performed as per the protocol of the QIAGEN extraction kit (QIAGEN, Germany). In brief, the sample in the tube was first vortexed and centrifuged to homogenize the sample. Then 200µL sample was transferred to a microcentrifuge tube. Then 20µL QIAGEN protease and 200µL AL Buffer were added and vortexed for 15sec. Then the mixture was incubated at 56^{0}C for 10 minutes to digest the bacterial cell. Then it was centrifuged at 14000rpm for 1min to separate cell debris and DNA. Then 200µL absolute ethanol was added and mixed again by vortex followed by centrifugation. Then the mixture was carefully transferred to the QIAamp spin tube (collection tube) without wetting the rim of the tube and centrifuged at 8000rpm for 60sec. The filtrate was then discarded and the QIAamp tube was put in another collection tube. Then AW1 washing buffer was added and centrifuged again at 8000rpm for 1min. Then washing buffer AW2 was added and centrifuged at 14000rpm for

3min. the filtrate was discarded and another collection tube was changed and centrifuged without adding any reagent (drying). Then 50µL AE buffer (elution buffer) was added to the QIAamp tube, incubated at room temperature for 5min, and then centrifuged at 8000rpm for 60sec. A second 50µL elution buffer was added and centrifuged again to increase the yield. The extract was then transferred to the amplification room for the next time.

Master Mix preparation -Master Mix was prepared in a separate room as per protocol to avoid contamination. Briefly, in a reaction mixture, 3µL nuclease-free water, 5µl of ×10 dream Taq buffer with MgCl2, 5µL mix of 2Mm each dNTP, 2µL of 10 pmol of each forward and reverse primers, 5µL of Dream Taq^{TM} DNA polymerase were used. The prepared master mix was then transferred to the amplification room.

PCR Amplification- Amplification of DNA for salmonella genus was performed by targeting oligonucleotide sequences corresponding to the *hto* gene (histidine transfer operon gene) with the following primers:

*F*w **5'-ACTGGCGTTATCCCTTTCTCTGGTG-3')** and

Rev **5'-ATGTTGTCCTGCCCCTGGTAAGAGA-3')** that amplifies a fragment of 496 bp predicted product size.

Extracted DNA was first vortexed to homogenize and then a 5µL DNA template was pipetted to each PCR tube to make the total volume 25µl and then centrifuged to be amplified. The amplification protocol was as described by Ngappa et al. (2007). An initial denaturation at 94 ^{0}C for 5min followed by 35 cycles of denaturation at 94^{0}C for 30sec, annealing at 55^{0}C for 30sec, elongation at 72^{0}C for 45sec, and final elongation at 72^{0}C for 5mins then the final product is stored at 4^{0}C until the machine is off.

The reaction was conducted by 25µL mixture and run on 2% Agarose gel for 1hr &20min, which was then detected by UV light. 100bp DNA ladder was used as standard.

Serotype specific amplification

S. Typhimurium detection was done using serotype-specific primer

PCR for the specific oligonucleotide sequences corresponding to the *SPY* gene was targeted for *S. Typhimurium* with the following sequences of primers.

***S. Typhimuium*: *SPY* gene primers**

F: 5'–TTGTTCACTTTTTACCCCTGAA-3' and

R: 5'-CCCTGACAGCCGTTAGGATATT-3' that amplifies a fragment of 401bp product size. The reaction was carried out in a 25μL amplification mixture consisting of 18.5μL of PCR master mix, 1.5μL Taq polymerase, and 5μL of each DNA template.

DNA Amplification-An initial denaturation at 94^0C for 5min, Followed by 35 cycles of denaturation at 94^0C for 60 sec, annealing at 57^0C for 60 sec, and elongation at 72^0C for 1.5mins, followed by 72^0C final elongation for 7mins. The final product was stored at 4^0C until the machines were off. The amplified product was separated on 2% Agarose gels and observed under UV light. A 100 bp DNA ladder was used as the DNA size standard.

Gel preparation-gel was prepared by weighing 2gm of Agarose gel powder and dissolving it in 100ml distilled water, which was then boiled in an oven at 180^0C for 5mins. After boiling, the Agarose was kept at room temperature to cool down to 60^0C. Then 5μL intercalating dye (Pronasafe, Himedia) was added and shaken to be mixed with Agarose. Then the gel was poured into the electrophoresis tank with the comb to make the well allowed to solidify for 20 minutes. Then, the comb was removed and the amplicon mixed with loading dye was loaded gently on each well together with negative and positive control and DNA ladder. The gel was run for 1hr and 20mins at 120V. Then the result was read under UV and the image was captured using the Gel Documentation System (Gel DocTM XR, Biorad, USA).

3.5.4. Antimicrobial Susceptibility testing

The antimicrobial susceptibility testing of the isolates was performed by the standard disk diffusion method according to the Clinical and Laboratory Standard Institute (CLSI, 2016). PCR confirmed serotype colonies grown on nutrient agar were transferred into tubes containing 5ml of saline water. The colonies were added until it achieved the 0.5 McFarland turbidity standards. A sterile cotton swab was dipped into the suspension and swabbed uniformly over the surface of the Muller Hinton agar plate. The plate was held at room temperature for 30 min to allow drying. Then, antimicrobial discs with known concentrations were placed on plates and incubated for 24 hr at 37ºC. The diameters of the zone of inhibition were recorded to the nearest millimeter. Interpretation of the results (i.e. categorization of isolates into susceptible, intermediate, or

resistant) was done according to NCCLS guidelines (NCCLS, 1999). The following commonly used antibiotics were tested for AST; Ampicillin, Amoxicillin, Ceftriaxone, Cefoxitin, Cefuroxime, Ceftazidime, Ciprofloxacin, Nalidixic acid, Trimethoprim-sulphamethoxazole, Sulphamethoxazole, Amikacin, Streptomycin, Spectinomycin, Oxytetracycline and Tetracyclin. The multiple antimicrobial resistance (MAR) index was calculated as previously described by Krumperman (1983) using the formula *a/b*, where *a* represents the number of antibiotics to which a particular isolate was resistant and *b* the total number of antibiotics tested.

3.6. Data management and analysis

Data were checked, coded, and entered into a Microsoft Excel spreadsheet. Descriptive statistics (frequency and percentages) were used to estimate prevalence of salmonella using SPSS statistical software Version 20. The sample level prevalence was calculated as the percentage of *Salmonella* positive samples among the total number of samples examined while farm level prevalence was computed as number of salmonella positive farm divided by total examined farm. A chi-square test was used to assess the association of various risk factors with the occurrence of *Salmonella*. Furthermore, univariate and multivariate logistic regression analysis was performed to reveal the strength of association of the potential risk factors with *Salmonella* occurrence. Association was considered statistically significant if the value was < 0.05.

3.7. Ethical Clearance

Ethical clearance for this research was obtained from Animal Ethics Review Committee of AAU, CVMA before the sample collection. The Ethical clearance reference number is AAU CVMA AERC/14/03/2022. The owner of the farm and shop were first asked to give their consent for sample collection. Their name and information they gave remained confidential. Filled and signed consent form was available at hands of researcher.

4. RESULTS

4.1. Prevalence of *Salmonella* in poultry and poultry products and its risk factors

Bacteriological analysis of 560 samples collected from cloacal swab (n=100), fresh chicken meat (n=300), frozen chicken meat (n=80), and egg (n=80) revealed that 11.3% (63/560) samples were positive for the presence of the *Salmonella.* Of the 18 farm examined, 83.3% (15/18) were *Salmonella* positive and three farms were negative for *Salmonella* species in this study (2 farms of egg swab sample and one farm for cloacal swab sample). The higher level of isolates was recorded in farms from Bishoftu (90%, 9/10) followed by Modjo (80%, 4/5) and L/Tafo (66.7%, 2/3).

The sample level isolation rate of *Salmonella* based on study area showed relatively higher percentages in Bishoftu (11.9) followed by L/Tafo (10.7%), Modjo (10%) and AA (7.5%). Based on the source, the rate of isolation of *Salmonella* spp. was 11.6%, 11%, 10%, and in Meat, cloacal swab, and egg samples, respectively. Thus, the higher percentage of *Salmonella* spp. was isolated from meat samples (11.6%, 44/380) (Table 2).

The rate of *Salmonella* positive (n=63) based on study area was higher from Bishoftu (46/63) followed by from Laga Tafo (8/63). The sample level isolation rate of *Salmonella* was relatively higher in Bishoftu (11.9) followed by 10.7%, 10%, and 7.5% in L/Tafo, Modjo and AA, respectively (Table 2).

Based on source of sample, the rate of isolation of *Salmonella* was 11%, 11.6%, 10%, and 11.3% in cloacal swab, Meat, and egg samples, respectively. Thus, the higher percentage of *Salmonella* spp. was isolated from meat samples (11.6%, 44/380) (Table 3). Additionally, all farms (100%) from where meat samples taken were positive for salmonella.

The level of salmonella among sample types revealed high contamination in fresh meat 12.7% followed by poultry 11% with frozen meat and egg 7.5%, 8%, respectively. Among overall chicken meat samples (n=380) taken in this study, 300 (200 swab and 100 organ) was from apparently healthy slaughtered chicken and 80 was FCM (60 FCM swab and 20 FCM carcasses). High prevalence was recorded in fresh meat 12.7% (38/300) followed by frozen meat swab (7.5%, 6/80).

The level of salmonella in egg showed that higher level for eggs from shop (13.3%) than eggs (8%) from farms. However sample level prevalence of salmonella didn't show association across risk factor (p>0.05)

Bacteriological analysis result of this study showed a different isolation rate of salmonella based on different associated risk factors. Accordingly, highest isolation rate of salmonella was recorded in swab from leg (14.6%), sample from Bishoftu (11.6%), sample from farms (11.6%) and fresh meat sample (12.7%). Besides, all risk factors was associated insignificantly with presence of salmonella in this study (P>0.05). Thus, the odds of salmonella increase in sources of sample and sample types (OR>1) (Table 3).

Table 2: Sample level prevalence and its associated risk factors for Salmonella

Variable	**Category**	**Establishment**		**Sample tested**	**Salmonella Positive**			
		Farm	**Shop**		**Farm**	**Shop**	**Samples N**	**X^2(P-value)**
					N (%)	**N (%)**	**N (%)**	
Study area	Bishoftu	(n=10)	(n=6)	385	9(90)	2(33.3)	46(11.9)	
	L/Tafo	(n=3)	(n=4)	75	2(66.7)	3(75)	8(10.7)	0.87
	Modjo	(n=5)	(n=2)	60	4(80)	1(50)	6(10)	(0.83)
	AA	(n=0)	(n=6)	40	NA	4(66.7)	3(7.5)	
Source	Farm	**18**	**0**	430	6(75)	NA	50(11.6)	
	Backyard	(n=8)	NA	20	5(100)	6(50)	3(15)	0.85
	Shop	(0)	(n=18)	110	3(60)	4(66.7)	10(9.1)	(0.65)
Sample type	Meat swab	5	12	260	5(100)	5()	36(13.8)	
	Organ	5	0	100	3(100)	0	7(7)	4.47
	Carcass	0	4	20	0	1(25)	1(5)	(0.34)
	C.swab	8	0	100	6(75)	NA	11(11)	
	E.swab	5	6	80	3(60)	4(66.7)	8(10)	
Total		**18**	**18**	**560**	**15(83.3)**	**10(55.6)**	**63(11.3)**	

CI=Confidence Interval OR= Odd Ratio C.swab= cloacal swab; E.swab= Egg swab

4.2. *Salmonella* prevalence in different poultry meat

A total of 380 meat samples tested for bacteriological analysis, 11.6% (44/380) prevalence was recorded out of which 12%, 13.3%, 7.5% from Bishoftu, L/Tafo and Addis Ababa, respectively. Higher *Salmonella* isolation rate was recorded in leg (14.7%), muscle swab (13.9%), breast (13.1%) and apparently slaughtered meat (12.5%). In contrast, low salmonella isolation rate of (7.5%), (7%), (5%) and (4%) was recorded in frozen meat, organ, carcass and spleen respectively (Table 4). The occurrence of salmonella was highly associated with sample item (X^2= 4.3, p=0.04). Among sample item salmonella isolated from leg, breast, liver and trimmed muscle was 14.7%, 13.1%, 10% and 5%, respectively.

Table 3: Prevalence of Salmonella in meat and its risk factors

Variables	**Category**	***Salmonella***			
		Sample	**Positive**	**(%)**	**X^2(P-value**
Study area	Bishoftu	325	39	12	
	L/Tafo	15	2	13.3	
	AA	40	3	7.5	4.2(0.3)
Establishment	Farm(n=5)	300	38	12.7	
	Shop(n=12)	80	6	7.5	2.3(0.6)
source	Fresh meat	300	38	12.7	1.6(0.9)
	Frozen meat	80	6	7.5	
Sample item	Breast	130	17	13.1	
	Leg	130	19	14.7	
	liver	50	5	10	4.3(0.04)
	Spleen	50	2	4	
	Trimmed muscle	20	1	5	
Sample type	Muscle swab	260	36	13.9	3.1(0.49)
	Organ	100	7	7	
	Carcass	20	1	5	
Total		380	44	11.6	

4.3. *Salmonella* prevalence among cloacal swabs from poultry

In this study, farm level prevalence of *Salmonella* in cloacal swab sample was 66.7% (6/8) whereas animal level prevalence was 15% (3/20) backyard chicken and 10% (8/80) in intensive farm chicken. *Salmonella* species were more commonly identified in poultry farms with higher flock sizes (12.5%) as compared to medium (10%) and small sized (10%) flocks of >1500 chickens than flock sizes 500-1500 and <500 chickens (Table 4). The isolation rate of *Salmonella* spp. across commodity was 11.7% in layers and 10% in broilers while across age groups was 15%, 13.3% and 3.3% in >18 months, <2months and 2-12months, respectively. Thus high prevalence was recorded in chicken of large flock size, >18months age and layers. However, there is no statistically significant difference in the isolation rate for *Salmonella* among the study area, age groups, commodity ($P > 0.05$). *Salmonella* occurrence was significantly associated with regular farm cleaning and presence of another farm within 1km radius ($p<0.05$) (Table 4).

Table 4: *Salmonella* prevalence in cloacal swab samples and associated risk factors

Variables	Category	Farm examined	Sample tested	Positive		
				No. of +ve farm N (%)	No. of +ve sample N (%)	X^2(P-value) sample level
Study area	Bishoftu	n=3	30	3(100)	4(13.3)	
	L/Tafo	n=2	40	1(50)	4(10)	0.24(0.89)
	Modjo	n=3	30	2(66.7)	3(10)	
Establishment	Farm	n=8	80	6(75)	8(10)	
	Backyard	10 hs	20	3(30)	3(15)	0.06(0.81)
Size of flock	<500	n=1	30	1(100)	3(10)	
	500-1500	n=3	30	2(66.7)	3(10)	0.15(0.93)
	>1500	n=4	40	4(100)	5(12.5)	
Commodity	Broiler	n=4	40	3(75)	4(10)	
	Layer	n=4	60	4(100)	7(11.7)	1.19(1)
Age	<=2month	n=4	30	3(75)	4(13.3)	
	2-18month	n=2	30	1(50)	1(3.3)	2.62(0.27)
	>18month	n=2	40	2(100)	6(15)	
Farm cleaned regularly	Yes	3	30	2(33.3)	1(3.3)	
	No	5	50	4(66.7)	7(14)	4.3(0.04)
Uses AM (last 6 months)	Yes	8	80	6(100)	8(10)	
	No	0	0	0(0)	0(0)	3.9(0.3)
Disposal of dead chicken	Bury	2	20	2(33.3)	2(10)	
	Burn	6	60	4(66.7)	6(12)	5.6(0.6)
Another farm within 1km	Yes	6	60	5(83.3)	6(10)	
	No	2	20	1(14.7)	2(10)	2.8(0.02)
Total		**n=8**	**100**	**6(75)**	**11(11)**	

AM= Antimicrobial; hs= houses

4.4. Sample level prevalence of *Salmonella* in Eggs and its risk factors

The isolation rate of *Salmonella* in the egg sample was 10% (8/80) with an egg overall prevalence of 1.4% (8/560). The isolation rate of salmonella on basis of study area was the same

(10%), however, the magnitude differ in which 3/8, 2/8, 3/8 from Bishoftu, L/Tafo and Modjo respectively. Farm level prevalence of salmonella in egg indicated that 60% (3/5) farm positive prevalence while shop had 66.7% (4/6) positive prevalence rate. The presence of Salmonella was found to be higher in eggs from shop (13.3%, x2=1.5; p=0.02) than eggs from farm (Table 6).

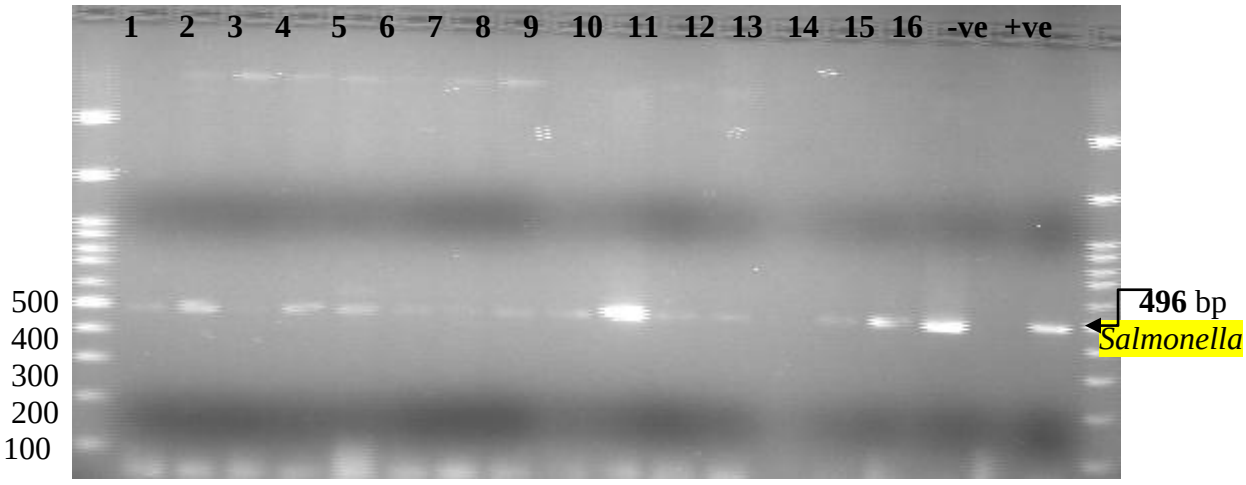
Table 5: *Salmonella* prevalence in eggs

Variables	Sample sources	Samples	Positive		
			+ve Sources N (%)	+ve samples N (%)	X^2 (P-value)
Bishoftu	Farm(n=2)	20	1(50)	2(10)	0 (1)
	Shop(n=2)	10	1(50)	1(10)	
	Total (n=4)	**30**	**2(50)**	**3(**10**)**	
Laga Tafo	Farm(n=1)	10	0(0)	0(0)	2.6(0.1)
	Shop(n=2)	10	2(100)	2(20)	
	Total (n=3)	**20**	**2(66.7)**	**2(**10**)**	
Modjo	Farm (n=2)	20	2(100)	2(10)	1.9(0.8)
	Shop (n=2)	10	1(50)	1(10)	
	Total (n=4)	**30**	**3(75)**	**3(10)**	
Subtotal	Farm(n=5)	50	3(60)	4(8)	1.5(0.02)
	Shop(n=6)	30	4(66.7)	4(13.3)	
Egg collected by hand	Farm=5	50	3(60)	4(8)	1.4(0.2)
Egg cont. by feces	Farm=4	50	4(100)	4(8)	2.3(0.03)
	Shop=4	20	4(100)		
Cracked egg presence	Farm=5	50	4(80)	4(8)	1.5(0.18)
	Shop=5	25	3(60)	3(12)	
Unsold egg stays for week	Shop=4	20	4(100)	4(20)	0.6(0.2)
Egg from d/t farm mixed	Shop=6	30	4(66.7)	4(13.3)	3.1(0.6)
Total	**(n=11)**	**80**	**7(63.6)**	**8(**10**)**	

4.5. Molecular Detection of Salmonella (PCR) and Serotype distribution

Out of 63 biochemically isolated *Salmonella* species, 21 were further characterized by PCR to identify the serotype of isolated salmonella. Thus, 11 S.Typhimurium were identified by targeting *SPY* gene (Figure 2) making 52.2% of the isolation rate. An effort was made to amplify S.Entritidis but it could not be landed on expected band size, which is 301bp. Another Spp. was

also not identified due to lack of primers. Thus, the proportion of S.Typhimurium in this study was 17.4 %
(11/63).



Line 1-16 samples. Line 17= negative control (nuclease free water); line 18= positive control (Salmonella spp.). 100bp DNA standard, and 496bp of expected nucleotide amplicon.

Figure 3 : *Salmonella* genus confirmed by PCR

The proportion of *S.* Typhimurium isolates based on sample type revealed higher five of the eleven isolates were from meat swab (45.5%) and three of the isolates were from cloacal swab (27.3%) while eggs, carcass and organs contributed to 27.3% of the isolates. Sample Source wise isolation rate of S.Typhimurium indicated that 60% 50%, and 50% of the isolates were from cloacal samples (3 out of 5), meat (7 out of 14) and egg (1 out of 2), respectively (Table 6). Based on study area, the highest number was recorded in Bishoftu (8/10) and L/Tafo 2(11) while none was isolated in samples from Modjo. Backyards (100%, 2/2) hold higher record for S.Typhimurium among cloacal swab sample when compared to poultry from farm (46.7%).Among meat samples, fresh meat was more positive for both *Salmonella* and S.Typhimurium with 6 Typhimurium serotype while the frozen meat have 1(50%). Leg swab (3) is more typhimurium positive than breast (2), liver (1), and carcass (1). But the sample level proportion was high for liver (100%) and carcass (100%) followed by leg (50%) and breast (40%) whereas none was from spleen (Table 6).

Table 6: The distribution of S.Typhimurium and its associated risk factors (n=21)

Variable	Category	S. Typhimurium		
		No. of isolate N(%)	**S.Typhimurium N (%)**	**X^2(P-value**
Study area	Bishoftu	15(3.9)	8(53.3)	
	L/Tafo	2(2.7)	2(100)	1.4 (0.6)
	Modjo	2(3.3)	0(0)	
	AA	2(5)	1(50)	
Establishment	Farm	15(3.5)	7(46.7)	
	Backyard	2(10)	2(100)	6.9 (0.03)
	Shop	4(3.6)	2(50)	
Sample source	Meat	14(3.7)	7(50)	
	Poultry	5(5.1)	3(60)	0.82 (0.66)
	Egg	2(2.5)	1(50)	
Meat Items	Breast	5(3.8)	2(40)	
	Leg	6(4.6)	3(50)	
	Liver	1(2)	1 (100)	2.9 (0.81)
	Spleen	1(2)	0(0)	
	Trimmed muscle	1(5)	1(100)	
Sample type	M. swab	11(4.2)	5(45.5)	
	Organ	2(2)	1(50)	2.21 (0.69)
	Carcass	1(5)	1(100)	
	Cloacal swab	5(5)	3(60)	
	Egg swab	2(2.5)	1(50)	
Total		**21(7)**	**11(52.3)**	

AA= Addis Ababa; M.swab= muscle swab

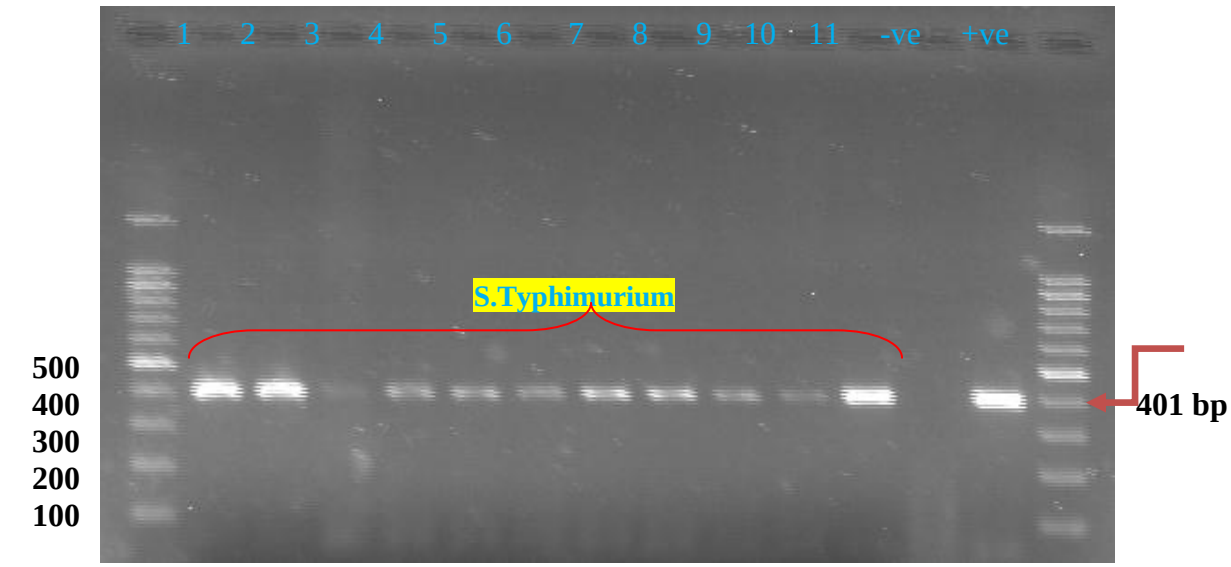


Figure 4: S.Typhimurium serotype (pooled amplicon)

(S.T DNA size 401bp; Rows: 1-11 samples; 12 is negative control (Sample without template DNA), 13 positive control (S.Typhimurium isolate). 100bp DNA size was used as standard marker

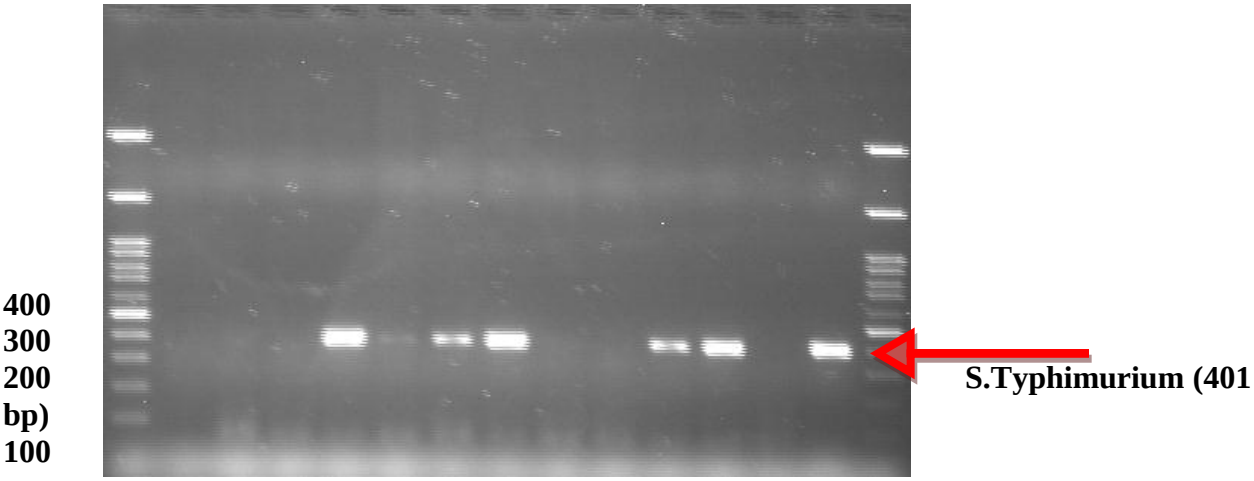


Figure 5: S.Typhimurium Isolate sample amplicon

Lane 1-11 is sample. Lane 12 –ve ctrl and Lane 13 +ve control
1, 2, 3, 5, 8 and 9 samples are negative; 4, 6, 7, 10 and 11 are positive samples

Generally, the current study showed that the detection rate of *S.* Typhimurium was higher in Laga Tafo, Backyard chicken, Liver, and carcass (Table 6). The data showed statistically

significant association of salmonella isolation in type of establishment (X^2=6.9, P=0.03) (Table 6).

4.6. Antimicrobial Resistance pattern of *Salmonella* Isolate

Antimicrobial susceptibility test was conducted for 11 S.Typhimurium isolate. The isolate showed resistant to multiple antimicrobial tested. Resistant was recorded in Ampicillin (81.8%), amoxicillin (81.8%), Oxytetracyclin (90.9%), Tetracyclin (72.7%), Nalixidic acid (72.7%) and sulphamethoxazole (54.5%) (Table 8). Isolate also showed resistant to cephalosporin, fluoroquinolones drug which commonly used in human infection.

Table 7: Phenotypic antimicrobial resistance profile of S.Typhimurium

Antimicrobial class	**Antim-icrobial agent**	**Drug concentration (µg)**	***Salmonella* spp. isolates**		
			Susceptible (%)	**Intermediate (%)**	**Resistant (%)**
Penicillin	AMP	25	0(0)	2(18.2)	9 (81.8)
	AML	25	1(9.1)	1(9.1)	9(81.8)
Cephalosporin	CRO	30	7(63.6)	2 (18.2)	2 (18.2)
	FOX	30	7 (63.6)	1 (9.1)	3 (27.3)
	CFX	25	5(45.4)	3(27.3)	3(27.3)
	CFZ	10	6(54.5)	3(27.3)	2(18.2)
Quinolones	CIP	5	6(54.5)	1(9.1)	4(36.4)
	NAL	30	1 (9.1)	2 (18.2)	8 (72.7)
Sulpha drugs	COTM	25	4(36.4)	3(27.2)	4(36.4)
	SXT	100	3 (27.3)	2 (18.2)	6(54.5)
	STR	10	1(9.1)	3(27.3)	7(63.6)
Amonoglycoside	SPM	30	4 (36.4)	4 (36.4)	3(27.2)
	KAN	30	2(18.2)	2(18.2)	7 (63.6)
Tetracycline	OXT	30	0(0)	1 (9.1)	10 (90.9)
	TET	30	1 (9.1)	2 (18.2)	8 (72.7)

AMP = Ampicillin; AMX = Amoxyxillin;CIP = Ciprofloxacin; KAN = Kanamycin;
NAL = Nalidixic Acid; OXT = Oxytetracycline; SPT = Spectinomycin; SXT = Sulfamethoxazol e; TET = Tetracycline, CRO = Ceftriaxone; CFZ= Ceftazidime; CFT= Cefoxitine; CFR= Cefuroxime; COTM= Cotrimoxazole; STR= StreptomycinTable 8: Multiple antimicrobial resistance patterns and Index of S. Typhimurium isolates

Table 8: Multi drug resistance patterns of salmonella isolate and MDR Index

Number of Antimicrobials	**Resistant pattern and number of isolates**	**Number of isolates (%)**	**MAR Index**
Three	NAL, OXT, TET	1(9.1)	0.2
Four	AMP, AMX, STR, SXT	1(9.1)	0.26
Five	AMP, AMX, KAN, OXT, SXT (1); AMP, AMX, , OXT, NAL, SXT (1)	2 (18.2)	0.33
Six	AMP, AMX, KAN, TET, OXT, STR, SXT(1); AMP, AMX, KAN, TET, OXT, SXT (1)	2 (54.5)	0.4
Eight	CIP, COTM, KAN, STR, NAL, OXT, TET, SXT	1(9.1)	0.53
Eleven	AMP, AMX, CIP, COTM, KAN, NAL, STR, OXT, TET, SPT, SXT (1); AMP, AMX, CRO, FOX, CFX, STR, NAL, OXT, TET, SPT, SXT (1)	2(18.2)	0.73
Fourteen	AMP, AMX, CIP, COTM, KAN, NAL, STR, OXT, TET, CRO, FOX, CFX, CFZ, SXT (1); AMP, AMX, CIP, COTM, KAN, NAL, FUR, OXT, TET, SPT, FOX, CFX, CFZ, SXT (1)	2(18.2)	0.93

AMP = Ampicillin; AMX = Amoxyxillin;CIP = Ciprofloxacin; KAN = Kanamycin; NAL = Nalidixic Acid; OXT = Oxytetracycline; SPT = Spectinomycin; SXT = Sulfamethoxazole; TET = Tetracycline, CRO = Ceftriaxone; CFZ= Ceftazidime; CFT= Cefoxitine; CFR= Cefuroxime; COTM= Cotrimoxazole; STR= Streptomycin

MDR pattern of S.Typhimurium showed that an isolate was at least resistant to three-tested antimicrobial. Two isolate showed resistant to 14/15 drug. The highest MRI recorded in this isolate was 0.93.

5. DISCUSSION

This study investigated the occurrence of Salmonella spp. in poultry value chain and antimicrobial resistance patterns of the isolated in selected towns around Addis Ababa. The detection of Salmonella in poultry products leads to rejection of large raw chicken meat, which could indirectly affect poultry trade with huge economic impacts. In developed country like USA and EU, the highest levels of non-compliance with Salmonella criteria generally occurred in foods of animal origin that are intended to be cooked before consumption. In the EU, human salmonellosis cases from poultry food sources i.e. eggs, broilers, and turkeys are estimated to be 17%, 10.6% and 2.6%, respectively (EFSA, 2012). Broiler-associated human salmonellosis were mainly due to serovars S. enteritidis (82%) and S. infantis (6.5%), while S.Entritidis and S. typhimurium serovars were most frequently associated with consumption of contaminated eggs and poultry meat (EFSA, 2000; EFSA, 2014).

In the current study, 77.8% of the 18 examined poultry farms were positive for Salmonella. This is in line with previous studies 76.7% (Ziyate *et al*., 2016), 73.5% (Arkali, 2020), 76.7% (Çetinkaya 2020) and 68.75% (Mridha *et al*., 2020) of *Salmonella* at farm level. However, it is very much higher compared to other studies from Ethiopia and Nigeria where 14.6% (Eguale, 2018) and 43.6% (Fagbamila *et al*., 2017) farms were reported positive for *Salmonella*, respectively.

Sample level prevalence of Salmonella in the current study (11.3%) is comparable to 8% study report by Aragaw *et al*. (2010) and 16.7% (Abdi *et al*., 2017) in Ethiopia. Nevertheless, higher prevalence 32.1% in South Africa (Ramtahal *et al*., 2022), 26.46% (Belachew *et al*., 2021) and 19.0% (Sarba *et al.*, 2020) and lower prevalence of 4.7% (Eguale, 2018) salmonella in poultry in central Ethiopia has been reported. This variation in prevalence could be due to the variation in isolation technique, sample size, sample type, sample item, geography, farm size, and farm management system and hygiene. This difference in prevalence may be due to difference in ecological geography, farm number examined, and season, hygiene status of the poultry farm and sample types.

Salmonella was more commonly isolated from poultry farms with larger flock size and in age group of 2– 6 months (Table 4). This finding is in agreement with previous report where high

prevalence of Salmonella was from large farms compared to medium and small farms (Barua *et al*., 2013; Dagnew *et al*., 2020; Mollenhorst *et al*., 2005; Eguale, 2018). From the total farms (n=18) examined in this study, all of the farms (100%) keep their birds on floor system (data not shown) and 50% were broilers. Salmonella was detected in 88.9%, 77.8% and 15% of broiler farms, layer farms and backyard chicken, respectively. This may be due to the fact that broilers farm environment are less in biosecurity than layers as the flock will be stocked out every 2 months in the former. but since layers stays for more than one year, the environment must be clean enough to prevent infection that cause economic loss.

Both farm level and pooled sample level prevalence of Salmonella and distribution of salmonella serovar S.Typhimurium was high in farms from Bishoftu compared to other areas (Table 2). However, the number of samples collected from Bishoftu was higher than samples from other areas. This could be because larger number of poultry farms were examined from this area compared to others as well as difference in agro ecology. Bishoftu is highly concentrated with large number of poultry farms compared to other study area. In addition, all fresh meat samples were collected from only Bishoftu, due to the absence of the broiler that was reached for slaughter in other study areas during the sample collection period.

This is in line with a previous study conducted by Eguale et al. (2018) but in contrary to reports of Belachew et al. (2021). In the current study, 11.6% prevalence of *Salmonella* in chicken meat is nearly comparable with earlier reports of 7% (Naik *et al*., 2015; Panda *et al*., 2012; Dahal, 2007; Hue *et al*., 2011; Patyal *et al*., 2012), 6.4% **(**Akeem *et al*., 2019**)** and 4% (Rabie *et al*., 2012). However, others reported a lower prevalence rate of 0.94% (Shekhar *et al*., 2013) and 1.5% (Kumar *et al*., 2008). On the other hand, a higher prevalence rate of 23.6% (Molla *et al*., 2003), 23.7% (Kaushik *et al*., 2014), 28.33% (Moon, 2011), 30% (Akhtar *et al*., 2010), 31.99% (Ruban *et al*., 2010), 44% (Fallah *et al*., 2013) and 46.29% (Sohail *et al*., 2021) salmonella in chicken meat samples. These differences in prevalence may be attributed to multiple factors, such as geographic and seasonal variation, variations in sampling procedures, sampling spot, and sample size, poultry management practices, hygienic conditions during production and processing of meat and meat products, or due to differences in the sensitivity and specificity of isolation methods used. The overall prevalence of *Salmonella* in retail meat (7.5%) in this study

is nearly in agreement with the 6.8% report from Yin et al., (2016), 10.5% (Zhou *et al*.**,** 2017) and 9.7% (Yu et al., 2014) in China.

Additionally, the high prevalence of *Salmonella* from meat in this study reveals that meat is more prone to contamination from handlers and the environment during processing if hygienic handling is not taken into considerations. In this study, all the meat was piled together during slaughtering processing, hence there is a chance of cross contamination between free and *Salmonella* positive meat. On the other hand, the condition of the meat processor at slaughtering site was not in line with food safety guideline. In this case, we cannot track the source of the *Salmonella*, as there is no sample taken from the chicken before slaughtering.

The liver and spleen was the primary target organs involved in *S.* Gallinarum infection, regardless of the route of infection. The characteristic lesions of the infected liver and spleen were hepatomegaly, discoloration of the liver, splenomegaly, congestion, and necrotic foci (Nazir *et al*., 2012). This is in line with the current finding of lesions in the positive liver and spleen (Annex iv). The organ level isolation rate of salmonella was 7% (5 from liver and 2 from spleen) in this study. While it is comparable with the report of Rahimi (2012), higher result of 19.7% (Li *et al*., 2013), 23% (Alali *et al*., 2012), and 47% (Uyttendaele *et al*., 1999) was reported.

The level of *Salmonella* in eggshells was 10% (8/80) in this study. In addition, salmonella isolation in eggs from the market was is higher than the those collected from farms. This is higher compared to report of *Salmonella* prevalence in eggshells 2.7% (Kemal *et al*., 2016) from Haramaya, eastern Ethiopia. This variation may be due to the difference in sample size, climate condition of the study area, storage condition of an egg at the shop, and hygienic status of the shop.

In Ethiopia, Prevalence of Salmonella serovar in poultry and poultry food has been reported (Ketema *et al.,* 2018; Dagnew *et al.,* 2020; Molla *et al*., 2003; Kemal *et al*., 2016). In addition, study also revealed differences in distribution of *Salmonella* serovar across geographic regions, establishment, poultry management systems, sample types, sources of sample, collection, and handling methods of poultry products (Ayalew *et al.,* 2017; Asfaw *et al.,* 2020; Kemal *et al*., 2016). *Salmonella* serovars frequently reported in Ethiopia include *S.* Typhimurium, *S.* Newport,

S. Saintpaul, *S.* Kentucky, *S.* Haifa, *S.* Enteritidis, *S.* Gallinarum, *S.* Anatum, and *S.* Pullorum (Aragaw *et al.,* 2010; Eguale, 2018; Ketema *et al.,* 2018; Dagnew *et al.,* 2020; Molla *et al*., 2003). Salmonella S.Typhimurium var Copenhagen has been also reported from in contact human in Ethiopia (Eguale, 2018).

The occurrence of *Salmonella* serovar S. Typhimurium in this study was 52.4% whereas 10/21 could be other serotypes that could not be typed due to lack of primer. This finding is comparable to Moawad et al. (2017) who reported 60% of the isolated to be S.Typhimurium from Egypt. In general, *S.* Typhimurium was the dominant serovar similar to reports from Cambodia and Thailand (Trongjit *et al*., 2017), and Brazil (Ristori *et al*., 2017). In contrast, it's different from study reports from Iran (Soltan-Dallal *et al*., 2010, Sodagari *et al*., 2015) and Hubei province, China (Zhou *et al*., 2019) in which the dominant serotype was S. Thompson. Another previous study from China (Yin *et al*., 2016) reports S. Hadar as the dominant serovar whereas S. Enteritidis was dominant in Canary Islands (Hernandez *et al*., 2005), China (Yang et al., 2013), and Spain (Capita *et al*., 2007). However, a simple conclusion could not be made from direct comparisons among studies due to regional differences, sampling seasons, and the types of products sampled. The differences in dominant serotypes may be due to geographical differences, sample types or seasons, and serotype pathogenicity. In this study, the higher contamination rate of S.Typhimurium serovar found may be related to less biosecurity and hygiene measures inside establishments and slaughtering environment.

Generally, differences in prevalence rates in various studies may be due to the method used to isolate the bacteria, types, and numbers of samples, animal management practices, and hygienic conditions of the farm and farm attendants. For instance, in this study, none of the poultry farm attendants had any form of formal training certificate on the safe handling practices of chickens. That is why there was no regular cleaning of chicken excreta on regular basis, which increase the chance to be mixed with chicken feed, which is prone to cross-contamination of chickens. Moreover, all the operations of bleeding, defeathering, skinning, evisceration and cutting are carried out in small premises without any demarcation of different slaughter and dressing operations. The hygienic practices of slaughtering process and cleanliness of the processing environment are at very minimum level. All processing's were carried out on a single plastic laid on the floor and the workers did not wear personal protective equipment.

Additionally, people who work as chicken processors during slaughter had no training in safety, quality, and proper meat processing. This may be one of the reasons why they eviscerate the intestinal content in the same place where they put the already finished one. This reveals that there is a high chance of cross contamination from chicken with *Salmonella*, and even from the handler, toward free one. The stage of processing the meat during slaughter must be different and by a different person to reduce the chance of contamination. However, in this study the one that removes the feather and eviscerates trims the muscle and packs it into the same working environment.

One thing as a risk factor for salmonella infection in human is sharing the same compound with the poultry. Especially small-scale poultry farm in this study live in the same compound with farm that may increase the chance of contamination by salmonellosis. The people can be also transfer salmonella to chicken by contaminating poultry feed and water through touching with bare hand.

Antimicrobial Resistance of *Salmonella Typhimurium*

Antibiotics are frequently utilized as a growth promotion and for prophylaxis at sub-therapeutic dose in animal feed that may be a reason for development of antimicrobial resistance to that particular drug (Chinasa *et al*., 2019) (Adhikari *et al*., 2018). Indiscriminate use of antimicrobials without prescription, improper dispensing and without disease indication favor selection pressure and help the bacteria to confer resistance genes (Arkali and Cetinkaya, 2020).

The Antimicrobial susceptibility test result of S.Typhimurium in this study indicated that all isolates (n=11) were resistant to at least three antimicrobials. This result agrees to a studies conducted in Ethiopia, which reports resistant to at least one drug (Eguale *et al.,* 2016; Geresu *et al.,* 2021). However, lower study results of about 58% (Taddese et al., 2019) and 72.72% (Odoch *et al.,* 2017) of the isolates resistant to at least one antimicrobial agent has been reported. The higher resistance shown in current study could have resulted from overuse of antimicrobials, as there is a poor regulation and monitoring of antimicrobial utilization in poultry farms (Gebremedhin *et al*., 2021b). The other possible reason behind this discrepancy could be the

number of serotypes. Whereas only *S.* Typhimurium serovar was tested in the current study, other than *S.* Typhimurium serotypes were investigated in the above studies.

The phenotypic antimicrobial resistance profile of *S.* Typhimurium isolates in the current investigation indicated that resistance to oxytetracycline, tetracycline, ampicillin, Amoxicillin, Streptomycin, Spectinomycin and Kanamycin with resistance rates of 90.9%, 72.7%, 81.8%, 81.8%, 63.3%, 63.3% and 27.3% respectively (Table 7). This indicated that higher resistance by isolate was against Oxytetracyclin which is in agreement with the previous studies report of 97.8% (Abdi *et al.,* 2017), 100% (Garedew *et al.,* 2015), 100% (Geresu *et al*., 2021) and 90% (Chibuzo and Okike, 2021) in oxytetracyclin. The emergence of *Salmonella* resistance to fore-mentioned antimicrobials could be due to irrational use of these drugs in both the livestock and human health sectors that resulted from weak regulation and easy accessibility of these drugs at pharmacy in Ethiopia (Abunna *et al.,* 2016; Gebremedhin *et al.,* 2021b).

Due to its broad spectrum properties, oxytetracycline is commonly used antibiotic in livestock sectors (Hiko et al., 2018) and in Ethiopia, it is widely used for the treatment and as prophylaxis in poultry farms (Dagnew *et al*., 2020). Use of antibiotic, especially broad spectrum, in flock when some birds get sick is being common practice among poultry farm (Eguale, 2018).

In current study, the finding revealed four resistant isolates to ciprofloxacin, which is in line with previous study (Moawad et al., 2017; Odoch et al., 2017; Siddiky et al., 2021) that reports ciprofloxacin resistant S.Typhimurim isolate in poultry. On other hand, study from Ethiopia (Abdi et al., 2017; Merera, 2018; Taddese et al., 2019) and others (Odoch et al., 2017 ; Siddiky et al., 2021) revealed that none of isolates was resistant against ciprofloxacin. The resistance profile of S.Typhimurium to cephalosporin ceftriaxone (18.2%), cefuroxime (27.3%), cefoxitin (27.3%) and ceftazidime (18.2%) in this study was in harmony with study by Moawad et al. (2017) which reported 20% and 33.3% of resistance in ceftriaxone and ceftazidime respectively. Sulphamethoxazole and cotrimoxazole resistance was recorded as 54.5% and 36.4% respectively. Resistances in these drugs are of great clinical significance because Sulfamethoxazole, cotrimoxazole, fluoroquinolones and cephalosporin antimicrobials are considered frontline therapeutic drugs for treatment of most bacterial infections in humans (Bradford, 2001). The report of study conducted on chicken farm in Ethiopia showed that the use of ciprofloxacin and cephalosporin antimicrobials was not commonly practiced (Eguale et al.,

2016). Nevertheless, it seems the bacteria conferred resistance in this study. However, since samples from meat are expected to have been contaminated from human handler, the isolate may be from human contaminant as the drug is most commonly being used in human. In addition, it suggests that there is transmission of resistance genes across species. However, study revealed that S.Typhimurium can naturally resist a variety of antimicrobial drugs and develop multi-drug resistance against many antimicrobial agents (Yang et al., 2020). The significance public health importance of this finding is related to the potential risk of transmission of these resistant strains to humans via contact with sick poultry or consumption of poultry products.

The MDR features of S.Typhimurium showed that 9.1% (1 isolate each) was resistant to three, four and eight antimicrobials, whereas 18.2% (2 isolate each) were resistant to five, six, eleven and fourteen antimicrobials used in the test. Almost all strain has the same resistance pattern to at least three drugs. This is in line with study in Nigeria (Moawad *et al*., 2017) and closely comparable with other studies (Pritha *et al.,* 2020; Zhou *et al*., 2019; Mridha *et al*., 2020; Siddiky *et al.*, 2021; Eguale *et al*., 2016; Geresu *et al*., 2021; Abdi *et al.*, 2017). The highest MAR indexes recorded in this study was 0.93 and the lowest index was 0.2 (Table 8).

6. CONCLUSION AND RECOMMENDATIONS

This study revealed poultry products are prone to contamination by *Salmonella* and becomes a potential public health risk. This affects the health of consumers unless intervention measures that reduce the level of contamination are implemented in the processing chain. Illegal slaughtering of chicken on the farm by people who do not have knowledge and training about zoonotic bacteria becoming usual. The hygienic status of the slaughtering area and chicken meat processors on the farm is very poor. Evisceration, trimming and final washing are carried out at a single spot, which may increases the possibility of the contamination of the meat from intestinal content, and the cross-contamination between *Salmonella*-free meats. Additionally, meat packaging was carried out in an open environment, which increases the chance of contamination of the meat from the environment. Finally, the higher prevalence of Salmonella in meat than other samples suggests existence of continual contamination of poultry meat during meat processing, and need special attention. Based on the above conclusion, the following recommendations are forwarded:

- Each step in poultry meat processing should be separate and clean from contaminants and packaging of meat should be under aseptic condition. Poultry product sellers should be those who have good knowledge about zoonotic *Salmonella* and its public health importance
- Farm hygiene should be in the form that prevents infection and thus reduce antibiotic usage in poultry. Timely quarantine and treatment of infected chicken from others should be practiced on the farm
- To reduce the risk of chicken meat contamination, implementation and monitoring of the Codex general principles of food hygiene and code of hygienic practice for meat and local food safety proclamations are required.
- Awareness and capacity building of workers at farms, slaughtering site and retail shops through provision of continuous professional development and educational sessions such as information campaign on zoonotic salmonellosis is needed.
- Further study is required to understand the epidemiological link among poultry production, poultry product consumption and salmonellosis in humans:

- An in-depth study is needed on the practice of antimicrobial usage in poultry farms as well as an investigation of the drivers and magnitude of the antimicrobial resistance
- Mapping of the environmental contamination in the poultry food chain to identify all possible sources for the contamination of poultry products should be performed.
- Cross-sectoral collaboration between animal health, human health and food safety and standard authority is needed to minimize food borne infection especially chicken meat.

Limitation of the study

In this study, the sample size collected was not equal for all study areas. For example, fresh meat swab and organ was collected only from Bishoftu. This is because broiler chicken that reached for slaughter was not found in other study sites. Additionally, even from Bishoftu, some of the farm owners were not willing to allow the sample collection. They complain that you might be among government organs and report us about the illegal slaughter on the farm so we may lose our license. Those who allowed me to take samples did not allow me to take a picture during sample collection as they worried about privacy.

Not all serotype were identified because of the scarcity of primers. This may affect the true prevalence of *Salmonella* serovar across different risk factors. Finally, only S.Typhimurium serotypes was identified, as it is the only primer available at hand. This affects the serotype distribution of *Salmonella* in the poultry food chain. There might be more serovars that could be isolated.

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8. ANNEX

Annex i: Bacteriological media preparation for *Salmonella* isolation and identification

a. Buffered Peptone Water (Himedia, Mubai, India)

Composition	**g/l**
Enzymatic digest of casein	10.0
Sodium chloride	5.0
disodium phosphate dodecahydrate	9.0
potassium dihydrogen phosphate	9.0

Preparation:

Suspend 20.07 grams of dehydrated components in 1000 ml of distilled water. Mix well and heat (if necessary) to dissolve the medium completely. distribute it into a universal bottle of suitable capacity to obtain the portions necessary for the test and sterilize in an autoclave at 121^0C for 15 minutes.

b. Modified Rappaport Vassiliadis (HIMEDIA, Mumbai, India)

Composition	g/l
soya peptone	4.5
sodium chloride	8.00
potassium dihydrogen phosphate	0.60
dipotassium phosphate	0.40
magnesium chloride, hexahydrate	29.00
malachite green	0.036

Preparation:

Suspend 27.11 grams of the hydrated medium in 1000ml distilled water. Heat if necessary to dissolve the medium completely. Dispense as desired into tubes and sterilize by autoclaving at 115 ℃ for 15 minutes. PH after sterilization: 5.2 + 0.2

c. Xylose Lysine Desoxycholate Agar (XLD) (HiMedia, India)

Composition	**g/l**
yeast extracts	3.0
l-lysine hydrochloric acid	5.0
Xylose	3.75
Lactose	7.5
Sucrose	7.5
L-Lysine hydrochloride	5.0
sodium chloride	5.0
sodium thiosulphate	6.8
ferric ammonium citrate	0.8
phenol red	0.08
Agar	15.0

Preparation:

Suspend 56.68 grams in one liter of distilled water. Heat with frequent agitation until the medium boils. **DO NOT OVER AUTOCLAVE**. Transfer immediately to a water bath at 500c. pour into plates as soon as the medium has cooled. It is important to preparing large volumes which will cause prolonged heating.

d. Nutrient Agar (CM 0003, OXOID, Basingstoke, England)

Composition	**g/l**
peptic digest of animal tissue	5.00
sodium chloride	5.00
beef extract	1.5
yeast extract	1.5
Agar	15

Preparation:

Suspend 28 grams in 100ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (1210c) for 15 minutes. Mix well and pour into sterile Petri dishes. Final PH (at 250c): 7.4 + 0.2.

e. Triple Sugar Iron Agar (Himedia, Mumbia, India)

Composition	**g/l**
meat extract	3.0
yeast extract	3.0
Peptone	20.0
sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
ferric citrate	0.3
sodium thiosulfate	0.3
phenol red	0.024
Agar	12.0

Preparation:

Suspend 64.52 grams in 1000ml of distilled water. Mix well and bring to boil to dissolve completely. distribute in 20 ml tubes (8-10 ml/tubes) and sterilize by autoclaving at 121℃ for 25 minutes. Allow the set as slope with 2.5 cm butts. PH: 7.4 + 0.2 at 25℃.

f. Simon Citrate Agar (Himedia, Mubia, India)

Composition	**gms/l**
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	1.0
Dipotasium phosphate	1.0
Sodium citrate	2.0
Sodium chloride	5.0
Bromo thymol blue	0.08
Agar	15

Preparation:

Suspend 24.28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired in tubes and sterilize by autoclaving at 121^0C for 15 minutes.

g. MR-VP Medium (HiMedia, Mumbai, India)

Composition	**grm/l**
pancreatic digest of casein	3.5
peptic digest of animal tissue; dextrose	5.00
dipotassium phosphate	5.00

Preparation:

Suspend 17.0 grams in one liter of distilled water. Heat to dissolve the medium completely (if necessary). Distribute in to test tubes 5ml amounts and sterilize by autoclaving at 15 Ibs pressure (121℃) for 15 minute

Reagent required for voges- proskauer reaction

α-Naphtanol, ethanolic solution, potassium hydroxide crystal, and distilled water

α-Naphtanol solution:

Composition	**Amount**
α-Naphtanol	5 grams
95 % ethano	100ml

Preparation:

Weight 5 gram α-Naphtanol crystal, dissolve in 250 volumetric flask containing 100 ml 95% ethanol and stored at $+4^0$C for about two to three weeks

Potassium Hydroxide Solution

Composition	**Amount**
potassium hydroxide	40 grams
distilled water	100ml

Preparation:

Weight 40 gm potassium hydroxide and dissolve in 250 ml polyethylene bottle. containing 100 ml distilled water and stir to dissolve the pellets and Keep the bottle in a cool water bath during preparation since the reaction procduce heat.

h. Tryptone Soya broth Preparation (Oxoid Ltd, Hampshire, England)

Composition	**g/L**
pancreatic digestion of casein	17
Enzymatic digest of soya bean	3.0
sodium chloride	5
Dipotassium hydrogen phosphate	2.8
Glucose	2.5

Preparation:

Dissolve 30 gm of powder in 1 liter of distilled water as required and distribute to 5ml test tube of the final container. Sterilized by Autoclaving at121^0c for 15 minutes. The broth was clear and yellow.

i. Lysine Decarboxylation Medium

Composition	**g/l**
Peptone	5.0
Beef Extract	5.0
Glucose	0.5
Bromcresol Purple	0.5
Cresol Red	5.0
Pyridoxal	5.0

Preparation:

Suspend 14 grams in a litter of distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired in tubes and sterilize by autoclaving at 1210C for 15 minutes.

Annex ii: Media and 0.5 McFarland standards preparation for drug susceptibility testing

a: Mueller-Hinton Agar (CM 0337, OXOID, Basingstoke, England)

Composition	**g/l**
beef, dehydrated infusion	300.00
casein hydrolysate	17.5
Starch	1.5
Agar	17.00

Preparation:

Suspend 38 grams in 1000 ml of distilled water. Bring to boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. PH: 7.3 + 0.1 at 25°C

b. 0.5 McFarland standards

Composition	**Amount**
1.17% BaCl.2H2O solution	0.05 ml
0.36N of 1% sulfuric acid (H2SO4).	9.95ml

Preparation:

Add approximately 85Ml of 1% H2SO4 to a 100ml of a volumetric flask, using a 0.5ml pipette add 0.05ml of 1.17% BaCl.2H2O dropwise to the 9.95ml of 1% H2SO4 while constantly swirling the flask. Bring to 100ml with 1% H2SO4. Place a magnetic stirring in the flask and place on the magnetic stirrer for approximately three to five minutes. Examine solution visually to make certain it appears homogeneous and free of visible clumps.

Annex iv: Preparation of agarose gel, molecular marker, and Loading dye for PCR

a. Agarose gel (1.5%)

Composition:

TAE Buffer (1:49 ratio of concentrated stock TAE buffer and distilled water);
agarose powder: 1.5 gm

Preparation:

First prepare Tris Acetate EDTA (TAE) buffer by mixing 980 ml distilled water and 20 ml concentrated stock TAE buffer. Then weigh 1.5 gm agarose powder and dissolve in 200 ml TAE buffer in a 250 ml flask and melt the agaross in the microwave for around 8-12 minutes. Repeat the boiling until it becomes clear and let the solution cool to about 50-55°C. Seal the ends of the casting tray with two lying of tape and place the combs in sealed gel casting tray. Pour on the gel try and wait for about 20 minutes until well solidified and carefully pull out the combs and remove the tape. Place the gel in the electrophoresis chamber and add enough TAE Buffer (about 2-3 mm of buffer over the gel).

b. Loading dye

Composition	**Concentration**
Loading buffer	6X
Gel Red	1000X

Procedure:
Mix 940 µl loading buffer and 60 µl Gel Red in 20 ml centrifuge tube by vortexing. Add 4 µl for 20 µl of PCR products, mix by pipeting and load 10 µl in to the Gel.

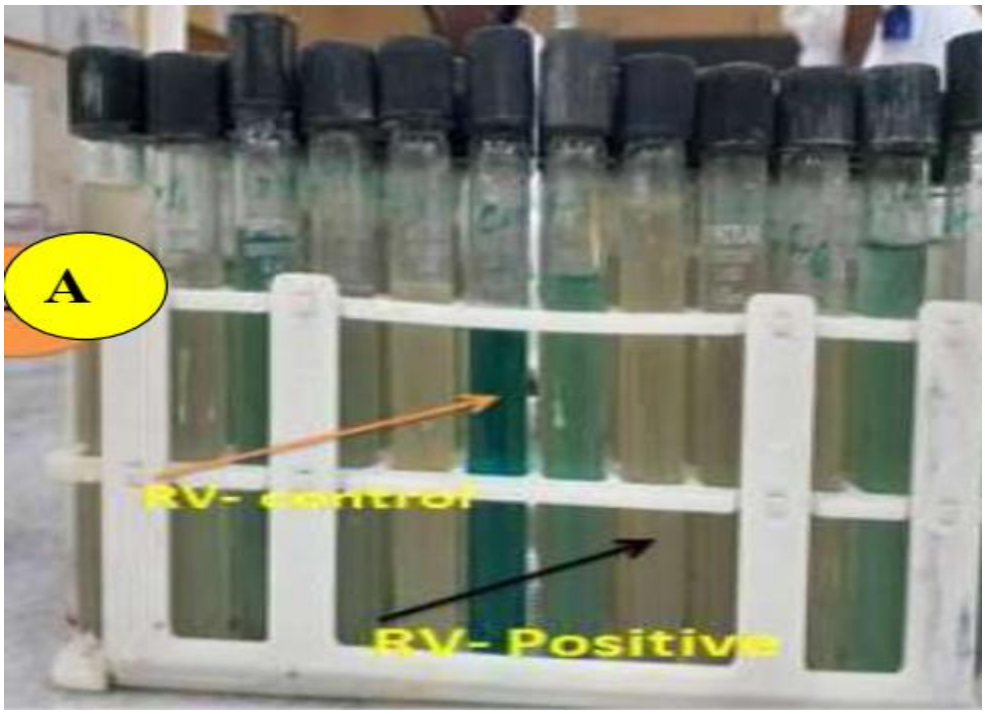
c. Molecular marker

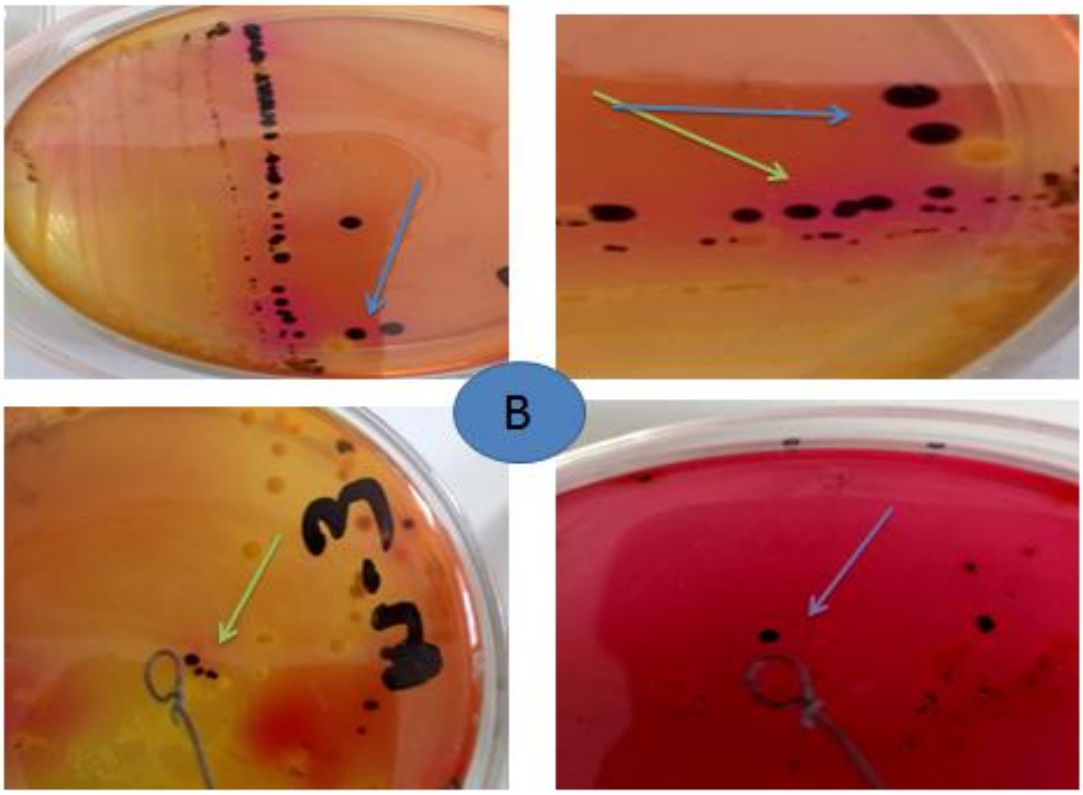
Composition	**Amount**
loading buffer	475 µl
Gel Red	25 µl
PCR water	265 µl

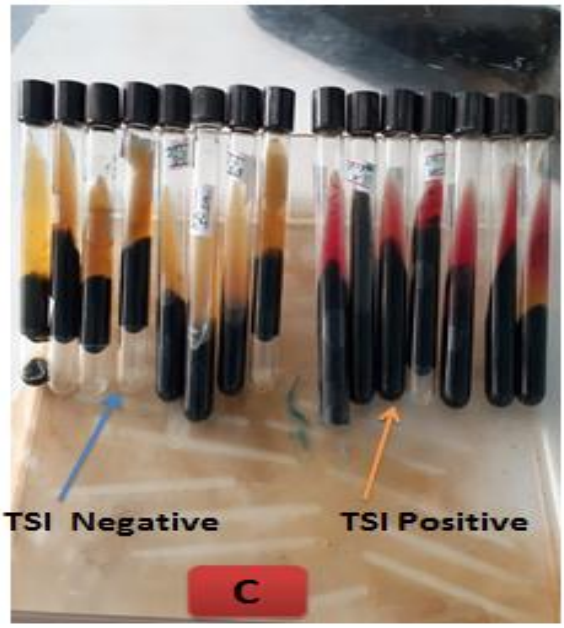
Procedure:

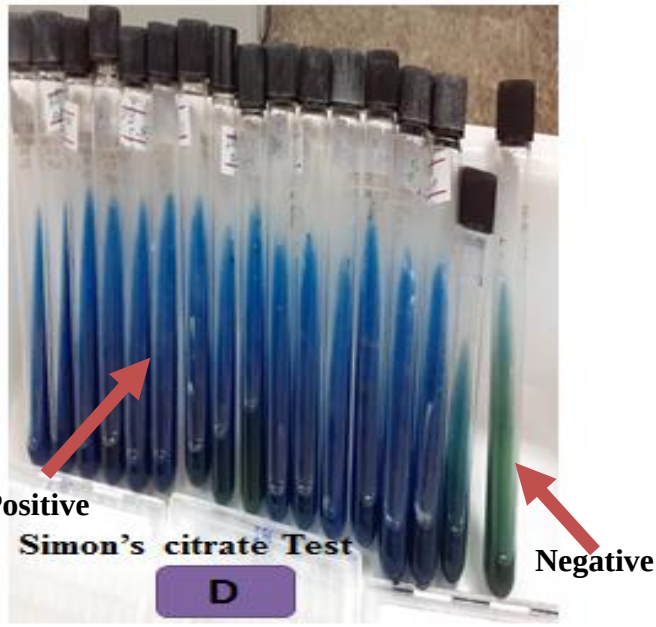
Mix 475 µl loading buffer and 25 µl Gel Red by vortexing. Again mixing **265** µl PCR water, 59 µl pre-prepared mixture of loading buffer and Gel Red and 27 µl of DNA ladder by vortexing. Finally add add 10 µl in to the gel as a molecular marker.

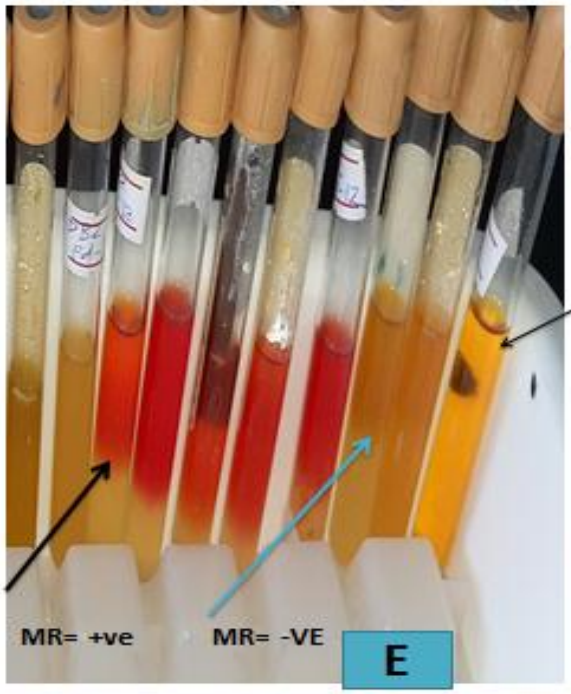
Annex iii: *Salmonella* colonies, biochemical results and antimicrobial inhibition zone

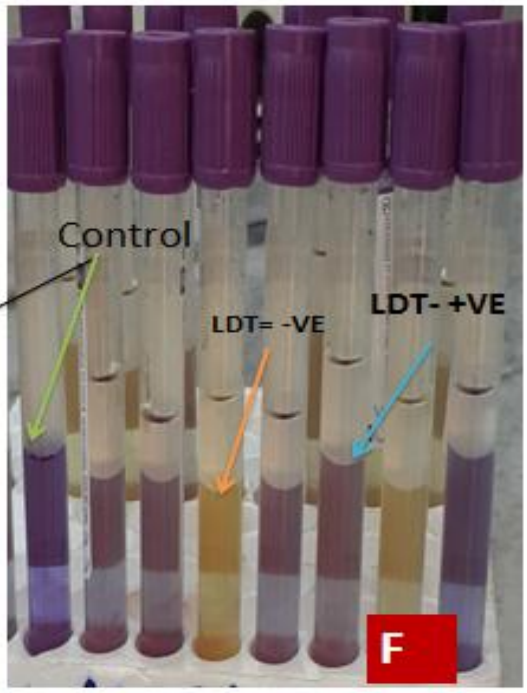


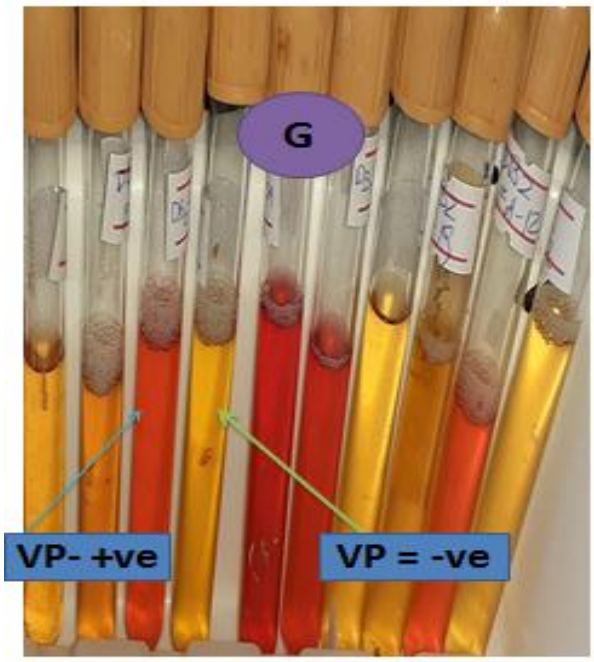


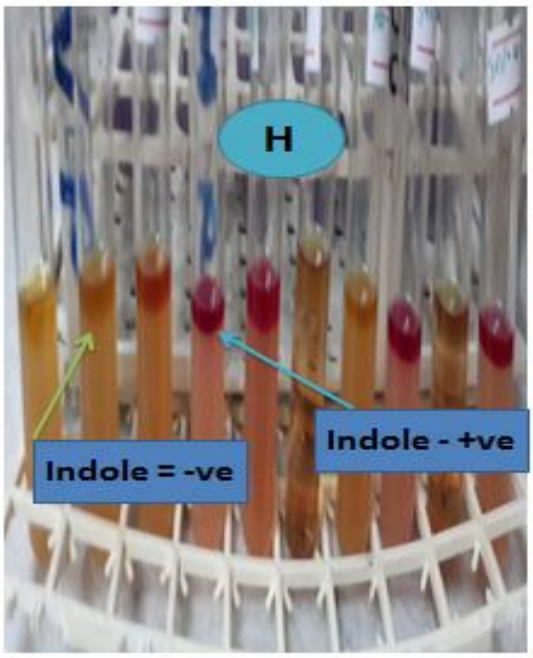

TSI Negative
TSI Positive
C

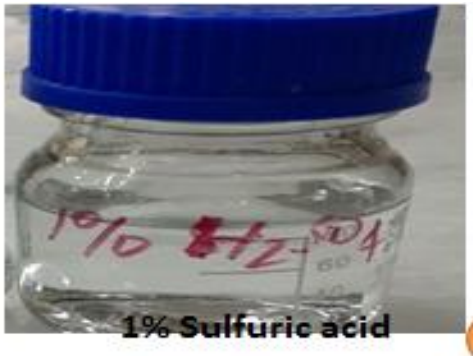

Positive
Simon's citrate Test
Negative
D

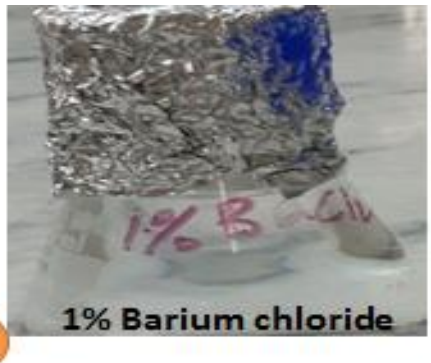

MR= +ve
MR= -VE
E


Control
LDT= -VE
LDT- +VE
F


G
VP- +ve
VP = -ve

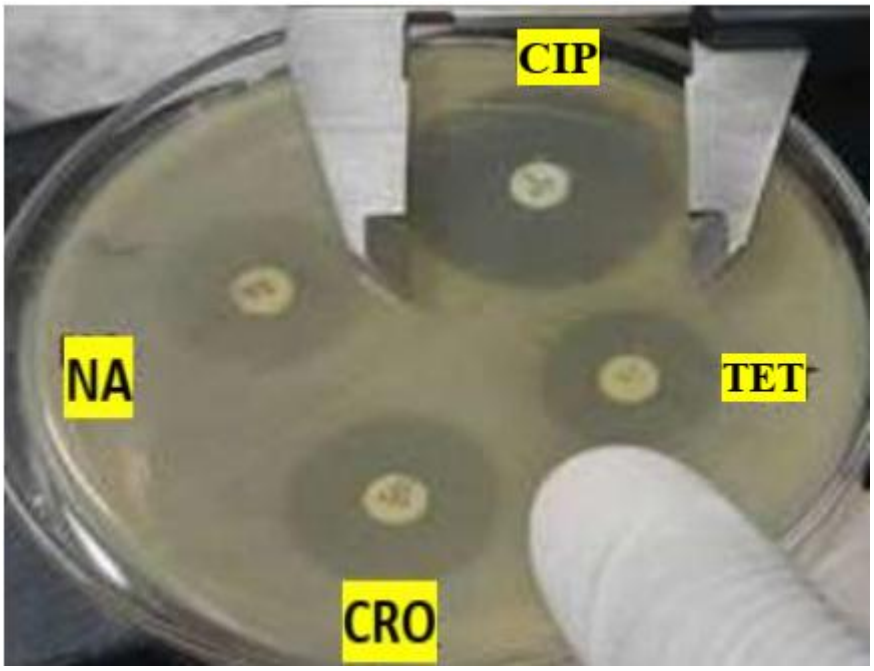

H
Indole = -ve
Indole - +ve


1% Sulfuric acid


1% Barium chloride

J


Sterile saline with Salmonella colonies
McFarland Standard



Annex iv: Data collection and laboratory result recording Sheets

a. Data collection sheet

Code	Site	Farm name	Age	Farm size	Source	Sample type
1						
2						
3						

b. Bacteriological tests result recording sheet

ID No.	RV	Plating media		Biochemical test results					PCR
		XLD	BGA	TSI	SCT	Indole	MR-VP	LDT	
1									
2									

XLD = Xylose Lysine Decarboxylate Test; BGA = Briliant Green Agar; TSI = Triple Sugar Iron Agar; SCT = Simon's Citrate Agar; MR-VP = Methyl Red and Vogas Prosker; LDT = Lysine Decarboxylation Test; PCR = Polymerase Chian Reaction

c. Drug sensitivity result recording sheet of Salmonella isolates in µg

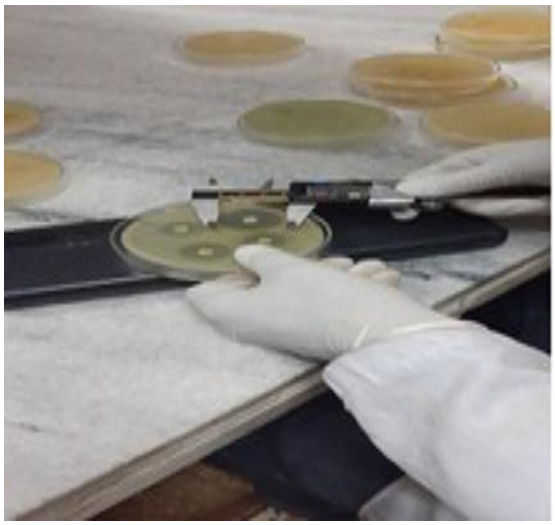
Isolates	AMP (10)	AMX (25)	CIP (5)	NA (30)	STR (10)	KAN (30)	TET 30	OXT (30)	SXT (100)	COT (25)	CRO 30	CFT 30	CFX 25	CFZ 10
1														
2														

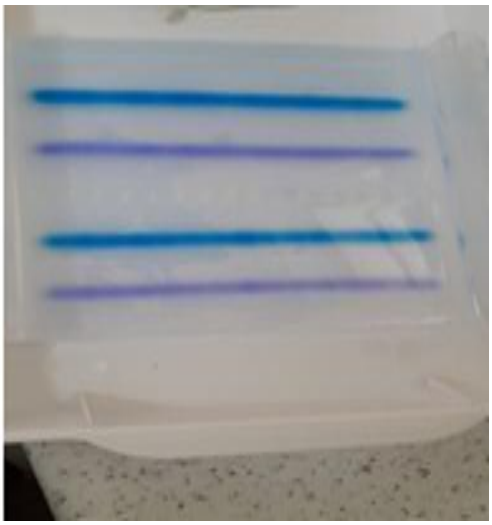
Annex v: Antimicrobial Susceptibility Breakpoints for *Enterobacteriaceae.*

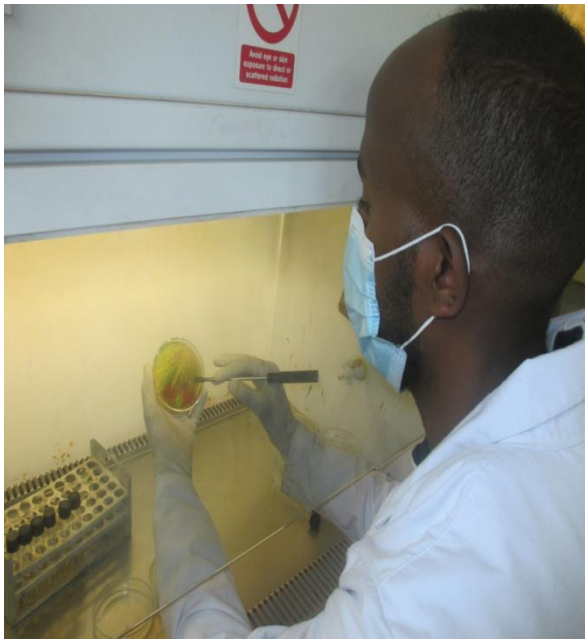
Types of antimicrobials	Concentration (µg/disc)	Susceptible	Intermediate	Resistant
Ampicillin (AMP)	25	≥17	14 – 16	≤13
Amoxycillin	25	18	14-17	≤13
Streptomycin	10	≥10	7-9	≤6
Spectinomycin	30	≥18	14 -17	≤13
Kanamycin (KNA)	30	≥18	14 -17	≤13
Ciprofloxacin	5	≥31	21-30	≤ 20
Nalidixic Acid (NA)	30	≥19	14 – 18	≤13
Oxytetracycline (OX)	30	≥16	12 – 16	≤12
Tetracycline (TET)	30	≥15	12 – 14	≤11
Sulfamethazole (SXT)	25	≥16	11 – 15	≤ 10
Cotrimoxazole	100	≥16	11-15	≤ 10
Ceftriaxone	30	≥23	20-22	≤19
Cefuroxime	25	≥18	15-17	≤14
Cefoxitin	30	≥18	15-17	≤14
Ceftazidime	10	≥21	18-20	≤17

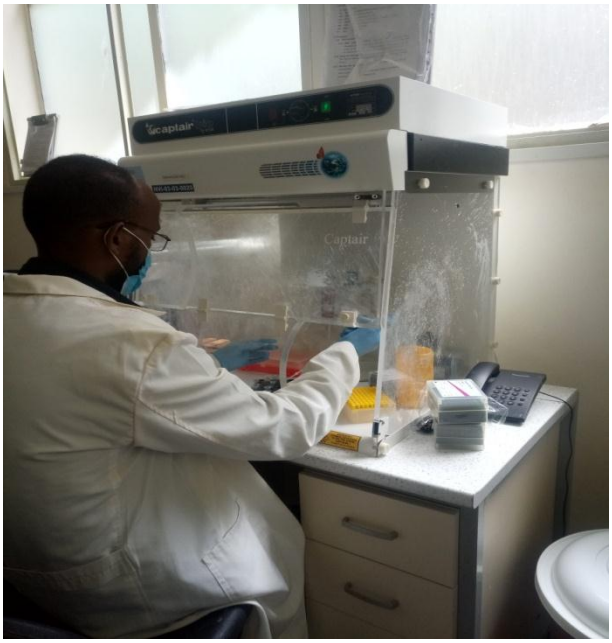
Source*:* Clinical and laboratory standard Institute (CLSI, 2016)

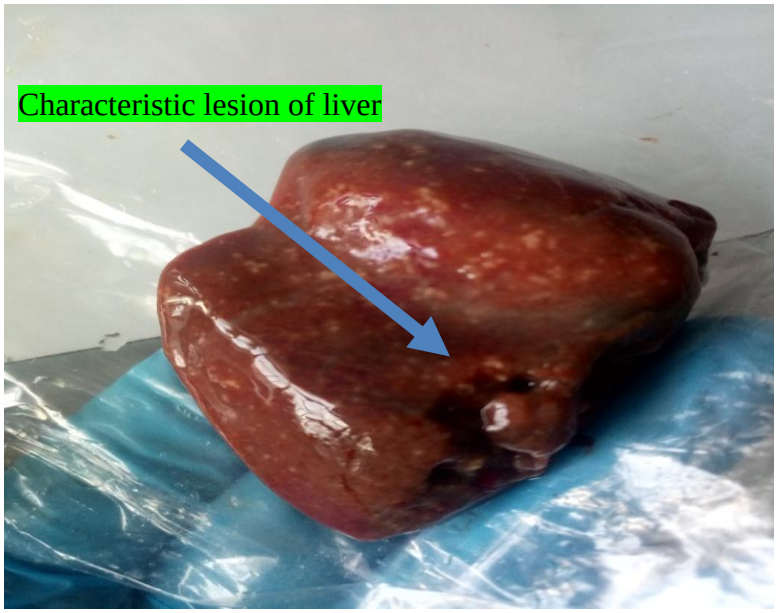
Annex vi: Different Laboratory Activities during the study period










Characteristic lesion of liver