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Review on Prevalence and Antimicrobial Resistance of Poultry *Salmonella* in Ethiopia

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Review Article

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Abstract

Salmonellosis is one of the major causes of morbidity and mortality in village and large scale poultry farms. This paper was aimed to review on the prevalence and antimicrobial resistance of poultry *Salmonella* in Ethiopia. The risk factors associated with *Salmonella* in laying hens that the presence of previous *Salmonella* infection, multi-age management, cage housing systems, rearing pullets on the floor, induced molting and in-line egg processing were factors associated with *Salmonella* infection. Among them, many isolates were *S. braenderup, S. typhimurium var. copenhagen*, *S. anatum and S. typhimurium* isolates were dominant *Salmonella* serotypes. Antimicrobial resistance is a global problem in general, but it might be more severe in Ethiopia where there is lack of antimicrobial resistance assessments of *Salmonella* and lack of rigorous regulations. During the last decade, there has been an alarming increase in the appearance of antibiotic-resistant bacteria as a result of poor management in antibiotic utilization. Poultry *Salmonella* are excellent examples of diseases that have decreased in prevalence in some of the developed countries or have been eradicated by application of basic management procedures or eradication programs. Every effort should be made to eradicate *Salmonella* and treatment should be the last option.

Keywords: Antimicrobial Resistance; Ethiopia; Poultry; *Salmonella*; Serotypes

Abbreviations: RFLP: Restriction Fragment Length Polymorphism; dNTP: Deoxynucleotides Triphosphates; PCR: Polymerase Chain Reaction; RVS: Rappaport-Vassiliadis Soya; HE: Hektoen Enteric; BG: Brilliant Green; MKTT: Muller Kauffman Tetrathionate; XLD: Xylose Lysine Deoxycholate.

Introduction

Background of Salmonellosis

Ethiopia has an estimated total population of 54,495,026 poultry; among them 90.85% indigenous, 4.39% exotic and 4.76% hybrid breeds [1]. In most developing countries, including Ethiopia, village chickens make up the largest proportion of the national poultry population [2]. However, currently semi-intensive chicken production system is widely expanding in urban, pei-urban and rural areas of Ethiopia. This type of chicken production system is better than free ranging production system since it uses inputs like supplemental feed, vaccine, etc. It has a small house which accommodate laying nest and feeders which serves as chicken house for night time. It contains flock size of 50-200 chicken/household which are improved breeds [3]. About 56% (9.6 million) of Ethiopian households have poultry holdings with varying range of flock size [4]. Young people of both urban and rural backgrounds have begun to establish microenterprises producing poultry and eggs, creating employment and income sources [2]. Further, it stimulates

local economic development of urban centers through the development of related micro-enterprises wholly or partly responsible for the provision of inputs and processing, packaging, and marketing of outputs as well as the provision of services to the sector [5].

However, chicken production is constrained by various factors that directly or indirectly influence productivity. This is due to different contributing factors, among which are low genetic potential of the indigenous breeds, high prevalence of infectious diseases and traditional feeding practice [6]. In Ethiopia, periodic disease outbreaks and constant presence of infectious disease can lead not only to illness and death but also reduce productivity. Infectious diseases, such as Newcastle disease, *salmonellosis*, Mark's disease, infectious bursal disease (Gumboro), fowl cholera, coccidiosis and fowl pox, are the major causes of morbidity and mortality in village and large scale poultry farms [2].

In recognition of its epidemiologic and pathogenic diversity, each of the more than 2800 serologic variants (serotypes, serovars) is treated. Each is capable of producing disease of the gastrointestinal tract as well as septicemia [7]. More recently, the genus has been divided into seven subgroups. The seven subgroups of genus *Salmonella* are subgroup I (enteric); subgroup II (salamae); subgroup IIIa (arizonae); subgroup IIIb (diarizonae); subgroup IV (houtenae); subgroup V (bongori); and subgroup VI (indica). Subgroup I contains most of the salmonellae organisms that are significant animal pathogens and most have been given names like "Dublin"or "Typhimurium". Subgroups IIIa and IIIb contain the bacteria once known as 'Arizona' and now called 'arizonae' if monophasic (IIIa) or 'diarizonae' if diphasic (IIIb) [8].

Salmonella are short bacilli, 0.7-1.5 x 2.5µm, gram-negative, aerobic or facultative anaerobic, oxidase-negative, catalase-positive, indole-negative, Voges-Proskauer (VP)-negative, methyl red-positive, citrate-positive, H_2S producing and urease negative bacteria [9]. They ferment sugars with gas production, non-spore forming, and are normally motile with peritrichous flagella, except *S. Pullorum* and *S. Gallinarum,* which are non-motile [10]. The optimal pH for multiplication is around 7.0; pH values above 9.0 or below 4.0 are bactericidal. The ideal temperature is between 35 to 37°C, with minimum of 5°C and maximum of 47°C. Concerning salt concentration, *Salmonella* serotypes do not survive at concentrations over 9% [11].

The reservoir for members of the genus *Salmonella* is the gastrointestinal tract of warm and cold-blooded animals. Sources of infection include contaminated soil, vegetation, water, and components of animal feeds such as, bone, meat, and fish meal, particularly those containing milk, meat, or egg-derived constituents, and the feces of infected individuals [12]. Other source of infection includes breeder, feed, environment, feather, human skin, floor and dirty equipment [7,13]. Lizards and snakes are commonly infected with several serotypes although these infections are usually subclinical [12].

Salmonella is primarily transmitted by the fecal-oral route, often through ingestion of contaminated feed and water for chicken. Infection occurs following the ingestion of viable *Salmonella*. The disease may follow infection immediately in an animal that are already infected and causes a change in the intestinal environment. The outcome of the interaction between host and *Salmonella* depends upon the state of the colonization resistance of the host, the infectious dose, and the particular serotypes of *Salmonella* [7]. Salmonellosis causes severe economic damage to chicken production by reducing production, causing 100% of morbidity and 20% of mortality in affected flocks [14].

"Paratyphoid" of poultry (in quotation marks because true paratyphoid is a disease of humans caused by paratyphoid serotypes of *Salmonella*) is *salmonellosis* produced by any of the motile strains of *Salmonella*. All salmonellae except *S. enterica* serotype *Pullorum* and *S. enterica* serotype *Gallinarum* are motile [12]. Pullorum disease has almost been eliminated in the United States due to a breeding flock testing program [7]. *Salmonella pullorum* infects the ova of turkeys and chickens. Thus, the embryo is already infected when the egg is hatched. The hatchery environment is contaminated following hatching of infected eggs, leading to infection of other chicks and poults. Mortality is due to septicemia and is greatest in the second to third weeks of life. Surviving birds carry the bacterium and may pass it to their offspring. It is difficult to detect infected breeding hens by bacteriologic means [12]. Fowl typhoid, caused by *S. gallinarum* is an acute septicemic or chronic disease of domesticated adult birds, mainly chickens. Fowl typhoid is rare now in the United States due to control programs [7].

Salmonella is spread by the trade of live animals within and between countries. *Salmonella* is additionally spread between countries by humans as a result of food-borne infections acquired abroad [10]. *Salmonella enterica* is the 3rd leading cause of foodborne illnesses globally; accounting for 78 million illnesses and 59 thousand foodborne-related deaths annually [15]. The overall importance of this route of transmission may reflect the prevalence of contamination of food by pathogenic *Salmonella* serotypes mainly food of animal origin in a particular country [10]. It is not yet clear as to which route is the most important for *Salmonella* to contaminate the egg contents that can be by vertical transmission and/or horizontal transmission.

The distribution of the pathogenic strains *Salmonella* were also causing a considerable loss in intensive, semi-intensive and extensive poultry farms. It is not only the main concern of poultry farms but, also it is the main concern of human beings due to the transmission of *Salmonella* to human beings through consumption of chicken origin foods, contact with infected chicken and products [16]. Currently, different approaches are undertaken in poultry farms to minimize the occurrence and distribution of the *Salmonella* strains by bio-security and antimicrobial therapy. As a result of the continuous use of some antibiotics for treatment of *Salmonella* pathogens antibiotic resistance has been common problem that blocks or limits the effective control of *salmonellosis*.

Objectives of the Review

- To review the prevalence of poultry *Salmonella* in different farms in Ethiopia.
- To review possible associated risk factors for the occurrence of chicken *salmonellosis* in the area.
- To review the antimicrobial susceptibility of the identified *Salmonella* pathogen *invitro*.

Litrature Review

Classification *Salmonella:* The bacteria of the genus *Salmonella* are responsible for illnesses in human beings and animals. The genus is divided into two species: *Salmonella enteric* and *Salmonella bongori* [17]. *S. enteric* is divided into six subspecies (*enterica, salamae, arizonae, diarizonae, houtenae* and *indica*) and each one of them has several serovars or serotypes [18]. Most pathogenic isolates from humans and other mammals belong to *S. enteric* subspecies *enterica*. Other *S. enteric* subspecies and *S. bongori* are more common in cold-blooded animals and the environment, with lower pathogenicity to humans and livestock [19]. A few serotypes are host-specific; i.e. *S. typhi* is implicated in typhoid fever in human beings, while *Salmonella* Pullorum and *S. gallinarum* are responsible for bacillary white diarrhea and fowl typhoid in poultry respectively [20]. *Salmonella* choleraesuis is host restricted to pigs, *Salmonella* serovar abortusovis is involved in sheep abortions and *Salmonella* dublin infects bovines [20].

Salmonellosis in Poultry: It has been well documented that poultry constitutes the largest single reservoir of *Salmonella* and that the non-host adapted serovars pathogenic to poultry are also potential pathogens of man and other animals [21]. *Salmonella gallinarum* and *S. gullorum* are both members of the family *Enterobacteriaceae* and are highly adapted to the poultry host. The bacteria belong to serogroup D according to the Kauffmann-White scheme. The majority of strains of *S. gallinarum* and *S. pullorum* are very similar at a chromosomal level [22]. Furthermore, *S.* enteritidis, another member of serogroup Group D, is thought to be closely related to *S. gallinarum* and *S. pullorum*, based on multi-locus enzyme electrophoresis [23]. According to one study, the most recent common ancestor of *S. gallinarum* and *S. pullorum* was non-motile. Since diverging from this ancestor, the *S. pullorum* lineage appears to have evolved more rapidly than the *S. gallinarum* lineage [24]. *Salmonella* is a leading cause of food-borne illness in many countries with eggs and poultry being important vehicles of transmission [25].

Aetiology: *Salmonella pullorum/gallinarum* are biovars within the genus *S. enteric* subspecies *enteria* within the family *Enterobacteriaceae*. They are the etiological agents of pullorum disease (*S. pullorum*) and fowl typhoid (*S. gallinarum*) and the two septicemic diseases of chickens are widely common in much of the world [8]. There are a several non host-specific serotypes that may infect several animal species, including humans, and these are generally responsible for food-borne diseases with foods of animal origin being the main source. Many non-typhoidal *Salmonella* strains, such as *Salmonella typhimurium* and *Salmonella enteritidis*, infect a wide range of animal host including poultry, cattle and pigs [26].

Pathogenesis: The clinical manifestation of *Salmonella* infection presents as *salmonellosis*, an enteric condition that ranges in severity from self-limiting gastroenteritis to septicemia. The severity of the disease depends heavily on the host susceptibility and the virulence of the serovar. Globally, *Salmonella* avoid host defense in the stomach and reach the intestines, and interact with the non-phagocytic cells such as the epithelial cells of the intestinal mucosa [27]. Those *Salmonella* organisms that survive the low-pH environment proceed to the lumen of the gastrointestinal tract (GIT) organs, including the small intestine, colon, and cecum (in poultry). Epithelial and immune cells lining these GIT organs provide the initial protective barrier against *Salmonella* in the gut. *Salmonella* competes with the gut microflora to make the initial contact with enterocytes to colonize the GIT [28]. They adhere to the intestinal epithelial cells by adhesive structures (fimbriae) that promote binding and invade epithelial cells to provoke gastroenteritis. The organisms have virulence factors such as virulence-plasmids, toxins, fimbriae and flagella that help in establishing an infection [29]. Pathogenicity is mediated by certain factors such as strain virulence, infectious dose, route of infection and host susceptibility. Some of the mechanisms of pathogenesis are bacterial mediated endocytosis, neutrophil recruitment and migration, epithelial cell cytokine secretion, fluid and electrolyte secretion, and systemic infection [26].

Clinical Signs: The clinical signs in chicks and poults include moribund and dead birds in the incubator or shortly after hatching if the chicks and poults are hatched from infected eggs [30]. Older birds show signs of anemia, depression, labored breathing and diarrhea causing adherence of feces to the vent. In older birds disease may be mild or inapparent [31]. In some situations, it may not be observed until five to ten days after hatching. The highest mortality usually occurs in birds of two to three weeks of age. Survivors may be greatly reduced in weight and poorly feathered, and may not mature into well-developed laying or breeding birds. Flocks that have experienced a severe outbreak will have a higher percentage of carriers at maturity. Other signs, including blindness, swelling of the tibio-tarsal joint and the humeral, radial and ulnar articulations may be observed [30]. Paratyphoid is a name given to infections of poultry by non-host-adapted *Salmonella* such as *Salmonella* enteritidis and *Salmonella typhimurium*. These infections are often subclinical in laying birds [32].

Transmission Routes: The gastrointestinal tracts of animals and humans are the primary sources of *Salmonella*. The bacteria are carried asymptomatically in the intestines or gall bladder of many animals and are continuously or intermittently shed in the feces [33]. Although, most infections cause mild to moderate self-limiting disease, serious infections leading to deaths do occur [34]. Its widespread presence in the environment is considered to be due to direct or indirect fecal contamination [9]. Chicken can become infected from contaminated feed, drinking water or close contact with an infected chicken (including humans). Trans-ovarian (vertical transmission) or trans-shell (horizontal transmission) occurs in poultry [35].

Trans-ovarian infection resulting in infection of the egg and hatched chicks or poults is one of the most important transmission routes of the disease [36]. In the first case, a contamination of the vitelline membrane, albumen and possibly the yolk of eggs occur. Following this route, *Salmonella* is introduced from infected reproductive tissues to eggs prior to shell formation. *Salmonella* serotypes with high importance to public health, associated with poultry reproductive tissues, include *S. enteritidis, S. typhimurium* and *S. heidelberg*. Among all the different serotypes, *S. enteritidis* may be more invasive and consequently, may be found more frequently in reproductive tissues [35]. Fecal contamination of egg shell is the primary cause of horizontal transmission. Bacteria can 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 by *Salmonella* [37].

Public Health Importance: The transmission to humans usually occurs through the consumption of food or water contaminated with animal faeces, but it can also happen through direct contact with infected animals or their environment and directly between humans [38]. Contamination with *Salmonella* in poultry products can occur at multiple steps along the food chain, which includes production, processing, distribution, retail marketing, handling and preparation [39]. In the same way, animals can become infected from contaminated feed (including pastures), drinking water or close contact with an infected animal (including humans). *Salmonella* has adapted to survive in a wide range of different environments, such that a large number of human infections are associated not only with food animal sources but also with pets, reptiles, fruits, vegetable, legumes, and other humans [38]. During the three and half decades *S.* Enteritidis has become a leading serotype causing human infections, with hen eggs being a principal source of the pathogen [25].

Isolation and Characterization of *Salmonella:*

- **Bacteriological isolation and identification**

In cases of intestinal infection, fecal samples are collected; in systemic disease, a blood culture. Spleen and bone marrow are cultured for the *Salmonella* responsible for postmortem diagnosis of systemic salmonellosis [7,40]. Fresh samples are inoculated onto one or more selective, indicator and differential media, including MacConkey agar, Xylose Lysine Deoxycholate (XLD) agar, Hektoen Enteric (HE) agar, *Salmonella-shigella* (SS) agar and Brilliant Green (BG) agar for isolation. However if we have suspicious that samples contain small number of *Salmonella* enrichment is commonly employed. Selenite cysteine and F broth, Muller Kauffman Tetrathionate (MKTT) broth and Rappaport-Vassiliadis Soya (RVS) broths are commonly used to enrich *Salmonella* before the specimens are inoculated onto agar plates. However, Rappaport broth is commonly used if the specimens are collected from poultry. *Salmonella* appears as lactose-non fermenting colonies on lactose-containing media like MacConkey agar. Colonies showing typical morphological appearance was presumptively identified as *Salmonella* by Gram's stain, catalase and oxidase, motility and OF tests [8]. *Salmonella* usually stained as gram-negative, rod-shaped appearance on Gram staining [41]. Since most serotypes of *Salmonella* produce H_2S, colonies on iron-containing media like XLD agar have a black center. Identified colonies with black center are confirmed biochemically using lysine and triple sugar iron agars and with API-20E [7].

Colonies of *S. gallinarum* on non-selective media are round, translucent, glistening, domed, smooth, and 1-2 mm in diameter after 24-48 hours of incubation. *Salmonella pullorum* colonies are slightly smaller and translucent. On selective media their appearance varies with the medium

[8,40]. *Salmonella pullorum* and *S. gallinarum* are non-motile and usually stained as gram-negative, rod-shaped appearance on Gram staining [41,42]. Biochemical tests such as, indole, methyl red, Voges-Proskauer, citrate utilization, urea hydrolysis, lysine decarboxylation, ornithine decarboxylation, maltose fermentation, dulcitol fermentation, inoculation into triple sugar iron (TSI) agar for acid and gas production is important for identification of *S. gallinarum* and *S. pullorum* [40]. Both organisms ferment arabinose, dextrose, galactose, mannitol, mannose, rhamnose and xylose by the production of acid with or without gas production [8].

- **Serological characterization**

For serological confirmation to serogroup level, colonies from non-selective media (nutrient or blood agar) are used [43]. Biochemically identified *Salmonella* isolates are further identified serologically according to Kauffman-White Scheme by slide agglutination test using specific polyvalent and monovalent antisera to O and H antigens [44]. *Salmonella* isolates are serotyped with their somatic antigen (O-antigen) and with their flagellar antigen (H-antigen) by using specific monoclonal anti-sera by latex agglutination according to the Kauffman-White scheme [45]. Phage typing is also important for the serotyping of *Salmonella* [7,46].

- **Molecular characterization of *Salmonella* serotypes**

The polymerase chain reaction is a techniques used to amplify the number of copies of a pre-selected region of DNA to a sufficient level to test for identification [9]. The DNA of *Salmonella* serotypes varies and specific DNA probes and primers for PCR assay have been developed for the detection of the organisms in samples like food, feces and water that may contain other microorganisms. DNA extraction from *Salmonella* isolates was done by 200µl of the bacterial suspension was incubated with 10µl of proteinase K and 200µl of lysis buffer at 56°C for 10 minutes. After incubation, 200µl of 100% ethanol was added to the lysate. The sample is then washed and centrifuged and the nucleic acid is eluted in 100µl elution buffer [47]. Polymerase Chain Reaction (PCR) is the latest techniques used to diagnose *Salmonella* in a molecular level based on the ability of *Salmonella* specific primers, through complementary DNA base pairing, to anneal only to the target sequence. Thermostable DNA polymerase (Taq polymerase) recognizes the template primer complex as a substrate, which results in the simultaneous copying of both strands of the segment of DNA between the two annealed primers. The denaturation, annealing and elongation steps take place in a cyclical fashion, relying on the thermostability of the Taq polymerase, until the target sequence is amplified to detectable amounts [48]. Before starting the first cycle in the thermocycler the DNA, primers, the polymerase, deoxynucleotides triphosphates (dNTP), and buffer are mixed in a reaction tube. The targeted region of the *Salmonella* genome is amplified by repetition of a three-step process: Denaturation of the double-stranded DNA into single strands by heating; annealing of specific complementary oligonucleotide primers to the single-stranded DNA by cooling and enzymatically extending the primers to produce an exact copy of the original double-stranded target sequence. This process is usually repeated for 30 to 40 cycles [48]. In the final step, detection of the amplified target DNA is done using agarose gel electrophoresis. Before PCR, it is often necessary first to grow the bacteria on an enrichment medium and then extract and purify the pathogen [48].

Standard molecular 'fingerprinting' techniques used for *Salmonella*, such as, plasmid profile analysis, pulsed-field gel electrophoresis PCR-restriction fragment length polymorphism (RFLP) or ribotyping can be used for investigating outbreaks of *S. pullorum* or *S. gallinarum*. It is often necessary to use in combinations of such methods and different restriction enzyme combinations to obtain maximum discrimination because of a high level of clonality. The most effective techniques may also vary by country because of the nature of circulating clones in that region. High throughput whole-genome sequencing has also been applied to *S. gallinarum*, but is not yet available or economically viable in all countries [49].

Distribution of *Salmonella*

Prevalence and major serotypes distributed in the world: The occurrence of *Salmonella* has been reported in a variety of chicken, turkey and other meat products. Compared to products such as ready-to-eat meat 3.1%, pasteurized eggs 14.6%, ground beef 7.5%, broiler chicken 20%, market hogs 8.7% and steers and heifers 1%, *Salmonella* was detected in 49.9% of ground turkey and 44.6% of ground chicken meat [50]. From the total *Salmonella* serotypes characterized worldwide, 15 serotypes have been reported to be commonly associated with poultry processing in the US [51,52].

Similar findings were also reported from different countries of the world. According to the CDC, in the United States, *S. enteritidis* was the serovar that most commonly implicated in human illness, overtaking *S. typhimurium* as the most common serovar [38]. Likewise, when data from the national veterinary services laboratory of the United States and from other studies examining the prevalence of *Salmonella* serovars were compared, *S. enteritidis* was associated most commonly with chickens and eggs and to a much lesser extent with other food animal species [53]. *Salmonella heidelberg* is found in most of the major food animal species, eggs, and retail meat samples and is among the top five most common serotypes associated with human illnesses [54]. Conversely, *Salmonella* serovars *kentucky* and

gallinarum rarely cause human infections in the United States although *S. kentucky* is an emerging serovar in Europe and North Africa [55]. *Salmonella serovar gallinarum* is a host-adapted serovar that is presently made up of two biovars, *gallinarum* and *pullorum* (which were previously considered two separate serotypes) [55]. Out of the total 365 chicken carcasses examined bacteriologically in Burkina Faso, 55% (n=192) were found to be contaminated by different *Salmonella* serotypes. It was found that *S.* Derby 51%, *S.* Cheser 31% and *S.* Hato 22% were found that the major serotypes were isolated from chicken carcass samples [56].

Prevalence and major serotypes distributed in Ethiopia: In Ethiopia many research reports showed that the prevalence of *Salmonella* serotypes affecting poultry was found to be different in many poultry industries. A recent study reports are included here to compare the prevalence poultry *Salmonella* serotypes with their distribution that have been identified in different poultry farms. According to Abunna, et al. [57] report, study that was conducted in selected poultry farms found in and around Modjo, out of the total 205 samples examined bacteriologically *Salmonella* serotypes were isolated in 15.12% (n=31) chickens. According to Eguale [58] report, the overall prevalence of chicken *salmonellosis* in central Ethiopia poultry farms was found to be 4.70%. The study that was conducted in selected poultry farms in and around Hawassa town by Kassaye, et al. [59] also reported that the prevalence of chicken *salmonellosis* caused by *S. gallinarum/ S. pullorum* serotypes was found to be 0.80% and 16.13% in cloacal swab samples and postmortem tissue samples respectively. Abdi, et al. [60] reported that the overall prevalence of *Salmonella* serotypes affecting poultry was found to be 16.70% (n=45) out of the 270 samples; a study conducted in selected poultry farms found in Southern Ethiopia.

The study conducted by Kasech [61] showed that the most predominant isolates were found to be *Salmonella* serotypes. Study showed that from the total of 150 omphalitis cases examined bacteriologically 34.2% (n=52) isolates were found to be *Salmonella* serotypes. Bacteriological study that was conducted by Zewudu, et al. [62] in selected supermarkets of Addis Ababa reported that out of the total chicken carcass examined bacteriologically 13.9% (n=29) were found to be positive for *Salmonella* serotypes. The bacteriological study that was conducted by TibaiJuka, et al. [63] on isolation of major bacteria on retail raw chicken products in Ethiopia showed that out of the total retail raw chicken products examined bacteriologically 17.90% were found to be contaminated by *Salmonella* serotypes.

Many studies conducted in Ethiopia show that chicken eggs can be infected by *Salmonella* serotypes. The study conducted in Gondar by Mebrat, et al. [64] reported that from the total eggs examined bacteriologically *Salmonella* serotypes were isolated and identified in 18% (n=9) raw egg samples sampled in and around Gondar town. The study that was conducted in Haramaya by Jelalu, et al. [65] reported that out of the total 300 chicken eggs examined bacteriologically 2.70% (n=8) was positive for *Salmonella* serotypes. It was found that 5.30 % isolates were found from market eggs while, an egg taken from poultry farm was found to be 0% [65]. The study conducted by Tessema, et al. [66] in Haramaya University poultry farm, Eastern Ethiopia reported that *Salmonella* serotypes were isolated from 2.90% (n=11) egg samples by conventional culture technique and all isolates were confirmed by the biochemical test. From a total of 11 isolates 9 *Salmonella* serotypes were identified from eggshells and 2 of them were recovered from egg content samples. The study conducted in local markets and poultry farms in Mekelle by Dawit, et al. showed that out of the total 156 chicken eggs examined bacteriologically for isolation of *Salmonella* serotypes affecting poultry, it was found that 15.38% and 8.33% of egg shells and egg contents respectively were positive.

The study conducted in Addis Abeba showed that the carcasses of chicken were contaminated by different serotypes of *Salmonella*. A study conducted by Zewudu, et al. [62] showed *S. braenderup, S. hadar, S. newport, S. typhimurium, S. kentucky* and *S. bovismorbificans* were the serovars isolated from chicken carcasses (Table 1). The study conducted by TibaiJuka, et al. [63] showed that *S. braenderup, S. anatum, S. saintpaul, S. uganda and S. typhimurium, S. haifa, S. roughform, S. II 4: 12 B*, and *S. virchow* were found the dominant serovars contaminating the retail raw chicken products (Table 1). Another study that was conducted by Eguale [58] showed that *S.* saintpaul was the dominant serotype and other serotypes, such as *S. typhimurium, S. kentucky* and *S. haifa* were also identified (Table 1).

According to Bayleyegn, et al. [67] report, around fifty *Salmonella* serotypes were identified (Table 1). Among them, many isolates were *S. braenderup, S. typhimurium var. copenhagen, S. anatum* and *S. typhimurium* isolates were dominant *Salmonella* serotypes (Table 1). Among them *S. typhimurium, S. braenderup* and *S. saintpaul* were reported by both three reporters while the other serotypes were reported by two or one reporter (Table 1). The major *Salmonella* serotypes that were isolated in different poultry farms in Ethiopia were indicated in Table 1.

Salmonella serotypes	Reporters, years and study areas			
	Bayleyegn, et al. [67] (Ethiopia)	TibaiJuka, et al. [63]	Zewudu, et al. [62]	Eguale, [58]
	(No.)	(Addis Ababa)(%)	(Addis Ababa) (No.)	(Central Ethiopia) (No.)
S. hadar	2	-	6	-
S. newprt	-	-	4	-
S. typhimurium	18	3.7	3	3
S. braenderup	52	31.5	12	-
S. anatum	22	25.9	-	-
S. saintpaul	8	14.8	-	20
S. uganda	6	11.1	-	-
S. kentucky	-	-	2	2
S. haifa	2	3.7	-	1
S. typhimurium var. copenhagen	24	-	-	-
S. infants	2	-	-	-
S. kottbus	5	-	-	-
S. bovismorbificans	1	-	1	-
S. enteritidis	4	-	-	-
S. virchow	1	1.8	-	-
Salmonella II 4: 12B	2	3.7	-	-
S. rough form	2	3.7	-	-

Table 1: Distribution of the Major Chicken *Salmonella* Serotypes in Ethiopia.

Factors Affecting the Epidemiology of *Salmonellosis* in Poultry

Several studies have studied the risk factors associated with *Salmonella* contamination in broiler chickens. The most important risk factors included contaminated chicks, size of the farm (related to increased human traffic among multiple sheds) and contaminated feed (the risk of *Salmonella* contamination of the flock was increased when feed trucks were parked near the entrance of the workers' change room and when feed meal, instead of small pellets) [68]. The risk factors associated with *Salmonella* in laying hens that the presence of previous *Salmonella* infection, multi-age management, cage housing systems, rearing pullets on the floor, induced molting and in-line egg processing were factors associated with *Salmonella* infection. Also, cleaning and disinfection, presence of rodents, pests with access to feed before movement to the feed trough, visitors allowed in the layer houses and trucks near farms and air inlets were risks identified to be associated with *Salmonella* contamination of laying hen premises [69].

Furthermore, contamination through environmental vectors, such as farmers, pets and rodents, feed, water, fluff, dust, shavings and straw, insects, equipment, and thus, many different serotypes of the genus *Salmonella* can be involved [70,71]. *Salmonella* contamination appeared to persist preferentially in association with dust particles swept from the floor and in food troughs, and *S.* enteritidis survived at least 26 months in artificially contaminated poultry feed [72].

Control and Prevention Strategies

Poultry *Salmonella* are excellent examples of diseases that have decreased in prevalence in some of the developed countries or have been eradicated by application of basic management procedures or eradication programs [30]. *Salmonella* can be effectively controlled by coordinated and simultaneous interventions on the problem from different

directions. At the farm level, eggs and chicks or poults can only be obtained from *Salmonella* free breeding flocks. Hatching eggs should be properly disinfected and hatched from farms adhering to stringent sanitation standards [73]. Since egg transmission plays an important role in the spread of the disease, only eggs from flocks known to be free of *Salmonella* should be introduced into hatcheries [30]. After depopulation, and when cleansing and disinfection have been carried out, buildings should be checked for persistence of *salmonellosis*. Samples should include large fabric swabs of earth floor surfaces or floor sweepings from concrete floors, nest-box floors, beams, pipes and electrical fittings [74]. Rodents and insect control measures should be incorporated into house design and management and verified by periodic testing. Rigidly enforced bio-security practices should be implemented, restricting entry onto poultry housing premises to only authorized personnel and equipment, preventing horizontal transmission of *Salmonella* between houses. Treatments such as medication, competitive exclusion cultures, or vaccination can be applied to reduce *Salmonella* susceptibility. Frequent testing of poultry and environmental samples has also reportedly been successful for *Salmonella* control in the poultry industry [73]. Every effort should be made to eradicate *Salmonella* and treatment should be the last option. Various sulphonamides, followed by nitrofurans and several other antibiotics like furaltodone, furazolidone, chloramphenicol, biomycin, apramycin, gentamicin and chlorotetracycline have been found to be effective in reducing mortality from the *Salmonella* [75].

Antimicrobial Resistance Pattern of Poultry *Salmonella* Serotypes

Global resistance patterns: Antimicrobial resistance is becoming an increasingly important issue in *salmonellosis* in both animals and humans [76]. Feeds have been responsible for the infection of poultry with multidrug-resistant non-typhoid *Salmonella* in several industrialized countries. In food animal production, antimicrobials are administered for therapeutic means, for treatment of infection, prophylactic and non-therapeutic purposes for growth promotion and improved feed efficiency [77]. Chronic low-level doses of antibiotics and characteristic of growth-promoting agents (GPAs) administered in the animal production environment encourage the elimination of susceptible bacteria and yield the selections and expansion of resistant-bacterial population. Many drugs used in veterinary medicine have identical analogs that are used in human medicine [78].

The use of GPAs in feed preparations or water supplements illustrate the largest segment of antibiotic use in poultry production. The usage of GPAs in food animal production is a major public health threat, because this practice can contribute to the emergence of antimicrobial-resistance worldwide [79,80]. As a consequence, chicken and chicken meat can harbor antimicrobial-resistant strains and function as a vehicle for dissemination of these to human. Today, antimicrobial resistant of *Salmonella* strains are frequently encountered in most of the world and the proportion of antimicrobial-resistant dramatically increased over the past decade [81]. In developing countries, household subsistence farming is common, which means that a large proportion of the population has close contact with food animals; therefore, if resistant organisms are common in animals, the chance that they will be transmitted to human beings is more likely [82].

Resistance patterns in Ethiopia: Antimicrobial resistance is a global problem in general, but it might be more severe in Ethiopia where there is lack of antimicrobial resistance assessments of *Salmonella* and lack of rigorous regulations, but there is easy access of antimicrobials for purchase of people without prescription and incomplete treatment courses as the result of patient non-compliance [83]. During the last decade, there has been an alarming increase in the appearance of antibiotic-resistant bacteria as a result of poor management in antibiotic utilization [84]. According to Abdi, et al. [60] 45 *Salmonella* serotypes isolates were subjected to the antimicrobial susceptibility test and it was found that all 45 isolates 100% were resistance to kanamycin and sulfamethoxazole-trimethoprim. Further, 44 isolates 97.80% also had resistance to ampicillin, cefoxitin, nalidixic acid, streptomycin and tetracyclines; and it was also observed that 91.10% and 31.10% of isolates were resistance to chloramphenicol and ciprofloxacin respectively. Among the common antimicrobials, oxytetracycline was used widely in 83.30% of the farms, followed by amoxicillin in 29.20% farms and sulfonamides in 22.90% poultry farms [60]. Other antimicrobials such as fluoroquinolones (enrofloxacin and ciprofloxacin) and florphenicol were also used in 22.90% and in 14.60% of poultry farms respectively in central Ethiopia [58].

In fact depending on the reports shown in different journal articles listed in Table 2 obtained from researchers, tetracycline was found to be the first antimicrobial drug to which most bacterial pathogens developed resistance followed by ampicillin. From all researchers five of them reported that most *Salmonella* serotypes developed resistance to sulphamethoxazole-trimethoprim (Table 2). Three reports showed that the development of resistance by most *Salmonella* serotypes to nalidixic acid, streptomycin, kanamycin, amoxicillin and nitrofurantoin (Table 2). However, it is important to note that these antimicrobials are commonly used in veterinary medicine and infections with these resistant *Salmonella* isolates could lower the efficiency of antimicrobial treatment [85].

	Reports					
Antibiotics	**Kasech [61]**	**Jelalu, et al. [65]**	**Mebrat, et al. [64]**	**Tessema, et al. [66]**	**Abunna, et al. [57]**	**Eguale [58]**
Gentamycin	0(0.00%)	1(12.50%)	0(0.00%)	-	0(0.00%)	2(7.70%)
Tetracycline	113(100%)	3(37.50%)	4(80.00%)	8(72.70%)	23(74.20%)	8(30.80%)
Ampicillin	-	3(37.50%)	1(20.00%)	8(72.70%)	17(54.0 5%)	11(42.30%)
Nalidixic acid	-	-	2(40.00%)	-	18(58.10%)	5(19.00%)
Cefoxitin	-	-	-	-	18(58.10%)	0(0.00%)
Streptomycin	13(11.50%)	-	-	0(0.00%)	6(19.40%)	24(92.30%)
Ciprofloxacin	-	-	-	-	0(0.00%)	2(7.70%)
Kanamycin	0(0.00%)	3(37.50%)	-	0(0.00%)	16(51.60%)	11(42.30%)
Sulphamethoxazole-trimethoprim	113(100%)	1(12.50%)	2(40.00%)	-	17(54.50%)	1(3.90%)
Chloramphenicol	0(0.00%)	-	-	0(0.00%)	6(19.40%)	11(42.30%)
Amoxicillin	113(100%)	3(37.50%)	1(20.00%)	-	-	-
Clindamycin	-	-	-	-	-	-
Erythromycin	-	6(75.00%)	-	-	-	-
Nitrofurantoin	-	2(25.00%)	2(40.00%)	-	-	7(26.70%)
Spectinomycin	-	2(25.00%)	-	-	-	-
Penicillin	113(100%)	-	-	-	-	-
Amikacine	0(0.00%)	-	-	-	-	-
Norfloxacin	-	-	-	-	-	-
Amoxicillin+ clavulinic acid	-	-	-	7(63.60%)	-	11(42.30%)
Cephalothin	-	-	0(0%)	-	-	12(46.2%)
Trimethoprim	-	-	-	-	-	1(3.9%)
Sulfisoxazole	-	-	-	-	-	24(92.3%)
Neomycin	-	-	-	-	-	3(11.5%)
Ceftriaxone	-	-	0(0%)	-	-	-

Table 2: Antimicrobial Resistance Profile of Poultry *Salmonella* of Ethiopia.

Conclusion and Recommendations

Salmonella infection remains a one of major poultry pathogen. Concerns due to the emergence of AMR in poultry farm which also creates public health concerns due to the presence of antimicrobial residues in poultry meat and eggs. Furthermore, AMR in poultry pathogens is likely to lead to economic losses, derived from the expenditure on ineffective antimicrobials, as well as the burden of untreated poultry disease. Therefore, this review provides the prevalence, associated risk factors and antmicrobial resistance patterns of *Salmonella* from chickens in the area.

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