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SALMONELLA ISOLATES AND DRUG RESISTANCE EPIDEMIOLOGY IN POULTRY RELATED SAMPLES IN SELECTED SITES OF CENTRAL AND SOUTH ETHIOPIA

MSc Thesis

By

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June, 2015

Bishoftu, Ethiopia

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A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Tropical Veterinary Epidemiology

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As member of the examining board of the final MSc open defense, we certify that we have read and evaluated the Thesis entitled ***"SALMONELLA* ISOLATES AND DRUG RESISTANCE EPIDEMIOLOGY IN POULTRY RELATED SAMPLES IN SELECTED SITES OF CENTRAL AND SOUTH ETHIOPIA",** prepared by Fisseha Mengstie and recommend that it be accepted as fulfilling the thesis requirement for the Degree of Master of Science in Tropical Veterinary Epidemiology.

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STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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LIST OF ABBREVIATIONS

AST	Antimicrobial Susceptibility Testing
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CDC	Center for disease control and prevention
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
EU	European Union
GSS	Global *Salmonella* Surveillance
H_2S	Hydrogen Sulphide
HACCP	Hazard analysis and critical control points
ISO	International Organization for Standardization
MDR	Multi-Drug Resistant
MKTTn	Muller-Kauffmann Tetrathionet with novobiocinnin
NCCLS	National Committee for Clinical Laboratory Standards
NHS	National Health Services
OIE	Office International des Epizooties
RV	Rappaport Vassiliadis broth
Spp.	Species
Subsp.	Subspecies
TSI	Triple Sugar Iron agar
VP	Voges-Proskauer
WHO	World Health Organization
WTO	World Trade Organization
XLD	Xylose lysine desoxycholate

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ABSTRACT

A cross-sectional study to determine the prevalence, distribution and associated risk factors of Salmonella and its drug resistance pattern as well as the risk factors associated with drug resistance to different antibiotics was conducted from November 2014 to June 2015 in poultry farms and abattoir of selected six areas of central and south Ethiopia. 200 samples from Addis Ababa, 184 samples from Bishoftu, 205 samples from Modjo, 199 samples from Adama, 170 samples from Hawassa and 100 samples from Bonga with total 1059 samples from different sources consisting of poultry (feces, swab), feed, water, personnel (hand swab), environment (litter, pooled feces) and carcass swab were randomly collected following the standard techniques and procedures. The isolation of Salmonella was conducted using standard bacteriological methods and the drug sensitivity testing was done by using disk diffusion methods. From these wide spectrum of samples, 27% from Bonga (P=0.000), 15.1% from Modjo, 11.5% from Addis Ababa, 10.6% from Hawassa, 7.5% from Adama and 6.5% from Bishoftu had Salmonella organisms. Out of the total sample collected, 126 (11.9%) are positive for Salmonella, indicating its ubiquitous distribution. Its distribution is associated with location, specific area and individual farms within broad area, sample source and breed indicating, which are important risk factors. Of the 126 isolates, 123 (97.8%) were developed resistance to all types of the 10 tested antibiotics although Gentamcycin and Ciprofloxacin demonstrated relatively a better efficacy. Alarmingly, multidrug resistance (86.5%) is very common. In this regard, 7 isolates to 2 drugs, 9 isolates to 3 drugs, 7 isolates to 4 drugs, 16 isolates to 5 drugs, 10 isolates to 6 drugs, 16 isolates to 7 drugs and 44 isolates to 8 drugs have developed resistance. Each specific drug in this study has its own specific risk factor for resistance development. In general, location, production type, flock size and housing system were important risk factors for drug resistance development. Significant wide distribution and multi drug resistance for routinely prescribed antimicrobial drugs both in veterinary and public health sectors poses considerable health hazards to poultry production industry as well as the consumers unless prudent antimicrobial usage, improvement of standards of hygiene and development and enforcement of suitable legislation are urgently instituted. Serotyping and molecular characterization of Salmonella is of significance.

Keywords*: Drug resistance, Epidemiology, Poultry, Prevalence, Salmonella*

1. INTRODUCTION

Foodborne diseases are among the most widespread global public health problems of recent times and their implication for health and economy is increasingly recognized (Van der Venter, 1999; Gomez *et al*., 1997) which affects development worldwide (WHO 1984). *Salmonella* remain among the leading causes of Foodborne illness throughout the world accounting for an estimated 27% of all Foodborne illnesses caused by known bacterial agents (Mead *et al.,* 1999) which approximately 99% humans and other warm blooded animals infection are caused by under subspecies *enterica* (Velge *et al.,* 2005).

Salmonella species associated with infection in humans can be divided into those causing typhoid fever (exclusively transmitted from human to human) and non-typhoidal species (transmission through contaminated food is thought to cause 85% human infection). The incidence of typhoid fever has decreased in recent years but food poisoning caused by non-typhoidal *Salmonella* strains has now reached higher proportions in many countries despite improvements in hygiene and sanitation (CDC, 2009). Thus, Non-typhoidal salmonellosis (NTS) is one of the most frequently reported food borne disease worldwide in developing as well as developed countries (WHO, 2002) which results millions of cases of enteric diseases, thousands of hospitalizations and deaths each year (CDC, 2009; Dunkley *et al*., 2009), substantial morbidity, mortality and significant economic impact (Abatcha *et al.,* 2014).

In addition to human health implications, *Salmonella* is a pathogen of significant importance in worldwide animal production (European Union, 2003; Wierup, 1994; WHO, 1993). All livestock species can be affected by *Salmonella* with young, debilitated and parturient animals are the most susceptible to clinical disease and the mortality rate is particularly high in young animals. It often leads to diminished feed conversion which in turn increases feed costs (Rushton, 2009), performance losses and mortality in cattle which again increases direct and indirect costs associated with treatment and infection

control. In poultry industry, *Salmonella* causes heavy economic loss through mortality and reduced production (Khan *et al.*, 1998) with the various direct expenses producers face as a consequence of infections in their flocks and in other animals, can lower milk production, weight loss, abortions and death. On the other hand, there are additional costs associated with the treatment of sick animals such as the price of medicines and productivity costs associated with increased labor for management of affected stock.

The extensive use of antimicrobials in human and animals results the increase in the number of resistant and multi drug resistant strains of bacteria has been recognized by the World Health Organization (WHO) and health authorities as one of the major problems in global public health, veterinary sector and are growing because of infections from resistant bacteria are more hard and costly to treat (Helmuth, 2001; European Union, 2003). The increasing single and multiple antimicrobial-resistant *Salmonella* strains isolated from human cases have been associated with widespread use of antimicrobial agents in food animal production in which they represents a public health risk by transfer of resistant *Salmonella* strains to humans through the consumption of contaminated food and food products (Bada-Alambedji *et al.*, 2006). Antimicrobial resistance can increase the morbidity, mortality and costs associated with disease. Moreover, it has social and economic consequences and requires strong scientific and public health efforts to improve the situation (Bada-Alambedji *et al.*, 2006).

Salmonella can be found in virtually every part of the world and carried by an extremely wide verity of hosts including humans and other mammals, birds, reptiles and insects (Austin and Wilkims, 1998; Gast, 1997; Kusters *et al.*, 1993; Sato *et al.*, 1999) with greater than 2,500 identified serotypes (Brenner *et al.*, 2000). The habitat of the genus *Salmonella* seems to be limited to the digestive tract of humans and animals but the presence in other habitats (water, food, natural environment, farm effluents and human sewage) is explained by fecal contamination. The majority of human salmonellosis cases are related to the consumption of contaminated food products (Mead *et al.*, 1999). A wide range of foods especially foods of animal origin such as poultry products, beef and pork have been implicated in foodborne illness (WHO, 2002; Annonymous, 2008) as a

consequence of subclinicaly infected food animals leading to contamination. Fruits and vegetables that have been fertilized or irrigated by fecal wastes are also recognized as source of infection (OIE, 2010).

The epidemiology of Foodborne 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, poverty and lack of safe food preparation facilities (Van der Venter, 1999; Altekruse *et al.,* 1998) which favors the dissemination, increase the incidence and severity of foodborne diseases worldwide (Gomez *et al.,* 1997; Kaferstein *et al.,* 1997; Todd, 1997).

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 (GMP), 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 (Gomez *et al.,* 1997).

In spite of the relatively considerable information of antimicrobial susceptibility of *Salmonella* in human infections and the contamination of foods of animal origin in many parts of the world, there is however a dearth of such information in Ethiopia. During the reviewing of the current literature, it was clearly documented that non-typhoid *Salmonella* are important public health problems in many parts of the world in which carrier food animals are the main reservoir through maintaining the life of the agent, continuous source of infection and transfer of MDR resistant genes to the public, to susceptible animal species and to the environment (which in turn become source of

infection to animals and human). Therefore, it is of importance to monitor antimicrobial resistance of *Salmonella* from the production setting of food animals of which poultry is a major concern as they are the most important sources of livelihood food and income (Alebachew and Mekonnen, 2013). Estimating the distribution of *Salmonella*, sources of infection, antibiotic resistance epidemiology and major risk factors contributing for the occurrence in poultry production sector helps to prevent and control the losses it caused as well as zoonotic transmission of drug resistant strains. These is because of that, poultry's are source of salmonellosis outbreak in humans (European Union, 2003; Wierup, 1994; WHO, 1993).

This study therefore attempts to analyze the epidemiology of *Salmonella* and its antibiotic resistance pattern isolated from different sources (poultry environment, poultry themselves and poultry personnel) in poultry farm and abattoir in selected sites of Central and south Ethiopia. Therefore, the study was designed with the following objectives.

- Determine the prevalence and distribution of *Salmonella* in poultry and poultry related samples.

- Identify the associated risk factors of *Salmonella* isolates in poultry.

- Determine the drug resistance pattern of the *Salmonella* isolates and their risk factors.

2. LITERATURE REVIEW

2.1. Historical Background of *Salmonella*

Salmon and Smith isolated a bacillus from diseased pigs in 1885 and they called it *Bacterium* Suipestifer, They wrongly considered it the causative agent of swine fever. This bacterium was later on called *Salmonella* Cholerasuis (Eliana *et al.,* 2012), it was named after Daniel Salmon, a veterinary bacteriologist at the United States Department of Agriculture (USDA) (Gast, 2003; Salyers and Whitt, 2002). In the late 19th century, serological tests utilizing agglutination with antiserum were developed as a result new serotypes were discovered and named after denoting syndrome (example *S.* Typhi), host-specificity (example *S.* Choleraesuis and *S.* Gallinarum) (Grimont *et al.,* 2000) and geographical origin of the first isolation (example *S.* Dublin) (PlymForshell & Wierup, 2006). Currently, *Salmonella* consists of different serotypes divided into two species namely *salmonella enterica* replacing the old name *Salmonella* Choleraesuis (Hohmann, 2001) and *Salmonella bongori*.

2.2. Taxonomy and Nomenclature

The genus *Salmonella* comprises two species namely *Salmonella enterica and Salmonella bongori*. *Salmonella enterica* also divided into six subspecies such as *Salmonella enterica* subspecies *enterica* (I), *Salmonella enterica* subspecies *salamae* (II), *Salmonella enterica* subspecies *arizonae* (IIIa), *Salmonella enterica* subspecies *diarizonae* (IIIb), *Salmonella enterica* subspecies *houtenae* (IV) and *Salmonella enterica* subspecies *indica* (VI). Each species contains multiple serotypes (Brenner *et al.,* 2000) (table 1), classified on the basis of extensive diversity of lipopolysaccharide (LPS) antigens (O), flagella protein antigens (H) and capsular (Vi) antigens (Selander *et al.,* 1996) in accordance with the Kauffmann-White scheme.

The O antigens are located in the cell wall and are shared with other members of the *Enterobacteriaceae*. It have been used to separate *Salmonella* into groups and based on these fifty serologically distinct groups have been assigned thus all of which have O antigen. H antigens are carried by flagella, these are composed of protein subunits called flagellin. H antigens are typically diphasic in *Salmonella*. The availability of two genetic systems expressing different flagellins could help the organism to survive the host's defences (Neidhardt *et al.,* 1987). The flagellar antigens are highly specific to *Salmonella.* Thus, a positive reaction, with polyvalent H antiserum is sufficient to presume that the organism is *Salmonella.* Capsular antigen is associated with virulence and is only expressed by serotype Typhi, Paratyphi C and Dublin (Rycroft, 2000; Grimont *et al.,* 2000). Currently, as recognized by Kauffman-White scheme there are a total of 2,610 serotypes (Grimont and Weill, 2007).

Table 1: *Salmonella* species, subspecies, number of serotypes and their usual habitats, Kauffmann-White scheme

Salmonella species and subspecies	No. of serotypes	Usual habitat
S. enterica subsp. *Enterica* (I)	1,454	Warm blooded animals
S. enterica subsp. *Salamae* (II)	489	Cold blooded animals and the environment
S. enterica subsp. *Arizonae* (IIIa)	94	Cold blooded animals and the environment
S. enterica subsp. *Diarizonae* (IIIb)	324	Cold blooded animals and the environment
S. enterica subsp. *Hautenae* (IV)	70	Cold blooded animals and the environment
S. enterica subsp. *Indica* (VI)	12	Cold blooded animals and the environment
S. bongori (V)	20	Cold blooded animals and the environment

2.3. General Characteristics of *Salmonella*

2.3.1. Phenotypic characteristics

Strains belonging to the genus *Salmonella* comply with the definition of the family *Enterobacteriaceae*: straight rods, generally motile with peritrichous flagella, except *S.* Pullorum and *S.* Gallinarum, which lack flagella (Holt *et al.,* 1997), facultative anaerobic

and gram-negative (Yan *et al.*, 2003). They are chemo-organotrophic organisms having both a respiratory and a fermentative type of metabolism and most are prototrophic, i.e. they have no growth factor requirement and can grow in a minimal medium with glucose as sole carbon and energy source and ammonium ion as nitrogen source but Some host-adapted serotypes are auxotrophic and require one or more growth factors.

2.3.2. Growth characteristics

Salmonella grows in the range 5^{o}C to 46^{o}C with an optimum between 35^{o}C to 43^{o}C, pH between 6.6 to 8.2 with value greater than 9 or lower than 4 inhibiting growth. Depending on the acid used, minimum pH for growth may be as high as 5.5 and Minimum water activity 0.94 in media with a neutral pH but higher values are required as pH decreases towards growth minimum *Salmonella* do not survive over 9% salt concentration It can survive for long periods under refrigeration and freezing does not ensure inactivation in foods. Survival for greater than10 weeks in butter stored at –23^{O}C and 25^{O}C has been noted. It can survive for 28 days on the surfaces of vegetables under refrigeration (Lake *et al.*, 2004), In minced chicken breast (pH 5.8), 60-83% of cells survived storage at -20ºC for 126 days, whereas at -2ºC and -5ºC only 1.3% to 5.8% were still viable after 5 days.

2.4. Methods of *Salmonella* Detection in Samples

Salmonella can be isolated either from tissues collected aseptically at necropsy, feces, rectal swabs, environmental samples, food products and feedstuffs. When infection of the reproductive organs, abortion occurs, it is necessary to culture fetal stomach contents, placenta and vaginal swabs and in the case of poultry, embryonated eggs. Individual samples for bacteriological tests should be collected as aseptically as possible by following the respective standards. Moreover, precaution should be taken to avoid cross contamination of samples during transit and at the laboratory. Packages should also be kept cool and accompanied by adequate information. Fresh feces are the most sensitive sample to detect the shedding of *Salmonella*. However, the detection in fecal

sample can be complicated because the organism may be greatly outnumbered by other competing micro flora (Cherrington and Huls, 1993b). As a consequence, isolation and detection of *Salmonella* either by conventional or rapid methods involves a selective enrichment step (Cherrington and Huls, 1993a). Thus, isolation and identification of *Salmonella* can be performed using techniques recommended by International Organizations for Standardization (ISO-6579, 2002), and those recommended by the Global *Salmonella* Surveillance (GSS) and National Health Services for Wales (NHS) (Zelalem *et al*., 2011).

2.4.1. Conventional isolation and identification methods

Culture

Conventional culture methods for the detection of foodborne *Salmonella* species generally consist of five distinct and successive steps. These are pre-enrichment in nonselective media and selective enrichment in broth media, plating on differential agar, biochemical screening and serological conformation (D'Aoust, 2001).

Pre-Enrichment Media

The number of *Salmonella* in feces from asymptomatic animals, environmental samples, animal feed and food is usually low and it is usually necessary to use pre-enrichment media such as buffered peptone water (BPW) to assist isolation. This may allow the small numbers of *Salmonella* which may otherwise be killed by the toxic effect of selective enrichment media, to multiply, or it may help to resuscitate *Salmonella* that have been sub-lethally damaged, for example by freezing, heating or desiccation (WHO, 2010).

Enrichment Media

A selective enrichment medium contains inhibitory reagents that allow *Salmonella* to grow while restricting the growth of other microflora. This selectivity is based on the

synergism between the inhibitory reagent and the incubation temperature which varies between 37°C and 43^{O}C (Tletjen and Fung, 1995) thus, 41.5°C is recommended for incubation of Rappaport–Vassiliadis broth-based media. Many inhibitors are in use but the most common are bile, tetrathionate, sodium selenite, brilliant green and malachite green dyes. Selective enrichment culture Medias includes Rappaport Vassaliadis broth (RV) (D'Aoust *et al.,* 1992), sodium tetrathionate as in Müller–Kaufman broth, selenite F and selenite cysteine. Additions such as Ferrioxamine E may be added to selective media to enhance isolation of *Salmonella* from iron or nutrient limited samples such as eggs, water or soil (Reissbrodt, 1995) or antibiotics such as novobiocin may be added to suppress most Gram-positive organisms or other Gram-negative bacteria such as *Proteus*. Specific antibiotics can be added to enhance the isolation of antimicrobial resistant *Salmonella* strains.

Selective Plating Media

These are solid selective agars that permit differential growth to varying degrees. They inhibit growth of bacteria other than *Salmonella* and give information on some of the principal differential biochemical characteristics usually non-lactose fermentation and hydrogen sulphide (H_2S) production. The results are read after 24 and 48 hours of culture at 37°C. *Salmonella* form characteristic colonies on such media that are usually distinguishable from the colonies of other bacteria on the plate, with the possible exceptions of *Proteus, Pseudomonas*, *Citrobacter* and *Hafnia*. Examples of selective media are brilliant green agar, xylose lysine desoxycholate agar, deoxycholate/citrate agar and bismuth sulphite agar (WHO, 2010). Finally colonies with appearances characteristic of *Salmonella* are subjected to biochemical and serologic tests to confirm their genus and serotype identity.

Biochemical Tests

Confirmation can be made using biochemical tests like triple sugar iron agar (TSI), urea agar, lysine iron agar (LIA), Voges Proskauer (VP), methyl red (MR) and Indole tests

(Zelalem *et al*., 2011). Typical *Salmonella* cultures in TSI show alkaline (red) slants and acid (yellow) butts with formation of hydrogen sulfide (blackening of the agar).

Serological confirmation

A number of serological tests have been developed for the diagnosis of *Salmonella* infections in animals. In poultry, the whole blood test, which uses a stained antigen, and the serum agglutination test (SAT) have been used successfully for the identification of flocks infected with *S.* Pullorum/Gallinarum. Because *S.* Enteritidis possesses the same group D somatic antigen as *S.* Pullorum/Gallinarum and is thought to originate from it, the whole blood test and related tests can be used for the diagnosis of *S.* Enteritidis infection, but the sensitivity is low. In recent years, other tests, such as the ELISA (Barrow, 1994) have been developed for the diagnosis of *S.* Enteritidis and *S.* Typhimurium infections in poultry and for other serotypes in farm animals. The ELISA has been used effectively to identify serologically *S.* Dublin carrier cattle and can be applied to bulk milk for screening dairy herds.

2.4.2. Epidemiological Salmonella typing and subtyping

The strain typing methods for bacterial pathogens play an important role in understanding infectious disease transmission, tracking and distribution (Gudmundsdottir *et al*., 2003; Kubota *et al*., 2005). Methods such as Phenotypic and genotypic characterization can provide information on the strain implicated; demonstrate an epidemiological link between cases and associate cases with a potential source. Furthermore, these typing schemes can also be used as diagnostic tools and for the assessment of the pathogenic properties of *Salmonella* (Gonzalez and Mendoza, 1995; Poppe *et al.*, 1993).

2.4.2.1. Phenotypic methods of *Salmonella* typing

Phenotypic methods are traditional typing methods for discriminating between bacteria from a single species based on phenotypes (Hunter *et al*., 2005).

Serotyping

Salmonella serotyping plays an essential role in determining species and subspecies. It is initial step for routine diagnosis of strains and this can be done with commercially available poly and monovalent antisera. Of the two species of *Salmonella*, *S. enterica* and *S. bongori* classified by Kaufmann-White scheme, over 99% of serotypes are grouped into species *S.* enterica, and nearly 60% of them belong to the subspecies enterica (subspecies I) (Brenner *et al*., 2000). In this method, a series of antisera was used to detect different antigenic determinants such as somatic (O), capsular (Vi) and flagellar (H) antigens on the surface of bacterial cell (Tenover *et al*., 1997). The O antigen reactivity toward specific antisera forms the basis of the *Salmonella* serotyping scheme (Grimont and Weill, 2007). Although Serotyping has a wide acceptance as a method to differentiate *Salmonella* strains (Abatcha *et al.,* 2014) and is highly discriminative (Olsen *et al.,* 2011). serotyping has limitations such as it is based on the use of expensive antisera; the procedure is time consuming; requires well-trained technicians; and some isolates are not typeable (Abatcha *et al.,* 2014).

Phage typing

This typing method has proven to be epidemiologically valuable in strains differentiation within a particular *Salmonella* serotype. In this subtyping approach, *Salmonella* strains are separated into different phage types based on their reactivity against a set of serotype specific typing phages (Abatcha *et al.,* 2014). It is a highly discriminatory method for the subdivision of serotypes into distinct phage types (PT) which facilitates the identification of the strains involved in human outbreaks. The advantage of phage typing resides in the simplicity of its implementation, which requires only basic laboratory equipment (Wattiau *et al.,* 2011).

2.4.2.2. Genotypic methods of *Salmonella* typing

Pulsed Field Gel Electrophoresis

It has highly discriminatory nature. PFGE is a molecular typing method, many reports indicate that PFGE is widely used in tracking the source of *Salmonella* infections for different serotypes and are well discriminatory in nature (Dionisirt *et al.,* 2006). PFGE typing is more time and labor-intensive, with lack of sensitivity for different serotypes. It is suitable as an epidemiological tool for investigating outbreaks. Moreover, it is considered as a gold standard for molecular typing of *Salmonella* and many other bacterial pathogens (Pierre *et al.,* 2011).

Ribotyping

This is a genotyping method that can clarify and classify bacteria on the basis of differences in rRNA. This method is known as rRNA gene restriction pattern determination or ribotyping. It involves digestion of bacterial DNA by a common restriction endonuclease and many fragments are generated. For bacterial typing, ribotyping has a relatively low discriminatory power and for that reason, it has not been commonly used for the outbreak epidemiological investigation and surveillance studies or epidemiological findings (Aarts *et al.,* 2001). Moreover, due to its complexity and technicality, it is not a preferred strain typing method for bacterial pathogens (Tenover *et al.,* 1997).

Randomly Amplified Polymorphic DNA

It is a type of PCR with a random DNA segment amplification. A number of studies have reported success in using RAPD assays to differentiate *Salmonella* serotypes or to distinguish strains within single serotypes. The advantage of RAPD is that, it is simple to perform, widely applicable and does not require previous knowledge of the genome. For a first-screen typing method, RAPD is generally considered to be an excellent choice

(Williams *et al.,* 1999). The main demerit of RAPD analysis is low reproducibility and difficulties occur in relating the different patterns.

Plasmid Fingerprinting

Plasmid fingerprinting provides a rapid and dependable means of identifying bacterial isolates of the same strain. The stability, wide distribution and diverse nature and size of extra-chromosomal elements make it suitable for virtually all bacterial genera (Tenover, 1985). Most of the plasmids harbor virulence and antimicrobial resistance properties in *Salmonella*. The plasmids found in *Salmonella* differ in size 2-200kb with different functionalities (Porwollik and McClelland, 2003; Rychlik *et al.,* 2006). This because plasmids are not stable and may lose or acquired by strains and some organisms contains few or no plasmids, this technique is not useful for subtyping *Salmonella.*

2.5. Distribution of *Salmonella*

The epidemiology of *Salmonella* is complex due to wide range of hosts and vectors (for effective use of transmission), the ability to survive for a long time in the environment, persist in the population due to efficient fecal shedding from carrier animals and abundant reservoir hosts thus, it have mastered virtually all of the attributes necessary to ensure wide distribution. In developed countries, non-typhoidal *Salmonella* (NTS) predominantly cause diseases (Gordon, 2008) and in sub-Saharan Africa is consistently the most common isolates both in adults and children presenting with fever (Reddy *et al*., 2010; Gordon *et al*., 2008; Gilks *et al*., 1990) and case fatality estimates for invasive NTS disease among hospitalized patients in Africa have been in the range of 4.4% to 27% for children (Enwere *et al*., 2006) and 22% to 47% for adults (Gordon *et al*., 2008). In most developed countries, salmonella Typhimurium and Enteritidis are the most commonly reported causes of human salmonellosis; however, other serotypes appear to be more prevalent in specific regions such as *Salmonella* Stanley and *Salmonella* Weltevreden in Southeast Asia (Herikstad *et al.,* 2002).

The global monitoring of *Salmonella* serotype distribution from the world health organization global foodborne infections network country data bank from 2001 to 2007 indicated that, Globally, the overall proportion of *Salmonella* Enteritidis and Typhimurium was 43.5% and 17.1% ranging from 40.6% in 2005 to 44.9% in 2003 and 15% in 2007 to 18.9% in 2001 respectively and the proportion of the distribution indicated *Salmonella* Enteritidis presented an increase in Africa (16.7% in 2001 to 32.2% in 2007), Oceania (5% in 2001 to 11.3% in 2007), North America (18.4% in 2001 to 25.7% in 2007) and Europe (66.3% in 2001 to 75.7% in 2005). *Salmonella* Typhimurium increased in Latin America (14.5% in 2001 to 24% in 2007). Concerning the other serotypes, Globally, the overall proportion of *Salmonella* Infantis ranged over the years, from 1.5% to 2.2%; *Salmonella* Agona was frequently observed in Latin America, Europe and North America; *Salmonella* Heidelberg was much more common among developed Countries like North America, Europe and Latin America; *Salmonella* Virchow also reported from Asia, Europe and Oceania regions; High frequencies of *Salmonella* Thompson in Europe and North America; *Salmonella* Newport has consistently been reported by the North American, European and Latin American regions; and *Salmonella* Hadar and *Salmonella* Montevideo were reported by almost all regions (Hendriksen *et al.*, 2011).

Shifts in the prevalence of specific serotypes in human and animal populations may follow the introduction of the serotype through international travel, human migration, food, animal feed and livestock trade (Crump *et al.*, 2002; Aarestrup *et al.*, 2007). Thus, failure to control *Salmonella* in one country presents a potential problem for other countries.

2.6. *Salmonella* Isolate Diversity Vs Host Diversity

Salmonella serotypes are normally divided into two groups on the basis of host range; host adapted and ubiquitous (non-adapted). Host-adapted serotypes typically cause systemic disease in a limited number of related species. For example, *S.* Typhi, *S.* Gallinarum and *S.* Abortusovis are almost exclusively associated with systemic disease in

humans (Edsall *et al.,* 1960), fowl (Barrow *et al.,* 1994) and ovine respectively. However, some host adapted serotypes can also cause disease in more than one host species: *S.* Dublin and *S.* Choleraesuis, for example, are generally associated with severe systemic disease in cattle and pigs respectively but may also infrequently cause disease in other mammalian hosts including humans (Nnalue, 1991; Smith and Jones, 1967; Wray and Sojka, 1977)

The degree of host adaptation of *Salmonella* serotypes can vary widely. Their fore, *Salmonella* serotypes which are almost exclusively associated with one particular host species, (for example, Typhi, Abortusequi, Gallinarum, Typhisuis, and Abortusovis) are referred to as host-restricted serotypes and Serotypes which are prevalent in one particular host species but which can also cause disease in other host species (for example Dublin and Choleraesuis) referred to as host-adapted serotypes. Ubiquitous serotypes, for example *S.* Typhimurium and *S.* Enteritidis, although capable of causing systemic disease in a wide range of host animals, usually in a broad range of unrelated host species and these serotypes will be referred to as un-restricted serotypes. *S.* Enteritidis, *S.* Typhimurium and *S.* Newport, were isolated from chicken ovaries and Feces (Uzzau *et al.,* 2000).

2.7. Reservoir and Carrier State of *Salmonella*

Salmonella are widely distributed in natural environment however, the main reservoir is the intestinal tract of infected or carrier animals particularly meat animals (Lax *et al.,* 1995) and humans (Gomez *et al.,* 1997). A number of animal species including ruminants, carnivores, birds and reptiles can play a major role as carriers in the spread of *Salmonella* and transmit them to other healthy animals and humans (Sanchez *et al*., 2002) and clinically normal carrier animal is a serious problem in all host species. Among warm-blooded animals, chickens, geese, turkeys and ducks are the most important reservoirs. Domestic animals, such as dogs, cats, turtles and birds may be carriers and pose great risk. Poultry are the most important reservoir of asymptomatic *Salmonella* excreters in the human food chain. Thus, Long-term carriers that can shed *Salmonella* in

feces continuously or intermittently, often in high numbers are common in most host species which is exacerbated by a long list of stressors including transportation, concurrent diseases and food deprivation (Uzzau *et al.,* 2000).

2.8. Incidence of *Salmonella* in Poultry Houses and their Environment

Salmonella transmission and contamination can be aggravated by the situation pertaining in poultry houses. Practices such as overcrowding, unhygienic farming activities, lack of adequate biosecurity measures and movement of birds and equipment from one farm to the other worsens the situation. Mice, wild birds, ants and snakes have been be showed by some researchers to be important agents for the transmission of *Salmonella* in among birds, flocks and farms (Carrique-Mas *et al.,* 2009; Davies *et al.,* 1997). In addition, farm pertaining samples and their environmental conditions including feces, soil, crevices, dusts, manure, litter, feeders and/or drinkers will harbour *Salmonella* and increased the rate of contamination (Mallinson *et al.,* 2000; Wales *et al.,* 2006).

Environmental sampling has been reported to be a good indicator for the presence of *Salmonella* in poultry flocks (Davies and Breslin, 2001). Van de Giessen et al. (1994) suggested that laying flocks were mainly infected from farm environmental samples including improperly cleaned and disinfected poultry houses and infected vermin present on the farm. The underlying principle is that once poultry farms, houses or the rearing environment is infested with *Salmonella* they are more likely to be transmitted to the birds which may subsequently end up in foods exposing humans to the risk of contracting Salmonellosis.

2.9. Incidence of *Salmonella* in Poultry Feeds

Poultry feeds can be sources of *Salmonella* and consequently serve as an indirect cause of human infection to people consuming poultry meats and meat products. Feeds are contaminated either from feed mills or on farms during feed formulation, feeding or handling and subsequently spread to poultry mostly through ingestion. *Salmonella* have

the ability to survive under prolong periods in dry conditions like feeds and may be recycled in all production stages in commercial feed preparation (Whyte *et al.,* 2003). This makes the impact of reducing *Salmonella* contamination in feeds and the risks of human infection cumbersome to assess (Davies *et al.,* 2004).

Hinton (1988) reported that the incidence of *Salmonella* carriage in poultry flocks will be under estimated if only cloacal feces are sampled without sampling poultry feeds. Several factors most especially ingredients used in preparing poultry feeds have been implicated to be the major source of contamination (Bale *et al.,* 2002; Maciorowski *et al.,* 2004; Okoli *et al.,* 2006). Persistence of the organism in feed mills and feed preparation environments are other predisposing factors. Henken et al. (1992) said poultry farms supplied with contaminated poultry feeds are 5.3 times more likely to produce *Salmonella* positive flocks compared to farms supplied with feeds free from *Salmonella.*

2.10. Source of Infection for Humans and Animals

Salmonella can be found in the feces of both infected animals with clinical signs and healthy carriers and when they slaughtered and dressed fecal contamination of the carcasses may occur. The primary source of *Salmonella* infection in humans worldwide is contaminated food and water (Tletjen and Fung, 1995). Food of animal origin such as poultry, egg, milk, beef and pork are the main sources and Contact with sick livestock is not an uncommon method of exposure for farm workers as CDC reported several outbreaks of multidrug-resistant *S.* Typhimurium infection associated with veterinary facilities. Poultry meat and its derivatives are among the food products that cause the most concern to public health authorities, owing to the associated risks of bacterial food poisoning (Luiz *et al.,* 2004), But zoonotic transmission is not limited to food animals alone as faeces of nearly all animal species may be potential sources; therefore, Pets, especially dogs that have close interaction with humans may be responsible (Kozak *et al*., 2003; Sanchez *et al*., 2002).

Salmonella infection in cattle originates from various sources, such as importation of infected animals or introducing infected or carrier animals to a healthy herd, contamination of feed and water and cross-infection from other domestic or wild animals. In poultry, the primary source of infection may be contaminated feed (Vela and Cuschler, 1995).

2.11. Source and Route of Contamination for Foods of Animal Origin

Contamination of poultry products can occur at multiple steps along the food chain, which includes production, processing, distribution, retail marketing, handling and preparation. Thus, the importance of red meat and poultry meat as vehicles of salmonellosis in human lies in their physical contamination with the organism in the processing plant and in failure of the consumer to handle the raw food so as to prevent infection (Cui *et al.,* 2005).

2.12. Mode of Transmission in Animals and Humans

Environment contaminated with *Salmonella* serves as the infection source because *Salmonella* can survive in the environment for a long time. Then it is transmitted to vectors such as rats, flies and birds where it can shed in their feces for weeks and even months. Following the direct transmission to animals through the feco-oral route such as swine, cows and chickens act as important source of infection due to these animal reservoirs can be infected as *Salmonella* is normally originates from contaminated environment and contaminated feed. Then after, humans can get infection when eating foods or drinking water that is contaminated with *Salmonella* through animal reservoirs. However, *S.* Typhi and *S.* Paratyphi A do not have animal reservoir, therefore infection can be happened by eating the improperly handled food by infected individuals (Newell *et al*., 2010).

Salmonella infection in animals can also occur via other routes, including the respiratory tract, by inhalation of aerosol. Raw meat obtained from rendering plants used to feed

dogs can be contaminated with *Salmonella* and have been associated with canine salmonellosis (Cantor *et al*., 1997) and Infection of breeder flocks with *Salmonella* leads to a rapid dissemination of the organism to progeny broiler and commercial egg laying flocks. In human, Apart from being foodborne, infections are also acquired through direct or indirect animal contact in homes, veterinary clinics, zoological gardens, farm environments or other public, professional or private settings (Hoelzer *et al*., 2011).

2.13. *Salmonella* Transmission in Poultry

The mechanism by which *Salmonella* contaminates eggs was proposed as shell penetration and transovarian transmission as three serotypes, *S.* Enteritidis, *S.* Typhimurium and *S.* Newport, were isolated from chicken ovaries and feces which are a result of infected laying hens. Vertical transmission of *Salmonella* in poultry is introduced from infected reproductive tissues to eggs prior to shell formation and many different *Salmonella* serotypes able to contaminate egg contents by migration through the egg shell and membranes. Such a route is facilitated by moist egg shells, storage at ambient temperature and shell damage. Horizontal transmission can occur by direct bird-to-bird contact, ingestion of contaminated feces or litter, contaminated water, infected personnel, farm and personal equipment, and a variety of other sources (Nakamura *et al.*, 1997). Backyard hens can also be infected through contact with wild animals, domestic mammals and commercial poultry that are carriers of *Salmonella* and consequently may play a role in the transmission of the organism to other animals and humans.

2.14. Virulence Factors and Pathogenesis of *Salmonella*

2.14.1. Salmonella virulence factors

The ability to cause disease relies on several virulence determinants. Some of these may be considered virulence determinants in the broad sense. Genes involved in nutrient biosynthesis/uptake, stress response (both in and outside the host) and repair of cell damage are among those. These genes may be considered housekeeping genes and are present in other closely related bacteria, such as *E. coli*. Another group of virulence genes

specific for the genus *Salmonella* encode adaptations to overcome host defense mechanisms and may therefore be called true virulence determinants. The expression of both groups of virulence genes is regulated in response to environmental signals in the host. The regulatory genes mediating this control may also be considered virulence determinants (Bäumler *et al*., 2000).

Many of the virulence genes of *S.* Enterica are chromosomal genes located on pathogenicity islands referred to as *Salmonella* Pathogenicity Islands (SPI). These genes are believed to have been acquired by *Salmonella* from other bacterial species through horizontal gene transfer and have functions such as host cell invasion and intracellular pathogenesis. The virulence of *Salmonella* relates to their ability to invade host cells, replicates in them and resist both digestions by phagocytes and destruction by the complement components of plasma. Following adherence, probably through fimbrial attachment to the surface of intestinal mucosal cells, the bacteria induce ruffling of cell membranes. The complex invasion process is mediated by the products of a number of chromosomal genes, whereas growth within host cells depends on the presence of virulence plasmids (van Asten and van Dijk, 2005).

Chromosomal virulence determinants

The ability of *Salmonella* to colonize relevant parts of the alimentary tract is the first stage of infection. Close association and penetration of the intestinal mucosa is a prerequisite for the pathogenesis (Lester *et al.*, 1995). The facultative intracellular growth and survival of *Salmonella* demand a large number of genes distributed around the chromosome, and at least 60 virulence genes including those responsible for nutrient biosynthesis have been identified (Groisman and Ochman, 1997).

Virulence plasmids

Salmonella species with the notable exception of *S.* Typhi often have a large plasmid (30-60MDa) which is serotype specific. This plasmid has been shown to be essential for

virulence in many *Salmonella* species (Finlay and Falkow, 1989). The plasmid is not required for initial interaction with the intestinal mucosa nor it is required for invasion and entry into deeper tissue as strains missing the plasmid reach the spleen, liver and lymph nodes. However, the large plasmid enables the organism to persist within the reticuloendothelial cells, while cured strains or strains that have no plasmid are quickly eliminated by the host immune system (Flnlay and Falkow, 1988; Swamy *et al.,* 1996).

2.14.2. Salmonella pathogenesis

The basic virulence strategy common to *Salmonella* species is to invade the intestinal mucosa and multiply in the gut-associated lymphoid tissue (GALT). From the infected intestinal tissues the pathogens are drained to the regional lymph nodes, where macrophages that line the lymphatic sinuses form a first effective barrier to prevent further spread. If this host defence mechanism successfully limits bacterial expansion, the infection remains localized to the intestine and the GALT. On the other hand, the macrophages located in the draining lymph nodes when unable to limit spread, can cause a systemic disease. The alimentary tract is a hostile environment, which imposes severe stress upon invading bacteria. The first host defence mechanism encountered after oral infection is the acid barrier of the stomach. The passage through this environment induces expression of a number of genes whose products are involved in pH homoeostasis and repair of macromolecules (Bearson *et al.*, 1997).The surviving bacteria then reach the small intestine, which contains bactericidal compounds, such as bile salts. *Salmonella* serotypes are well adapted to cope with these stress conditions. A still greater challenge than survival in the lumen of small and large intestines appears to be resisting removal by peristalsis and gaining a foothold at the preferred niche in the intestinal wall, the GALT.

The phenomenon of the indigenous flora being able to prevent colonization by exogenous bacteria is known as bacterial interference. Several mechanisms of bacterial interference have been proposed. These include production of inhibitory substances, as well as competition for tissue adhesion sites and limiting nutrients. The main strategy used by *Salmonella* serotypes to evade bacterial interference is to escape the competitive

environment of the gut by penetrating the intestinal mucosa. One of the hallmarks of *Salmonella* infection is the preferential invasion of lymph follicles that are located in the intestinal wall of the ileum (Hohmann *et al.*, 1978).

In mammals, lymph follicles of the small intestine are clustered in organs known as Peyer's patches. The Peyer's patches serve as the main port of entry for *Salmonella* serotypes, and their colonization contributes to the development of disease during both localized and systemic infection. Intestinal perforations at areas of Peyer's patches are the most frequent cause of death during typhoid fever. The protection against oral challenge is due to immune exclusion at the mucosal surface, which prevents entry of *Salmonella* into epithelial cells. Additionally *Salmonella* have several virulence factors that contribute to causing diarrhea, bacteremia, and septicemia. These factors include the lipopolysaccharide of the outer wall, pili, flagella, cytotoxin, and enterotoxin (Michetti *et al.*, 1994).

2.15. Risk Factors for Infection Acquisition

All animals are at increased risk of developing disease if their normal flora is disrupted (for example stress, antibiotics). These circumstances render animals susceptible to exogenous exposure or activation of silent infections. Young animals are more susceptible to salmonellosis than older ones. Poor sanitation, overcrowding, unfavorable weather, stress of hospitalization and surgery, parturition, parasitism, transportation and concurrent viral infections are all factors which predispose animals to clinical salmonellosis. Many animals suffer inapparent infections during their lifetimes. This is especially true of swine and poultry fed rations that contain *Salmonella*. In the subclinical form, the animal may have a latent infection and harbor the pathogen in its lymph nodes, or it may be a carrier and eliminate the agent in its fecal material briefly, intermittently or persistently. Large herd size represents an important risk factor for salmonellosis and the risk of *Salmonella* shedding seems to vary by production system, housing type, general hygiene level, management type and animal age. Clinical disease usually appears when animals are stressed by factors such as transportation, crowding, food deprivation,

weaning, parturition, exposure to cold, a concurrent viral or parasitic disease and sudden change of feed or overfeeding following a fast (Nollet *et al.,* 2004).

The physiology of *Salmonella* has contributed to difficulty in controlling environmental contamination and transmission of the organism. For example, *Salmonella* are hardy bacteria with several potential vehicles, vectors and reservoirs within a poultry flock. They are facultative anaerobes and can grow well under both aerobic and anaerobic conditions. The optimum temperature to support growth is 37°C, but they can grow over a range of 5 to 45°C and can grow within a pH range of 4.0 to 9.0, with an optimum pH 7.0 (Gast, 1997).

Cockroaches and lesser mealworms could carry *Salmonella* internally and externally and spread them throughout the poultry house (Kopanic *et al.,* 1994; McAllister *et al.,* 1994). Non-biting flies, fleas, ticks and bread beetles were also reported to be *Salmonella* vectors in feed and feed mixtures (Bidawid *et al.,* 1978). Mice have been also important vectors for *S.* Enteritidis in laying hens (Henzler and Opitz, 1992). Wild birds and lizards were recorded as reservoirs of *Salmonella* and can directly and indirectly cause transmission to poultry flocks. Insect and animal vectors are widely believed to be sources of bacterial contamination in feed, poultry products and even directly to human salmonellosis. The farm environment can also be a main reservoir. The physical conditions and feed constitute major reservoirs in which *Salmonella* can survive for extended periods of time. Infections can spread among chickens in flocks through direct contact with infected birds and with the contaminated environment. Contaminated food or water is a major source of *Salmonella* infections (Craven *et al.,* 2000).

The infective dose for one to be infested with Salmonellosis has been reported to range from 1-10^9 cfu g^{-1} depending on the type of food consumed, ingested amount, immune status of the host and virulence factor of the bacteria. Infectious dose decreases if consumed with liquid food (e.g., milk), foods that neutralizes gastric acid (e.g., cheese), higher number of cells are ingested, immuned challenged individuals and when the pathogen carries a high virulence genes (Bhunia, 2008).

The growing movement of people, live animals, and food products across borders; rapid urbanization in developing countries; increasing numbers of immunocompromised people; changes in food handling and consumption; and the emergence of new or antibiotic-resistant pathogens all contribute to increasing food safety risks (Unnevehr, 2003).

2.16. Antimicrobials and their Mechanism of Action

Antimicrobial agents are generally categorized according to their four major principal mechanisms of action. These are inhibition of cell wall synthesis and disruption of bacterial membrane structure, inhibition of protein synthesis, interference with nucleic acid synthesis and inhibition of a metabolic pathway (Hooper, 2001; Tenover, 2006). Inhibition of cell wall synthesis is a common mechanism for broad-spectrum antimicrobials such as the member of the ß-lactam group (penicillin, cephalosporin and carbapenems) and glycopeptides groups (Tenover, 2006).

Several classes of antimicrobials including macrolides, aminoglycosides, tetracycline, chloramphenicol, streptogramins, and oxazolidones produce their antimicrobials effects by inhibiting protein synthesis. Macrolides, aminoglycosides, and tetracyclines bind to the 30S subunit, whereas chloramphenicol binds to the 50S ribosomal subunit and cause misreading or termination of translation, effectively inhibiting a functional protein synthesis (Hooper, 2001; Tenover, 2006). The quinolones and fluoroquinolones target DNA synthesis and cause double strand DNA breaks during DNA replication by inhibiting gyrase (topoisomerase), which is an enzyme required in folding and super coiling for bacterial DNA (Drlica and Zhao, 1997). Sulfonamides and trimethoprim target the pathway for folic acid metabolism by competitively inhibiting the synthesis of folic acid for de novo amino acid biosynthesis, which ultimately inhibit DNA synthesis (Hooper, 2001; Tenover, 2006).

2.17. Antimicrobial Susceptibility and Testing Methods

There are three antimicrobial susceptibility test methods (disk diffusion, broth dilution and agar dilution) that have been shown consistently provide reproducible and repeatable results when followed correctly (CLSI, 2008).

Disk diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from disks, tablets or strips, into the solid culture medium that has been seeded with the selected inoculums isolated in a pure culture. Disk diffusion is based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The diffusion of the antimicrobial agent into the seeded culture media results in a gradient of the antimicrobial. When the concentration of the antimicrobial becomes so diluted that it can no longer inhibit the growth of the test bacterium, the zone of inhibition is demarcated. The diameter of this zone of inhibition around the antimicrobial disk is related to minimum inhibitory concentration (MIC) for that particular bacterium/antimicrobial combination; the zone of inhibition correlates inversely with the MIC of the test bacterium. Generally, the larger the zone of inhibition, the lower the concentration of antimicrobial required to inhibit the growth of the organisms. However, this depends on the concentration of antibiotic in the disk and its diffusibility (CLSI, 2008).

Broth and agar dilution methods- The aim of the broth and agar dilution methods is to determine the lowest concentration of the assayed antimicrobial that inhibits the visible growth of the bacterium being tested (MIC, usually expressed in µg/ml or mg/litre). However, the MIC does not always represent an absolute value. The ″true″ MIC is a point between the lowest test concentration that inhibits the growth of the bacterium and the next lower test concentration. Therefore, MIC determinations performed using a dilution series may be considered to have an inherent variation of one dilution. Antimicrobial ranges should encompass both the interpretive criteria (susceptible, intermediate and resistant) for a specific bacterium/antibiotic combination and appropriate quality control reference organisms. Antimicrobial susceptibility dilution methods appear to be more

reproducible and quantitative than agar disk diffusion. However, antibiotics are usually tested in doubling dilutions, which can produce inexact MIC data (CLSI, 2008).

2.18. Antimicrobial Resistance

Multidrug resistance among many organisms has become a big challenge to infectious disease management. It is increasingly being reported in bacteria and is often mediated by genetic mobile elements such as plasmids, transposons, and integrons (Dessen *et al.,* 2001). In recent years, testing of *Salmonella* isolates has shown that an increasing proportion of isolates are resistant to several antimicrobial agents both in developing and developed countries and also an increased antimicrobial resistance *Salmonella* isolate from humans and animals was observed (Foley and Lynne, 2008).

2.18.1. Mechanism of antimicrobial resistance in Salmonella

The mechanisms for antibiotic resistance can be categorized as (i) modification or destruction of the antimicrobial agent, (ii) pumping the antimicrobial agent out from the cell by efflux pumps, (iii) modification or replacement of the antibiotic target, and (iv) decrease in cell membrane permeability. Thus, microorganisms are developing resistance mechanisms by developing mutations in the gene locations of target proteins or acquiring mobile genetic elements carrying resistance genes such as plasmid, integrons and transposons. The resistant plasmids in bacteria's like *Salmonella*, Escherichia coli, Shigella and other genera of Enterobacteriaceae is which encodes antibiotic resistance, some of which are self-transmissible whereas others may be Co-transferred by conjugative plasmids (Poppe *et al.,* 1996). Antibiotic resistance in *Salmonella* has also been found to be encoded by chromosomal gens (Threlfall and Frost, 1990) and Development of resistance to new antibiotic is not uncommon. Although antibiotic resistance is not considered a virulence factor, it may be related to the bacterial existence and persistence in a host (Brackelsberg *et al.,* 1997). It has also been proven that the dissemination of virulence gens could occur under selective pressure of antibiotic use.

The antibiotic resistance patterns may vary from one geographical area to another (Balls *et al.,* 1996).

Aminoglycosides include a group of drugs which are characterized by the presence of an aminocyclitol ring linked to amino sugars in their structure and have a broad spectrum of activity against bacteria. Examples of these drugs include streptomycin, kanamycin, gentamycin, tobramycin, and amikacin, which are commonly used in the treatment of infections by both gram-negative and gram-positive organisms. Their bactericidal activity is attributed to the irreversible binding to the ribosomes but effects resulting from interaction with other cellular structures and metabolic processes are also known. There are three mechanisms by which *salmonella* become resistant to aminoglycosides, these are: reduction in antibiotic uptake or decreased permeability, alteration of ribosomal binding sites and antibiotic modifications. The *Salmonella* uses mechanisms such as expression of plasmid-mediated aminoglycoside modifying enzymes against aminoglycoside (Gebreyes and Altier, 2002; Guerra, 2002), these enzymes are categorized into three groups and are named based on reactions they perform; this includes acetyltransferases, phosphotransferases and nucleotidyltransferases (Shaw et al., 1993).

Aminoglycoside acetyltransferases (AAC), catalyze acetyl CoA-dependent aceytylation of an amino group (Mascaretti , 2003; Shaw et al., 1993). There are four groups of this enzyme based on the areas that they alter: AAC (1), AAC (2'), AAC (3), and AAC (6') (Mascaretti, 2003). Also, genes encoding these enzymes are typically designated aac. These enzymes provide resistance to tobramycin, gentamicin and kanamycin (Mascaretti, 2003). Aminoglycoside phosphotransferases, catalyzes ATP-dependent phosphorylation of a hydroxyl group (Mascaretti, 2003; Shaw et al., 1993). depending on the specific sites of phosphorylation, it is classified to Groups APH (3") and APH (6) which provide resistance to streptomycin (Mascaretti, 2003) and have been found encoded on plasmids harbored by Salmonella (Gebreyes and Altier, 2002) and the genes having these encoding enzymes are designated as aph. The third type of enzyme which is nucleotidyl transferase provides aminoglycoside resistance (Mascaretti, 2003, Shaw *et al.,* 1993), and is divided

into several groups based on the site of modification and. Genes encoding these enzymes are usually designated aad (Vanhoof *et al.*, 1998) and some are also designated as ant Which providing resistance for streptomycin (Mascaretti, 2003), tobramycin and gentamicin (Mascaretti, 2003).

Chloramphenicol is a broad-spectrum antibiotic against both the gram negative and gram positive bacteria and it is effectiveness and ability to cross the blood-brain barrier makes it the drugs of choice for systematic infections therapy. Chloramphenicol is specific and potent inhibitor of protein by binding to the peptidyltransferase center of the 50s ribosomal unit, thus preventing formation of peptide bonds (Mascaretti, 2003). There are two mechanisms in which *Salmonella* resistance to chloramphenicol is conferred: (i) by the plasmid-located enzymes called chloramphenicol acetyltransferases (CAT) or non enzymatic chloramphenicol resistance gene cm1A and (ii) Efflux pump in which the antibiotic is removed. Chloramphenicol binds to the 50S ribosomal subunit and inhibits the peptidyl transferase step in protein synthesis. Resistance to chloramphenicol is generally due to inactivation of the antibiotic by a chloramphenicol acetyltransferase (Traced *et al.,* 1993). Various enzymes have been described and are coded for by the cat genes found in gram-negative and gram-positive bacteria and usually show little homology. Sometimes decreased outer membrane permeability or active efflux is responsible for the resistance in gram-negative bacteria (Butaye *et al.*, 2003).

Tetracyclines are one of the very commonly used antimicrobial agents in both human and veterinary medicine in developing countries because of their availability and low cost as well as low toxicity and broad spectrum of activity. They are broad-spectrum agents, exhibiting activity against a wide range of gram-positive and gram-negative bacteria. Resistance to these agents occurs mainly through three mechanisms. These are: Efflux of the antibiotics, Ribosome protection, and Modification of the antibiotic (Roberts, 1996). Tetracyclines mostly act by stopping the binding of tRNA to the A site of the 30S ribosomal subunit by inhibiting protein synthesis (Mascaretti, 2003). Many finding indicated that tetracycline resistance in *Salmonella* can be attributed to the production of an energy dependent efflux pump to remove the antibiotic from within the cell.

Mechanisms of bacterial resistance to quinolones such as naldixic acid fall in to two principal categories: these are alterations in drug target enzymes and alterations that limit the permeability of the drug to the target (Hooper, 1999).

Trimethoprim resistance is caused by a number of mechanisms including overproduction of the host DHFR, mutations in the structural gene for DHFR, and acquisition of a gene (dfr) encoding a resistant DHFR enzyme which is the most resistant mechanism in clinical isolates (Thomson, 1993). *Salmonella* drug resistance mechanism is summarized in Table 2.

2.18.2. Transfer of antimicrobial resistance factors in Salmonella

It is well recognized that antimicrobial resistance genes can be transferred among bacterial populations. *Salmonella* becomes antimicrobials resistant either through spontaneous mutation or via horizontal resistance genes transfers by transformation, transduction, or conjugation. Bacterial transformation is the process in which a recipient cell takes up bacterial free DNA molecules from the environment. The transduction is the process involves the transfer of DNA from one cell to another through the replicating process of bacteriophage. Unlike the processes of transformation and transduction, bacterial conjugation is the transfer of genetic material between bacteria (Courvalin, 1996).

Table 2. Resistance mechanisms of the different antibiotics

Class and type of antibiotic	Resistance mechanism	Reference
	Alteration of target site	
β-Lactam	Altered a new penicillin-binding proteins	Quinn, 1999
Aminoglycosides	Alterd ribosomal protein	Quinn, 1999
Erythromicine	Ribosomal RNA methylation	Quinn, 1999
Tetracyclines	Ribosomal protection	Roberts, 1996
Naldixic acid	Altered DNA gyrase	Hooper, 1999
Sulphonamides	New drug insensitive dihydropteroate	Quinn, 1999
Trimethoprim	New drug insensitive dihydrofolate reductase	Quinn, 1999
	Drug destroying mechanism	
Aminoplycosides	Acetyltransferase, nucleotidyltransferase and phophotransferase	Mascaretti , 2003; Shaw et al., 1993
Chloramphenicol	Acetyltransferase	Traced *et al.*, 1993
	Decreased uptake (decreased permeability)	
β-Lactams antibiotics	Alteration in the permeability of the bacterial cell envelope	Quinn, 2004
	Other mechanisms	
Folate	Altered target	Wray (page 92)
Tetracycline	Efflux and modification of antibiotics	Roberts, 1996

2.18.3. Risk factors for Salmonella antimicrobial resistance development

The use of antibiotics together with the improvement of sanitation and hygiene as well as immunization and proper nutrition has provided major benefits in human life expectancy (WHO, 2002). However, the increased utilization of antibiotics in both public and veterinary settings has led to the emergence of antibiotic resistance and as a consequence poses a serious threat to public health safety (WHO, 2002). In general, the persistent use of drugs initiates selective pressure that encourages the development of antibiotic

resistant pathogens. One of the most important factors necessary for the development of antibiotic resistant strains of microorganisms is the irrational use of such antibiotics in the animal production settings, which has brought about the emergence of bacteria strains that were otherwise infective only to animals to now become infective to humans as well due to the acquisition of the antibiotic resistance traits (Akond *et al.,* 2012). Animals that have become infected with antibiotic resistant strains of *Salmonella* are important sources of resistant determinants that give room for the *Salmonella* serotypes to become infective to humans (Akoachere *et al.,* 2009). Antibiotic resistant strains of *Salmonella* have been frequently recovered from food of animal origin of which poultry is of major concern (Alambedji *et al.,* 2006; Okoli *et al.,* 2009).

In animal husbandry, antimicrobial agents are used for three purposes: therapy, prophylaxis and growth enhancement. In all instances, a selective pressure is imposed on bacterial populations and antibiotic resistances are selected. The major selective pressures on *Salmonella* stem from the overuse of antimicrobials for prophylaxis and therapy. In particular, the wide use of mixtures of antimicrobials, antimicrobials mixed into animal feeds and therapy without diagnosis leads to a long-lasting, strong selective pressure in animal husbandry and on *Salmonella* in intensive production units. It is now accepted that antimicrobial use is the single most important factor responsible for increased antimicrobial resistance (Aarestrup *et al.,* 2001; Byarugaba, 2004). And antibiotic incorporated into animal feeds are at sub-therapeutic concentrations which in consequence creates a favorable ground for the development of antibiotic resistance within the animal production setting (Angulo *et al.,* 2004).

The pool of resistance genes is thus spread in the environment (WHO, 2004). This has resulted in the dissemination of resistance genes to sensitive species and the emergence of new resistance determinants. Thus, the widespread use of antibiotics as feed additive in poultry, cattle and pigs facilitates intestinal colonization by resistant *Salmonella*. Such practice often leads to emergence and persistence of resistant strains of *Salmonella* and increases the potential for cross-contamination of animals through prolonged fecal shedding (Poppe *et al.,* 1996; D'Aoust, 1989).

The frequency of isolation of *Salmonella* strains resistant to several antimicrobial agents has increased in several countries worldwide (Yoke-Kqueen *et al.,* 2007; Pui *et al.,* 2011) and the association between the veterinary use of antibiotics and the development of resistant strains of microorganisms against these antibiotics in human infection is now an established fact (Begum *et al.,* 2010).

2.19. Clinical Features of Salmonellosis

Different infectious syndromes can be caused by *Salmonella* serotypes, e.g. serotype Typhi causes typhoid in humans, serotype Typhimurium causes diarrhoea in humans and other animal species, serotype Abortusovis is responsible for abortion in ewes and serotype Dublin has been associated with different extra-intestinal infections in AIDS patients.

2.19.1. Clinical features in humans

In human, salmonellosis may occur as an acute, self-limiting gastroenteritis or as systemic infection characterized by septicaemia and ultimate localization in extra-intestinal sites. The gastrointestinal form is often referred to as the "food poisoning syndrome." This is a misnomer as the disease is an infection rather than intoxication. The systemic disease is referred to as enteric fever, with typhoid being the classic form. All *Salmonella* serotypes are presumably pathogenic to humans can cause important morbidity, mortality and economic burden and are particularly severe in infants, the elderly and immunocompromised individuals (Lax *et al.,* 1995; D'Aoust, 1991).

The dose required to cause disease varies with many factors. Low attack rates have been observed in one outbreak where 4-45 cells were consumed and another where the dose was 6 cells/65g (Anonymous, 1996). Different serotypes may have different dose responses and doses generally recognized to cause disease at high attack rates are in the range of 10^5 to 10^7 cells. It has been repeatedly reported that the infectious dose is lower when the implicated food has a high fat or protein content (Lake *et al.,* 2004).

2.19.2. Clinical features in animals

Salmonellosis in animals is characterized clinically by one or more of three major syndromes; septicemia, acute enteritis and chronic enteritis. The incidence has increased with the intensification of livestock production. Young calves, piglets, lambs, and foals usually develop the septicemic form. Adult cattle, sheep, and horses commonly develop acute enteritis, and chronic enteritis may develop in growing pigs and occasionally in cattle. Pregnant animals may abort. Dogs generally seem to be resistant to *Salmonella* infection and most cases are latent and non-clinical (Kozak *et al*., 2003). Salmonellosis is a serious infectious disease of cattle causing enteritis, septicaemia, pneumonia and abortion (Macke *et al.,* 1996).

2.20. Treatment of Salmonellosis

Antimicrobial agents should not be used routinely to treat uncomplicated non-typhoid *Salmonella* gastroenteritis. However, antimicrobial therapy is essential in the treatment of serotype Choleraesuis infection, in view of the high rate of extra intestinal infections caused by this organism. Because of the increasing prevalence of resistance to conventional antimicrobial agents such as ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, empirical therapy for life-threatening bacteremia or focal infection suspected to be caused by non-typhoid *Salmonella* should include a broad-spectrum cephalosporin or a fluoroquinolone until susceptibility patterns are known (Chiu *et al.,* 2004).

2.21. *Salmonella*: a Public Health Perspective

In many countries incidence of human *Salmonella* infection has increased drastically over the years (Chiu *et al.,* 2004), causing substantial morbidity, and thus also has a significant economic impact. Although most infections cause mild to moderate self-limited disease, serious infections leading to deaths do occur (Jong and Ekdahl, 2006). Besides the importance for public health, another aspect is the cost generated by human

Salmonellosis. During 1999, the cost linked to foodborne Salmonellosis ranged between 560 million and 2.8 billion € in Europe, where *Salmonella* was estimated to be responsible for nearly 166 000 cases (Korsak *et al.*, 2006), in the United States is between 15 to 20 cases per 100 000 people (Oscar, 2004).

2.22. Prevention and Control

Salmonella prevention and control may be achieved by adopting Good Agricultural Practices and Hazard Analysis Critical Control Point (HACCP), and general measures on hygiene and biosecurity procedures in poultry production, in combination with the additional measures, where appropriate. No single measure used alone will achieve effective in *Salmonella* control and the control in meat animals and derived products is a most challenging task because of the complexity and interdependence of various aspects of animal husbandry, slaughtering, and food processing. Adequate nutrition and hygiene prior to and during transport and within the abattoir could be an important intervention to prevent spread of salmonellosis (OIE, 2010).

Vaccination

Vaccination can be done using live or inactivated vaccines, and vaccines should reduce or prevent the intestinal colonization resulting in reduced fecal shedding and thus egg shell contamination and prevent systemic infection resulting in a decreased colonization of the reproductive tissues, in this way reducing internal egg contamination. It is very well documented that both killed and live vaccines can reduce shedding of *Salmonella* in poultry (Van Immerseel *et al*., 2005). It might be possible to administer to newly hatched chicks live *Salmonella* vaccine strains such that they would colonize the gut extensively and very rapidly, inducing a profound resistance to colonization by other *Salmonella* strains of epidemiological significance, which may be present in the poultry house or may also have arisen from the hatchery. Colonization of the gut by the colonization-inhibition strains (live vaccines strains) would prevent gut colonization by virulent strains, while

invasion in the gut tissue would evoke an inflammatory response that would prevent invasion to the internal organs by virulent strains (Van Immerseel *et al*., 2005).

Hygiene

Good farming and hygienic practices need to be implemented, in order to avoid introduction of *Salmonella* on the farm or reduce the infection pressure when *Salmonella* is present. Hygienic measures at all levels of the production chain pre-harvest (during life), harvest (catching and transport) and post-harvest are essential for successful *Salmonella* control. Hygienic measures should take into account feed, birds, drinking water, environment, management, cleaning and disinfection. This can imply physical and chemical decontamination treatments of feed, drinking water, the environment of the birds (Graham, 2005).

Use of probiotics

Probiotics are non-digestible feed ingredients that beneficially affect the host by selectively stimulating the growth and activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health. In this definition it is understood that certain bacterial species multiplying in the colon can have a beneficial effect on host health. These bacterial species can also be administered through the feed. Most probiotics are carbohydrates (Tuohy *et al*., 2005).

Acidification

Acidic compounds are more and more used to combat *Salmonella* infections. Not only drinking water acidification, but also acid release in the proximal gastrointestinal tract (powder as feed additive) or the distal parts of the gastrointestinal tract (coated or encapsulated acids in feed) is widely used. Medium chain fatty acids (MCFA) are strongly bactericidal towards many Gram-positive and Gram-negative bacteria, including *Salmonella* (Nakai and Siebert, 2003). Even at concentrations as low as 10 milli mole

MCFA still show a bacteriostatic effect on *Salmonella* (Van Immerseel *et al*., 2004). Short chain fatty acids (SCFA) are the major bacterial fermentation products in the large intestine. SCFA are also commonly added to feed and drinking water. At high concentrations (1%) these products have an antimicrobial effect in moist environment. This microbial growth inhibition is traditionally explained by the ability of these acids to pass across the bacterial cell membrane in undissociate form, dissociate in the neutral at bacterial cell interior and thereby acidify the bacterial cell cytoplasm (Tuohy *et al*., 2005).

2.23. Economic Impact of *Salmonella*

Salmonellosis is a significant cause of economic loss in farm animals because of the costs of clinical disease that include deaths in a small proportion of cases, decreased milk and meat production, reduced value of contaminated products, diagnosis and treatment of clinical cases, diagnostic laboratory costs, the costs of cleaning and disinfections, and the costs of control and prevention. It can occur as a severe systemic disease responsible for heavy economic losses to the commercial poultry industry through morbidity, mortality and reduced egg production. *S.* Enteritidis has been most frequently involved in salmonellosis outbreaks. It causes millions of dollars in losses to the industry, mainly in cattle, swine and poultry production, both in local and international trade (Barrow, 1989).

Financial costs are not only associated with investigation, treatment and prevention of human illness but may affect the whole chain of food production. Thus, the costs of salmonellosis, as with other foodborne illness, fall into both the public and private sectors and may be surprising, both in terms of the level of costs incurred and the variety of areas affected. In the public sector, resources may be diverted from preventative activities into the treatment of patients and investigation of the source of infection. In the private sector considerable financial burdens may be imposed on industry in general and on the food industry in particular and last but not least, on the affected individual and his or her family. Infections with *Salmonella* were associated with increased long-term mortality. Costs of individual outbreaks can be very high and may range from thousands to millions

of dollars depending on the type of outbreak implicated. In the USA alone, nontyphoidal salmonellosis is estimated to be responsible for the deaths of more than 500 people each year, with costs of $1.1billion to $1.5 billion annually (Mead *et al.,* 1999). Medical costs and lost production are examples of tangible costs which are easily measured in monetary terms, while costs such as loss of leisure and pain and discomfort are intangible costs difficult to measure. Costs to society include costs of illness which fall directly on the ill persons and their immediate family; costs to the national economy which relate to sickness absence from work; and cost to producers, manufacturer or retailer when food products are implicated in food poisoning outbreak.

2.24. *Salmonella* in Ethiopia

Salmonella isolates in Ethiopia may have similar phenotypic and genotypic characteristics with isolates elsewhere in the world and non-typhoidal *Salmonella enterica* infection in children in Ethiopia is a major health problem and is caused by similar serotypes to these reported from elsewhere in Africa: *S.* Typhimurium and *S.* Enteritidis (Getenet, 2008). *Salmonella* infection most commonly occurs in countries with poor standards of hygiene in food preparation and handling and where sanitary disposal of sewage is lacking (Muleta and Ashenafi, 2001; WHO, 2003). In recent years the number of out breaks of *Salmonella* in humans has increased considerably in the country. In addition, the very young, elderly and immunocompromized individuals are particularly more susceptible to *Salmonella* infections at a lower infective dose than healthy adults. This is more important in developing countries such as Ethiopia where HIV/AIDS is highly prevalent and *Salmonella* is an important opportunistic infection in HIV/AIDS patients (Catherine *et al*., 2001).

2.25. Relevance of the current work to fill gap

Advances in poultry production practices changes in consumer lifestyles and preferences, and heightened nutritional awareness have all combined to make poultry products a leading source of protein for much of the world. Thus, the incidence of *Salmonella*

infection in poultry flocks and associated incidence of *Salmonella* contamination of poultry products are of considerable public health significance. Furthermore, routine assessment of patterns of emerging antibiotic resistant *Salmonella* strains is of paramount importance because such information channeled to physicians and veterinarians help to timely redirect drug use so as to diminish the development and spread of resistance (Tollefson *et al.* 1998). In recent years, testing of *Salmonella* isolates has shown that an increasing proportion of isolates are resistant to several antimicrobial agents both in developing and developed countries. The issue of antimicrobial resistance is more complex in developing countries (Leegaard *et al.,* 1996) like Ethiopia where *Salmonella* is no routine surveillance and resistance to commonly used antimicrobial drugs in veterinary and public health sector not regularly monitored. Therefore, the present study was undertaken to investigate the epidemiology and susceptibility of *Salmonella* isolates from poultry settings to commonly use antimicrobial agents.

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted in Addis Ababa, Adama, Bishoftu, and Mojo from Central Ethiopia, Hawassa and Bonga from South Ethiopia from November 2014 to June 2015 in poultry farms and slaughter houses.

Addis Ababa: Addis Ababa is the capital city and administration center for the Federal Democratic Republic of Ethiopia. It is situated at latitude of $9^{o}3'$ North and $38^{o}43'$ East (ILCA, 1994). It lies in the central highlands of Ethiopia at an altitude of 2500 m.a.s.l. It has an average rainfall of 1800 mm per annum. The annual average maximum and minimum temperature is $26^{O}C$ and $11^{O}C$, respectively; with an overall average of 18.7 ^{O}C. highest temperatures are reached in May. The main rainy season extends from June to September. Addis Ababa has a relative humidity varying 70% to 80% during the rainy season and 40% to 50% during the dry season.

Bishoftu: Bishoftu is located in Oromia National Regional State about 45 km South East of Addis Ababa; just on the escarpment of the Great Rift Valley and the geography of the area is marked by creator lakes. It is found at $9^{O}N$ latitude and $40^{O}E$ longitude and at an altitude of 1850 meter above sea level in the central highlands of Ethiopia. It has a human population of about 95,000. It experiences a bimodal pattern of rainfall with the main rain season extending from June to September (of which 84% of rain is expected) and a short rainy season from March to May with an average annual rainfall of 800mm. the mean annual minimum and maximum temperature are $12.3^{O}C$ and $27.7^{O}C$, respectively with an overall average of $18.7^{O}C$. the highest temperature are recorded in May and the mean relative humidity is 61.3%. Bishoftu is the center of Ada'a Woreda and it has a total land area of about $1610.56Km^2$ and is divided in to three agro ecological zones midland (94%), highland (3%) and lowland (3%) (CSA, 2007).

Modjo: Modjo town is the center of Lume District in Eastern Showa administrative zone of Oromia Regional State. It is located 73 kms southeast of Addis Ababa at an altitude of 1777 meters above sea level. It experiences a bimodal pattern of rainfall with the main rainy season extending from June to September and a short rainy season that extends from March to May with an average annual rainfall of 800mm. The average maximum and minimum temperatures are 28°C and 18°C respectively (ILRI, 2005).

Adama: Adama is located in the rift valley, about 95Km southeast of Addis Ababa (8.33^{O}N and 39.17^{O}E) with an altitude of 1622m above sea level. It receives an annual rainfall ranging from 400 to 800mm. the temperature range is 13.9 to 27.7OC (NMSA, 2006).

Hawassa: The study was conducted in poultry farms in Hawassa, Southern Ethiopia, located 275 km south of Addis Ababa, from November 2014 to May 2015. The annual rainfall and temperature of the area varies from 800-1000 mm and 20.1-25^{O}C, respectively (CAA, 2004).

Bonga: Bonga is the administrative city of the Kaffa zone, located (460 km far from Addis Ababa) in the south western part of Ethiopia in between 6^{0}24’ to 7^{0}70’ N and 35 69’ to 36^{0}78’ E. The area receives rainfall almost all the year round. From March to September. The mean annual rainfall reaches to 1710 mm. The mean monthly temperature ranges between 18 – 21^{o}C. From January to March the difference between mean minimum and mean maximum temperature received increases to gradually lower in April (Bekele, 2003).

3.2. Study Design

A cross-sectional study was conducted from November 2014 to June 2015 in randomly selected areas with purposive selection of poultry farms and abattoirs based on farm availability and willininghess of the owners (Table 3). During the study period

microbiological analysis of different samples taken from poultry farms and chicken slaughter houses with Simple random sampling technique was used.

Table 3. Studied number of poultry farms and abattoir along with the breed types in study areas.

Study Location	Study Area	selected farms	Selected abattoir	Breed
Central Ethiopia	Addis Ababa	6	-	Bovans and white leghorn
	Bishoftu	2	1	Bovans and white leghorn
	Modjo	4	-	Bovans, ISA brown and white leghorn
	Adama	2	-	White leghorn, Bovans and Fayomin
South Ethiopia	Hawassa	2	-	White leghorn
	Bonga	1	-	White leghorn
Total	6	17	1	

3.3. Study Population and Sample Type

The study populations were chickens from commercial and small scale poultry farms, and slaughter houses. For the isolation and identification of *salmonella*, sampling was carried out regularly and different sample types from different sources were collected. Cloacal swabs, fresh feces, pooled fecal samples, carcass swab, environmental swab, working equipments swab, water samples and personnel's hand swab (farm attendant) were among the collected samples for this study.

The commercial production system is highly intensive production system where birds are kept under indoor conditions with a medium to high bio-security level. This system heavily depends on imported exotic breeds that require intensive inputs such as feed, housing, health, and modern management systems. This system is characterized by higher level of productivity where poultry production is entirely market oriented to meet the large poultry demand in major cities.

Small-scale intensive production system is characterized by medium level of feed, water and veterinary service inputs and minimal to low bio-security. Most small-scale poultry farms obtain their feed and foundation stock from large-scale commercial farms.

3.4. Sample Size Determination

The sample size was calculated based on the prevalence's reported in previous studies as shown in Table 4 below. Accordingly, 5% desired absolute precision at 95% confidence interval using the formula recommended by Thrusfield (2005) was used. But for personnel's hand swab, the available workers found were included for sampling.

$$n = Z^2 \, p \, exp \, (1\text{-}pexp) \, / d^2$$

Where n = required sample size; pexp = expected prevalence and a desired absolute precision (d) of 0.05, Z = 1.96. This gave 176 samples to be collected from each of the six study areas involved in this study with the overall total sample size 1056. The actual collected sample size for each study area was shown in Table 5.

Table 4. Number of calculated samples.

Source	Type	Reported prevalence (%)	Authors	Total sample
Poultry	Cloacal swab and fresh feces	15.4	Bekele & Ashenafi, 2009	118
	Carcass swab	7.7	Molla *et al.*, 2003	58
Total				176

3.5. Sample Collection and Transportation

Swab samples were taken according to the method described in ISO-17604 (2003) from abattoir and farms as the distribution is shown in Table 4. A sterile cotton tipped swab (2X3 cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water (BPW) and rubbed over the delineated area horizontally and then vertically several times. Up on completion of the rubbing process, the swab was placed into the

buffered peptone water used to wet the swab, breaking off the wooden shaft pressing against the inside of the universal bottle and disposed leaving the cotton swab in the universal bottle. Similarly, other sample types also collected according to the above recommended procedures. Finally, by using ice boxes with ice packs the samples were transported to Addis Ababa University College of Veterinary Medicine and Agriculture for lab analysis.

Table 5. Distribution of collected sample type by study areas

Sample type	Study areas						Total
	AA	Bishoftu	Modjo	Adama	Hawassa	Bonga	
Cloacal swab	169	104	100	154	149	95	771
Fresh feces		10	75	25	-	-	110
Feed	5	-	8	3	-	-	16
Litter	12	-	10	9			31
Personnel hand swab	8	6	4	8	6	3	35
Pooled feces	6	-	8		15	2	31
Carcass swab	-	64	-	-	-	-	64
Pooled instrument swab	-	1	-	-	-	-	1
Total	200	185	205	199	170	100	1059

3.6. Bacteriological Examination

Culture methods

Salmonella was isolated and identified according to the technique recommended by the international organization for standardization (ISO-6579, 2002) (Annex 1) and the bacteriological media were prepared according to manufacturer's recommendations (Annex 2).

3.6.1. Pre-enrichment and selective enrichment

The swab samples were pre-enriched in appropriate amount of buffered peptone water in (1: 9) ratio and incubated at 37°C for 24 hrs. Rappaport- Vassiliadis medium (RV) broth and Müller Kauffman Tetrathionate broth were used for selective enrichment of the samples. About 0.1 ml of the pre-enriched sample was transferred into a tube containing 10 ml of Rappaport- Vassiliadis medium (RV broth) and incubated at 42 °C for 24 hours. Another 1ml of the pre-enriched broth was transferred into a tube containing 10ml of MKTTn broth and incubated at 37°C for 24 hours.

3.6.2. Plating out and identification

Xylose lysine desoxycholate (XLD) agar and brilliant green agar (BGA) plates were used for plating out and identification. A loop full of inoculums from each RV and MKTTn broth cultures were plated onto XLD and BGA plates and incubated at 37 ^{O}C for 24 hours. After incubation, the plates were examined for the presence of typical and suspect colonies. Typical colonies of *Salmonella* grown on XLD-agar have a black centre and a lightly transparent zone of reddish color due to the color change of the media (ISO 6579, 2002) while HS negative variants grown on XLD agar are pink with a darker pink center. Lactose-positive *Salmonella* grown on XLD agar are yellow with or without blackening. Typical colonies of *Salmonella* on BGA are pink, 1 mm to 2 mm in diameter, and cause the color of medium to change to red. Five typical or suspected colonies were selected from the selective plating media, streaked onto the surface of pre dried nutrient agar plates and incubated at 37^{O}C for 24hrs.

3.6.3. Biochemical tests

All suspected non-lactose fermenting *Salmonella* colonies were picked from the nutrient agar and inoculated into the following biochemical tubes for identification: triple sugar iron (TSI) agar, Simmon's citrate agar, urea broth, tryptone water, MR-VP broth and incubated for 24 or 48 hours at 37^{o}C. Colonies producing an alkaline slant with acid

(yellow color) butt on TSI with hydrogen sulphide 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 to be *Salmonella*-positive (Annex) (Quinn *et al.*,1999).

3.7. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the isolates was performed by using the disc diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2002) and (CLSI, 2012). Well isolated colonies from nutrient agar plates were transferred into tubes containing 5 ml of Tryptone soya broth (Oxoid, England). The broth culture was incubated at 37ºC for 4 hours until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension, rotated several times, pressing firmly on the inside wall of the tube above the fluid level to remove excess inoculums and swabbed uniformly over the surface of Muller Hinton agar plate (Oxiod, England). The plates were held at room temperature for 30 min to allow drying.

The susceptibilities of the isolates were tested for the following antibiotic discs: Ampicillin (AMP) 10µg, Gentamicin (CN) 10µg, Kanamycin (K) 30µg, Ciprofloxacin (CIP) 5µg, Chloranphenicol (C) 30µg, sulphamethoxazole trimethoprim (SXT) 25 µg, Tetracycline (TE) 30µg, Naldixic Acid (NA) 30µg, Streptomycine (S) 10µg and cefoxitin (FOX) 30µg, were placed at least 15 mm apart and from the edge of the plates to prevent overlapping of the inhibition zones. The plates were incubated at 37ºC for 24 h. The diameter of the zones of inhibition was classified as resistant, intermediate, or susceptible according to the interpretive of the Clinical Laboratory Standards Institute (CLSI, 2012). All the antibiotics are Oxoid, England made and their expired date was 2018. The interpretive standards and the drugs mechanism of action and resistance are indicated bellow in the Table 6.

Table 6. Performance standard for antimicrobial susceptibility testing of *Salmonella.*

No	Antimicrobial Agent, Disc code and Potency	Cutoff value			Mechanism of action of drugs	Reference
		R	IM	S		
1	Ampicillin (AMP),10µg	≤13	14-16	≥17		
2	Chloranphenicol (C),30µg	≤12	13-17	≥18	Inhibiting protein synthesis by bind to 50S ribosomal subunit	Hooper, 200; Tenover, 2006
3	Ciprofloxacin (CIP), 5µg	≤20	21-30	≥31	inhibit DNA synthesis by targeting DNA gyrase	Hooper, 200; Tenover, 2006
4	Gentamicin (CN), 10µg	≤12	13-14	≥15	Inhibiting protein synthesis by bind to the 30S subunit	Wray and Wray, 2000
5	Kanamycin (K), 30µg	≤13	14-17	≥18	Inhibiting protein synthesis by bind to the 30S subunit	Wray and Wray, 2000
6	Naldixic Acid (NA),30µg	≤13	14-18	≥19	Inhibit bacterial DNA gyrase, an enzyme essential for DNA replication	Pumbwe and Piddock, 2002;
7	Streptomycine (S), 10µg	≤11	12-14	≥15	Inhibiting protein synthesis by bind to the 30S subunit	Wray and Wray, 2000
8	Tetracycline (TE), 30µg	≤11	12-14	≥15	Inhibiting protein synthesis by bind to the 30S subunit	Hooper, 2001; Tenover, 2006
9	SXT* (SXT), 25µg	≤ 10	11-15	≥ 16	Inhibiting the synthesis of folic acid metabolism which ultimately inhibit DNA synthesis	Hooper, 2001; Tenover, 2006
10	Cefoxitin (FOX), 30µg	≤ 14	15-17	≥18	Inhibiting protein synthesis by bind to the 30S subunit	Wray and Wray, 2000

The source for drug sensitivity testing cutoff value was CLSI (2012). R = resistant; IM = intermediate; S = susceptible

3.8. Data Management and Analysis

Data of laboratory results of the collected samples were entered in Microsoft - Excel 7 and analyzed using SPSS version 20 software programs. Descriptive statistics such as percentage and frequency distribution was used to describe the nature and the characteristics of the data. Bacterial isolates and antimicrobial susceptibility were expressed as percent of resistant, intermediate and susceptible using positive isolates from total examined. In addition, the proportion of bacteria resistant to at least one of the ten antibiotics and resistant to two or more was calculated. Moreover, comparisons between each risk factor were analyzed using x^2test, then, confirmed by univariate logistic regression test. Univariate and multivariate logistic regression analysis was performed to reveal the strength of association of the potential risk factors with positivity of sample using odds ratio (OR). The probability of type one error was set at 5% with 95% confidence level to establish biological and statistical association between dependent and independent variables.

4. RESULTS

4.1. Prevalence and Distribution of *Salmonella*

A total of 1059 samples from central and south Ethiopia were cultured for *Salmonella* isolation and detected in 126 (11.9%) samples. The types of samples that gave positive results were shown in Table 7. The prevalence of *Salmonella* in south Ethiopia was significantly higher than that of central Ethiopia (P = 0.005). The prevalence in Bonga was significantly higher than the other study areas (P = 0.000) even though there were significantly different prevalence (p=0.000) among the farms within each of the six study areas as shown in Table 8.

4.2. Univariate Analysis for Association between Risk Factors and *Salmonella* Isolation

South Ethiopia had significantly higher odds of *Salmonella* infection than Central Ethiopia. Study area, sample source and breed had significant association with *Salmonella* isolation (Table 9). The univariate analysis results for detailed risk factors were indicated in Annex 3.

There was significant association between individual farms and *Salmonella* infection indicating different farms within a given area had different level of exposure and infection to Salmonellosis (Annex 4).

4.3. Multivariate Logistic Regression Analysis for Association between Risk Factors and *Salmonella* Infection

The risk factors that strongly had effect on *Salmonella* infection were area and source of sample after the effect of the other risk factors removed using multivariate logistic regression (Table 10).

Table 7. Prevalence and distribution of *Salmonella* isolated from poultry, human and environment in central and south Ethiopia.

Risk factors		N	Positive	Prevalence (%)	95% CI	x^2 test	p-value
Location	South Ethiopia	270	45	16.7	16.2, 17.2	7.862	0.005
	Central Ethiopia	789	81	10.3	10.1, 10.5		
Category	Farm	995	123	12.4	12.2, 12.6	3.379	0.066
	Abattoir	64	3	4.7	4.2, 5.2		
Area	Bonga	100	27	27	26.0, 28.0	32.876	0.000
	Modjo	205	31	15.1	14.6, 15.6		
	Addis Ababa	200	23	11.5	11.0, 12.0		
	Hawassa	170	18	10.6	10.1, 11.1		
	Adama	199	15	7.5	7.1, 7.9		
	Bishoftu	185	12	6.5	6.1, 6.9		
Housing	Litter	768	86	11.2	11.0, 11.4	14.317	0.001
	Cage	186	16	8.6	8.2, 9.0		
Breed	ISA Brown	73	13	17.8	16.8, 18.8	26.09	0.000
	White leghorn	397	51	12.8	12.4, 13.2		
	Bovans	431	33	7.7	7.4, 8.0		
	Fayomin	48	3	6.3	5.6, 7.0		
Source	Environment	69	17	24.6	23.4, 25.8	15.548	0.004
	Water	15	3	20	17.7, 22.3		
	Personnel	35	7	20	18.5, 21.5		
	Feed	16	2	12.5	10.8, 14.2		
	Poultry	924	97	10.5	10.3, 10.7		
Sample type	Pooled feces	17	6	35.3	32.5, 38.1	22.309	0.002
	Litter	31	7	22.6	20.9, 24.3		
	Water	15	3	20	17.7, 22.3		
	Hand swab	35	7	20	18.5, 21.5		
	Fresh feces	110	18	16.4	15.6, 17.2		
	Poultry Feed	16	2	12.5	10.8, 14.2		
	Cloacal swab	770	80	10.4	10.2, 10.6		
	Carcass swab	64	3	4.7	4.2, 5.2		
	PIS*	1	0	0	0.0		
Flock size	Medium size	215	26	12.1	11.6, 12.6	17.218	0.000
	Large size	735	74	10.1	9.9, 10.3		
Production type	Broiler	208	22	10.6	10.2, 11.0	16.136	0.000
	Layer	741	78	10.5	10.3, 10.7		
Total		1059	126	11.9	11.7, 12.1		

PIS*=pooled instrument swab and is not included in the analysis

Table 8. Prevalence and distribution of *Salmonella* isolated from different poultry farms in central and south Ethiopia.

Risk factors		N	Positive	Prevalence (%)	95% CI	x^2 test	p value
Farms	Addis Ababa 5*	47	14	29.8	28.2, 31.4	61.09	0.000
	Bonga1	100	27	27	26.0, 28.0		
	Modjo 3	50	10	20	18.8, 21.2		
	Modjo 4	53	9	17	15.9, 18.1		
	Hawassa 2	80	11	13.8	13.0, 14.6		
	Bishoftu 2	31	4	12.9	11.6, 14.2		
	Modjo 2	55	7	12.7	11.8, 13.6		
	Modjo1	47	5	10.6	9.7, 11.5		
	Addis Ababa 1	20	2	10	8.6, 11.4		
	Addis Ababa 2	43	4	9.3	8.4, 10.2		
	Adama1	140	12	8.6	8.1, 9.1		
	Hawassa 1	90	7	7.8	7.2, 8.4		
	Bishoftu 3	82	6	7.3	6.7, 7.9		
	Addis Ababa 6	33	2	6.1	5.3, 6.9		
	Adama2	59	3	5.1	4.5, 5.7		
	Addis Ababa 4	24	1	4.2	3.4, 5.0		
	Bishoftu1	72	2	2.8	2.4, 3.2		
Total		1059	126	11.9	11.7, 12.1		

*Name of the farms in each study areas

Table 9. Univariate risk factor analysis for occurrence of *Salmonella* in poultry farms, abattoir, and environment in central and south Ethiopia

		OR	95% CI.OR	p-value
Study Location	South/Central Ethiopia	1.748	1.179, 2.593	.005
Study area	Addis Ababa vs. Modjo	.729	.409,1.301	.285
	Adama vs. Modjo	.458	.239, .877	.018
	Bishoftu vs. Modjo	.389	.194, .783	.008
	Bonga vs. Modjo	2.076	1.158, 3.722	.014
	Hawassa vs. Modjo	.665	.357, 1.236	.197
Sample source	Water vs. environment	.765	.193, 3.035	.703
	Feed vs. environment	.437	.090, 2.121	.304
	Personnel vs. environment	.765	.283, 2.064	.596
	Poultry vs. environment	.359	.200, .645	.001
Breed	Bovans vs. White leghorn	.563	.355, .892	.014
	Fayomin vs. White leghorn	.452	.136, 1.509	.197
	ISA Brown vs. White leghorn	1.470	.754, 2.866	.258

Table 10: Multivariate logistic regression analysis for association between risk factors and *Salmonella* infection

Risk factor		OR	95% CI. for EXP(B)	P value
Area	Adama vs. Modjo	.408	.210, .790	.008
	Bishoftu vs. Modjo	.447	.220, .908	.026
	Bonga vs. Modjo	2.360	1.299, 4.285	.005
Source	Poultry vs. Environment	.356	.206, .616	.000

4.4. Antimicrobial Resistance

4.4.1. Susceptible and resistant Salmonella isolates by antimicrobials

One hundred twenty six *Salmonella* isolates were subjected to antimicrobial susceptibility test. The highest level of resistance (95 isolates, 75.4%) was observed for kanamycin followed by Sulphamethoxazole- Trimethoprim (92 isolates, 73%) and Tetracycline (90 isolates, 71.4%) and the lowest resistance was observed against Gentamicin (5 isolates, 4%) and ciprofloxacin (15 isolates, 11.9%). The response of the isolates to 10 different antimicrobials was shown in Table 11.

Table 11. Number of Susceptible and resistant isolates of *Salmonella* by antimicrobials
* Sulphamethoxazole- Trimethoprim

Type of Antibiotics	Number of *Salmonella* isolates			
	N	Susceptible (%)	Intermediate (%)	Resistant (%)
Gentamicin (CN) 10 µg	126	108 (85.7)	13 (10.3)	5 (4)
Tetracycline (TE) 30 µg	126	26 (20.6)	10(7.9)	90 (71.4)
Ampicillin (AMP) 10 µg	126	47 (37.3)	2(1.6)	77(61.1)
Nalodixic Acid (NA) 30 µg	126	26(20.6)	14(11.1)	86(68.3)
Cefoxitin (FOX) 30 µg	126	42(33.3)	5(4)	79(62.7)
Ciprofloxacin (CIP) 5 µg	126	97(77)	14(11.1)	15(11.9)
Streptomycine (S) 10 µg	126	25(19.8)	27(21.4)	74(58.7)
Kanamycin (KAN)30 µg	126	27(21.4)	4(3.2)	95(75.4)
(SXT)* 25 µg	126	28(22.2)	6(4.8)	92(73)
Chloranphenicol (C) 30 µg	126	33(26.2)	19(15.1)	74(58.7)

4.4.2. Prevalence of resistant Salmonella isolates by sources in six locations

Of 126 isolates, 123 had developed drug resistance. Most the resistant isolates were recovered from Cloacal swab (79 of 123). The distribution of the resistant isolates by location and sample type was indicated in Table 12. Resistant *Salmonella* isolate

recovered from personnel's, poultry and poultry house environment showed resistance to all types of tested antibiotics whereas those isolated from water and poultry feed showed less resistance for Gentamicine (Table 13).

Table 12. Distribution of antibiotic resistant *Salmonella* isolates by sample type in six locations

Area	CS	ClS	PF	FF	Li	PHS	PoF	W	Grand Total (%)
Bonga		22				3	2		27(21.9)
Adama		7	2	3	1	2			15(12.2)
Modjo		11		14	2			2	29(23.6)
Hawassa		14					4		18(14.6)
Bishoftu	3	5		1		2		1	12(9.8)
Addis Ababa		20			2				22(17.9)
Total	3	79	2	18	5	7	6	3	123(100)

CS=carcass swab, ClS=cloacal swab, PF=poultry feed, FF=fresh feces, Li=liter, PHS=personnel hand swab, PoF= pooled feeces, W= water.

Table 13. Resistance prevalence of *Salmonella* isolated from poultry sources against different antibiotics.

Antibiotics	Resistant *Salmonella* isolates from sources				
	Water (%) (n=3)	PF (%) (n=2)	PHS (%) (n=7)	Poultry (%) (n=97)	PHE (%) (n=17)
Gentamicin (CN) 10µg	0(0.0)	0(0.0)	2(28.6)	1(1.0)	2(11.8)
Tetracycline (TE) 30 µg	2(66.7)	0(0.0)	5(71.4)	72(74.2)	11(64.7)
Ampicillin (AMP) 10 µg	3(100)	0(0)	5(71.4)	60(61.9)	9(52.9)
Naldixic Acid (NA) 30µg	3(100)	0(0)	6(85.7)	69(71.1)	8(47.1)
Cefoxitin (FOX) 30µg	2(66.7)	0(0)	6(85.7)	64(66)	7(41.2)
Ciprofloxacin (CIP) 5µg	0(0)	2(100)	2(28.6)	7(7.2)	4(23.5)
Streptomycine (S) 10µg	2(66.7)	1(50)	5(71.4)	57(58.8)	9(52.9)
Kanamycin (KAN)30µg	3(100)	2(100)	6(85.7)	74(76.3)	10(58.8)
(SXT)* 25µg	3(100)	1(50)	6(85.7)	70(72.2)	12(70.6)
Chloranphenicol (C) 30µg	1(33.3)	0(0)	6(85.7)	57(58.8)	10(58.8)

* Sulphamethoxazole- Trimethoprim, PF= poultry feed, PHS= personnel hand swab, PHE= poultry house environment.

4.4.3. Double antimicrobial resistances of the isolated Salmonella

Out of 126 *Salmonella* isolates tested for antimicrobial resistance pattern, 123 had drug resistance trait. In this regard, 95 isolates for Kenamycin, 92 isolates for Sulphamethoxazole- Trimethoprim, 90 isolates for Tetracycline, 15 isolates for Ciprofloxacin and 5 isolates for Gentamycin (Table 14, diagonal line). Of 95 Kenamycin resistant isolates, 79 of them were resistant to Sulphamethoxazole- Trimethoprim while of 15 isolates were Ciprofloxacin resistant, only 4 isolates were resistant to Gentamycin (Table 14, below diagonal line).

Table 14. *Salmonella* isolates (n= 126) drug resistance pattern as assessed for single (green diagonal), double drug resistance (below diagonal) and the unshared isolate number in the double resistance (above diagonal).

	CN	TE	AMP	NA	FOX	CIP	S	K	SXT	C
CN	5	4(89)*	4(76)	3(84)	3(77)	1(11)	4(73)	1(91)	1(88)	3(72)
TE	1	90	22(9)	20(14)	73(62)	90(15)	25(9)	18(23)	24(26)	88(72)
AMP	1	68	77	5(14)	11(13)	77(15)	16(13)	6(24)	4(19)	20(17)
NA	2	70	72	86	11(4)	82(11)	19(7)	7(16)	11(17)	29(17)
FOX	2	17	66	75	79	74(10)	18(13)	5(21)	13(26)	20(15)
CIP	4	0	0	4	5	15	12(71)	5(85)	2(79)	7(66)
S	1	65	61	67	61	3	74	5(26)	11(29)	17(17)
K	4	72	71	79	74	10	69	95	16(13)	29(8)
SXT	4	66	73	75	66	13	63	79	92	25(7)
C	2	60	57	63	59	8	57	66	67	74

*The isolate number in the outside of the brackets represented the drugs listed in the column/vertical whilst the number in the brackets represented the drugs listed in the row/horizontal.

CN = Gentamycin; TE = tetracycline; AMP = Ampicilline; NA = Naldixic acid; FOX = cefoxitin; CIP = ciprofloxacine; S = streptomycin; K = kanamycin; SXT = sulphamethoxazole-trimethoprime; C = chloramphenicole

4.4.4. Multidrug resistant Salmonella isolates by different sources

Out of 126 isolates, 109 (86.5%) had multi- drug resistance indicating one isolates was resistant to two or more drugs. In this regard, 7 isolates to 2 drugs, 9 isolates to 3 drugs, 7isolates to 4 drugs, 16 isolates to 5 drugs, 10 isolates to 6 drugs, 16 isolates to 7 drugs and 44 isolates to 8 drugs have developed resistance. Those *Salmonella* isolates recovered from poultry sources showed the highest percentage of multi- drug resistance (85 isolates, 78%) involving two to eight resistant antibiotic pattern. Isolates collected from poultry environment, also had muti-drug resistance traits (16 isolates, 14.7%) as shown in Table 15.

Table 15. Melti drug resistant *Salmonella* isolates and their pattern of resistance by different sources in central and south Ethiopia

Nr. of Drug	Drug R pattern (Nr. of isolate)	Source
Two	TEK(2)	Layer
	TENA (1)	
	KNA (1)	
	KC (1)	
	CIPSXT (1)	
	CIPSXT (1)	PHE*
Three	TESXTC (1)	Layer
	AMPKSXT (1)	
	AMPSK (1)	
	TEAMPC (1)	
	KSXTC (1)	
	NAKSXT (1)	PF***
	CIPSK (1)	
	CIPSXTS (1)	FA**
	CIPSXTC (1)	
Four	AMPNAFOXSXT (1)	Layer
	TEAMPKSXT (1)	
	TEFOXSK (1)	
	NASKC (1)	
	CIPSKSXT (1)	
	NAFOXSXTC (1)	Broiler
	CIPSCCN (1)	FA**

Nr. of Drug	Drug R pattern (Nr. of isolate)	Source
Five	AMPNAFOXKSXT (1)	Layer
	FOXCIPKSXTC (1)	
	TEAMPNAFOXSXT (1)	
	AMPNAFOXKSXT (1)	
	TENAFOXSK (1)	
	TEFOXSKC (1)	
	TEAMPNAKSXT (1)	
	TENAFOXKSXT (1)	
	TENASKC (1)	
	TENAFOXSK (1)	
	TEAMPNASC (1)	
	FOXCIPKSXTC (1)	
	CN NAFOXCIPK (1)	
	CNCIPKSXTC (1)	PHE*
	TEAMPNASK (1)	
	AMPNAFOXKSXT (1)	Water
Six	TEAMPNAFOXSSXT (1)	Layer
	TEAMPNAFOXKSXT(2)	
	AMPNAFOXSKSXT(2)	
	NAFOXSKSXTC (1)	
	NAFOXSKSXTC (1)	
	NAFOXCIPKSXTC (1)	
	CNNAFOXCIPKSXT (1)	FA**
	CNTEAMPSKSXT (1)	PHE*

Nr.of Drug	Drug R pattern (Nr. of isolate)	Source
Seven	AMPNAFOXSKSXTC (1)	Layer
	TENACIPSKSXTC (1)	
	TEAMPNASKSXTC (1)	
	TEAMPNAFOXSSXTC (1)	
	TEAMPNAFOXKSXTC (5)	
	TEAMPNAFOXSKSXT (3)	
	TEAMPNASKSXTC (1)	Broiler
	TEAMPNASKSXTC (2)	Water
	TEAMPNAFOXSKC (1)	FA**
Eight	TEAMPNAFOXSKSXTC (34)	Layer
	TEAMPNAFOXSKSXTC (7)	PHE*
	TEAMPNAFOXSKSXTC (3)	FA**

Source	MDR (%)
FA**	8 (7.3%)
PHE*	11(10 %)
Layer	83 (76%)
Broiler	2 (1.8%)
Water	3 (2.8 %)
Feed	2 (1.8%)
Total	109(100%)

PHE* = poultry house environment; FA**= farm attendant personnel; ***poultry feed

4.4.5. Univariate and distribution analysis drug resistant isolates by risk factors

Significant association was observed between locations, production type, flock size and housing system against drug resistance development. The cause of resistance development for different drugs was different risk factors indicating specific drug has its own specific risk factor for resistance development (Table 16).

Table 16. Univariate Risk factors analysis of *Salmonella* drug resistances

Risk factor	Risk factor	Resistant drug	OR	95% CI	P-value
Location	Central vs. South Eth.	Tetracycline	.030	.004, .227	.001
		Ampicillne	.016	.002, .119	.000
		Naldixic acid	.024	.003, .186	.000
		Cefoxitin	.017	.002, .132	.000
		Streptomycin	.013	.002, .102	.000
		Chloranphenicol	.047	.013, .163	.000
Production type	Broiler vs. Layer	Ampicillne	.286	.106, .767	.013
		Naldixic acid	.345	.130, .917	.033
		Cefoxitin	.290	.109, .771	.013
		SXT*	.198	.072, .545	.002
Flock size	Large vs. Medium	Kanamycin	3.143	1.190, 8.303	.021
		Streptomycin	5.655	2.093, 15.278	.001
		SXT	2.658	1.023, 6.907	.045
		Chloranphenicol	6.416	2.361, 17.435	.000
Housing system	Cage vs. Litter	Streptomycin	14.318	1.810, 113.234	.012
		Chloranphenicol	3.768	1.002, 14.176	.050

*sulphamethoxazole-trimethoprime

The distribution of resistant, intermediate and susceptible *Salmonella* isolates was indicated across the different potential risk factors in Table 17 below for some selected drugs. The same distribution analysis was presented in Annex 5, 6, 7 for the remaining drugs.

Table 17. Distribution of resistant and susceptible *Salmonella* isolates (n = 126) across the different risk factors for selected tested antibiotics.

Risk factors		N	Streptomycin				Kanamycin			
			S	IM	R	%R	S	IM	R	%R
Study location	Central Ethiopia	81	25	26	30	37	27	4	50	61.7
	South Ethiopia	45	0	1	44	98	0	0	45	100
Study area	Addis Ababa	23	5	5	13	57	6	0	17	73.9
	Adama	15	5	8	2	13	6	0	9	60
	Bishoftu	12	2	2	8	67	4	0	8	66.7
	Bonga	27	0	1	26	96	0	0	27	100
	Hawassa	18	0	0	18	100	0	0	18	100
	Modjo	31	13	11	7	23	11	4	16	51.6
Category	Abattoir	3	0	1	2	67	1	0	2	66.7
	Farm	123	25	26	72	59	26	4	93	75.6
Housing system	Cage	16	0	1	15	94	1	0	15	93.8
	Litter	86	20	22	44	51	21	3	62	72.1
Flock size	large size	74	11	13	50	68	14	0	60	81.1
	medium size	26	9	10	7	27	8	3	15	57.7
Breed	Bovans	33	6	10	17	52	7	2	24	72.7
	Fayomine	3	1	1	1	33	0	0	3	100
	ISA Brown	13	5	4	4	31	5	1	7	53.8
	White leghorn	51	8	8	35	69	10	0	41	80.4
Poultry type	Broiler	22	7	5	10	46	9	0	13	59.1
	Layer	78	13	18	47	60	13	3	62	79.5
Sample source	Water	3	0	1	2	67	0	0	3	100
	Feed	2	1	0	1	50	0	0	2	100
	Personnel	7	0	2	5	71	1	0	6	85.7
	Poultry	97	17	23	57	59	20	3	74	76.3
	Poultry house	17	7	1	9	53	6	1	10	58.8
Sample type	Carcass swab	3	0	1	2	67	1	0	2	66.7
	Cloacal swab	80	12	15	53	66	15	1	64	80
	Feed	2	1	0	1	50	0	0	2	100
	Fresh feces	18	8	7	3	17	6	2	10	55.6
	Litter	7	4	1	2	29	4	1	2	28.6
	Hand swab	7	0	2	5	71	1	0	6	85.7
	Pooled feces	6	0	0	6	100	0	0	6	100
	Water	3	0	1	2	67	0	0	3	100

S=susceptible; IM= intermediate; R= resistant; %R= percent of resistant isolate

5. DISCUSSION

5.1. Prevalence and Distribution of *Salmonella*

The overall prevalence of *Salmonella* in the present study was 11.9% (126/1059) which is lower than previous studies in Ethiopia: 15.4% in four intensive poultry farms of Addis Ababa (Bekele and Ashenafi, 2010) and 42.7% in indoor chicken flocks of Jimma town (Kindu and Addis, 2013). Higher prevalence of *Salmonella* was also reported from different countries of Africa and elsewhere that showed great difference with the present result in poultry farms. Among these, from Netherlands 94% in broiler farms and 47% in layer farms (Edel, 1994); from United States (southern US), 100% in layer farms and 86% from several regions Of US (Ebel *et al.* (1992); from Italy 17.9% in layer farm (Adeline *et al.*, 2009); from Japan 36.1% in broiler (Ishihara *et al.*, 2009).

The higher prevalence which was reported by different studies from different country could be due to the variation in the prevalence of *Salmonella* contamination, differences in sample type, locations, breed, sampling techniques and the detection methods employed (Dominguez *et al.*, 1994; Bryan and Doyle, 1995; Rusul *et al.*, 1996; Uyttendaele *et al.*, 1998). Additionally, the higher prevalence of *Salmonella* infection in layer might be attributed with the reality that they are physiologically stressed during egg production and molting which significantly depress the immune response and increase the susceptibility to *Salmonella* infection (Landers *et al.*, 2005). In this study, layers were also highly infected. Thus, in such countries intensification of large number of chickens for production might cause physiological stress.

The present study result was slightly higher than the 8% report from smallscale chicken flock farms of Hawassa detected using direct swab plating technique (Kassaye *et al.*, 2010). The higher prevalence reported in this study may be due to the different sample types used. Moreover, prior to subjected in selective enrichment media for isolation, use of pre enrichment media is recommended by ISO-6579, 2002, which can allow the small number of *Salmonella* in samples to multiply, to resuscitate that have been sub-lethally

damaged by freezing, heating or desiccation. Therefore, direct plating samples to selective enrichment media can reduce the prevalence by reducing positive samples. The difference of prevalence between South (16.7%) and Central Ethiopia (10.1%) in this study may be due to poor biosecurity to the farm which can increase the level of *Salmonella* contamination as observed during sampling.

Areal distribution of *Salmonella* in the present finding indicated that, highest prevalence was observed in Bonga which was 27% followed by Modjo (11.5%) and the least prevalence was in Bishoftu (6.5%). The differences among the study areas might be due to the fact that, the poor management experienced by the owners along with poor biosecurity exposes birds to numerous potential sources of *Salmonella* contamination on chicken flocks which were kept indoor (Liljebjelke *et al.,* 2005) and the risk of being contaminated by *Salmonella* was higher in farms with multiage management of layers than all-in/all out practice (Adeline *et al.,* 2009). Thus poultry farms those observed in South Ethiopia was not practicing all in all out strategy rather placement of new layers from pullet grower houses when they are old enough to lay eggs to those layers were observed and which will cause cross contamination of flocks with *Salmonella.*

The prevalence of *Salmonella* was significantly higher in chickens kept in litter (11.2%) than kept in cage system (8.6%). This result was controversial with the study by Adeline et al. (2009) in French who stated that, the apparent prevalence was significantly higher in caged flocks than in on-floor flocks (30.9% in caged flocks and 7.9% in on-floor flocks). The same author suggested that, the higher prevalence in cage housing might be related to the higher sensitivity of pooled feces samples taken in cage poultry houses than that of boot swabs taken from on on-floor flocks. Therefore, the higher prevalence might be due to the sensitivity of samples to isolate *Salmonella*. This statement was confirmed by Aho *et al.* (1992) that pooled samples were more effective to predict contamination than individual samples (Eriksson *et al.,* 2001). Another author stated that, fecal samples are more sensitive indicators of *Salmonella* contamination (Mallinson *et al.,* 2000).

The rate of *Salmonella* isolation have significant differences among different sources observed in poultry, poultry personnel and poultry environment. Although *Salmonella* was detected in all types of sources in the present study, there was a substantial difference in prevalence. The highest number of *Salmonella* isolates was recovered from poultry environment sources (24.6%). This finding was lower than the study by Yhiler and Bassey (2015) in Nigeria who reported from poultry environmental sources was 58.8% and Irwin et al. (1994) 87% of turkey flocks in Canada. The difference may be due to environmental persistence of *Salmonella* is a significant factor in the epidemiology in poultry by creating opportunities for horizontal transmission of infection within and between flocks (McIlroy *et al.,*1989) and *Salmonella* can be introduced into poultry flocks from many different sources.

There were a lot of possible risk factors regarding the source of *Salmonella* contamination in poultry flocks that cockroaches and lesser mealworms could carry *Salmonella* internally and externally and spread them throughout the poultry house (McAllister *et al.,* 1994; Kopanic *et al.,* 1994). Mice have been also important vectors for *S.* Enteritidis in laying hens (Henzler and Opitz, 1992).

Significantly higher isolates were detected in south Ethiopia than central Ethiopia (OR = 1.75) particularly higher in Bonga than Modjo (OR = 2.08). In the farms of south area, poultry production and multiplication centers rear varying age groups. The higher prevalence in the south could be explained by management practice of mixed age group rearing in south area whilst single age rearing is common in central Ethiopia in private commercial farms. Single age rearing practice has reportedly prevented several infectious diseases in poultry. Furthermore, the risk of *Salmonella* contamination in flock reared on litter was higher with multiage management than on farms with an all-in/all out practice or farms with a single flock Davies and Breslin (2004). The susceptibility of hens to *Salmonella* infection varies in relation to the age of the birds.

5.2. Antimicrobial Resistance of *Salmonella*

In the present study, antibiotic resistance was observed for kanamycin (75.4%), Sulphamethoxazole-Trimethoprim (73%), Tetracycline (71.4%), Naldixic Acid (68.3%), Cefoxitin (62.7%), Ampicillin (61.1%), Chloranphenicol and Streptomycine (58.7%), Ciprofloxacin (11.9%) and Gentamicin (4%).

The present finding was higher than previous reports on *Salmonella* drug resistance which were isolated from food animals, food products of animal origin and personnel. Such as 41.2% to tetracycline, 30% to chloramphenicol and 22.5% to streptomycin (Molla *et al.*, 2003a), 75% to streptomycin, 46.9% to tetracycline (Zewdu and Cornelius, 2009), chloramphenicol 45.5% and 100% to Tetracycline, , 79.5% to kanamycin, 18.2% to Gentamicine Naldixic acid 56.8% (Ferede, 2014). The high level of *Salmonella* resistance to antibiotic may be due to the fact that, in many of the sub-Saharan African countries antimicrobials can be purchased without any prescription and indiscriminate use of antimicrobial agents by unskilled practitioners both in the veterinary and public health sectors is common (Okeke *et al.,* 2005). And there is lack of quality compliance and monitoring of drugs at all levels. Furthermore, this high level of resistance could be due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products (Molla *et al*., 2003a; Molla *et al*., 2006 and Zewdu and Cornelius, 2009).

Gentamycin (4.0%) and Ciprofloxacin (11.9%) have demonstrated lower resistance in this study implying both drugs are relatively the most effective against *Salmonella* in the poultry production. The effectiveness of such drugs like ciprofloxacin could be because of that they are not widely used in countries like Ethiopia and other African countries (Zelalem *et al*., 2011). In addition to this, effectiveness of this drug could be because of this drug is not well distributed to all societies and not simply prescribed rather than it is used as drug of choice in antibiotic resistant person was considered to be the reason. The

11.9% resistance for Ciprofloxacin in this study disagrees with previous report of 100% susceptibility in raw meat of goat in Diredawa (Ferede, 2014), sheep and goat meat in central Ethiopia (Molla *et al*., 2006), from human isolates in Nigeria (Akinyemi *et al*., 2011) and isolates of *Salmonella* from dairy farms in Addis Ababa (Zelalem *et al*., 2011). The variation of their result from the current finding could be explained by difference in host population studied and time of the study as the drug circulates in the market, the likelihood of resistance development might increase.

The present study revealed that, 109 (86.5%) were multi drug resistant isolates with different resistant pattern. Explicitly, 7 isolates to 2 drugs, 9 isolates to 3 drugs, 7 isolates to 4 drugs, 16 isolates to 5 drugs, 10 isolates to 6 drugs, 16 isolates to 7 drugs and 44 isolates to 8 drugs have developed resistance.

The level of multidrug resistant result in the current study was lower than the 97.7% (Ferede, 2014), higher than the 32.65% from food items and personnel (Zewdu and Cornelius, 2009); 44.8% (Molla *et al*., 2004) and 83.3% (Zelalem *et al*., 2011); as they isolated from food of animal sources, animals and humans. Molla et al. (2003b) reported that from chicken carcass and giblet 52.5% displayed multiple-drug resistance. This reveals a wide range of antibiotics which *Salmonella* become resistant against can be attributed to the gross indiscriminate use of antimicrobial agents in the poultry production setting (Zha *et al.*, 2003; Angulo *et al.*, 2004). Furthermore, the incorporation of antibiotics in the diet of animals at sub therapeutic concentrations as prophylaxes and as growth promoters has invariably contributed to the development of antibiotic resistant strains of *Salmonella* (Shah and Korejo, 2012). In this study, the pattern of resistant to eight antibiotics out of ten indicate the potential importance of chicken as a source of single and multiple antimicrobial resistant *Salmonella* infection and a serious public health concern.

5.3. Risk Factors Associated with *Salmonella* Drug Resistance

Salmonella drug resistance development and distribution in south Ethiopia were higher than central Ethiopia. It was higher there for Tetracycline (OR = .030); Ampicillne (OR =

.016); Naldixic Acid (OR = .024); Cefoxitin (OR = .017); Streptomycine (OR = .013) and Chloranphenicol (OR = .047).

The unregulated usage of the drugs in livestock and poultry in the area could lead to the release of antibiotic residues into the environment. This subsequently causes *Salmonella* isolates to pick the resistance gene from other bacteria species or from the environment. The excessive misuse of drugs subjects to selective pressure for resistance traits to develop against these drugs (Levy, 1997). *Salmonella* may also develop resistance through conjugation (i.e. the transfer of genetic material between living bacteria), transformation (i.e., obtaining genetic material from the environment), or transduction (i.e., the transfer of genetic material between bacteria via a bacteriophage (Courvalin, 1996).

Drug resistant S*almonella* isolates were lower in broiler types than layer chickens against Ampicillne, Naldixic Acid, Cefoxitin and Sulphamethoxazole- Trimethoprim. This could be related to short lifespan of broilers in the farm. Significantly higher drug resistance was observed in larger poultry flocks (greater than 500 chickens in a house) against Kanamycin, Streptomycine, Sulphamethoxazole- Trimethoprim and Chloranphenicol.

6. CONCLUSION AND RECOMMENDATION

Of 1059 samples tested, 126 (11.9%) are positive for *Salmonella*. *Salmonella* was isolated from a wide spectrum of samples, namely poultry (feaces, swab), environment (litter), feed, water, carcass and humans indicating its ubiquitous distribution. Its distribution is associated with location, specific area and individual farms within broad area, sample source and breed indicating these factors are important risk factors. Of 126 isolates, 123 (97.8%) have developed resistance to all types of the 10 tested antibiotics although gentamcycin and Ciprofloxacin demonstrated relatively a better efficacy. Alarmingly, multidrug resistance (86.5%) is very common. In this line, 7 isolates to 2 drugs, 9 isolates to 3 drugs, 7 isolates to 4 drugs, 16 isolates to 5 drugs, 10 isolates to 6 drugs, 16 isolates to 7 drugs and 44 isolates to 8 drugs have developed resistance. Each specific drug in this study has its own specific risk factor for resistance development. In general, however, location, production type, flock size and housing system were important risk factors for drug resistance development. Based on the above conclusions, the following recommendations are forwarded.

- ✓ Regular epidemiological surveillance should be undertaken in monitoring the occurrence and distribution of *Salmonella* with periodical antibiotic sensitivity check up.

- ✓ Training of workers in poultry farms as well as slaughter houses regarding sanitation, use of clean drinking water, promotion of safe food handling practices, awareness for zoonotic pathogens and precautions is mandatory.

- ✓ Restrictions on the irrational use of antibiotics and establishment of standardized monitoring systems in poultry farms are required.

- ✓ *Salmonella* serotyping and molecular genotyping should be performed starting from farm.

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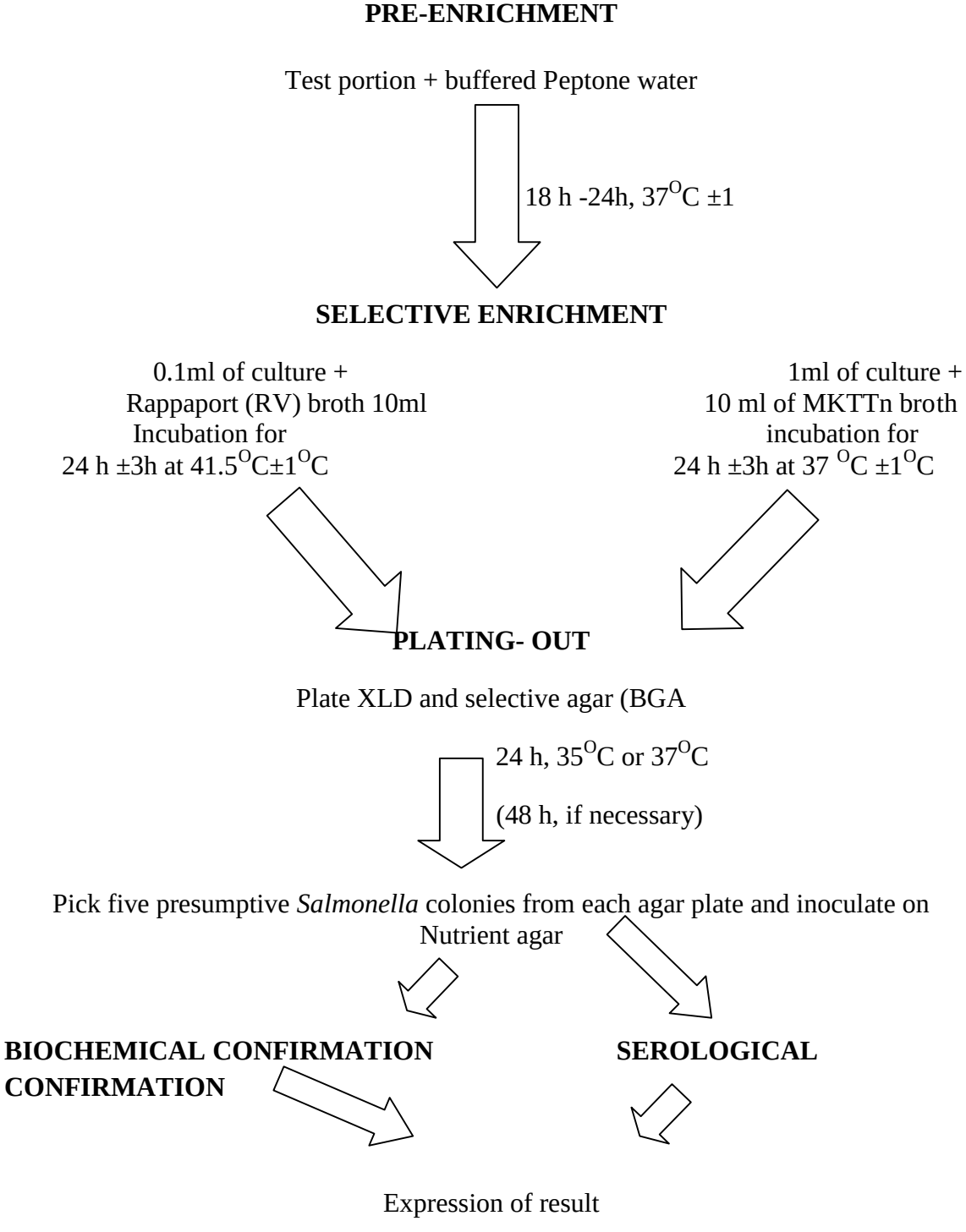
8. ANNEXS

Annex 1. Flow diagram showing ISO method for detection of *Salmonella*



Sources: ISO-6579, 2002

Annex 2. Composition and preparation of culture media and reagents

A) Buffered peptone water

Composition (g/Litre):

Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$)	9.0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.5 g
Water	1 000 ml

Preparation: Add 15 gram of the components in the 1000 ml of distilled water, Mix well and distribute into universal bottle of suitable capacity to obtain the portions necessary for the test. Sterilize by autoclaving for 15 min in the autoclave set at 121 °C.

B) Rappaport -Vassiliadis (RV) enrichment broth of 500 g (Oxoid, England)

Composition (g/Litre):

- Soya peptone …….5.0 g
- Sodium chloride……..8.0 g
- Potassium dihydrogen phosphate …..1.6 g
- Magnesium chloride …….40.0 g
- Malachite green…….0.04 g

Preparation: Weigh 30 g (the equivalent weight of dehydrated medium per Litre) and add to 1 Litre of distilled water. Heat gently until completely dissolved. Dispense 10 ml volumes into screw capped bottles or tubes and sterilize by autoclaving at $115^{O}C$ for 15minutes.

C) Muller-Kauffman Tetrathionate (Novobiocine enrichment broth) (Oxoid Ltd., Basingstoke Hampshire , England)

Composition (g/Litre):

Tryptone 7.0; Soya peptone 2.3; Sodium Chloride 2.3; calcium carbonate 25.0; Sodium thiosulphate 40.7 and ox bile 4.75

Preparation: Suspend 89.5g in one litre of demineralized water, heat briefly to boiling. Do not autoclave! After cooling, add 20ml iodine potassium-iodide solution. Dispense evenly any precipitate. Potassium iodine solution: (5g KI, 4g I, PH 8.0 ±0.2 at 25^{O}c).

D) Xylose lysine deoxycholate agar (XLD agar) 500 g (Sifin, Berlin,Germany)

Composition (g/Litre):

Yeast extract…….. 3.0

- L-Lysine hydrochloride……..5.0
- Xylose…….3.75
- Lactose……....7.5
- Sucrose……7.5
- Sodium deoxycholate……..1.0
- Sodium chloride……..5.0
- Sodium thiosulphate……6.8
- Iron (III) ammonium citrate……0.8
- Phenol red……...0.08
- Agar……..16.5

Preparation: Suspend 56.68gm in 1000 (1 Litre) of distilled water by heating, with frequent agitation, until the medium starts to boil. Avoid overheating. Adjust the pH, if necessary, so that after sterilization it is 7.4 at 25 °C. Heat with frequent agitation until the medium boils and the agar dissolves. Do not overheat. Transfer immediately to a water bath at 50 °C, agitate and pour into plates. Allow to solidify. Immediately before use, dry the agar plates carefully (preferably with the lids off and the agar surface downwards) in the oven set between 37 °C and 55 °C until the surface of the agar is dry. It is advisable not to prepare large volumes which will require prolonged heating.

E) BRILLIANT GREEN AGAR

Preparation: Suspend 29.0 g of the medium in one 500ml of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 ibs pressure (121°C) for 15 minutes. Avoid overheating

F) Nutrient agar

Composition (g/Litre):

- Lab-Lemco powder……..1.0
- Yeast extract ……2.0
- Peptone…….5.0
- Sodium chloride……....5.0
- Agar……15.0

pH: 7.4 ± 0.2

Preparation: Dissolve 28g of the components or the dehydrated complete medium in 1000ml of distilled water, by heating if necessary. Sterilize for 15 min in the autoclave set at 121 °C. Transfer about 15 ml of the melted medium to sterile small Petri dishes and proceed.

G) Triple sugar/iron agar (TSI agar)

Composition (g/Litre):

'Lab-Lenco' powder 3.0; yeast extract 3.0; peptone 20.0; sodium chloride 5.0; lactose 10.0; sucrose 10.0; glucose 1.0; ferric citrate 0.3; sodium thiosulfate 0.3;Phenol red 9.5; agar 12.0. pH=7.4± 0.2 at 25^0C

Preparation: Suspend 65 grams in one Litre of distilled water and bring to the boil to dissolve completely. Sterilize in the autoclave set at 121 °C for 15 minutes. Dispense the medium into test tubes or dishes in quantities of 10 ml Allow to set in a slopped form to give a butt of depth 2.5 cm to about 5 cm

H) L-Lysine Decarboxylation Broth of 500 g (Difco, Detroit, USA).

Composition (g/Litre):

- Bacto peptone……5 g
- Bacto yeast extract……3 g
- Bacto dextrose……...1 g
- L-lysine…….5 g
- Bacto Brom Cresol Purple… ……...0.02 g

Preparation: Suspend 14 grams in 1 litre distilled water or deionized water and boil to dissolve completely. Sterilize at 121 – 124 ^{O}C for 15 minutes. Final pH: 6.8 ± 0.2 at 25^{O}C.

I) Tryptophan Broth for Indole test

Composition (g/Litre):

Casein enzymic hydrolysate	10.0
Sodium chloride	5.0
DL- Tryptophan	1.0

Preparation: Dissolve 30gm of the components of tryptone broth in one litre distilled water. Dispense 3 to 5 ml of the medium into each of tubes. Sterilize for 15 min in the autoclave set at 121 °C.

J) 0.5 McFarland standard

Composition: 1.17% $BaCl.2H_2O$ solution and 0.36N of 1% sulfuric acid (H).

Preparation: Add approximately 85Ml of 1% H_2SO_4 to a 100ml of volumetric flask, using a 0.5ml pipette add 0.5ml of 1.17% $BaCl.2H_2O$ dropwise to the H_2SO_4 while constantly swirling the flask. Bring to 100ml with 1% H_2SO_4 place a magnetic stirring in the flask and place on the magnetic stirrer for approximately three to five minutes. Examine solution visually to make certain it appears homogeneous and free of visible

clumps. Dispense three to seven ml, cub tube tightly and seal with paraffin and keep at dark and room temperature.

K) Muller – hinton agar (Oxoid, England

-Beef, dehydrated infusion from 300.0
-Casein hydrolysate 17.5
-Starch 1.5
-Agar 17.0
-pH 7.3 ± 0.1 at 25°C

Preparation: Add 38g to one litre of distilled water. Bring to the boil to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes.

L) Urea broth

Composition (g/l):

- Yeast extract ……0.1
- Potassium dihydrogen phosphate…….9.1
- Disodium hydrogen phosphate…….9.5
- Urea…….20.0
- Phenol red ……..…0.01

Preparation: 38.5 g/l was dissolved in Sterilize by filtration or dispense aliquots of approximately 3 ml into test tubes and sterilize for 5 minutes in a current of steam under mild conditions. Don‟t autoclaved. pH = 6.8.±0.1. The broth was clear and yellow-orange.

Annex 3. Univariate risk factor analysis for occurrence of *salmonella* in poultry farms, abattoir, and environment in central and southern Ethiopia

	B	S.E.	Wald	Df	OR	95% CI.OR	Sig.
Study Location							
Central Ethiopia					1		Ref
South / Central Ethiopia	-.559	.201	7.718	1	.572	.386, .848	.005
Abattoir							
Abattoir					1		Ref
farm / abattoir	-1.054	.599	3.092	1	.349	.108,1.128	.079
Study area							
Addis Ababa (AA)					1		Ref
Adama /AA	-.316	.295	1.143	1	.729	.409,1.301	.285
Bishoftu / AA	-.782	.332	5.551	1	.458	.239, .877	.018
Bonga / AA	-.943	.357	7.000	1	.389	.194, .783	.008
Hawassa / AA	.730	.298	6.012	1	2.076	1.158, 3.722	.014
Modjo / AA	-.408	.316	1.666	1	.665	.357, 1.236	.197
Housing system							
Cage					1		Ref
litter / cage	-.293	.285	1.050	1	.746	.427, 1.306	.305
Sample source							
Water					1		Ref
feed /water	-.268	.703	.145	1	.765	.193, 3.035	.703
Fa* / water	-.828	.806	1.055	1	.437	.090, 2.121	.304
poultry / water	-.268	.507	.280	1	.765	.283, 2.064	.596
poultry house / water	-1.025	.299	11.731	1	.359	.200, .645	.001
Sample type							
Carcass swab (Crs)					1		Ref
Cloacal swab / Crs	-1.626	.875	3.450	1	.197	.035, 1.094	.063
feed / Crs	-.768	.656	1.371	1	.464	.128, 1.678	.242
Fresh feces / Crs	-.560	.994	.317	1	.571	.081, 4.009	.573
Litter / Crs	-.245	.695	.124	1	.783	.200, 3.056	.724
Hand swab / Crs	.154	.775	.040	1	1.167	.255, 5.333	.842
Equipment swab / Crs	.000	.772	.000	1	1.000	.220, 4.536	1.000
Pooled feces / Crs	-19.817	40192.970	.000	1	.000	0.000	1.000
Water / Crs	.780	.821	.903	1	2.182	.436, 10.908	.342
Flock size							
Large size					1		Ref
Medium/ large size	-.206	.242	.722	1	.814	.506, 1.309	.395
Poultry type							
Broiler					1		Ref
Layer/ Broiler	.005	.255	.000	1	1.005	.610, 1.658	.983
Breed							
BB					1		Ref
fa / BB	-.575	.235	5.984	1	.563	.355, .892	.014
ISA B / BB	-.793	.615	1.665	1	.452	.136, 1.509	.197
WLH / BB	.385	.341	1.278	1	1.470	.754, 2.866	.258

Annex 4. Univariate risk factor analysis of poultry farm for occurrence of *salmonella* in poultry farms in central and southern Ethiopia

		B	S.E.	Wald	Df	OR	95% CI.OR	Sig.
Farm level	Addis Ababa1 (AA1)					1		Ref
	Addis Ababa 2 / AA1	-.610	.830	.540	1	.543	.107, 2.765	.462
	Addis Ababa 3 /AA1	-.690	.640	1.164	1	.501	.143, 1.757	.281
	Addis Ababa 4 / AA1	-19.616	6996.698	.000	1	.000	0.000	.998
	Addis Ababa 5 / AA1	-1.549	1.085	2.037	1	.213	.025, 1.783	.154
	Addis Ababa 6 / AA1	.730	.485	2.259	1	2.074	.801, 5.370	.133
	Adama1 / AA1	-1.154	.816	1.999	1	.315	.064, 1.562	.157
	Adama2 / AA1	-.780	.474	2.705	1	.458	.181, 1.161	.100
	Bishoftu1 / AA1	-1.340	.696	3.701	1	.262	.067, 1.026	.054
	Bishoftu2 / AA1	-1.968	.805	5.978	1	.140	.029, .677	.014
	Bishoftu3 / AA1	-.323	.649	.247	1	.724	.203, 2.583	.619
	Bonga1 / AA1	-.952	.560	2.889	1	.386	.129, 1.157	.089
	Hawassa1 / AA1	.592	.430	1.901	1	1.808	.779, 4.197	.168
	Hawassa2 / AA1	-.886	.537	2.718	1	.412	.144, 1.182	.099
	Modjo1 / AA1	-.249	.489	.260	1	.779	.299, 2.033	.610
	Modjo2 / AA1	-.541	.598	.819	1	.582	.180, 1.879	.365
	Modjo3 / AA1	-.338	.545	.385	1	.713	.245, 2.077	.535
	Modjo4 / AA1	.201	.509	.156	1	1.222	.451, 3.313	.693

Annex 5. Distribution of resistant and susceptible isolates (n = 126) across the different risk factors for the 10 drugs tested.

			FOX				CIP			
		N	S	IM	R	%R	S	IM	R	%R
Study location	Central Ethiopia	81	41	5	35	43.2	52	14	15	18.5
	South Ethiopia	45	1	0	44	97.8	45	0	0	0
study area	Addis Ababa	23	12	5	6	26.1	18	4	1	4.3
	Adama	15	10	0	5	33.3	1	0	14	93.3
	Bishoftu	12	6	0	6	50	12	0	0	0
	Bonga	27	1	0	26	96.3	27	0	0	0
	Hawassa	18	0	0	18	100	18	0	0	0
	Modjo	31	13	0	18	58.1	21	10	0	0
Category	Abattoir	3	1	0	2	66.7	3	0	0	0
	Farm	123	41	5	77	62.6	94	14	15	12.2
Housing system	Cage	16	5	0	11	68.8	13	2	1	6.3
	Litter	86	28	5	53	61.6	67	9	10	11.6
Flock size	large size	74	23	3	48	64.9	61	3	10	13.5
	medium size	26	9	1	16	61.5	18	8	0	0
Breed	Bovans	33	13	1	19	57.6	0	0	3	100
	Fayomine	3	2	0	1	33.3	11	2	0	0
	ISA Brown	13	5	0	8	61.5	48	3	0	0
	White leghorn	51	12	3	36	70.6	21	1	0	0
Poultry type	Broiler	22	10	3	9	40.9	58	10	10	12.8
	Layer	78	22	1	55	70.5	18	3	5	6.4
Sample source	Water	3	1	0	2	66.7	2	1	0	0
	Feed	2	2	0	0	0	0	0	2	100
	Personnel	7	1	0	6	85.7	5	0	2	28.6
	Poultry	97	29	4	64	66	79	11	7	7.2
	Poultry house	17	9	1	7	41.2	11	2	4	23.5
Sample type	Carcass swab	3	1	0	2	66.7	3	0	0	0
	Cloacal swab	80	22	4	54	67.5	66	7	7	8.8
	Feed	2	2	0	0	0	0	0	2	100
	Fresh feces	18	9	0	9	50	11	4	3	16.7
	Litter	7	6	1	0	0	4	2	1	14.3
	Hand swab	7	1	0	6	85.7	5	0	2	28.6
	Pooled feces	6	0	0	6	100	6	0	0	0
	Water	3	1	0	2	66.7	2	1	0	0

Annex 6. Distribution of resistant and susceptible isolates (n = 126) across the different risk factors for the 10 drugs tested.

Risk factor		N	Sulphamethoxazole-trimethoprim				Chloramphenicole			
			S	IM	R	%R	S	IM	R	%R
Studylocation	Central Ethiopia	81	28	6	47	58	30	19	32	39.5
	South Ethiopia	45	0	0	45	100	3	0	42	93.3
study area	Addis Ababa	23	11	3	9	39.1	12	4	7	30.4
	Adama	15	0	2	13	86.7	5	3	7	46.7
	Bishoftu	12	3	1	8	66.7	0	0	12	100
	Bonga	27	0	0	27	100	2	0	25	92.6
	Hawassa	18	0	0	18	100	1	0	17	94.4
	Modjo	31	14	0	17	54.8	13	12	6	19.4
category	abatoir	3	0	0	3	100	0	0	3	100
	Farm	123	28	6	89	72.4	33	19	71	57.7
Housing system	Cage	16	2	1	13	81.3	3	0	13	81.3
	Litter	86	22	4	60	69.8	26	14	46	53.5
Flock size	large size	74	12	4	58	78.4	18	4	52	70.3
	medium size	26	11	0	15	57.7	9	10	7	26.9
Breed	Bovans	33	6	1	26	78.8	10	4	19	57.6
	Fayomi	3	0	1	2	66.7	1	1	1	33.3
	ISA Brown	13	6	0	7	53.8	4	7	2	15.4
	White leghorn	51	11	2	38	74.5	12	2	37	72.5
Poultry type	broiler	22	10	2	10	45.5	9	2	11	50
	layer	78	13	2	63	80.8	18	12	48	61.5
Sample source	water	3	0	0	3	100	0	2	1	33.3
	Feed	2	0	1	1	50	1	1	0	0
	personnel	7	0	1	6	85.7	0	1	6	85.7
	Poultry	97	23	4	70	72.2	26	14	57	58.8
	Poultry house	17	5	0	12	70.6	6	1	10	58.8
Sample type	Carcass swab	3	0	0	3	100	0	0	3	100
	Cloacal swab	80	17	4	59	73.8	21	8	51	63.8
	Feed	2	0	1	1	50	1	1	0	0
	Fresh feces	18	6	0	12	66.7	6	6	6	33.3
	litter	7	5	0	2	28.6	5	1	1	14.3
	Hand swab	7	0	1	6	85.7	0	1	6	85.7
	Pooled feces	6	0	0	6	100	0	0	6	100
	water	3	0	0	3	100	0	2	1	33.3

Annex 7. Distribution of resistant and susceptible isolates (n = 126) across the different risk factors for the 10 drugs tested.

			Gentamicin				Tetracycline				Ampicilline				Naldixic acid			
Risk factors		N	S	IM	R	% R	S	IM	R	%R	S	IM	R	%R	S	IM	R	%R
Study location	Central Ethio.	81	66	10	5	6	25	10	46	57	46	2	33	41	25	14	42	52
	South Ethio.	45	42	3	0	0	1	0	44	98	1	0	44	98	1	0	44	98
study area	Addis Ababa	23	22	0	1	4	5	3	15	65	16	0	7	30	10	2	11	48
	Adama	15	1	10	4	27	15	0	0	0	15	0	0	0	6	6	3	20
	Bishoftu	12	12	0	0	0	0	3	9	75	2	1	9	75	1	1	10	83
	Bonga	27	24	3	0	0	1	0	26	96	1	0	26	96	1	0	26	96
	Hawassa	18	18	0	0	0	0	0	18	100	0	0	18	100	0	0	18	10
	Modjo	31	31	0	0	0	5	4	22	71	13	1	17	55	8	5	18	58
Category	Abattoir	3	3	0	0	0	0	2	1	33	1	1	1	33	0	0	3	10
	Farm	123	105	13	5	4	26	8	89	72	46	1	76	62	26	14	83	67
Housing system	Cage	16	16	0	0	0	2	1	13	81	4	0	12	75	1	1	14	88
	Litter	86	75	9	2	2	17	9	60	70	35	2	49	57	21	9	56	65
Breed	Bovans	33	26	7	0	0	9	3	21	64	15	0	18	55	7	4	22	67
	Fayomin	3	1	0	2	67	3	0	0	0	3	0	0	0	0	2	1	33
	ISA Brown	13	13	0	0	0	2	2	9	69	5	1	7	54	2	3	8	62
	White leghorn	51	50	1	0	0	4	5	42	82	15	1	35	69	12	1	38	75
Sample source	Water	3	3	0	0	0	1	0	2	67	0	0	3	100	0	0	3	100
	Feed	2	0	2	0	0	2	0	0	0	2	0	0	0	1	1	0	0
	Personnel	7	4	1	2	29	2	0	5	71	2	0	5	71	1	0	6	86
	Poultry	97	90	6	1	1	15	10	72	74	35	2	60	62	19	9	69	71
	Poultry house	17	11	4	2	12	6	0	11	65	8	0	9	53	5	4	8	47
Sample**	Carcass swab	3	3	0	0	0	0	2	1	33	1	1	1	33	0	0	3	10
	Cloacal swab	80	73	6	1	1	14	5	61	76	28	1	51	64	15	7	58	73
	Feed	2	0	2	0	0	2	0	0	0	2	0	0	0	1	1	0	0
	Fresh feces	18	15	2	1	6	4	3	11	61	9	0	9	50	6	3	9	50
	Litter	7	5	1	1	14	3	0	4	57	5	0	2	29	3	3	1	14
	Hand swab	7	4	1	2	29	2	0	5	71	2	0	5	71	1	0	6	86
	Pooled feces	6	5	1	0	0	0	0	6	100	0	0	6	100	0	0	6	10
	Water	3	3	0	0	0	1	0	2	67	0	0	3	100	0	0	3	100
Flock	large	74	64	8	2	3	16	6	52	70	28	1	45	61	16	6	52	70
	medium	26	26	0	0	0	2	4	20	77	10	1	15	58	5	4	17	65
Prod*	Broiler	22	22	0	0	0	3	5	14	64	13	1	8	36	10	1	11	50
	Layer	78	68	8	2	3	15	5	58	74	25	1	52	67	11	9	58	74
Total		126	108	13	5	4	26	10	90	71	47	2	77	61	26	14	86	68