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ANTIBIOTIC USE AND ANTIBIOTIC RESISTANCE OF *SALMONELLA* SPECIES IN DIFFERENT POULTRY FARMS IN SELECTED DISTRICTS OF EAST SHEWA ZONE, OROMIA REGIONAL STATE, CENTRAL ETHIOPIA

Ph.D. DISSERTATION

BY

MEZENE WOYESSA BUSSA

Addis Ababa University, College of Veterinary Medicine and Agriculture
Department of Microbiology, Immunology and Veterinary Public health
Ph.D Program in Veterinary Public Health

June, 2021

Bishoftu, Ethiopia

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A dissertation submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Veterinary public health

By

Mezene Woyessa Bussa

June, 2021

Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology and Veterinary Public health

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As members of the Examining Board of the final PhD open defense, we certify that we have read and evaluated the dissertation prepared by Mezene Woyessa Bussa entitled: "Antibiotic use and antibiotic resistance of *Salmonella* species in different Poultry Farms in selected districts of East Shewa Zone, Oromia Regional State, central Ethiopia', and recommend that it be accepted as fulfilling the dissertation requirement for the Doctor of Philosophy in Veterinary public health.

Prof. Bekele Megersa	_____	_____
Chairman	Signature	Date
Dr. Bedaso Mammo	_____	_____
Internal Examiner	Signature	Date
Prof. Adem Hiko	_____	_____
External Examiner	Signature	Date
Prof. Gezahegn Mamo	_____	_____
Major advisor	Signature	Date
Dr Balako Gumi	_____	_____
Co-advisor	Signature	Date

I hereby certify that I have read the revised version of this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dr Gezahegn Mamo	_____	_____
Major advisor	Signature	Date
Dr. Balako Gumi	_____	_____
Co-advisor	Signature	Date

STATEMENT OF THE AUTHOR

I first, declare that this dissertation is my original work and that all sources of materials used for this dissertation have been duly acknowledged. This dissertation has been submitted to the requirements for PhD degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University's Library to be made available to borrowers under rules of the Library. I seriously declare that this dissertation is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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Name: Mezene Woyessa Bussa Signature: _____

College of Veterinary Medicine and Agriculture

Date of Submission:_____

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LIST OF ABBREVIATIONS

AAU	Addis Ababa University
AGP	Antimicrobial growth promoter
AIDS	Acquired immunodeficiency syndrome
AMR	Antimicrobial resistance
AMU	Antimicrobial use
BGA	Brilliant green agar
BPW	Buffered peptone water
CAT	Chloramphenicol acetyltransferases
CDC	Centre for Disease Control and Prevention
CI	Confidence interval
CIA	Critically important antimicrobials
CLSI	Clinical Laboratory Standards Institute
DCA	Desoxycholate citrate agar
DHFR	Trimethoprim inhibits dihydrofolate reductase
DHPS	Sulfonamides inhibit dihyrdropteroate synthetase
DNA	Deoxyribonucleic acid
DSE	Delayed secondary enrichment
DTs	Definitive phage types
ECDC	European Centre for Disease Control and Prevention
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
HACCP	Hazard Analysis Critical Control Point
HIV	Human immunodeficiency virus
iNTS	Infectious non typhoidal salmonella
ISO	International standard organization
LMICs	Low and middle income countries
MDR	Multidrug resistance
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing

MLVA	Multiple-locus variable-number tandem repeat analysis
MOA	Ministry of agriculture
MR-VP	Methyl red-Voges-Proskauer
NTS	Non typhoidal *Salmonella*
OIE	World Organization for Animal Health
OR	Odds ratio
PBP	Penicillin-binding proteins
PCR	Polymers chain reaction
PCU	Population correction unit
PFGE	Pulsed-field gel electrophoresis
RNA	Ribonucleic acid
RV	Rappaport-Vassiliadis
SPSS	Statistical process for Social Sciences
SSI	Statens Serum Institute, Copenhagen
TSIA	Triple sugar iron agar
TSI	Triple sugar iron
USA	United States of America
WHO	World Health Organization
XLD	Xylose lysine deoxycholate

DEDICATION

This dissertation is dedicated to my father and my mother for their nursing me with affection and love and dedication in the success of my life.

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Mezene Woyessa Bussa

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ABSTRACT

Antibiotics are limited resources. The more antibiotics are used today, the lesser it is likely they will still be effective in the future. Antibiotic resistance is considered one of the major threats to the world's health as antibiotic resistant infections are increasing in humans, animals and the environment from time to time. Misuse and overuse of antibiotics is a primary contributor for the development of antibiotic resistance. The World Health Organization estimates that in the past decade the number of deaths attributed to antibiotic resistant bacteria exceeded the combined number of deaths due to influenza, human immunodeficiency virus and traffic accidents. Salmonella is the major cause of foodborne zoonotic bacterial infections worldwide and poultry is a major source of this zoonotic infection. Drug resistant Salmonella, such as quinolone and the higher generation cephalosporin resistant strains are regarded by World Health Organization as a critically important highest priority pathogen. Hence, a cross sectional study was conducted from September, 2017 to January, 2021 to determine the prevalence and antibiotic resistance of Salmonella species isolated from poultry and to evaluate a prudent use of antibiotics in intensive and semi-intensive commercial and backyard poultry farms in a selected districts (Ada'a, Lome, Akaki and Adama) of East Shewa zone of Oromia regional state, central Ethiopia. A multi-stage sampling technique was used to select districts, peasant association and poultry farms. A total of 780 samples (500 chicken fecal, 153 egg, 100 chicken meat and 27 hand swab from poultry farm workers) were collected for Salmonella isolation and identification. Three hundred eighty eight individuals (n=388) were included in the study for the assessment of prudent use of antibiotics using structured questionnaire surveys. Based on microbial isolation and identification, the prevalence of Salmonella was 18.4% (95% CI=14.6-21.3) in the fecal samples, 14.8% (95% CI=1.5-28.5) in the hand swabs of poultry farm workers, 4.5% (95% CI=1.2-7.7) of eggs and 6% (95% CI=1.3-10.7) of meat samples with the overall prevalence of 13.97% (95% CI=11.6-16.4).

The highest prevalence of salmonella infection was observed in intensive production system (16.9%) and the lowest was found in backyard scavenging system (7.4%). One of the serious zoonotic strains (S. typhmurium) was recorded as a dominant serovar (69.7%) followed by S. Saintpaul (18.2%), S. Kentucky (6%), S. Newport (3%) and S. Anatum (3%) during this study. Risk factors such as poultry production system (P=0.006), production type (P=0.001), breed of chicken (P=0.005) and sample type (P=0.001) were significantly associated with Salmonella prevalence. A total of 37 Salmonella isolates were tested for their resistance against 15 antibiotics using disc diffusion method. Majority of the isolates (64.86%) were resistant or intermediately resistant to at least one antibiotic. The prevalence of resistance was high to chloramphenicol (62.2%), tetracycline (59.5%), ampicillin (54.1%) and streptomycin (51.4%). More than half of the isolates (56.8%) were multidrug resistant. The widespread occurrence of drug resistant Salmonella in poultry farms clearly demonstrated that, there is lack of awareness about prudent use of antibiotics, which highly contributed to the development of antibiotic resistant bacteria. All farms used one or more antibiotics and administered them mainly through feed/water. Tetracycline (100%) and sulfadiazine + trimethoprim (94.1%), fluroquinolones (41.5%) and cloxacillin + ampicillin (29.1%) were the most frequently used antibiotics. Antibiotics were used for disease treatment (100%), for disease prevention (56%) and /or for growth promotion (32.2%). Veterinary pharmacies (100%), veterinary clinic (51.0%), human pharmacies (26.8%) and illegal open market (16.2%) were the sources of antibiotics for the poultry producers. Sixty two percent of the farms obtained antibiotics through prescription from veterinarian and the rest were as self-prescription (32.2%) or as recommended by friends with prior experience (11.9%). Only 37.9% of the prescribed antibiotics administered by veterinary professional while majority of the farms administered antibiotics by themselves, based on the drug labels or as directed by a prescriber or pharmacist. Antibiotic stewardship programs such as removing the use of antibiotics for growth promotion, controlling illegal open market access as well as increasing veterinary and diagnostic services will help to mitigate antibiotic resistance. Measures to control of Salmonella infections in poultry are needed to reduce foodborne infections in humans.

***Keywords**: Antibiotic use, antibiotic resistance, poultry, Salmonella*

1. INTRODUCTION

Poultry is one of the most widespread food animal industries worldwide. Chicken is the largest farmed animal species, with over 90 billion tons of chicken meat produced per year(FAO, 2018). Large amounts of antimicrobials are used to raise poultry in most countries(Awogbemi *et al.*, 2018). Large numbers of such antimicrobials are considered essential in human medicine(Furgasa & Beyene, 2018). Indiscriminate use of essential antimicrobials in animal production is likely to accelerate the development of antibiotic resistance in pathogenic and commensal bacteria (Langata *et al.*, 2019). Antimicrobial resistance (AMR) would result in treatment failures, economic losses and could act as source of gene pool for transmission to humans(Antunes *et al.*,2016).

The problem of bacterial resistance to antibiotics is a burning question throughout the world(Chattopadhyay *et al.*, 2014). According to an estimate by the World Health Organization (WHO), during the past decade the number of deaths caused by some resistant strains exceeded the combined number of deaths caused by influenza, human immunodeficiency virus and traffic accident (Chattopadhyay *et al.*, 2014). Over the past few decades, new antibiotics have not been produced and almost all known antibiotics are increasingly losing their activity against pathogenic microorganisms (Paulson *et al.*, 2015). The levels of multi-drug resistant bacteria have also increased (Adesiji *et al.*, 2014). It is known that worldwide, more than 60% of all antibiotics are used in animal production both for therapeutic and non-therapeutic purposes(Christian *et al.*,2018).

According to the World Organization for Animal Health (OIE) annual report on antimicrobial agents intended for use in animals (OIE, 2018), in many countries antibiotics were widely available virtually with no restriction or control. Of 143 OIE member countries and three non-OIE member countries assisted through the OIE performance of veterinary service pathway, as of 2019, seven countries did not yet have completed relevant legislation to ensure appropriate condition for import, distribution, manufacturing and use of veterinary medicinal products including antimicrobial agents.

As a result, these products circulate freely, like ordinary goods, and are often falsified or substandard. Inappropriate use of antimicrobials creates condition for high risk for the development and spread of antibiotic resistance(Nhung *et al*., 2017). In recent years, enough evidence highlighting a link between excessive use of antimicrobial agents and antibiotic resistance from animals as a contributing factor to the overall burden of antibiotic resistance has emerged (Odeyemi and Sani, 2016). The extent of usage is increased markedly over coming years due to intensification of farming practices in most of the developing countries(Christy *et al.*, 2018). In general livestock producers are using antibiotics in their farm animals for different purpose (prevention of infections, treatment of infections and growth promotion) particularly in commercial poultry and dairy farms(Van Boeckel *et al*.,2015).

Poultry are important reservoirs for many zoonotic bacterial pathogens such as *Salmonella* (Mir *et al.*,2015). The presence of *Salmonella* in healthy poultry is suggested as the main risk factor for its transmission to humans through table eggs and poultry meat (Antunes *et al*., 2016). The prevalence of *Salmonella* associated with poultry and poultry products is high and this high prevalence has both public health and economic implications(Cosby *et al*. 2015). The globalization of the food supply and the increased movements of people, animals and goods have increased the burden of *Salmonella* infections in several countries (Shrestha *et al*., 2016).

Rational of the study

Currently, there is a rapidly growing commercial poultry production in Ethiopia(FAO, 2008). The government of Ethiopia has prepared a five-year strategic development plan for 2015 to 2020 identifying the poultry production as an important sub-sector (MOA, 2015). The plan was to increase the total broiler production by 235% and the total egg production by 828% in 2020. The goal of the plan was to reduce poverty, improve household nutrition as well as increased income. Such transformative production systems may however lead to unrecognized risks to human health in terms of antibiotic use and

antibiotic resistance as a result of emerging intensified production systems, which often rely on antibiotics as a stopgap for disease control and prevention in low and middle income countries in place of improving hygiene and sanitation in large-scale operations and other management inadequacies leading to increased exposure to bacterial pathogens(Nhung *et al.*, 2017).

As several studies from different developed countries reveal that, *Salmonella* serotypes isolated from foods of animal origin have multidrug resistance profiles (Benacer *et al*., 2010). The role of poultry products in the dissemination of antimicrobial-resistant zoonotic bacterial pathogens also well documented in developed countries(Basler *et al*., 2016). A review made by(Omulo *et al*., 2015) of 40 years (1974 and 2013) of enteric antimicrobial resistance research both in humans and animals in Eastern Africa summarized that despite the high burden of disease in sub-Saharan Africa and the potential health and economic consequences, the level of research on antimicrobial resistance in the region remains unknown. Local knowledge on the prevalence of *Salmonella*, serotype distribution and associated risk factors is important to implement appropriate control strategy to reduce wider dissemination of important zoonotic serovars. While antibiotic resistance is recognized as a 'one health' issue, and international organizations such as WHO, OIE, Food and Agriculture Organization of the United Nations (FAO) have been adopting global action plan to combat antibiotic resistance(WHO, 2015) data on antibiotic use and antibiotic resistance in poultry production system in Ethiopia is limited. This study is therefore aimed with the following objectives:

General objective:

- To assess rational use of antibiotics and investigate antibiotic resistance using *salmonella* as a model in different poultry production system in selected districts of East Shewa Zone (Ada'a, Lome, Akaki and Adama) of Oromia Regional State, central Ethiopia.

Specific objective:

- To assess rational use of antibiotics in different poultry production system in selected districts of East Shewa Zone (Ada'a, Lome, Akaki and Adama) of Oromia regional state, central Ethiopia (Paper I).
- To investigate the prevalence, antibiotic resistance and zoonotic aspect of *Salmonella* species isolated from chicken feces, meat, egg and hand swabs poultry farm worker (Paper II).

2. LITERATURE REVIEW

PART I- ANTIMICROBIAL USE AND ANTIMICROBIAL RESISTANCE

2.1. Antimicrobial use in livestock

Antimicrobial agents, especially antibacterial agents, are used throughout the world, across a diverse array of extensive and intensive livestock production systems, to protect the health and welfare of livestock and to improve their performance. Many bacterial diseases of livestock cause devastating losses of animal life and productivity. As a result, their keepers can lose their livelihoods and see a dramatic reduction in income, so there is often a great sense of urgency to treat affected animals early (Hao *et al.*, 2014).

Even though, there are a large number of bacterial pathogens that cause disease and death in animals, still there are many ways in which existing uses of antimicrobial agents can be reduced, amongst the most important are increased utilization of veterinary professional services, the introduction of enhanced infection control measures, improved point-of-care diagnostic tests, and the application of physiologically based population pharmacokinetic-pharmacodynamic-modelling (You and Silbergeld, 2014).

Of great concern, the uses, types, and mode of actions of the antibiotics employed in agriculture and veterinary practice are closely related or the same to those prescribed to humans (Hao *et al.*, 2014). Clearly, the choice of antibiotics and the antimicrobial consumption pattern demonstrates geographical variation across the continents being influenced by the food animal species, regional production patterns and types of production system, purpose of farming, lack of clear legislative framework or policies on the use of antibiotics, as well as the size and socioeconomic status of the population, and the farmers in particular (Bester and Essack,2012).

2.1.1. Therapeutic use of antimicrobial agents

Treatment, prophylaxis and metaphylaxis are applied at different times during pathogen challenge and categorized as therapeutic use. In intensive livestock operations the objective is to minimize the occurrence of disease, and significant resources are generally applied to mitigate disease risk factors by ensuring each animal is as robust and resistant to pathogen challenge as possible (Cameron and Mcallister, 2016).

The value of prophylaxis in reducing pathogen challenge and disease and enhancing animal health and welfare has been demonstrated repeatedly for necrotic enteritis in broilers and bovine respiratory disease (Mcewen and Fedorka-cray, 2002). As diagnostic capacity improves, selective metaphylaxis is likely to become more common with reduced use of antimicrobial agents consistent with high animal health. There is abundant evidence that metaphylaxis has significant benefits in reducing the impact of bovine respiratory disease, lameness in ewes and diarrhoea in pre-weaned calves (Lorenz *et al.*, 2011).

The prominent use of the penicillins, tetracyclines, macrolides and aminoglycosides is notable, especially since each of these classes has been in use for more than 50 years (Van Boeckel *et al.*,2015). For instance, in terms of used daily doses, a survey of 32 Belgian broiler farms showed that the main antimicrobial agents employed were macrolides, lincosamides, sulphonamide-trimethoprim and penicillins, with necrotic enteritis and the poorly defined ‘dysbacteriosis’ the major reasons for use. In Danish, the antimicrobial agents most commonly used to treat enteric disease in nursery or weaner pigs were pleuromutilins, tetracyclines, macrolides and the combination of a sulphonamide and trimethoprim. Worldwide the treatment of mastitis is one of the most common reasons for administering antimicrobial agents to dairy cattle (Marshall and Levy, 2011; Van Boeckel *et al.*,2015)

2.1.2. Nutritional use of antimicrobial agents

Antimicrobials especially antibiotics as growth promotant was discovered in the 1940s, when it was observed that chicken improve in growth when fed bacterial shells of Streptomyces aureofaciens from which antibiotics had been extracted. Because the amount of antibiotic that can provide growth enhancement was extremely small, the effect was regarded as largely nutritional by producers and authorities in the food industry (Krishnasamy *et al.*, 2015). In the years to follow, other countries also allowed the use of antibiotics in animal feeds. For the nutritional uses of antimicrobial agents (antibiotic growth promotion), it is not possible to select a dose regimen based on the minimum inhibitory concentration (MIC) of any particular bacterial species as the specific mode (s) of action remains elusive (Furgasa and Beyene, 2018).

There are abundant theories to explain the mode of action. Among the hypotheses tested the mechanism action of growth promoters are the following: Stimulation of intestinal synthesis of vitamins by bacteria, reduction in total numbers of bacteria in the intestinal tract with a lowering of competition between microorganisms and host animals for nutrients, inhibition of harmful bacteria which may be mildly pathogenic or toxin-producing, inhibition of bacterial urease, improved energy efficiency of the gut, inhibition of bacterial cholytaurin hydrolase activity, nutrient sparing, improved nutrient absorption from morphological changes to small intestinal epithelium, modification of intestinal enzyme activity, reduced immune stimulation and modification of rumen microbial metabolism(Furgasa & Beyene, 2018).

2.2. Global trend, its concern and action plan on antimicrobial use in livestock

2.2.1. Antimicrobial use trend

Global antimicrobial consumption in terrestrial and aquatic food animal production is accelerating, associated with expanded production to meet increasing demand for animal-

source nutrition (Van Boeckel *et al.*, 2017). In South Asia, for example, demand for poultry between 2000 and 2030 is expected to increase by 725% (FAO, 2018). Overall, growth in demand for animal source nutrition through 2030 is anticipated to be higher in low and middle income countries (LMICs) as compared with high income countries (FAO, 2018). Efforts to meet rising demand for animal-source nutrition in LMICs are driving a shift in animal production from small holder, mixed crop, and livestock operations to increasingly intensive, large-scale, and specialized commercialization (FAO, 2009). Intensive production systems have historically employed nontherapeutic antimicrobial use, particularly during transitionary stages. Nontherapeutic antimicrobial use includes both mass administration for prevention and control of disease and antimicrobial growth promoter (AGP) use(Van Boeckel *et al.*, 2017).

In 2013, the global consumption of all antimicrobials in food animals was estimated at 131,109 tons and is projected to reach 200, 235 tons by 2030. Consumption levels varied considerably between countries, ranging from 8 mg/population correction unit (PCU) (a kilogram of animal product) in Norway to 318 mg/PCU in China (Van Boeckel *et al.*,2015).

In relative terms, humans and animals use comparable amounts of antimicrobials 118 mg/PCU and 133 mg/kg, respectively, but given that the biomass of animals raised for food exceeds by far the biomass of humans, new resistant mutations are more likely to arise in animals. Furthermore, a central distinction between animals and humans is the purpose of antimicrobial use. Unlike in humans, antimicrobial use in animals is primarily intended for growth promotion and mass prophylaxis. These uses are often administered both through feed, directly targeting the gut, and in low-dose patterns that promote the evolution of resistance. These factors suggest that the food animal reservoir is a greater source of resistance genes than humans. However, the subsequent spread of those genes to humans follows complex pathways, and recent work has highlighted that curtailing antimicrobial use in animals alone will not suffice to contain antimicrobial resistance (AMR) in humans (Van Bunnik and Woolhouse, 2017).

2.2.2. Antimicrobial use concern: antimicrobial resistance development

Today, antibiotic resistance has become a significant and increasing public health problem internationally. Owing to antibiotic resistance, infections that normally responded to antibiotic treatment have become difficult and sometimes impossible to cure. This causes treatment failures and increased morbidity, mortality and costs to society (Enright *et al*., 2002).

The use of antibiotics for any purpose-in people, animals and plants anywhere in the world affects everyone. Antibiotic use in one individual can promote the local survival of resistant strains that can subsequently spread from that individual to others and potentially, over time, to any community worldwide. It is known that worldwide, more than 60% of all antibiotics that are produced find their use in animal production for both therapeutic and non-therapeutic purposes. The use of antimicrobial agents in animal husbandry has been linked to the development and spread of resistant bacteria (Kariuk and Dougan, 2014).

A surge in the development and spread of antibiotic resistance has become a major cause for concern. Over the past few decades, no major new types of antibiotics have been produced and almost all known antibiotics are increasingly losing their activity against pathogenic microorganisms. The levels of multi-drug resistant bacteria have also increased (Woolhouse *et al*., 2015).

The epidemiology of antibiotic resistance is made more complicated by the ability of genes responsible for such resistance to spread between different types of bacteria. Also, antibiotic-resistant bacteria can spread across sectors, settings and geographical borders. This spread can be attributed to people, animals, animal products or environmental contamination. This bacterial ability is mainly facilitated by irrational use of antibiotics in different sectors including misuse in food animal industry, chronic clinical over-prescription and public misconceptions (Fair and Tor, 2014).

Misuse by food animal industry

Treatment, prophylaxis and growth promoters are the commonly uses of antibiotics in food-producing animals and is essential for a sustainable and economically sustainable animal industry. However, the application of these antibiotic drugs in animals, particularly in food animals, may lead to a selection of resistant strains of bacteria, which in turn may proceed to infect both animals and human (Moyane *et al.*, 2013).

Several authors have demonstrated the indiscriminate use of antibiotics by farmers, and attributed it to lack of knowledge on the prudent use of these drugs, and the possible adverse effects associated with their abuse, non-adherence to manufacturer's instructions, and the antibiotic withdrawal periods, unavailability of veterinarians and their services. Furthermore, inexperienced farmers relied on the knowledge and advice of experienced farmers and local drug sellers for drug administration, and above all, wealthy farmers tended to employ multiple antibiotics, since they have the potential to acquire the drugs. Also, very few animals are referred to the laboratory for diagnosis to identify the causative agent and to assess the antibiotic susceptibility testing prior to antibiotic application(Odeyemi and Sani, 2016; Schar *et al.*, 2018).

The extent of indiscriminate antibiotic usage in livestock industry is increasing markedly over the past years due to intensification of farming practices particularly in almost all of the developing countries (Chattopadhyay *et al.*, 2014). On top of this, enough evidence

highlighting a link between excessive use of antimicrobial agents and antimicrobial resistance from animals as a contributing factor to the overall burden of antimicrobial resistance (Paulson *et al.*, 2015).

Generally, in the developing countries, the level and rate of antibiotic utilization in the farming sector might be influenced by the manner in which the farmers acquire (over the counters) and use these antibiotics (multidrug practices), and also, the presence of existing factors. The existing factors include a high prevalence or level of infections, profound scarcity of state management and development strategies, shortfall in husbandry zone planning, negligible hygienic practices in livestock husbandry in conjunction with the presence of an integrated agricultural system (Chowdhury *et al.*, 2009).

According to Woolhouse *et al.*, (2015), antibiotic resistance in livestock farming can be looked at from four different viewpoints, i.e., the animals, animal derived products, farm workers, and farm environmental sites. All these constitute the several compartments and different niches in the farm described as an ecosystem. Undoubtedly, farm animals are a very important component in understanding the interplay between humans, animals, and the environment regarding bacteria, antibiotics, and antibiotic resistance gene movement (Woolhouse *et al.*, 2015).

Chronic clinical over-prescription and public misconceptions

The other factor fueling antibiotic resistance is the evolution and dissemination of resistance factors within bacterial populations. There are a plethora of means by which humans have inadvertently accelerated the evolution of bacterial resistance. The over prescription of antibiotics by doctors for symptoms that in many cases may not be caused by bacteria has historically been one such problematic policy (Friedman *et al.*, 2016). A lack of public knowledge about antibiotics has also led to their overuse. In a 2009 European survey, of those who had taken antibiotics within the last year, 20% claimed to have taken them for influenza, a viral malady, and only 36% of those surveyed answered correctly that antibiotics do not kill viruses. This particular variety of misuse is especially

problematic in countries where antibiotics can be obtained without prescriptions(BEUC, 2014).

Diminished pharmaceutical investment

A flagging interest in antibiotics by the pharmaceutical industry is one factor that has contributed to an increased occurrence of hard to treat bacterial infections. In 2004 for example, only 1.6% of drugs in clinical development by the world's 15 largest drug companies were antibiotics. This reduced output of antibiotics has several causes. Antibiotics regimens are typically administered for very limited durations making them far less profitable than drugs used to treat chronic ailments. Further, newly approved drugs for most other ailments are immediately prescribed, whereas new antibiotics are typically held in reserve and only prescribed for infections that more established antibiotics can't treat. This policy helps delay the emergence of resistant strains, but it also limits initial investment return. A market saturated with generic competitors and the inevitable growth of bacterial resistance exacerbates this profit disparity as compared to other drugs in the long term (Landers, 2012; Hao *et al.*, 2014).

Regulatory hurdles have also muted the interest of major pharmaceutical companies. The tolerance of adverse side effects has recently been decreased for many drug classes, including antibiotics. Approval requirements during clinical trials have escalated in most cases from demonstration of non-inferiority to superiority, and at times a lack of clear trial guidelines for antibiotics, in particular, have stifled development. Pharmaceutical companies are presented with a paradox where in federal agencies issue calls for antibiotic development while concomitantly other federal agencies enact policies limiting the appeal of that very development(Spellberg, 2014; Friedman *et al.*, 2016).

2.2.3. Global action plan against antimicrobial resistance

Antimicrobial resistance threatens the very core of modern medicine and the sustainability of an effective, global public health response to the enduring threat from infectious diseases. Effective antimicrobial drugs are prerequisites for both preventive and curative measures, protecting patients from potentially fatal diseases and ensuring that complex procedures, such as surgery and chemotherapy, can be provided at low risk. Yet systematic misuse and overuse of these drugs in human medicine and food production have put every nation at risk. Few replacement products are in the pipeline. Without harmonized and immediate action on a global scale, the world is heading towards a post-antibiotic era in which common infections could once again kill (Friedman *et al*., 2016).

Alert to this crisis, the World Health (WHO, 2015) assembly adopted a global action plan on antimicrobial resistance, which outlines five objectives: To improve awareness and understanding of antimicrobial resistance through effective communication, education and training; To strengthen the knowledge and evidence base through surveillance and research; To reduce the incidence of infection through effective sanitation, hygiene and infection prevention measures; To optimize the use of antimicrobial medicines in human and animal health; To develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines, diagnostic tools, vaccines and other interventions.

FAO's Thirty-ninth Conference (FAO, 2018) adopted Resolution 4/2015 on AMR which recognized that it poses an increasingly serious threat to public health and sustainable food production, and that an effective response should involve all sectors of government and society.

To support the implementation of Resolution 4/2015, the FAO Action Plan on AMR addresses four major Focus Areas: Improve awareness on AMR and related threats; Develop capacity for surveillance and monitoring of AMR and antimicrobial use (AMU)

in food and agriculture; Strengthen governance related to AMU and AMR in food and agriculture; Promote good practices in food and agricultural systems and the prudent use of antimicrobials. This Action Plan supports the WHO-led Global Action Plan on Antimicrobial Resistance in highlighting the necessity of adopting a "One Health" approach, with the involvement of public health and veterinary authorities, the food and agriculture sectors, financial planners, environmental specialists, and consumers.

All action plans of WHO, FAO and OIE (2015) in one way or the other reflect the following principles:

Whole of society engagement including a one health approach: Antimicrobial resistance will affect everybody, regardless of where they live, their health, economic circumstances, lifestyle or behavior. It will affect sectors beyond human health, such as animal health, agriculture, food security and economic development. Therefore, everybody in all sectors and disciplines should be engaged in the implementation of the action plan, and in particular in efforts to preserve the effectiveness of antimicrobial medicines through conservation and stewardship programs (WHO, FAO and OIE, 2015).

Prevention first: Every infection prevented is one that needs no treatment. Prevention of infection can be cost effective and implemented in all settings and sectors, even where resources are limited. Good sanitation, hygiene and other infection prevention measures that can slow the development and restrict the spread of difficult to treat antibiotic resistant infections are a "best buy" (WHO, FAO and OIE, 2015).

Access: The aim to preserve the ability to treat serious infections requires both equitable access to and appropriate use of the existing and new antimicrobial medicines. Effective implementation of national and global action plans to address antimicrobial resistance depends also on access, inter alia, to health facilities, health care professionals, veterinarians, preventive technologies, diagnostic tools including those which are "point of care", and to knowledge, education and information (WHO, FAO and OIE, 2015).

Sustainability: All countries should have a national action plan on antimicrobial resistance that includes an assessment of resource needs. The implementation of these plans will require long-term investment, for instance in surveillance, operational research, laboratories, human and animal health systems, competent regulatory capacities, and professional education and training, in both the human and animal health sectors. Political commitment and international collaboration are needed to promote the technical and financial investment necessary for effective development and implementation of national action plans (WHO, FAO and OIE, 2015).

Incremental targets for implementation: Member States are at very different stages in terms of developing and implementing national plans to combat antimicrobial resistance. To enable all countries to make the most progress towards implementing the global action plan on antimicrobial resistance, flexibility will be built into the monitoring and reporting arrangements (WHO, FAO and OIE, 2015).

2.3. Consequences of antimicrobial resistance

Broadly speaking, infections caused by resistant bacterial strains lead to up to two-fold higher rates of adverse outcomes compared with similar infections caused by susceptible strains. These adverse outcomes may be clinical (death or treatment failure) or economic (costs of care, length of stay) and reflect both treatment delays and the failure of antibiotic treatment to cure infections. The magnitude of these adverse outcomes will be more pronounced as disease severity, strain virulence, or host vulnerability increase. It is the cost of these treatment delays and failures to patients and the healthcare system that forms the basis of the negative impact of antibiotic resistance(Nji *et al.*, 2021).

2.3.1. Impact on public health and healthcare system

The negative impacts of antibiotic resistance on healthcare systems as a whole are substantial (Wassenberg *et al.*, 2010). Resistant bacterial spread reflects both additional infections caused by resistant strains and replacement of susceptible strains by resistant strains. There is evidence of additional infections caused by resistant strains rather than

merely a replacement of susceptible strains(Grace *et al.*, 2016). In other words, if before the onset of antibiotic resistance there were 100 cases of infection caused by susceptible strains, the onset of antibiotic resistance would result in 90 infections caused by susceptible strains, and 30 infections caused by resistant strains. The end result is 20 additional infections. The emergence and spread of epidemic clones of both vancomycin-resistant Enterococcus faecium and Acinetobacter spp. are good examples of additional infections caused by previously harmless commensals of the gastrointestinal tract and the environment, which became important pathogens causing nosocomial infection(Johnson *et al.*, 2010).

Increasing antibiotic resistance potentially threatens the safety and efficacy of surgical procedures and immunosuppressive chemotherapy. It is estimated that between 38.7% and 50.9% of pathogens causing surgical site infections and 26.8% of pathogens causing infections after chemotherapy are resistant to standard prophylactic antibiotics in the USA(Wassenberg *et al.*, 2010).

Within the healthcare system, there are cases in which antibiotic resistance may therefore limit available and often lifesaving treatment options. There is also an enormous impact of antibacterial resistance on day-to-day hospital activity (Wong *et al.*, 2014). Total closure of an affected ward or unit is one of the most expensive infection control measures that may be required to contain a nosocomial outbreak. In addition to these costs, are the consumable, microbiology and staff costs associated with the implementation of infection control measures, such as screening and contact isolation, intended to both prevent and eradicate multidrug resistance (MDR) bacteria from healthcare facilities(Doyle *et al.*, 2013).

2.3.2. Impact on animal health, welfare and food production

Globally, animals kept for food production are important sources of food, and food production is a significant contributor to world economy(FAO, 2013). The consequences of antibiotic resistance in bacteria are basically the same in human and veterinary

medicine. Loss of effective antibiotic treatments through resistance will cause suffering for the affected individual, regardless of whether it is a human being or an animal. There will also be economic consequences through increased treatment costs in animal and human health care. Loss of access to effective therapy will also lead to economic losses due to reduced productivity of the animals, and loss of effective therapy in human health care is also associated with losses of productivity and subsequently to societal costs (Page and Gautier, 2012).

A variety of contagious bacterial diseases cause illness and suffering of the animals and thereby bring on economic and welfare losses in food production (Cameron and Mcallister, 2016). Respiratory and enteric diseases are among the most important in several species, and mastitis is common in animals kept for milk production, mainly cows but also goats, sheep, and buffaloes (Page and Gautier, 2012). Bacterial diseases are important also in aquaculture where aquatic animals such as fish and shrimps are raised in large numbers with close contact between individuals (Oliva-Teles, 2012).

Lack of effective treatment for diseases will lead to suffering for the animals and welfare problems, which in turn lead to emotional stress for the keeper of the animals. Moreover, there will be financial losses directly through higher mortality and indirectly through decreased feed conversion, reduced production and growth, as well as early culling of breeding animals and dairy cows. Eventually this leads to higher costs of commodities from animal food production for the end consumer(Van Bunnik and Woolhouse, 2017).

2.4. Methods of determining antimicrobial resistance of bacterial isolates

The antibiotic resistance profile of bacterial isolates to available antibiotics can be determined by using multiple culture-based methods, with the key feature to isolate the target organism through growth on general multipurpose or selective and/or enriched microbiological media, and subsequent evaluation of their growth in response to specific antibiotic concentrations. The culture-based methods offer a link between antibiotic resistance measurement both in the environment and human clinical setting (Brown and

Macgowan, 2010). These cultured-based techniques are designed as susceptibility tests, and the resistance of the bacterium can be deduced directly from the susceptibility testing. The antibiotic susceptibility test involves both qualitative diffusion and quantitative dilution methods, amongst which is the Kirby–Bauer disc diffusion technique that implements the guidelines adopted from Clinical Laboratory Standards Institute (CLIS, 2019) In this methodology, the size (diameter) of the zone of inhibition developed around each disc placed on plates of microbiological medium inoculated with the pure culture of bacteria isolate is considered as the degree of sensitivity (Brown & Macgowan, 2010). The antibiotic susceptibility test with disc diffusion test is, however, regarded as a qualitative test to classify an organism as being susceptible or resistant, and paves the way for the better quantitative tests(CLIS, 2019).

Determining the MIC of an antibiotic against a bacterial isolate ensures the best quantitative estimate of susceptibility. The MIC describes the lowest or least concentration of a drug that is required to inhibit visible bacterial growth after overnight incubation. The MIC value gives an insight into the degree of resistance, the resistance mechanisms, as well as the resistance genes. It can be determined both by micro broth dilution in microplates, and agar dilution (ECDC, 2016).

In between, the E-test (Epsilometer test), combines the diffusion and dilution theories in susceptibility testing, whereby it determines whether the isolate is resistant or susceptible, based on the clear zone of inhibition. At the same time, it quantifies the sensitivity of the isolate by giving a discrete MIC value at the point where the clear zone of inhibition intersects on the test strip that harbors a predefined gradient of continuous antibiotic concentration ranges (Limbago, 2001).

In general, both the agar-based and broth dilution methods are faced with challenges, including cost, and that they are time-consuming and labour intensive. It is worth mentioning that the resistance level of a bacterium greatly depends on the type of test and test conditions applied for the determination of resistance, as well as the kind of antibiotics and its mode of action. It is necessary to conduct continuous surveillance of

the antibiotic resistance profile of bacteria, since bacteria are vulnerable to develop unpredictable resistance patterns, based on their genetic plasticity. In addition, the susceptibility patterns of particular bacteria changes with time, geographical location, country, and the prevailing environmental condition. The recovered information gives knowledge and serves as guidelines in antibiotic selection for treatment, to reduce the use of broad-spectrum antibiotics, and also to slow down resistance development, hence predict future resistance in bacterial isolates (Antunes *et al.*, 2016).

2.5. Tackling antimicrobial resistance in animal industry

2.5.1. Reduced need for the use of antimicrobial in animal husbandry

Antibiotics are probably the most valuable drugs in animal production, and maintaining their beneficial effect is therefore of utmost importance, if this is supported by prudent use. Focus should be given to the continuous implementation of appropriate measures for disease prevention, to decrease the need for antibiotics. This can be achieved by improving hygiene, biosecurity, health management on farms and by use of alternatives to uses of antibiotics (Mcewen and Fedorka-cray, 2002).

Management practices can both prevent disease and its transmission and enhance animal productivity. Improving living conditions for animals can lead to less stress and hence better immunity. Stress reduction can occur through better heat, humidity, and ventilation management, limiting the introduction of new pen mates, and increasing space per animal. Heat stress can be reduced through evaporative cooling and design changes in production buildings (Christy *et al.*, 2018).

Measures to promote biosecurity (the protection of agricultural animals from any type of infectious agent) can also reduce the incidence of disease at production facilities, reducing the need for antibiotics. One biosecurity measure is exclusion of potential pathogen carriers from the premises, including wild animals, domestic pets, and workers

not essential to the operation. Other measures include prompt removal of dead animals, increased hygiene within production facilities, and locating barns away from potential sources of infection. Optimizing nutrition can also encourage animal immunity. High-energy food rations can be fed to animals to increase growth and to reduce the effects of stressors. Adapting nutrients in feed to specific stages of growth can also boost immunity. In addition, supplementation with specific micronutrients (vitamins and minerals) has been shown to reduce disease(Van Boeckel *et al.*, 2017).

With the intelligent use of the panoramic range of disease prevention measures, a good health situation and improved economy of animal production can be achieved without the use of antibiotics, unless needed to treat sick animals. This can minimize the risk of spreading antibiotic resistance to human beings (Hao *et al.*, 2014).

Many methods and substitutes have been investigated for reducing the use of antibiotics in animal husbandry. Generally, these alternatives (Probiotics, Prebiotics, phytobiotics, Bacteriophage, etc.) can be divided into those used to prevent disease and those used for growth promotion. These two goals may overlap, as animals that are not fighting disease may grow faster or display greater feed efficiency (Sneeringer *et al.*, 2015).

2.5.2. Ensuring prudent use of antimicrobial in animal husbandry

As animals far outnumber people worldwide, the misuse of antibiotics in food-animal production has a broad adverse effect on environmental bacterial flora. Such misuse enhances the risk of developing antibiotic resistance and the associated risks for animal and public health. Ensuring the prudent use of antibiotics in animals is therefore of utmost importance (Spellberg, 2014).

WHO defines appropriate use as “the cost-effective use of antimicrobials which maximizes clinical therapeutic effect while minimizing both drug-related toxicity and the development of antimicrobial resistance” (WHO, 2015). In the context of food-animal

production, prudent use means eliminating non-therapeutic uses, including for growth promotion and as feed additives. Another definition of prudent antibiotic use is: the right drug for the right condition for the right amount of time(Spellberg, 2014).

International interaction is important. All parties must recognize that resistance caused by practices for using antibiotics in food animals in one country travels very quickly to other countries through food exports. A strong national policy for prudent antibiotic use is a necessary first step to minimize misuse of antibiotics in food animals and to reduce problems related to the occurrence of antibiotic resistance in the food-chain. The responsible and prudent use of antibiotics in food animals is intended to minimize potential harm to human health-particularly the development of antibiotic resistance-while ensuring the safe and effective use of antibiotics in veterinary medicine(OIE/FAO/WHO, 2004). The main principles of responsible and prudent use of veterinary antibiotics in food animals include:

- The need for antibiotics in food animals should be reduced by improving animal health through biosecurity measures (to prevent the introduction of harmful bacteria and the development of infections), disease prevention (including the introduction of effective vaccines, prebiotics and probiotics), and good hygiene and management practices.
- Antibiotics should be administered to food animals only when prescribed by a veterinarian.
- Antibiotics should be used only therapeutically, and use should be based on the results of resistance surveillance (microbial cultures and antibiotic susceptibility testing), as well as clinical experience.
- Use of antibiotics as growth promoters should be eliminated.
- Narrow-spectrum antibiotics should be the first choice when antibiotic therapy is justified.
- Antibiotics identified as critically important for human medicine-particularly fluoroquinolones and third and fourth-generation cephalosporins-should only be used in animals if their use is justified.

- The use of antibiotics in food animals should be limited to their approved and intended uses, take into consideration on-farm sampling and testing of isolates from food animals during their production, where appropriate and include adjustments to treatment when problems become evident.
- International guidelines on prudent use of antibiotics, adapted to countries, circumstances, should be followed at the national level. Veterinarians' professional societies should establish guidelines on the appropriate usage of antibiotics for different classes of food animals, including indications of first, second and last-resort choices for treating different bacterial infections.
- Economic incentives that facilitate the inappropriate prescription of antibiotics should be eliminated.

Part-II- *SALMONELLA* AND AMR

2.6. General Characteristics of *Salmonella*

The morphology of *Salmonellae* is similar to that of other enterobacteria. They are Gram-negative bacilli which are non-acid fast and non-sporing. These organisms usually occur as short rods, measuring 2-4 micrometer in length and 0.5 micrometer in diameter. Occasionally, they develop into longer pleomorphic forms or very short coccobacilli after prolonged culture on laboratory media. With the exception of S. Pullorum and S. Gallinarum, all strains normally possess peritrichous flagella and are actively motile. Occasionally non motile variants may be encountered in any serotype as a result of the occurrence of dysfunctional flagella. Many serotypes are also known to develop fimbriae(Antunes *et al*., 2016; Uro 2019).

The genus *Salmonella* contains two species; *S. enterica* and *S. bongori*, which was formerly subspecies. Six subspecies are differentiated within *S. enterica* based on their biochemical and genomic characteristics, a roman numeral and a name are used for the designation of these six subspecies as follows: I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*, and VI, *S. enterica* subsp. *indica*). With regard to food safety *S. enterica* subsp. *enterica* is the subspecies of most concern because the strains within these serogroups are known to cause 99% of *Salmonella* infections in humans (Pui, *et al*., 2011). *Salmonella* constitute more than 2500 serotypes that are highly adapted for growth in both humans and animals and that cause a wide spectrum of disease(De, Freitas *et al*., 2010).

Based on their association with human and animal hosts *Salmonella* can be classified into three main groups. The first group comprises S. Typhi and Paratyphi A and C, which infect only man, in whom these organisms cause enteric (typhoid) fever. The second group includes serovars that are host adapted for particular species of vertebrates,

example *S*. Gallinarum and S. Pullorum in poultry, *S*. Dublin in cattle, *S*. Abortus equi in horse, *S*. Abortus ovis in sheep and *S*. Choleraesuis and *S*. Typhisuis in swine. Some of these are also pathogenic for man (especially *S*. Dublin and *S*. Choleraesuis). The third group contains the majority of other *Salmonella* serovars with no particular host preference that infect both animals and man. The second and third group of *Salmonella* serotypes, referred to as non-typhoidal *Salmonella,* can colonize the gastrointestinal tracts of a broad range of animals, including mammals, reptiles, birds, and insects. More than 200 of these serotypes are pathogenic to humans, in which they often cause gastroenteritis and can also be associated with localized infections and/or bacteremia (Pui, *et al*., 2011; Mąka and Popowska, 2016)

Most *Salmonella* serotypes grow at temperature range of 5 to 45°C with optimum temperature of 35 to 37°C but some can grow at temperature as low as 2 to 4°C or as high as 54°C. They are sensitive to heat and often killed at temperature of 70°C or above. *Salmonella* grow in a pH range of 4 to 9 with the optimum between 6.5 and 7.5. They require high water activity between 0.99 and 0.94 (pure water, aw =1.0) yet can survive at water activity less than 0.2 such as in dried foods. Complete inhibition of growth occurs at temperatures less than 7°C, pH less than 3.8 or water activity less than 0.94 (Pui *et al*.,2011).

The organisms grow in selective enrichment media such as selenite-F-broth and tetrathionate broth, and on differential plating media such as MacConkey, bismuth sulfite, and brilliant green agars. The optimum incubation times for *Salmonella* enrichment cultures were obtained by inoculation of enrichment broth onto plating media after 24 hours incubation at 37ºC, after 48 hours at 37ºC, after a 3-day delayed secondary enrichment (DSE), and after a 5-day DSE procedure. Inoculations of the enrichment broth onto plating media after 24 hours incubation followed by 5-day (DSE) enable the detection of 96-98% of *Salmonella* positive samples and were the best combination of condition (Kim *et al*., 2012).

The *Salmonella* colonies appear with different shapes and colors on different media. On nutrient agar they appear small, smooth, circular and translucent while S. Gallinarum colonies are blue gray. On MacConky agar they are colorless, smooth, round, shiny and up to 2mm in diameter. S. Gallinarum produce colonies larger than S. Pullorum and have a characteristic odour. On selenite-F- broth the growth is turbid with heavy flocculent sediment. On desoxycholate citrate agar (DCA) the colonies are slightly opaque, dome shaped with central black spot. S. Pullorum is a lactose fermenter producing pink colonies with a precipitate in surrounding media. On triple sugar iron agar (TSI) S. Pullorum *and* S. Gllinarum produce a red slant with a yellow butt that show delayed blackening from H_2S production (Pui *et al*., 2011).

The genus *Salmonella* produce usually gas from glucose except S. Typhi which ferments glucose and manitol without gas production. Hydrogen sulphide is usually produced on triple sugar iron agar but some strains of S. Choleraesuis and most stains of S. Paratyphi *A* do not, and *S. arizonae* utilizes manitol. Nitrate is reduced to nitrite and citrate is usually utilized by *Salmonella* as a carbon source. The members are urease, indole and oxidase negative but catalase positive. Sucrose, salicin and lactose are not generally fermented by *Salmonella*. However many strains of *S. arizonae* ferments lactose rapidly or slowly as well as having activity to B-glactosidase enzyme (Mikoleit, 2015).

2.7. Foodborne salmonellosis

Foodborne illnesses, including salmonellosis, are widespread and have an impact on communities in both the developing and developed world(Zelalem *et al.*, 2011). In industrialized countries the incidence of salmonellosis is on the rise due to the emergence and increase of S. Enteritidis and S. Typhimurium DT 104 (Uro, 2019). Interest in *Salmonella* has heightened in recent years due to the increased susceptibility of acquired immunodeficiency syndrome (AIDS) patients to salmonellosis, the devastating effects of S. Enteritidis in the poultry industry and the globalization of agricultural trade(Phagoo and Neetoo, 2015).

The epidemiology of foodborne problems like salmonellosis is complex and expected to vary with change in the pathogens themselves, industrialization, urbanization and change of lifestyles, knowledge, belief and practices of food handlers and consumers, demographic changes (increased susceptible population), international travel and migration, international trade in food, animal feed and in animals, and poverty and lack of safe food preparation facilities (Tadesse, 2015).

Salmonella is a leading cause of foodborne illnesses(Omulo *et al*., 2015). According to the WHO reports of 1995, 88% of all foodborne diseases were caused by *Salmonella* (Marshall and Levy, 2011). Salmonellosis, the disease caused by infection with *Salmonella*, is a common intestinal illness caused by numerous *Salmonella* serovars and manifested clinically in animals and humans as an acute enteritis and chronic enteritis, an acute septicaemic disease or as subclinical infections (Abatcha *et al*., 2014).

The disease appears to be most prevalent in areas of intensive animal husbandry, especially of poultry, pigs and dairy cattle reared in confinement. There are pandemics of *S*. Enteritidis and *S*. Typhimurium DT 104 which resulted in enacting regulations in many countries to control the prevalence of salmonellosis in farm animals in order to prevent foodborne infection (Tadesse, 2015). For instance, a total of 88,715 confirmed human cases of salmonellosis were reported in the European Union (EU) in 2014 and of these, 34.4% were hospitalized (hospitalization status was provided for 10.4% of all confirmed cases) (Messens *et al*., 2013). The United States alone reported an estimated 1.4 million total cases of non-thyphoidal *Salmonella* per year (Doyle *et al*., 2013). The large number of outbreaks in developed and developing countries produced by this bacteria indicates its importance and impact(Tadesse, 2015). Salmonellosis is not only responsible of a large number of illnesses but also there is a cost associated with these outbreaks which in United States has been estimated to range from $600 million to $3.5 billion each year (Mąka and Popowska, 2016).

In developing countries a rapidly growing industry of intensive animal production is accompanying the process of urbanization with all its environmental and behavioral

changes favorable for *Salmonella* to prevail. Most food industries in developing countries are not well aware of food safety issues, and knowledge of modern technologies, good manufacturing practices, hygiene, Hazard Analysis Critical Control Point (HACCP) system, and quality control is often limited or absent. Cold storage facilities are inadequate and quality of water used for food processing may not be suitable. The vast numbers of laborers that handle food in factories, as well as on farms, are illiterate and untrained(Andoh *et al*. 2016) .

In countries like, Ethiopia, foodborne illnesses are perceived as mild and self-limiting diseases. Their severe and chronic health consequence is often overlooked, as are their consequences on trade and the economy. In such countries lack of information leads to lack of appreciation of the health significance of unsafe food and this, in turn, leads to low priority and sometimes no resources assigned to food safety (Garedew *et al*., 2015).

2.8. Methodologies for *Salmonella* Subtyping

Classically, *Salmonella enterica* isolates are identified and typed by phenotypic methods such as biochemical profiling, serotyping and phage typing. Serotyping is an 80-year old method which serves as the basis for the present classification of *Salmonella enterica* subsp. *enterica* subtypes (Arrach *et al*., 2008). Serotyping deciphers the antigenic makeup of the organism by identifying the somatic (O) and flagellar (H) antigens through reactions with specific antisera. Its usefulness in surveillance programs has long been recognized, in spite of the fact that it does not have the capacity to fingerprint strains in a sensitive manner (Grimont and Weill, 2009).

For the latter purpose, pulsed-field gel electrophoresis (PFGE), which was adapted to *Salmonella* in the 1990s, demonstrated the capacity to identify strains at the origin of an outbreak and rapidly became very popular, to the point where it is considered the gold standard for *Salmonella* molecular subtyping. During the last 2 decades, surveillance techniques have become easier following the development of alternative methods, most of them DNA based. These new methods improved the performance of the historical

methods by displaying higher subtyping sensitivity or facilitated the analysis by requiring no specific technical expertise and by making use of common laboratory reagents. Such a method if it turns out to exist should be rapid, robust, portable, and sensitive, producing objective results, differentiating epidemiologically unrelated strains from each other, and grouping all isolates associated with the same source while staying as close as possible to the present classification of *Salmonella* into subspecies and serovars. The implementation of this ideal method has to be balanced against the budget constraints and invariably associated with a technology that needs to be conducted on a massive scale in the routine laboratory (Arrach *et al*., 2008).

Salmonella serotyping by slide agglutination test (Kauffmann-White-Le Minor scheme)

Salmonella serotyping is based on the Kauffmann-White-Le Minor (KW) scheme which is a modification of the original scheme from the 1930s (Grimont and Weill, 2007). Serotyping is based on the agglutination of bacteria with specific sera to identify variants of the somatic (O) and flagellar (H) antigens. These antigens are highly variable, with 64 "O" and 114 "H" variants identified (Grimont and Weill, 2009). The O antigen is the saccharidic component of the lipopolysaccharide exposed on the bacterial surface. Its reactivity toward specific antisera forms the basis of the *Salmonella* serotyping scheme(Grimont and Weill, 2007). Several O antigens may be expressed together at the surface of a single cell. In contrast, although most salmonellae possess two different copies of the gene encoding the flagellar protein, these bacteria have the unique property of expressing only one flagellar protein at a time (Grimont and Weill, 2009). Most isolates are therefore termed diphasic (phase I and phase II, also called H_1 and H_2) with respect to the flagellar antigens. Monophasic *Salmonella* are not rare; triphasic and quadriphasic subtypes are exceptional. Although only one H antigen is expressed at a time, both H_1 and H_2 can usually be detected in pure cultures because the two phases are expressed by discrete bacterial populations in the same culture or colony. However, if one H phase is undetectable, a method called "phase inversion" is used, which consists of inhibiting the dominant phase by a specific antiserum on a special medium which will

favor the growth of the bacterial population expressing the other H antigen (Grimont and Weill, 2007).

By convention, the antigens identified in a given strain are reported in a so-called antigenic formula in which the subspecies number followed by the O, H_1, and H_2 antigens separated by colons is reported (e.g., I 6,7,14 : r : 1,2). Historically, serovar names were attributed to groups of *Salmonella* isolates displaying a common unique antigenic formula. It was noticed later on that some strains had the same antigenic formula but differed in their biochemical profiles(Grimont and weill, 2007).

The latter were associated with different subspecies names (*S. enterica* subsp. *salamae* - subsp. II; subsp. *arizonae* - subsp. IIIa; subsp. *diarizonae* - subsp. IIIb; subsp. *houtenae*- subsp. IV; subsp. *Bongori* - subsp. V; subsp. *Indica* - subsp. VI). Serotyping thus defines subtypes (i.e., serovars or serotypes) within a subspecies. In this review, typing of strains belonging only to *Salmonella enterica* subsp. *enterica* (subsp. I) is considered, acknowledging that more than 1,500 different serovars are described just for this particular subspecies (Grimont and weill, 2007). The slide agglutination test is an early typing technique reflecting the technology available at the time. It is exclusively based on phenotypic characteristics. False-positive reactions may occur as a result of weak, nonspecific agglutination (Wayne, 2019). Autoagglutination and loss of antigen expression, such as that observed with rough, non-motile, and mucoid strains, may occasionally lead to strain untypeability, but these strains typically have little epidemiological significance. The method is intended neither to provide a sensitive fingerprint (e.g., for tracing during an outbreak) nor to define phyletic relationships. It requires the use of over 150 specific antisera and carefully trained personnel. It is still defined as the reference method and is commonly used as an initial screening, followed by molecular subtyping to identify outbreak-related strains (Wayne, 2019).

2.9. Antimicrobial resistance in *Salmonella*

Since the early 1960s, several MDR clones of *S.* Typhimurium have emerged. These are typically resistant to a wide range of antibiotics, including some of those listed as critically important antibiotics for human medicine. Such clones which include such definitive phage types (DTs) as 29, 204, 193 and 104 have become widely disseminated in both food production animals and people(Greeson *et al.*, 2013).

Multidrug-resistant non-typhoidal salmonella (NTS) isolates are associated with increased morbidity compared to antibiotic-sensitive strains and are an important health and safety concern in both humans and animals (Crump *et al.*, 2013). Previously in developing countries where NTS is endemic the first line treatment choices for enteric and infectious NTS (iNTS) disease were co-trimoxazole, ampicillin or chloramphenicol. However, from the late 1980s due to an increasing prevalence of resistance to commonly available antibiotics, extended-spectrum cephalosporins and fluoroquinolones replaced these older agents for the management of iNTS disease (Msefula *et al.*, 2012). Ceftriaxone resistant *S.* Typhimurium have previously been reported in other countries in Europe, (Yang *et al.*, 2014), Asia (Hoa *et al.*, 2014) and in the USA(Msefula *et al.*, 2012).

Food, primarily of animal origin, is an important reservoir of antibiotic-resistant *Salmonella* that can spread from food animals to people (Kariuki and Dougan, 2014). Foodborne disease in people caused by antibiotic resistant *Salmonella* is well documented. The foods implicated in these infections are typically beef, pork, poultry and dairy products, but eggs and fresh produce are also culprits. The resistance patterns in *Salmonella* in animals often reflect the selective pressure caused by antibiotic usage in animals. Moreover, EU data show that the occurrence of resistance in *Salmonella* from pigs, cattle and broiler chickens largely resembles the occurrence of resistance reported for *Salmonella* in corresponding foodstuffs and in people(Uro, 2019).

For foodborne *Salmonella*, the resistances of most concern are those against quinolones and cephalosporins, both on the WHO list of critically important antibiotics for human medicine. Further, multi resistance is widespread in several *Salmonella* serovars and particularly *S.* Typhimurium, worldwide (ECDC, 2016). Resistance to quinolones in *Salmonella* from food animals and their products has increased substantially in many countries around the world over the last few years(Grace *et al*., 2016).

In the EU, there is considerable variance among the countries, the serovars and the different animal species (resistance is especially high in poultry) and their products. The rise in quinolone resistance in animal *Salmonella* isolates has been partly linked to a subsequent increase in human infections with resistant *Salmonella* species; in particular, this is associated with the consumption of contaminated eggs and egg products(Grace, 2016).

The prevalence of resistance to third-generation cephalosporins in *Salmonella* from animals and people is not low at present. A further concern is the increasing emergence of strains of *Salmonella* of animal origin that contain transferable AmpC-like β-lactamases that also inactivate extended-spectrum cephalosporins. The human infection with cephalosporin-resistant Salmonella is usually related to foreign travel (ECDC, 2016).

2.10. *Salmonella* prevalence and its resistance status in Ethiopia

In Ethiopia, as in other developing countries, it is difficult to evaluate the burden of salmonellosis because of the limited scope of studies and lack of coordinated epidemiological surveillance systems. In addition, under-reporting of cases and the presence of other diseases considered to be of high priority may have overshadowed the problem of salmonellosis (Tadesse and Gebremedhin, 2015).

Studies on human Salmonellosis in Ethiopia began in the 1970s. However, the number of studies so far carried out is small and do not include all segments of the population and

geographic regions of the country (Tadesse, 2014). Most studies were carried out in Addis Ababa and risk factors that potentially influence the occurrence of the disease including the diversity of serovars were not sufficiently addressed to provide a comprehensive picture of the epidemiology of the disease. In addition to the small number of studies, the aggregate reporting and lack of distinct data by sample type and age group were constraints in estimating the prevalence of serogroups and serotypes (Tadesse, 2014).

A meta-analysis of the prevalence of Salmonella in food animals in Ethiopia reported by Tadesse and Tessema, (2014) were 7.07%, 8.41%, 9.01% and 43.81% in slaughtered cattle, sheep, goats and pigs respectively. The recent research output by some of the investigators clearly showed that, there is an increasing of both the incidence and antimicrobial resistance of salmonella in different parts of Ethiopia in different hosts of food animals. For instance, Eguale, (2018) reported that, *Salmonella* was recovered in 14.6% of the poultry farms located in three districts around Addis Ababa.

S. Saintpaul was the dominant serotype detected by Eguale, (2018), accounting for 76.9% of all isolates. Other serovars, such as S. Typhimurium (11.5%), S. Kentucky (7.7%) and S. Haifa (3.8%) were also detected. Of all the Salmonella isolates tested, 92.3% were intermediately or fully resistant to sulfisoxazole and streptomycin, 46.2% to cephalothin, while 42.3% were resistant to ampicillin, amoxicillin+clavulanic acid, kanamycin and chloramphenicol. Multidrug resistance (MDR) to several drugs was common in S. Kentucky and S. Saintpaul.

According to Abdi *et al.*, (2017), Salmonella was isolated from 16.7% of the total samples collected at Bonga and Hawassa. All of the 45 isolates (100%) exhibited resistance to kanamycin and sulfamethoxazole-trimethoprim, nalidixic acid (97.8%), ampicillin (97.8%), cefoxitin (97.8%), streptomycin (97.8%) tetracycline (97.8%), chloramphenicol (91.3%) and ciprofloxacin (31.1%). Alarmingly, 42 isolates (93.4%) exhibited multidrug resistance (MDR) to ≥ 8 drugs and all 45 isolates had resistance to ≥ 3 drugs.

The study conducted on butcher shop premises and utensils sanitation and personnel's hygiene in north Ethiopia by (Garedew *et al.*, 2015) revealed that 17.3% overall prevalence with the highest in meat sample (35.6 %) and followed by 23.2%, 9.1% and 5.6% in hand swabs, knife swabs, chopping board surface swabs respectively. Of the total 53 *Salmonella* isolates subjected to antimicrobial susceptibility test, 88.7 %, 62.3 %, 35.8 %, 32.1 % and 30.2 % of them exhibited resistance to Ampicillin, Amoxicillin, Nitrofuranthoin, Tetracycline, and Sulfamethoxazole-Trimethoprime, respectively. Furthermore 28.3 % of the isolates were multidrug-resistant.

Of the total 21 *Salmonella* isolates obtained from animal origin food sample purchased from restaurants, hotels, cafeterias, and pastry and retail shops, including egg sandwich, minced and raw meat, burger patties, cottage cheese, cream cake, and beef pizza and subjected to antimicrobial susceptibility test using nine different antimicrobials by (Ejo *et al.*, 2016), a total of 10 (47.6%) *Salmonella* isolates were found to be resistant to one or more than one (multidrug) of the antimicrobials, and 6 (28.6%) isolates showed an intermediate sensitivity to one or more antimicrobials, and 5 (23.8%) isolates were susceptible to all tested antimicrobials.

In general, from this limited studies and reviews, it can be concluded that the prevalence of *Salmonella* in animals, animal origin foods and human is increasing. High proportion of *Salmonella* isolates were resistant to two or more of the antimicrobials that are commonly used in the veterinary and public health set up. Salmonellosis is one of the major diseases in Ethiopia and several factors including under and mal-nutrition and HIV-AIDS, the unhygienic living circumstances and the close relations between humans and animals may substantially contribute to its occurrence. Despite its importance, surveillance and monitoring systems are not in place and a comprehensive picture of its epidemiology is not available.

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in Ada'a, Akaki, Adama and Lome districts because of large concentrations of poultry farms in the area. Most of the large-scale commercial poultry farms in Ethiopia are found in East Shewa Zone of Oromia regional state. In addition, there are also emerging intensive small-scale poultry farms in the urban and peri-urban areas in which small number of exotic breeds of chicken (50-1,000) are produced along commercial lines using relatively modern management methods (Demeke, 2008).

East Shewa Zone is situated from 38° 03′ to 40° 05′ E longitude and from 7° 04′ to 9° 10′ N latitude covering a total land area of about 13766.5 km^2. The altitude of the study area ranges from 538 to 3,101 m above sea level. East Shewa Zone is characterized by semi-arid and sub-humid climate based on the moisture index climate classification. Considering the long-term average seasonal (June - September) rainfall, the area receives 458 - 518 mm rain. Based on mean annual rainfall and temperature of the area, the major climatic classes of the zone are dry climate and tropical rainy climate (Legesse and Suryabhagavan, 2014).

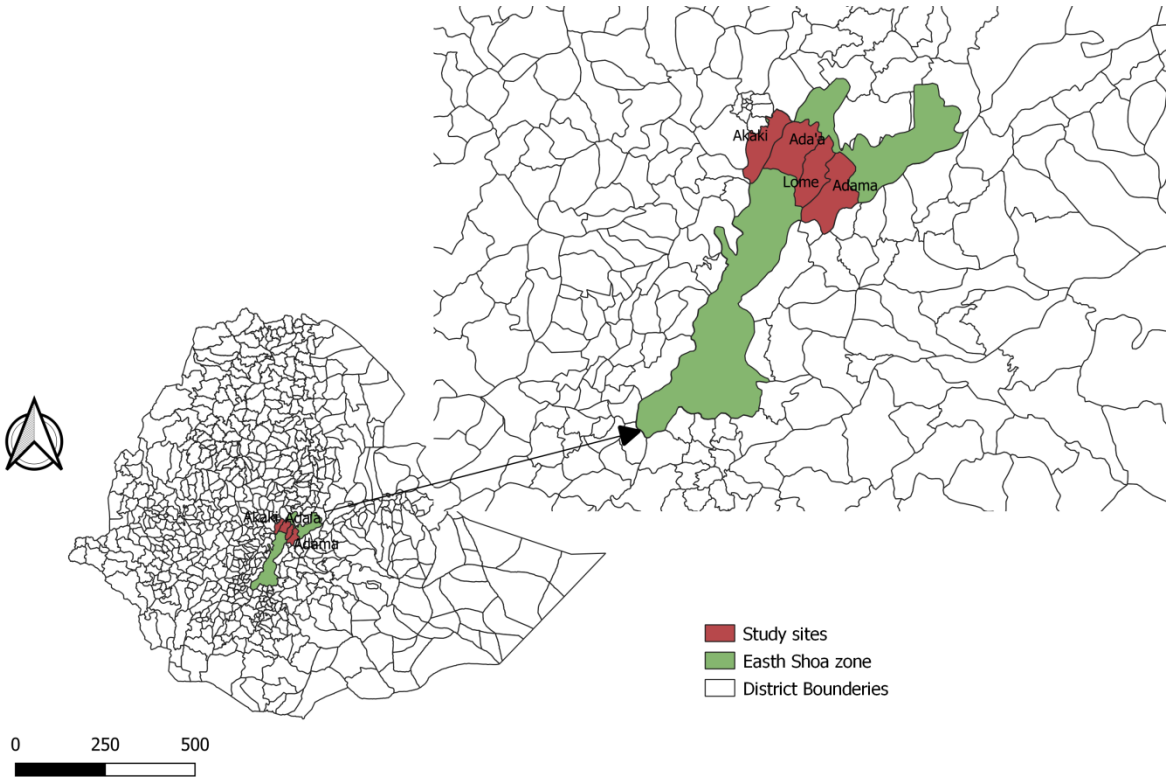

Fig 1. Map of the study area

3.2. Study design and sample size determination

A cross-sectional study was conducted in four districts of East Shewa Zone of Oromia regional state, central Ethiopia from September, 2017–January, 2021.

Sample size was determined by using an average (12.1%) of the three *Salmonella* prevalence, 16.67% (Abdi *et al.*, 2017), 4.7% (Eguale, 2018) and 15.12% (Abunna *et al.*, 2016) recorded in previous studies in the country on poultry by product, product and hand swab of farm worker samples. The absolute precision was decided to be 5% at 95% confidence level and the sample size was calculated according to Thrusfield, 2005 formula as shown below:

$$N = \frac{(Z\alpha/2)^2 \times P(1-P)}{d^2}$$

Where, n=required sample size, P_{exp}= expected prevalence, d^2= desired absolute precision Thus, based on the above formula the calculated sample size was 163.

3.3. Sampling strategy

3.3.1. Biological sample collection strategy, transportation and storage

The four districts of East Shewa zone, one town and one peasant association from each district were purposively selected because of concentrated poultry production in the area, agro-ecological suitability and accessibility. Within each district, the backyard and commercial production system were included. Households with more than 10 chickens were also purposively selected. For the backyard, selected households were sampling unit while farms were for commercial production. All commercial farms in the major cities of the districts were selected stratified by the size of the farms (small$\leq$1000 or large> 1000) (FAO, 2018).

Fecal, egg, chicken meat and hand swab of the farm worker were collected according to the method described in ISO-17604. For chicken fecal samples, 25g pooled fresh feces were collected from the floor by sterile spatula into sterile universal bottle (Oxoid Ltd., London, England). The egg samples were collected from the farms included in the study. Samples were marked with a permanent marker stating the date of collection, the farm and identification number for each egg. For the chicken meat, 25 g of tissues were collected from the commercial farms at slaughterhouses and placed into plastic freezer bags. Hand swabs of poultry farm workers were collected by rubbing the palm and fingers using pre-moistened swabs (Oxoid Ltd.) in 15 ml conical tubes (Oxoid Ltd.) containing 10ml of buffered peptone water (BPW).

Precautions were taken at all stages to ensure that the equipment used during sampling, transportation and storage to avoid any contamination and cross contamination. All types of samples were transported to CVMA, Veterinary public health laboratory in cooler containing icepacks and stored refrigerated at 4 °C until processed (Annex 2).

3.3.2. Questionnaire data collection

Questionnaire data was collected from 388 respondents to assess the rational use of antibiotic on different poultry production system. All large and medium scale commercial type poultry farms included due to their limited number and one respondent from each farm based on their responsibility for farm management was selected. The owners of backyard poultry production included if they had more than 10 chicken based on the information gained from the local development agencies. Structured questionnaire survey and in-depth interviews with poultry owners and key-informants (Veterinarians, animal production experts working on the farm) were performed. Questionnaire survey focused on the demography of the respondents, farm characteristics, common antibiotics used, common poultry diseases encountered, antibiotics used (types, doses, administration routes, formulation, reason for use, time and duration) and information from direct observation of the farms also collected to identify indicators for the use of antibiotics on the farms (Annex 3).

3.4. Study Methods

3.4.1. Isolation and identification of *Salmonella*

3.4.1.1. Isolation of salmonella

Isolation of *Salmonella* isolates was conducted following the laboratory procedure of "isolation of *Salmonella* spp. from food and animal faces (WHO, 2010) based on the ISO protocol 6579:2002. Raw meat samples (25 g) were sliced into small pieces on sterile metal trays using sterile scalpel and forceps and placed into flasks containing 225 ml BPW and incubated for 24 hr at 37 ^{o}C. Fecal samples (25 g) and the contents of individual eggs were placed into a sterile stomacher bag (Oxoid Ltd.) and 225 ml of BPW was added in 1:9 ratio and homogenized using a laboratory blender (Oxoid Ltd.) for 2 minutes and incubated for 24 hrs at 37 ^{o}C. Hand swabs were transported in 10ml of BPW and incubated for 24 hrs at 37^{o}C. Following incubation, 1 ml and 0.1 ml of the pre enrichment broths were aseptically transferred to 10 ml of tetrathionate broth (Oxoid Ltd.) and 10 ml of Rappaport-Vassiliadis (RV) broth (Oxoid Ltd.), mixed, and incubated for 18 to 24 hr at 37°C and 42°C, respectively. Following incubation, a loopful of each broth culture was streaked onto xylose lysine deoxycholate (XLD) agar (Oxoid Ltd.) and brilliant green agar (BGA) (Oxoid Ltd.) and incubated at 37°C for 24 to 48 hrs. The XLD and BGA plates were examined for the presence of *Salmonella* colonies. Red colonies with black centers on XLD, and pink colonies with a red zone on BGA plates were considered presumptive positive and further purified by culturing on nutrient agar (Oxoid Ltd)(Annex 2).

3.4.1.2. Identification of salmonella species based on biochemical tests

For biochemical identification(Mikoleit, 2015), presumptive *Salmonella* colonies were picked from the nutrient agar and inoculated into triple sugar iron (TSI) agar(Oxoid Ltd.), lysine decarboxylate broth(Oxoid Ltd.), Simon's citrate agar(Oxoid Ltd.), urea broth(Oxoid Ltd.), tryptone water(Oxoid Ltd.), MR-VP broth (Oxoid Ltd.) and incubated for 24 or 48 hours at 37 °C. Colonies producing an alkaline slant with acid (yellow color)

butt on TSI with hydrogen sulfide production, positive for lysine (purple color), negative for urea hydrolysis (red color), negative for tryptophan utilization (indole test) (yellow-brown ring), negative for Voges-Proskauer, and positive for citrate utilization were considered *Salmonella*-positive.

Identified *Salmonella* isolates were sent to OIE and National reference laboratory for *salmonella*" within the Instituto Zooprofilattico Sperimentale Delle Venezie, Italy for further analysis.

3.4.2. Serotyping of *Salmonella* isolates

The serotyping of *salmonella* isolates(Grimont & Weill, 2007) previously confirmed as specified by ISO 6579 and inoculated in a tube of tryptose agar (SSI, Copenhagen). The *salmonella* isolates were further characterized based on slide agglutination test as described in ISO/TR 6579-3:2014 at "OIE and National reference laboratory for *salmonella*" within the instituto Zooprofilattico Sperimentale delle Venezie, Italy. A small drop (approximately 20 µL) of Salmonella antisera from SSI (Statens Serum Institute, Copenhagen) was added on a glass slide and mixed with the *salmonella* culture. The slide tilted for 5-10 seconds. A positive reaction was seen as visible agglutination with the naked eye in front of a light source against a black background, whereas a negative reaction was seen as homogeneous milky turbidity. For a control purpose a loop of growth with a loop full of saline was mixed on a slide. If within the drop lumps appeared, the culture is auto agglutinating and no further typing is possible, while if the drop is an opaque suspension the strain is not auto-agglutinating.

Somatic-O antigen typing: First, the strains were tested in the O-sera-pools (SSI, Copenhagen) by placing a drop of poly O antisera on a slide. From the inoculated nutrient agar plate, a loop full of growth removed and mixed into the antisera drop on the slide. Antisera and culture (antigen) was mixed with a loop and the slid was rocked with the slide gently for a maximum of 2 minutes. A homogenous suspension indicates a

negative reaction, while lumping was a positive reaction. When the sample was positive to O-sera pools, the strains were tested in the individual O-sera represented in the positive O-pool.

Flagellar-H antigens typing: The identification of first phase was performed by using the same producer described for somatic antigens. A homogenous suspension is a negative reaction and lumping is a positive reaction. Flagella agglutination is more'' floccular'' in appearance than somatic agglutination and may formed only around the edge of the drop. If the strain were first positive to the H-antisera-pools (SSI, Copenhagen), then the strains were further tested in the individual H-antisera represented in the positive H-pool. Once the first phase has been identified, the second one could be detected by" phase inversion" which is carried out by using specific antisera inhibiting the identified first phase and allowing the expression of second phase. To perform this, 5ml of a melted Sven Gard medium (SSI, Copenhagen) placed into a small sterile petridish, until it solidify. Then, some drops of antiserum against the detected H-antigen added in order to block the first phase and allow the expression of the second phase. The strain was inoculated and incubated overnight in one spot at the center of the Sven Gard plate at 37 ^{o}c. Remove a loop full of growth from the edge of the motility zone on Sven Gard and mix carefully with the H-antiserum on the slide. Rock the slide gently for a maximum of 2 minutes. The agglutination appears as the one for the first phase.

3.4.3. Antibiotic susceptibility testing

Antibiotic susceptibility was determined by Kirby-Bauer disc diffusion method (Hudzicki, 2016) based on the Clinical and Laboratory Standards Institute (CLSI)(CLIS, 2019) with commercially available antibiotic discs. Refreshed pure isolate colonies from the nutrient agar plates were transferred into tubes containing 5 mL of tryptone soya broth (Oxoid Ltd.). The broth culture was incubated at 37 °C for 4 h until it achieved 0.5 McFarland turbidity standards. A sterile cotton swab was used to swab the inoculum

uniformly over the surface of Muller Hinton agar plate (Oxoid Ltd.). Plates were held at room temperature for 30 min to dry. The pre-determined antibiotic disks(all from Oxoid Ltd.): norfloxacin (10µg), nalidixic acid (30µg), ciprofloxacin (5µg), amoxicillin-clavulanic acid (20/10µg), tetracycline (30µg), chloramphenicol (30µg), ampicillin (10µg), gentamicin (10µg), streptomycin (10µg), ceftazidime (30µg), cefotaxime (30µg), trimethoprim + sulfamethoxazole (1.25/23.75µg), meropenem (10µg), doxycycline (30µg) and tigecycline (15µg) were then dispensed into the bacterial lawn by means of a sterile pair of forceps and gently pressed to ensure complete contact with the agar. The discs were positioned 15 mm away from the edge of the plate and 25 mm away from each other. The plates were incubated at 35-37°C for 18 to 24 hrs. Zones of inhibition around the discs were measured to the nearest millimeter using a digital caliper, the size of the inhibition zone was compared with the disc manufacturer's standard and classified as sensitive (S), intermediate (I) or resistant (R) to the given drug. *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853) and *E.coli* (ATCC 25922) were used as control strains. *Salmonella* colonies that showed resistance to more than or equal to three different classes of antibiotics were considered MDR.

3.5. Ethical consideration

For sample collection, ethical clearance certificate (Annex-7)) was obtained from the College of Veterinary Medicine and Agriculture (Addis Ababa University). Informed consents were obtained from each participant after informing the purpose of the study, the procedure, the risk, benefit and their rights.

3.6. Data analysis

Data obtained through both laboratory work and questionnaire survey were entered into Microsoft Excel, cleaned, organized and sorted. Data were then coded and imported into IBM SPSS statistics 22 for data analysis.

A Chi-square test was used for initial analysis to determine differences in the proportions of positive results among districts, breed, production type, production system and sample types. Multivariable logistic regression was used depending on the nature of the outcome measures. Significant statistical difference was considered at $P<0.05$.

Descriptive analysis was done to generate frequencies and percentages for an outcome variable from the independent variables (demographic characteristics of the respondent, farm characteristics, common antibiotics used, major poultry disease and antibiotic use practice and administration).

Index of poultry production constraints = Sum (n x number of households ranked first) + (n-1) x number of households ranked second + (n-2) x number of households ranked third +…+ 1 x number of households ranked last) for one factor divided by the sum of (n x number of households ranked first+ (n-1) x number of households ranked second +…. +1x number of households ranked last) for all factors, and where n= value given for the least ranked factor. The variable with the highest index value is the highest economically important (Kosgey, 2004).

4. RESULTS

4.1. Result of the biological samples

4.1.1. Prevalence of *Salmonella* and its associated risk factors

The overall prevalence of *Salmonella* in the current study was 13.97% (95% CI =11.6-16.4) isolated from 92 of 500 (18.4%, with 95% CI = 14.6- 21.3) fecal samples, 7 of 153 (4.5%, 95% CI = 1.2-7.7) eggs and 6 of 100 (6.0%, 95% CI = 1.3-10.7) meat samples and 4 of 27 (14.8% with 95% CI = 1.5-28.5) hand swabs sampled from farm workers. As indicated in Table 1 farm type, production type, breed and sample type were significantly (P<0.05) associated with Salmonella prevalence. *Salmonella* prevalence was significantly (P= 0.001) higher in the intensive farms (16.9%) than in the back yard farms (7.4%). The prevalence of *Salmonella* was significantly (P=0.024) higher in layer chicken (20.4%) than in broiler chicken (11.1%), and it was twice in the exotic breeds (15.8%) as compared (P= 0.021) to local breeds (7.4%). Fecal sample had significantly (P =0.000) the highest (18.4%) prevalence than poultry products (5.13%) and farm workers (14.8%).

Table 1. The overall prevalence and risk factors for the occurrence of *Salmonella* in poultry farms

Risk factor		No of sample	No of Positive	Prevalence (%)	$\chi 2$	P-value
Study district	Akaki	123	13	10.6	1.785	0.618
	Ada'a	339	44	12.9		
	Lome	116	21	18.1		
	Adama	202	31	15.3		
Farm type	Intensive	467	79	16.9	14.444	0.001
	Semi-intensive	125	16	12.8		
	Backyard	188	14	7.4		
Prod. type	Layers	313	64	20.4	7.466	0.024
	Broilers	279	31	11.1		
	Dual	188	14	7.4		
Breed	Exotic	606	96	15.8	5.340	0.021
	Local	174	13	7.4		
Sample type	Feces	500	92	18.4	24.696	0.000
	Hand swab	27	4	14.8		
	Egg	153	7	4.5		
	Meat	100	6	6		

Further analysis by using multivariable logistic regression analysis showed that, farm type (P=0.047) and sample type (P=0.001) were significantly associated with *Salmonella* prevalence (Table 2). In this regard, semi-intensive farms had higher odds of *Salmonella* prevalence than the backyard scavenging system (Odds ratio [OR] = 2.25; 95% confidence interval [CI: 1.15- 4.40; P= 0.018]. Fecal samples had three times higher Odds ratio than meat samples [OR = 3.34; 95% CI for OR = 1.39- 8.01; P = 0.007] for fecal samples.

Table 2. Associated risk factors for the occurrence of *Salmonella* in poultry by products, products and farm workers based on logistic regression analysis

Variables		No of Samples	No of Positive	d.f.	P-Value	OR	95% CI for OR	
							Lower	Upper
Farm type				2				
	Intensive	467	79	1	0.277	1.39	0.77	2.52
	Semi-intensive	125	16	1	0.018	2.25	1.15	4.4
Sample type				3				
	Fecal	500	92	1	0.007	3.34	1.39	8.01
	egg	27	4	1	0.795	0.86	0.27	2.73
	Hand swab	153	7	1	0.236	2.28	0.58	8.92
Constant				1	0	0.05		

4.1.2. *Salmonella* prevalence in fecal samples

Higher fecal prevalence were observed in Ada'a district (20.1%), in semi-intensive farm (25.9%), in broiler chicken (20.1%) and in exotic breed (19.6%) as compared to their counterparts, although these differences were not statistically significant except marginally significant (P=0.037) effect of farm type (Table 3).

Table 3. Fecal prevalence of *Salmonella* by study characteristics

Risk factor		Sample size(n)	Positive	Prevalence (%)	$\chi 2$	P-value
Study district	Akaki	76	11	14.5	2.217	0.529
	Ada'a	199	40	20.1		
	Lome	70	10	14.3		
	Adama	155	31	20		
Farm type	Intensive	288	51	17.7	6.577	0.037
	Semi-intensive	108	28	25.9		
	Backyard	104	13	12.5		
Prod. type	Layers	242	48	19.8	3.247	0.197
	Broilers	159	32	20.1		
	Dual	99	12	12.1		
Breed	Exotic	403	80	19.6	2.913	0.088
	Local	97	12	12.4		

4.1.3. *Salmonella* prevalence in egg samples

The prevalence of *Salmonella* in the egg samples was higher in Lome district (10.3%), in semi-intensive farms (25.0%), in layers (5.8%) and in exotic breeds (5.3%) as compared to other districts, farm types, production types and breed respectively (Table 4). Only farm type was marginally significant (P=0.022).

Table 4. Prevalence of *Salmonella* in chicken egg samples by study characteristics

Risk factor		Sample size(n)	Positive	Prevalence (%)	χ2	P-value
Study district	Akaki	44	1	2.3	3.486	0.357
	Ada'a	34	2	5.9		
	Lome	29	3	10.3		
	Adama	46	1	0.021		
Farm type	Intensive	61	1	1.6	8.855	0.022
	Semi-intensive	8	2	25		
	Backyard	84	4	4.8		
Production type	Layers	52	3	5.8	0.746	0.885
	Broilers	12	0	0		
	Dual	89	4	4.5		
Breed	Exotic	76	4	5.3	0.164	0.493
	Local	77	3	3.9		

4.1.4. Serotype of *Salmonella* isolates

The serotype result showed that a high proportion of the isolates (69.7 %) were S. Typhmurium followed by S. Saintpaul (18.2%). Even though with very low proportion, S. Kentucky, S. New port and S. Anatum were also recorded (Table 5).

Table 5. *Salmonella* serotype isolated from poultry farms

No	Study site	Sample type	Sample code	Isolate code	Serotype
1	Ada'a	Hand swab	HS-7	N.19 SAL/1022	S. Typhmurium
2	Ada'a	Fecal	F-10	N. 19SAL/1023	S. Saintpaul
3	Ada'a	Fecal	F-11	N. 19SAL/1024	S. Typhmurium
4	Ada'a	Fecal	F-15	N. 19SAL/1025	S. Kentucky
5	Ada'a	Hand swab	HS-16	N. 19SAL/1026	S. Typhmurium
6	Ada'a	Fecal	F- 17	N. 19SAL/1027	S. Typhmurium
7	Ada'a	Hand swab	HS-18	N. 19SAL/1028	S. Newport
8	Ada'a	Fecal	F-19	N. 19SAL/1029	S. Saintpaul
9	Ada'a	Fecal	F-21A	N. 19SAL/1030	S. Typhmurium
10	Ada'a	Fecal	F- 23	N. 19SAL/1032	S. Typhmurium
11	Lome	Hand swab	HS-24	N. 19SAL/1033	S. Typhmurium
12	Lome	Fecal	F-71A	N. 19SAL/1034	S. Saintpaul
13	Lome	Fecal	F-74	N. 19SAL/1036	S. Typhmurium
14	Lome	Fecal	F-75	N. 19SAL/1037	S. Saintpaul
15	Akaki	Fecal	F-76A	N. 19SAL/1038	S. Typhmurium
16	Akaki	Hand swab	HS-78	N. 19SAL/1041	S. Typhmurium
17	Akaki	Fecal	F-81A	N. 19SAL/1042	S. Kentucky
18	Akaki	Fecal	F-81C	N. 19SAL/1043	S. Typhmurium
19	Akaki	Hand swab	HS-89	N. 19SAL/1044	S. Typhmurium
20	Akaki	Fecal	F-90	N. 19SAL/1045	S. Typhmurium
21	Akaki	Fecal	F-95	N. 19SAL/1046	S. Typhmurium
22	Akaki	Fecal	F-96	N. 19SAL/1047	S. Typhmurium
23	Akaki	Fecal	F-100	N. 19SAL/1048	S. Saintpaul
24	Ada'a	Egg	E-103	N. 19SAL/1049	S. Typhmurium
25	Ada'a	Egg	E-105	N. 19SAL/1050	S. Typhmurium
26	Ada'a	Egg	E-107	N. 19SAL/1051	S. Typhmurium
27	Ada'a	Egg	E-112	N. 19SAL/1052	S. Typhmurium
28	Lome	Egg	E-117	N. 19SAL/1053	S. Typhmurium
29	Lome	Egg	E-119	N. 19SAL/1054	S. Typhmurium
30	Akaki	Egg	E-133	N. 19SAL/1055	S. Saintpaul
31	Ada'a	Meat	M-137	N. 19SAL/1056	S. Typhmurium
32	Ada'a	Meat	M-152	N. 19SAL/1057	S. Typhmurium
33	Ada'a	Meat	M-168	N. 19SAL/1058	S. Anatum

4.1.5. Antibiotic resistance profile

The overall antibiotic resistance profiles of *Salmonella* isolates from feces (n=20), raw meat (n=6), raw eggs (n=7) and hand swabs of farm workers (n=4) were performed. All positive isolates of hand swab, meat and egg samples were included due to their small number while 20 positive isolates of fecal samples selected by simple random method. All isolates tested were resistant to at least one drug. About fifty-seven percent (n=21) of the isolates tested were resistant to more than or equal to three antibiotic classes indicating MDR (Tables 6 & 7). The prevalence of resistance was high for chloramphenicol (62.2%), tetracycline (59.5%), ampicillin (54.1%) and streptomycin (51.4%). All isolates were susceptible to meropenem, norfloxacin, nalidixic acid, ciprofloxacin, amoxicillin-clavulanic acid, gentamicin, ceftazidime and tigecycline.

Table 6. Antibiotic resistance of *Salmonella* isolates

Antibiotics	**Number (%)**		
	Susceptible (%)	**Intermediate (%)**	**Resistance (%)**
Norfloxacin (10µg)	37(100)	0(0.0)	0(0.0)
Nalidixic acid (30µg)	32(86.5)	5(13.5)	0(0.0)
Ciprofloxacin (5µg)	34(91.9)	3(8.1)	0(0.0)
Amoxicillin-clavulanic acid (20/10µg)	35(94.6)	2(5.4)	0(0.0)
Tetracycline (30µg)	10(27.0)	5(13.5)	22(59.5)
Chloramphenicol (30µg)	12(32.4)	2(5.4)	23(62.2)
Ampicillin (10 µg)	13(35.1)	4(10.8)	20(54.1)
Gentamicin (10µg)	22(59.5)	5(13.5)	0(0.0)
Streptomycin (10µg)	15(40.5)	3(8.1)	19(51.4)
Meropenem (10µg)	37(0.0)	0(0.0)	0(0.0)
Doxycycline (30µg)	25(67.6)	5 (13.5)	7(18.9)
Trimethoprim + sulfamethoxazole (1.25/23.75µg)	22(59.5)	8(21.6)	7(18.9)
Tigecycline (15µg)	36(97.3)	1(2.7)	0(0.0)
Cefotaxime (30µg)	36(97.3)	0(0.0)	1(2.7)
Ceftazidime (30µg)	35(94.6)	2(5.4)	0(0.0)

Table 7. Multi-resistance profiles of the *Salmonella* isolates

Number of antibiotics	Pattern of antibiotics	No of resistant isolate	Total no of resistant isolate (%)
Three	CHL,AMP,SXT	1	9(42.9)
	TET,CHL,AMP	2	
	CHL,AMP,STR	3	
	AMP,STR,SXT	1	
	CHL,AMP,DOX	1	
	TET,CHL,STR	1	
Four	TET,CHL,AMP,STR	4	9(42.9)
	CHL,STR, DOX,SXT	1	
	CHL,AMP,STR,SXT	1	
	CHL,AMP,STR,DOX	2	
	CHL,AMP,DOX,CTX	1	
Five	TET,CHL,AMP,STR,DOX	1	2(9.5)
	TET,CHL,AMP,STR,SXT	1	
Six	TET,CHL,AMP,STR,DOX, SXT	1	1(4.8)
Total MDR		**21**	**21(100)**

† Nalidixic acid (NAL), † Ciprofloxacin (CIP), †Tetracycline (TET), †Chloramphenicol (CHL), †Ampicillin (AMP), †Gentamicin (GMN), †Ceftazidime (CAZ), †Cefotaxime (CTX), †Trimethoprim + sulfamethoxazole (SXT), †Meropenem (MEM), † Doxycycline(DOX), †Tigecycline (TGC),† Streptomycin (STR), †Norfloxacin (NOR)

4.2. Result of the questionnaire survey

4.2.1. Demographic characteristics of the respondents

As shown in Table 8, majority (81.4%) of the poultry farmers interviewed were male, were 31-50 years of age (48.4%), completed primary school (35.8%), secondary school (31.2%), or post-secondary education (26.0%). Bishoftu and Adama are the potential cities where poultry production has been growing. Almost all poultry farming activities were carried out by the farm owners (88.9% of farms). About 41.2% of the respondents had over 10 years of experience in poultry production.

Table 8. Demographic characteristics of the respondents

Characteristics of the respondent		No of respondents	Percentage (n=388)
1. Location:			
District	**Town/kebele**		
Ada'a			
	Bishoftu	83	21.4
	Udde	37	9.5
Akaki			
	Dukam	36	9.3
	Ensilale	63	16.2
Lome			
	Modjo	28	8.5
	Tsededilima	33	7.2
Adama			
	Adama	71	18.3
	Adulala hate haroret	37	9.5
2. Age category:			
	Less than 30 years	107	27.6
	31-50 years	188	48.4
	Greater than 50 years	93	24
3. Sex:			
	Male	316	81.4
	Female	72	18.6
4. Education level:			
	Cannot write and read	27	7
	Primary school (1-8)	139	35.8
	Secondary school (9-12)	121	31.2
	TVET	92	23.7
	University graduate	9	2.3
5. Work experience:			
	Less than 5 years	126	32.5
	5-10 years	102	26.3
	Greater than 10 years	160	41.2

4.2.2. Farm characteristics

Over half of the respondents (56.4%) were rearing different improved breeds of chicken for commercial purpose while the rest of the respondents (43.6%) had different local breeds with less than 100 chickens being reared under backyard production system for subsistence life. The commercial producers kept either layers (27.8%) or broilers (24.5%) by using semi-intensive (44.6%) and intensive (8.8%) production system. Very few farms (1.3%) were using cage system of housing while more than half of the producers (56.2%) were using deep litter system of housing (Table 9). The backyard scavenging production systems were using different housing system (traditional type) different from the two commercial production systems.

Table 9. Poultry farm characteristics

Characteristics and/or management	No of respondents	Percentage
1. Chicken breed		
Local	169	43.6
Exotic	219	56.4
2. Number of chicken		
Less than 100	181	46.6
101-1000	173	44.6
More than 1000	34	8.8
3. Production type		
Layers	108	27.8
Broilers	95	24.5
Dual purpose	185	47.7
4. Production system		
Backyard scavenging	181	46.6
Semi-intensive	173	44.6
Intensive	34	8.8
5. Housing type		
Deep litter system	218	56.2
Cage system	5	1.3
Traditional housing	165	42.5

4.2.3. Constraining factors of poultry production in the study area

As presented in (Table 10), the major constraints encountered by the poultry farmers in the study area were high prevalence of disease (1^{st}), inadequate animal health services (2^{nd}), lack of enough feed resource(3^{rd}), inadequate land(4^{th}), inadequate finance (5^{th}) and presence of predators(6^{th}), according to their importance.

Table 10. Constraining factors of the poultry production in the study area

Constraints	1st	2nd	3rd	4th	5th	6th	Index	Rank
Lack of enough feed resource	123	50	113	8	94	0	0.169	3
Presence of predators	54	10	49	58	67	150	0.151	6
High prevalence of disease	373	13	2	0	0	0	0.201	1
Inadequate animal health services	307	53	18	10	0	0	0.189	2
Inadequate finance	13	42	39	57	103	134	0.153	5
Inadequate land	3	0	14	101	136	134	0.168	4

4.2.4. Common chicken disease for which antibiotics used

The commonly encountered poultry diseases which necessitated frequent use of antibiotics were Newcastle disease (100%), Gumboro disease (54.4%), coccidiosis (53.4%), Marek's disease (49.7%), foul typhoid (45.9%) and foul pox (39.2%) in decreasing order (Table 11). Almost three-fourths (71%) of the diseases encountered in the farms were attributed to viral diseases (Fig 2).

Table 11. Common infectious disease encountered within the past one year in poultry farms

Disease	Disease category	Number of farms	Percentage
Newcastle	Viral	388	100
Gumboro disease	Viral	211	54.4
Marek's disease	Viral	193	49.7
Foul typhoid	Bacterial	178	45.9
Foul pox	Viral	152	39.2
Coccidiosis	Protozoal	207	53.4

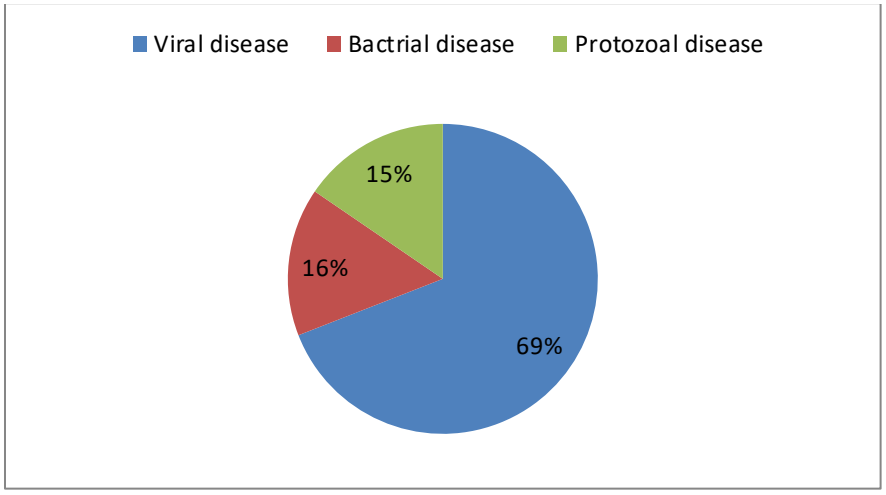

Figure 2. Percentages of the frequency of disease occurrence by causative agent

4.2.5. Antibiotic used in poultry farms

All the poultry farms (n=388) used one or more antibiotics on their farms and administered mainly by mixing them with feed or water (Personal observation and information from the Pack). The antibiotics used are in different commercial products with a wide variety of trade names (not reported in this study for ethical reasons). Tetracycline group (oxytetracycline + doxytetracycline) (100 % farms) and sulfadiazine + trimethoprim (94.1%) and fluroquinolones (enrofloxacin + norfloxacin) were the most frequently used antibiotics (Table 12).

Table 12. Types and frequency of antibiotics used in poultry farms

Antibiotics	**No of Respondent**	**Percentage**
Oxytetracycline + doxytetracycline	388	100
Lactaclox (Cloxacillin+ ampicillin)	113	29.1
Amoxycillin	85	21.9
Colistin sulphate	65	16.6
Enrofloxacin + norfloxacin	161	41.5
Sulfadiazine+ trimethoprim	365	94.1
Tylosin	55	14.2

4.2.6. Purpose of antibiotic used

Antibiotics were used on all farms for different purposes. All farms frequently used antibiotics for the treatment of sick chicken; 50% for disease prevention; 32.2% for growth promotion while 98.7% reported using antibiotics for all three purposes i.e. for treatment, prevention and growth promotion (Figure 3).

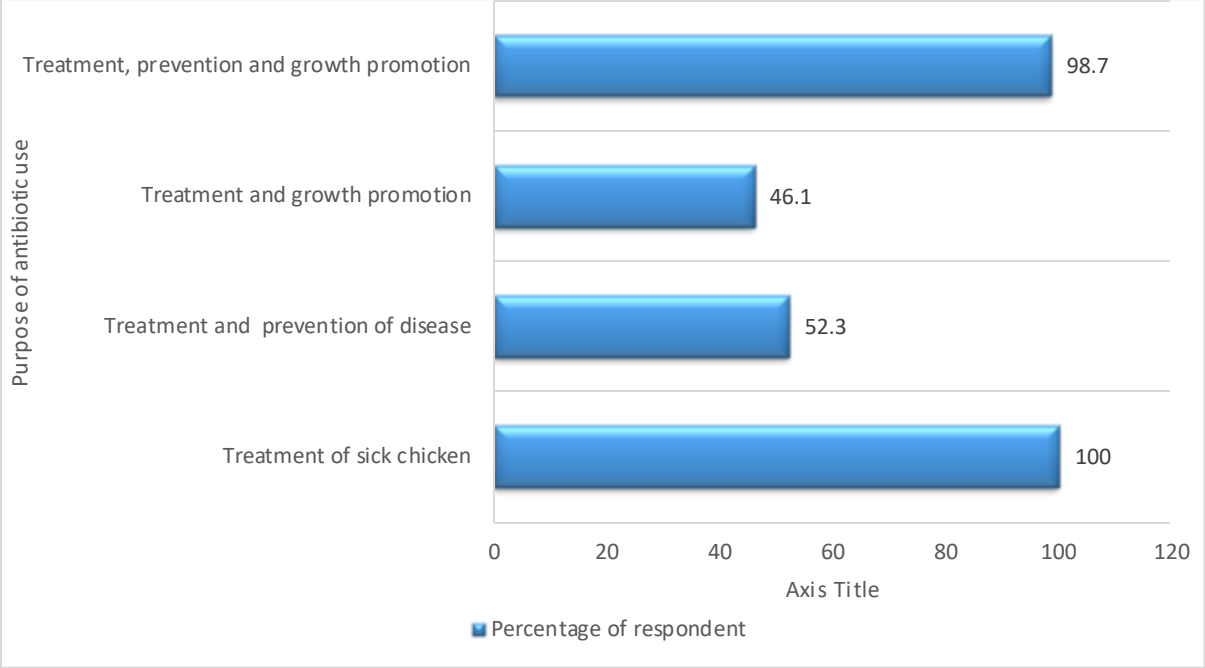

Figure 3. Proportion of farmers by the purpose of antibiotic used

4.2.7. Source, prescription and administration of antibiotics

Even though all (100%) farms reported getting antibiotics from veterinary drug shops, in addition they also obtained from public veterinary clinic (51.0%), human pharmacies (26.8%) and open markets (16.2%) (Table 13). While the majority (61.9%) of the farms reported obtaining antibiotics by prescription or recommendation by veterinarians or veterinary assistants at the drug stores or hired by the farms, 32.2% reported self-prescription, and 11.9 % of farms reported using antibiotics based on advice from friends or other farm owners with previous experience.

Only 147 (37.9%) of the farms reported that antibiotics were administered by animal health workers. However, on overwhelming majority (297/388, 76.5%) of farms antibiotics were administered by farm owners themselves based on instruction given by veterinary drug stores or by prescriber (28.1%), after reading a leaflet insert (48.5%), or by assumption based on prior experience (29.1%). All (100%) farms reported that they

incorporate antibiotics in to either feed or water, but only few intensive farms had sensitive balance or measuring cylinder for accurate dosages (Table 13).

Table 13. Source, prescription and administration of antibiotics on the poultry farms

Questions	No of respondent	Proportion
1. From where you get drugs used on your poultry farm?		
Veterinary drug shop	388	100
Public Veterinary clinic	198	51.0
Human pharmacy	104	26.8
Open market/hawkers	63	16.2
2. Who prescribe (recommend to the use) antibiotics in your farm for intended use?		
Animal health personnel	240	61.9
Friends with experience	46	11.9
Self-prescription	125	32.2
3. Who administer antibiotics to your chicken?		
Self-administer	297	76.5
Animal health personnel	147	37.9
4. How is an antimicrobial dosage determined before usage in birds in your farms?		
After reading a leaflet	188	48.5
From the prescription paper	109	28.1
With assumption	113	29.1
5. What is/are the common route of antibiotic administration?		
Injection	19	4.9
In feed or water	388	100
Drop into the mouth	72	18.6

5. DISCUSSION

In current study, the overall finding on the prevalence of salmonella (14%) was moderate and similar to 16.7% *Salmonella* isolated from three chicken multiplication centers in Southern Ethiopia (Abdi *et al.*, 2017), 15.12% recorded in and around Modjo town, central Ethiopia (Abunna *et al.*, 2016) and 12% from chicken droppings in Nairobi, Kenya(Langata *et al.* 2019). The result of the current study was higher than the one reported by Eguale, (2018) from Addis Ababa and its surrounding districts (4.7%) in Ethiopia, while it is lower than 25.4% reported in poultry and poultry products in Dhaka, Bangladesh(Karim *et al.*, 2017). The difference in the results may be attributed to differences in sample size, sampling techniques, geographical difference, breed, and/or age of the chickens and production types among other factors.

Based on the $\chi 2$ test, all the expected risk factors (farm type, production type, breed, sample type) were associated with the occurrence of *Salmonella* (Table 1). *Salmonella* prevalence was higher in exotic breeds and in intensive farms than local breed that are usually kept under scavenging backyard system. This may be due to the difference in genetic composition and stress of high physiologic activity in intensive farming systems. The higher prevalence of *Salmonella* in layers (20.4%) than in broilers (11.1%) could be due to physiological stress of egg production and molting, which significantly depresses the immune response and increases the susceptibility to *Salmonella* infection (Holt, 2003)

Multivariate logistic regression analysis was conducted to see the strength of association between risk factors and *Salmonella* prevalence. After the effect of other risk factors were adjusted for, only farm type and sample type had a significant effect on *Salmonella* recovery (table 2). Sample types as a risk factor reveal that, *Salmonella* prevalence was three times higher in fecal sample than in meat sample. This is as a result of *Salmonella* spp are commonly found in the alimentary tract of animals (Garedew *et al.*, 2015). Even though feces is a major source, poultry products account for a higher percentage of *Salmonella* outbreaks in humans as a result of its most widespread food commodity

worldwide(Kagambèga *et al.,* 2013) and raw/undercooked consumption of these products (Mąka and Popowska, 2016).

Salmonella Typhmurium was the dominant serotype in this study, which is in contrary to the result reported by Eguale, (2018) in which S. Saintpaul was the dominant serotype detected in poultry farms around Addis Ababa accounting for 76.9% of all isolates. The difference may be contribute to the introduction of new serovar following the import of day old chicken or pullets from different countries and there is likelihood of contamination of poultry farms from other farms in Ethiopia due to lack of biosecurity (dairy, swine and beef farms). Previous studies in different countries showed that, different serovars are dominant in different countries. For instance S. Kentucky was the dominant serovar in studies conducted in Nigeria (Fagbamila *et al.*, 2017) and Bangladesh (Barua *et al.*, 2012). S. Typhimurium was the dominant one reported in china (Kuang *et al.,* 2015)

Antibiotic-resistant *Salmonella* were observed in 65% (n=37) of *Salmonella* isolates tested, which was higher than previous studies by Ejo *et al.*, (2016) (47.6%) and was lower than what other studies reported, 83% by Zelalem *et al*. (2011), 72.7% by Tessema *et al*., (2017) and 82.1% by Hiko *et al*.,(2018) in Ethiopia. The resistance result was relatively in agreement with 60.6% recorded in Ghana by (Andoh *et al*. 2016). This resistance variation could be due to indiscriminate use of antibiotics in animal production, variation in implementation of biosecurity, lack of policy on antibiotic use and disease prevention, difference in awareness about antibiotic use, level of livestock intensification and others which might favor selection pressure that increased the advantage of maintaining resistance genes in bacteria(Pui *et al*.,2011; Kim *et al*. 2012; Lamas *et al*., 2016; Van Boeckel *et al*., 2017)

The prevalence of resistance was high to chloramphenicol (62.2%), tetracycline (59.5%), ampicillin (54.1%) and streptomycin (51.4%). This is inconsistent with the report of Tessema *et al*., (2017) in which all isolates were resistant to tetracycline and ampicillin but in contrary to the current result chloramphenicol was effective against most of the

Salmonella isolates. In addition to this Phagoo & Neetoo, (2015) also reported 60% and 80% of isolates were resistant to chloramphenicol and erythromycin respectively. On the other hand, the current result was in contrary to the report from Brazil by(De Oliveira *et al.*, 2006), in which the highest sensitivity was demonstrated for ampicillin (94.9%), tetracycline (91.1%) and chloramphenicol (98.7%). The possible reason for high resistance rate to chloramphenicol, tetracycline, ampicillin and streptomycin in Ethiopia could be due to long time availability of these antibiotics on the market and these antibiotics were the most commonly used antibiotics in both human and veterinary medicine in the country(Tufa *et al.*, 2018). High sensitivity of isolates was also recorded for norfloxacin (100%), meropenem (100%), tigecycline (97.3%), cefotaxime (97.3%), ceftazidime (94.6%) and amoxicillin-clavulanic acid (94.6%) which may associated with limited access and the high price to get the newly developed antibiotics like cephalosporins and quinolones in the country(Tadesse and Tessema, 2014).

Majority (56.8%) of the isolates in the current study were resistant or intermediately resistant to more than two antibiotic classes, which is very similar to 57.4% of the S. Typhimurium strains were multidrug resistant (MDR) and showed high resistance rates to tetracycline (70.2%), sulfonamides (57.4%), streptomycin (53.1%), and ampicillin in Malaysia (Benacer *et al.*, 2010). Our result of MDR was higher than the record by Garedew *et al.*, (2015) in Ethiopia (28.3%) and by Kuang *et al.*, (2015) in China in which 34.72% were resistant to more than three classes of antibiotics. The high occurrence of MDR in *Salmonella* may be due to irrational use of antibiotics in livestock.

The current result revealed that poultry faming was mainly practiced by the medium age groups ranging from 31 to 50 years of age (Table 8). Similarly, Bamiro *et al.*, (2013) reported that most of the poultry farmers in peri-urban areas were 31-50 years old. In contrast to this, Adebayo and Adeola, (2005) reported that majority (74%) of the poultry farmers fall within the age group of 20-40 years.

The result also showed that 81.4% of the poultry farmers were male that is in agreement with the results of Bamiro *et al.*, (2013) in peri-urban areas of Legos, where the majority

of the poultry farmers were male and Akintunde *et al*, (2015) who reported modern poultry farming was predominantly a male occupation in southwest Nigeria.

In the present study most poultry farmers attended primary and secondary education and very few of them had tertiary education. In contrast to this, Jatto, (2012) and Bamiro *et al*., (2013) reported that most of the poultry farmers had tertiary education. The less involvement of highly educated personnel in poultry farming in the study area might be due to shortage of capital, land, time and less attractiveness of the business.

Most poultry farmers had more than 10 years of experience which was comparable with the report of Ojo, (2003) (9.67 years) and higher than the value reported by Bamiro *et al*., ,(2013). According to Onyebinama, (2004) previous experiences in farm business management enable farmers to set realistic time and cost targets, allocate, combine and utilize resources efficiently and identify production risks.

Majority of the respondents were rearing exotic breed, which contradicted the report of Durmuş *et al.,* 2012 where local chicken were mostly preferred by consumers due to its natural, flavor and taste. But the current result was in agreement with Tikasz *et al*. (2009) that reported as consumers prefer the farm chicken due to high quality and production of their products.

The dominance of bovans brown layer breed and sasso broiler breed of bird in the study area (52.3%) is not by choice, but due to the fact that these are the only available breeds in the supplier farms and kept by the poultry farmers during the study period. The supply of the required layer breed is determined by the supplier farms. The present study revealed that small scale intensive poultry farmers of the study area obtain foundation and replacement breed from privately owned large scale poultry farms. Similarly Desalew *et al*.,(2013) reported that the majority of poultry farmers in Ada'a and Lome districts purchased improved chicks from private hatcheries

Disease was listed as the most constraining factor by the poultry producers, reducing both the number and productivity of the birds (FAO, 2004; Weyuma *et al*., 2015) Similarly, the current result revealed that, high disease prevalence was the major problem often encountered by poultry farmers during this study period, which is also inconsistence with reports from different Nigerian states (Bukar-Kolo,*et al*., 2006) in Gombe state and (Ovwigho *et al*. 2009) in Delta state.

A high proportion of the disease complained by poultry producer was caused by viral agents. All respondents including veterinarians recommended administration of antibiotics following any disease outbreak whether it was bacterial, viral or protozoan origin purposefully to combat secondary bacterial complications by animal health professionals or unknowingly by poultry producers in response to disease outbreaks. This is not in line with the OIE guideline on responsible and prudent use of antimicrobial agents in veterinary medicine (Annex 4). According to OIE guideline on judicious use of antimicrobials, viral, fungal and other non-bacterial infections are not treated with antibiotics.

The most frequently used antibiotics in the current study were tetracyclines, sulfadiazine and trimethoprim combination, fluroquinolones, amoxycillin, colistin sulphate and tylosin. Our finding is consistent with that of Gumphol *et al*., (2017) from Thailand in poultry farms and Fagbamila *et al*., (2017) in commercial poultry laying hens in Nigeria, who reported the use of enrofloxacin, amoxicillin, colistin, doxycycline, and oxytetracycline. Antibiotic resistance to the listed antibiotics has been reported in bacteria of food animal origin in Ethiopia and other countries (Spellberg *et al*., 2016; Ejo *et al*., 2016). Particularly, in low and middle income countries, the use of antibiotic in food producing animals is unregulated which can contribute to the development and spread of antibiotic resistant bacteria(Schar *et al*., 2018).

Tetracycline, amoxicillin, fluroquinolones and colistin are highly important antibiotics in human medicine(WHO, 2014). The use of quinolones in poultry is worrisome as this drug is classified by the WHO as a priority for risk management and as critically

important drug for the treatments of enteric diseases in humans and has been associated with increased resistant bacteria in humans exposed to it from farm animals. Several countries have therefore banned fluoroquinolone use in poultry. Since 2003, fluoroquinolones were withdrawn for use in animals in Denmark and it has also been banned for poultry production in the United States since 2005 (BEUC, 2014). The increased use of this drug has been attributed to several factors that include its broad-spectrum activity, its easy application in water and feed, and its lack of restrictions.

Colistin is another critically important drug and considered to be the last defense against multidrug resistant bacteria especially those resistant to carbapenem antibiotics. The increased use of colistin in livestock production in China resulted in high selection pressure leading to the acquisition of the mobilizable colistin resistance *mcr-1* gene by *Escherichia coli*. China already banned the use of colistin as a growth promoter and released a mandate controlling the use of colistin only for the treatment of disease in animals (Gumphol *et al.*, 2017).

The antibiotic survey showed that poultry farmers in the study area were highly relied on antimicrobial medications. Most farms used combinations of antibiotics, and all farms used one or more antibiotics for therapeutic purpose followed by prophylactic and to a lesser extent for growth promotion. The result of the current study was higher than similar study from Nigeria (Awogbemi *et al.*, 2018) in which antibiotics were commonly administered for therapy followed by, prophylaxis and growth promotion. Another study from the same country uniquely (Adelowo *et al.*, 2014) reported that 86% of the poultry farms used antibiotics for growth promotion. The dependence of poultry farmers on antibiotics for therapeutic and/or prophylactic purposes may also be due to poor environmental sanitation, unhygienic practices, lack of biosecurity and other management inadequacies leading to increased exposure to bacterial pathogens (Christian *et al.*, 2018).

The current study revealed that, human pharmacy and illegal open markets were also a source of antibiotics for poultry farms. This is in agreement with Desta, (2015) from Eastern part of Ethiopia who reported as there have been illegal actors selling veterinary

drugs together with vegetables in local markets called ‘gulit’, inside local shops called ‘sheketa sheket suk’ together with substances used for cooking, in boutiques together with clothing and inside medical pharmacy shops without any legal permission. Tufa *et al.*, (2018) also reported as a packet of different drugs were sold on open market in direct sunlight violating the drug handling and storage recommendations by the WHO, and possibly causing substantial changes to the drug active ingredients. This study also advances our understanding of the factors that contributed to antibiotic misuse and the results showed that there is a need for collaboration between animal agriculture and the public health sectors to assess risk factors developed and distribute protocols to monitor the use of antibiotics, and improve antibiotic resistance surveillance in animals and humans.

Based on responses received from respondents, it is evident that most of the poultry farms do not obtain information on antibiotics they use from qualified personnel. Some of them admitted antibiotics relied on directives from drug store vendors while others depended on their own experience for antibiotic administration. This is relatively better than the report of Dishon *et al.*, (2019) in which all veterinary drug stores sold antibiotics without a prescription in Nairobi, Kenya. The knowledge gap is lower among Ethiopian poultry producers as compared to the report of Fagbamila *et al.*, (2017) in commercial poultry laying hens in Nigeria in which 78.6% agreed that antibiotics used in poultry should be regulated and used when prescribed by veterinarians only. According to the principles of prudent use of antibiotics, administration on prescription-only and under veterinary supervision were recommended (McDonald, 2015).

The respondents also mentioned that observation of a single sick chicken can lead to a blanket prescription of antimicrobials to the entire flock at the farm. This is not in compliant with WHO/OIE guideline on responsible use of antibiotics since Poor feeders, especially those that are ill, may naturally limit their food intake and thus receive lower doses than intended. According to the guideline, treating only sick individual animals is recommended. Blanket administration may result from, fear that, it may be followed by

an outbreak and/or rapidity of disease spread in intensively reared animals and for animal welfare reasons or stress of catching and restrain (Abdi *et al*., 2017).

The field observation divulged that, almost all antibiotics ready for poultry use are formulated for use as in-feed and in-water. Respondents also complained that, making a balanced ratio between the drug and feed/water was difficult. Only few intensive poultry farms had sensitive balance or measuring cylinder to make a correct ratio between the drugs and feed/water. On top of that, resistance is more likely to be selected where the concentration and diversity of bacterial population is high, such as bowel, mouth and throat(Nchawa *et al*., 2019)**.**

According to the respondents, duration of antibiotic administration was dependent on the duration of clinical sign of the disease. This means, as soon as the clinical sign disappears, antibiotic administration was stopped. This is also contradicting the principles of responsible and prudent use of antibiotics. Similar findings of low knowledge of antibiotic stewardship have been reported in countries such as in Vietnam (Pham-duc *et al*., 2019). Low and middle-income countries are often more challenged in allocating adequate resources and instituting policies to address the gaps in knowledge and practices in food production industries(Odeyemi and Sani, 2016). Antibiotic use in food animals remains unregulated, leading to inappropriate use of the drugs and widespread increase in antibiotic resistance (Chattopadhyay *et al*., 2014).

Limitation of the research:

- Initially, the study planned to include antibiotic residue analysis for selected antibiotics and genomic characterization of resistant isolates. But due to budget constraint, lack of laboratory facility and unavailability of chemical agents on domestic market the residue analysis part was omitted.
- For the molecular analysis, even though, FDA accepted our request and has been sent us Meta-data registration form, material transfer agreement form and others to fill and sent back, due to the pandemic occurrence of COVID-19, all the process interrupted. Hence, these activities will be considered in the future research outlook.
- The questionnaire we used for data collection may be difficult to some uneducated respondents in some cases. To fill this gap and avoid data collection bias different techniques had been used.

6. CONCLUSIONS AND RECOMMENDATIONS

In Ethiopia several factors including under and malnutrition, HIV-AIDS, unhygienic living circumstances, consumption of raw/undercooked animal products and the close contact between humans and animals can substantially contribute to the occurrence of foodborne human infection including salmonellosis. Poultry products and farm workers can acquire *Salmonella* from intestinal contents or from cross-contamination during product collection, transportation, slaughtering processes and other farm management activities. Even though, booming poultry production in the current study area provides a major source of income in and around the major towns including Bishoftu, Dukam, Adama and Modjo, it needs special attention from foodborne zoonotic infection perspective, since most of the supply of eggs and dressed poultry carcasses to Addis Ababa and other major cities supermarkets come from small and large commercial farms located in these towns.

However, the overall prevalence of salmonella in the current study was moderate, very important zoonotic *salmonella* serovars were identified. S. Typhimurium, which is the most serious agent involved in outbreak of salmonellosis worldwide was the dominant serovar recorded in this study. A high prevalence of this serovar warrant improved farm hygienic measures to reduce its dissemination to humans and to the environment through all steps of product collection, processing, transportation and storage.

The prevalence of antibiotic resistant salmonella isolates was very high in the current study to most of antibiotics tested, even to those identified by World health organization as priority for risk management and critically important antibiotics for the treatments of enteric diseases in humans including quinolones and high generation of cephalosporin. A single most important action needed to greatly slow down the development and spread of antibiotic-resistant infections is to change the way antibiotics are used. To prescribe a powerful new antibiotic is easy. To protect it is hard. But if we want to break the cycle

and stop repeating the same mistakes of the past over and over, the stakeholders' must learn how to not use and abuse new antibiotics.

A very important constraining factor identified in poultry production in the current study was the prevalence of disease. But, very few large scale poultry farms have a guideline for disease prevention and control including the major biosecurity program.

This study clearly demonstrated that, there was irrational use of antibiotics in almost all poultry farms. Totally, the guideline for responsible and prudent use of antibiotics was not in practices. Remarkable number respondents confirmed as antibiotics used without prescription based on their own and their friends past experiences. The vast majority of the respondents administered antibiotics by themselves based on the instructions given from the veterinary drug shops. Almost all antibiotics ready for poultry use are formulated for use as in-feed and in-water. Only few large scale commercial poultry farms do have sensitive balance to make a correct ratio of antibiotic to feed/water. The respondents also mentioned that observation of a single sick chicken can lead to a blanket prescription of antimicrobials to the entire flock at the farms because of the fear that, it may be followed by an outbreak. Maintaining the status quo and continuing to misuse antibiotics as we have been doing will jeopardize our ability to effectively treat infectious diseases in the future.

Based on the above conclusions the following are forwarded:

- Personnel training on personal hygiene, biosafety and farm biosecurity for all farm attendants and visitors, education on the dangers of indiscriminate use of antibiotics in livestock, zoonotic pathogens, major risk factors that affect awareness on safe handling and management of animal origin food should get focus.

- ❖ Adoption of hazard analysis critical control point principle at various critical points from farm to table, as a tool to determine the precise sources of contamination, is of paramount importance in designing appropriate strategies to substantially reduce the prevalence and associated risk for consumer and trade partners.

- ❖ It would be beneficial to extend this study nationally in order to investigate the perspectives of antibiotic use and other antimicrobials particularly, conducting interviews and focus group sessions with veterinary staff and veterinary students could provide an in depth understanding of the perspectives of veterinary professionals regarding antibiotic resistance as well as aid in the development of strategies for the prudent use of antibiotic drugs.

- ❖ National regulation and improved surveillance are needed to ensure that antibiotics are used prudently and are not routinely fed to animals for nontherapeutic purposes. Responsible authorities should immediately kick off implementation of regulations associated with antimicrobial administrations in poultry production and monitoring programs.

Future research outlook:

- Identifying the knowledge and attitude gap of Veterinary professionals in prescribing and administering of antibiotic from prudent use of antibiotics perspective.
- Investigation of transmission dynamics of *Salmonella* species among human, animal and environment to intervene the expansion of the agent through one health approach.
- Genomic characterization of antibiotic resistant *Salmonella* species of different food sources is very essential to plan national control strategy.

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8. ANNEXES

Annex 1. Biographical Sketch of the researcher

I, the author of this PhD dissertation, was born in December 1974 at a town called Kombolcha which is located in Horro Guduru Wollega Zone, Oromia regional state, Ethiopia. I attended Elementary school at Kombolcha Junior Secondary school and High School Education at Shambo senior secondary School. After a successful completion of my High School Education, I joined Addis Ababa University (AAU), College of Veterinary Medicine and Agriculture and graduated with diploma in animal health in 1995. I was employed as an assistance Veterinarian in Ministry of Agriculture and rural development office of Oromia regional state to serve as a field Veterinarian. I joined AAU again with the opportunity of an advanced standing given by this University for top performers during their diploma program studies. Then after, I graduated in Doctor of Veterinary Medicine degree in 2007 and assigned by Ministry of education to serve as an instructor in Wollega University. After one year of services in 2009, I joined AAU to pursue my Master of Science in Veterinary Epidemiology. Then, I was served as instructor and a dean of the school of Veterinary Medicine in Wollega University until I joined AAU for this PhD programme.

Annex 2. Laboratory procedure for isolation and identification of Salmonella isolates

Materials

Equipment

- Erlenmeyer flasks (500 ml) etc. sterile (for pre-enrichment)
- Disposable inoculation loops (1 µl and 10 µl)
- Plastic petri dishes (9 cm diameter) sterile
- Balance
- Incubators at 37^{o}C and 41.5^{o}C

- Bunsen burner
- Pipettes for 0.1 ml (e.g. 1 ml pipettes)
- Wood spatulas

Media

- Buffered peptone water 225 ml
- Tetrathionate broth (Müller-Kauffmann) 10 ml
- Rappaport Vassiliadis soy peptone broth 10 ml
- Xylose Lysine Desoxycholate (XLD) agar plates
- Brilliant Green (BGA) agar plates
- Nutrient agar plates

Bacterial strains

- Food samples
- Animal Faecal samples

Safety: Carry out all procedures in accordance with the local codes of safe practice.

Specimen Collection and Transport

Ideally, at least 25g of food or animal faeces should be submitted. However, smaller samples may be submitted if larger samples cannot be obtained. *Salmonella* spp. may not be evenly distributed within a sample. Specimens should be mixed prior to testing and specimens should be obtained from several locations within the sample. Food samples should be transported to the laboratory at the appropriate temperature. Foods should be maintained at their recommended storage temperature during transport: Frozen foods (example: ice cream) should be remain frozen for transport; cold foods (example: milk) should be kept cold (not frozen) for transport; and room temperature foods (example: powdered formula) should be transported at room temperature. Faecal samples must be

submitted in a clean, container with no soap or disinfectant residue. Small faecal samples (example: swabs from small animals) may be placed in transport media. The sample must be kept cold and transported to the lab within 8 hours of collection. If the sample cannot reach the laboratory within 8 hours; the sample should be frozen at 70°C or stored on dry ice.

Procedure

Day 1: Non-selective pre-enrichment

Weigh out 25 g food or animal faeces with a sterile wood spatula, place the sample into an Erlenmeyer flask and add 225 ml buffered peptone water to obtain 1 part sample + 9 part buffer. Mix. Incubate at 36°C (+/- 1°C) overnight (16-20 hours).

Day 2: Prepare selective enrichment (I) and (II)

Use a pipette to transfer 1 ml of the pre-enrichment broth to 10 ml Tetrathionate broth (Müller-Kaufmann). (Label as Tube I).
Use a micro-pipette to transfer 0.1 ml (100 ul) of the pre-enrichment broth to 10 ml Rappaport-Vassiliadis soy peptone (RVS) broth. (Label as Tube II)
Incubate Tube I: Tetrathionate broth (Müller-Kaufmann) at 36.0°C ± 1°C and Tube II: Rappaport-Vassiliadis soy peptone (RVS) at 41.5°C± 0.5°C overnight (18-24 hours).

Day 3: Spread on selective agar plates

Spread a 10 µl loop full from the inoculated and incubated Tetrathionate broth (I) and RVS broth (II) on XLD and on BGA agar plates and incubate at 36.0°C ± 1°C overnight (18-24 hours).

Day 4: Selection and subculture of suspect *salmonella* colonies

Examine the XLD plates:

A typical *Salmonella* colony has a slightly transparent red halo and a black centre, a pink-red zone may be seen in the media surrounding the colonies. Note the presence of typical *Salmonella*- like colonies on XLD with a + in the record sheets.

Examine the BGA plates:

Typical *Salmonella* colonies on a BGA agar plate appear red and impart a red/pink colour to the surrounding agar. Other enterics typically appear green or yellow. Note the presence of typical Salmonella-like colines on BGA with a + in the record sheets.

Plate two suspect colonies from XLD agar and BGA onto non-selective media (e.g. nutrient agar) for biochemical confirmation and serotyping.

Day 5-7: Biochemical Identification and Serotyping:

Composition and preparation of culture media and reagents:

The media and reagents are available from several companies including Oxoid, Merck and Difco. The composition of the dehydrated media given below is an example and may vary a little among the different manufacturers. Also, the media should be prepared according to the manufacturer's description if it differs from the description given here.

Brilliant Green Agar (BGA)

Proteose peptone	10.0 g
Yeast extracts	3.0 g
Sodium chloride	5.0 g
Lactose	10.0 g
Sucrose	10.0 g
Phenol red	0.09 g
Brilliant green	0.0047 g

Agar	12.0 g
Water	1000 ml

Preparation

Dissolve 50g of the dehydrated medium in water by heating to the boiling point for 1 minute, adjust pH to 6.7 - 7.1 if necessary and transfer to sterile 1000 ml bottles. Do not autoclave.

Description

Brilliant green is a selective agent. Its indicative principle is based on the ability to ferment lactose and sucrose. Phenol red is the pH indicator, which changes from yellow to red at pH 6.8 - 8.4. Therefore, lactose negative and sucrose negative bacteria like *Salmonella* grow as red-pink, white opaque colonies surrounded by brilliant red zones in the agar.

Proteus and *Pseudomonas* species may grow as small red colonies. Lactose and/or sucrose fermenting organisms are normally inhibited but may grow as yellow to greenish-yellow colonies surrounded by intense yellow-green zones in the agar. These may belong to *E. coli* or the *Klebsiella/Enterobacter* group.

Buffered peptone water

Peptone	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$)	9.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.5 g
Water	1000 ml

Preparation

Dissolve the peptone and chemicals in water; adjust pH to 7.0 after sterilisation. Dispense into suitable flasks and autoclave at 121oC for 20 min.

Nutrient agar

Meat extracts	3.0 g
Peptone	5.0 g
Agar	12 g to 18 g
Water	1000 ml

Preparation

Dissolve the dehydrated medium in the water by heating if necessary. Adjust pH to 7.0 after sterilisation, transfer into bottles and autoclave at 121^{o}C for 20 min. Pour 15 ml of melted medium in each plate.

Rappaport-Vassiliadis Soy Peptone (RVS) Broth

Base

Soy peptone	5.0 g
Sodium chloride	8.0 g
Potassium dihydrogen phosphate (KH2PO4)	1.4 g
Dipotassium hydrogen phosphate (K2HPO4)	0.2 g
Distilled water	1000 ml

Heat to about 80^{o}C to dissolve all ingredients. Prepare this solution the same day as the complete RVS medium is prepared.

Magnesium chloride solution

Magnesium chloride (MgCl2.6H2O)	400 g

Water	1000 ml

Dissolve the salt in the water. Because this salt is very hygroscopic, it is advisable to dissolve the entire contents of a newly opened container in distilled water. The magnesium chloride solution can be stored unsterilised, in a dark bottle with screw cap, at room temperature for up to 2 years.

Malachite green solution

Malachite green oxalate	0.4 g
Distilled water	100 ml

Dissolve the salt in the water. The solution can be stored unsterilized, in a dark bottle with screw cap, at room temperature for up to 8 months.

Complete medium

Base	1000 ml
Magnesium chloride solution	100 ml
Malachite green solution	10 ml

Preparation

Mix the solutions well and distribute the solution in portions of 10 ml per tube with screw cap. Autoclave at 115^{o}C for 15 min. Adjust the pH so that after sterilization, Store at about 4^{o}C for a maximum of 4 months.

Description

This medium is used as a selective enrichment medium for the isolation of *Salmonella* from food, environment specimens and from faeces. Malachite green is the selective agent.

Salmonella species have the following characteristics when compared with other *Enterobacteriacea*. Ability to survive at relatively high osmotic pressure; Multiply at relatively low pH values; Are more resistant to malachite green and have less demanding nutritional requirements.

Tetrathionate broth

Brilliant green solution	
Brilliant green	0.1 g
Sterile distilled water	100 ml

Iodine - Potassium iodine solution

Iodine double sublimated	16 g
Potassium iodide	20 g
Sterile distilled water	80 ml

Base

Meat extracts	0.9 g
Peptone from meat	4.5 g
Yeast extracts	1.8 g
Sodium chloride	4.5 g
Calcium carbonates	25.0 g
Sodium thiosulfate	40.7 g
Ox bile, dried	4.75 g
Sterile water	1000 ml
Brilliant green solution	1:1000 10 ml
Iodine-Potassium iodine solution	20 ml

Preparation

Dissolve the tetrathionate bouillon in sterile water in a flask by shaking. Aseptically add brilliant green solution and then iodine-potassium iodine solution. Adjust pH to 7.4 - 7.8 at 25^{o}C. Store bouillon at about 4^{o}C.

Description

Tetrathionate broth is used for selective enrichment of *Salmonella*. According to ref. 2 Mueller-Kauffman Tetrathionate broths (CM343) has improved selectivity compared with Tetrathionate broth (USP) (CM671) and Tetrathionate broth (CM29) all from Oxoid, but it is inhibitory to *S*. Typhi, *S*. Pullorum, and *S*. Gallinarum.

Xylose lysine desoxycholate (XLD) agar	
Yeast extracts	3.0 g
Sodium chloride	5.0 g
Xylose	3.75 g
Lactose	7.5 g
Sucrose	7.5 g
L-lysine hydrogen chloride	5.0 g
Sodium thiosulphate	6.8 g
Iron (III) ammonium citrate	0.8 g
Phenol red	0.08 g
Sodium desoxycholate	1.0 g
Agar	15.0 g
Distilled water	1000 ml

Preparation

Dissolve the components in the water. Heat under constant stirring until the medium starts to boil. Avoid over-heating. Avoid preparing too large a volume of medium, as this requires prolonged heating. Immediately transfer the solution to a water bath at about 50oC, continue stirring until the medium has reached about 50oC. Adjust the pH so that after heating it is 7.4 □ 0.2 at 25^{o}C. Poured agar plates can be stored for a maximum of 14 days, if stored in plastic bags in the dark at about 4oC. Add 10 ml of a 0.15% filter sterilized solution of sodium novobiocin to increase the selectivity.

Description

Sodium desoxycholate is the selective agent and phenol red is the pH indicator. The indicative principle is based on lactose, sucrose and xylose fermentation, H_2S production and lysine decarboxylation. If H_2S is produced from sodium thiosulphate, black FeS (Ferrosulfide) will develop. *Salmonella* ferments xylose, but not lactose and sucrose, decarboxylate lysine and produces H_2S. *Salmonella* suspect colonies grow as red colonies with a black center. Other bacteria that may grow on XLD agar are usually yellow and the agar will also turn yellow. Other bacteria such as *Edwardsiella* may mimic *Salmonella*.

Flow diagram for isolation/identification of *Salmonella* from Food / Animal Faceces

Non-selective pre-enrichment

25 g food / faeces in 10% phosphate buffer (36° +/-1°C, 24 h.)

↓

Selective enrichment *

0.1 ml in 10 ml Rappaport-Vassiliadis Soy Broth (41.5° +/- 0.5°C, 24 h.)

1 ml in 10 ml Tetrathionate broth (Müller-Kauffman) (36° +/-1°C, 24 h.)

↓

Isolation

XLD with an inoculation loop (36° +/-1°C, 24 h.)

BGA with an inoculation loop (36° +/-1°C, 24 h.)

↓

Suspect colonies to nutrient agar (36° +/-1°C, 24 h.)

↓

Biochemical confirmation (36° +/-1°C, 24 h.)

Triple Sugar Iron Agar (TSI) or Kliger Iron Agar (KIA)

Urea broth

Lysine Iron Agar (LIA)

Motility-Indol-Ornithine (MIO)

Citrate

↓

Serotyping

O-antigens

H-antigens

Phase I (37°C, overnight)

Phase II

* If *Salmonella* serovars Typhi or Paratyphi A are suspected: inoculate 1mL of pre-enrichment broth into 10mL of Selenite Cystine (or Selenite F) broth and incubate at 36°C (+/-1°C) for 18-24 h. Following incubation, it is advisable to inoculate the selective broth onto bismuth sulphate agar (in addition to XLD and BGA).

(Source: WHO, 2010)

Annex 3. Questionnaire about antibiotic use in poultry farms

Part I: Characteristics of the respondent

1. Name/code of the respondent_____

 Address: District_____ Kebele _____

2. Is your residential place characterized by: ☐ urban ☐ Peri-urban ☐ rural?
3. Gender of the respondent: ☐ Male☐ Female
4. How old are you? (Age in years)_____
5. To what level have you attended school?

 ☐ Cannot write and read in any language

 ☐ Primary school (grades 1-8) ☐Secondary school (grades 9-12)

 ☐ Technical and vocational school degree) ☐College/university (at least BSc

6. For how long you have been working in this chicken farm? _____

7. What is your responsibility or position in this poultry farm (multiple responses possible)

☐ Farm owner and/or manager ☐ Hired manager

☐ Hired employee (state your responsibility): _____

8. Do you have training related to biosecurity, antibiotic use and effects related to antibiotic use? ☐ Yes ☐ No

II. Characteristics and management of the farm

9. To which production system is the farm grouped?

☐ Backyard scavenging (family owned) for subsistence

☐ Semi-intensive commercial farms

☐ Intensive commercial

10. Since when this chicken farm was established? (years): _____

11. What is the type of the chicken? ☐Layers ☐broilers ☐ Both (mixed egg and meat)

12. How many chickens are found on your farm currently?_____

☐Less than 100 ☐101-1000 ☐greater than 1000

13. Do you follow all-in-all-out production system? ☐ Yes ☐ No

14. Which breed of chicken are you keeping on your farm? ☐ Local ☐ Exotic/Crossbred

15. What is the type of housing you use for your poultry?

☐ Deep litter system ☐ cage system ☐ other (specify) _____

16. What is the approximate space size allocated for your chicken? (No of chicken per m^2)

17. What source of water are you using for chicken on your farm?

☐Well water ☐ Pipe water ☐other (specify) _____

18. Which of the following factors are considered problems of your chicken farm? Please rank them according to the extent of the problems (1= most problematic and continue).

Constraints	Rank (1, 2…)
☐ lack of enough feed resource	
☐ presence of predators	
☐ high prevalence of diseases	
☐ inadequate animal health services	
☐ inadequate finance	
☐ inadequate land (house space)	
☐ Other (specify):	

19. In your view, what role do the following management practices play in reducing disease occurrence? [Use the following scale of 1-5 where 1 = No Role and 5 = Substantial Role]

Management Practices	**No Role**	**Minimal role**	**Not Sure about the role**	**Moderate role**	**Maximal role**
Regular veterinary inspection of the birds					
Appropriate nutrition of the chicken					
Programmed vaccination					
Keeping bio-security (Keeping chicken from contact with others and disinfecting of premises)					
Provision of good quality water					

Good air circulation					
Clean bedding/housing					
Regular use of antibiotics on healthy birds					

Part III: Health condition and antibiotic use

20. Mention common diseases which affect your chicken? ____ ____ _____ _____
21. Do you have ☐ your own veterinarian or ☐ other animal health personnel or ☐ do you have access to any veterinary service
22. Do you routinely use vaccines? If so for which diseases? _____ _____ _____
23. For what purposes are modern drugs used in poultry production, in your farm case? ☐For the treatment of sick chicken ☐ For prevention of diseases in healthy chicken☐ For growth promotio ☐ other (specify) _____
24. Which of the following medicine which I will show you the picture do you use in your farm? Kindly, tell me how you give each of the medicine to the chicken.

Type of antibiotics	Means of administration				
	Injection	Adding to feed/water	Spray	drop into mouth	Other (specify)
☐ Amoxycillin					
☐ Collistinsulphate					
☐ Oxytetracycline					
☐ Doxycycline					
☐ Norfloxacin					
☐ Enrofloxacin					
☐ Tylosin tartrate					
☐ Trimethoprime					
☐ Sulpha dizine					
☐other(specify)					

25. From where do you get drugs used in your poultry farm?

☐ Veterinary drug shop ☐ Human Pharmacy/drug shop

☐ Public Veterinary Clinic ☐ Open market (hawkers)

26. Who prescribe (recommend to use) drugs in your farm for intended use?

☐ Animal health personnel ☐ Self-prescription (by your own)

☐ Friends/ people in your acquaintance ☐ other (specify) _____

27. If you consult animal health professional to prescribe medication for your chicken, how consistent it is? ☐ Always ☐ Sometimes

28. Who administer drugs on your birds? ☐ Self - administer ☐ Animal health personnel ☐ Other (specify) _____

29. How is antimicrobial dosage determined before usage in birds in your farm?

☐ After reading the instructions on labels ☐ From prescription paper

☐ With assumption ☐ Other (specify) _____

30. Please indicate the extent to which you agree or disagree with the following statements about the use of drugs in poultry farms

Respondent opinion	**Strongly disagree**	**disagree**	**Neither agree nor disagree**	**agree**	**Strongly agree**
It is always recommended to consult animal health professional before using drugs in poultry farm					
It is good treating birds only when they become sick					
Preventative use of antibiotics in the poultry farm helps to meet production goals					
Consuming eggs from chicken under treatment may cause					

effect on human health					
The frequent use of antibiotics in poultry farm can lead to antibiotic resistant germs in animals					
Use of antibiotics in poultry farming can contribute to the occurrence of pathogen resistance against drugs in the human population					
Eggs or meat from animals being treated with antibiotics should not be sold for consumers					

IV. Antimicrobial residue

31. Do you think antibiotics can pass in to animal product (egg, meat, milk) if the animal is under treatment? ☐ Yes ☐ No
32. Have you heard of drug withdrawal period (the time administering the last dose of a drug to the animals to the time it is possible to use produce of the animals being treated)? ☐ Yes ☐ No
33. If yes, from which source did you get?
    ☐ From drug leaflet ☐ From Veterinarian
    ☐ From poultry product buyer ☐ From friends
34. What do you know about drug misuse in poultry production?

☐ When given under-dose

☐ When given over-dose

☐ Use of drugs in the absence of disease

☐ When stopped before the end of the recommended duration

☐ I don't know

35. What will happen to the consumer health if drug withdrawal period is not followed/kept?

V. Antimicrobial resistance

36. Do you know the resistance of germs causing animal diseases to medicines?
☐ Yes ☐ No

37. If yes to above question, what is your source for the information?
☐ Mass media (Radio/TV) ☐ Friends/Relatives
☐ Animal health personnel ☐ Human health personnel
☐ Other (Specify) _____

38. Can antimicrobial misuse results in the development antimicrobial resistance in animal pathogenic germs? ☐ Yes ☐ No

39. If yes to above question, what do you think the causes for the occurrence of the resistance?
☐ Use of drug over the recommended dose ☐ Use of drug under the recommended dose
☐ Continuous supply of drugs in animal feed
☐ Use of human preparation drugs for animals

40. What consequences do have drug resistance in pathogens affecting animals?
☐ A threat for human health ☐ A non-responsive treatment and death of animal
☐ Increased in the treatment cost due to repetitive use
☐ Other (specify) _____

Annex 4. Guideline on responsible and prudent use of antibiotics in animal Production

General principles of appropriate antibiotic use

A. Pretreatment

Disease Prevention

- Appropriate (best practice) husbandry and hygiene, routine health monitoring, vaccination, nutrition, etc
- Codes of Practice, Quality Assurance Programmes, Flock Health Surveillance Programmes (FHSP) and Education Programmes should promote the responsible and prudent use of antimicrobial agents

Professional Intervention

- All uses (labelled and extra-label) of antimicrobials meet all the requirements of a valid veterinarian-client-patient relationship.

Distribution of Antimicrobial Agents

- Veterinarians should work with those responsible for the care of animals to use antimicrobials judiciously regardless of the distribution system through which the antimicrobial was obtained.

Alternatives to Antimicrobial Agents

- Efficacious, scientific evidence-based alternatives to antimicrobial agents can be an important adjunct to good husbandry practices

Diagnosis - Accurate diagnosis

- Diagnosis of a bacterial infection (clinical diagnosis complemented with laboratory diagnosis and epidemiological information as appropriate).

Therapeutic objective & plan

- Develop outcome objectives (for example clinical or microbiological cure) and implementation plan (including consideration of therapeutic choices, supportive therapy, host and other factors)

Drug selection

Justification of antimicrobial use

- Other therapeutic options should be considered prior to antimicrobial therapy
- Antimicrobials are a complement to good husbandry practices and should never be used to compensate for or mask poor farm or veterinary practices.
- Informed professional judgment balancing the risks and benefits for humans and animals

Guidelines for antimicrobial use

- Disease specific guidelines for antimicrobial selection and use should be consulted.

Critically important antimicrobial agents

- All antimicrobial agents, including those considered important in treating refractory infections in human or veterinary medicine, should be used in animals only after careful review and reasonable justification.

Culture and sensitivity testing

- Utilize culture and susceptibility when clinically relevant results to aid in the selection of antimicrobials, especially if initial treatment has failed.

Spectrum of activity

- Use narrow-spectrum in preference to broad-spectrum antimicrobials whenever appropriate.

Extra-label (off label) antimicrobial therapy

- Must be prescribed only in accordance with prevailing laws and regulations.
- Should be confined to situations where medications used according to label instructions have been ineffective and where there is scientific evidence, including residue data, supporting the off-label use pattern

Drug use

Dosage Regimens

- Regimens for therapeutic antimicrobial use should be optimized using current pharmacological (pharmacokinetic-pharmaco-dynamic [PK/PD]) information.

Duration of Treatment

- Therapeutic exposure to antimicrobials should be minimized by treating only for as long as needed to meet the therapeutic objective.

Labelling and Instructions

- Written instructions about the drug use regimen must be given to the end user by the veterinarian with clear details of method of administration, dose rate, frequency and duration of treatment, precautions and withholding period.

Target Animals

- Limit therapeutic antimicrobial treatment to ill or at risk animals, treating the fewest animals possible.

Record Keeping

- Accurate records of treatment and outcome should be used to evaluate therapeutic regimens.

Compliance

- Encourage and ensure that instructions for drug use are implemented appropriately

- **Monitor Response to Treatment**

- Report to appropriate authorities any reasonable suspicion of an adverse reaction to the medicine in either the treated animals or farm staff having contact with the medicine, including any unexpected failure to respond to the medication.

B. Post treatment considerations

Environmental contamination

- Minimize environmental contamination with antimicrobials whenever possible.

Surveillance of antimicrobial resistance

- Susceptibility surveillance should be undertaken periodically and the results provided to the prescriber, supervising veterinarians and other relevant parties.

Continuous evaluation

- Veterinarians should continuously evaluate their prescribing practices, based on such information as the main indications and types of antimicrobials used in different animal species and evaluated in relation to available data on antimicrobial resistance and current use guidelines.

Source: RUMA (Responsible Use of Medicines in Agriculture Alliance) (2005).

OIE guideline on responsible and prudent use of antimicrobial agents in Veterinary Medicine

A. The responsible use of antimicrobials in veterinary medicine

The Ad hoc Group described responsible use as follows:

a) Represents the scientific and technically directed use of these compounds that are the responsibility of professionals with the required expertise

b) Is part of good veterinary and animal husbandry practice and takes into consideration disease prevention practices such as the use of vaccination and improvements in husbandry conditions when disease problems become evident

c) Aims to reduce the use of antimicrobial agents to their approved and intended uses

d) Takes into consideration on-farm sampling and testing of isolates from food-producing animals during their production (where appropriate), and makes adjustments to therapy when problems become evident

e) Should be based on the results of resistance surveillance and monitoring (bacterial cultures and antimicrobial sensitivity testing)

f) Is aimed at all the relevant professionals, including the following:

- Administrative and scientific authorities
- The veterinary pharmaceutical industry
- Distributors and others handling antimicrobials
- Veterinarians, pharmacists and livestock producers.

B. Responsibilities of veterinarians

The use of antimicrobials is no substitute for good management practices and the prime concern of the veterinarian is to encourage good farming practice in order to minimize the need for antimicrobial use in livestock. In the frame of good management practice, the veterinarian is responsible for identifying recurrent disease problems and developing alternative strategies to prevent or control disease. These may include changes in husbandry conditions and vaccination

programmes where vaccines are available. Veterinarians should only prescribe antimicrobials for animals under their care, which means that:

- The veterinarian must have been assigned responsibility for the health of the animal or the
  herd/flock by the producer or an agent of the producer
- That responsibility must be real and not merely nominal
- That the animal(s) or herd/flock must have been examined immediately before the prescription and supply or sufficiently recently or frequently for the veterinarian to have personal knowledge of the condition of the animal(s) or current health status of the herd or flock to make a diagnosis and prescribe
- The veterinarian should maintain clinical records of the animal(s)/herd/flock.

It is recommended that veterinary professional organizations develop for their members, species specific clinical practice guidelines on the responsible use of antimicrobials, with particular reference to the choice of product, disease prevention strategies and treatment protocols.

The responsibilities of veterinarians in this area are described below.

C. Use of antimicrobial agents when necessary

The appropriate use of antimicrobials in practice is a critical decision which, where possible, should be based on the following:

- The experience and local expertise of the prescribing veterinarian
- An accurate diagnosis, based on adequate diagnostic procedures.

On certain occasions, a group of animals which may have been exposed to pathogenic bacteria may need to be treated without recourse to an accurate diagnosis and antimicrobial susceptibility
testing, to prevent the development of clinical disease and for reasons of animal welfare.

D. Determination of the choice of an antimicrobial

The expected efficacy of the treatment

The expected efficacy of the treatment is based on the following:

- The clinical experience of the veterinarian
- The activity towards the pathogenic bacteria involved
- The epidemiological history of the rearing unit, particularly in relation to the antimicrobial resistance profiles of the pathogenic bacteria involved. Ideally, the antibiotic profiles should be established before the commencement of treatment. Should a first line antibiotic treatment fail or should the disease recur, the use of a second line antimicrobial agent should be based on the results of the microbiological tests
- The appropriate route of administration
- Results of initial treatment
- known pharmacokinetics/tissue distribution to ensure that the selected therapeutic agent is active at the site of infection
- Prognosis.

To minimize the likelihood of antimicrobial resistance developing, it is recommended that antimicrobials be targeted to bacteria likely to be the cause of infection.

Absence of selection or limited selection of antimicrobial resistant bacteria

The absence of selection or limited selection of antimicrobial resistant bacteria is influenced by the following:

- The choice of the activity spectrum of the antimicrobial
- The targeting of specific bacteria
- Known or predictable susceptibilities using antimicrobial susceptibility testing
- The correct dosing regimens
- The use of combinations of antimicrobial agents
- The importance of the drug to human and/or veterinary medicine. Antimicrobials which are considered important to treat critical diseases in humans and/or animals, should be used only when other therapies are unavailable or inappropriate
- The route of administration.

Combinations of antimicrobials

Combinations of antimicrobials are used for their synergistic effect to increase therapeutic efficacy or to broaden the spectrum of activity. Furthermore, the use of combinations of antimicrobials can be protective against the selection of resistance in cases in which bacteria exhibit a high mutation rate against a given antimicrobial. However, a bad choice of a combination of antimicrobials may, in certain cases, lead to an increase of the selection of resistance. If the use of a combination of antimicrobials is justified, the veterinarian should ensure that there is no antagonism between the chosen antimicrobials and should check the ability of these antibiotics to reach the infection site under similar time and concentration conditions, to maintain effective therapeutic concentrations as long as required.

E. Appropriate use of the antimicrobial agent chosen

A prescription for antimicrobial agents must precisely indicate the treatment regime, the dose, the dosage intervals, the duration of the treatment, the withdrawal period and the amount of drug to be delivered, depending on the dosage and the number of animals to be treated. All medicinal products should be prescribed and used according to the conditions of the marketing authorization, which are reflected in the summary of product characteristics provided by the manufacturer. If the label conditions allow for some flexibility, the veterinarian should consider a therapeutic regimen that is sufficiently long to allow the effective recovery of the animal, but sufficiently short to limit the selection of resistance in food-borne and/or commensal bacteria.

'Off label use' (extra-label use) of veterinary medicinal products

Although all medicinal products should be prescribed and used in accordance with the specifications of the marketing authorization, the prescribing veterinarian should have the discretion to adapt these in exceptional circumstances. The 'off label use' of an

antimicrobial agent may be permitted in appropriate circumstances and should be in agreement with the national legislation in force. The veterinarian has the responsibility to define the conditions of responsible use in such a case, including the therapeutic regimen, the route of administration and the duration of the treatment.

Recording

All available information should be consolidated into one form or database, such that this information should:

- Allow monitoring of the quantities of medication used
- Contain a list of all medicines supplied to each livestock holding
- Contain a list of medicine withdrawal periods and a system for allowing information to be updated
- Contain a record of antimicrobial susceptibilities
- Provide comments concerning the response of animals to medication
- Allow the investigation of adverse reactions to antimicrobial treatment, including lack of response due to antimicrobial resistance. Suspected adverse reactions should be reported to the appropriate regulatory authorities.

Labeling

All medicines supplied by a veterinarian should be adequately labelled with the following minimum information:

- The name of the owner/keeper or person who has control of the animal(s)
- The address of the premises where the animal(s) is kept
- The name and address of the prescribing veterinarian
- The date of supply
- The indication 'For animal treatment only'
- The warning 'Keep out of the reach of children'
- The relevant withdrawal period, even if this is nil.

The label should not obscure the expiry date of the preparation or any important information supplied by the manufacturer.

Training

Veterinary professional organizations should participate in the training programmes as defined in the earlier section entitled 'Training of antibiotic users'.

F. Responsibilities of producers

Producers are responsible for preventing outbreaks of disease and implementing health and welfare programmes on their farms. They may, as appropriate, call on the assistance of their veterinarian in undertaking these duties. All those involved with the livestock on the farm have an important role to play in ensuring the responsible use of antimicrobials. Therapeutic antimicrobial Products should be regarded as complementing good management, vaccination and farm hygiene. Efforts should be made to ensure that environmental contamination both by antimicrobials and by resistant bacteria is kept to a minimum.

Livestock producers have the following responsibilities:

a) To draw up a health plan with the veterinarian in charge of the animals that outlines preventative measures (mastitis plan, worming and vaccination programmes, etc.)

b) To use antimicrobial agents only on veterinary prescription and according to the provisions of the prescription

c) To use antimicrobial agents in the species, for the uses and at the doses on the approved/registered labels and in accordance with product label instructions or the advice of a veterinarian familiar with the animals and the production site

d) To isolate sick animals, when appropriate, to avoid the transfer of resistant bacteria

e) To comply with the storage conditions of antimicrobials in the rearing unit, according to the provisions of the leaflet and package insert

f) To address hygienic conditions regarding contacts between people (veterinarians, breeders, owners, children) and the animals treated

g) To comply with the recommended withdrawal periods to ensure that residue levels in animal derived food do not present a risk for the consumer

h) To dispose of surplus antimicrobials under safe conditions for the environment. Partially-used medicines should only be used within the expiry date, for the condition for which they were prescribed and, if possible, in consultation with the prescribing veterinarian

i) To maintain all the laboratory records of bacteriological and susceptibility tests. These data should be made available to the veterinarian responsible for treating the animals to optimise the use of antimicrobials in that unit

j) To keep adequate records of all medicines used, including the following:

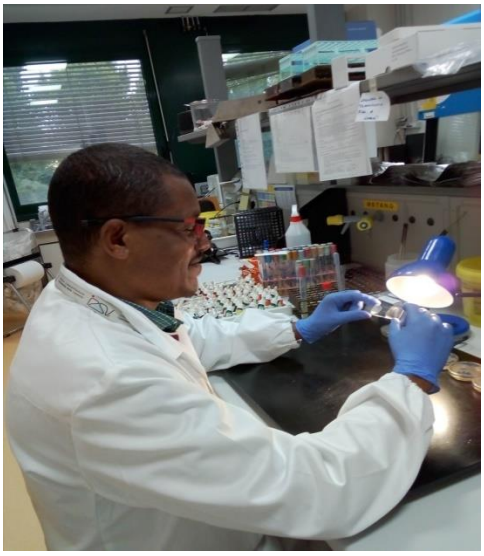
- Name of the product/active substance and batch number
- Name of supplier
- Date of administration
- Identification of the animal or group of animals to which the antimicrobial agent was administered
- Diagnosis/clinical conditions treated
- Quantity of the antimicrobial agent administered
- Withdrawal periods
- Result of laboratory tests
- Effectiveness of therapy

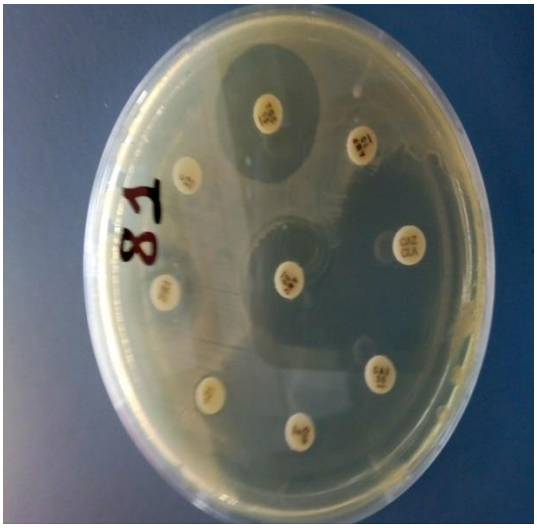
k) To inform the veterinarian responsible for the unit of recurrent disease problems.

Source: Anthony *et al.*, 2001

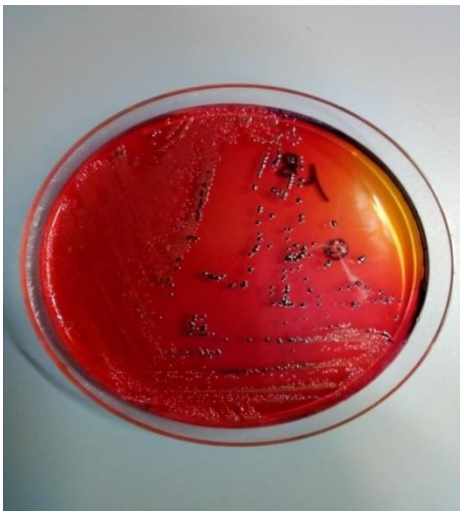
Annex 5. Photos of different poultry farms, laboratory activity and training

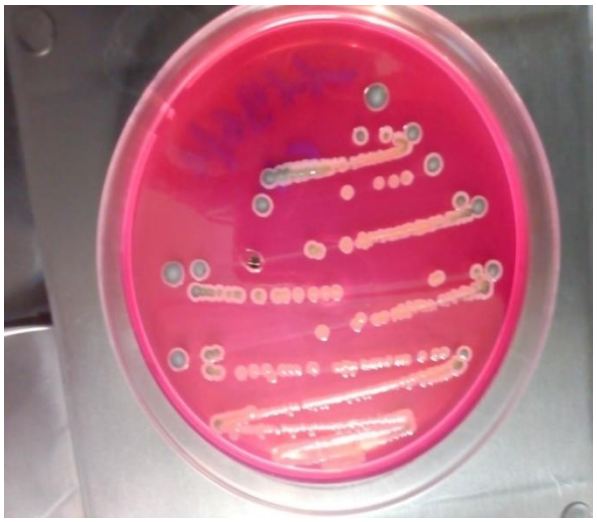
Different poultry production systems

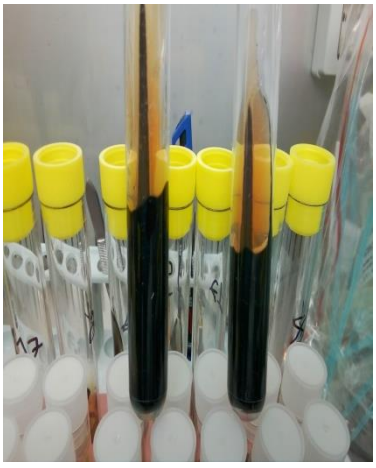


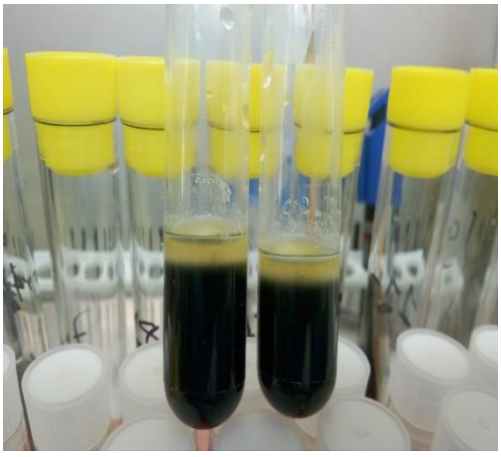


Laboratory activity and training at Italy









Salmonella isolation and identification activity

Annex 6. Publications of the research output

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Antibiotic Use in Poultry Production in Selected Districts of East Showa Zone, Central Ethiopia: From Antibiotic Stewardship Perspective

[1]Mezene Woyessa, [2]Getahun E. Agga, [3]Balako Gumi, [4]Dinka Ayana and [5]Gezahegn Mamo

[1]School of Veterinary Medicine, Wollega University, P.O. Box: 395, Nekemte, Ethiopia
[2]USDA, Agricultural Research Service, Food Animal Environmental Systems Research Unit, 2413 Nashville Road, B-5 Bowling Green, KY 42101, USA
[3]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P.O. Box: 1176, Addis Ababa, Ethiopia
[4]College of Veterinary Medicine and Agriculture, Addis Ababa University, P.O. Box: 34, Bishoftu, Ethiopia

Abstract: Misuse and overuse of antibiotics is a primary contributor for the development of antibiotic resistance. The World Health Organization estimates that in the past decade the number of deaths attributed to antibiotic resistant bacteria exceeded the combined number of deaths due to influenza, human immunodeficiency virus and traffic accidents. Antibiotic stewardship plays a major role for the control of antibiotic resistance. For the assessments of antibiotic usage on the poultry farms, structured questionnaire surveys and in-depth interviews with poultry producers, poultry managers and key-informants (veterinarians, animal production experts working on the farm) were performed. All farms used one or more antibiotics and administered them mainly through feed/water. Tetracycline (100% of farms) and sulfadiazine + trimethoprim (94.1%), fluoroquinolones (41.5%) and cloxacillin + ampicillin (29.1%) were the most frequently used drugs. Antibiotics were used for disease treatment (100% of farms), for disease prevention (56%) or for growth promotion (32.2%). Diseases for which antibiotics were frequently used include Newcastle disease (100% of farms), Gumboro disease (54.4%), coccidiosis (53.4%), Marek's disease (49.7%), foul typhoid (45.9%) and foul pox (39.2%). Majority (61.9%) of the farms obtained antibiotics by prescription from veterinarian, over the counter as self-prescription (32.2%) or as recommended by friends with prior experience (11.9%). Veterinary pharmacies (100% of farms), veterinary clinic (51.0%), human pharmacies (26.8%) and open market (16.2%) were the sources of antibiotics. Although on 37.9% of farms antibiotics were administered by veterinary professionals, majority of the farms administered antibiotics by themselves based on the drug labels or as directed by a prescriber or pharmacist. This study clearly demonstrated lack of awareness about antibiotic use that in turn can lead to the propagation of antibiotic resistant bacteria and foster accumulation of antibiotic residues in poultry products in the study area. There is also lack of polices and regulatory system on antibiotic use, in the country as a whole. Antibiotic stewardship programs such as removing the use of antibiotics for growth promotion, open market access as well as increasing veterinary and diagnostic services will help to mitigate antibiotic resistance.

Key words: Antibiotic Use · Poultry · Antibiotic Stewardship · Antibiotic Resistance

INTRODUCTION

The problem of bacterial resistance to antibiotics is a burning question throughout the world. According to an estimate by the World Health Organization (WHO), during the past decade the number of deaths caused by some resistant strains exceeded the combined number of deaths caused by influenza, human immunodeficiency virus and traffic accidents [1]. Over the past few decades, new antibiotics have not been produced and almost all known

Corresponding Author: Mezene Woyessa Bussa, School of Veterinary Medicine, Wollega University, P.O. Box: 395, Nekemte, Ethiopia. E-mail: mezeneghimbi@yahoo.com.

antibiotics are increasingly losing their activity against pathogenic microorganisms [2]. The levels of multi-drug resistant bacteria have also increased [3-5]. It is known that worldwide, more than 60% of all antibiotics are used in animal production both for therapeutic and non-therapeutic purposes [6].

According to the World Organization for Animal Health (OIE) annual report on antimicrobial agents intended for use in animals [7], in many countries antibiotics were widely available virtually with no restriction or control. Of 143 OIE member countries and three non-OIE member countries assisted through the OIE performance of veterinary service pathway, as of November 2017, seven countries did not yet have completed relevant legislation to ensure appropriate condition for import, distribution, manufacturing and use of veterinary medicinal products including antimicrobial agents. As a result, these products circulate freely, like ordinary goods and are often falsified or substandard. Inappropriate use of antimicrobials creates condition for high risk for the development and spread of antibiotic resistance [8-10]. The rapid surge in the development and spread of antibiotic resistance is the main cause for concern [11]. In recent years, enough evidence highlighting a link between excessive use of antimicrobial agents and antibiotic resistance from animals as a contributing factor to the overall burden of antibiotic resistance has emerged [12-14]. The extent of usage is expected to increase markedly over coming years due to intensification of farming practices in most of the developing countries[15]. The main reasons for the use of antibiotics in food-producing animals include prevention of infections, treatment of infections, promotion of growth and improvement in production in the farm animals [16, 17].

Poultry is one of the most wide spread food animal industries worldwide. Chicken is the largest farmed animal species, with over 90 billion tons of chicken meat produced per year [18]. Large amounts of antimicrobials are used to raise poultry in most countries. Large number of such antimicrobials are considered essential in human medicine. Indiscriminate use of essential antimicrobials in animal production is likely to accelerate the development of antibiotic resistance in pathogenic and commensal bacteria. Antimicrobial resistance(AMR) would result in treatment failures, economic losses and could act as source of gene pool for transmission to humans [19]. In addition, there are also human health concerns about the presence of antimicrobial residues in meat, eggs and other animal products [20].

It has been planned to increase the total broiler production by 235% and the total egg production by 828% from 2015 to 2020 through improved family poultry as semi-scavenging crossbreeds and working towards commercial specialized layer and broiler operations in Ethiopia [21]. Even though the goal of the plan is to reduce poverty and improve household nutrition as well as to increase national income, such transformative production systems may lead to unrecognized risks to human health in terms of antibiotic resistance and drug residues as a result of intensified production systems.

While antibiotic resistance is recognized as a 'one health' issue and international organizations such as WHO, OIE, Food and Agriculture Organization of the United Nations (FAO) have been adopting global action plan to combat antibiotic resistance [22], data on antibiotic use in poultry production system in Ethiopia is limited. Despite limited antibiotic resistance studies in poultry, studies assessing the knowledge, attitude and practices related to antibiotic use in poultry farms are lacking in Ethiopia. Our objective was to collect baseline data on the use of antibiotics in poultry production and to provide a greater understanding of the potential impact of veterinary practices on public health. We used structured and semi structured questionnaire survey in major poultry producing districts in East Shewa Zone of Oromia, Ethiopia.

MATERIALS AND METHODS

Relevance and Description of Study Area: Almost all large-scale commercial poultry farms in Ethiopia are found in East Shewa Zone of Oromia regional state. In addition, there are also emerging intensive small-scale poultry farms in the urban and peri-urban areas in which small number of exotic breeds of chicken (50-1, 000) are produced along commercial lines using relatively modern management methods [23]. Booming poultry production in this area provides a major source of income in and around the major cities including Bishoftu, Dukam, Adama and Modjo. Most of the supply of eggs and dressed poultry meat to Addis Ababa supermarkets come from small and large commercial farms located in these cities. The study was conducted in Ada'a, Akaki, Adama and Lume districts because of large concentrations of poultry farms in the area.

East Shewa Zone is situated from 38° 03' to 40° 05' E longitude and from 7° 04' to 9° 10' N latitude covering a total land area of about 13766.5 km^2. The altitude of the study area ranges from 538 to 3, 101 m above sea level.

East Shewa Zone is characterized by semi-arid and sub-humid climate based on the moisture index climate classification. Considering the long-term average seasonal (June - September) rainfall, the area receives 458-518 mm rain. Based on meanannual rainfall and temperature of the area, the major climatic classes of the zone are dry climate and tropical rainy climate [24].

Questioner Survey: For the assessments of antibiotic usage on the poultry farms, structured questionnaire surveys and in-depth interviews with poultry producers, poultry managers and key-informants (veterinarians, animal production experts working on the farm) were performed from January, 2019 to November, 2020. Questionnaire survey focusing on the demography of the respondents, farm characteristics, common antibiotics used, common poultry diseases encountered, antibiotics used (types, doses, administration routes, formulation, reason for use, time and duration, etc.) and a researcher's own observation were used to identify indicators for the use of antibiotics on the farms. It was planned to include 100-120 respondents from each district based on the availability of poultry farms from every poultry production system. All large and medium scale commercial type poultry farms were included due to their limited number and one respondent from each farm based on their responsibility for farm management were selected. The respondents owning backyard poultry were included if they had more than 10 chicken based on the information gained from the local development agencies.

Data Analysis: Data collected through the questionnaire survey were entered into Microsoft Excel, cleaned, organized and sorted. Data were then coded and imported into IBMSPSS statistics 22 for data analysis. Descriptive analysis was done to generate frequencies and percentages for an outcome variable from the independent variables (demographic characteristics of the respondent, farm characteristics, common antibiotics used, major poultry disease and antibiotic use practice and administration).

RESULTS

Demographic Characteristics of the Respondents: As shown in Table 1, majority (81.4%) of the poultry farmers interviewed were male, were 31-50 years of age (48.4%), completed primary school (35.8%), secondary school (31.2%), or post-secondary education (26.0%). Bishoftu and Adama are the potential cities where poultry production has been growing. Almost all poultry farming activities were carried out by the farm owners (88.9% of farms). About 41.2% of the respondent's had over 10 years of experience in poultry production.

Over half of the respondents (56.4%) were rearing different improved breeds of chicken for commercial purpose while the rest of the respondents (43.6%) had different local breeds with less than 100 chicken being reared under backyard production system for subsistence life. The commercial producers kept either layers (27.8%) or broilers (24.5%) by using semi-intensive (44.6%) and intensive (8.8%) production system. Very few farms (1.3%) were using cage system of housing while more than half of the producers (56.2%) were using deep litter system of housing (Table 2). The backyard scavenging production systems were using different housing system (traditional type) different from the two commercial production systems.

All the poultry farms (n=388) used one or more antibiotics on their farms and administered mainly by mixing them with feed or water (Personal observation and information from the leaflet). The antibiotics used are in different commercial products with a wide variety of trade names (not reported in this study for ethical reasons). Tetracycline group (oxytetracycline + doxytetracycline) (100 % farms) and sulfadiazine + trimethoprim (94.1%) and fluroquinolones (enrofloxacin + norfloxacin) were the most frequently used antibiotics (Table 3).

Antibiotics were used on all farms for different purposes. All farms frequently used antibiotics for the treatment of diseased chicken; 50% for disease prevention; 32.2% for growth promotion while 98.7% reported using antibiotics for all three purposes i.e. for treatment, prevention and growth promotion (Figure 1).

The commonly encountered poultry diseases which necessitated frequent use of antibiotics were Newcastle disease (100%), Gumboro disease (54.4%), coccidiosis (53.4%), Marek's disease (49.7%), Fowl typhoid (45.9%) and Fowl pox (39.2%) in decreasing order (Table 4). Almost three-fourths (71%) of the diseases encountered in the farms were attributed to viral diseases (Figure 2).

Even though all (100%) farms reported getting antibiotics from veterinary drug shops, in addition they also obtained from public veterinary clinic (51.0%), human pharmacies (26.8%) and open markets (16.2%) (Table 5). While the majority (61.9%) of the farms reported obtaining antibiotics by prescription or recommendation by veterinarians or veterinary assistants at the drug stores or hired by the farms, 32.2% reported self-prescription and 11.9% of farms reported using antibiotics based on advice from friends or other farm owners with previous experience.

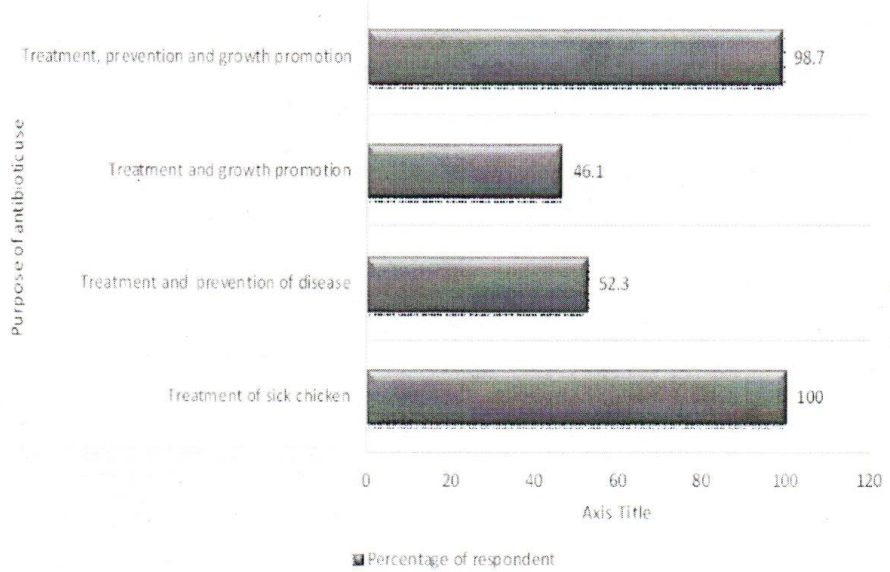

Fig. 1: Proportion (n=388) of farmers by the purpose of antibiotic use

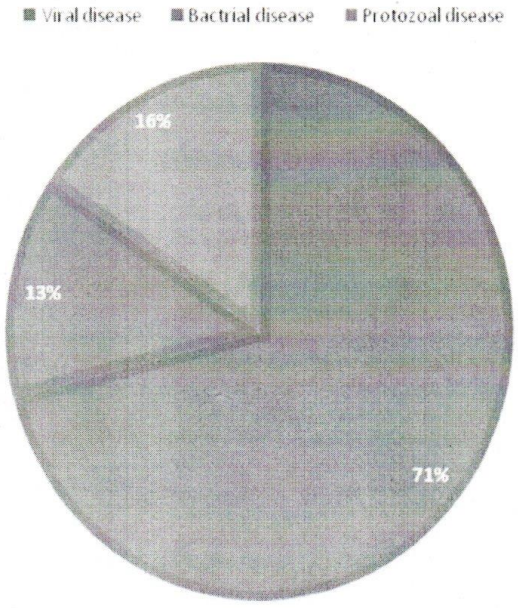

Fig. 2: Percentages of the frequency of disease occurrence by causative agent

Table 1: Demographic characteristics of poultry farm respondents

Characteristics of the respondent		No of respondents	Percentage (n=388)
1.Location:			
District	Town/kebele		
Ada'a	Bishoftu	83	21.4
	Udde	37	9.5
Akaki	Dukam	36	9.3
	Ensilale	63	16.2
Lume	Modjo	28	8.5
	Tsededilima	33	7.2
Adama	Adama	71	18.3
	Adulala hate haroret	37	9.5
2.Age category:			
	Less than 30 years	107	27.6
	31-50 years	188	48.4
	Greater than 50 years	93	24.0
3.Sex:	Male	316	81.4
	Female	72	18.6
4.Education level:			
	Cannot write and read	27	7.0
	Primary school (1-8)	139	35.8
	Secondary school (9-12)	121	31.2
	TVET	92	23.7
	University graduate	9	2.3
5.Work experience:			
	Less than 5 years	126	32.5
	5-10 years	102	26.3
	Greater than 10 years	160	41.2
6.Responsibility of the respondent in poultry farms:			
	Farm owner	345	88.9
	Hired manager	43	11.1

Table 2: Characteristics and/or management of the poultry farms

Characteristics and/or management		No of respondents	Percentage (n=388)
1.Chicken breed			
	Local	169	43.6
	Exotic	219	56.4
2.Number of chicken			
	Less than 100	181	46.6
	101-1000	173	44.6
	More than 1000	34	8.8
3.Production type			
	Layers	108	27.8
	Broilers	95	24.5
	Dual purpose	185	47.7
4.Production system			
	Backyard scavenging	181	46.6
	Semi-intensive	173	44.6
	Intensive	34	8.8
5.Housing type			
	Deep litter system	218	56.2
	Cage system	5	1.3
	Traditional housing	165	42.5

Table 3: Types and frequency of antibiotic use in poultry farms

Antibiotics	No of Respondent	Percentage (n=388)
Oxytetracycline + doxytetracycline	388	100.0
Lactaclox(Cloxacillin+ ampicillin)	113	29.1
Amoxycillin	85	21.9
Colistinsulphate	65	16.6
Enrofloxacin + norfloxacin	161	41.5
Sulfadiazine+ trimethoprim	365	94.1
Tylosin	55	14.2

Table 4: Common infectious diseases encountered at the poultry farms within the past one year

Disease	Disease category	Number of farms	Percentage (n=388)
Newcastle	Viral	388	100.0
Gumboro disease	Viral	211	54.4
Marek's disease	Viral	193	49.7
Fowl typhoid	Bacterial	178	45.9
Fowl pox	Viral	152	39.2
Coccidiosis	Protozoal	207	53.4

Table 5: Source, prescription and administration of antibiotics on the poultry farms

Questions	Frequency of respondent	Proportion (n=388)
1. From where you get drugs used on your poultry farm?		
Veterinary drug shop	388	100.0
Public Veterinary clinic	198	51.0
Human pharmacy	104	26.8
Open market/hawkers	63	16.2
2.Who prescribe (recommend to the use) antibiotics in your farm for intended use?		
Animal health personnel	240	61.9
Friends with experience	46	11.9
Self-prescription	125	32.2
3.Who administer antibiotics to your chicken?		
Self-administer	297	76.5
Animal health personnel	147	37.9
4. How is an antimicrobial dosage determined before usage in birds in your farms?		
After reading a leaflet	188	48.5
From the prescription paper	109	28.1
With assumption	113	29.1
5.What is/are the common route of antibiotic administration?		
Injection	19	4.9
In feed or water	388	100.0
Drop into the mouth	72	18.6

Only 147(37.9%) of the farms reported that antibiotics were administered by animal health workers. However, on over whelming majority (297/388, 76.5%) of farms antibiotics were administered by farm owners themselves based on instruction given by veterinary drug stores or by prescriber (28.1%), after reading a leaflet insert (48.5%),

or by assumption based on prior experience (29.1%). All (100%) farms reported that they incorporate antibiotics in to either feed or water, but only few intensive farms had sensitive balance or measuring cylinder for accurate dosages (Table 5).

DISCUSSION

Antibiotic usage in farm animals has raised many concerns among which the potential transfer of antibiotic resistant pathogens from animals to humans. This transfer can have severe health implications including treatment failures, which may lead to death and increased cost of human therapies [25]. Furthermore, overuse of antibiotics leads to the occurrence of harmful residues in edible poultry tissues (meat and eggs) and other animal products, which consequently are detrimental to health when such products are consumed by the public [26]. In low and middle income countries, the use of antimicrobials in food producing animals is not well regulated which can contribute to the development and spread of antibiotic resistant bacteria [27]. This study is the first knowledge and practice-based study in central Ethiopia and the results provide vital information on the potential risk factors that can increase the occurrence and spread of antibiotic resistance. The findings also have implications for public health in other parts of the country and the world since there is unlimited movement of people, goods and animals which provide a ready environment for the propagation and spread of antibiotic resistant bacteria [28].

The most frequently used antibiotics observed in the present study were tetracyclines, sulfadiazine and trimethoprim combination, fluroquinolones, amoxycillin, colistinsulphate and tylosin (Table 3). Our finding is consistent with that of GumpholWongsuvan *et al.* [29] from Thailand in poultry farms and Adebowale *et al.* [30] from Nigeria in commercial poultry laying hens, who reported the use of enrofloxacin, amoxicillin, colistin, doxycycline and oxytetracycline. Multidrug resistance to the listed antibiotics has been reported in bacteria of food animal origin in Ethiopia and other countries [31, 32] and the studies have attributed this to the uncontrolled use of antibiotics by farmers because of a lack of antibiotic control policies specially in developing countries [33].

Tetracycline, amoxicillin, fluroquinolones and colistin are highly important antimicrobials in human medicine [34]. The use of quinolones in poultry is worrisome as this drug is classified by the WHO as a priority for risk management and as critically important drug for the treatments of enteric diseases in humans and has been associated with increased resistant bacteria in humans exposed to it from farm animals. Several countries have therefore banned fluoroquinolone use in poultry. Since 2003 fluoroquinolones were withdrawn for use in animals in Denmark and it has also been banned for poultry production in the United States since 2005, BEUC [35]. The increased use of this drug has been attributed to several factors that include its broad-spectrum activity, its easy application in water and feed and its lack of restrictions. Colistin, another critically important drug, is currently considered to be the last defense against multidrug resistant bacteria especially those resistant to carbapenem antibiotics. The increased use of colistin in livestock production in China resulted in high selection pressure leading to the acquisition of the mobilizable colistin resistance *mcr-1* gene by *Escherichia coli*. China recently banned the use of colistin as a growth promoter and released a mandate controlling the use of colistin only for the treatment of diseases in animals [36].

The antibiotic usage pattern observed in this surveys showed that poultry farmers in the study area heavily relied on antimicrobial medications. Most farms used combinations of antibiotics and all farms used one or more antibiotics for therapeutic (100%), prophylactic (56%) and to a lesser extent for growth promotion (Fig. 1). Our result is higher than similar study from Nigeria [37] in which antibiotics were commonly administered for therapy (36.2%), prophylaxis (29.3%) and growth promotion (7%). Another study from Nigeria [38] reported that 86% of the poultry farms used antibiotics for growth promotion. The dependence of poultry farmers on antibiotics for therapeutic and/or prophylactic purposes may also be due to poor environmental sanitation, unhygienic practices, lack of biosecurity and other management inadequacies leading to increased exposure to bacterial pathogens [39]. Ensuring these measures is important for judicious use of antibiotics.

Disease was listed as the most important problem by the poultry producers, reducing both the number and productivity of the birds [40]. Some farmers had given up rearing poultry because of an increase in disease problems. Even though it was difficult to identify poultry diseases based only on clinical signs and symptoms, the most common diseases complained by the farmers were Newcastle, Gumboro disease, Marek's disease, Fowl typhoid, Fowl pox and coccidiosis (Table 4). The overall proportion of the disease caused by viral agent comprised about 71% as indicated in Fig. 2. All respondents

including veterinarians recommended administration of antibiotics following any disease outbreak whether it was bacterial, viral or protozoan origin purposefully to combat secondary bacterial complications by animal health professionals or unknowingly by poultry producers in response to disease outbreaks. This is not in line with the OIE guideline on responsible and prudent use of antimicrobial agents in veterinary medicine [41]. According to OIE guideline on judicious use of antimicrobials, viral, fungal and other non-bacterial infections are not treated with antimicrobials. Veterinarians must pay special attention to disease outbreaks to determine if and when antimicrobial therapy is warranted. Every effort should be made to address disease outbreaks with other disease management strategies prior to the initiation of antimicrobial therapy. Mortality and morbidity on farms must be closely monitored; diagnostic evaluations are performed to confirm bacterial involvement prior to antimicrobial therapy.

The veterinary drug supply chain starts from importers in Addis Ababa (the capital city of the country) and finally to veterinary drug shops and animal health posts found in each district [42]. In the current study, poultry farmers obtain antibiotics from veterinary drug stores (100%) and additionally from public veterinary clinic (51.0%), human pharmacy (26.8%) and open market (16.2%) (Table 5). This is in agreement with Takele *et al.* [43], who reported the presence of drug venders in Bishoftu town who distribute medicines in a packet in direct sunlight, violating the drug handling and storage recommendations by the WHO [44] and possibly causing substantial changes to the drug active ingredients. This study also advances our understanding of the factors that contribute to antimicrobial misuse and the results show there is a need for collaboration between animal agriculture and the public health sectors to assess risk factors, develop and distribute protocols to monitor the use of antibiotics and improve antimicrobial resistance surveillance in animals and humans.

In this study although 61.9% of the respondents, reported that antibiotics were prescribed or recommended for use by veterinarians and veterinary assistants, the rest of the respondents (38.1%) used antibiotics without prescription based on their own and their friends past experiences (Table 5). This is relatively better than the report of Dishon Muloi *et al.* [45], in which all veterinary drug stores sold antibiotics without a prescription in Nairobi, Kenya. The knowledge gap is relatively higher among Ethiopian poultry producers as compared to the report of Adebowale *et al.* [30] in commercial poultry laying hens in Nigeria in which 78.6% agreed that antibiotics used in poultry should be regulated and used when prescribed by veterinarians only. To minimize the risk of resistance selection during veterinary therapeutic use and to safe guard the future utility of antibiotics in veterinary medicine, a document titled 'Principles for the Responsible Use of antibiotics in Veterinary Medicine', was published by concerned global organizations like WHO, OIE and FAO [46]. According to these principles, administration on prescription-only and under veterinary supervision were recommended.

It is probable that, the method or regimen of drug administration has a significant influence on the likelihood of resistance strains emerging in an individual host [47]. The vast majority (76.5%) of the respondents administered antibiotics by themselves based on the instructions given from the veterinary drug shops or prescriber, long years' of experience and using an information on the leaflet insert about the dosage, duration and route of administration (Table 5). Treating only sick individual animals reduces the population of bacteria exposed to antibiotics. However, this is not always practical, especially during disease outbreaks because of labor requirements, expense, rapidity of disease spread in intensively reared animals and for animal welfare reasons or stress of catching and restrain [48]. To overcome this challenge, almost all antibiotics ready for poultry use are formulated for use as in-feed and in-water. But making a balanced ratio between the drug and feed/water is an area of irrationality in antibiotic use. Only few intensive poultry farms had sensitive balance or measuring cylinder to make a correct ratio between the drugs and feed/water. Thus, lower concentration (lower doses) and longer duration of exposure (course) appear to provide the highest selection pressure for resistance [49]. Resistance is more likely to be selected where the concentration and diversity of bacterial population is high, namely in some areas of normal flora such as bowel, mouth and throat. At normal flora site with mixed bacterial population there is the additional risk of selecting for resistance in potential pathogens by the transfer of resistance genes from bacteria sharing that niche [50].

The other risk factor for the development of resistance may be adjusting the amount of antibiotics to the total number of chickens that need therapy or prophylaxis. The respondents also mentioned that observation of a single sick chicken can lead to a blanket prescription of antimicrobials to the entire flock at the farms because of the fear that, it may be followed by an

outbreak. This is not consistent with a theoretical complication of administrating antibiotics in the feed in the variable dose that each animal may receive. Poor feeders, especially those that are ill, may naturally limit their food intake and thus receive lower doses than intended [51]. According to the respondents, duration of antibiotic administration is dependent on the duration of clinical sign of the disease. This means, as soon as the clinical sign disappears, antibiotic administration is stopped. The low level of knowledge among the respondents is not surprising in this geographical context. Since low and middle income countries are often more challenged in allocating adequate resources and instituting policies to address the gaps in knowledge and practices in food production industries. Antibiotic use in food animals remains unregulated, leading to inappropriate use of the drugs and widespread increase in antibiotic resistance [52]. Similar findings of low knowledge of antibiotic stewardship have been reported in countries such as in Vietnam [53].

CONCLUSION

Antibiotics are limited resources. The more antibiotics are used today, the lesser it is likely they will still be effective in the future. Antibiotic resistant infections are increasing in humans, animals and the environment. Antimicrobial resistance is considered one of the major threats to the world's health. This study clearly demonstrated lack of awareness about antibiotic use that in turn can lead to the propagation of antibiotic resistant bacteria and foster accumulation of antibiotic residues in poultry products in the study area. The misuse and overuse of antibiotics in poultry production is a major source of the problem observed. Antibiotics identified by WHO, as critically important for human treatment are commonly using in poultry for treatment, prevention and growth promotion. About half of the respondents confirmed as antibiotics used without prescription based on their own and their friends past experiences. The vast majority of the respondents administered antibiotics by themselves based on the instructions given from the veterinary drug shops. Almost all antibiotics ready for poultry use are formulated for use as in-feed and in-water. Only few large scale commercial poultry farms do have sensitive balance to make a correct ratio of antibiotic to feed/water. The respondents also mentioned that observation of a single sick chicken can lead to a blanket prescription of antimicrobials to the entire flock at the farms because of the fear that, it may be followed by an outbreak. There is also lack of polices and regulatory system on antibiotic use, resistance and residues in the country as a whole. Surveillance and research on antibiotic use and antibiotic resistance are very infant. Maintaining the status quo and continuing to misuse antibiotics as we have been doing will jeopardize our ability to effectively treat infectious diseases in the future. National authorities, veterinarians, physicians and farmers all have a role in "preserving the power of antibiotics". Perhaps the single most important action needed to greatly slow down the development and spread of antibiotic-resistant infections is to change the way antibiotics are used. Therefore, there is an urgent need for action on the issue of antibiotic resistance particularly in developing countries. National regulation and improved surveillance are needed to ensure that antibiotics are used prudently and are not routinely fed to animals for nontherapeutic purposes.

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Prevalence and Antimicrobial Resistance of *Salmonella* from Poultry, Poultry Meat, Eggs and Farm Workers

[1]*Mezene Woyessa,* [2]*Gezahegn Mamo,* [3]*Balako Gumi,* [2]*Bersissa Kumsa,*
[4]*Boka Tesfaye,* [4]*Takele Sori and* [5]*Getahun E. Agga*

[1]School of Veterinary Medicine, Wollega University, P.O. Box: 395, Nekemte, Ethiopia
[2]College of Veterinary Medicine and Agriculture, Addis Ababa University,
P.O. Box: 34, Bishoftu, Oromia, Ethiopia
[3]Aklilu Lemma Institute of Pathobiology, Addis Ababa University,
P.O. Box: 1176 Addis Ababa, Ethiopia
[4]College of Agriculture and Veterinary Medicine,
Jimma University, P.O. Box: 307, Jimma, Ethiopia
[5]U.S. Department of Agriculture, Agricultural Research Service,
Food Animal Environmental Systems Research Unit,
2413 Nashville Road, B-5 Bowling Green, KY 42101, USA

Abstract: *Salmonella* is the second major cause of foodborne bacterial infections in humans worldwide and poultry is a major source of infection. Drug resistant *Salmonella,* such as quinolone and 3rd and higher generation cephalosporin resistant strains, are regarded by World Health Organization as a critically important highest priority pathogen. Studies from developing countries like Ethiopia are scarce. We conducted a cross sectional study to determine the prevalence and antimicrobial resistance of *Salmonella* in intensive and semi-intensive commercial and backyard poultry farms, poultry meat and eggs and farm workers. The prevalence of *Salmonella* was 18.4% in the fecal samples, 14.8% in the hand swabs of farm workers, 4.5% of eggs and 6% of meat samples. The highest prevalence was observed in intensive production system (16.9%) and the lowest was found in backyard scavenging system (7.4%). Risk factors such as farm type (P=0.006), production type (P=0.001), breed (P=0.005) and sample type (P=0.001) were significantly associated with *Salmonella* prevalence. *Salmonella* isolates (n=37) were tested for their resistance against 15 antimicrobials using disc diffusion method. Majority of the isolates (24/37) were resistant or intermediately resistant to at least one antimicrobial. The prevalence of resistance was high to chloramphenicol (62.2%), tetracycline (59.5%), ampicillin (54.1%) and streptomycin (51.4%). More than half of the isolates (56.8%) were multidrug resistant. Our results showed a widespread occurrence of drug resistant *Salmonella* in poultry farms in the study area. Measures to control of *Salmonella* infections in poultry are needed to reduce foodborne infections in humans.

Key words: Antimicrobial resistance · Egg · Poultry · *Salmonella*

INTRODUCTION

Salmonellos is represents a serious threat to public health resulting in considerable economic consequences in many parts of the world [1]. The emergence of antimicrobial resistant *Salmonella* and other enteric pathogens has become a major concern [2]. The use of antimicrobials for therapeutic purposes, disease prevention and growth promotion in food animal production can potentially lead to a widespread dissemination of antimicrobial resistant bacteria [3, 4]. Salmonellosis is an important bacterial disease which affects diverse host species including poultry [5]. Poultry are important reservoirs for many zoonotic bacterial pathogens such as *Salmonella* [6]. The presence of *Salmonella* in healthy poultry is suggested as the main

Corresponding Author: Mezene Woyessa, School of Veterinary Medicine, Wollega University, P.O. Box: 395, Nekemte, Ethiopia.

risk factor for its transmission to humans through table eggs and poultry meat [7, 8]. The prevalence of *Salmonella* associated with poultry and poultry products is high and this high prevalence has both public health and economic implications [9]. The globalization of the food supply and the increased movements of people, animals and goods have increased the burden of *Salmonella* infections in several countries [10-12].

Currently, there is a rapidly growing commercial poultry production in Ethiopia [13]. The government of Ethiopia has prepared a five-year strategic development plan from 2015 to 2020 identifying the poultry production as an important sub-sector. The plan was to increase the total broiler production by 235% and the total egg production by 828% in 2020. The goal of the plan is to reduce poverty, improve household nutrition as well as increased income. Such transformative production systems may however lead to unrecognized risks to human health in terms of antibiotic use and antibiotic resistance as a result of emerging intensified production systems, which often rely on antibiotics as a stopgap for disease control and prevention in low and middle income countries in place of improving hygiene and sanitation in large-scale operations and other management inadequacies leading to increased exposure to bacterial pathogens [14]. Studies from different countries reveal that *Salmonella* serotypes isolated from foods of animal origin have multidrug resistance profiles [15]. The role of poultry products in the dissemination of antimicrobial resistant zoonotic bacterial pathogens also is well documented in developed countries [16]. A review made by Sylvia *et al.* [17] of 40 years (1974 and 2013) of enteric antimicrobial resistance research both in humans and animals in Eastern Africa summarized that despite the high burden of disease in sub-Saharan Africa and the potential health and economic consequences, the research output on antimicrobial resistance in the region was limited. Little data exists to quantify the contribution of different factors to the current levels of antimicrobial resistance. Local knowledge on the prevalence of *Salmonella*, serotype distribution and associated risk factors is important to implement appropriate control strategy to reduce wider dissemination of important zoonotic serovars. The objectives of this study were to investigate the prevalence and antimicrobial resistance of *Salmonella* in backyard and commercial poultry farms, farm workers, poultry meat and eggs in rapidly expanding poultry production areas of central Ethiopia.

MATERIALS AND METHODS

Relevance and Description of the Study Areas: Almost all large-scale commercial poultry farms in Ethiopia are found in East Shewa zone of Oromia regional state. In this zone, there are also an emerging intensive and semi intensive small-scale poultry farms in the urban and peri-urban areas. This activity is being undertaken as a source of income in Adama, Lome, Ada'a and Akaki districts. Most of egg and dressed poultry carcasses supply to supermarkets comes from commercial farms located in the major towns of these districts [18].

Sampling Methods, Sample Collection, Transportation and Storage: A multi-stage sampling procedure was used in which the East Shewa Zone and the four districts were purposively selected because of concentrated poultry production in the area, agro-ecological suitability and accessibility. Within each district for the backyard production system, we selected households. All commercial farms in the major cities of the districts were selected stratified by the size of the farms (small<1000 or large> 1000). For sample collection, ethical clearance certificate (Ref. No: VM/ERC/07/01/12/2020) was obtained from the College of Veterinary Medicine and Agriculture (Addis Ababa University).Written informed consents were obtained from each participant after informing the purpose of the study, the procedure, the risk, benefit and their rights.

Samples were collected according to the method described in ISO-17604 [19]. For chicken fecal samples, 25g pooled fresh feces were collected from the floor by sterile spatula into sterile universal bottle (Oxoid Ltd., London, England). Hand swabs were collected to check for direct transmission of *Salmonella* to farm workers. Swabs were done by rubbing the palm and fingers using pre-moistened swabs (Oxoid Ltd.) in 15 ml conical tubes (Oxoid Ltd.) containing 10ml of BPW. The egg samples were collected from the farms included in the study. Samples were marked with a permanent marker stating the date of collection, the farm and identification number for each egg. For the poultry meat, 25 g of tissues were collected from the commercial farms at slaughterhouses and placed into plastic freezer bags. Precautions were taken at all stages to ensure that the equipment used during sampling, transportation and storage to avoid any contamination. All types of samples were transported to laboratory in cooler containing icepacks and stored refrigerated at 4°C until processed.

Isolation and Identification of *Salmonella*: ISO protocol 6579: 2002 [20] was also used for the isolation and identification of *Salmonella*. Raw meat samples (25 g) were sliced into small pieces on sterile metal trays using sterile scalpel and forceps and placed into flasks containing 225 ml BPW and incubated for 24 hr at 37°C. Fecal samples (25 g) and the contents of individual eggs were placed into a sterile stomacher bag (Oxoid Ltd.) and 225 ml of BPW was added in 1:9 ratio and homogenized using a laboratory blender (Oxoid Ltd.) for 2 minutes and incubated for 24 hrs at 37°C. Hand swabs were transported in 10ml of BPW and incubated for 24 hrs at 37°C. Following incubation, 1 ml and 0.1 ml of the pre enrichment broths were aseptically transferred to 10 ml of tetrathionate broth (Oxoid Ltd.) and 10 ml of Rappaport-Vassiliadis (RV) broth (Oxoid Ltd.), mixed and incubated for 18 to 24 hr at 37°C and 42°C, respectively. Following incubation, a loopful of each broth culture was streaked onto xylose lysine deoxycholate (XLD) agar (Oxoid Ltd.) and brilliant green agar (BGA) (Oxoid Ltd.) and incubated at 37°C for 24 to 48 hrs. The XLD and BGA plates were examined for the presence of *Salmonella* colonies. Red colonies with black centers on XLD and pink colonies with a red zone on BGA plates were considered presumptive positive and further purified by culturing on nutrient agar (Oxoid Ltd.) [21].

For biochemical identification, presumptive *Salmonella* colonies were picked from the nutrient agar and inoculated into triple sugar iron (TSI) agar (Oxoid Ltd.), indol-ornithine agar (Oxoid Ltd) Simon's citrate agar (Oxoid Ltd.), urea broth (Oxoid Ltd.), MR-VP broth (Oxoid Ltd.) and incubated for 24 or 48 hours at 37°C [21]. Colonies producing an alkaline slant with acid (yellow color) butt on TSI with hydrogen sulfide production, positive for lysine (purple color), negative for urea hydrolysis (red color), negative for tryptophan utilization (indole test) (yellow-brown ring), negative for Voges-Proskauer and positive for citrate utilization were considered *Salmonella*-positive [22]. *Salmonella* colonies were tested for sero-grouping by rapid slide agglutination test using *Salmonella* polyvalent O antiserum "I" and "II"(Oxoid Ltd.) according to the manufacturer's instructions [23].

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion method based on the Clinical and Laboratory Standards Institute (CLSI) [24] with commercially available antibiotic discs. Refreshed pure isolate colonies from the nutrient agar plates were transferred into tubes containing 5 mL of tryptone soya broth (Oxoid Ltd.). The broth culture was incubated at 37°C for 4 h until it achieved 0.5 McFarland turbidity standards. A sterile cotton swab was used to swab the inoculum uniformly over the surface of Muller Hinton agar plate (Oxoid Ltd.). Plates were held at room temperature for 30 min to dry. The pre-determined antibiotic disks (all from Oxoid Ltd.): norfloxacin (10µg), nalidixic acid (30µg), ciprofloxacin (5µg), amoxicillin-clavulanic acid (20/10µg), tetracycline (30µg), chloramphenicol (30µg), ampicillin (10µg), gentamicin (10µg), streptomycin (10µg), ceftazidime (30µg), cefotaxime (30µg), trimethoprim + sulfamethoxazole (1.25/23.75µg), meropenem (10µg), doxycycline (30µg) and tigecycline (15µg) were then dispensed into the bacterial lawn by means of a sterile pair of forceps and gently pressed to ensure complete contact with the agar. The discs were positioned 15 mm away from the edge of the plate and 25 mm away from each other. The plates were incubated at 35-37°C for 18 to 24 hrs. Zones of inhibition around the discs were measured to the nearest millimeter using a digital caliper, the size of the inhibition zone was compared with the disc manufacturer's standard and classified as sensitive (S), intermediate (I) or resistant (R) to the given drug. *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922) were used as control strains. *Salmonella* colonies that showed resistance to more than or equal to three different classes of antibiotics were considered multidrug resistant (MDR).

Data Analysis: The data was recorded in Microsoft Excel, cleaned, organized and sorted. Descriptive analysis was performed using percentages for each prevalence and resistance to each drug. A Chi-square test was used for initial analysis to determine differences in the proportions of positive between districts, breed, purpose of production, production setting and sample types. Multivariate logistic regression was used depending on the nature of the outcome measures. Significant statistical difference was considered at $P<0.05$.

RESULTS

Prevalence of *Salmonella*: *Salmonella* was isolated from 92(18.4%) fecal samples, 4 (14.8%) hand swabs sampled from farm workers, 7(4.5%) eggs and 6(6.0%) meat samples. As indicated in Table 1, farm type, production

Table 1: The overall prevalence and risk factors for the occurrence of *salmonella* in poultry, products and farm workers

Risk factor		Sample size	Positive	Prevalence (%)	χ^2	P-value
Study district	Akaki	123	13	10.6	1.785	0.618
	Ada'a	339	44	12.9		
	Lome	116	21	18.1		
	Adama	202	31	15.3		
Farm type	Intensive	467	79	16.9	14.444	0.001
	Semi-intensive	125	16	12.8		
	Backyard	188	14	7.4		
Production type	Layers	313	64	20.4	7.466	0.024
	Broilers	279	31	11.1		
	Dual	188	14	7.4		
Breed	Exotic	606	96	15.8	5.340	0.021
	Local	174	13	7.4		
Sample type	Feces	500	92	18.4	24.696	0.000
	Hand swab	27	4	14.8		
	Egg	153	7	4.5		
	Meat	100	6	6		

Table 2: Associated risk factors for the occurrence of *Salmonella* in poultry by products, products and farm workers based on logistic regression analysis

							95% CI for OR	
Variables	Coef.	S.E.	Wald	df	*P*-value	OR	Lower	Upper
Farm type			6.134	2	0.047			
Intensive	0.329	0.303	1.18	1	0.277	1.39	0.77	2.52
Semi-intensive	0.809	0.342	5.579	1	0.018	2.25	1.15	4.40
Sample types			17.144	3	0.001			
Fecal sample	1.204	0.447	7.252	1	0.007	3.34	1.39	8.01
Hand swab	0.824	0.696	1.404	1	0.236	2.28	0.58	8.92
Egg sample	-0.154	0.591	0.068	1	0.795	0.86	0.27	2.73
Constant	-3.08	0.519	35.277	1	0	0.05		

Table 3: Fecal prevalence of *Salmonella* by study characteristics

Risk factor		Sample size(n)	Positive(#)	Prevalence (%)	χ^2	P-value
Study district	Akaki	76	11	14.5	2.217	0.529
	Ada'a	199	40	20.1		
	Lome	70	10	14.3		
	Adama	155	31	20		
Farm type	Intensive	288	51	17.7	6.577	0.037
	Semi-intensive	108	28	25.9		
	Backyard	104	13	12.5		
Production type	Layers	242	48	19.8	3.247	0.197
	Broilers	159	32	20.1		
	Dual	99	12	12.1		
Breed	Exotic	403	80	19.6	2.913	0.088
	Local	97	12	12.4		

type, breed and sample type were significantly ($P<0.05$) associated with prevalence. *Salmonella* prevalence was significantly ($P= 0.001$) higher in the intensive farms (16.9%) than in the back yard farms (7.4%). The prevalence of *Salmonella* was significantly ($P=0.024$) higher in layer chicken (20.4%) than in broiler chicken (11.1%) and it was twice in the exotic breeds (15.8%) as compared ($P= 0.021$) to local breeds (7.4%). Fecal sample had significantly ($P =0.000$) the highest (18.4%) prevalence than poultry products (5.13%) and farm workers (14.8%).

Using multivariable logistic regression analysis, farm type ($P=0.047$) and sample type ($P=0.001$) were significantly associated with *Salmonella* prevalence (Table 2). In this regard, semi-intensive farms had higher odds of *Salmonella* prevalence than the backyard

Table 4: Prevalence of *Salmonella* in chicken egg samples by study characteristics

Risk factor		Sample size(n)	Positive(#)	Prevalence (%)	χ^2	P-value
Study district	Akaki	44	1	2.3	3.486	0.357
	Ada'a	34	2	5.9		
	Lome	29	3	10.3		
	Adama	46	1	2.1		
Farm type	Intensive	61	1	1.6	8.855	0.022
	Semi-intensive	8	2	25		
	Backyard	84	4	4.8		
Production type	Layers	52	3	5.8	0.746	0.885
	Broilers	12	0	0		
	Dual	89	4	4.5		
Breed	Exotic	76	4	5.3	0.164	0.493
	Local	77	3	3.9		

Table 5: Antimicrobial resistance among *Salmonella* isolates

	Number (%)		
Antibiotics	Susceptible (%)	Intermediate (%)	Resistance (%)
Norfloxacin (10µg)	37(100)	0(0.0)	0(0.0)
Nalidixic acid (30µg)	32(86.5)	5(13.5)	0(0.0)
Ciprofloxacin (5µg)	34(91.9)	3(8.1)	0(0.0)
Amoxicillin-clavulanic acid (20/10µg)	35(94.6)	2(5.4)	0(0.0)
Tetracycline (30µg)	10(27.0)	5(13.5)	22(59.5)
Chloramphenicol (30µg)	12(32.4)	2(5.4)	23(62.2)
Ampicillin (10 µg)	13(35.1)	4(10.8)	20(54.1)
Gentamicin (10µg)	22(59.5)	5(13.5)	0(0.0)
Streptomycin (10µg)	15(40.5)	3(8.1)	19(51.4)
Meropenem (10µg)	37(100)	0(0.0)	0(0.0)
Doxycycline (30µg)	25(67.6)	5 (13.5)	7(18.9)
Trimethoprim + sulfamethoxazole (1.25/23.75µg)	22(59.5)	8(21.6)	7(18.9)
Tigecycline (15µg)	36(97.3)	1(2.7)	0(0.0)
Cefotaxime (30µg)	36(97.3)	0(0.0)	1(2.7)
Ceftazidime (30µg)	35(94.6)	2(5.4)	0(0.0)

Table 6: Multiple antimicrobial resistance profiles and proportion of *Salmonella* isolates from samples in poultry farms

Number of antibiotics	Pattern of antibiotics	No of resistant isolate	Total no of multi resistant isolate (%)
Three	CHL, AMP, SXT	1	9(42.9)
	TET, CHL, AMP,	2	
	CHL, AMP, STR,	3	
	AMP, STR, SXT	1	
	CHL, AMP, DOX	1	
	TET, CHL, STR	1	
Four	TET, CHL, AMP, STR	4	9(42.9)
	CHL, STR, DOX, SXT	1	
	CHL, AMP, STR, SXT	1	
	CHL, AMP, STR, DOX,	2	
	CHL, AMP, DOX, CTX	1	
Five	TET, CHL, AMP, STR, DOX	1	2(9.5)
	TET, CHL, AMP, STR, SXT	1	
Six	TET, CHL, AMP, STR, DOX, SXT	1	1(4.7)
Total MDR		21	21(100)

† Nalidixic acid (NAL), † Ciprofloxacin (CIP), †Tetracycline (TET), †Chloramphenicol (CHL), †Ampicillin (AMP), †Gentamicin (GMN), †Ceftazidime (CAZ), †Cefotaxime (CTX), †Trimethoprim + sulfamethoxazole (SXT), †Meropenem (MEM), † Doxycycline(DOX), †Tigecycline (TGC), † Streptomycin (STR), †Norfloxacin (NOR)

scavenging system (Odds ratio [OR] = 2.25; 95% confidence interval [CI= 1.15- 4.40; *P*= 0.018]. Fecal samples had three times higher Odds ratio than meat samples [OR = 3.34; 95% CI for OR = 1.39- 8.01; *P* = 0.007] for fecal samples.

Higher fecal prevalence were observed in Ada'a district (20.1%), in semi-intensive farm (25.9%), in broiler chicken (20.1%) and in exotic breed (19.6%) although these differences were not statistically significant (Table 3).

The prevalence of *Salmonella* in the egg samples (Table 4) was relatively higher in Lome district (10.3%) than the other study sites. From the comparison of farm types, production types and breed of chicken, the highest prevalence were 25.0%, 5.8% and 5.3% in semi-intensive farms, layers and exotic breeds respectively as indicated in Table 4. Only farm type was marginally significant (*P*=0.022).

Antimicrobial Resistance Profile: The overall antimicrobial resistance profiles of *Salmonella* isolates from feces (n=20), hand swabs of farm workers (n=4), raw meat (n=6) and raw eggs (n=7) are given in Table 5. Twenty four of the total isolates tested (64.8) were resistant to at least one drug. About fifty-seven percent (21/37) of the isolates tested were resistant to more than or equal to three antibiotic classes indicating multidrug resistance (Table 6). The prevalence of resistance was high for chloramphenicol (62.2%), tetracycline (59.5%), ampicillin (54.1%) and streptomycin (51.4%). All isolates were susceptible to meropenem, norfloxacin, nalidixic acid, ciprofloxacin, amoxicillin-clavulanic acid, gentamicin, ceftazidime and tigecycline.

DISCUSSION

In Ethiopia several factors including under and malnutrition, HIV-AIDS, the unhygienic living circumstances and the close contact between humans and animals can substantially contribute to the occurrence of human salmonellosis [25]. Chicken products and farm workers can acquire *Salmonella* from intestinal contents, fecal matter or from cross-contamination during slaughtering processes, egg collection and other farm management activities [26, 27].

Our finding is nearly similar to 16.7% *Salmonella* isolated in Southern Ethiopia [28], 15.12% recorded in central Ethiopia [29] and nearly 12% in Kenya [30]. However, our result is higher than reported from Addis Ababa (4.7%) in Ethiopia [31] while it is lower than 25.35% reported in poultry in Bangladesh [32]. The difference in the results may be attributed to differences in sampling procedure, sample type, sample size, locality and other factors.

Based on the univariate analysis using χ^2 test, all the investigated risk factors except district of the study area were associated with the presence of *Salmonella* (Table 1). Finally, multivariable logistic regression analysis was conducted to see the strength of association between risk factors and *Salmonella* isolation. After the effect of other risk factors were adjusted for, only farm type and sample type had a significant effect on *Salmonella* recovery (Table 2). Sample types as a risk factor reveal that, *Salmonella* prevalence was three times higher in fecal sample than in meat sample. This is as a result of *Salmonella* spp. are commonly found in the alimentary tract of animals [33]. The chicken product surface and farm workers can acquire *Salmonella* from intestinal contents, fecal material or from cross-contamination during slaughtering processes, egg collection and other farm management activities [34, 35].

The strength of association indicated that, odds ratio was twice in semi-intensive farms than backyard scavenging system. *Salmonella* prevalence was higher in exotic breeds and in intensive farms than local breed that are usually kept under scavenging backyard system. This may be due to the difference in genetic composition and stress of high physiologic activity in intensive farming systems. The higher prevalence of *Salmonella* in layers (20.4%) than in broilers (11.1%) could be due to physiological stress of egg production and molting, which significantly depresses the immune response and increases the susceptibility to *Salmonella* infection [36].

Antimicrobial-resistant *Salmonella* were observed in 65% of 37 *Salmonella* isolates tested, which is higher than previous studies, 47.6% by Ejo *et al.* [37] in Ethiopia and 19.7% in Spanish by Lamas *et al.* [38]. This observed resistance is lower than what other studies reported, 83% by Zelalem. *et al.*[39] in Ethiopia and 87.2% by Kim *et al.* [40] in Korea. The overall resistance result was relatively in agreement with 60.6% recorded in Ghana by Andoh *et al.* [41]. This resistance variation could be due to indiscriminate use of antimicrobials in animal production without prescription in the animal health sector especially in developing countries, duration of exposure to antibiotics and others which might favor selection pressure that increased the advantage of maintaining resistance genes in bacteria.

Majority (56.8%) of the isolates in the current study was resistant or intermediately resistant to more than two

antimicrobial classes and the prevalence of resistance was high to chloramphenicol (62.2%), tetracycline (59.5%), ampicillin (54.1%) and streptomycin (51.4%). This is consistent with the report of Phagoo & Neetoo [42] in which all isolates were resistant to tetracycline while 60% and 80% of isolates were resistant to chloramphenicol and erythromycin respectively. On the other hand, the current result is in contrary to the report from Brazil by Arboite *et al.*[43], in which the highest sensitivity was demonstrated for ampicillin (94.9%), tetracycline (91.1%) and chloramphenicol (98.7%). The possible reason for high resistance rate to chloramphenicol, tetracycline, ampicillin and streptomycin in Ethiopia could be, these antibiotics are the most commonly used in veterinary medicine in the country [44].

High sensitivity of isolates was recorded for norfloxacin (100%), meropenem (100%), tigecycline (97.3%), cefotaxime (97.3%), ceftazidime (94.6%) and amoxicillin-clavulanic acid (94.6%) which can be associated with no or limited use of these antibiotics in poultry production in Ethiopia. Due to the relatively limited access and high price to get the newly developed cephalosporins and quinolones, the high prevalence of antimicrobial-resistant *Salmonella* to relatively low-priced and commonly available antibiotics is alarming for a low-income society living in most developing countries, like Ethiopia. However, it is important to note that these antibiotics are commonly used in veterinary medicine and infections with these resistant *Salmonella* isolates could lower the efficiency of antibiotic treatments in human infections that require antibiotic therapy [45].

In current study, more than half of the isolates (57%) were MDR. The remarkably high occurrence of MDR in *Salmonella* is probably an indication of their availability and frequent use of the antibiotics in the poultry production system in Ethiopia. Studies conducted elsewhere in Ethiopia [46] also indicated high proportion of drug-resistant *Salmonella* isolates that could be due to the irrational use of antimicrobials and inappropriateness of the prescription and dispensing methods in both the public and private veterinary services.

CONCLUSIONS

This study showed about 20% fecal prevalence of non-typhoidal *Salmonella* in the chicken sampled at poultry farms. This finding warrants improved farm hygienic measures to reduce its dissemination to humans and to the environment through all steps of product collection, processing, transportation and storage. The overall prevalence of MDR isolates (56.8%; n= 37) indicate the importance of chicken as a source of MDR *Salmonella* infections in humans. There are inappropriate practices and attitudes associated with improper antibiotic handling and management issues in the professionals, awareness problems in the community and easy accessibility of the drugs in the black markets that could potentially affect the drug effectiveness. Personnel training on personal hygiene, biosafety and farm biosecurity for all farm attendants and visitors, education on zoonotic pathogens, major risk factors that affect awareness on safe handling and management of veterinary drugs, restrictions on uncontrolled use of antimicrobials and establishment of a standard pathogen and AMR monitoring system in poultry production are recommended. Overall, we hypothesize that control of *Salmonella* infections in poultry is the important step to control *Salmonella* infections in humans.

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Conflict of Interest: The authors declare that they have no competing interests.

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Annex 7. Ethical clearance

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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
Bishoftu/Debre Zeit

Animal Research Ethics Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/07/01/12/2020

Name of Applicant: Mezene Woyesa (MVSc in Vet. Epidemiology, PhD fellow)

Address: College of Veterinary Medicine and Agriculture (Addis Ababa University)

Title of the project: *Antibiotic use and antimicrobial resistance in different poultry farms in selected areas of East Showa Zone, Oromia Regional State, Ethiopia*

Date of application:	**4/11/2019**
Nature of the project:	**mildly invasive**
Target animal species:	**chicken**
Number of animals involved:	**400**
Study area:	**East Showa, Ethiopia**

Minutes No. and date of review: **VM/ERC/01/12/020, 03/01/2020**

The above indicated research project is acceptable from ethical perspective, relevance, originality and technical competence points of view. Hence the project is ethically sound to be executed provided that:

1. All procedures and conditions stipulated in the proposal are respected, minor comments are corrected and any deviation or changes be reported to the committee
2. The project activities be open for occasional supervision by the committee when this is deemed necessary

Dr Getachew Terefe
Chairman

Signature

Addis Ababa University College of Veterinary Medicine and Agriculture

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Fax 251-11-4339933 Tel. +251 114338450 P.o.x. Box}34 Bishoftu/Debre Zeit, Ethiopia