Determination of the sources and antimicrobial resistance patterns of Salmonella isolated from the poultry industry in Southern Ethiopia

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Abstract

Background: Ethiopia set an ambitious masterplan to increase chicken meat and egg production from 2015 to 2020. Poultry breeding, multiplication and distribution centers in the country have received executive order to import, amplify and distribute commercial chickens to end users. The biosecurity and the pathogen fauna of the centers have not been evaluated as to whether the centers could implement the mission effectively without any risk. Thus, the aim of this study was to evaluate the biosecurity practices and the pathogen prevalence, risk factors and their antimicrobial resistance (AMR) using Salmonella as case study.

Methods: Routine farm workers of the centers were interviewed about the different management (biosecurity) practices using a checklist. Samples (n = 270) from different sources consisting of chicken’s cloacal swab (n = 244), personnel hand swab (n = 9) and bedding (n = 17) were collected from three chicken multiplication centers. Standard bacteriological methods were used for the isolation of Salmonella. Disk diffusion method was used for drug sensitivity testing.

Results: Antimicrobials were often over prescribed without confirming the cause of ill health and without susceptibility testing. The general biosecurity and flock management practices were substandard. Salmonella was isolated from 45 (16.7%) of the 270 samples. Its prevalence was significantly (p<0.05) associated with location of the multiplication center, 27% at Bonga and 10.6% at Hawassa. Sample type was also significantly (p<0.05) affected in that it was higher in the bedding (35.3%) and personnel hand swabs (33.3%) than in the chicken cloaca (14.8%), which demonstrates the poor biosecurity and personnel hygienic practices in the centers. All of the 45 isolates (100%) exhibited resistance to kanamycin and sulfamethoxazole-trimethoprim, nalidixic acid (97.8%), ampicillin (97.8%), cefoxitin (97.8%), streptomycin (97.8%), tetracycline (97.8%), chloramphenicol (91.3%), ciprofloxacin (31.1%), and gentamicin (0%). Alarmingly, 42 isolates (93.4%) exhibited multidrug resistance (MDR) to ≥ 8 drugs and all 45 isolates had resistance to ≥ 3 drugs. The high rate of Salmonella isolation from (i) bedding, (ii) personnel hand swabs (iii) chickens, (iv) presence of more MDR isolates, (v) coupled with poor biosecurity practices in the centers could pose a risk for spreading of pathogens and drug resistant genes to the smallholder chicken producers and the public.

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Conclusions: We conclude that the poultry breeding, multiplication and distribution centers in Ethiopia, as they stand currently, seem to be a source of pathogens and AMR isolates at least for *Salmonella*. Therefore, strict biosecurity, personnel safety, prudent drug use, regular monitoring and traceability of *Salmonella* serotypes or genotypes and AMR are recommended.

Keywords: Antimicrobial resistance, Biosecurity, Multiplication centers, Risk factors, Poultry, *Salmonella* prevalence

**Background**

Ethiopia has about 56.87 million chickens comprising mainly indigenous chickens, with the majority (95%) kept in low-input low-out-put village chicken production systems [1, 2]. Chicken provides an important source of income in addition to offering eggs and meat for the poor smallholder households [1]. However, the 0.5 kg annual per capita egg consumption in Ethiopia is five times lower than the average 2.3 kg per capita for other sub-Saharan African countries [3]. The Ethiopian indigenous chickens cannot be produced and fill the national demand for chicken meat and eggs despite the fact that they harbor suitable phenotypic traits for ecological, social and cultural context of Ethiopia [1, 4]. Rhode Island Red, Australorp, New Hampshire and White Leghorns chickens were imported to Haromaya, Jimma, Bishoftu and Shashemene in 1950s [5]. This led to the establishment of modern poultry farms in 1959 able to distribute commercial chickens to urban producers. Shortly thereafter, seven poultry multiplication and distribution centers were established in different locations under the Ministry of Agriculture to distribute commercial chickens [6]. Although Ethiopia started utilization of imported high producing commercial chicken’s years ago, the supply to fill the demand has never been met, even though the number of multiplication centers has increased to 15 [7]. Poultry multiplication centers are defined as those government owned centers engaged in importation of improved commercial chickens from developed countries, rear, multiply and distribute chicks of mostly up to 3 month old all over the country. The centers are not for profit as chicks are sold with around 50% below real market prices unlike the private commercial poultry farms [7]. However, the poultry subsector has remained at subsistence level. The national demand for chicken products remains still under-met, and hence malnutrition and poverty is widespread. Thus, Ethiopia has launched recently a master plan for livestock development. The plan aims to increase chicken meat production by 247% and egg production by 827% in five years, 2020 [8].

Improved commercial laying hens, which produce about 325 eggs per year and broilers that can reach 2.5 kg in 42 days with high feed efficiency and product quality are available elsewhere [9]. These animals are targeted for importation and incorporation in the current Ethiopian livestock development master plan based on preliminary experiences on these animals in the country [10–12].

In Ethiopia, farm hygiene, biosecurity, diagnostic capabilities, monitoring, tracing and notification of pathogens and AMR at the farm and national level are not well developed [7, 13] and uncontrolled antimicrobial use and misuse is common [14]. Thus, the burden of pathogens in poultry multiplication centers is poorly known in Ethiopia, which may entail risk of distributing infected chickens unless appropriately handled.

One of the major pathogens devastating the poultry industry is *Salmonella* [15]. Salmonellosis causes severe economic damage to chicken production by reducing production, 100% morbidity, and 20% mortality in affected flocks [15]. Furthermore, the vertical and horizontal transmission ability of *Salmonella* complicates the spread and control of the disease; hence, it remains a problem on the farm for prolonged time [16]. *Salmonella* is also known as one of the major food-borne zoonotic pathogens. In United State, it is responsible for 1.4 million cases of human illness, 20,000 hospitalizations and over 500 deaths annually [17]. The ability of *Salmonella* to acquire antimicrobial resistance (AMR) is another capability of this pathogen rendering it difficult to eliminate. The emergence and spread of such resistant strains among food animals is life threatening and a global public health concern, as they are often non-treatable with currently available antimicrobials [18]. Animal agriculture such as poultry farming and antibiotic usage on the farms are a hot debate topic, because the overuse may be a contributing factor for the emergence of AMR pathogens and AMR genes to the food chain [19]. Thus, *Salmonella* in poultry remains a political issue due to its impact on food production, animal welfare, public health and antimicrobial resistance.

A study in northern Ethiopia reported that the prevalence of *Salmonella* in the local and commercial breeds was 39.3% and 29.2%, respectively [20], 42.7% in indoor chickens and 40.8% in free ranging in western Oromia at Jimma [21]. The limited studies have reported the presence of multidrug resistant *Salmonella* strains in chicken farms [22, 23], which cannot be killed by antimicrobials and hence increase the morbidity, mortality and costs.
associated disease. Overall, studies conducted on *Salmonella* in Ethiopia are scarce. It is difficult to understand the real picture in the country because of the diverse chicken population growing in the different agro-ecologies of the country [1, 4]. Considerable differences in level of susceptibility to *Salmonella* have been reported among different chicken breeds [24]. Moreover, controlling *Salmonella* in poultry operations is complicated because there are numerous potential sources of contamination, including chicks, feed, rodents, wild birds, equipment and personnel in the poultry processing plant [25] as well as hatcheries [26]. One of the key areas for controlling *Salmonella* is to know its source and dissemination pathways. Ethiopia has developed research-extension programs that interact to transfer improved technology packages from agricultural research centers (e.g. commercial chicken breeding, multiplication and distribution centers) to end users [27]. However, little is known about where *Salmonella* establishes at the poultry breeding, multiplication and/or distribution centers in Ethiopia. Therefore, the aim of this study was to (i) identify poor farm practices in biosecurity that trigger pathogen flare-ups, (ii) determine the prevalence and (iii) AMR patterns of *Salmonella* isolates in three poultry breeding, multiplication and distribution centers in Southern Ethiopia.

**Methods**

**Description of study areas**

This study was conducted in Hawassa and Bonga, Southern Nations, Nationalities and Peoples Region (SNNPR), from November 2014 to May 2015. Hawassa is the capital of the SNNPR and is located at 275 km south of Addis Ababa. The annual rainfall and temperature of the area varies from 800 mm to 1000 mm and 20 °C to 25 °C, respectively. Bonga is an administrative city for Kaffa zone and is situated at 460 km south-west of Addis Ababa. The mean annual rainfall in the area is about 1710 mm, and the monthly temperature varies from 18 °C to 21 °C [2, 28].

**Study design and samples**

A cross-sectional study was used, and sampling was conducted only once. Of four poultry multiplication centers in SNNPR, two (i.e. Bonga and Hawassa centers) were randomly selected. White leg horn breed was reared at both Bonga and Hawassa multiplication centers. Bonga Poultry Multiplication Center (BPMC) is mandated mainly to distribute improved commercial chickens to zones in the south and western part of the region, such as Kaffa, Shaka and Bench Maji zones. Hawassa Poultry Multiplication Center (HPMC) serves zones in the northeastern of the region. In addition, one commercial farm for Bovans brown breed was included from Hawassa for comparison. The Bonga center had three houses for layer and eight houses for growers. HPMC had 12 houses for layers and 4 houses for growers and chicks. Hawassa commercial farm had houses for layers alone. These multiplication centers disseminate growers and chicks of white leghorn and brown Bovans breeds for producers and farmers. The average number of chickens per house was 1000.

The minimum required sample size was calculated based on an expected prevalence of 15.4% [23] with 95% desired confidence interval and 5% absolute precision using the formula described in Thrusfield et al. [29]. Accordingly, 270 chickens (100 from BPMC, 90 from HPMC and 80 from Hawassa commercial farm) were sampled. In addition, environmental (bedding) samples were collected from these farms. Hand swabs of six personnel working in the poultry houses were sampled from Hawassa multiplication center and three from Bonga. The personnel were healthy and had no symptoms of infection. Human samples were collected based on an ethical approval (Ref. RD/LT/194/2013) and sampled based on their consent. The aim of hand swabbing was to evaluate the hygiene and biosecurity practices of workers in order to use the results as a risk indicator for the development of zoonoses and involvement of the workers in the dissemination of the pathogens on the farm(s). Along with the hand swab samples, farm attendants were interviewed in relation to the routine management practices of the multiplication centers and farm that could be risk factors for salmonellosis.

**Sampling and sample transportation**

A sterile cotton tipped swab (2 × 3 cm) was first soaked in approximately 10 mL buffered peptone water (BPW, Oxoid Ltd., England). Then cloacal/fecal samples were collected using the pre-moistened swab. In addition, both sides of the right and left hands of farm attendants were rubbed using pre-soaked swab. Each swab sample was placed in a separate universal bottle with 4 mL BPW. The wooden shaft was broken off; and the cotton swab was left inside the bottle. The swab samples were taken according to the method described in ISO-17604 (2003) [30]. The collected samples were transported in icebox to the Laboratory of Microbiology in College of Veterinary Medicine and Agriculture, Addis Ababa University.

**Bacteriological isolation and identification**

The isolation was performed using the conventional bacteriological methods for the detection of *Salmonella* standard guidelines [31]. The bacteriological culture media and biochemical tests were prepared according to manufacturer's recommendations.
For the confirmation of the typical Salmonella colonies using biochemical tests, typical and suspected colonies of Salmonella were selected from the selective plating media, streaked onto the surface of nutrient agar (Oxoid Ltd., England) plates and incubated at 37 °C for 24 h according to ISO 6579 [31]. All suspected non-lactose fermenting Salmonella colonies were picked from the nutrient agar and inoculated into triple sugar iron (TSI) agar, Simmon’s citrate agar, urea broth, tryptone water, methylene red and Voges-Proskauer (MR-VP) broth (Oxoid Ltd., England) and incubated for 24 at 37 °C. A colony was considered Salmonella if an alkaline slant (Red) with acid butt (yellow) on TSI with hydrogen sulfide was produced, positive for lysine (purple), negative for urea hydrolysis (red), negative for tryptophan utilization (indole test) (yellow-brown ring) and negative for Voges-Proskauer (colorless) [32].

Non-selective pre-enrichment
The swab samples were pre-enriched in BPW in the ratio of one to nine (i.e. 25 mL of the sample was inoculated into 225 mL of BPW) and incubated at 37 °C for 24 h.

Selective enrichment
Rappaport-Vassiliadis medium (RV, Oxoid Ltd., England) broth and Müller Kauffman Tetrathionate (MKTT, Oxoid Ltd., England) broth were used for selective enrichment of the samples. From the pre-enriched sample, 0.1 mL was transferred into a tube containing 10 mL of RV broth and incubated at 42 °C for 24 h. Another 1 mL was transferred into a tube containing 10 mL of MKTT broth and incubated at 37 °C for 24 h.

Plating out and identification
Xylose lysine deoxycholate (XLD, Oxoid Ltd., England) agar and brilliant green agar (BGA, Oxoid Ltd., England) plates were used for plating out and identification. A loop full of inoculums from each RV and MKTT broth cultures were plated onto XLD and BGA plates and incubated at 37 °C for 24 h. After incubation, the plates were examined for the presence of typical and suspect Salmonella colonies. Typical colonies of Salmonella grown on XLD agar have a black center and a lightly transparent zone of reddish color due to the color change of the media [31] while hydrogen sulfide-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 change the color of medium to red.

Biochemical tests
For the confirmation of the typical Salmonella colonies using biochemical tests, typical and suspected colonies of Salmonella were selected from the selective plating media, streaked onto the surface of nutrient agar (Oxoid Ltd., England) plates and incubated at 37 °C for 24 h according to ISO 6579 [31]. All suspected non-lactose fermenting Salmonella colonies were picked from the nutrient agar and inoculated into triple sugar iron (TSI) agar, Simmon’s citrate agar, urea broth, tryptone water, methylene red and Voges-Proskauer (MR-VP) broth (Oxoid Ltd., England) and incubated for 24 at 37 °C. A colony was considered Salmonella if an alkaline slant (Red) with acid butt (yellow) on TSI with hydrogen sulfide was produced, positive for lysine (purple), negative for urea hydrolysis (red), negative for tryptophan utilization (indole test) (yellow-brown ring) and negative for Voges-Proskauer (colorless) [32].

Antimicrobial susceptibility testing
The antimicrobial susceptibility testing was performed using the disc diffusion method. The result was interpreted as per the recommendation of National Committee for Clinical Laboratory Standards Institute (CLSI) [33]. In short, isolated colonies from the nutrient agar plates were transferred into tubes containing 5 mL of tryptone soya broth (Oxoid Ltd., England). The broth culture was incubated at 37 °C for 4 h until it achieved 0.5 McFarland turbidity standards. A sterile cotton swab was used to swab the inoculum uniformly over the surface of Muller Hinton agar plate. The plates were held at room temperature for 30 min to allow drying and tested for ampicillin (AMP) 10 μg, gentamicin (CN) 10 μg, kanamycin (K) 30 μg, ciprofloxacin (CIP) 5 μg, chloramphenicol (C) 30 μg, sulfamethoxazole-trimethoprim (SXT) 25 μg, tetracycline (TE) 30 μg, nalidixic acid (NA) 30 μg, streptomycin (S) 10 μg, and cefoxitin (FOX) 30 μg. The plates were incubated at 37 °C for 24 h. The diameter of the zones of inhibition was classified as resistant, intermediate, or susceptible according to the CLSI [33]. All antibiotics were Oxoid Ltd., England made and the expiry dates were 2018.

Data management and analysis
Data of laboratory results were entered in Microsoft Excel spread sheet and analyzed using SPSS version 20. Descriptive analysis was conducted to determine the prevalence of Salmonella in the centers and the farm. Association of risk factors with Salmonella isolation frequencies was analyzed using χ² test (Fisher’s exact test). Furthermore, univariate and multivariate logistic regression analyses were performed to reveal the strength of association of the potential risk factors and Salmonella prevalence. The significance level was set at α = 0.05.

Results
Substandard biosecurity and personnel hygiene practices in the centers
Interviews with center supervisors revealed that the management practices at the breeding and multiplication centers were generally poor as summarized in Table 1. The hygiene of the poultry houses was also substandard, as litter changes and pest control were not carried out regularly. Working and protective materials were commonly used between houses. Personnel engaged in poor safety practices (i.e. hand washing, etc.). Antibiotics were prescribed irrationally based on symptoms without diagnosing the causative agent for the sick animals on the farms. In this regard, suspicion of a single sick chicken in the centers often led to blanket prescription of antimicrobials to all the chickens in the centers. Over prescription of drugs was practiced to
avoid the risk of the undetected spread of pathogens and onset of disease outbreak at the centers (Table 1).

Prevalence of Salmonella distribution
Salmonella was isolated from 45 of the 270 samples (16.67%); 36 of 244 cloacal swabs (14.8%), 6 of 17 pooled bedding sample (35.3%), and 3 of 9 hand swabs sampled from farm attendants (33.3%). Both bedding and hand swab samples had significantly \((p = 0.028)\) higher Salmonella isolation rate than cloacal samples. The prevalence of Salmonella was significantly higher at Bonga than at Hawassa \((p = 0.000)\). The Bonga poultry multiplication center had a significantly higher prevalence of Salmonella than the other two multiplication centers \((p = 0.001)\). Cage versus litter nor Leghorn versus Bovans did not significantly \((p > 0.05)\) affect the prevalence of Salmonella detected (Table 2). Young age groups (chicks and cocks) had a significantly \((p = 0.003)\) higher prevalence followed by layers than the other age groups.

Univariate analysis of risk factors indicated that samples from Bonga had a significantly \((p = 0.001)\) higher odds of being Salmonella positive than those from Hawassa. Study areas, multiplication centers and sample types had a significant association with Salmonella prevalence (Tables 2 and 3). Finally, multivariate logistic regression analysis was conducted for association between risk factors and Salmonella isolation. After the effect of other risk factors were adjusted for, only sample type had a significant effect on Salmonella recovery. In this regard, bedding had higher Salmonella contamination than chicken (cloacal) samples \([\text{OR for bedding vs. poultry cloacal} = 7.5; 95\% \text{ CI for OR} = 2.0–25; p = 0.002]\).

Antimicrobial susceptibility test
Forty-five Salmonella isolates were subjected to the antimicrobial susceptibility test. All of the 45 isolates (100%) were resistance to kanamycin and sulfamethoxazole-trimethoprim. Almost all (44 isolates, 97.8%) also had resistance to ampicillin, cefoxitin, nalidixic acid, streptomycin, and tetracycline. Resistance to chloramphenicol and ciprofloxacin was 91.1% and 31.1%, respectively. All isolates (100%) were susceptible to gentamicin with three isolates manifested intermediate breakpoints (Table 4).

### Table 1

| No. | Risk factors related to Salmonella emergence, maintenance and spread | Bonga center | Hawassa center | Hawassa commercial farm |
|-----|---------------------------------------------------------------------|--------------|----------------|-------------------------|
| 1   | Litter change when new flock introduced to the house                | No           | No             | No                      |
| 2   | Poultry-house disinfection before placement                         | Yes          | Yes            | Yes                     |
| 3   | Presence of pests in the houses (cockroaches, rats, etc.)           | Yes          | Yes            | No                      |
| 4   | Pest control                                                        | No           | No             | No                      |
| 5   | Presence of other animal species in the farm                        | No           | No             | No                      |
| 6   | Farm access to wild birds                                           | Yes          | Yes            | Yes                     |
| 7   | Boots for workers                                                   | Yes          | Yes            | Yes                     |
| 8   | Common use of working and protective materials between houses        | Yes          | Yes            | Yes                     |
| 9   | Always boots dipping before entering poultry houses                  | No           | No             | No                      |
| 10  | Free access to visitors and other workers of the farm               | Yes          | Yes            | Yes                     |
| 11  | Source of water for chickens                                        | Well         | Well           | Pipe                    |
| 12  | Conservation of sick chickens together with layer house             | Yes          | Yes            | Yes                     |
| 13  | Storage of manure inside the farm compound                          | Yes          | Yes            | Yes                     |
| 14  | Antibiotic use without identification of disease etiology           | Yes          | Yes            | Yes                     |
| 15  | Poultry management types                                            | Litter       | Litter         | Cage                    |
| 16  | Washing of hands before handling chickens                            | No           | No             | No                      |
| 17  | Regular cleaning of watering and feeding trough                     | No           | No             | No                      |
| 18  | All in-all out practice                                             | No           | No             | No                      |
| 19  | Disposal of a dead chicken in the compound                          | Yes          | Yes            | No                      |
| 20  | Rearing of different age groups of chickens in the farm             | Yes          | Yes            | No                      |
| 21  | Regular health checkup for workers                                  | No           | No             | No                      |
| 22  | Recent history of workers having signs of diarrhea                   | Yes          | Yes            | No                      |
Phenotypic patterns to antimicrobial resistance

All 45 isolates (100%) were multi-drug resistant to ≥3 antimicrobials. Forty-two isolates (93.3%) exhibited resistance to ≥8 antimicrobials simultaneously (Table 5). The 45 Salmonella isolates manifested nine different phenotypic patterns to 10 antimicrobials. The most widely distributed pattern was resistance to AMP*C*CIP-I*FOX*K*NA*S*SXT*TE with 24 (53.3%) of the 45 isolates exhibiting this pattern. Nine of the 45 isolates (20.0%) displayed the AMP*C*CIP*FOX*K*NA*S*SXT*TE pattern.

Three Salmonella isolates from hand swabs displayed disturbing AMR to 8 antimicrobials (two isolates) and 9 antimicrobials (one isolate). All isolates from personnel and poultry bedding were resistant to at least eight antimicrobials, but susceptible to gentamicin and ciprofloxacin (Table 6). The six isolates from bedding samples revealed resistant to eight or nine antimicrobials (four and two isolates respectively). The distribution of resistance patterns of the isolates across different locations, management, breed and sample type in southern Ethiopia is indicated in Table 6.

Table 2 Prevalence of Salmonella in chickens, farm attendants and bedding in Hawassa and Bonga chicken breeding and multiplication centers in Southern Ethiopia

| Risk Factors                  | # sample | Positive | Prevalence (%) | p-value |
|------------------------------|----------|----------|----------------|---------|
| Study area                   |          |          |                |         |
| Bonga                        | 100      | 27       | 27             | 0.000   |
| Hawassa                      | 170      | 18       | 10.6           |         |
| Farms                        |          |          |                |         |
| Bonga multiplication center   | 100      | 27       | 27             | 0.001   |
| Hawassa multiplication center | 80       | 11       | 13.8           |         |
| Hawassa commercial farm 2    | 90       | 7        | 7.8            |         |
| Sample type                  |          |          |                |         |
| Bedding (environment)        | 17       | 6        | 35.3           | 0.028   |
| Hand swab (personnel)        | 9        | 3        | 33.3           |         |
| Cloacal swab (chickens)      | 244      | 36       | 14.8           |         |
| Housing                      |          |          |                |         |
| Litter                       | 190      | 34       | 17.9           | 0.404   |
| Cage                         | 80       | 11       | 13.9           |         |
| Breed                        |          |          |                |         |
| White leghorn                | 190      | 34       | 17.9           | 0.404   |
| Bovans                       | 80       | 11       | 13.8           |         |
| Age group                    |          |          |                |         |
| Chicks                       | 21       | 10       | 47.6           | 0.003   |
| Cock                         | 19       | 4        | 21.1           |         |
| Cockerel                     | 3        | 0        | 0              |         |
| Layer                        | 194      | 29       | 14.9           |         |
| Pullet                       | 33       | 2        | 6.1            |         |
| Total                        | 270      | 45       | 16.7           |         |

Table 3 Univariate analysis of Salmonella infection using various risk factors in Hawassa and Bonga chicken breeding and multiplication centers in Southern Ethiopia

| Risk Factors          | Variables     | OR  | 95% CI for OR | p-value |
|-----------------------|---------------|-----|---------------|---------|
| Study area            | Bonga vs. Hawassa | 3.1 | 1.6–6.0       | 0.001   |
| Breeding center       | Bonga vs. Hawassa | 2.3 | 1.1–5.0       | 0.033   |
| Sample source         | Bedding vs. Chicken | 8.3 | 2.5–25.0      | 0.000   |

Discussion

Salmonella is one of the important pathogens in the poultry industry [34]. Additionally, it is one of the major cause of bacterial foodborne gastroenteritis of humans with poultry being indicated as the major source for human infection [17, 35]. Salmonella can enter the food supply at many points in the food production chain (processing, distribution, preparation, or handling steps). Thus, food safety should involve intervention at all of the steps along the production chain in order to safeguard the health of consumers [36]. Some intervention have been successful against Salmonella in the processing plants [37]. The effect of Salmonella intervention on the farms to reduce Salmonella prevalence along the food chain requires further evaluation. Understanding of the ecology of Salmonella helps to reduce horizontal and vertical transmission. Furthermore, tracing the origin of Salmonella outbreaks and the antimicrobial resistant patterns of isolates are fundamental to containing the risk. However, little is known about the Salmonella problem in chicken breeding, multiplication and distribution centers in Ethiopia. The present study was conducted to determine the prevalence of Salmonella and their AMR in poultry production and multiplication centers in southern Ethiopia.

The overall prevalence of Salmonella was 16.7% in this study. Our finding is similar to the 17.9% prevalence on layer farms in Italy [38], but lower than the 42.7% reported from Jimma in western Ethiopia [21] and 36.1%
Table 4  Inhibition ranges and number of resistant, intermediate and susceptible isolates of *Salmonella* to 10 antimicrobials tested as per the CLSI (2012) [34]

| Inhibition diameter (mm)* | SXT | S | TE | C | CN | AMP | K | NA | FOX | CIP |
|--------------------------|-----|---|----|---|----|-----|---|----|-----|-----|
| 0                        | 27  | - | 26 | - | 21 | 24  | 23| -  | -   | -   |
| 6                        | -   | 2 | 1  | 2 | 4  | -   | - | -  | -   | -   |
| 7                        | 4   | 10| 1  | 16| 14 | 16  | - | -  | -   | -   |
| 8                        | 8   | 1 | 6  | 5 | 5  | 2   | 4 | -  | -   | -   |
| 9                        | 5   | 13| 16 | - | 15 | -   | - | -  | -   | -   |
| 10                       | 1   | 19| 7  | - | 21 | 1   | - | -  | -   | -   |
| 11                       | -   | 11| 9  | - | 2  | -   | - | -  | -   | -   |
| 12                       | -   | - | 1  | 1 | 1  | -   | - | -  | -   | -   |
| 13                       | -   | - | 1  | - | -  | -   | - | -  | -   | -   |
| 14                       | -   | 1 | 3  | - | 1  | -   | - | -  | -   | -   |
| 15                       | -   | 1 | 8  | - | 12 | -   | - | -  | -   | -   |
| 16                       | -   | - | 15 | - | -  | -   | - | -  | -   | -   |
| 17                       | -   | - | 1  | 5 | 1  | -   | - | -  | -   | -   |
| 18                       | -   | - | 1  | 2 | 1  | -   | - | -  | -   | -   |
| 19                       | -   | - | 1  | 2 | 1  | -   | - | -  | -   | -   |
| 20                       | -   | - | 1  | 2 | -  | -   | - | -  | -   | -   |
| 21                       | -   | - | 2  | - | 1  | -   | - | -  | -   | -   |
| 22                       | -   | - | 1  | 3 | 3  | -   | - | -  | -   | -   |
| 23                       | -   | - | 5  | - | 3  | -   | - | -  | -   | -   |
| 24                       | -   | - | -  | 1 | 1  | -   | - | -  | -   | -   |
| 25                       | -   | - | -  | 1 | 4  | -   | - | -  | -   | -   |
| 26                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |
| 27                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |
| 28                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |
| 29                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |
| 30                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |
| 31                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |
| 32                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |
| 33                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |
| 34                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |
| 35                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |
| 36                       | -   | - | -  | 1 | -  | -   | - | -  | -   | -   |

No. isolates 45 45 45 45 45 45 45 45 45 45
No. resistant isolates 45 44 44 41 0 44 45 44 44 14
Prevalence of resistance (%) 100 97.8 97.8 91.1 0 97.8 100 97.8 97.8 31.1

AMP ampicillin; C chloramphenicol; CN gentamicin; CIP ciprofloxacin; FOX cefoxitin; K kanamycin; NA nalidixic acid; S streptomycin; SXT sulfamethoxazole-trimethoprim; TE tetracycline

*The diameter indicated zone of inhibition of the antimicrobials during disc diffusion test (mm). The top dark shaded part within the table indicated breakpoints for resistance along with the frequency of isolates. The middle unshaded part indicated breakpoints for intermediate and the lower green shaded part indicated susceptible isolates

Table 5  Lists of AMR patterns of *Salmonella* isolates from chicken breeding and multiplication centers in response to 10 antimicrobials in Southern Ethiopia

| The nine AMR pattern | Number antimicrobials resistance | Number of isolates | Prevalence (%) |
|----------------------|----------------------------------|--------------------|----------------|
| C*K*S-I **SXT        | (+S-I)                           | 1                  | 2.2            |
| AMP*C*i*FOX*K*NA*S*SXT*TE | (+C-I)                      | 1                  | 2.2            |
| AMP*C*FOX*K*NA*S*SXT*TE | (+C-I)                      | 1                  | 2.2            |
| AMP*C*FOX*K*NA*S*SXT*TE | (+CIP-I)                     | 4                  | 8.9            |
| AMP*C*FOX*K*NA*S*SXT*TE | (+CIP-I)                     | 2                  | 4.4            |
| AMP*C*FOX*K*NA*S*SXT*TE | (+CIP-I)                     | 24                 | 53.3           |
| AMP*C*FOX*K*NA*S*SXT*TE | (+CIP-I)                     | 1                  | 2.2            |
| AMP*C*FOX*K*NA*S*SXT*TE | (+CIP-I)                     | 9                  | 20.0           |
| AMP*C*FOX*K*NA*S*SXT*TE | (+CIP-I)                     | 2                  | 4.4            |
| AMP*C*FOX*K*NA*S*SXT*TE | (+CIP-I)                     | 45                 | 100.0          |

AMP ampicillin; AMR antimicrobial resistance; C chloramphenicol; CN gentamicin; CIP ciprofloxacin; FOX cefoxitin; K kanamycin; NA nalidixic acid; S streptomycin; SXT sulfamethoxazole-trimethoprim; TE tetracycline

*I indicates intermediate
in broilers in Japan [39]. The variation in prevalence reported could be due to the variation in the sample type, location, breed, and/or age of the chickens [40]. Given that observation of a single sick chicken can lead to a blanket prescription of antimicrobials to the entire flock (as per the respondents), the intensive antimicrobial use currently could also contribute to the lowered prevalence in our findings. The present finding was higher than the 8% prevalence reported from small-scale chicken farms from Hawassa [41]. It has been reported that farm type and chicken flock density might have a significant impact on the prevalence of Salmonella, particularly about 7 times higher on large-scale versus small-scale farms [42] and nearly 8 times higher on conventional compared to organic farms [43].

In the current study, breed, age, sample type and location have been evaluated as triggering factors for Salmonella occurrence. In this regard, both White Leghorn and Bovans Brown chicken breeds were equally susceptible. However, some researchers have reported that breed differences on the level of susceptibility to Salmonella exists among different chicken lines [24, 44]. In this regard, resistant lines to S. Typhimurium have also shown resistance to S. Gallinarum, S. Pullorum, and S. Enteritidis, and lines susceptible to S. Typhimurium are more susceptible to the other Salmonella serotypes [44]. This suggests that a general mechanism of resistance that may apply to all serotypes of Salmonella in chicken [44]. Three levels of susceptibility have been noticed among chicken lines to Salmonella. These include (i) highly susceptible chicken lines which reveal greater localization of Salmonella in the reproductive tract (vertical transmission) in addition to harboring Salmonella in other body parts, (ii) moderately resistant which reveal greater localization of Salmonella in the intestine along with long term persistence in the liver and spleen, and (iii) highly Salmonella resistant chicken lines [45]. As prophylactic measures, vaccination and use of antibiotics are insufficient to eradicate salmonellosis in poultry stocks, some researchers have tried to control Salmonella infection, its carrier-state and drug resistant Salmonella strains through rearing Salmonella resistant chicken lines [46].

### Table 6 Antimicrobial resistance patterns of Salmonella isolates collected from chicken multiplication centers of different locations, management, breed and sample type in Southern Ethiopia

| AMR pattern (No. of drugs showing resistant + intermediate) | Study area (N = 45) | Management type | Breed type | Sample type |
|-------------------------------------------------------------|---------------------|----------------|----------|-------------|
| AMP*C-FOX*K*NA*S*SXT*TE (7 + 9)                             | Hawassa 1 2.2       | Litter 1 2.2   | WLH 1 2.2 | Cloacal swab 1 2.2 |
| AMP*C*CIP*FOX*K*NA*S*SXT*TE (8 + 1)                         | Bonga 10 22.2       | Cage 8 17.8    | Bovans 8 17.8 | Cloacal swab 18 40.0 |
|                                                           | Hawassa 14 31.1     | Litter 16 35.6 | WLH 16 35.6 | Hand swab 2 4.4 |
|                                                           | Total 24 53.3       | Total 24 53.3  | Total 24 53.3 | Total 24 53.3 |
| AMP*C*CIP*FOX*K*NA*S*SXT*TE (9)                             | Bonga 8 17.8        | Cage 1 2.2     | Bovans 1 2.2 | Cloacal swab 8 17.8 |
|                                                           | Hawassa 1 2.2       | Litter 8 17.8  | WLH 8 17.8 | Bedding 1 2.2 |
|                                                           | Total 9 20.0        | Total 9 20.0   | Total 9 20.0 | Total 9 20.0 |
| AMP*C-CN*CIP*FOX*K*NA*S*SXT*TE (8 + 2)                      | Bonga 1 2.2         | Litter 1 2.2   | WLH 1 2.2 | Cloacal swab 1 2.2 |
| AMP*C-CN*CIP*FOX*K*NA*S*SXT*TE (9 + 1)                      | Bonga 2 4.4         | Litter 2 4.4   | WLH 2 4.4 | Hand swab 1 2.2 |
|                                                           | Bedding 1 2.2       | Total 2 4.4    | Total 2 4.4 |
| AMP*C*FOX*K*NA*S*SXT*TE (8)                                 | Bonga 3 6.7         | Cage 1 2.2     | Bovans 1 2.2 | Cloacal swab 4 8.9 |
|                                                           | Hawassa 1 2.2       | Litter 3 6.7   | WLH 3 6.7 |     |
|                                                           | Total 4 8.9         | Total 4 8.9    | Total 4 8.9 |
| AMP*C-CN*FOX*K*NA*S*SXT*TE (7 + 1)                          | Bonga 1 2.2         | Litter 1 2.2   | WLH 1 2.2 | Cloacal swab 1 2.2 |
| AMP*C-CN*FOX*K*NA*S*SXT*TE (8)                              | Bonga 1 2.2         | Cage 1 2.2     | Bovans 1 2.2 | Cloacal swab 2 4.4 |
|                                                           | Hawassa 1 2.2       | Litter 1 2.2   | WLH 1 2.2 |     |
|                                                           | Total 2 4.4         | Total 2 4.4    | Total 2 4.4 |
| C*K*S*I*SXT (3 + 1)                                         | Bonga 1 2.2         | Litter 1 2.2   | WLH 1 2.2 | Cloacal swab 1 2.2 |

#Number of isolates exhibiting the indicated resistance pattern in the respective rows. The “(+)” in the first column indicated number of drug types that manifested intermediate response category to the isolates. The number of isolates tested for AMR from Hawassa (n = 18), Bonga (n = 27), Cage (n = 11), Litter (n = 34), Bovans Brown (n = 11), White Leghorn (WLH) (n = 34), Cloacal swab (n = 36), bedding (n = 6) and personnel hand swab (n = 3). AMP, ampicillin; AMR, antimicrobial resistance; C, chloramphenicol; CN, gentamicin; CIP, ciprofloxacin; FOX, cefoxitin; K, kanamycin; NA, nalidixic acid; S, streptomycin; SXT, sulfamethoxazole-trimethoprim; TE, tetracycline

##Indicates intermediate breakpoints
Young age groups (chicks and cock) harbored higher Salmonella prevalence in this study. This suggests a higher susceptibility of younger chicks to Salmonella. In this regard, it has been reported that the ability to survive infection increases with age, and mortality is greatest in newly hatched chicks [47]. Unfortunately, once Salmonella infects chicks at a young age, they remain in a carrier state for a prolonged period of time [48]. The high susceptibility and inability of the young chicks to clear the infection (persistent carrier) could be due to the fact that infection at a younger age is less effective in producing protective immunity than infection in older chickens [48]. Chicks infected at 4-days of age remain (i) a carrier until they start lay eggs, (ii) produced Salmonella infected eggs; and hence, (iii) the progeny is infected via vertical transmission [45]. Following young chicks, laying hens were the next most susceptible age group in this study. The higher prevalence of Salmonella infection in laying hens might be due to physiological stress of egg production and molting, which significantly depresses the immune response and increases the susceptibility to Salmonella infection [49]. The interesting part of Salmonella is its ability to infect chicks at early age, persist for long time in the infected chickens and allows for vertical transmission to the progeny through contaminated eggs. This may complicate the epidemiology and control of Salmonella in chicken multiplication centers of Ethiopia, as Salmonella was highly abundant among young chicks followed by laying hens in this study.

The abundance of Salmonella distribution was higher in Bonga (27%) than Hawassa (10.6%) area in the present study. Significantly more isolates were detected in Bonga (27%) than Hawassa (10.6%) area in the present study. Significantly more isolates were detected in Bonga (27%) than Hawassa (10.6%) area in the present study. Our finding is lower than the 58.8% reported from poultry bedding in Nigeria [51]. The difference may be due to the environmental persistence of Salmonella which affects its epidemiology in poultry by creating opportunities for the horizontal transmission of Salmonella among chickens within the flocks [52] and Salmonella can be introduced into the flocks from many different sources [25, 26]. There are a lot of possible sources of Salmonella contamination in poultry flocks from cockroaches and lesser mealworms which can carry Salmonella internally and externally and spread Salmonella throughout the flock [53, 54]. Mice have also been important vectors for Salmonella in laying hens [55]. Practices such as unhygienic farming activities, lack of adequate biosecurity measures and movement of birds and equipment from one house or farm to another often worsens the situation. Mice, wild birds, ants and other insects have been shown to be important agents for Salmonella transmission among chickens, flocks and farms [56].

Currently, 33.3% of the 9 attendants carried Salmonella on their hands contributing three isolates (6.7%) of the 45 Salmonella isolates collected in this study. Personnel hand contamination is a real concern due to limited supply of personnel hygiene supplies, poor hand washing practices and the high levels of Salmonella in the beddings of this study. These factors could lead to (i) self-infection of personnel from the Salmonella on their hands and possible contamination of their families, (ii) spread of infection to individuals in the centers and guests by routine hand shaking as part of the cultural greetings in Ethiopia and (iii) spreading infection between poultry houses in the centers. Prompt attention is necessary to address the contamination of personnel hands in order to safeguard the safety of the personnel and the animals on the farms. Salmonella is responsible for 1.4 million cases of human illness, 20,000 hospitalizations and over 500 deaths annually in US [17] of which 46.4% of the isolates are from animal sources mainly poultry [35]. Of concern in this study is that all of the isolates from hands were multidrug resistant.

Fifty percent of the isolates in this study were resistant to at least one antimicrobial. This is in agreement with the 57–66% of the isolates in Europe [57] and 79.8% of isolates from processed poultry in US [18]. Another study of a national monitoring system reported AMR of 30.5% among the total US isolates [58].

In this study, the most effective drug was gentamicin with 0/45 (0%) isolates resistant, followed by ciprofloxacin.
with 14/45 (31.1%) were resistant. No reports of resistance to gentamycin or ciprofloxacin have been made in sheep and goats isolates in central Ethiopia [59], dairy farm isolates in Addis Ababa [60] or human isolates in Nigeria [61]. Gentamycin is still effective against Salmonella irrespective of the time or location of the study. We have not found information on whether gentamycin is or is not widely used, prescribed or distributed in Ethiopia or other African countries. Lack of use of these two antimicrobials could explain the lack of resistance in the recovered isolates. In two previous studies conducted in Ethiopia, 41.2 and 46% of isolates were resistant to tetracycline; 22.5 and 75% were resistant to streptomycin; and 30% were resistant to chloramphenicol. In our study, 100% of isolates were resistant to kanamycin and sulfamethoxazole-trimethoprim; 97.8% of isolates were resistant to ampicillin, cefoxitin, nalidixic acid, streptomycin and tetracycline; and 91.1% of the isolates were resistant to chloramphenicol. These findings represent a potential increase in the antimicrobial resistance in Ethiopian flocks. This increase could be due to the unregulated increase in the use of prescription antimicrobials [14], often purchased and used by unskilled practitioners in the veterinary and public health sectors [62]. There is a lack of compliance and monitoring for antimicrobials at all levels in Ethiopia. Additionally, the use of antimicrobial drugs at sub-therapeutic or prophylactic dosages in food animals may promote on farm selection of antimicrobial resistance genes in Salmonella as well as other potential human and animal pathogens.

Overall, 42 isolates (93.4%) exhibited a MDR phenotype to more than 8 antimicrobials simultaneously. These MDR isolates had different resistant pattern. Specifically, 11 isolates were resistant to 9 antimicrobials and 31 were resistant to 8 antimicrobials while two were resistant to only 7 antimicrobials and one isolate was resistant to only three antimicrobials. This level of MDR was higher than the 32.65% [63], 44.8% [64], and 83.3% [60] reported previously in Ethiopia. Interestingly, the number of MDR Salmonella isolates has increased in many countries. In the US, 53.4% isolates from processed poultry are MDR [18] and 15–18% of the European isolates [57] are MDR. The widespread resistance of Salmonella isolates in this study may suggest that the gross indiscriminate use of antimicrobial agents in the poultry production setting is responsible for this increase, which has also been suggested by other researchers [65, 66]. In fact, multiplication centers indiscriminately prescribe drugs for the entire flock if ill health is noticed in a single chicken on the ground in order to prevent the spread of infection to healthy chickens at the centers. This excessive misuse of antimicrobial drugs subjects all bacteria present to selective pressures and could lead to the development of resistance to the drugs currently in use and proposed for future use [67]. The incorporation of antibiotics in the animal diets at sub-therapeutic levels to serve as a prophylaxes and/or a growth promotants has invariably contributed to the development of antibiotic resistant strains of Salmonella [68]. Overall, abundance the MDR Salmonella isolates from poultry multiplication centers render poultry meat and eggs a very important potential source of resistant bacteria and hence a serious potential public health concern. However, the limitation of lack of serotype and genotype data in this study precludes the analysis of the relatedness of the specimens collected from different centers including from humans.

Conclusions

Of 270 samples tested, Salmonella was isolated from 45 (16.7%) of the samples. Salmonella was recovered from chicken cloaca, the environment (bedding) and human hands in the multiplication centers. Its distribution was associated with location of the individual center/farm, age of the chickens and the sample type indicating these factors are important potential factors for intervention. Substandard management practices and poor biosecurity exposes the birds to numerous potential sources of Salmonella. Overall, 93.4% of the 45 isolates exhibited resistance against ≥8 antimicrobials while two isolates were resistant to 7 antimicrobials and one was resistant to three antimicrobials. This implies that all isolates are MDR (100%). The high percentage of MDR isolates recovered indicate the potential importance of chickens as a source of MDR Salmonella infections and a serious public health concern, because the multiplication centers rely on the over use of drugs while practicing poor biosecurity and sub-standard management. We conclude that the poultry breeding, multiplication and distribution centers in Ethiopia, as they currently stand, are a source for the dissemination of pathogens and drug resistant pathogens, at least Salmonella. Therefore, we recommend personnel training on personal hygiene, biosafety and farm biosecurity for all farm attendants and visitors; education on zoonotic pathogens; restrictions on the uncontrolled use of antimicrobials; and establishment of a standard pathogen and AMR monitoring system in poultry production. Overall, we hypothesize that control of Salmonella infections in poultry will be one of the important steps to control Salmonella infections in public health.

Abbreviations

AMP: ampicillin; AMR: antimicrobial resistance; BPMC: Bonga poultry multiplication center; C: chloramphenicol; CN: gentamicin; FOX: cefoxitin; HPMC: Hawassa poultry multiplication center; K: kanamycin; MDR: Multi-drug resistance; NA: nalidixic acid; S: streptomycin; SNPR: Southern Nations, Nationalities and Peoples Region; SXT: sulfamethoxazole-trimethoprim; TE: tetracycline
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Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

Conceived and designed the experiments: RDA, TB, AFB, BM, DA, FA. Performed the experiments: RDA, FMT, TB, BM, FA. Analyzed the data: RDA, FMT, AFB. Contributed reagents/materials/analysis tools: RDA, HW, BM, DA, FA. Wrote the paper: RDA, FM. Improved the paper: RDA, TB, AF. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Permission on ethical approval was obtained from the College of Veterinary Medicine and Agriculture of Addis Ababa University (Ref: RD/LT/194/2013). Additionally, human samples were collected following prior explanation of the objectives of the study to the farm workers, and sampled based on their consent.

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References

1. Duguma R. Understanding the role of indigenous chicken during the long walk to food security in Ethiopia. Livestock Res Rev Dev. 2009;21(116). http://www.lrrd.org/lrrd21/8/dugu21116.htm. Accessed 07 Feb 2017.
2. CSA (Central Statistical Agency). Agricultural Sample Survey 2014/15 [2007 E.C]. Volume II report on livestock and livestock characteristics (private peasant holdings). Addis Ababa, 2015.
3. Boere a, Vernooij a, duns H, Legesse M, Kidane D. Business opportunities for backward transformation. A contribution to the Growth and Transformation Plan II (2017/2025) (Private peasant holdings, Meher Season). Addis Ababa; 2015.
4. Duguma R. Phenotypic characterization of some indigenous chicken ecotypes of Ethiopia. Livestock Res Rev Dev. 2006;18(131). http://www.lrrd.org/lrrd18/9/dugu18131.htm. Accessed 07 Feb 2017.
5. Wiggins LL. IECA and JATS staff report. Agriculture of Ethiopia. 1958:58–61.
6. Herdick J. IECA and JATS staff report. Agriculture of Ethiopia. 1961:8.p.
7. Pagani F, Wossene A. Review of the new features of the Ethiopian poultry sector biosecurity implications. Food and Agriculture Organization of the United Nations. FAO. 2008;29. http://www.fao.org/docrep/013/a837e/a837e00.pdf Accessed 08 Feb 2017.
8. Ethiopia Livestock Master Plan (ELMP), Roadmaps for growth and transformation. A contribution to the Growth and Transformation Plan II (2015–2020). ILRI Editorial and Publishing Services. Addis Ababa, Ethiopia; 2015.
9. Biesbes B, Durecq V, Fouille JL, Protas M, Tavernier A, Tixierboichard M, et al. Box-cox transformation of egg-production traits of laying hens to improve genetic parameter estimation and breeding evaluation. Livestock Prod Sci. 1993;33(3):413–26.
10. Dana N, Duguma R, Teklewold H, Aluye S. Transforming village poultry systems into small agro-business ventures: a partnership model for the transfer of livestock technologies in Ethiopia. Livestock Res Rev Dev. 2006;18(12). http://www.lrrd.org/lrrd18/12/dana18169.htm. Accessed 08 February 2017.
11. Reta D, Negussie D, Alemu Y. Comparative production performance of two exotic chicken breeds under two different feed regimes in three agro-ecologies of central Oromia, Ethiopia—a step forward for distribution or contract rearing of day old exotic chicks under rural setting. Livestock Res Dev. 2012;24(9). http://www.lrrd.org/lrrd12/9/ret12145.htm. Accessed 08 Feb 2017.
12. Teklewold H, Dada L, Yami A, Dana N. Determinants of adoption of poultry technology: a double hurdle approach. Livestock Res Rev Dev. 2006;18(40). http://www.lrrd.org/lrrd18/3/tek18040.htm. Accessed 08 Feb 2017.
13. Wossene A. Poultry bio-security study in Ethiopia. A consultancy report for Food and Agriculture Organization of the United Nations, Addis Ababa, Ethiopia. 2006:35
14. Belaye T, Endalameaw E, Tolossa Y, Feysia A. Evaluation of rational use of veterinary drugs in Bishoftu. Central Ethiopia BMC. Res Notes. 2015;8:482.
15. Jordan FTV, Pattison M. Poultry diseases. London: WB Saunders Company Ltd; 1996.
16. Liljebjelke K, Hofacre T, White S, Ayers S, Maurer J. Vertical and horizontal transmission of Salmonella within integrated broiler production system. Foodborne Pathog. 2005;2:90–102.
17. Need PS, Slutsker L, Dietz V, McGal LF, Breeze JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis. 1999;6:275–7.
18. Parveen S, Taabodi M, Schwarz JG, Oscar TP, Hartter-Dennis J, White DG. Prevalence and AMR of Salmonella recovered from processed poultry. J Food Prot. 2007;70(11):2466–2472.
19. Davis MF, Price LB, Liu CMH, Silbergeld EK. An ecological perspective on US industrial poultry production: the role of anthropogenic ecosystems on the emergence of drug-resistant bacteria from agricultural environments.Curr Opin Microbiol. 2011;14(3):244–50.
20. Berhe N, Afera B, Abebe N, Tesfaya A, Kalayou S. Serorelevance of Salmonella pullorum infection in local and exotic commercial chicken from Mekelle areas, northern Ethiopia. Rev Electron Vet. 2012;13(9):091204.
21. Kindu A, Addis D. A survey on Salmonella infection among chicken flocks in Jimma town. Ethiopia World Appl Sci J. 2013;21(10):1415–9.
22. Molla B, Medfin A, Alemayehu D. Multiple antimicrobial-resistant Salmonella serotypes isolated from chicken carcass and giblets in Debre Zeit and Addis Ababa. Ethiop J Health Devip. 2003;17:131–9.
23. Bekele B, Ashenafi M. Distribution of drug resistance among enterococci and Salmonella from poultry and cattle in Ethiopia. Triop Anim Health Prod. 2010;4:2851–64.
24. Wiegley P. Genetic resistance to Salmonella infection in domestic animals. Res Vet Sci. 2004;76(3):165–9.
25. Kim A, Lee YJ, Kang MS, Kwag SI, Cho JK. Dissemination and tracking of Salmonella spp. in integrated broiler operation. J Vet Sci. 2007;8(2):155–61.
26. Svaramalingam T, Pearl DL, McEwen SA, Oijik D, Guerin MT. A temporal study of Salmonella serovars from food samples from poultry breeder hatcheries in Ontario between 1998 and 2008. Can J Vet Res. 2013;77(1):12–8.
27. Teshome A, Agriculture, growth and poverty reduction in Ethiopia: policy processes around the new PRSP (PASDEP). In a paper for the Future Agricultures Consortium Workshop, Institute of Development Studies, University of Sussex, UK; 2006. p. 20–22.
28. CSA (Central Statistical Agency). Agriculture sample survey 2014/2015 (2007 E.C). Volume VII. Report on crop and livestock product utilization (Private peasant holdings). Meher Season). Addis Ababa; 2015.
29. Thrusfield M. Veterinary Epidemiology. 2nd ed. Black well, Cambridge; 2005.
30. Quinn PJ, Carter ME, Markey B, Carter GR. Enterobacteriaceae spp. in integrated broiler operation: the role of anthropogenic ecosystems on the emergence of drug-resistant bacteria from agricultural environments. Curr Opin Microbiol. 2011;14(3):244–50.
31. Kim A, Lee YJ, Kwag SI, Cho JK. Dissemination and tracking of Salmonella spp. in integrated broiler operation. J Vet Sci. 2007;8(2):155–61.
32. Thrusfield M. Veterinary Epidemiology. 2nd ed. Black well, Cambridge; 2005.
33. CLSI (Clinical Laboratory Standards Institute). Performance Standards for Antimicrobial Susceptibility Testing: Twenty Second Informational Supplement. CLSI document M100-S22, Wayne PA; 2012.
49. Holt PS. Molting and Salmonella

45. Berchieri JA, Murphy CK, Marston K, Barrow PA. Observations on the

44. Bumstead N, Barrow P. Resistance to Salmonella

47. Gast RK, Beard CW. Age-related changes in the persistence and pathogenicity

46. Calenge F, Kaiser P, Vignal A, Beaumont C. Genetic control of resistance to

40. Uyttendaele MR, Debevere JM, Lips RM, Neyts KD. Prevalence of

55. Henzler DJ, Opitz HM. The role of mice in the epizootiology of Salmonella

53. McAllister JC, Steelman CD, Skeeles JK. Reservoir competence of the lesser

52. McIlroy SG, McCracken RM, Neilland SD, O

54. Kopanic RJ, Sheldon BW Jr, Wright CG. Cockroaches as vectors of

39. Ishihara K, Takahashi T, Morioka A, Kojima A, Kijima M, Asai T, Tamura Y. Infection

37. Dórea FC, Cole DJ, Hofacre C, Zamperini K, Mathis D, Doyle MP, et al. Effect

34. Cosby DE, Cox NA, Harrison MA, Wilson JL, Buhr R, Fedorka-Cray PJ. Salmonella and antimicrobial resistance in broilers: a review. J Appl Poult Res. 2015;24(3):408–26.

35. Sanchez S, Hofacre CL, Lee MD, Maurer JI, Doyle MP. Animal sources of salmonellosis in humans. J Am Vet Med Assoc. 2002;221(4):492–7.

36. CDC (Centers for Disease Control and Prevention). The food production chain-how food gets contaminated. Foodborne Outbreaks. 2015. https://www.cdc.gov/foodsafety/outbreaks/investigating-outbreaks-production-chain.html. Accessed 07 Feb 2017.

37. Dórea FC, Cole DJ, Hofacre C, Zamperini K, Mathis D, Doyle MP, et al. Effect of Salmonella vaccination of breeder chickens on contamination of broiler chicken carcasses in integrated poultry operations. App Environ Microbiol. 2010;76(23):7820–5.

38. Adeline HS, Marianne C, Sophie Le B, Franc L, Isabelle P, Sandra R, et al. Risk factors for Salmonella enterica subsp. enterica contamination in 519 French laying hen flocks at the end of the laying period. Prev Vet Med. 2009;89(1):51–8.

39. Ishihara K, Takahashi T, Morioka A, Kojima A, Kijima M, Asai T, Tamura Y. National surveillance of Salmonella enterica in food-producing animals in Japan. Acta Vet Scand. 2003;44(3):53.

40. Uyttendaele MR, Debevere JM, Lips RM, Neys KD. Prevalence of Salmonella in poultry carcasses and their products in Belgium. Int J Food Microbiol. 1998;40:1–8.

41. Kassaye A, Lencho T, Mesale A. Prevalence of Salmonella infection in intensive poultry farms in Hawassa and isolation of Salmonella species from sick and dead chickens. Ethiop Vet J. 2010;14(1):11–24.

42. Makaya PV, Matope G, Phukenyi DM. Distribution of Salmonella serovars and antimicrobial susceptibility of Salmonella enteritis from poultry in Zimbabwe. Avian Pathol. 2012;41(2):221–6.

43. Aliu WQ, Thakur S, Berghaus RD, Martin MP, Gebreyes WA. Prevalence and distribution of Salmonella in organic and conventional broiler poultry farms. Foodborne Pathog Dis. 2010;7(11):1363–71.

44. Bumstead N, Barrow P. Resistance to Salmonella gallinarum, S. pullorum, and S. enteritidis in inbred lines of chickens. Avian Dis. 1993;3:189–193.

45. Berchieri JA, Murphy CK, Marston K, Barrow PA. Observations on the persistence and vertical transmission of Salmonella enterica enterica serovars Pullorum and Gallinarum in chickens: effect of bacterial and host genetic background. Avian Pathol. 2001;30(3):221–31.

46. Calenge F, Kaiser P, Vignal A, Beumont C. Genetic control of resistance to salmonellosis and to Salmonella carrier-state in fowl: a review. Genet Sel Evol. 2010;42(1):11.

47. Gast RK, Beard CW. Age-related changes in the persistence and pathogenicity of Salmonella typhimurium in chicks. Poult Sci. 1989;68(1):1454–60.

48. Beal RK, Wrigley P, Powers C, Hulme SD, Barrow PA, Smith AL. Age at primary infection with Salmonella enterica serovar typhimurium in the chicken influences persistence of infection and subsequent immunity to re-challenge. Vet Immunol Immunopathol. 2004;100(3):151–64.

49. Holt PS. Molting and Salmonella enterica enterica serovar Enteritidis infection: the problem and some solutions. Poult Sci. 2003;82(6):1008–10.

50. Davies R, Breslin M. Environmental contamination and detection of Salmonella enterica serovar enteritidis in laying flocks. Vet Rec. 2001;149:699–704.

51. Yhiler NT, Bassey BE. Antimicrobial susceptibility patterns of Salmonella species from sources in poultry production settings in Calabar, Cross River state, Nigeria Am J Health Res. 2015;3(76-81).

52. McLoy SG, McCracken RM, Neilland SD, O'Brien JJ. Control, prevention and eradication of Salmonella enteritidis infection in broiler and broiler breeder flocks. Vet Rec. 1989;125:5445–548.

53. McAllister JC, Steelman CD, Skeeeles DK. Reservoir competence of the lesser worm (Coleoptera: Tenebrionidae) for Salmonella typhimurium (Eubacteriales: Enterobacteriaceae). J Med Entomol. 1994;31:369–72.

54. Kopanic RJ, Sheldon BW Jr, Wright CG. Cockroaches as vectors of Salmonella: laboratory and field trials. J Food Prot. 1994;57:125–32.

55. Henzler DJ, Opitz HM. The role of mice in the epizootiology of Salmonella enteritidis infection on chicken layer farms. Avian Dis. 1992;36:625–31.

56. Carrique-Mas JJ, Breslin M, snow L, McLaren I, Sayers, Davies RH. Persistence and clearance of different Salmonella serovars in buildings housing laying hens. Epidemiol Infect. 2009;137:857–46.

57. Meakins S, Fisher IS, Berghold C, Gemmery-Smith P, Tischape H, Cormican M, et al. 2008. Antimicrobial drug resistance in human nontyphoidal Salmonella isolates in Europe 2000-2004: a report from the enter-net international surveillance network. Microb Drug Resist. 2008;14(1):31–5.

58. Crump JA, Medalla FM, Joyce KW, Krueger AL, Hoekstra RM, Whichard JM, et al. Antimicrobial resistance among invasive nontyphoidal Salmonella enterica in the United States, National Antimicrobial Resistance Monitoring System, 1996-2007. Antimicrob Agents Chemother. 2011;55(3):1148–54.

59. Molla W, Molla B, Alemayehu D, Muckie A, Cole L, Wille E. Occurrence and antimicrobial resistance of Salmonella serovars in apparently healthy slaughtered sheep and goats of central Ethiopia. Trop Anim Health Prod. 2006;38:455–62.

60. Zealem A, Nigatu K, Zufan W, Haile G, Alehegne Y, Tesfu K. Prevalence and antimicrobial resistance of Salmonella isolated from lactating cows and in contact humans in dairy farms of Addis Ababa. BMC Infect Dis. 2011;11:222.

61. Akinyemi KO, Iwabokun BA, Foli F, Oshodi K, Coker AO. Prevalence of multiple drug resistance and screening of enterotoxin (stn) gene in Salmonella enterica serovars from water sources in Lagos. Nigeria Public Health. 2011;12:65–71.

62. Okeke IN, Ruminaranay R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, et al. Antimicrobial resistance in developing countries. Part i: recent trends and current status. Lancet Infect Dis. 2005;5:481–93.

63. Zewdu E, Cornelius P. Antimicrobial resistance pattern of Salmonella serotypes isolated from food items and personnel in Addis Ababa. Ethiopia Trop Anim Health Prod. 2009;41:241–9.

64. Molla W, Mohammed A, Salah W. Salmonella prevalence and distribution of serotypes in apparently healthy slaughtered camels (Camelus dromedaries) in Ethiopia. Trop Anim Health Prod. 2004;36:451–8.

65. Zhao S, Datta AR, Ayers S, Friedman S, Walker RD, White DG. Antimicrobial-resistant Salmonella serovars isolated from imported foods. Int J Food Microbiol. 2003;84:887–92.

66. Angulo FJ, Nargund VN, Chiller TC. Evidence of an association between use of antimicrobial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. J Vet Med. 2004;51:374–9.

67. Levy SB. Antibiotic resistance: an ecological imbalance. In: Antibiotic Resistance: Origin, Evolution, Selection and Spread. Ciba Foundation Symposium 207, Wiley, Chichester; 1997. p.1–14.

68. Shah AH, Korejo NA. Antimicrobial resistance profile of Salmonella serovars isolated from chicken meat. J Vet Anim Sci. 2012;2:40–6.