



**ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY TEST OF *SALMONELLA*
SPECIES AND OWNERS KNOWLEDGE, ATTITUDE AND PRACTICES ON
ANTIMICROBIAL RESISTANCE IN SELECTED POULTRY FARMS OF ADDIS
ABABA AND BISHOFTU, ETHIOPIA**

MVSC Thesis

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**A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis
Ababa University in partial fulfillment of the requirements for the degree of Masters
of Veterinary Public health**

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First, I declare that this thesis is my work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for Masters of veterinary science degree at Addis Ababa University, College of Veterinary Medicine and Agriculture. It has been submitted to the college library for anyone's use under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial Resistance
AMU	Antimicrobial Use
BGA	Brilliant Green Agar
CLSI	Clinical and Laboratory Standards Institute
ENTAG	Ethio-Netherlands Trade for Agricultural Growth
<i>Hto</i> gene	Histidine transport operon gene
KAP	Knowledge, Attitude and Practice
MDR	Multi Drug Resistant
MRL	Maximum Residue Level
MR VP	Methyl Red Vogues Parker
NCCLS	National Committee for Clinical Laboratory Standards
NTS	Non Typhoidal <i>Salmonella</i>
PCR	Polymerase Chain Reaction
PFGE	Pulse Field Gel Electrophoresis
RVS	Rappaport-Vassiliadis soy peptone
SIM	Sulfide-Indole-Motility test
<i>Spy</i> gene	Spheroplast Protein Y
TSI	Triple Sugar Iron agar
XLD	Xylose Lysine Deoxycholate

ABSTRACT

Salmonellosis in poultry cause mortality and high morbidity with economic consequences and has become public health concern as cause of food borne disease and due to antimicrobial resistance (AMR). The global deaths speculated from antimicrobial resistance threat is estimated to be over 300 million per year unless significant steps are taken by 2050. Little is known of contributing factors in poultry sector for the threat in Central Ethiopia. A cross sectional study carried out from December 2021 to May 2022 to investigate the distribution of *Salmonella* isolates, antimicrobial resistance using Kirby–Bauer disk diffusion assay in twelve intensive layer and broiler poultry farms of Bishoftu town. Also surveyed 72 poultry farm owner’s knowledge, attitude and practice (KAP) towards AMR development and risks to public health in Addis Ababa and Bishoftu, Ethiopia. A total of 214 samples collected in Bishoftu town; 191 cloaca swabs and 23 environmental samples (litter and drinking water) were processed for bacteriological isolation. *Salmonella* found in twelve farms (n=42), 19.6% (95% CI= 0.14-0.25) of which 90% were from the cloaca swabs. Out of 42 isolates, 30 were processed using Polymerase Chain Reaction (PCR) and (n=29) 13.5% were detected for *Salmonella* and of that (n=16) 53.3% were detected for *Salmonella* Typhimurium. Antimicrobial resistance test was done on nine isolates detected and all were resistant to at least five antimicrobials, indicating a multidrug resistance (MDR). Using multivariable logistic regression analysis, production type and age of the chicken were significantly associated with *Salmonella* positives (P<0.05). With regard to KAP of farm owners on AMR, the cumulative average score was less than 50% in both Addis Ababa and Bishoftu; despite 70% owners had high school plus background. Association of farm owner’s knowledge and attitude with education levels and farming experience years were significant except, practices were only significantly associated in production types. The biosecurity status found good in 47.2% of poultry farms visited. In conclusion, the predominance occurrence of *Salmonella* Typhimurium, detection of MDR isolates in poultry farms and identified poor knowledge of the owners about AMR and potential risk to public health in this study, suggests the need for designing feasible Salmonellosis control strategies and in-depth detection of MDR genes. Furthermore, recommended improving awareness of poultry farm owners on AMR and investigations of other contributing factors to AMR development in the poultry sector.

Key words: Addis Ababa; Antimicrobial Resistance; Bishoftu; Knowledge; Poultry farm owners; *Salmonella*,

1. INTRODUCTION

Salmonellosis is one of the most commonly reported zoonotic food-borne diseases in the world, and it has had a significant economic and public health impact in many countries (Hugas and Beloeil, 2014; González *et al.*, 2019). Normally, the birds' digestive tract is a reservoir for *Salmonella* and *Campylobacter* spp., which are usually spread by cross-contamination during defeathering and evisceration (Wahyono and Utami, 2018; González *et al.*, 2019).

The poultry industry continues to face challenges such as disease, high feed costs, and antibiotics in the treatment of Salmonellosis, which are proving less effective due to the rise of antibiotic-resistant *Salmonella* strains, effects on normal gut microflora, and antibiotic-associated diarrhea. Moreover, with 100% morbidity and 20% mortality in affected flocks, Salmonellosis causes severe economic damage to chicken production (Gut *et al.*, 2018; Abdi *et al.*, 2017). Although poultry production industry has a high economic return in short term productions every year, improved cleaning and disinfection, biosecurity, and the use of vaccines for breeding and laying flocks have helped to reduce the prevalence of *Salmonella* in chickens (Atterbury *et al.*, 2020; Ricke, 2021). Infection at the site of primary production (e.g., parent stock, incubator, and farm); cross-contamination in the handling of food or byproducts; and consumption of undercooked poultry meat, eggs, or egg products are some of the diverse sources of contamination in the case of poultry products. All of these sources have been linked to *Salmonella* infection in humans (Castro-Vargas *et al.*, 2020).

Worldwide gastroenteritis is the most common *Salmonella* infection, accounting for 93.8 million cases which result in 155,000 deaths per year. Despite improvements in hygiene and sanitation, the incidence of NTS human infections continues to increase, creating a burden in both industrialized and underdeveloped countries (Teferi, 2020). In US Nowadays on top infections in human, emergence of AMR *Salmonella* strains effect estimated almost 10 million people could die of bacteria that are resistant to the antibiotic by 2050 according to a report commissioned by the United Kingdom (UK). The risk prompted the European Union in 2006 to place a ban on the use of antibiotic feed additives to uphold food safety (Akintayo and Alagbe, 2020; Selaledi *et al.*, 2020).

Correspondingly intensive production systems worldwide had made Africa face the spread of zoonotic disease, environmental change, and change in livelihood with AMR risk in humans beyond the weak regulation and surveillance system to manage. Resistance to ampicillin,

tetracyclines, and trimethoprim/sulfamethoxazole is widespread in Africa; the genes that confer resistance in animals, humans, and the environment have been identified in several African countries (Selaledi *et al.*, 2020; Mshana *et al.*, 2021).

Studies in around districts of Addis Ababa *Salmonella* was found on 14.6% of farms prevalence in (Egualé, 2018). And a 28.8 percent prevalence in Adama and Modjo town also 14.6 percent in Bishoftu and Modjo investigated recently (Asfaw *et al.*, 2020; Dagneu *et al.*, 2020). While a small hold broiler supply chains study found 22 percent and 26 percent in Bishoftu and Modjo (Belachew *et al.*, 2021) respectively, emphasizes the persistence of the problem on top of multidrug resistance. Only a substantial change seen from Asfaw *et al.* (2014) report of 86% to Dagneu (2020) 76% resistant reported in the particular area (Asfaw *et al.*, 2020; Dagneu *et al.*, 2020).

A knowledge, attitude and practice of animal producers in Ethiopia reports revealed the knowledge of farmers on AMR might contribute on the irrational use of antimicrobials in food producing animals (Gemedá *et al.*, 2020; Gebeyehu *et al.*, 2021; Geta and Kibret, 2021). Unlike Ethiopia, other countries conducted studies in particular to poultry farm owners' knowledge, attitude and practice on AMR and AMU accordingly emphasized the lack of awareness on effects of AMR and irrational AMU corresponded with farmers educational and experience levels (Ozturk *et al.*, 2019; Hassan *et al.*, 2021; Siddiky *et al.*, 2022). As KAP survey is meant to be a representative survey of a target population; aiming to elicit what is known (knowledge), believed (attitude), and done (practiced) in the context of the topic of interest. It is ideally important to design an awareness program or an intervention program as the results of the survey provide the inputs needed for designing an effective program, and baseline data for the future evaluation of the success (Andrade *et al.*, 2020).

Although previous researches on isolation and identification of *Salmonella* studies conducted in poultry farms of central Ethiopia by Belachew *et al.* (2021), Dagenew *et al.* (2020) and Egualé (2018), alarmingly indicated the emergence threat of this food borne disease prevalence in poultry and antimicrobial resistance patterns which potentially lead to resistant strain in animal and public health. As far as the known the direct or indirectly contributing factors to AMR at farm level in association with a farm owners' knowledge, attitude and practice of AMU and AMR has not assessed yet. In fact studies have assessed the knowledge and practice of animal producers in general on AMR and AMU in different parts of Ethiopia.

Poultry farming is attracting considerable interest due to high turnover, affordable production business and management in recent years. Thereof, it has become important to reduce burden of infections, control antimicrobial use, and monitor resistance at a nutshell in the poultry production. WHO recommended on global action plan 2015 (WHO, 2015) to improve awareness strengthen the knowledge through surveillance, education and training on AMR control. As matter of fact to meet the goal of monitoring AMU and contributing factors to AMR from end users, surveys conducted on target population can provide input for better recommendation and policy improvement in the context of once country.

Knowledge is a master key to change antibiotic use and build a desirable attitude towards AMU and public health protection hence farm owners or managers, veterinarians, feed sellers, consumers, environmentalist and respective concern bodies under the one health circles; either are victims or contributing factors of the threat in one way or another. Consequently, AMR awareness undeniably will create a behavioral change at the country level. Though to know where to start or what to implement under the education and training plans of owners', surveillance of the existing gap is crucial in developing effective and feasible strategy that improves awareness, practice of end users and enforce regulations not only to control and monitor but to promote investigations of alternative resolutions that prevent unnecessary use of antimicrobials and AMR development in poultry and public health. Thus objectives of this study were;

- To isolate and detect *Salmonella* species in poultry farms of Bishoftu town Ethiopia
- To investigate the antimicrobial resistance pattern of the *Salmonella* isolates.
- To assess the knowledge of poultry farm owners or managers towards use of antimicrobials, AMR development and risk to public health in Addis Ababa and Bishoftu with the assessment of overall biosecurity status of their poultry farms.

2. LITERATURE REVIEW

2.1 General characteristics of *Salmonella*

A *Salmonella* spp. is a Gram-negative rod-shaped bacterium that is one of the most common infectious agents in the tropics, especially in areas with poor hygiene. The source of infection with *Salmonella* spp. is from human or animal feces or urine which contaminates drinking water and food so that it becomes a source of infection, besides fish, flies, and dust can also act as intermediaries for salmonellosis (Acevedo-villanueva *et al.*, 2021). The genus is classified into two species: *Salmonella enterica* and *Salmonella bongori*. Whereas a biochemical and genomic analysis of *Salmonella enterica* has led to further classification into subspecies six types. The clinically important *Salmonella* species are classified under *Salmonella enterica*, further classified into more than 2,579 serovars based on their antigenicity (Gut *et al.*, 2018).

Major antigens are somatic (O), capsule (K), and flagellum (H). Somatic antigen (O) is positioned outside the membrane of the bacterial cell and has stable heat to form the lipopolysaccharide (LPS) oligosaccharide portion of the bacterial cell (Abdi *et al.*, 2017). The thermally-labile H antigen is used to activate the immune response of the host and is found mainly in bacterial flagella. With two different genes that flagellar encode the bacteria can be diphasic (phase I and II), so they can release one protein at a time only. The K antigen surface is rarely found between most serotypes of *Salmonella* and heat sensitive polysaccharides mainly located on the surface of the bacterial capsule. Only *Paratyphi C*, *Dublin*, and *Typhi* serotypes were found for the K antigen subtype virulence (VI) (Gut *et al.*, 2018; Selaledi *et al.*, 2020).

2.2 Epidemiology of Poultry Salmonellosis

2.2.1 Geographical distribution

Fowl typhoid and Pullorum disease are widespread and economically significant in many countries around the world. They are primarily found in Latin America, the Middle East, India, Africa, and possibly other parts of the world. Salmonellosis has also been reported in several South-East Asian countries, including Bangladesh, India, Pakistan, and Nepal. Both backyard chickens and commercial poultry are susceptible to fowl typhoid. The bacteria are passed out through the faeces and lateral spread takes place through the fecal contaminated feeds, water, and litter. Age perspective prevalence of avian salmonellosis showed the highest infection rate

in adult layers (53.25%) in comparison to brooding (14.55%), growing (16.10%), and pullet (16.10%) chickens (Kabir, 2010).

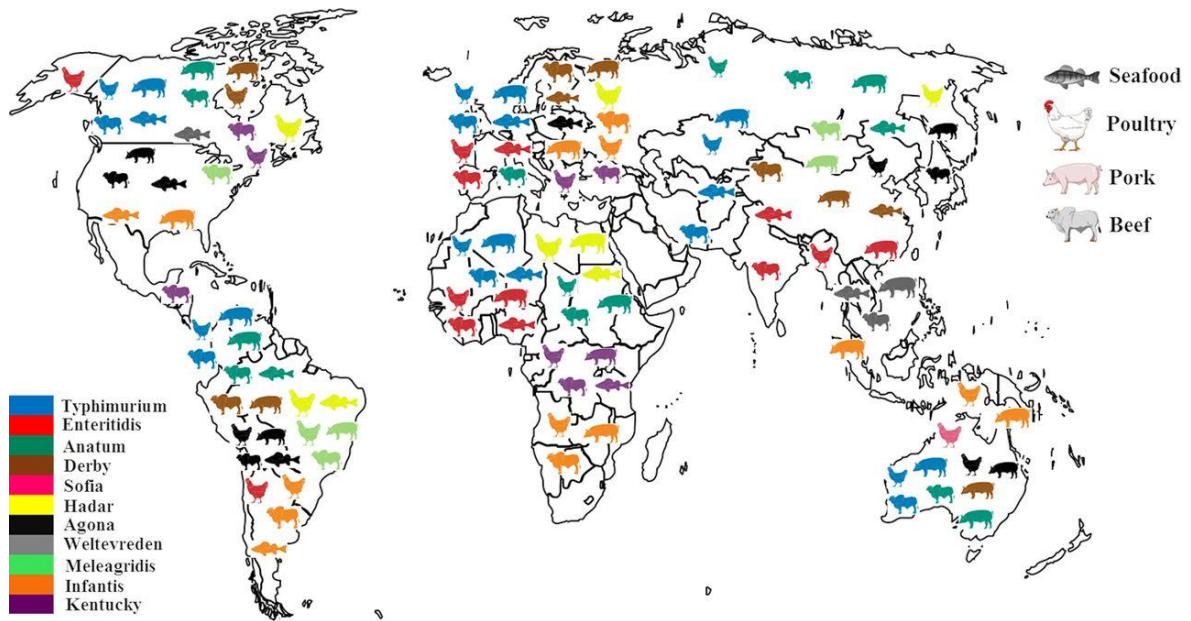


Figure 1 World distribution of *Salmonella* strains by matrix

Source: (Ferrari *et al.*, 2021)

2.2.2 Host range

Pullorum disease is host-specific that affects mainly chicks under 3 weeks of age and the initial indication is usually excessive numbers of dead in shell chicks and death immediately after hatching, sometimes it may appear in adult birds. An infection can be found in many avian species including chickens, turkeys, quail, guinea fowl, pheasants, ducks, pigeons, sparrows canaries, bullfinches, and parrots however, Pullorum disease is uncommon except in chickens, turkeys, and pheasants (Markos and Abdela, 2016).

In humans, *Salmonella*, which is infectivity, has three distinct infections: typhoid, paratyphoid, and non-typhoid *Salmonella* (NTS). In human beings, *Salmonella* is the cause of typhoid and paratyphus fever also *Salmonella* Typhi, *S. Enterica* serovar Paratyphi, a fever characteristic, and complications, including septicemia, immunological symptoms, leukopenia, and neurology. This typhoid and paratyphoid complications may cause death (Wibisono *et al.*, 2020).

The morbidity and mortality vary by age, the strain of chicken, management, nutritional status route and dose of exposure, and other disease stresses in the flock. Pullorum disease in chicks

for example can have up to 100 percent mortality with the highest losses in the second week. Fowl typhoid has a 10-90 percent infection rate with higher morbidity than mortality (Berhanu and Fulasa, 2020).

2.2.3 Transmission

Salmonella spread is common and persistent in dry environments, but it can survive in water for months. *Salmonella* Enterica has a complicated host/parasite relationship reservoir of pathogens that can infect both humans and animals. *Salmonella* can cause clinical or sub-clinical infection in asymptomatic animals in animals that are sometimes considered "carriers." Previous research has discovered that chickens can suffer from subclinical infection for up to 22 weeks. Transports in farms and climate are not also to ignore as the bacteria can constantly and routinely be excreted in feces without clinical symptoms (Wibisono *et al.*, 2020).

Even if chicks emerge from the hatchery virtually free of *Salmonella*, there are numerous ways for birds to get it during the production cycle. A variety of carriers, including rodents, cats, and insects, among others could transmit *Salmonella* to birds. *Salmonella* can survive for long periods of time in some of these reservoirs, including aerosols and water, as well as contaminated litter and feed. For example, *Salmonella* spp. have been shown to survive for several weeks in low water activity poultry feeds and litter (O'Bryan *et al.*, 2022). Vertical and horizontal spread through eggs (chicken embryos) through the reproductive system of poultry when it colonizes the fallopian tubes contaminating fresh eggs and contaminated chicken embryos may be lost due to *Salmonella's* pathogenicity. After hatching survived chicken embryos will carry *Salmonella* and result in infection in healthy chicks (Cui *et al.*, 2021).

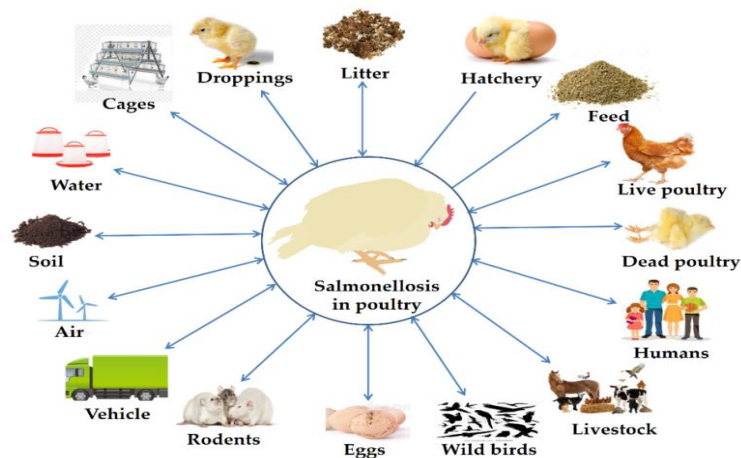


Figure 2: Possible transmissions of Salmonellosis in poultry

Source: (Hossain *et al.*, 2021)

2.3 Pathogenesis of Salmonellosis

Various virulence factors in *Salmonella* play many roles in the pathogenicity (e.g. plasmids, flagella, adhesion systems, capsule and type III secretion systems) Typhoid fever illness caused by *Salmonella typhi* and *Salmonella Paratyphi A* and *B* that classified as human-adapted species. Furthermore, strains of *Salmonella Typhi* and *Salmonella Typhimurium* produce a thermolabile enterotoxin which is similar to cholera toxin and *Escherichia.coli* heat labile enterotoxin. However, *Salmonella* have a thermostable cytotoxin that inhibits synthesis of protein eukaryotic cell and disrupts host cell monolayers. It has been expected that endotoxine has a role in symptoms of typhoid. Moreover, *Salmonella* pathogenicity island (SPIs) encodes some virulence factors that responsible for invasion, adhesion and toxin genes (Kadhim, 2020).

Salmonella outer membrane LPS is an important toxin that interacts with the host immune system to cause inflammation, septic shock, fever, and death. It can also change the structure of the O antigen to dampen the host's innate immune response, which increases the microorganism's ability to persist and survive in the host. From blood, bacteria are seeded into cells and tissues of different organs such as liver, lung, spleen, kidney, different parts of reproductive tracts of hens, and testes of male and other tissues producing pathological lesions. It is also confirmed that the bacteria invade the ovary and egg follicles and this infection persists in the ovary and egg follicles and transmits into laid eggs then hatched in chicks (Markos and Abdela, 2016).

2.4 Detection and Identification of *Salmonella*

Current testing of food and environmental samples for the presence of *Salmonella* can be divided into three stages: detection of the pathogen; identification of the isolate as *Salmonella* and its specific serovar designation; and subtyping of the isolate for association with any clinical cases of salmonellosis. Traditional isolation method take 5 days and confirmation relies on biochemical testing even with newer automated technologies that permit simultaneous testing needed at least 24 hrs for confirmation (Bell *et al.*, 2016).

2.4.1 Culture method and Biochemical testing

Culture method is recommended method by both by US FDA and FSIS of US Department of Agriculture for the detection of *Salmonella* and still center cultural selection both in selective liquid media and on agar plates (Maciorowski *et al.*, 2006). Conventional culture methods serve

as the basis in food testing laboratories despite rather laborious and time-consuming protocols. Pre-enrichment uses a nutritious nonselective medium to recover sublethally injured *Salmonella* cells while inhibiting the growth of competing flora. The incubated pre-enrichment media are inoculated into selective media containing two or more inhibitory reagents such as bile salts, brilliant green, thiosulphate, deoxycholate, malachite green, novobiocin, tetrathionate, cycloheximide, nitrofurantoin, and sulphacetamide (Lee *et al.*, 2015). Characteristics of colonies done stab inoculation into triple sugar iron and lysine iron agars and biochemically characterized using the urease and indole tests followed by serological typing with both Somatic(O) flagellar (H) antisera (Maciorowski *et al.*, 2006).

2.4.2 Genotyping of *Salmonella*

Conventional PCR-based assays for detecting a wide range of pathogenic agents associated with animals have been developed. Commercial kits for identifying these and other pathogenic organisms are available. The detection of both viable and non-viable cells is a potential limitation of DNA-based diagnostic methods. This limitation can be overcome by performing an enrichment step prior to nucleic acid extraction or by performing an RNA-based detection method known as RT-PCR. Because RNA is only produced by living cells, only viable cells are detected (Quinn *et al.*, 2011).

The PCR technique provides a new strategy for rapid and sensitive detection of *Salmonella* strains. Oligonucleotide primers of 25 bp defined the amplified region of a 496-bp segment of the histidine transport operon of *Salmonella* Typhimurium. The gene considered highly conserved among species of *Salmonella* and Cohen in 1992 compared with other enterobacteriaceae and identified *Salmonella* spp at 496 base pair (Cohen *et al.*, 1993). Bacterial adherence is generally believed to be a prerequisite for infection, and there is evidence that many bacteria have surface appendages, such as fimbriae or pili, that mediate binding to specific receptors on the epithelial cell surface, to urinary tract or intestinal mucus. The *Salmonella* Typhimurium *fimA* gene has been cloned and sequenced. The nucleotide sequences of the *fimA* genes of *E.coli* and *Klebsiella.pneu-moniae* have significant homology but are not fully homologous (Cohen *et al.*, 1996).

Transcription of the *S. enterica serovar* Typhimurium *spy* gene is the ortholog of which in *Escherichia.coli* encode *Spy* (spheroplast protein y). *Spy* is a chaperone protein expressed and localized in the periplasm of *E.coli* during spheroplast formation, or by exposure to protein

denaturing conditions. Spy expression in *Salmonella* Typhimurium was examined by constructing a spy-gfp transcriptional fusion. *Salmonella* Typhimurium spy transcription was strongly induced during spheroplast formation, and also when exposed to membrane-disrupting agents, including ethanol and the antimicrobial peptide polymyxin B. Moreover, spy induction required the activity of regulator proteins BaeR and CpxR, which are part of the major envelope stress response systems BaeS/BaeR and CpxA/CpxR, respectively (Jeong *et al.*, 2017).

2.4.2.1 Pulse-field Gel electrophoresis (PFGE)

Pulsed-field gel electrophoresis (PFGE) is a common molecular method that is able to further distinguish *Salmonella* species on the basis of their DNA. In New Zealand this technique is usually only applied during cluster or outbreak investigations where it is used to determine whether salmonellosis cases had become ill with the same strain of *Salmonella* and to help link these cases with a source of infection. If PFGE does not adequately discriminate *Salmonella* Typhimurium, another molecular-based test called multiple-locus variable-number tandem repeat analysis (MLVA) can be used (King *et al.*, 2015).

2.5 Measures to reduce Salmonellosis in poultry farms

2.5.1 Biosecurity

Biosecurity is a broad concept to deal even though in order to reduce cross-infection, growers should provide protective clothing, such as boots, coveralls, and headgear, to any visitors who work with, or have recently had contact with, poultry or a poultry farm. Contact with other animals, wild birds, and poultry should be limited because cross-contamination is high and unmanageable unless poultry housing is guarded. Rodents and pest control or reduction are also important because they act as disease vectors. Maintaining litter in a moderately dry state (i.e. 20%-30% moisture) and providing adequate ventilation will aid in the control of microorganism numbers inside the house as well as hygiene around the outside of the house (Cunningham and Fairchild, 2020). In More than 25 years documented practice of stringent biosecurity measures targeting *Salmonella* spp. of poultry at herd level in Finland, Norway, and Sweden was successful in controlling this pathogen (da Costa *et al.*, 2021).

2.5.2 Vaccination

A vaccination role involving surveillance and biosecurity in the decrease of salmonellosis cases in Europe was a successful control measure used in poultry/egg production. The vaccination concentrated on main public health concern serotypes (e.g. *Salmonella* Enteritidis and *Salmonella* Typhimurium) (Foley *et al.*, 2011; Hugas and Beloeil, 2014; Antunes *et al.*, 2016). The two groups of vaccines inactivated (bacterins) and attenuated live vaccines both are serotype specific. However, the ability of the live vaccine strain to revert to its virulent form the killed or inactivated vaccines preferred to reduce risks despite the administration drawbacks resulting in focal inflammatory myositis and decrease quality of the product (Acevedo-villanueva *et al.*, 2021).

At this point, vaccination with an attenuated *Salmonella* Typhimurium strain has proven to prevent *Salmonella* Typhimurium infection in layer chickens. In addition provides cross-protective immunity against infections with *Salmonella* Enteritidis, and *Salmonella* Gallinarum serovars (Abdul-Rahiman *et al.*, 2021). Commercially killed vaccines for *Salmonella* are POULVAC® SE and POULVAC® SE-ND-IB intended for broiler and/or layer use, via intramuscular injection, and live vaccines, POULVAC® ST and SALMOVAC® SE (Acevedo-villanueva *et al.*, 2021).

2.5.3 Antimicrobial therapeutics and Alternatives to antibiotics

Antimicrobial use (AMU) in food-producing animals has benefited by improving health, productivity, and economic returns by lowering disease incidence, morbidity, and mortality from therapeutic levels, while growth promotion and feed efficiency are considered nontherapeutic purposes (Hassan *et al.*, 2021).

In the field of animal husbandry antibiotics use for growth promotion, therapy and disease prophylaxis have long practice counts, and 80% of pharmaceutical products are utilized in food production. However, indiscriminate use of antibiotics leads to threaten animal and human health with emerging multidrug-resistant pathogens (Low *et al.*, 2021). Compel to promote alternative interventions to reduce the use of antimicrobials such as good hygiene practices, biosecurity measures, and improved vaccination are important to mitigate the public health effects. Probiotics, prebiotics, and phytobiotics are likely to be the alternatives to antibiotics as growth promoters in food-producing animals (Mshana *et al.*, 2021).

Many recommendations to reduce the burden of infections in animals are based on creating or promoting clean environments within farms and animal facilities (Pinto *et al.*, 2020).

2.6 Economic Impacts of Salmonellosis

Poultry diseases are responsible for several adverse economic and social impacts high mortality and morbidity of chickens, high medication costs, loss in production and market, and can pose risk to public health through zoonoses (Asfaw, *et al.*, 2021). It cause severe economic damage to chicken production by reducing production with 100% morbidity, and 20% mortality in affected flocks (Abdi *et al.*, 2017).

Poultry meat and its products have served as major sources of *Salmonella* spp., which have caused human and animal diseases as well as economic losses to the poultry industry (Pelyuntha *et al.*, 2021). In the US, annual economic losses due to *Salmonella* infections run from \$1.18 billion to over \$11.58 billion, based on an estimated 1.92 million cases also associated medical costs amounted to \$2.17 billion (for 1.4 million infections) in 2010 (Andino and Hanning, 2015; Gut *et al.*, 2018).

Worldwide gastroenteritis is the most common *Salmonella* infection, accounting for 93.8 million cases which result in 155,000 deaths per year. Despite improvements in hygiene and sanitation, the incidence of NTS infections which commonly associated with consumption of contaminated food of animal origin such as poultry products, beef and pork as well as contact with infected animals, continues to increase, creating a burden in both industrialized and underdeveloped countries (Egualé *et al.*, 2017; Teferi, 2020). In Ethiopia 44.7% and 12.8% of the reported cases assigned to the gastrointestinal disease with bloody diarrhea in young chickens indicating, e.g., coccidiosis, Newcastle disease, and other gastrointestinal diseases white diarrhea sticky to vent suggesting Pullorum diseases (Asfaw, *et al.*, 2021).

2.7 Public Health Impacts and Antimicrobial Resistance

Public health impact apart from economic aspect emerged serotypes in America and Europe, *Salmonella* Heidelberg, *Salmonella* Kentucky, *Salmonella*. Infantis, and *Salmonella* Stanley other than *Salmonella* Enteritidis and *Salmonella* Typhimurium raised as predominant serotypes of chicken and turkey meat with human infection consequence (Antunes *et al.*, 2016;

Wibisono *et al.*, 2020). Poultry and poultry products are considered to be main sources of human infection and healthy adult chickens generally show no clinical disease following oral infection with these serovars. The bacterium can grow in range of 5 to 46⁰C and it can survive under refrigeration with in a butter stored 23⁰C- 25⁰C more than 10 weeks. Although *Salmonella* are killed at ordinary cooking temperature of 70⁰C, the bacteria that cause human salmonellosis are characterized by fever and bacteraemia, mild watery diarrhoea, dysentery and bloody diarrhoea, focal infections (cholecystitis, hepatitis, nephritis) and typhoid fever (King *et al.*, 2015; Kadhim, 2020).

In the United States, it is estimated that 17.9 percent of all foodborne illnesses are associated with poultry, with *Salmonella* accounting for 19 percent of poultry-associated foodborne illnesses. The incidence of salmonellosis (most recent data show 15.3 cases per 100,000) remain well above the CDC's Healthy People 2030 target of 11.5 cases per 100,000 population and have not decreased significantly over the last two decades (O'Bryan *et al.*, 2022).

The persistent challenge of Salmonellosis become a public health concern due AMR which refers to the situation in which microbe-caused infections no longer respond to treatment. Drug resistance's emergence and spread poses a serious threat to modern medicine. According to World Bank research, AMR could cost low-income countries more than 5% of their GDP and push 28 million people into poverty by 2050, predominantly in the developing world (WHO, 2018).

Salmonella can develop antibiotic resistance by defending the antibiotic's target site, which can be an enzyme or a specific cell structure, drug inactivation for instance the plasmid-encoded quinolone resistance protein (Qnr), for example, confers quinol resistance by acting as a DNA homolog that competes for the binding of DNA gyrase and topoisomerase IV. Another mechanism is a decrease in membrane permeability and prevent drug entry as a result of new genetic information, which alters the membrane transport system pores and thus prevents antibiotics from passing through (Castro-Vargas *et al.*, 2020).

In intensive agriculture and food production systems, antimicrobial use (AMU), intended to maintain animal health and productivity (Mshana *et al.*, 2021). The obvious fact is that antimicrobials are used for the treatment in farms to mention in North America poultry farms use Salinomycin, tetracycline, bacitracin, tylosin, virginiamycin, and bambarmycin these and

other antimicrobials used around the globe caused multiple antibiotic-resistant *Salmonella* strains (Haque *et al.*, 2021; Pelyuntha *et al.*, 2021).

A comprehensive data of NARMS in 2013 reported non typhoidal *Salmonella* from humans were resistant to tetracycline (12.6%), streptomycin (11.5%), ampicillin (10.4%), and sulphonamides (10.3%) small number of isolates to fluoroquinolones and third-generation cephalosporins and nalidixic acid with *Salmonella*. Enteritidis representing and to ceftriaxone with *Salmonella* Typhimurium, *Salmonella* Dublin, *Salmonella* Newport and *Salmonella* Heidelberg (9 isolates) representing the most common serovars revealing the resistance pattern interpenetration characteristics (Michael and Schwarz, 2016).

Human health has been threatened by antimicrobial resistance as emergences of antibiotic-resistant *Salmonella* to first-line antibiotics. This lead to the use of fluoroquinolones (ciprofloxacin) and extended-spectrum cephalosporins thus *Salmonella* strains become resistant to fluoroquinolones and third-generation cephalosporins considered as an emerging problem worldwide. However, continued emerging resistance results in increased severity, morbidity, and mortality of diseases and the need for the use of last-line antimicrobials (e.g. carbapenems) in therapy (Antunes *et al.*, 2016 ; Asfaw *et al.*, 2021).

Globally, the speculation deaths due to AMR consequences will be more than 300 million per year, unless significant steps are taken by 2050. In developed countries like the US, *Salmonella* Enteritidis accounted for 50 % of ciprofloxacin-resistant infections, whereas *Salmonella*. Newport, *Salmonella* Typhimurium, and *Salmonella* Heidelberg were reported to be responsible for 75 % of ceftriaxone and ampicillin antibiotic-resistant infections found (Gut *et al.*, 2018). World critics have warned that the low and middle-income countries will face the worst impacts of AMR (Talukder *et al.*, 2021). A systematic review revealed in Africa Tetracycline contributed 63% of the quantity used in 2016, 11.6% in 2017, 31.7% in 2018 and 28.7% in 2020. A report in Malawi reported that 7 % of *Salmonella* Typhi infection cases were multi-drug resistant in 2010, and the figure increased in four years to 97 % (Feasey *et al.*, 2015; Mshana *et al.*, 2021).

AMR genes found across large numbers of *Salmonella* genomes accounts for 72% and 74% of produce and animal food products respectively. A study in Belachew et al showed 24% of *Salmonella* were resistant to most antimicrobials in central Ethiopia (Belachew *et al.*, 2021;

Brown *et al.*, 2021). A research in Central Ethiopia of 43 resistant isolates to one or more beta-lactam antimicrobials (ampicillin, amoxicillin + clavulanic acid, cephalothin, ceftriaxone), bla genes detected in 79% and the dominant bla gene responsible for resistance to beta-lactam antimicrobials in the majority of *Salmonella* isolates was found to be variants of blaTEM gene there is a likelihood of emergence of resistant gene in poultry sector (Egualé *et al.*, 2017).

2.7.1 Contributing factors in farm management

Antimicrobial use in food producing animals undeniably benefited in improving health, productivity and economic returns by reducing disease incidence, morbidity and mortality in therapeutic purpose. Nevertheless misuse creates a favorable niche for AMR bacteria in livestock farms, wildlife and the environment which can affect public health through food contamination or direct contact and occurrence of intolerant level of drug residues result in accumulation of toxic and harmful residues causing allergic reactions. Though the growing concern of emergence has led various authorities worldwide to implement measures to decrease antimicrobial use in livestock production (Geta and Kibret, 2021; Hassan *et al.*, 2021).

Types of resistance observed in poultry and chicken meat isolates were broader, with notable additional resistance to quinolones and third-generation cephalosporins, both of which are critical in human medicine. There was an increase in AMR prevalence and MDR among isolates from commercial abattoirs sourced from medium- and large-scale commercial farms. Tetracycline resistance was most prevalent along the meat value chain, beginning with small-scale farms and correlating with farmers frequently reporting its use (FAO, 2016). An interesting preliminary work demonstrated anaerobic digestion of tetracycline in livestock and poultry manure, thus found tetracycline persistence and stated a potential risk to emergence and persistence of antibiotic resistant bacteria in the environment when anaerobic digestion byproducts land applied for crop showing a potential risk under one health circles (Kasumba *et al.*, 2020).

Antibiotic resistance is influenced by livestock farmers' knowledge, attitudes, and behaviors regarding the use of antibiotics in animals. Previous reports, however, indicated that a significant portion of the farming community lacked adequate information about antibiotic knowledge, antibiotic use and resistance. Hence, directly or indirectly this suggested to be influencers of AMR development (Ozturk *et al.*, 2019).

Caudell et al KAP study across five countries of Africa reported significant variation across the countries and in between poultry farmers holding a better of knowledge, attitudes, and particularly practices than pastoralist households. According to their study “a bottom-up understanding of antimicrobial use at the farm-level” in low and middle income countries is important to establish the understanding across cultures and production systems to implement intervention that could bring behavioral change and reduce antimicrobial resistance globally (Caudell *et al.*, 2020).

2.7.2 Ethiopian status of AMR in Poultry

Many studies of antimicrobial resistance confirmed MDR in poultry sector of different areas of Ethiopia, alarming the emergence and existence of the threat in this sector too (Abdi *et al.*, 2017; Eguale *et al.*, 2017; Asfaw *et al.*, 2020; Dagneu *et al.*, 2020) . A study in Amhara region on animal farm owners indicated as poor awareness on AMR by (76.9%) as a most important factor with inclusion of delayed and non-effective diagnostic techniques (67%), sub-standard quality of antibiotics (64.8%) and use of antimicrobials for as growth promoter (60.4%) (Geta and Kibret, 2021). Since antimicrobial resistance (AMR) has been identified as a global public health issue, information on antimicrobial end users provides input for Antimicrobial use monitoring (AMU) and policy development to mitigate AMR risks and is thus recommended by international organizations (Gemedu *et al.*, 2020).

3. MATERIALS AND METHODS

3.1 Description of the study area

This study was conducted in Central Ethiopia Addis Ababa and Bishoftu town selected poultry farms. Addis Ababa the capital city of Ethiopia lies at an elevation of 2,355 meters (7,726 ft) and is a grassland biome, located at 9°1'48"N 38°44'24"E. The city lies at the foot of Mount Entoto and forms part of the watershed for the Awash and with a subtropical highland climate. The other study area was Bishoftu town found 45 kilometers southeast of Addis Ababa. The town is located at 9°N latitude and 4°E longitude and an altitude of 1,850 meters above sea level in the central high lands of Ethiopia. Farmers near Bishoftu town practice a mixed crop-livestock farming system and significant commercial poultry productions are available in the town.

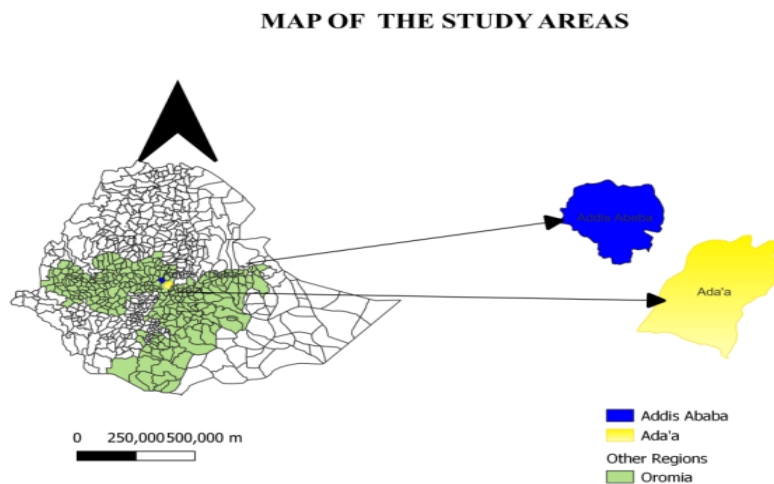


Figure 3 Study area map developed by on QGIS

In Ethiopia the commercial poultry sector has taken the lead according to ENTAG,2020 data collected about 71,000 broiler breeder stock, 87,300-layer breeder stock, and 141,700 dual-purpose parent stock which are imported (ENTAG, 2020). All the large-scale and medium-scale poultry farms depend on their parent stock to ensure sustainable production of day-old chicks (DOCs). All parent stocks are imported and popular parent stock breeds for broilers include Cobb-500, Hubbard, and Rose 308. Likewise, common layer and dual-purpose breeders in Ethiopia include Bovans Brown, ISA, Lohmann, TETRA-SL, and Sasso dual-purpose breeds. Poultry meat production is conservatively estimated to be 7,750 tons per year and annual egg

production is estimated at 1.83 billion eggs or about 73,357 tons (Mekuriaw and Harris-Coble, 2021). Large, medium, and small-scale intensive broiler and layer farms are concentrated in the central part of Ethiopia. The main commercial poultry farms in Oromia, such as Elflora, Agro Industry, Genesis, and Alema, are located around Bishoftu (Surra *et al.*, 2018; Ismael *et al.*, 2021). Many poultry productions are available in the for indicated almost half of the poultry producers (48.9%) located in Babogaya whereas 17.0% and 10.6% of them were located in Dembi and Chelekleka, respectively with Larger percentages of male poultry producers (69.2%) in Bishoftu (Ebsa *et al.*, 2019).

3.2 Study unit and population

The poultry farms found in Bishoftu town that the city municipal administration livestock and fishery development office provided lists of thirty registered commercial poultry farms. The current study included twelve commercial poultry farms purposively selected regarding voluntariness for sampling, time and resource accessibility, and owners who consent to KAP interviews. Under the two production types intensive layers and broilers poultry farming systems farms which have flocks above 5 weeks of age (the decisive advantage of age counted in weeks was to track broiler farms before stock out and to increase probability of finding poultry product producers for consumption in the study period), a flock size greater than 100, a minimum one year of experience in farming, and are producer of poultry and poultry products for consumption were selected for random sampling of chickens and environmental sample collection.

The KAP survey conducted in poultry farm owners found in Addis Ababa city and Bishoftu town with the above inclusion criterion (a flock size greater than 100, at least one year of farming experience and produce poultry and poultry products for consumers) that were accessible during the study period. One farm owner respondent per each farm interviewed on Salmonellosis case history, management practice, observational and conceptual biosecurity status with ten indicators and a KAP on AMR development after verbal or written consent. The accessible and selected farms flock size in this study ranged from 500-3,000 flock size. GPS location of visited farms collected using GPS essential android application see the map in Figure 4.

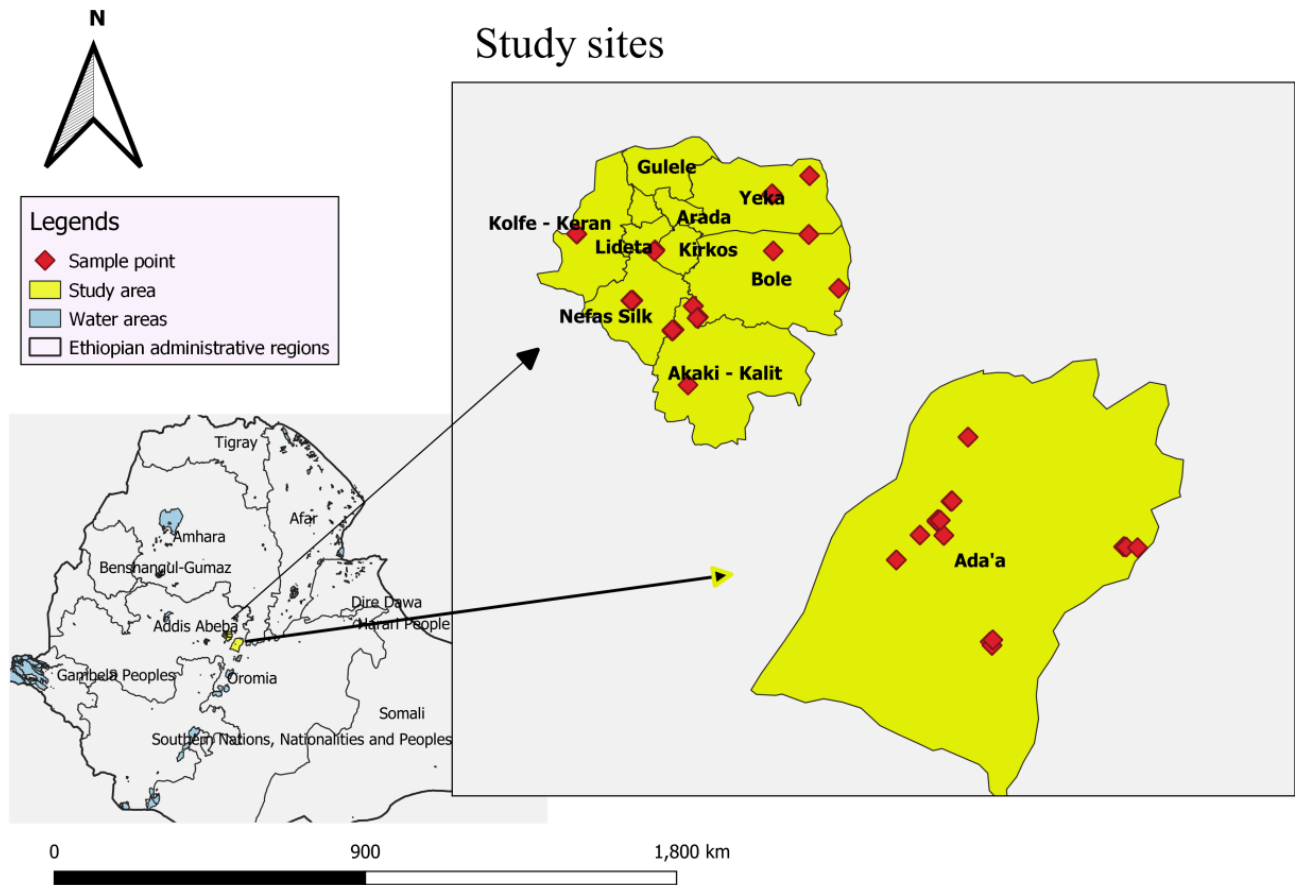


Figure 4 Study Sites location map developed on Q GIS

3.3 Study design and Sample size

A cross-sectional study conducted from December 2021 to May 2022 in purposively selected Bishoftu commercial intensive layers and intensive broiler poultry farms list of city municipal administration office to isolate, determine antimicrobial susceptibility and molecular detection of *Salmonella* species and zoonotic important spp in poultry product producer farms. And also conducted a KAP survey on poultry farm owners' found in Addis Ababa and Bishoftu town of their knowledge, attitude and practices of antimicrobial use and resistance in related to Salmonellosis management and control.

Sample size determined the formula described by (Thrusfield, 2018) with a 95% confidence interval.

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

Where n = required sample size, 1.96 = the value of Z at 95% confidence interval, p = expected prevalence of at farm level which will be (0.146) from the previous study on the prevalence of *Salmonella* (Eguale, 2018) conducted in Central Ethiopia and d = desired absolute precision, Therefore, the calculated sample size was 191 for cloaca swabs and environmental samples in Bishoftu but collected 214 samples in total.

A Questionnaires data sample size calculated based on (Arsham, 2002) $N = 0.25/SE^2$ N = sample size, SE (standard error) =5% and 100 questionnaire data calculated. Depended on the accessibility and convenience, poultry farms in Addis Ababa and Bishoftu town visitation and owner's interviews conducted. Depending on the time availability only 72 farms were reached to collect data.

3.4 Sample Collection and Sampling techniques

3.4.1 Laboratory samples

In Bishoftu town 12 poultry farms purposively allocated and selected based on accessibility in the limited time and resource with required criteria to conduct the study and randomly taken 191 cloaca swabs of chickens and 23 environmental samples from both intensive layers and intensive broiler farms. Twenty litter samples collected from each corner of the house and the middle, three drinking water samples were from drinking pot or the water sources. Sampling inclusion criteria was poultry farms produce for consumption of public, flock size greater than 100 and farm establishment minimum of one year in the two production types.

The twelve poultry farms from the two production types asked to hand over randomly selected chickens for cloaca swab samples and number of chickens per farms were dependable on full owners consent however, a minimum of 10 chicken samples were the bottom line. A total of 191 chickens and 23 environmental samples (litter, drinking water) summed to 214 samples collected.

The poultry farms biosafety protection was secured during the sample taking procedure and reached consensus with farm owners on the method. Hence, visitation of farms in between 48 hrs avoided and proper PPEs (coveralls or gowns, gloves, and shoe covers) were used when collecting the samples, to reduce stress due to strangers entry and to keep the biosecurity, handlers handed and hold the chickens to take the swabs. Also with proper guidance and

collecting gloves given, litter samples taken from corners and middle with the assistance of the handlers'. See Annex 3

Chicken cloaca swabs were collected by gentle rotation with sterile, moistened cotton swabs in 10 ml of sterile buffered peptone water and the farm handlers asked to assist though the collector will not enter the house of the chickens to keep the biosafety. A 25gm of litter samples from different corners of the house and a 50 ml drinking water sample were collected and transported with an ice box to the College of Veterinary Medicine and Agriculture Microbiology laboratory for sample processing. The number of chickens to be sampled is determined by the flock sizes and for some by the farm owners' limits. Plastic bags used for litter and drinking water samples are labeled and transported in an ice box to the laboratory. And biohazard materials were incinerated and disposed of at the disposal area found on the college of veterinary medicine and agriculture campus.

3.4.2 KAP surveys

Thirty commercial poultry farm lists registered at Bishoftu City Administration Livestock and fisheries development office were taken and 23 poultry farms selected including the twelve farms samples taken and owners who volunteer and consented to participate. Biosecurity status of the farms assessed with ten indicator checklist.

Although the poultry farm lists was not available at Addis Ababa urban agricultural development office, only the 'poultry beneficiaries' data that contain over five thousand lists categorized by woredas was provided. The list was used to estimate the probable chance of getting a high number of poultry and poultry product producer farms and selected sub-cities with the higher number of poultry beneficiaries' to contact on their perspective sub-city offices. Unfortunately, the active and not active poultry farms in the shades given by the woreda's at the time of the survey and specific locations of private poultry farms information were inadequate. A purposive and non-probability sampling technique was used in selecting poultry farms in Addis Ababa for the KAP survey. Thus a 'snow ball' sampling technique used as farmers identify other poultry farms, feed seller companies or agents used to get poultry farm owner's contact or locations of the farms. The active commercial farms in the study period with greater than 100 flock size, active poultry farms working in the shades given by the woreda's, and private farms found in Nifas silk, Akaki Kality, Bole, Lideta and Yeka sub-cities were totally 49 poultry farms and farm owner/managers included in this study.

Volunteer farm owners/managers were interviewed with consent confirmation for the survey and filled-in the data on the Epi5 collection tool for their knowledge, attitude, and practice on AMR in Salmonellosis management, and antimicrobial use questions. And hard copies of the overall biosecurity measures questions were filled in addition to the respondents' responses, the interviewee made observational judgments and cross-checks for many of the questions about the premises' hygiene status, restrictions, record keeping, changing rooms, and quarantine room availability. The total time spent on each farm was about 40 minutes to 1-hour utmost and interviews were conducted face to face by the same person.

Site visits of the observational data of restriction to visitors, farm visits in 24hrs, changing room and isolation room availability by observing the appropriate partitions of the housing in general, rodent and pest controls, history recordings, farm house and equipment cleaning, litter disposal methods and frequencies included in hard copies. KAP questions were based on Hassan et al (2021) with significant modifications to meet the objective of the study and developed in Epicollect 5 tool. For the survey farm characteristics such as production type, production system, flock size, number of handlers, Whether the farm has professional contractual or permanent, and relevant biosecurity practices were included in the questionnaire. Specific data included the demography of farm owners (gender, education level, experience in running chicken farms and market supply chains and characteristics of the farms (premises, farm capacity, and farm type), and GPS locations on GPS essentials android application.

Questions included knowledge on salmonellosis its zoonotic importance and AMR effects on public health due to residues, AMR strains, indiscriminate antimicrobial use practices on the farm, drug administration, with prescriptions or without, keeping withdrawal periods, and attitude questions on reducing AMR effects in public health questions prepared. Questionnaires had minor adjustments after a few interviews of owners which showed overlapping antimicrobials as any drugs or prophylaxis preparations, to avoid confusion of antimicrobials with vitamins or minerals and vaccines respondents asked first to rule out commonly used 'drugs' including vaccines minerals and antimicrobial treatments along with the observational data. Then referred back to the mentioned antimicrobials to distinguish what AMR resistance, antimicrobial residues, and treatment failure experiences were asked. A cumulative of 51 questions was developed for the interview.

3.5 Sample Processing and Survey Methodologies

3.5.1 Bacteriological Isolation and Identification

The samples collected aseptically from the poultry farms transferred to the college of veterinary medicine and agriculture microbiology laboratory processed for isolation through bacteriological isolation and molecular detection at National Veterinary Institute molecular biology laboratory. Antimicrobial sensitivity test was done in microbiology laboratory of the college from the molecular detected isolates

3.5.1.1 Non- Selective and Selective enrichments of samples

A pre enriched BPW samples of 10ml cloaca swabs, 225 ml of litter and 50ml of water samples incubated at 37⁰C for 24hr. Then 100μL mixtures was transferred to 10ml Rappaport – Vassiliadis (HIMEDIA, India) medium and incubated at 41-42⁰C for 18-24hr, for better recovery of *Salmonella* and inhibition of other Enterobateriace. All the enriched samples inoculated to selective media to be investigated according to (ISO-6579, 2017).

3.5.1.2 Isolation of *Salmonella* species

A loop full of broth samples cultured on to selective media Xylose Lysine Deoxycholate /XLD agar (Alpha Chemika, India) and Brillinat Green (BGA) agar (HIMEDIA, India) and incubated at 37⁰c for 24-48h. XLD media prepared and dispensed to petri dishes after heating to dissolve without autoclaving and BGA autoclaved at 121⁰C for 15 minutes and dispensed to petri dish. Plates was observed for colony morphology and characterization pinkish colony with black and without black centered on XLD and pinkish mucoid colonies from BGA was taken as suspected colonies for *Salmonella* spp. and inoculated to Nutrient agar to be stored at 4⁰Cfor further identification and characterization.

3.5.1.3 Biochemical test identification

Biochemical identification of isolates were performed using Triple sugar iron agar (TSI) to identify three sugar fermentation Sucrose, glucose and lactose fermentation ability of the colonies. *Salmonella* species mostly are non-lactose fermenters and the slant of the TSI produced alkaline/red slant with acidic/yellow but with or without H₂S production and gas there are strains that ferment lactose and appeared on the slant acidic/acidic with H₂S or gas production taken for further biochemical confirmation. Simmon Citrate agar (HIMEDIA, India)

to see citrate utilization of the bacteria commonly utilized by *Salmonella* colonies and color changed from green to blue slant after 37⁰C incubation of 24-48h with exceptions of *Salmonella*.Typhi. The decomposing ability of the amino acid tryptophane and indole production detected with KOVAC's reagent a yellow or yellow brownish ring formed as *Salmonella* spp. are indole test negative. In the methyl red test (MR test) used to detect if the bacteria has the ability to utilize glucose with production of a stable acid a colour change indicates positive reaction *Salmonella* spp. are positive and negative for VP test we used Methyl Red Voges Prosakuer Broth(MR-VP) (BioMarks Laboratories, India).

Many enterobacteriace organisms' differentiation using IMVic test is a standard, additionally the following tests were done in this study. Christenes urease agar used to observe hydrolysis of urea by the bacteria and ammonia and CO₂ production in Urease slant. The ammonia alkalizes the medium and the pH shift indicator phenol red showed a color change to pink seen on Annex 3 (ISO-6579, 2017).

The ability of some bacteria to cleave L-lysine to cadaverine under the liberation of carbon dioxide observed on Lysine decarboxylation broth inoculation. The colonies inoculated in broth and topped with paraffin oil. After incubation of 48h at 37⁰C a positive result of purple or unchanged color taken. Observation before 48h may give misinterpretation with the pale discoloration as decomposition of glucose in the first few hours occurs to decrease the PH of medium, in order to decarboxylation start. The test could differentiate commonly *Salmonella* spp from *Shigella* spp. A motility test to differentiate zoonotic and non-zoonotic species done using SIM Medium (TM MEDIA, India) that motile bacteria 'swarm' and give a diffuse spreading growth in semi-solid agar media confirmed by observing the diffusion (ISO-6579, 2017).

3.5.2 Molecular detection of Isolates

Strongly suspected positive isolates was sub cultured on nutrient agar and incubated at 37⁰C overnight and inoculated into 5 ml Tryptone soya broth (TPC, India) with 37⁰C of 18-24h incubation time. The broth culture was preserved with 40% glycerol in 1.8ml cryovial tubes and taken to National Veterinary Institute molecular biology laboratory for further detection.

3.5.2.1 DNA Extraction

The DNA extraction was using QIAGENTM Mini columns with final 50µL volume output to increase DNA concentration. A 20 µL of QIAGEN protease and 200 µL of AL lysis buffer added to 200 µL sample mixed by vortex 15 sec or short centrifuged. Incubation of the mixture with 56⁰C for 20 minutes supports the lysis of membranes and to get a maximum DNA yield. The lysate centrifuged briefly and 200 µL 97% ethanol added to precipitate the colony and transferred to QIAmp spin column. A white precipitate was shown in some collection tubes. The spin column placed in a clean 2ml collection tube and 500 µL AW1 buffer and AW2 washing buffers added with 1 min and 3 min centrifugation for each washing buffers respectively. This can clear all the debris except the DNA nucleic acids. The final Elution buffer with a volume of 50 µL incubated at room temperature for 5 minutes and transferred to PCR room.

3.5.2.2 Master mix preparation

The master mix kit used (Thermo scientific, Germany) to prepare 23.5µL mixture for one reaction by Rnase free water 3 µL, 10XDream TaqTM buffer with Mgcl₂, 5 µL dNTP mix 2Mm, Dream Taq TM DNA polymerase, Forward and reverse universal primers (Primer-salm-Fow-5pm/µl 5'-ACTGGCGTTATCCCTTCTCTGGTG- 3' and Primer-Salm-Rev-5pm/µl 5'-ATGTTGTCCTGCCCTGGTAAGAGA- 3') 2 µl each added to detect Histidine transport operon (*hto*) gene which is found in most *Salmonella* spp. (Cohen *et al.*, 1993;) (Rychlik and Rhoads, 1989) with software program developed and 5 µL extracted DNA templates vortexed and centrifuged. Also specific primers for *Salmonella* Typhimurim (Primer –SPY-Fow-5pm/ µl 5' TTGTTCACTTTTTACCCCTGAA 3' and Primer –SPY-Rev-5pm/ µl 5' CCCTGACAGCCGTTAGATATT 3') added for molecular typing (Can et al., 2014).

3.5.2.3 DNA Amplification

The extracted DNA with master mix amplified in 2720 Thermal Cycler (*Applied bio system*) at 94⁰C initial and second denaturation for 5 min, 30sec respectively. A 30 cycle amplification with Annealing temperature of 56⁰C for 30sec and 72⁰C elongation for 45sec. Final elongation for 5min with 72⁰C. For *Spy* gene detection in *Salmonella* Typhimurium at 94⁰C initial and second denaturation for 5 min and 1 min respectively, annealing temperature of 55⁰C 1 min following elongation at 72⁰C for 1.30 min with 35 cycles final elongation for 10 min at 72⁰C.

3.5.2.4 Gel electrophoresis

The amplified PCR product electrophoresed by (Bio-Rad Sub-cell GT, California, USA) with 2% agarose gel (CSL-AG500,) and loading dye of prepared poured on a plate with combs and after 20-30 minutes cooling the comb removed carefully to get perfectly lined wells. Then 1X TAE (SIGMA-ALDRICH, India) loading buffer used in the electrophoresis bath. A 4µl Bromoside or Bromosafe(Mol Bio product, MBT 130-200LN, HIMEDIA, India) and 10 µl PCR product mixed to be loaded into the wells carefully with two lanes loaded by marker Ladder (Mol Bio product, MBT 130-200LN, HIMEDIA, India). Amplified products by electrophoresis performed on 120V for about 50 minute. A positive isolate of *Salmonella* detected and read at 496bp segment, *Salmonella* Typhimurium at 401bp on the gel using a UV transilluminator.

3.5.3 Antimicrobial sensitivity test

Depending on resource and time availability from PCR confirmed isolates 10 samples tested for antimicrobial susceptibility according to (CLSI, 2020) by using the standard Kirby-Bauer disk diffusion method on Muller Hinton agar medium (ACCUMIX, India). Each isolates four to five colonies grown on nutrient agar transferred with a sterile loop into tubes containing autoclaved 5 ml broth of Tryptone soya. The broth culture incubated at 35°C for 4 hr to achieve the 0.5 McFarland turbidity standards. With a sterile cotton swab the bacteria swabbed uniformly over the surface of Muller Hinton agar plate and allowed to dry at room temperature for 3 minutes.

Antibiotic discs (Tetracycline (TE) (30µg), Ampicillin (AMP) (10µg), Chloramphenicol (C) (30µg), Ciprofloxacin (CIP) (5µg), Nalidixic acid (NA) (30µg), Azithromycin(AZM)(15 µg), Ceftizadime (Caz) (30 µg), Ceftaxime (CTX) (30µg), Colistin (CT) (10 µg) and Sulphamethoxazole-trimethoprim (SXT) (1.25/23.75µg), OXOID, England) placed on the plates and incubated for 24 h at 37°C. The diameters of inhibition zones measured with a caliper (Lacy et al., 2004). All ten zone of inhibition against ten antimicrobial agents for each isolate were recorded and compared with standards and interpreted as resistant, intermediate, or susceptible according to published interpretive chart (CLSI, 2020).

Table 1 Antimicrobial susceptibility test reference of *Salmonella*

Antimicrobial disc	Zone diameter break points nearest mm				
	Disc content	S	SDD	I	R
AMP 10/Ampicilin	10µg	≥17	14-16	16	≤13
Caz30/Ceftizadime	30 µg	≥21		18-20	<17
TE30/Tetracycline	30µg	≥15		12-14	≤11
AZM15/ Azythromycin	15 µg	≥13			≤12
Cip5/Ciproflaxicin	5 µg	≥26		22-25	≤21
C30/chloramphenicole	30 µg	≥18		13-17	≤12
NA30/ Nalidix acid	30 µg	≥19		14-18	≤13
CTX30/ceftaxime	30 µg	≥26		23-25	≤22
SXT25/Sulphamethaxinone	25 µg	≥17		13-16	≤12
CT10/ Colistin	10 µg	≥21			≤16*

Source: (CLSI, 2020)

*Colistin sulphate agent zone of inhibition break points were in CLSI 2003

3.6 Data Analysis and Management

Data of laboratory results of the collected samples were entered in Microsoft - Excel 7 and analyzed using R software program. Descriptive statistics such as percentage and frequency distribution was used to describe the nature and the characteristics of the data. Bacterial isolates and antimicrobial susceptibility were expressed as percent of resistant, intermediate and susceptible using positive isolates from total examined. In addition, the proportion of bacteria resistant to at least one of the ten antibiotics and resistant to two or more was calculated.

Moreover, comparisons between each risk factor were analyzed using χ^2 test, then by univariable logistic regression test. Univariable and multivariable logistic regressions analysis was performed to reveal the strength of association of the potential risk factors with positivity of sample using odds ratio (OR). The probability of type one error was set at 5% with 95% confidence level to establish biological and statistical association between dependent and independent variables. The KAP data analyzed as descriptive using Microsoft excel and R software used for Chi square and pearson correlation tests to associate the knowledge, attitude and practices scores of the farm owners with demographic characteristics in contributing to

AMR development. The scoring was done by two ranges (0 and 1) then categorized based on knowledge 'correct' and 'incorrect' towards AMR knowledge, Attitudes of owners on AMR effects on public health 'favorable' and 'unfavorable' and there practices 'Good' or 'Bad' as protective practices and contributing towards AMR development respectively.

3.7 Ethical Approval

Permission on ethical approval was obtained from the College of Veterinary Medicine and Agriculture of Addis Ababa University (Ref. VM/ERC/26/02/14/2022). A consent declaration obtained from all respondents interviewed for knowledge, attitude and practice on AMR survey in Bishoftu and Addis Ababa.

4. RESULTS

4.1 Descriptive data of Bishoftu poultry farms

The volunteered poultry farms selected in Bishoftu town were six intensive layer farms and six broilers (n=12) with greater than one year farming experience or establishment year and were producers of poultry and poultry products for consumers in different markets like Addis Ababa, Hawassa and Adama. The poultry farms sampled at Bishoftu town were 52% layers and 48% broiler chicken farms equally distributed scale according to the above classification. Degree and above qualification was the dominant one with 75% figure.

Table 2: Farm descriptions and demographics of sample collected.

Descriptions	Category	N% (n= 12)
Flock size	Small scale	50%
	Large scale	50%
Breed /Production type	Intensive Layers	48%
	Intensive Broilers	52%
Managers education level	Degree/above	76%
	Diploma/TVET	11%
	High school	8%
	Elementary	4%

*Small scale= Layers 100-500, Broilers 200-1000

*Large scale= Layers ≥ 500 , Broilers ≥ 1000 (Mekuriaw and Harris-Coble, 2021)

All the farms use a deep litter system, litter disposal claimed to be sold out for livestock farms and restrict new visitors from entering the chicken house observed and cross checked on site visits. Also observational checked for changing room compartments and isolation rooms along with foot wear or bath used at the entry. The overall biosecurity status is relatively good in restrictions of new visitors and visits of other farms in 24 hrs.

Table 3: Over all biosecurity indicators of the (n= 12) poultryfarms

Categories		N% (n= 12farms)
Restrictions	Restrictions new visitors	100%
	Restriction visits between farms 24h	75%
	Restrict Vehicle entry to the farm	42%
Structures	Use foot wear / bathing at entrance	58%
	Changing room	42%
	own quarantine room compartmented	33%
House conditions	Deep litter /flooring system	100%
	House cleaning/litter disposal	92%
		(batch out)
Operational practices	Litter disposal	100%
	(Sold out)	
	Rodent/pest control management (Good)	8.3%
	Record keeping	75%

4.1.1 Isolation and identification

In this study *Salmonella* was isolated from 42 (19.6%, 95% CI, 0.14_0.25) of the 214 samples tested and 19.2% farm level prevalence. In layers and broiler chickens *Salmonella* was found equally 21 in layers (9.8%, 95% CI, 0.12_0.28) and broilers (9.8%, 95% CI, 0.12_0.26). The non typhoidal strain *Salmonella* Typhimurium isolated and detected molecularly in 16 (7.4%, 95% CI, 0.04_0.11) of the 214 samples tested. In between breed type distribution (8%, 95% CI, 0.03_0.14) were taken from broilers and (6%, 95% CI, 0.02_0.11) were from layers. Distribution within the positive isolate by sample type was 17.7% *Salmonella* isolated from cloaca swab samples and 1.8% from Litter and drinking water samples i.e 90% of the positive samples was from cloaca swabs.

At the farm level prevalence of each twelve farms of samples taken presented in Figure 7 and demographic farm descriptions in table 2. The biosecurity indicators scored and greater than 70% classed as good and poor less than scores for biosecurity status. Seventy percent of farm owners were educated and of which three farm owners were veterinarians. All the farms were for commercial purpose except the dual purpose one, although poultry and poultry products provided for consumers.

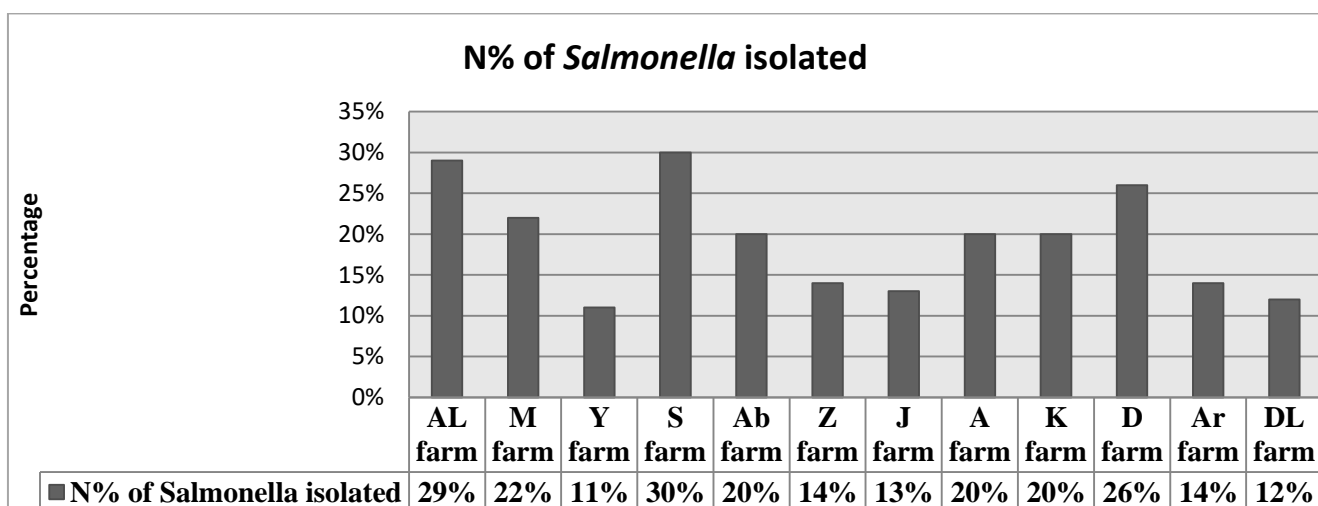


Figure 5 Farm level isolates of *Salmonella* spp

4.1.2 Molecular detection of Isolates

Further of isolation characterization biochemically 30 samples were molecularly detected (*hto*) gene and confirmed 29 (13%) *Salmonella*, 16(53.3%) of *Salmonella* Typhimurium using (*SPY*) gene and at farm level at least one isolate were detected in all the farms and from 8(66.6%) farms *Salmonella* Typhimurium molecularly detected. Number of isolates in layers and broilers on *Salmonella* isolates found to be 18% for both with difference of frequency on *Salmonella* Typhimurium 8% and 6% respectively. PCR molecular detection taken isolates to NVI molecular biology laboratory and confirmed by a protocol reference of Cohen et al 1993 used to detect the Histidine transport operon gene (*hto*) by Salm-F-5'ACTGGCGTTATCCCTTCTCTGGTG-3' and Salm-R-5'-ATGTTGTCCTGCCCTGGTAAGAGA- 3' at 496bp segment shown on Figure 6. The protocol used in this study was as per manufacturers (QIAGENTM Mini columns) instructions with 30 cycles and 56⁰C annealing temperature as described on the methodology. The results were 29 for *Salmonella* positive that was 96.6% of accuracy.

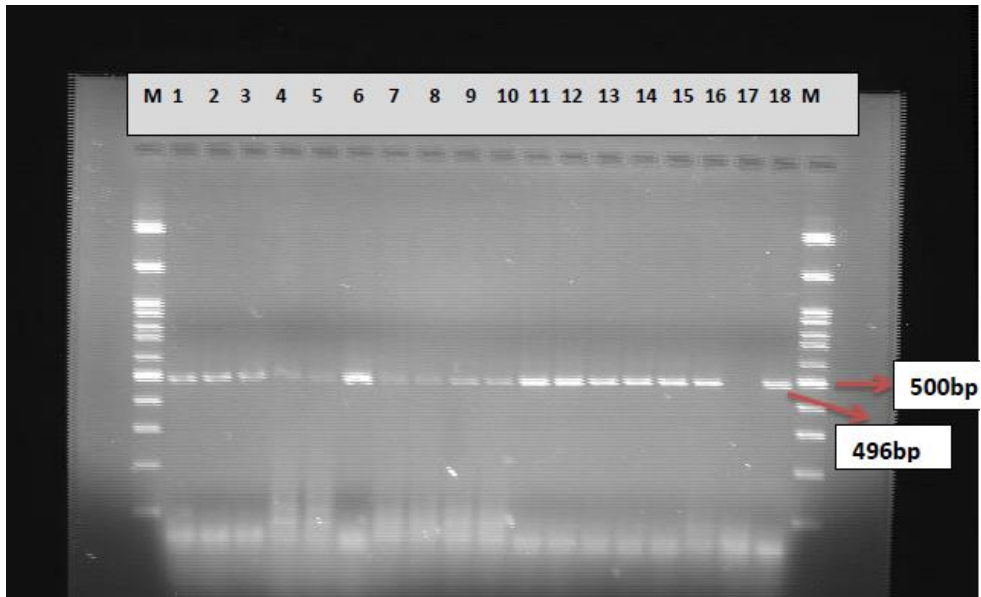


Figure 6: PFGE finger print result of *Salmonella* isolates.

The M lanes both sides DNA ladders/ marker, Lanes (1-16) samples, lane 17 Negative control and lane 18 a positive control of *Salmonella* (detected at 496bp segment).

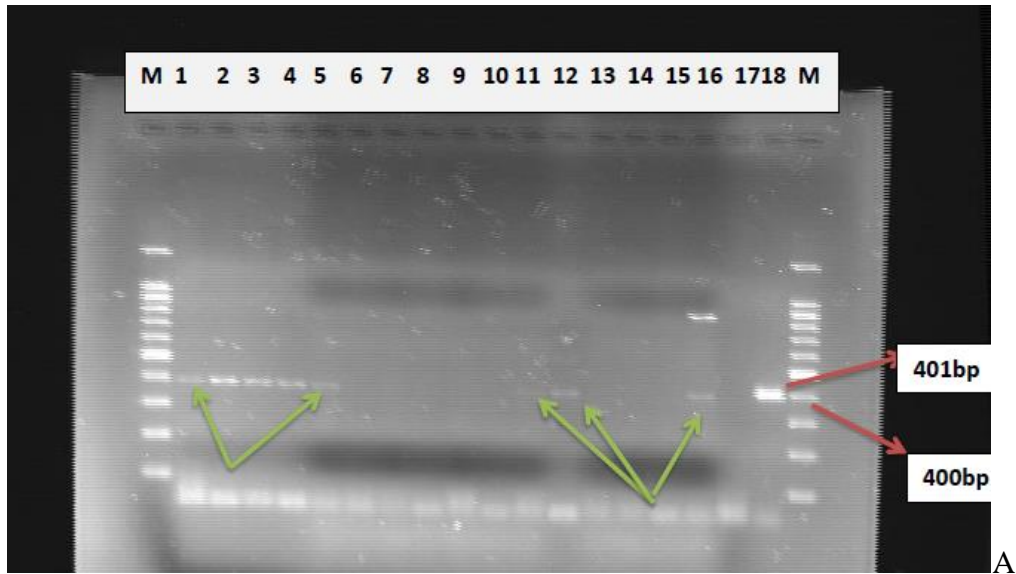


Figure 7: PFGE finger prints results of *Salmonella* Typhimurium.

- a) The M lanes both sides DNA ladders/ marker Lanes (1,3,6,7,9,10,12,13) positive samples for *Salmonella* Typhimurium, lane 15 Negative control and lane 16 a positive control at 401bp segment
- b) The M lanes both sides DNA ladders/ marker Lanes (1-5,10,11,16) positive samples for *Salmonella* Typhimurium, lane 17 Negative control and lane 18 a positive control at 401bp segment

Salmonella Typhimurium detected outputs were not clearly revealed on the Gel electrophoresis finger prints like the universal primer used for *Salmonella*. A non-specific band shown on the 13th lane of finger print indicating there was mutation change of various sero types or mixed colony of the sample. Fainted bands were seen on (a) *Salmonella* Typhimurium (annealing

temp of 55⁰C) because of the low concentrated colony counts in the samples. Optimized trials of the procedure with 57⁰C annealing temperature failed to give a perfect output result which had double bands annexed on **Annex3**.

4.1.3 Antimicrobial sensitivity test results

Antimicrobial susceptibility test results of nine isolates using ten antimicrobial discs interestingly all the isolates tested were resistant to one or two drugs and more than five drugs was the sky limit per the drugs tested on the samples. Azithromycin AZM15 shown the highest susceptibility rate with 89% and second chloramphenicol C30 with 44% rate that isolates were susceptible. The most resistant drug stating the obvious was Tetracycline TE30 and Ampicillin AMP10 with 100% rate followed by 67% rate CTX30. The current study found Cip5 and Nalidixic acid NA30 resistance rate in the isolates tested to be 100% like the prior ones.

The nine isolates from molecularly detected by PCR were tested for antimicrobial sensitivity test four were from *Salmonella* Typhimurium detected isolates and one litter sample positive for *Salmonella*. The finding was no less than five antimicrobial resistance found in each isolates tested. All the nine isolates were indicating multidrug resistance MDR in this study. The antimicrobial agents that were susceptible, intermediate and resistance to each isolates shown in table 4.

Table 4: Antimicrobial sensitivity test results

	Isolates n=8 (Cloaca swabs) n=1 (Litter sample) n(%)		
Antimicrobial discs	Susceptible	Intermediate	Resistance
Azithromycin	7(78%)	-	2(22%)
Chloramphenicol	4(44%)	3(33%)	2(22%)
Sulphamethaxinone	1(11%)	1(11%)	7(78%)
Ceftizadime	-	4(44%)	5(56%)
Ceftaxime	-	2(22%)	7(78%)
Tetracycline	-	-	9(100%)
Ampicilin	-	-	9(100%)
Colistin	-	-	9(100%)
Nalidix acid	-	-	9(100%)
Ciproflaxicin	-	-	9(100%)

4.1.4 Analysis results of risk factors and *Salmonella* isolates

The positive isolates of each farms taken was described and associated risk factors for the findings of *Salmonella* isolates analyzed and univariate and multiple logistic regressions as a final model developed on R studio software. Possible risk factors sorted were age, production type, flock size; biosecurity status classed 'good or bad' based on the ten indicators used in the study and owners/ managers education level with regard to number of *Salmonella* and *Salmonella* Typhimurium positive isolates.

Table 5: Association of *Salmonella* positives with demographic and farm characteristics

Variables	Category	(n) of samples=214 N% positives	X ²	P-value
Age in week	≤5weeks	(28)14.2%	5.89	0.659
	>5 weeks	(186)20.4%		
Breed type	Broiler	(111)18.9%	0.00964	0.92
	Layer	(103) 20.3%		
Flock size	≤500	(25) 12%	3.2748	0.85
	>500	(189) 20.6%		
Sample type	Cloaca swabs	(191) 20%	0..00	0.99
	Environmental(litter and drinking water samples)	(23) 17%		
Manager education level	Degree/above	(162) 19.1%	2.23	0.524
	Diploma/TVET	(24) 29.1%		
	High school	(18) 11.1%		
	Elementary	(10)20%		
Biosecurity status	Good	(116) 23.2%	1.663	0.197
	Poor	(98)15.3%		

None of the variables has a significant association with *Salmonella* isolates and a logistic regression implemented to further investigate the correlations of dummy variables with positive results and *Salmonella* Typhimurium isolates in the tables below.

Table 6: Univariable logistic regression of positive isolates of *Salmonella* and *Salmonella* Typhimurium in the poultry farms

Variables	Categories	No. <i>Salmonella</i> (n=42)and <i>Salmonella</i> Typhimurium (n=16) samples	Frequency % (n=214) total sampled	OR (CI 95%)	P value
Age_week	5weeks-33wks range	<i>Salmonella</i> (42)	19.6%	1.03(1.02_1.06)	0.049*
		<i>Salmonella</i> Typhimurium (16)	7.4%	1.02(0.97_1.07)	0.384
Breed type	Broilers			Ref	
	Layers	<i>Salmonella</i> (21) <i>Salmonella</i> Typhimurium (7)	9.8% 3.27%	1.09(0.55_2.16) 1.42(0.02_0.13)	0.787 0.501
Flock size	<500			Ref	
	>500	<i>Salmonella</i> (38) <i>Salmonella</i> Typhimurium(16)	17.7% 7.4%	1.00(0.99_1.00) 0.99(0.99_1.00)	0.362 0.7877
Max time Litter kept undispose d.	Over 10 month			Ref	
	2 months	<i>Salmonella</i> (21) <i>Salmonella</i> Typhimurium (7)	9.8% 3.2%	0.72(0.36_1.42) 0.57(0.19_1.59)	0.341 0.288
Rodent/pe st control managem ent	Good			Ref	
	None	<i>Salmonella</i> (34) <i>Salmonella</i> Typhimurium(16)	15.8% 7.4%	0.62(0.26_1.59) 0.00(0.0_0.0)	0.2983 0.989
Managers education level	Elementary			Ref	
	Degree/above	<i>Salmonella</i> (31)	14.4%	0.94(0.22_6.47)	0.946
		<i>Salmonella</i> Typhimurium (9)	4.2%	0.52(0.08_10.3)	0.566
	High school	<i>Salmonella</i> (2)	0.93%	0.5(0.05_4.79)	0.524
<i>Salmonella</i> Typhimurium (1)		0.46%	0.52(0.01_14.4)	0.665	
Diploma/TVE T	<i>Salmonella</i> (7)	3.27%	1.6(0.30_12.75)	0.583	
	<i>Salmonella</i> Typhimurium (5)	2.3%	2.3(0.31_48.9)	0.460	
Biosecurit y status	Good ^a			Ref	
	Poor	<i>Salmonella</i> (17) <i>Salmonella</i> Typhimurium (2)	6.54% 0.93%	0.6(0.30_1.19) 0.34(0.10_0.97)	0.151 0.0142*

^a scored >70% 'Good'

In the univariable logistic regression significant variables were age > 5 weeks of age had higher *Salmonella* contamination than lesser aged (OR = 1.02, 95% CI = 1.02_1.06. P = 0.049). And biosecurity status with in *Salmonella* Typhimurium isolation less in good biosecurity status

farms (OR=0.34, 95% CI =0.10_0.97, P= 0.014). Maximum time litter kept in the farm, flock size, breed type, and rodent/pest control management variables were P>0.05 in both dependent variables. However, *Salmonella* positive frequency of farms with none permanent rodent control is higher than have permanent control management 34(15.8%) and also 16(100%) *Salmonella* Typhimurium isolates were from farms under category of none permanent Rodent/pest control. The highest isolation rate recorded in management education level Degree/above category was Diploma/TVET proportionally despite 31(74%) of the total positive samples were from under degree/ above category as shown in **Table 4**.

All the variables run in univariate logistic regression analysis combined in a multivariable logistic regression model on R studio to develop the final model for final analysis. Using step wise model simplification of R studio the model simplified by the significant and marginal significant variables identified from univariable logistic regression. The simplified final model compared with lmrtest and chisquare p value was insignificant implying reduction of the dummy variables hardly affect the significance value. Goodness fit of the model tested by ROCR command on R and auc value was greater than 0.5.

Table 7: Multivariable logistic regression of *Salmonella* positive isolates

Variables	No of sampled	N(%)positives	OR (95% CI)	P-Value
Breed type				
Broilers			Ref	
Layers	<i>Salmonella</i> 103	21(20.3%)	0.30(0.10-0.89)	0.0306*
Age in weeks				
<5weeks			Ref	
>5weeks	<i>Salmonella</i> 186	21(20.4%)	1.07(1.024- 1.133)	0.003 **

In the output *Salmonella* Age and production types were significantly associated with presence of *Salmonella* isolates in the farms studied. In layers less likely to get *Salmonella* positive by (OR 0.30 95%CI=0.105_0.904, P= 0.036) than intensive broiler farms sampled. Increasing Ages of chickens likely increase *Salmonella* positivity by (OR 1.07, 95%CI=1.02-1.13, P= 0.003) than younger chickens in this study.

Table 8: Multivariable logistic regression of *Salmonella* Typhimurium positive isolates with associated variables

Variables		No of sampled	N(%) positives	OR(95%CI)	P-value
Management education					
Level					
Elementary				Ref	
Degree/Above	<i>Salmonella</i>	162	9(5.5%)	0.81(0.12_16.29)	0.860
Diploma/TVET	Typhimurium	24	5(20.8%)	10.2(0.65_391.8)	0.141
High school		18	1(5.5%)	0.99(0.03_30.13)	0.998
Flock size					
500				Ref	
500-2600	<i>Salmonella</i>	186	15(8.06%)	0.99(0.99_1.00)	0.169
	Typhimurium				

Unlike *Salmonella* isolates *Salmonella* Typhimurium were not significant with management education level and flock size. The farm under management of Degree/above and High school education level were low likely (OR= 0.81 and OR= 0.99 respectively) positive to *Salmonella* Typhimurium than elementary level managed farms. Despite this Diploma /TVET has inverse likely hood by OR= 10.2 than elementary group. Again the highest isolation rate of *Salmonella* Typhimurium was under this category.

Taking as the one isolate found multidrug resistance as a farm positive indicator, nine farms three fourth of the farms were positive for MDR. Arguably the knowledge score on AMR of the farms tested for Salmonellosis were 81%, Attitude and practice 46% on KAP results. Comparatively the practice and attitude scores were less than the knowledge scores.

4.2 Descriptive data of Farms and Farm owners

In this study (n=72) farms visited and 72 farm owners interviewed with semi structured KAP questionnaire on AMR development and effects on human health their contributing factors assessed to associate the risk factors in poultry farm management. Forty nine farms in Addis Ababa and twenty three in Bishoftu town were visited and one owner/manager interviewed per each farm. For every farm owners interviewed in Bishoftu and Addis Ababa which provide poultry and poultry products to consumers interviewed after their verbal or written consent to participate in the research. The respondents asked their position or role in the farm for people who were not available at the time of interview interviewed through phone calls in assistance of

available handler found. The 'manager' refers for poultry farms that obtain associate partners and one of the members delegated as a manager. Demographic characteristics of owners shown in **Table 8** 70% of owner's education level were greater than high school which presumptively considered able to read and write. Male respondents were high proportion regardless of the gender involved in the business. Hence associate partners might include females but the manager or respondent found at the time of interview was recorded.

Poultry farms found in Addis Ababa were mostly concentrated in extension sub cities see **Figure 4** and in specific areas where the woreda given as job opportunity for the community organized as associations and after taken competence exam of the trainings given for basic poultry farming skills and principles by the program. Litter disposal management was in wide variation some dispose on nearby river, sewerage and public garbage. The survey included demographic data of respondent's age level of education and their experience in poultry farming also for interviewee's volunteered phone numbers were collected. Farming experience ranges in 1-3years proportionally higher and respondents aged from 20-30 year were 27.7% and 30-35 year were 26.3% respondents out of the total seventy two. It is detailed in Table 9.

Table 9: Demographic data of respondents

Variables	Categories	N% (n=72)
Age of farm owners	20-30	20(27.7%)
	30-35	19(26.3%)
	35-40	15(20.8%)
	40-50	11(15.2%)
	>50 more	7(9.7%)
Gender of farm owners	Male	50(69.4%)
	Female	22(30.5%)
Experience in farming(years)	1-3years	48(66.6%)
	4-7years	14(19.4%)
	7-10years	6(8.3%)
	>10years	4(5.5%)
Level of Education	Degree/above	21(29.1%)
	Diploma/TVET	5(6.9%)
	High school	25(34.7%)
	Elementary	3(4.1%)

As one respondent owner/mangers interviewed per each farm for the KAP survey interviewees were in Addis Ababa 68% and less compared to Bishoftu farms. Layers, deep litter system and large scale categories has the greater numbers comparatively. Male respondent proportion was two times high than female respondents. The biosecurity score falls to 47.2% “Good” biosecurity status based on the evaluations conducted in this study.

Table 10: Farm description of KAP surveyed

Descriptions	Categories	N% (n=72)
Production/breed type	Broiler	9(12.5%)
	Layer	62(86.1%)
Production system	Deep litter	68(94.4%)
	Cage system	4(5.5%)
Farm size	Small scale	19(26.3%)
	Large scale	53(73.6%)
Address	Addis Ababa	49(68%)
	Bishoftu	23(32%)
Have animal professional	Permanently	14(19%)
	Contractual	32(44%)
	None	26(36%)
Biosecurity status	Good	34(47.2%)
	Bad	38(52.7%)

*Small scale= Layers 100-500, Broilers 200-1000

*Large scale= Layers \geq 500, Broilers \geq 1000 (Mekuriaw and Harris-Coble, 2021)

4.2.1 Biosecurity measures assessments

Over all biosecurity data with site visits interviewed to understand the practices and actual experiences used by the farmers to prevent and reduce poultry disease infections. Biosecurity indicators used in this study were some structural and operational biosecurity restriction to new visitors, vehicles in the farm, avoid farm visits between 24 hours, foot wear or foot bath used on entry for strangers and handlers asked also observed if it was available at the time of visitation. House compartments included changing room and quarantine or isolation room for sick birds separately or far enough to the actual farm house also observed and recorded. Rodent or pest control mechanisms record keeping and documentation of diseased, recovered, mortality, feeding records asked if available to support the biosecurity measures practiced in each poultry farm.

Table 11: Biosecurity evaluations of farms visited

Biosecurity indicators	Yes (%) =1	No (%) =0	Grading system >70%=Good <70%=Poor
Restriction of new visitors to the farm	72(100%)	0(0%)	
Restriction of other farm visits between 24hr	66(91.6%)	6(8.3%)	
Restrict vehicle entry to the farm	51(70.8%)	21(29.1%)	Good
Foot wear/bathing and clothes used in the house	63(87.5%)	9(12.5%)	=34(47.2%)
Quarantine isolation room if compartmented	34(47.2%)	38(52.7%)	
Regular cleaning house /observed cleaning condition	67(93%)	5(7%)	Poor=38(52.7%)
Farm equipment regular cleaning	72(100%)	0(0%)	
Permanent Rodent and pests/ wild birds control	28(38.8%)	44(61.1%)	
Cleaning and sanitation condition (judgemental)	68(94.4%)	4(5.5%)	
Documentation and record keeping of sick, mortality and feed information	42(58.3%)	20(27.7%)	

Restriction to visitors in all the farms observed at the time of interview the practice well trained among the poultry farms visited in this study. The lowest number found on rodent and pest control management which 61% of the respondents answered the lack of controlling rodents out the farm house. In this scoring respondents answered “use pets as a controlling management” also given =0 pets not allowed in the actual farm house in biosecurity measures. The closeness of the farm houses in some places made it vulnerable to rodent infestation whether they control in their own farm house or not. Commonly experienced disease in the farm and drugs were collected presented in the figures below.

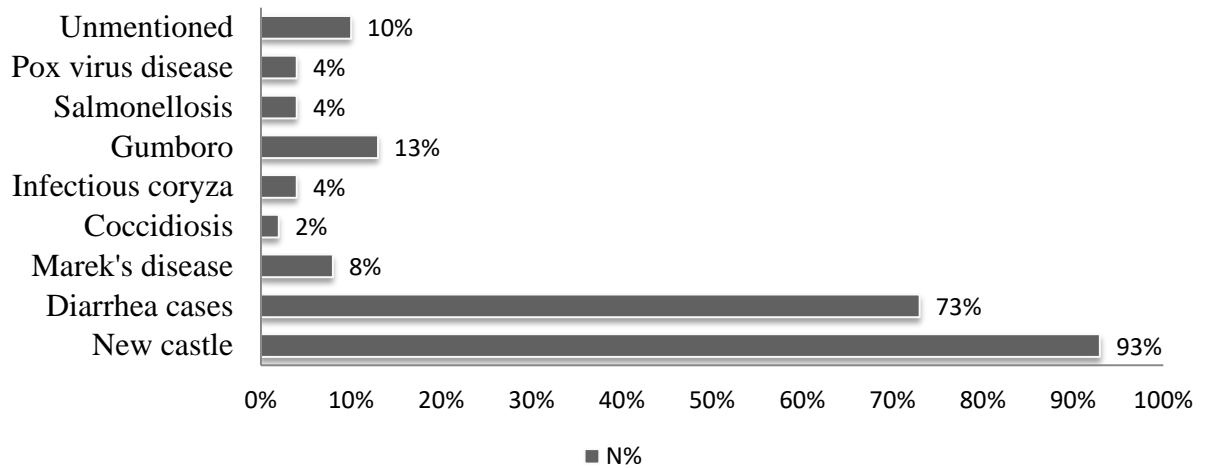


Figure 8: Common diseases experienced in the farms

The estimation of diseases identified from clinical signs given and commonly reported cross matched to specify to which disease type they are referring to or otherwise put as per their expressions like ‘Diarrhea cases’ for instance. The same was true for drugs cover pictures and drug brand names searched to correlate the types. To avoid confusion of vaccines, vitamins and antimicrobials they were asked on observational interview to list commonly used ‘drugs’ in the farm (vitamins and vaccines also called drugs at communication level) then in reference to the antimicrobials listed explained the KAP questions. The common used drugs mentioned were tetracycline, sulphonamides, enrofloxacin, ciprofloxacin, amprolium and alternatives used as a treatment were garlic, onion, ginger, lemon, acheto, ‘mitmita’(chilli pepers), ‘tena adam’(Rue), alpha alpha herbs, lettuce and cabbage in general considered for infection reduction or prevention reasons. Some were believed and used to reduce viral diseases like New castle disease. As a mite control and litter disinfectant onion and garlic were mentioned by the farm owners. Garlic and ginger also has recommended to them by some veterinarians in a balanced dose.

4.2.2 Knowledge, Attitude and Practice of poultry farm owners on AMR

The demographic factors considered to influence farm owners knowledge practice and attitude towards management of AMR development in poultry productions were Age, experience years in farming, production type, location or town and education levels the same was considered in Hassan et al KAP survey in Bangladesh. Location variation and production type included in the study hence exposure, access and management difference might influence KAP scores. The following three tables’ presents the questions asked and scored to scale the KAP scores accordingly.

Table 12 Knowledge questions correct towards AMR and public health

Knowledge on AMR and public health risk	Frequency (%)	Mean (CI-95%)
K.1 Do you know poultry diseases could transmit to human? yes(1)	26(36%)	0.36 [0.25;0.47]
K2. Do you know withdrawal periods of drugs? A minimum and maximum ranges mentioned approximately (1)	23(32%)	0.32 [0.21;0.43]
K3. Is it possible drug residues transmitted to human through consumption? Yes (1)	26(36%)	0.36 [0.25;0.47]
K4. What is Antimicrobial resistance? If Understood mechanism judgmental score (1)	25(35%)	0.40 [0.29;0.52]
K5. What Possible reasons to develop AMR? If mentioned, a. Using prescribed drugs without diagnosis(1) b. Under dosage of antimicrobials(1) c. Repeated treatments(1) d. Sub therapeutic use of drugs(as additives)(1)	10(14%)	1.97 [1.65;2.29]
K.6 Do you know possible reasons to treatment failure? a. Due efficacy of drugs (1) b. Misdiagnosed and inappropriate medication(1) c. The disease might be unmanageable inherently(1) d. Might be due AMR(1) e. Inappropriate administration(1)	22	2.39 [2.07;2.71]

The knowledge questions K5 and K6 considered one value for every multiple choices of all possible answers regarding AMR development in flock and reasons to treatment failures respectively. The challenge in filling replies for the K4 question was a multiple choices prepared were not directly used as an answer rather most respondents explain the concept in their general conversation through any examples they experienced in the farm or heard of in the other. As result K4 “What is AMR?” question amended after the first trials to get the answers from interviewee’s attempts to explain in relation to general medications to human for example “comparing with Paracetamol intake repetition will create non responsiveness or to develop adaptations of the drugs” or some explained in relation to their experience of Newcastle vaccine resistance in which vaccination intervals change through time be a resistance development.

There was a confusion understanding between vaccination and other drugs to explain antimicrobial resistance. So the researcher scored through professional judgments of the answers if they responded the concepts like ‘bacteria change over time and no longer respond to medicines, difficulty to treat and increasing the risk of disease spread and severe illness and death’ which implicates clear understanding about the matter and marked as “Understand the mechanism”. The survey revealed a 45% correct response with most were by The total knowledge scored out of 13 points.

Respondents attitudes towards biosecurity measures could prevent poultry disease were 49% the left options were “it couldn’t without antimicrobials” used as prophylaxis Repeated treatment effects explained by the respondents in decreasing productivity in egg and meat production. The 21% respondents think “it can only improve health of the chicken” or it wouldn’t affect at all. The most respondents who claim to have repeated treatment effects reasoned with egg production decrease and growth reduction. See Table 13 below.

Table 13 Attitudes favorable towards AMR and public health

Attitudes	Frequency (%)	Mean (CI-95%)
A.1 Biosecurity measures can prevent disease. Yes it could(favorable) It couldn’t without antimicrobials/ it can’t (unfavorable)	23(32%)	0.32[0.21;0.43]
A.2 Do you think treatment repetitions have effects on poultry? Yes (favorable)	57(79%)	0.79[0.70;0.89]
A.3 Do any alternatives used as medicinal drugs? Yes (favorable)	54(75%)	0.75[0.65;0.85]
A.4 AMR could affect human health. Agree(favorable)	12(17%)	0.17[0.08;0.25]
A.5 Would rather hold marketing before the end of withdrawal periods to reduce public health? Yes (favorable)	8(11%)	0.11[0.04;0.19]

Practice questions in this study carefully developed to avoid the need of cross check confirmatory. In between the conversations or what they will suggest for instance if recovery delayed or improved early would it be a problem to increase dose or halt medication to get the actual practices they trended. Strikingly eating poultry product at end stage treatments without

keeping withdrawal periods was 89% and drugs administration by farm owner or handler 90% and using as prophylaxis other than treatment was 96%, were the highest percentages from other practices. Antimicrobials used as a prophylaxis and feed additives. See Table 14 below.

Table 14: Practices risky towards AMR development and public health

Practices	Frequency	Mean (CI-95%)
P.1 Do antimicrobials used in the farm? Yes (exposure risk)	72(100%)	1.00 [1.00;1.00]
P.2 Are all antimicrobials always prescribed by veterinarian? Not always= 0	40(55.5%)	0.46 [0.34;0.58]
P.3 Who could administer drugs? Farm owner or handler (0)	65(90%)	0.10 [0.03;0.17]
P.4 Other than treatment for what purpose are used? Prophylaxis Additives =0	69(96%)	0.04[-0.01;0.09]
P.5 Any experience of treatment failure? Yes= 0	24(33%)	0.67 [0.56;0.78]
P.6 Do you order to halt medication if early or delayed recovery signs in the flock? Yes= 0	32(44%)	0.56 [0.44;0.67]
P.7 Do you increase dose or frequency if no recovery signs seen? Yes= 0	28(39%)	0.61 [0.50;0.73]
P.8 Do you eat the meat or egg of birds that are given antimicrobials at end stage? Yes =0	64(89%)	0.11 [0.04;0.19]

Demographic factors of respondent's age, educational background, farming experience in years were taken as variables and associated with KAP scores. The other variables were production type and location hence Addis Ababa and Bishoftu were the study areas. The Chisquare analysis using R software presented in Table 15.

Table 15: Association of knowledge, attitude and practice scores with demographic characteristics.

Variables	Knowledge			Attitudes			Practices		
	Correct	Incorrect	P-value	Favorable	Unfavorable	P-value	Good	Bad	P-value
Age									
20-30	9(45%)	11(55%)	0.414	1(5%)	19(90%)	0.159	4(50%)	16(50%)	0.103
30-35	11(63%)	8(37%)		4(21%)	15(79%)		6(36%)	13(68%)	
35-40	3(33%)	12(67%)		5(33%)	10(77%)		7(67%)	8(33%)	
40-50	4(45%)	7(56%)		3(27%)	8(73%)		10(27%)	1(73%)	
>50 more	2(29%)	5(71%)		0(0%)	7(100%)		0(29%)	7(71%)	
Experience years									
1-3years	14(29%)	34(71%)	0.001*	7(14%)	41(86%)	0.025*	12(25%)	36(75%)	0.956
4-7years	10(71%)	4(29%)		2(14%)	12(86%)		4(29%)	10(71%)	
7-10years	1(17%)	5(83%)		1(0)	5(100%)		1(17%)	5(83%)	
>10years	4(100%)	0(0)		3(75%)	1(25%)		1(25%)	3(75%)	
Education level									
Degree/above	16(80%)	4(20%)	0.000*	3(15%)	17(85%)	0.002*	9(47%)	10(47%)	0.083
e	3(50%)	3(50%)		4(67%)	2(33%)		0(0%)	6(100%)	
Diploma/TVET	8(38%)	17(80%)		2(8%)	23(92%)		6(24%)	19(76%)	
High school <Elementary	1(5%)	20(95%)		4(19%)	17(81%)		3(14%)	18(86%)	
Farm type									
Layers	23(32%)	40(68%)	0.156	13(21)	50(79%)	0.076	13(21%)	50(79%)	0.032*
Broiler	6(67%)	3(33%)		0(0)	9(100%)		4(44%)	5(56%)	
Location									
Bishoftu	18(78%)	5(22%)	*	4(17%)	19(83%)		6(26%)	17(74%)	
Addis Ababa	11(22%)	38(78%)	0.001	9(18%)	40(82%)	0.885	12(24%)	37(76%)	0.840

>50% Knowledge = Correct , Attitude =favorable, Practice= Good

As a non-parametric test, Chi square test can be used to determine if categorical data shows dependency or the two classifications are independent. Also to compare between theoretical populations and actual data of categories this analysis used for KAP analysis in the present study. The scores converted to percentages and >50% classed 'Correct', 'favorable' and 'good' for knowledge, attitude and practice scores respectively. According to Chi square test significant association between education level an experience years in farming with knowledge and attitude but there was ($P < 0.05$) no significant association in any of demographic characteristics with practice scores except with production types ($P=0.0329$) farm owners of intensive broiler farm have twice better practice than intensive layers farm owners . Knowledge scores also significantly associate with location though 78% of Bishoftu respondent have good knowledge.

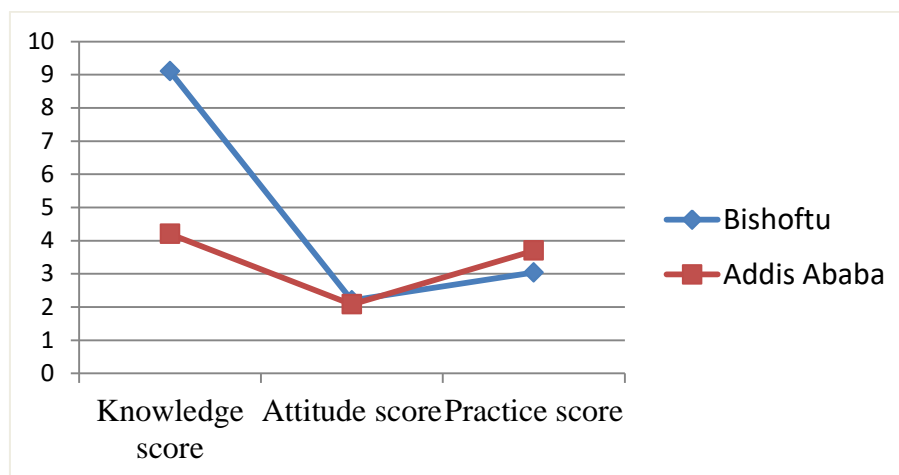


Figure 9: KAP scores between Bishoftu and Addis Ababa

The knowledge was significantly associated with location difference in the statically analysis. The graph in Figure10 shows the mean score of knowledge, attitude and practice scores of the two study area. Relatively in Bishoftu town 78% respondents have correct knowledge however the average scores in attitude and practice have no significant difference.

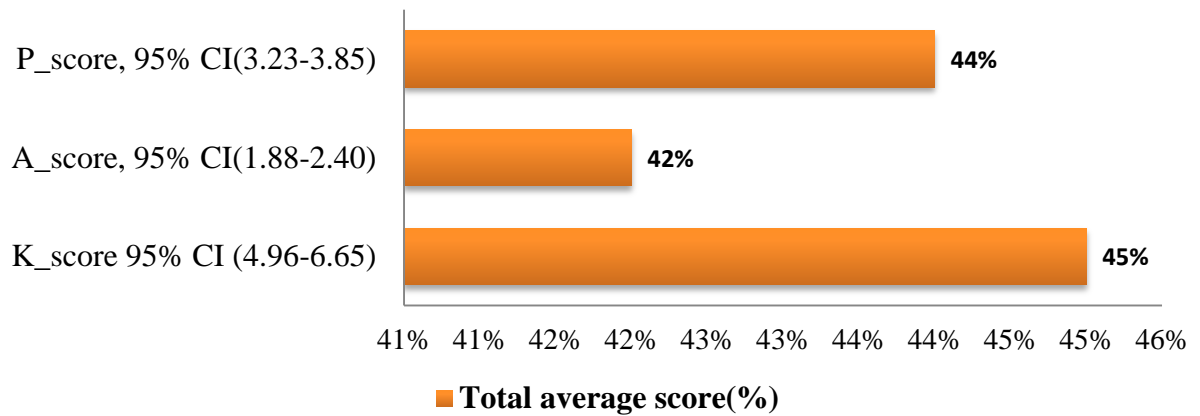


Figure 10: Cumulative Knowledge, attitude and practice scores

Taken together the mean scores for knowledge 5.8 to 13 questions mean score of attitude 2.1 for 5 and mean scores of practices to 8 number of questions on AMR and public health related questions studied in this research, the average percentage score were less than half percent in cumulative sum scores under all categories. None of the scores pass the >50% score indicating a knowledge, attitude and practice gap of the subject matter studied.

The spearman correlation coefficient between knowledge score with attitude scores and practice scores with attitude scores shown a positive correlation that the value increment in one of the scores might increase the second score the knowledge and attitude correlation is higher than practice and attitude correlations. In reverse knowledge and practice scores are negatively correlated which implicated a negative relations between the knowledge score and practice score one value inversely proportion to the other.

Table 16: Correlation between Knowledge, Attitude and Practice

Variables	Rho	p-value
Knowledge score-Practice score	-0.097	0.4155
Knowledge score -Attitude score	0.25	0.0339
Practice score-Attitude score	0.007	0.948

5. DISCUSSION

5.1 Distribution of Salmonellosis and AMR in poultry farms

The main reason for controlling the presence of *Salmonella* in the poultry industry is food safety issues hence, these food-borne diseases affect public health. Further *Salmonella* is important in terms of antimicrobial resistance and the bacterium could cause international restrictions on imports and exports of chickens and eggs. Infections could occur after eating undercooked meat, poultry, and eggs, as well as fresh-cut produce that is readily consumed raw and has been contaminated with it. The last but not very least of its effects is on the health of the birds (Pulido-Landínez, 2019; Brown *et al.*, 2021).

The overall proportion of samples yielding *Salmonella* isolates in the current study was 19.6% with biochemical tests and 13.5% were detected with molecular technique (PCR). The average farm-level prevalence was 19.2% with equal distribution between layers and broiler chickens found to be 9.8% isolates. This prevalence figure is higher than those (Abdi *et al.*, 2017; Eguale, 2018; Asfaw *et al.*, 2020) reported 16.7%, 14%, and 14.6% respectively but lower compared to (Kindu and Addis, 2013; Dagneu *et al.*, 2020) 41.9% reported in Jimma town and 28.8% in Adama and Modjo respectively.

This difference might be due to three reasons; sampling techniques over 80 percent of samples in the current study were cloaca swabs where the intestine is a natural reservoir of the bacterium, environmental contamination difference, and the number of samples taken per farm. Not to ignore the confounding factor, three of the farms had an outbreak of Marek's disease during the study period, and two farms with a history of Salmonellosis outbreak six months before sampling. The lower finding could be due to spatial differences, production system diversity, and breed types (Alali *et al.*, 2010; Abdi *et al.*, 2017; Jibril *et al.*, 2020).

Birds infected by *Salmonella* Typhimurium or *Salmonella* Enteritidis when a few days old result in asymptomatic cecal colonization with persistent shedding of organisms, which leads to carcass contamination at slaughter and entry into the human food chain (Harvey *et al.*, 2011). Considerably in this study NTS group, *Salmonella* Typhimurium was found in 53.3% of molecularly detected cloaca swab samples which attributed to what opted for this research investigation of public health risks through consumption of poultry and poultry products. Post-

harvest poultry food safety can be impacted by the status of the live broiler flocks entering the processing plant due to cross-contamination risk in processing (Ricke, 2021).

In the current study, age was significantly associated with *Salmonella* isolates distribution in the study farms. Chickens with higher age in weeks (> 5 weeks) have a high proportion of *Salmonella* isolates than the lower ages (<5 weeks). This finding is in good agreement with the age significance in (Abdi *et al.*, 2017) study. Similarly speaking in Eguale's (2018) findings *Salmonella* was comparatively higher between 2-6 months age than 7-12 months at farm level with no significant association (half percent of isolates were in the prior group) (Eguale, 2018). A production type variation significantly showed a difference in line with (Jibril *et al.*, 2020) study hence, broiler chickens were more likely to have *Salmonella* isolates distribution with (P=0.03) in this case. Nevertheless, the public health risk of the poultry products is indistinguishable because of *Salmonella* isolation remarks food safety issue mutually in either of the production types.

All the farms randomly investigated for *Salmonella* presence were using a deep litter system in this study and preliminary findings of litter disposal time variation depended on production types thus broiler farms stock out less than 2 months and layers might keep it long for a year. As 90 percent of farms visited trended a batch out cleaning, intervals were taken as risk variables in this study. Though this study has not confirmed previous research compared deep litter to cage systems that reasoned the deep litter system prone to salmonellosis infection than the second one (Mollenhorst *et al.*, 2005; Jibril *et al.*, 2020). Probably increasing number of farms and production systems can illustrate the association enough than interval variation of litter disposal. However, Eguale's (2018) findings with higher farm numbers were not significant between deep litter and cage system comparison.

There might be other natural explanations for only found 2(0.9%) isolates of *Salmonella* and an 8.6% rate from 23 environmental samples collected than less contamination of the litter or disinfections used. Taken as a whole, sample processing techniques, collecting methods as handlers assisted in taking for sake of biosafety, and processing techniques might be the reasons for less isolation rate. A sample size might be a factor but a study in India found only two NTS molecularly detected of 363 environmental samples collected (Saravanan *et al.*, 2015).

Reports justified a hostile environment for *Salmonella* sources like feed, water, and litter might get contaminated with rodent excrements containing a variety of serotypes and can survive up to 120 days in rat feces (Pulido-Landínez, 2019). Despite the permanent rodent or pest control variable has not shown a significant correlation with *Salmonella* isolation in the current study aligning with (ADESIYUN *et al.*, 2014; Jibril *et al.*, 2020), the 92% of farms lack controlling methods or are fed up to try under the current study findings. The approach difference might be the reason for those studies and the current study failed to significantly correlate with *Salmonella* infection. However, some author's found the prevalence of *Salmonella* Enteritidis in mice from environmentally positive houses was nearly four times that in mice from environmentally negative houses (Garber *et al.*, 2003).

Notably in this study, Some isolates tested using Kirby–Bauer disk diffusion assay for AMR representing the farms was resistant to more than five drugs that are multi-drug resistant, and similar studies found high percentages of MDR from isolates reported (Abdi *et al.*, 2017; Dagneu *et al.*, 2020). The nine isolates were resistant to Tetracycline, Ampicillin, Nalidixic, Fluoroquinolones drug and ciprofloxacin though shared in human medications. Azithromycin with the highest susceptibility and Chloramphenicol followed for 78% and 44% of isolates tested. According to Beyene et al report, Tetracycline next to ampicillin and streptomycin most frequently used veterinary drug by animal farmers in Bishoftu town. This support the high resistance found in tetracycline and ampicillin compared to other drugs in the current scenario (Beyene *et al.*, 2015).

One litter sample was resistant to seven drugs (TE30, AMP 10, CTX30, Caz30, SXT25, NA30, and Cip5) and in most of the farms litter disposals claimed to be sold for livestock farms. Given that the limited number and not molecularly detected for resistant gene here, a note of caution should be taken from this, as concerns rose due to the irrational use of antibiotics in humans, animals, communities, and associated environments that emerged reservoirs of resistance resulting persistence of drug residues or resistance genes in the environment (Aslam *et al.*, 2021).

Four of *Salmonella* Typhimurium isolates were resistant to more than three drugs same was true in the (Eguale, 2018; Dagneu *et al.*, 2020) study and over 50% were multi-drug resistant in the present study. Underlining a very characteristics behavior of *Salmonella* Typhimurium, the genomic element that carries resistance to five antimicrobials (Ampicillin, Chloramphenicol,

Streptomycin, Sulphonamides, and Tetracycline) can be spread horizontally among other serotypes and acquire additional resistance determinants (Mouttotou *et al.*, 2017). A comparison study of a resistant gene from retail chicken meat and human pulse net database found the exact resistant gene for the same group of drugs used in both human and animal medications (M'ikanatha *et al.*, 2010). The present findings emphasized the possibility of public health threat by antimicrobial resistance through NTS of poultry and poultry products as all *Salmonella* Typhimurium isolates were from cloaca swabs.

5.2 Knowledge, Attitude, and Practice of poultry farm owners on AMR

In poultry products, MDR strain *Salmonella* infection sources include at the site of primary production (e.g., parent stock, incubator, and farm); cross-contamination in the handling of food or byproducts; and consumption of undercooked poultry meat, eggs, or egg products are all potential sources of contamination in poultry products. All of these sources have been linked to *Salmonella* infection in humans (Castro-Vargas *et al.*, 2020). The widespread use of antimicrobials in animal production is believed to be one of the major factors driving the emergence of antimicrobial resistance in bacterial strains (Gemedo *et al.*, 2020).

In the current study, 72 poultry farms were assessed and owners' were interviewed on knowledge, attitude, and practice of antimicrobial use and antimicrobial resistance in Bishoftu and Addis Ababa. In terms of the KAP survey of poultry farm owners on AMR, as far as known specific study of this kind are limited or scarce. Around 40% have the correct knowledge, 18% have favorable attitudes, and 53% have good practice toward AMR and public health. Remarkably, the average score out of corresponding questions falls under 50% seen in Figure 10. This study has shown a significance association between Knowledge and attitude scores of respondents with education level and years of experience in farming and locations in Knowledge scores. The significant association with education levels were consistent with those (Caudell *et al.*, 2020; Moffo *et al.*, 2020; Gebeyehu *et al.*, 2021; Geta and Kibret, 2021; Hassan *et al.*, 2021) findings cumulatively emphasizing the education level of farm owners positively contribute to the relatively higher KAP scores on AMR and AMU.

Hassan et al (Hassan *et al.*, 2021) have shown the association difference in both farming experiences and age of respondents hence, reasoned increased age with years of experience may lead to the development of expertise in poultry farming, gaining knowledge for exploring

veterinary services and exposure to continuous training, awareness programs, and other learning processes of AMU and AMR. The significant values of association in farming experience years of owners in the current study fairly well correlate with (Ozturk *et al.*, 2019; Hassan *et al.*, 2021) but in contrast to the study in Cameroon (Moffo *et al.*, 2020) not found significant with farming experience. In our study knowledge and attitude scores with > 10 years of experienced farmers had the highest correct knowledge and favorable attitudes proportion than the lower then 4-7 years of experience follow the rank. This might be because of frequent experience of disease and control management exposure through years uplifted the knowledge of the farm owners on AMR and AMU. In contrast to the above researchers' findings, age increase was inversely related to knowledge and attitudes in our study which aligned with (Ozturk *et al.*, 2019).

Biosecurity measures' role in prevention and infection burden reduction could not be ignored in poultry farming sector. Though here assessed using biosecurity indicators scored in percentile and classed as 'good' for farms scored greater than 70% and 'poor' to lesser scores used as determinant of the biosecurity status of the farm. Of that category, 47.2% of the farms fall under 'Good' biosecurity status. The discrepancy of scoring percentages with Ismael *et al.* classed 'good' for greater than 50% scoring using 72 indicators (Ismael *et al.*, 2021) might not be a factor for less number of well-practiced farms recorded in this study.

Here assumed the indicators compiled to the least minimum required for prevention and reduce infections at the farm level and incorporate basic biosecurity measures to reduce Salmonellosis, thus taken from (Cunningham and Fairchild, 2020) and custom-built to applicable and short checklists. Paid in interpretations of biosecurity status in a limited number of indicators, the results from such analysis might not outlet the associations. However, the reasons for using basic indicators were to balance the bias, time, and resource availability needed to take all the measurements in the farms. Successful animal production, including poultry, requires the adoption of good biosecurity practices, which is the most effective and inexpensive method of disease burden reduction (Ismael *et al.*, 2021).

Studies demonstrated that acquiring antibiotics was a preferred response to disease outbreaks over other disease reduction or prevention measures in Vietnam (Pham-Duc *et al.*, 2019). A marked observation in the current study was that 54 % of respondents believe antimicrobials combined with biosecurity measures could be more effective in prevention than biosecurity measures alone. In the same way, the farms use antimicrobials as prophylaxis which may

increase the risk of AMR development at farm level. Environmental pollutions and other animals' health risks are under question hence, improper litter disposal was found in 47% of poultry farms in Addis Ababa. The litter disposal sold to livestock farms or disposed of wastes to a nearby river, sewerage, or public garbage. Poultry waste contains high moisture with organic materials, which create environmental problems such as fly breeding, odor nuisance, and greenhouse gas emission if not disposed of or managed properly (Siddiky *et al.*, 2022).

A considerable number of respondents know about antimicrobials and antimicrobial resistance than (Tufa *et al.*, 2018) research on animal producers in Central Ethiopia, this might be due to the specificity of the current KAP study only on poultry farm owners and 70% of the respondents were educated that were higher than secondary level. The reverse might be true if handlers were considered in this study with the same level of education and variations parallel with Tufa et al findings result in the values. All the farmers interviewed had at least used one antimicrobial as prophylaxis if not for therapeutic, like oxytetracycline, sulfa drugs, and enrofloxacin drugs take the consecutive ranks of commonly used drugs. Assessments done on animal producers reported oxytetracycline (100%), amoxicillin (71%), ciprofloxacin (29%), and sulfa drugs (29%) are used as prophylaxis and/or control of bacterial infections used in Bishoftu and Modjo town (Beyene *et al.*, 2015).

Contributing factors, practices, and attitudes to AMR development are not solely dependent on poultry farm owners. Risky practices of professionals, drug sellers, and feed sellers might drop their part in this AMR threat. The KAP survey on drug and feed sellers indicated unauthorized prescribers might not suggest antimicrobial withdrawals to the customers and sold drugs near to expiry date with the lowest price to get profits (Kalam *et al.*, 2021). Stating the obvious 54% of the poultry farm owners interviewed in this study mentioned that drugs are not always prescribed by professionals after diagnosis but rather purchased from drug stores without prescription and understanding of AMR in general were low. Studying risky practices and attitudes of sellers as a contributing factor in AMR development is undeniably important as in Bangladesh feed sellers suggest antimicrobial drugs to the farmers and drug companies promote their products to feed and drug sellers even for farmers to get the profits from (Kalam *et al.*, 2021).

Further investigation of knowledge and understanding of the labeled notification on the drugs was assessed by asking the owners' to mention one minimum and maximum ranges of

withdrawal periods recommended for the commonly used drugs in the respective farms. Surprisingly (69.4%) respondents do not even know or what for written as ‘withdrawal periods’ on leaflets and late alone use it accordingly. Aside from the knowledge gap reasons for understanding withdrawal period importance, this result eliciting details of a label to be read on drug covers lacks clarity or the reason may be similar to Tufa et al study in Central Ethiopia livestock farmers including poultry farm owners, found 57 percent of them could not read and understand to check the expiry date of drugs due to a lack of English language understanding (Tufa *et al.*, 2018). Even though assessing language efficiency is outside the scope of the current study, none of the drugs encountered during the investigation period were clearly labeled in the local language. End-user notifications are critical, which may have contributed to the failure to keep withdrawal periods for market and consumption of poultry products regardless of recommended withdrawal periods.

The above finding appeared to be supported by ninety percent of respondents who consume poultry products at the end-stage without keeping the recommended withdrawal periods. The results were close to (Kalam *et al.*, 2021) findings and 23% of owners think it wouldn't affect human health because of the assumption that residues "might be lost through proper cooking" which is a conspicuous result in this study. Researches mentioned ARs are relatively stable in the environment, and even high heat treatment cannot destroy antibiotics completely. Eventually, these ARs can come into contact with the human intestine through the food chain. A possible harmful residues that produce potential threats of direct toxicity in humans and alteration of micro-flora with possible development of resistant strains due to low and continuous exposure to antibiotics, thus causing failure of antibiotic therapies (Mund *et al.*, 2017; Haque *et al.*, 2021).

In conclusion, not only in Ethiopia the case of developing and poor countries, guidelines for judicious use of antibiotics also are not demonstrated to farmers, which could be a major reason behind AR in foods from animal sources (Haque *et al.*, 2021). The upshot of these results of MDR isolates in cloaca swabs and environmental samples, disseminated in the livestock island and overall KAP of farm owners’ survey result on antimicrobial residue and resistance are explicit indicators of the possible risk to face in public health, animal health, and environmental safety. Detection of MRL on poultry products is indispensable for monitoring antimicrobial residues and to use legal enforcements. Most farm owners of the current study use organic herbs traditionally as alternatives promised a favorable attitude to decrease the irrational use of antimicrobials in poultry farms.

6. CONCLUSION AND RECOMMENDATIONS

The current study revealed a *Salmonella* and *Salmonella* Typhimurium distribution in selected poultry farms of Bishoftu town with bacteriological isolation and molecular techniques 42(19.6%) and 29(13.5%) respectively. Age and breed types were significant risk factors for higher isolation rate between the poultry farms. Detection of 16 (7.4%) *Salmonella* Typhimurium in chicken cloaca swabs were indication of cross-contamination risks in slaughter and horizontal contamination risk of poultry and poultry products marketed for consumption to public. The emergence threat of antimicrobial-resistant strains of *Salmonella* becomes public health concern beyond the economic loss at the farm level and trade restrictions of poultry and poultry products. A drug resistance test using kirby disc diffusion assay on nine isolates indicated a multidrug resistance in all and of which four were *Salmonella* Typhimurium and one litter sample that bold the public health risk and environmental spread possibilities. Remarkably, the current study incorporated the KAP investigation of indisputable contributors to AMR development in poultry farms 'farm owners' found in Bishoftu and Addis Ababa. Based on the findings from KAP of poultry farm owners towards AMR and public health protection were substantial less than 50% of average score and multi-sectorial contributors to the low understanding and practices pointed out in the survey. Unless specific measures planned to improve the awareness and practices towards AMR development in this sector, folding measures in general to food animal producers may not brought the required behavioral change. This study highlighted at the surface importance of a one health approach on all sides to monitor and control AMR at the country level. Therefore, the following recommendations are forwarded:

- Further investigations and studies of the risk factors in Salmonellosis distribution with emphasized finding of biosecurity measures that possibly reduce burden of infections and transmission.
- Investigate antimicrobial resistant strain and molecular detection of MDR strain genes shared at the interface of poultry, human and the environment, to mitigate evident monitoring plans.
- Exploratory investigation of knowledge, attitude and practice gaps in the poultry farming sector and further contributors to implement effective education and training program.

- Implementing feasible and applicable training and education to the poultry farm owners' based on the existed status of the farmers on AMR development and risks.
- Creating awareness of environmental pollution factors and risks in the poultry sectors under one health approach to collaborate all sided protection plan to the human health, animal health and environmental safety.
- VDFACA to enforce drug companies for clear and understandable labeling with local language to end users on antimicrobial drug usages and withdrawal periods.

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8. ANNEXES

Annex 1

Media preparations for isolation and identification

Buffered Peptone Water Preparation

Composition (g/l): Enzymatic digest of casein 10.0; Sodium chloride 5.0; disodium phosphate dodecahydrate 9.0 and potassium dihydrogen phosphate 9.0 2.

Preparation:

- 20 grams of BPW components will be suspended in 1000ml of distilled water Mix well and distribute into universal bottle of suitable capacity to obtain the portions necessary for the test and
- sterilize in autoclave at 121 °C for 12 minutes. Final PH is 7.0 + 0.2 at 25°C.

Modified Rappaport Vassiliadis *Salmonella* enrichment Broth

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:

- 49.17 grams of hydrated medium RVS broth will be suspended in 1000ml distilled water.
- Heat if necessary, to dissolve the medium completely. Dispense as desired in to tubes and sterilize by autoclaving at 115 °C for 15 minutes. PH after sterilization: 5.2 + 0.2.

Tetrathionate (TT) *Salmonella* enrichment Broth

Composition(g/L): Polypeptone 5g, Bile salts 1g, Calcium carbonate 10g, Sodium thiosulfate•5H₂O 30 g, Distilled water 1 liter.

Preparation:

- Suspend ingredients in 1-liter distilled water, mix, and heat to boiling. Do not autoclave. (Precipitate will not dissolve completely.)
- Cool to less than 45°C. Store at 5-8°C. The Final pH, 8.4 ± 0.2. 32

Xylose Lysine Desoxycholate Agar (XLD)

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 53grams in one liter of distilled water. Heat with frequent agitation until the medium boils. DO NOT OVERHEAT.
- Transfer immediately to a water bath at 50°C. Pour into plates as soon as the medium has cooled. It is important to preparing large volumes which will cause prolonged heating. PH: 7.4 + 0.2 at 25 °C

Brilliant Green Agar

Composition(g/L): Peptone 5.000, Tryptone 5.000, Yeast extract 3.000, Lactose 10.000, Sucrose 10.000, Sodium chloride 5.000, Phenol red 0.080, Brilliant green 0.0125, Agar 20.000.

Preparation:

- Suspend 29.05grams in 500 ml purified /distilled water.
- Then Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid overheating. Cool to 45-50°C. Mix well and pour into sterile Petri plates. Final pH (at 25°C): 6.9±0.2

Nutrient Agar

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 and dissolve the medium completely.
- Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour in to sterile petridishes. Final PH (at 25°C): 7.4 + 0.2.

Triple Sugar Agar

Composition (g/l): 'meat extract 3.0; yeast extract 3.0g; 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 24.28 grams in 1000ml of distilled water. Bring to boil to dissolve completely.
- Mix well and distribute into containers. Sterilize by autoclaving at 121°C for 25 minutes. Allow the set as slope with 2.5 cm butts. PH: 7.4 + 0.2 at 25°C.

MR-VP Medium

Preparation:

- suspend 17.0 gram in 1000ml distilled water. Heat if necessary, to dissolve the medium completely.
- Distribute in to test tubes 10ml amounts and sterilize by autoclaving at 15 Ibs pressure (121°C) for 15 minutes.

Composition (g/l): buffered peptone 7.00; dextrose 5.00; dipotassium phosphate 5.00 34

Reagent required for voges- proskauer reaction

➤ α -Naphtanol, ethanolic solution

Composition (g/l): α -Naphtanol 6 grams; ethanol 96 % (volume fraction) 100ml.

Preparation: dissolve α -Naphtanol in ethanol

➤ Potassium hydroxide solution

Composition (g/l): potassium hydroxide 40 grams; distilled water 100ml

Preparation: dissolve potassium hydroxide in distilled water

Sulfide, Indole, Motility (SIM) Medium

Composition(g/L): Pancreatic Digest of Casein 20.0gm, Peptic Digest of Animal Tissue 6.1gm, Ferrous Ammonium Sulfate 0.2gm, Sodium Thiosulfate 0.2gm, Agar 3.5gm.

Preparation:

- Suspend 20grams of medium in 1000 ml of demineralized water. Then Heat to boiling with agitation to completely dissolve.
 - Dispense into tubes and sterilize by autoclaving at 121°C for 15 minutes. Final pH 7.3 +/- 0.2 at 25°C.
1. Touch a straight needle to a colony of a young (18- to 24-hour) culture growing on agar medium.

2. Stab once to a depth of only $1/3$ to $1/2$ inch in the middle of the tube. Be sure to keep the needle in the same line it entered as it is removed from the medium.
3. Incubate at 35° - 37° C and examine daily for up to 7 days.
4. Observe for a diffuse zone of growth flaring out from the line of inoculation.

Mueller-Hinton Agar

Composition (g/l): beef, dehydrated infusion 300.00; casein hydrolysate 17.5; starch 1.5; agar 17.00

Preparation:

- Suspend 38 grams in 1000ml 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.

0.5 McFarland standards

Composition: 1.17% $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ solution and 0.36N of 1% sulfuric acid (H_2SO_4).

Preparation:

- Add approximately 85ml of 1% H_2SO_4 to a 100ml volumetric flask, using a 0.5ml pipette
- Add 0.5ml of 1.17% $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ drop wise to the H_2SO_4 while constantly swirling the flask.
- Bring to 100ml with 1% H_2SO_4 . Place a magnetic stirrer 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. Dispense three to seven ml, cub tube tightly and seal with paraffin and keep at dark and room temperature.

Urea Agar

Composition: Peptone 1,0 g Glucose 1,0 g Sodium chloride (NaCl) 5,0 g Potassium dihydrogen phosphate (KH_2PO_4) 2,0 g Phenol red 0,012 g Agar 9 g to 18 g Water 1 000 ml

Preparation:

The components or the dehydrated complete base dissolved in the water by heating with frequent agitation. Adjust the pH, if necessary, so that after sterilization, it is $6,8 \pm 0,2$ at 25° C.

Pour the base medium into tubes or flasks of appropriate capacity and Sterilized for 15 min in the autoclave set at 121 °C. The base medium may be stored in closed tubes or flasks at 5 °C for up to three months

- Urea solution preparation

Composition: 400gm urea and 1000ml water to prepare 40% concentration.

Preparation: The urea dissolved t in the water and sterilized by filtration through a filter with a pore size of 0,22 µm.

- Complete medium preparation

Composition: Urea solution 50ml and urea agar medium 950ml

Under aseptic conditions, the urea solution to the base previously melted and then cooled to 47 °C to 50 °C added. Dispensed complete medium into sterile tubes in quantities of 10 ml allowed to set in a sloping position.

L-Lysine Decarboxylation Medium

Composition (g/l): L-Lysine monohydrochloride 5.0, yeast extract 3.0, glucose 1.0, bromocresol purple 0.015.

Preparation:

- 5.25 g/500ml and 5g/500ml decarboxylase base moller and L-Lysine monohydrochloride respectively were dissolved together by heating if necessary and,
- dispense 5ml into test tubes and sterilize at 121°Cfor 10 minutes. The broths were clear and yellow to amber.

Simmons Citrate Agar

Composition (g/L): Magnesium sulphate 0.200, Ammonium dihydrogen phosphate 1.000, Dipotassium phosphate 1.000, Sodium citrate 2.000, Sodium chloride 5.000, Bromthymol blue 0.080, Agar 15.000

Preparation:

- Suspend 24.28 grams in 1000 ml distilled water.
- Heat to boiling, to dissolve the medium completely.
- Mix well and distribute in tubes or flasks. For tubes, dispense 4.0 to 5.0 ml into 16-mm tubes.

- Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool in slanted position (long slant, shallow butt). Tubes should be stored in a refrigerator to ensure a shelf life of 6 to 8 weeks. The uninoculated medium will be a deep forest green due to the pH of the sample and the bromothymol blue. Final pH (at 25°C) 6.8±0.2 36

Tryptone broth

Composition (g/L): Tryptone 10.0, Sodium Chloride 5.0, DL-Tryptophan 1.0.

Preparation:

- 10g/1000ml, 5g/1000 and 3g/1000ml of Tryptone, sodium chloride and DL_Tryptophan respectively were dissolved together by heating if necessary and
- dispense 5ml into test tubes and sterilize at 121°C for 15 minutes. The broth was clear and yellow.

Conventional PCR

DNA extraction using QIAGENTM mini columns

Reagents:

- Water bath at 56°C.
- Buffer AE or dd.H2O for elution.
- Buffer AW1, Buffer AW2, and QIAGEN Protease.
- If a precipitate has formed in Buffer AL, dissolve by incubating at 70°C
- All centrifugation steps should be carried out at room temperature

1. A 20µl of QIAGEN Protease Pipetted into the bottom of a 1.5ml microcentrifuge tube and 200 µl sample to the microcentrifuge tube.
2. Then 200µl buffer AL to the sample. Mixed by pulse-vortexing for 15sec and Incubated at 56°C for 10 min. DNA yield reaches a maximum after lysis for 10 min at 56°C.
3. Briefly centrifuged and the 1.5ml microfuge tube to remove drops from the inside of the lid.
4. A 200µl ethanol (96-100%) was added to the sample and mixed again by pulse-vortexing. After mixing, briefly centrifuged the 1.5ml microfuge tube to remove drops from the inside of the lid then carefully transferred the mixture from to the QIAamp spin column (in a 2ml collection tube) without wetting the rim and closed centrifuged at 6000xg (8000rpm) for 1 min.
5. The tube containing the filtrate discarded and placed the QIAamp spin column in a clean 2

ml collection tube provided.

6. Carefully opened the QIAamp spin column and added 500µl Buffer AW1 without wetting the rim and centrifuged at 6000xg (8000rpm) for 1min. Again repeated step 5 and added 500µl Buffer AW2 without wetting the rim. Centrifuged at full speed (20,000xg; 14000rpm) for 3 min. Continue directly with step-10, or to eliminate any chance of possible buffer AW2 carryover, perform step 9a, and then continue with step 10.
7. Finally placed the QIAamp spin column in a clean 1.5ml microfuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp spin column and add 200µl Buffer AE or distilled water. Incubate at room temperature for 5min, and then centrifuge at 6000xg (8000rpm) for 1min.

*A second elution step with a further 200µl Buffer AE will increase yields by up to 15%.

For calculating DNA concentration;

Pipette 2µl in a clean 1ml tube and add to it 198µl of water to give 1/100 dilution factor

Take the OD at A260 x 100 x 50 = µg/ml of DNA

Master mix Preparation with universal *Salmonella* primer:

Type of reagent	For one reaction	Total reaction 36
○ RNase free water	3 µl	108µl
○ Primer- salm-Fow-5pm/ µl 5'-ACTGGCGTTATCCCTTTCTCTGGTG-3'	2 µl	72µl
○ Primer-Salm Rev-5pm/ µL 5'ATGTTGTCCTGCCCTGGTAAGAGA-3'	2 µl	72µl
○ 10x Dream Taq™ buffer(with Mgcl2)	5µl	180 µl
○ dNTP mix ,2Mm each	5µl	180µl
○ Dream Taq™ DNA polymerase	1.5µl	54 µl
○ Add Template (DN A)	5 µl	

*Total volume of 18.5µl mixture added in eppindrof tubes and RNase water as a negative control all in master mix room.

Master mix preparation for *Salmonella* Typhimurum

Type of reagent	For one reaction	Total 36 reaction
○ RNase free water	3 µl	108µl
○ Primer-SPY-FOW-5pm/ul 5'TTGTTCACTTTTACCCCTGAA 3'	2 µl	72 µl

- Primer-SPY-REV-5pm/ul
5'CCCTGACAGCCGTTAGATATT 3' 2 µl 72 µl
- 10x Dream Taq™ buffer(with Mgcl2) 5µl 180 µl
- dNTP mix ,2Mm each 5µl 180 µl
- Dream Taq™ DNA polymerase 1.5µl 54µl
- Add Template (DNA) 5µl

*Total volume of 18.5µl mixture added in eppindrof tubes and RNase water as a negative control all in master mix room.

PCR amplification

	Temperature	Time	Cycle
Initial Denaturation	94oc.....	5mints.....	1-Cycle
Denaturation	94oc.....	1 mint	35 Cycles
Annealing	55oc.....	1 mint	
Elongation	72oc.....	1:30 mint	
Final Elongation	72oc.....	10 mints....	1-Cycle
Put at 4oc	Until machine off		

Agarose gel preparation

Composition:

A 2% Agarose gel prepared

-Add 4µ Gel red with Loading dye, 10PCR product and 10 µl markers (Ladder)

-Run Electrophoresis for 1 hour at 120V

-Red the result by using UV –light

-It is around 401bp for SpY (*S.thyphimurium*) and 304bp for sdf1 (*S. enteritidis*) positive result.

Annex 2

KAP survey of Poultry farm owners on AMR development and human health risks

Informed consent

Research Title: Isolation, Susceptibility Test of *Salmonella* and Owners Knowledge, Attitude And Practices of Antimicrobial Resistance in Selected Poultry Farms Of Addis Ababa and Bishoftu , Ethiopia

Researcher: Hayat Kelifa (DVM)

University: Addis Ababa University, College of Veterinary Medicine and Agriculture

Sponsor: A thematic project sponsored

You are being selected to take part in this research because you as a poultry farm owner /manager or professional working in Addis Ababa and Bishoftu Ethiopia are eligible for the survey on knowledge attitude and practice of AMR development in poultry and human health. If your farm is located in Bishoftu town some chickens will be sampled for cloaca swabs, litter sample and drinking water with strictly kept farm biosafety your own handlers' cooperation. Before you decide, it is important for you to understand the research purpose is to isolate and identify *Salmonella* species and AMR patterns at some farms and assess knowledge of farm owners, managers and professionals on AMR development risk factors. This study is to provide input for a better intervention and recommendations to prompt rationale antibiotic use in poultry farms of Addis Ababa and Bishoftu Ethiopia. Please feel free to ask for any unclear issues or to discuss it with anyone you wish.

Please take time to decide whether or not you want to take part in this research and note withdrawal at any time is possible. I would like to stress that taking part in this study is entirely voluntary and responses are confidential will be used only for the purpose of the study.

Do you consent to participate in this interview and study?

Yes I consent to participate in this study.

Consent declaration

Name_____ Signature _____

If No, Thank the individual.

Observational data will be collected during site visits

General information

- a) Establishment year of the poultry farm _____
- b) Size of flocks in the poultry farm _____
- c) Scale/level of poultry farm _____
- d) Production system and type of the poultry farms _____
- e) Number of attendants in the farm _____
- f) Rooms/area/ facilities of the farm _____
- g) House cleaning condition _____

Biosecurity Checklists

➤ Restrictions

Restrict new visitors' _____ Restrict visits between farms in 24hrs/share
equipment's _____ restrict vehicle entry to farm area or Drivers to entry _____

➤ Changing room at entrance _____ Footwear/bath and hand washing before
entry _____

➤ Isolation and quarantine room (compartmented) for new birds/ sick birds

➤ Cleaning and sanitation

Farm equipment's _____

Poultry house cleaning / frequency _____

Permanent rodents or pest control management _____

➤ Personnel hygiene _____

➤ Disposal of poultry manure and dead chickens _____

➤ Documentation and record keeping of flocks Mortality _____ Feeding and drinking
_____ Disease and recovery history _____

h) Commonly used antimicrobials/ drugs in the farm

i) Market chain or supply information / Most clients are from where

- a) Addis Ababa
- b) Bishoftu
- c) Adama
- d) Other, Mention _____
- e) information is unavailable/ unknown

Semi Structured Questionnaire to interview poultry farm owners or managers

Background

Name _____ Age _____ Educational level _____ Animal health
background Yes/No Address _____

Farm management and operation

1. How often do you attend the farm?
 - a. Everyday
 - b. Sometimes
 - c. Every week
 - d. Every month

2. Experiences in the poultry farm production?
If less than a year stop the survey
3. Does the farm have animal professional?
 - a) Yes, 1. contractual 2. Permanent
 - b) None
4. Do you use all-in all-out system?

5. Ask if there is no professional to interview, what are the common diseases experienced in the farm?

6. Common diarrhea cases in the farm? Which type?

Questions K1. Salmonellosis in the farm

7. Do you know about Salmonellosis?
Yes No
8. How could you suspect birds with Salmonellosis disease?

- a. If Weakness , loss of appetite and poor growth
 - b. If had White sticky diarrhea
 - c. I don't know how to differentiate
9. Do you know poultry diseases could be transmitted to humans?
Yes No

Questions P.1 Salmonellosis control practices

10. What managerial decisions you usually made to control Salmonellosis in your farm?

11. Any alternatives used in your farm to improve the gut health of chickens or reduce infection?

Questions P.2 Treatment and management practices

12. Are all treatments for cases prescribed by a veterinarian?
a. Yes always b. Not always
13. Who can administer medications in the farm to flocks?
a. Farm owner or handler
b. A veterinarian or animal professional
c. Other than injections Farm owner or handler
14. Other than treatment purposes for what reasons will antimicrobials prescribed in the farm?
a. Prophylaxis
b. To increase productivity(as feed additives)
c. To increase growth (as feed additives)
15. Are there experiences of treatment failure in the flocks?
Yes No
16. If you order to halt medication before the end of prescribed periods What might be the reasons?
a. Because of recovery and so to reduce costs
b. If recovery delayed to shift to other treatments between the course.

- c. I don't halt medications.
17. Do you increase dose and frequency when there are no recovery signs?
- a. Yes b. No
18. Have you eaten the meat or egg of chickens given antimicrobials at the end stage?
- a. Yes b. No

Question K.2 AMR knowledge questions

19. How long should the chicken products avoided after treatment? Minimum and maximum range.
- a. _____ b. I don't know
20. Is it possible that Antimicrobial residues can pass to human through consumption of poultry meat and egg?
- a. Yes b. No
21. Do you know about antimicrobial resistance?
- a. Yes b. No
22. What do you think are the effects of repeated antimicrobial treatment?
- _____
23. What are the possible reasons for treatment failures?
- a. Due efficacy of drugs
 - b. Misdiagnosed and inappropriate medication
 - c. The disease might be unmanageable inherently
 - d. Might be due AMR
 - e. Inappropriate administration
24. What are the possible reasons of AMR to develop in poultry?
- a. Prescribed drugs without diagnosis
 - b. Under dosage of antibiotics
 - c. Repeated treatments
 - d. Sub-therapeutic use of drugs as additives
 - e. I don't know how AMR develop

Questions A.1 Attitudes on prevention and management of salmonellosis AMR

25. Biosecurity measures could prevent poultry disease or infections?

- a. Yes it could
- b. No it couldn't without antimicrobials
- c. No it can not.

26. Would you rather hold marketing before withdrawal period end to reduce residual effects on human health?

- a. I don't think it will affect humans I would rather stock out
- b. I don't know about residues and I stock out before it ends
- c. Yes I keep withdrawal periods as recommended by veterinarians
- d. Yes I understand the residual effects and keep withdrawal periods before marketing

27. Repeated antimicrobial treatments predispose to drug resistance?

- a. Yes it can lead to resistance
- b. No it can improve the chicken health and production

28. Antimicrobial resistance could affect human health?

- a. I agree
- b. Moderately agree
- c. I don't think it affects

29. Would you take responsibility to reduce the effects of AMR on consumers by using less and rationale use of antimicrobials?

- a. Yes
- b. No
- c. I don't think it will contribute

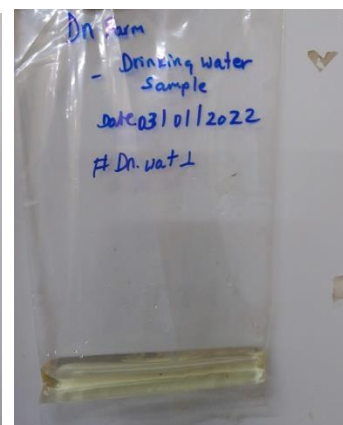
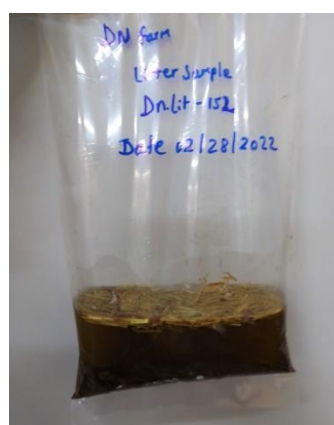
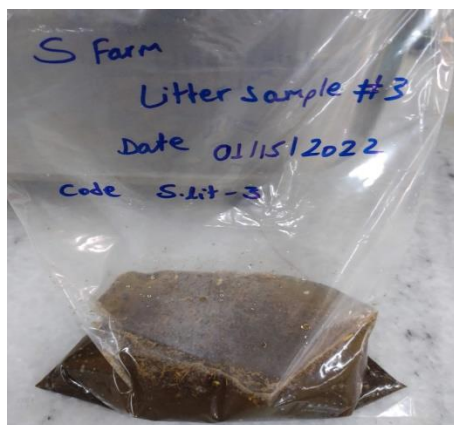
30. What else could be done to improve the gut health of birds other than antibiotics?

Annex 3

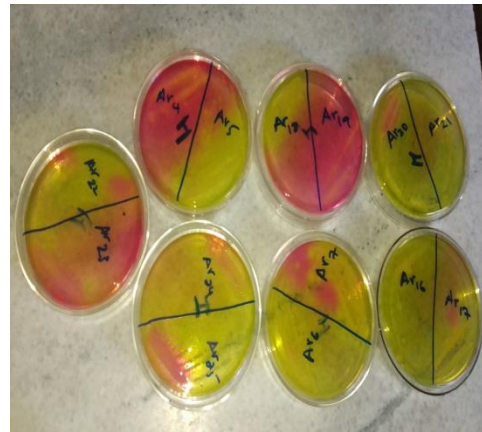
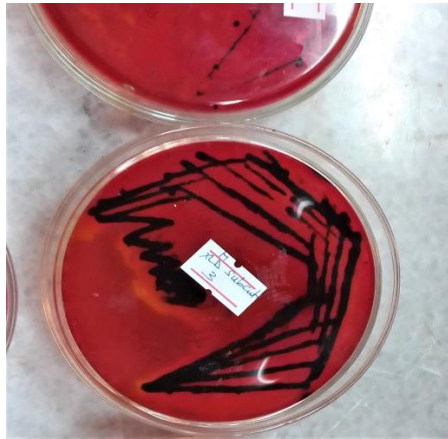
Pictures of laboratory related works



Lovely farm handler helping me to take cloaca swabs and the swabs put into BPW test tubes to transport in ice box.



Environmental samples taken from poultry farms added 225 ml BPW from the left and 45ml BPW for water sample in the last right.



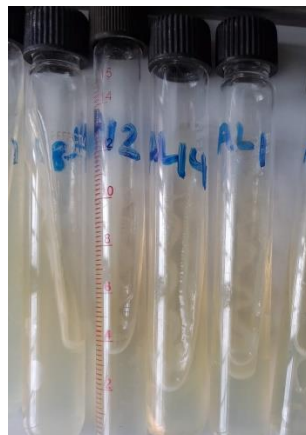
a) XLD agar inoculated suspected to confirm via biochemical test to b) To the right BGA agar inoculates greenish colony taken as a negative pinkish positive for *Salmonella* further biochemical tests done.



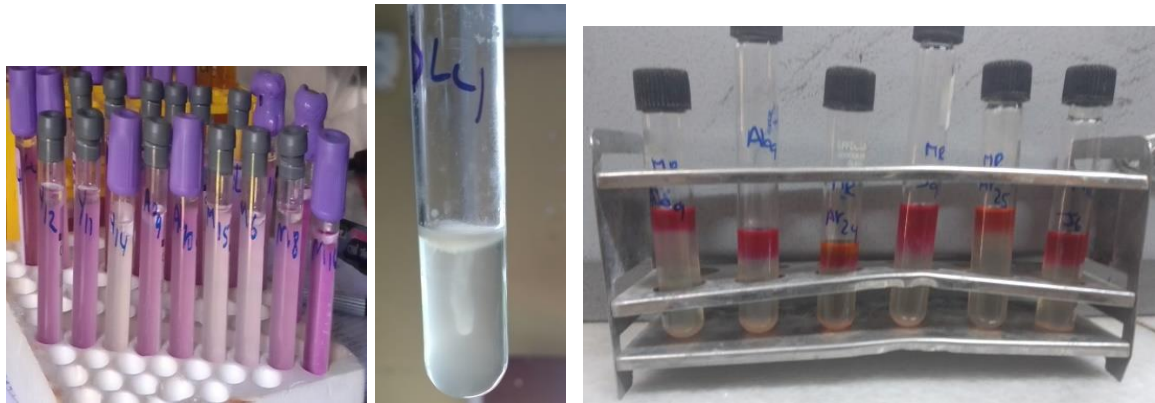
XLD agar inoculate mucoid like colony



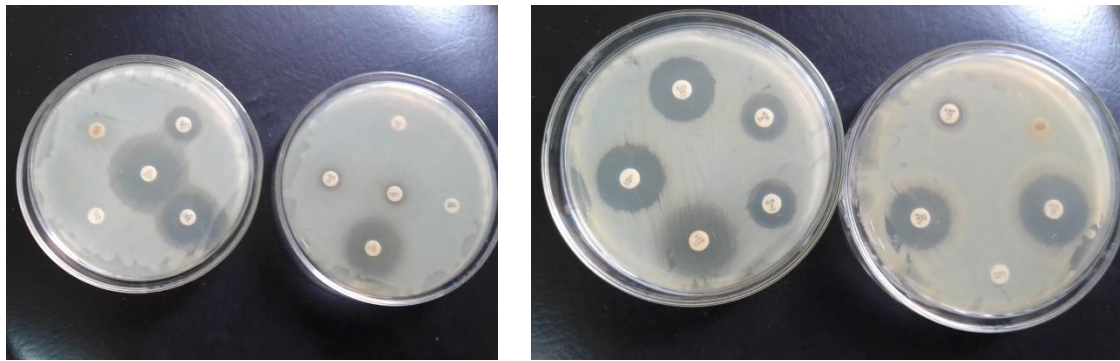
XLD agar inoculates NGs for *Salmonella*



TSI slant test, Urease agar slant, Indole test from left to the right respectively



Lysine decarboxylase, Motility test, MRVP tests



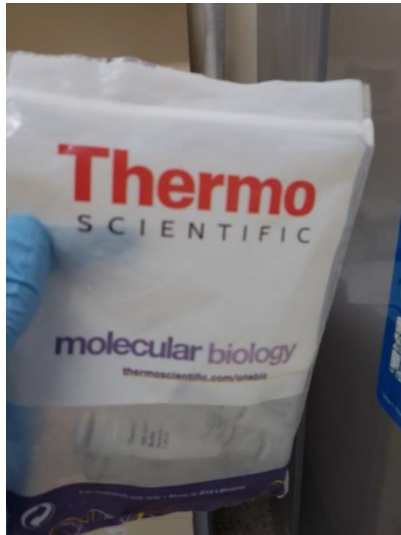
Muller Hilton agar inoculates with antimicrobial discs for antimicrobial sensitivity test



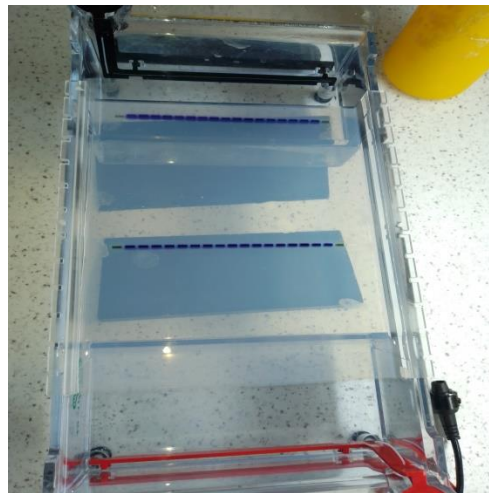
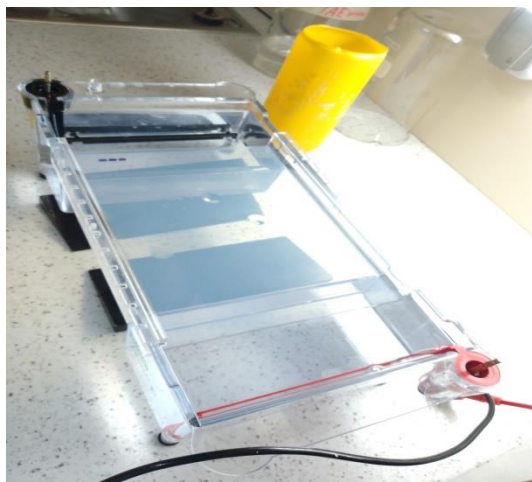
My samples at reception of NVI



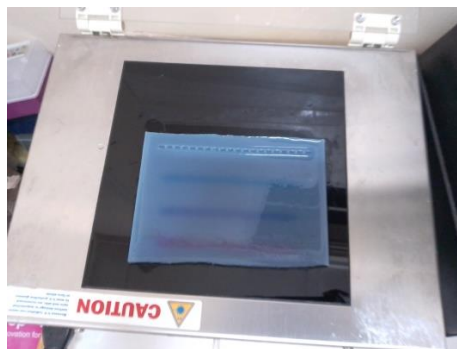
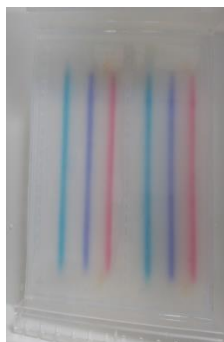
Molecular biology lab DNA extraction room NVI



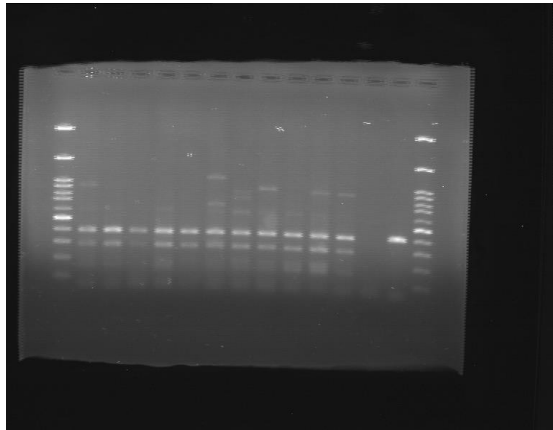
Master mix reagents (Thermo scientific) and Mr. Getaw putting the mixture on conventional PCR for amplification at NVI molecular biology laboratory



Loading the amplified and dye loaded amplicon to the 2% Agarose Gel wells formed



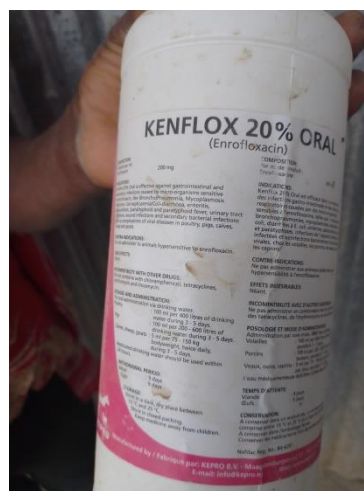
Pictures showing Completed Gel electrophoresing, Cut and put on Transilluminator, and camera out put on computer to the right end



Unsuccessful trial of re amplified the DNA extract used for the first Salmonella.Typhimurium finger print but the output has double bands and none specific bands again even with adjusted annealing temperature of 57⁰C.



Postmortem diagnose of the Marek's incident in one of the farms went for sampling. Some of the samples confirmed for Marek's at NAHDIC by my friend.



Farm owner's asked to show antimicrobials or the drug they are referring, Even I have gone to the garbage to get the names and confirm of antimicrobials



One of Poultry farm shades in Saris adis sefer Nifas silk subcity A.A