Chicken Salmonellosis Prevalence and Its associated risk factors : The case of Asossa town, Benishangul Gumuz Regional State, Western Ethiopia

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SUMMARY: Cross - sectional study on Chicken Salmonella prevalence, and associated risk factors in Asossa town, Benishangul Gumuz Regional State was carried out from September 2024 to Febuarary 2025 with the objectives to determine the prevalence of Salmonellosis from small householder farmstead/ local poultry farm, and assessment of the risk factors associated with salmonella infection. For this purpose, a total of 384 cloacal swab samples were collected and were subjected to various cultural and biochemical examinations, 87(22.65%) of the positive isolates were identified. In this study, previous treatment history, body conditions and salmonella species were potential risk factors, which were statistically significant for salmonella infection ($p<0.05$) whereas origin/sites/ and sex groups were not significant ($p>0.05$). The antimicrobial susceptibility profile of all isolates were assessed against six antimicrobials by disk diffusion technique; almost all isolates were resistant to one or more of the tested antimicrobials. 73.07%, 65.38%, 30.76 %, 26.92%, 15.38%, and 11.53% of the isolates were resistant to Tetracycline, Streptomycin, Trimthoprim, Chloramphenicol, Gentamycine, and Ciprofloxacillin respectively. However, the majority of the isolates were susceptible to Ciprofloxacin and Gentamycin, Chloramphenicol, followed by Trimethoprim. This is a significant threat to public health particularly to those who have direct or indirect contact to poultry and poultry products so that hygienic management of poultry and its products in order to reduce the risk and selection of antimicrobials by antimicrobial sensitivity test were also suggested.

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1. INTRODUCTION

Salmonellosis is an important zoonotic disease caused by the genus *Salmonella* which constitutes a major public health burden and represents a significant cost in many countries. The prevalence of *Salmonella* in animals is a continuous threat to human health (Murugkar *et al.*, 2005). Salmonellae are widely distributed in nature and cause a spectrum of diseases in man, animal and birds. Poultry eggs, meat and their products are the commonest vehicles of *Salmonella* to humans (Nagappa *et al*., 2007). Every year millions of human cases are reported worldwide and the disease results in thousands of deaths (Herikstad *et al*., 2002).

Members of the genus *Salmonella* are Gram-negative, motile, facultatively anaerobic organisms belonging to the family *Enterobacteracae* (Ellermeier and Slauch, 2006). The genus *Salmonella* contains two species, *Salmonella enterica*, which consists of six subspecies, and *Salmonella bongori*. Currently the genus includes a total of more than 2,500 serotypes (Popoff *et al*., 2004). *Salmonella* nomenclature is complex, and is based on names for serotypes in subspecies I. For example, *Salmonella enterica* subsp. *enterica* serotype Enteritidis, is shortened to *S.* Enteritidis (Brenner *et al*; 2000). *Salmonella enterica* subspecies *enterica* (subspecies I) is responsible for 99.5 % of infection in man and animal (Martin *et al*., 2006). Most of the infections are zoonotic in origin but some serotypes like *S.* Typhi and *S.* Paratyphi infect only humans (Yan *et al*., 2003). The infectious dose, incubation period, symptoms and mode of transmission of salmonellosis caused by different serotypes are similar. Symptoms include diarrhea, fever and abdominal cramps with incubation periods ranging from 12 to 72 hours. The illness usually lasts from 4 to 7 days and most people recover without treatment. The elderly, infants and those with impaired immune systems are more likely to have a severe illness (Hans and Dean, 2006).

Egg contents may be contaminated with salmonellae by two routes: vertical transmission (transovarian) or horizontal (trans-shell) transmission (FAO/WHO, 2002). In transovarian transmission, *Salmonella* are introduced from infected reproductive tissues to eggs prior to shell formation. *Salmonella* serotypes associated with poultry

reproductive tissues and that are of public health concern include *S.* Enteritidis, *S.* Typhimurium and *S.* Heidelberg (ICMSF, 1996; ACMSF, 2001). *S.* Enteritidis may be better able to achieve invasion (ACMSF, 2001).
Horizontal transmission is usually derived from fecal contamination on the egg shell with penetration after the egg is laid. It also includes contamination through environmental vectors, such as farmers, pets and rodents. Many different serotypes of the genus *Salmonella* may be able to contaminate egg contents by migration through the egg shell and membranes. Such a route is facilitated by moist egg shells, storage at ambient temperature and shell damage (ACMSF, 2001).

Food Standards Agency (FSA, 2003) of the United Kingdom has drawn attention to the risk associated with eating raw and lightly cooked eggs and issued public health advice on the safe handling and use of eggs. It is estimated that, in the United States, *Salmonella* transmission through contaminated shell eggs or egg products results in 700,000 cases of salmonellosis and costs 1.1 billion United States dollar annually (Rodriguez and Yousef, 2005). In many countries, *Salmonella* spp. is controlled in egg production chain. In addition, storing eggs in a cool area (below 15oC) and keeping eggs separate from other foods is important to avoid possible *Salmonella* cross contamination and keep eggs safe (Hans and Dean, 2006). One study in Ethiopia showed from the total 400 chicken eggs examined for *Salmonella* prevalence, 46 (11.5 %) were positive, from which 25 (6.3 %) and 27 (6.8 %) were found from egg shell and egg content, respectively (Minte *et al.*, 2011).

The use of antibiotics in food animals selects bacteria which are resistant to antibiotics used in humans. These might be spread via the food to humans and cause human infection (Phillips, 2004). Amongst *Salmonella* spp., antimicrobial resistance is a well confirmed phenomenon and antimicrobial-resistant *Salmonella* are increasingly associated with the use of antimicrobial agents. Antimicrobials are substances that have significantly contributed to the prevention and treatment of infectious diseases in humans, as well as to many animal species (CDC, 2008). However, the excess or overuse of antimicrobials can generate genomic selective pressures to enable microbes to adapt and acquire resistance (Witte, 2001).

Ultimately, increases in bacterial antimicrobial resistance pose a considerable threat to public health, especially for vulnerable populations including young children (Shea, 2003), the elderly and immune compromised individuals (Hitti and Wolff, 2005). Concentrated animal feeding operations (CAFOs) in agricultural practices have evolved to accommodate food consumption rates with increased agricultural output at the risk of introducing antimicrobial resistant pathogens into the environment. In addition, several studies have suggested that characteristics of agricultural environmental settings, including animal crowding, CAFO hygiene, temperature, ventilation control and stress, can influence antimicrobial resistance and pathogen risk (Silbergeld *et al*., 2008).

There are reports of high prevalence of resistance in *Salmonella* isolates from countries such as Taiwan (Lauderdale *et al.*, 2006), India (Mandal *et al.*, 2004, 2006), the Netherlands (Duijkeren *et al.*, 2003), France (Weill *et al.*, 2006), Canada (Poppe *et al.*, 2006) and Ethiopia (Mache, 2002; Molla *et al.*, 2003, 2006; Ayalu *et al.*, 2011; Beyene *et al.*, 2011; Sibhat *et al*., 2011). The presence of resistant organisms in the poultry and poultry products for consumption is a safety concern to the population (Schlundt *et al.*, 2004) and therapeutic concern for the physicians which might pose prolonged treatment in cases of outbreaks, delayed recovery or treatment failure (Silbergeld, 2008). There is a scarcity of knowledge concerning poultry farm development associated with antimicrobial resistance and foodborne bacteria. Information on the antimicrobial resistance pattern of the *Salmonella* isolates from chicken table eggs could be useful for successful treatment, as well as planning strategic use of drugs to minimize resistance in the future.

In Ethiopia as in other developing countries, it is difficult to evaluate the burden of salmonellosis because of the limited scope of studies and lack of coordinated epidemiological surveillance systems. In addition, under-reporting of cases and presence of other diseases considered to be of high priority may have overshadowed the problem of salmonellosis (Beyene *et al.*, 2011). Salmonellosis is endemic in the country and there is a desire to strengthen the monitoring and surveillance of salmonellosis using suitable diagnostic tools so as to prevent and control its occurrence. Besides this, the extent of *Salmonella* contamination of cloacal swab, egg shell surface swab and antimicrobial profile of the *Salmonella* isolates has not been adequately studied and limited information exists in the Asossa District. Therefore, the present study focusing with the objectives of determining the prevalence of *Salmonella* isolated from cloacal swab , to identify risk factors associated with salmonellosis and to determine the antimicrobial susceptibility profile of the salmonella in the study area.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Assosa town, BenishangulGumuz Regional State, west Ethiopia. According to CSA (2007), before a year, Assosa town (the capital city/town of BenishangulGumuz Regional State) had four kebeles but currently, the town has changed its administrative structure to two districts (district-1 and district-2). Each district has five "ketenas". According to BGRSMSC (2020), the town is located at 10º 00'and 10º 03' north latitude and 34º 35' and 34º 39' east longitude. The total population of the town is 62,632 of which 32, 100 are male and 30,532 are female (projection made for the CSA, 2020). The total area of the town is 2361.34 hectares with an altitudinal difference that ranges between 1461- 1641 meters above sea level (BGRSEIB, 2020). The mean annual temperature of Assosa town ranges from a minimum of 14-33^{0}C. However, there is a slight variation of temperature by month. February to May is the hottest months while November to December is the cold months). The total amount of rain fall recorded at Assosa town during the last nine months of (2020) is 1,119 mm (BGRSMSC, 2020). The rainy season starts in March and extends to November with the highest concentration in June, July, and August. The population size of different livestock species in Assosa town are cattle 569, goat 1545, sheep 739, poultry 17676 donkeys 122, and pig 8 total 20,659 livestock populations are found in the town (ATAOA, 2020).

Figure 1: Administrative map of Assosa town (ATAO, 2021)

2.2 Study population

The study animals was apparently health chickens kept under intensive farming system. The farms were categorized as small (1000 chickens), medium (1001-2000 chickens) and large scale (>2000 chickens) farms according to flock size (Edge *et al.*, 2023). Chickens included in this study was Saso and Bovans brown breeds and grouped into young (<24 weeks) and adult (>24 weeks) (Sarba *et al*.,2020).

2.3 Study Design

Across- sectional design was conducted from September 2024 to Febuarary, 2025 in Assosa town to determine prevalence, associated risk factors from cloacal swabs of chickens kept under intensive farming system.

2.4 Sample size Determination

The sample size was estimated using Thrusfied, (2018) formula with an expected prevalence of 23.2% as studied by Asmamaw Aki (2018) in Asossa and Bambasi districts with 0.05 precision and the sample size at a 95% confidence interval. Therefore, the total sample size for the study was calculated using the following formula for each sampling units.

$$n = \frac{(1.96)^2 x\ Pexp\ (1-Pexp)}{d^2}$$

Where: n = the total sample size, P = expected prevalence (50%), d = desired absolute precision (5%), (0.05) at 95% CI

n = (1.96) x (1.96) x (0.232) x (1-0.232)/((0.05) x (0.05) = **274** samples .

2.5 Sampling methods

Simple random sampling technique was used, to select the farm included in the study and collect swab samples from chickens kept under intensive farming system in Assosa town. A total of 274 samples from cloacal swab and egg shell swab was collected from chicken of different categories of the poultry farms. Non proportional sampling was used to collect each sample due to availability of limited layers chicken farms. A total of 274 respondents from poultry farms was interviewed by using semi structured questionnaire which covers demographic data, biosecurity measures, feeding, housing and overall managements of the farm. Additionally, information including, flock size, age, sex, farm type, breed type, sanitary/management status and other related information was recorded from each farms.

2.6 Questionnaire survey

Semi structured questionnaires was prepared in English language and translated to local language and interviewed to obtain information. Data on each sampled chicken swabs was collected using a properly designed questionnaire format for determining the associated risk factors. This includes demographic data, feeding and watering, environmental contamination, management factors, housing/ventilation, health status, vaccination status, disease conditions, handling practices/ egg storage, egg/ chicken transportation, breed, previous history of treatment, bio security measures, hygienic/sanitary condition and other relevant information related to salmonellosis was gathered .

2.7 Sample collection , Transportation and Processing

A total of 274 samples was collected from every local poultry farmstead. Purposive sampling technique was used based on the availability of chickens in the study area/ kebeles. Simple random sampling techniques may be used for selection of each chickens in small holder poultry farmstead of Asossa local poultry farmstead. Aseptic procedure was followed when collecting faecal samples. The sterile plastic bags was used for containing selected cloacal faeces. The cloaca/ vulva/ surface was sterilized by swabbing in 70 % alcohol for 2 min. The cloacal swab/ faecal/ samples was collected in sterile ice box, Therefore, samples was properly transported immediately in an ice box to Regional Veterinary Laboratory of Benishangul Gumuz, Asossa, for microbiological examination. The isolation was conducted utilizing the conventional methods for the detection of *Salmonella* following the standard guidelines from ISO 6579 (ISO, 2002).

2.8 Laboratory Analyses

2.8.1 Isolation and Identification of Salmonella

The isolation and identification of salmonella was performed at the Asossa regional animal health diagnostic microbiology laboratory by using techniques recommended by international organizations for standardization (ISO-6579, 2017). It involves four steps: pre enrichment in pre-enrichment broth media, enrichment in selective media, plating on selective media and biochemical confirmation of suspected colonies from selective media.

Primary enrichment in non- selective liquid medium (pre-enrichment): All samples will be pre enriched separately with an appropriate amount of buffered peptone water (Himedia M021, India) (1:9) and will be homogenized using a rotary shaker at 50-350 revolutions per minute for 2min and then enriched by incubating aerobically at 37oc for18 to 24 hrs (ISO-6579, 2017).

Secondary enrichment in selective liquid media *:* Following non selective pre enrichment, 0.1ml (100µ) of cultured broth was inoculated aseptically into tubes containing 10ml of Rappaport vassiliadis soy broth after mixing by using vortex. Inoculated Rappaport vassiliadis soy broth (Himedia, M880) was mixed well and incubated at 42 oc for 18-24 hrs (ISO-6579,2017).

Plating out and identification : Xylose- lysine deoxycholate (XLD) agar plate was used forplating and identification purpose. A loop-full of inoculum from each clocal swab/ faeces/ sample was transferred and streaked separately onto the surface of XLD agar. The plates was incubated at 37 oC ± 1 oC for 24 ± 3 hours. After proper incubation, the plates was examined for the presence of suspected *Salmonella* colonies, which on XLD agar were pink with a darker center and a lightly transparent zone of reddish color due to the color change of the indicator whereas lactose positive salmonellae are yellow with or without blackening. Five *Salmonella* presumptive colonies were transferred to nonselective solid media for further confirmatory tests. Confirmation was done by using biochemical test according to ISO 6579 (ISO, 2002).

2.8.2 Biochemical Characterization

Triple sugar Iron Agar *:* The Triple sugar iron agar slants was prepared with a thick butt. A loopful culture of pure growth from nutrient agar will be stabbed into the butt and streaked on the slant and was incubated for 24 hours at 37ºC. Typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas production (bubbles) and formation of hydrogen sulfide (blackening of the agar) (ISO 6579:2002(E); Quinn *et al*., 2002).

Urease test : The hydrolysis of urea releases ammonia and production of ammonia increases the pH of the medium that change color of phenol red (pH indicator) to rose pink, and later to moderate red. The basal medium will be sterilized by autoclaving at 121 ºC for 15 minutes. When it has cooled to about 50 ºC, 100 ml of a 20 percent solution of pure urea previously sterilized by filtration will be added and poured into test tubes. The isolates will be inoculated into the urea to determine urease production. The inoculated tubes can be incubated at 37 oC for up to 96 hours. The observations may be made at an interval of 4, 24, 48 and 96 hours. Urease positive cultures changed the color of the indicator to red (ISO 6579:2002(E); Quinn et al., 1999).

Simmon's citrate Utilization test : simmon's citrate agar was sterilized by autoclaving at 121 °C for 15 minutes at 15 lb pressure and cooled for slant formation. The strains was cultured on the prepared Simmon's citrate agar medium, incubated at 37 °C for 48 hours and observations will be recorded. Opacity and change in color of bromothymol from green to blue indicated a positive reaction (Quinn *et al*., 2002).

Indole test *:* Indole is a nitrogen-containing compound that can be formed from the degradation of the amino acid tryptophan by certain bacteria. Tryptone was used as a substrate because it contains much tryptophan. The indole reacts with aldehyde compound of Kovac's reagent and forms red coloured compound that is more soluble in alcohol. For indole test peptone water was prepared and the ingredients were dissolved in distilled water, dispensed in test tubes and sterilized by autoclaving at 121 oC for 15 minutes. The tubes of the medium were inoculated with test isolates using sterile platinum loop and incubated at 37 oC aerobically for up to 96 hours. Finally, 0.5 ml of Kovac's reagent was added to each of the inoculated and un inoculated controls. The tubes were shaken gently and the results were recorded. Positive results were indicated by the development of red colour in the alcoholic layer of the reagent and no colour in the control tube (Quinn *et al*., 2002).

2. 9 Antimicrobial Susceptibility Testing

The agar disc diffusion method, as published by the Clinical and Laboratory Standards Institute, will be used to determine antimicrobial susceptibility patterns (CLSI, 2015). A digital caliper was used to determine the zone of inhibition. Spectinomycin (10 µg) Vancomycin (30µg) Norfloxacin (50µg) Kanamycin (30µg) Chloramphenicol (50 µg) Tetracycline (10 g), Erythromycin (15 g), and Penicillin (10 g) are used to test the antibiotic susceptibility of Escherichia coli isolates. In a nutshell, the bacteria were suspended in a 0.85 percent sterile normal saline solution in a 0.5 McFarland standardized suspension. A sterile cotton swab was dipped in the standardized bacteria suspension and then streaked uniformly across the Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, England) surface. The paper discs impregnated with a set concentration of antibiotics are then placed on the agar surface and inverted for 24 hours at 37 °C. The bacterial growth and diffusion of the antibiotics are going to produce obvious zones of inhibition after 24 hours of incubation and are measured in millimeters using a caliper and characterized as susceptible, intermediate, and resistant (CLSI, 2015). The media were checked for free of contamination by incubating 5% of the batch at 37 °C for 18-24 hrs, and ice box was used during sample collection, and transportation and the performance of the media were checked using E. coli (ATCC, 25922).

Table 1: Antimicrobial susceptibility test interpretive criteria for Enterobacteriaceae

Antibiotics Disc	Disc code	Potency	Zone of diameters		
			Susceptibility (mm)	Intermediate (mm)	Resistance (mm)
Erythromycin	E	15 μg	≥17	12-16	≤14
Vancomycin	VA	30 μg	12	10-11	≤9
Kanamycin	KF	30 μg	≥18	13–17	≤13
Tetracycline	T	10 μg	≥15	12–14	≤11
Chloramphenicol C	C	50 μg	≥18	13–17	≤12
Penicillin G	P	10 μg	29	-	28

3. DATA ANALYSIS

Processing of data was done by computer software. Data was coded and entered to MS Excel spreadsheet and checked for accuracy. After validation, it was transferred and processed using computer software STATA version 12 for analysis. Pearson's chi-square tests will be used when appropriate to analyze the proportions of categorical data. Odd ratio and 95% CI will be computed, the 95% confidence level was used, and results was considered significant at ($P < 0.05$).

4. Ethical Considerations

This protocol was presented for initial and continuing review and approval by JUCAVM institutional ethical review board. Any changes to the protocol or consent form were approved by all ethics committees. Since the study involves domestic animals subject, the ethical approval for the study were obtained from Ethical Research Board (ERB) of the JUCAVM for domestic animal.

5. RESULTS

5.1 Prevalence of Salmonella in poultry

A total of 274 Sasu and Bovans poultry samples were randomley collected at Assosa district 01 and 02 during the study period. Samples were processed microbiologically for isolation and identification of *Salmonella*. Based on the bacteriological culture and biochemical test, 26/ 274 (9.48%) *Salmonella spp* were isolated and it was found to be statistically significant (P>0.08, Chi2=3.06). The highest salmonella distribution were observed in district 02 (12.41%) while the lowest prevalence was reported in district01 (6.20%) as shown in (Table 2**).**

Table 2**: Origin based prevalence of salmonella by culture and biochemical test**

Factors	Asossa town	No examined	Prevalence(%)	Chi2	p-value
Study areas	District 1	129	8(6.20)	3.06	0.08
	District 2	145	18(12.41)		
Sample type	Cloacal swab	212	21(9.90)	0.18	0.66
	Egg shell swab	62	5(8.06)		

(274 Chicken swab were examined, overall salmonella prevalence at different study site level were (n=274, 9.48%), by bacteriological method. The prevalence of salmonella amongst study sites has significant difference (X^2=3.06, Pr=0.08) (Table 2).

5.2. Risk Factors Associated with salmonella Prevalence

Prevalence of salmonella related to the specific risk factors were determined as the proportion of affected chickens out of the total examined. As indicated in (Table 3), the questionnaire survey and observation data result shows disease history, management/ sanitary status , breed, floor system, previous treatment history and vaccination history were amongst the potential risk factors, which are associated with salmonella disease in poultry/chicken farmstead. Accordingly, salmonella prevalence showed significant variation among different disease history groups (p = 0.000), floor system (p=0.000), breed(p=0.02),vaccination status (p=0.006), sanitary and management status (p=0.000) and

previous treatment history and vaccination history(p=0.000). However, flock size, age, district and sample type have no significant difference with salmonella (p>0.05) as indicated in Table 3.

Table 3: Association of potential risk factors with *Salmonella prevalence* in poultry population in Assosa "woreda"

Factor	Level	No examined	Prevalence (%)	X^2	P-value
Age	Young(<24wks)	187 (	15(8.02)	1.47	0.22
	Adult (>24wks)	87	11(12.64)		
Sanitary	Poor	69	14(20.28)	12.52	0.000
	Good	205	12(5.85)		
Housing system	Floor bedding	202	11(5.44)	14.63	0.000
	Cemented and cages	72	15(20.83)		
Previous treatment history	Yes	38	10(26.31)	14.54	0.000
	No	236	16(6.77)		
Disease history	Yes	28	24(85.71)	210.98	0.000
	No	246	2(0.81)		
Management status	poor	58	12(20.68)	10.74	0.001
	Good	216	14(6.48)		
Breed	sasu	146	8(5.47)	5.85	0.02
	Bovans brown	128	18(14.06)		
Flock size	small	91	6(5.59)	5.37	0.06
	Medium	51	2(3.92)		
	large	132	18(13.63)		
Sample type	Cloacal swab	212	21(9.90)	0.18	0.66
	Egg shell swab	62	5(8.06)		
District	District 01	129	8(6.20)	3.06	0.08
	District 02	145	18(12.41)		
Vaccination status	Yes	141	20(14.18)	7.45	0.006
	No	133	6(4.51)		

5.3. In vitro antimicrobial Susceptibility Test

Antimicrobial susceptibility tests were performed on 26 isolates and were tested for antimicrobial sensitivity for six types of antibiotics. The present study has demonstrated the existence of the levels of resistance of *Salmonella* to commonly used antimicrobial agents in the study area. The antimicrobial susceptibility profile of all isolates were assessed against six antimicrobials by disk diffusion technique; almost all isolates were resistant to one or more of the tested antimicrobials. 73.07%, 65.38%, 30.76 %, 26.92%, 15.38%, and 11.53% of the isolates were resistant to Tetracycline, Streptomycin, Trimthoprim, Chloramphenicol, gentamycine, and ciprofloxacillin respectively (Table-4). However, the majority of the isolates were susceptible to ciprofloxacin and gentamycin, chloramphenicol, followed by Trimethoprim(Table-4).

Table 4: Antimicrobial susceptibility test result for Salmonella isolates (n = 46).

Antimicrobial agents	Disc content (µg)	No of isolates	Resistance	Intermediate	Susceptible
			No (%)	No (%)	No (%)
Tetracycline(TE)	30	26	19 (73.07)	3 (11.53)	4 (15.38)
Gentamycin(CN)	10	26	4 (15.38)	6 (23.07)	16 (61.53)
Streptomycin(S)	10	26	17(65.38)	3(11.53)	6 (23.07)
Chloramphenicol(C)	30	26	7(26.92)	3(11.53)	16(61.53)
Trimethoprim(w)	2	26	8(30.76)	3(11.53)	15(57.69)
Ciprofloxacin(CIP)	5	26	3(11.53)	-	23(88.46)

Key: *S- Susceptible, I- Intermediate, R- Resistant profile of Salmonella isolated from cloacal swab of chicken.*

6. DISCUSSION

In the present study, the overall prevalence of salmonella was 9.48 % in chickens/ poultry, which was statistically significant and associated with the infection (p>0.08). This finding is inline with the previous findings of Solomon T *et al*., 2016) in Alage, Ziway and Shashemene area, Endrias ZG (2004) in Addis Ababa supermarkets, Liyuwork T *et al*. (2013) in Addis Ababa and F. Abunna *et al*. (2016) in and around Modjo, Central Oromia, (Aseffa *et al*., 2011) from chicken table eggs by bacteriological methods in Ethiopia, (Hassanain *et al*., 2012) in Egypt, and (Urji *et al*., 2005) in Nigeria by bacteriological methods, 13.3%, 14%, 1.6%, 15.2%, 11.5%, 11.4%, and 12.5% salmonella infection in poultry farm respectively. The difference might be difference in farming system, poor hygienic practice in semi-intensive farm might contribute the major problem for high prevalence rate of salmonellosis

This result was in lower as compared with the previous studies made by Kasech M. (2015) in DAZARC poultry farm, at Bishoftu, Central Oromia, by Beshatu F. (2014) in Dire Dawa municipal abattoir, Ashwani *et al*. (2014) by serology method in Ethiopia and Asmamaw A(2018) in Asossa and bambasi in BG, 30.4 % , 18% , 20%, and 23.2% salmonella infection respectively. Besides this, the prevalence of *Salmonella* in chicken samples was concord with the results of earlier studies made by Molla *et al*. (1999a) who reported 28.6%, 22.6%, and 15.4% in chicken gizzard, liver, and heart, respectively, Molla and Mesfin (2003) who detected (21.1%) *Salmonella* in chicken carcass and giblets samples in central Ethiopia, Tibaijuka *et al.* (2003) who indicated 18 % prevalence (54/301) in chicken meat and edible offals and Hang'ombe (1999) who published 20.5% frequency of isolation for *Salmonella* from dressed chicken carcass in Lusaka, Zambia. This variability in prevalence of salmonellosis between different reports could be attributed to differences in farms management practice. As poultry salmonellosis is a complex disease involving interactions of various factors such sanitary problems, environmental conditions, animal risk factors, and causative agents, contamination in the farm during collection, transportation and poor hygiene of workers as well as farms and different in different farming system. Different authors reported that the presence of chickens of different ages in the farm, the presence of arthropod pests, wet and soiled litter in the farm (Smeltzer T *et al*., 1979) and the housing system and flock size could be important reasons for egg contamination with various micro-organisms. Chicken feeds and hatcheries also possible sources of *Salmonella* infections in the farm.

Higher prevalence than present finding was also reported in Ethiopia and in other counties as 41.9% (Kindu and Addis, 2013) from fecal sample by bacteriological method, 35.7% (Endris *et al*., 2013) of *S. Gallinarum* and *S. pullorum* from cloacal swab by serology and culture, 55% (Kagambega *et al*., 2013) in Burkina Faso, 56.5% (Khan *et al*., 2014) in Pakistan, 45% v 60% (Jahan *et al*., 2012) in Bangladesh in cloacal poultry swab samples, 66% (Jerngklinchan *et al.* 1994) from Thailand, 29.7% by Plummer *et al.* (1995) from whole bird in UK, 38.3% (Rusal *et al.*, 1996) in Malaysia from poultry carcass arising from wet markets and processing plants, and Arumugaswamy *et al., (*1995) from Malaysia also reported a much higher *Salmonella* isolation rate from chicken portions (39%), liver (35%) and gizzard (44%).

Likewise, lower prevalence than the present finding was also reported in Ethiopia and other countries. Few examples include 0.8% (Kassaye *et al*., 2010) of *Gallinarum* and *S. pullorum* from cloacal swabs by culture technique, 10.9% (Agada *et al*., 2014) in Nigeria, 9.2% (Al-Abadi and Al-Mayah, 2012) in Iraq in culture techniques in cloacal swab samples and 32(16%) of the 198 skin samples (Whyte *et al*. (2000) in Ireland, using the culture methods. These differences above (higher or lower prevalence) from present finding might be resulted from the difference in isolation technique, and difference in geographical location, difference in bio secuity measure like cross – contamination and poor housing system.

Occurrence of salmonella was significantly associated with hygienic practice. Poultry at farms with poor hygiene/ sanitary/poor management / standard are severely affected than those with good hygiene/ sanitary/ management practices. (20.28%) higher prevalence of salmonella infection was recorded in poor housing system whereas (5.85%) lower infection was investigated in good housing sysem which was significantly associated with infection (p=0.000). This might be due to absence of good sanitary / bedding of poultry house and feed, water contamination infected ones faceas and egg as well. This result was consistent with Deen *et al.(* 2001) who indicated, stresses due to transport, improper feeding and poor hygiene, etc. might happen to these animals considering the prevailing socioeconomic conditions, knowledge and awareness of the people, particularly those from rural areas. Different

authors (Deen *et al.*, 2001; Wray and Davies, 2000) have attributed various stress factors to be in favor of increased *Salmonella* prevalence. Besides this, the present finding supports the report of Davies and Hinton (2000) "Even though feed, sanitary is widely accepted as a source of possible contamination, the incidence of outbreaks being attributed to feed is very low". The detection were more or less in harmony with AL-Iedani *et al.* (2014) finding that 14% from cloacal swab, 37% from litter, 10% from water and 20% from ration of *Salmonella* isolate had identified. And also the level of contamination of dressed chicken meat was found to be slightly higher than the 11.5% prevalence report by Živkovic *et al.* (1997) on market ready dressed chicken meat, in Zagreb, Croatia and 4.2% by Zhao *et al.* (2001) from Greater Washington D.C. area. Variation in the frequency of isolation of *salmonella* between the present and earlier studies in Ethiopia might stem from either actual difference in prevalence of *Salmonella* in carrier chicken in the flock of origin or the fact that, unlike our studies, giblets were included in previous studies, which contributed substantially for the difference in prevalence.

Similarly, according to D'Aoust (1989) high prevalence of *Salmonella* in chicken carcass is attributable to problems associated with poultry husbandry, processing, and cross-contamination of carcasses in slaughtering plant through common scalding, de-feathering, and chilling processes. The same author also showed that cross-contamination from the hands of workers, equipment and utensils can spread the bacterium to uncontaminated carcass and parts. The relatively high prevalence of *Salmonella* in dressed chicken carcass might have emerged, in part, from their feeding habits i.e., their daily ration comprises of animal proteins, as source of essential amino acids and minerals, that might have been contaminated with *Salmonella* (D'Aoust, 1989; Pegram, 1981). Similar result was reported by Netsanet *et al.* (2012), who indicated , the low prevalence in the intensive farms might be due to a relatively good management practice including ventilation, proper spacing and relatively trained workers whereas high prevalence of infection in semi-intensive system due to due to economic reason to accommodate good housing with trained personnel.

The findings of (5.44%) prevalence of salmonella in farms with floors bedding was diagnosed whereas (20.83%) infection was recorded in cemented and cages types, which influence the occurrence of salmonella, and was statistically significant ($p<0.000$), this result was concord with finding of Al-Abadi and Al-Mayah (2012) 19.1 % salmonella isolated from fecal samples. Comparably low result was reported by Tessema K. *et al.*, (2017) in Haramaya poultry farm, 2.3% and 3.3% salmonella positive egg samples were recorded from cage and floor house system respectively; however, there was no statistically significant difference ($P>0.05$) in the prevalence of *Salmonella* among the two house systems. The slight increase of prevalence might be due to poor housing system which have access to entrance of carriers of salmonella like rodents, birds and pests to poultry farm and cross contamination also associated with farm workers, hygienic status, air quality, confinement of birds and dust originated from feed and faeces may contain large number of microorganisms and this poor system favor the proliferation and transmission of salmonella pathogens. It could also be due to contamination from equipment, floor and hands of personnel, as has been reported by various authors (Baird-Parker, 1990; Smeltzer *et al.*, 1980b; Smeltzer *et al*., 1980a; Smeltzer *et al.*, 1979; Watson, 1975). Comparably, Baird-Parker (1990) reported that, the main sources of infection are infected chickens transferred via environment contamination.

The prevalence of salmonella in sasu chicken was (5.47%) whereas infection in bovans breed was (14.06%) which was significantly associated with the occurrence of salmonellosis ($p<0.02$). This finding was lower when compared with the reports made by by Zhao *et al.* (2001) from Greater Washington D.C. area, it is of interest to note that 69.2% of dressed chicken carcass sampled, originated from indigenous backyard local chicken with different management from commercial farms. Unlike the previous studies made on chicken in Ethiopia, it is of interest to note that 144 (69.2%) of dressed chicken carcass sampled in this research work originated from indigenous backyard local chicken with different management from commercial farms. Comparably lower research was reported by Tessema K. *et al.*, (2017) in Haramaya poultry farm , who indicated, the prevalence of *Salmonella* in eggs on the bases of chicken breed sources was 2.9%, 3.8% and 2% for Bovans, Fayoumi and White leg horn, respectively; the prevalence difference was not show statistical significance ($P>0.05$) between the rate of detecting *Salmonella* spp., and non-significant analytical situation was observed in eggs sampled from different chicken breeds. This is presumably due to unequal exposure to the risk factors as the breeds were housed in different house system. This difference might be due to Fayoumi breed was kept in the floor house system in which there is lower hygienic and high cross contamination between the flock eggs at laying than the cage house system. Other study also reported that one day- old chicks orally infected with *S. pullorum* produced contaminated eggs frequently during the period of sexual maturity as a consequence of reproductive tract colonization (Wigley p. *et al.*, 2001)

The effect of different risk factors such as disease history, age categories, study sites, previous treatment history and vaccination history on prevalence of chicken salmonellosis was studied and, statistically significant associations were observed in disease history, previous treatment and vaccination history ($p<0.05$) while age groups, study districts, flock size, sample type were not found to be statistically significant ($P>0.05$). This result is in agreement with previous reports of (Wigley p. *et al*., 2001). The fact that salmonellosis do not depend on gender could possibly be hypothesised that both male and female animals have virtually equal chance of being in contact with infection and ultimately developing the disease.

Many reports on treatment trials of *Salmonella* infection do not contain detailed descriptions of host factors of the treated animals, or of the strains causing the infections that are treated (Barkema *et al*., 2006). In the previously infected animals, the Salmonella isolates which were responsible to the previous infection were not eliminated by the effect of various antibiotics which was related to the development of drug resistance by Salmonella organisms. But mainly, salmonellosis is a complex disease involving interactions of several factors, mainly of management, environment, and factors relating to animal and causative organisms (Tessema K. *et al*., (2017). Previous treatment history of poultry had a significant influence on the occurrence of salmonella infection, 22.2 % of salmonella was reported in previously treated poultry where as 23.87% infection was registered in not previously treated case of salmonella, which were higher. This result was not significant (Chi=0.14, pr>0.05). Similarly, 24.06% salmonella infection was recorded in treated poultry whereas 22.7% infection was recorded in not treated case of salmonella, which were not statisticall significant difference(Chi=0.08, pr> 0.05). The possible fair judgment for this could be that inappropriate implementation of antibiotics to treat salmonella case in some part of the study area leading to occurrence of an isolate which had a potential of drug resistance.

Of all 26 *Salmonella* isolates screened for antimicrobial susceptibility test against six antimicrobials. All the isolates were susceptible to Ciprofloxacilin, Gentamycin and Chloramphenicol and Trimthoprim. The reason why these antimicrobials were less resistant/susceptible/ might be that they are not used in the study area in veterinary clinics or services and even not frequently used (infrequent use of therapeutics) perhaps in human medicine.
This finding is similar with finding of Begum *et al*. (2010) on *Salmonella* isolates from chicken eggs, intestines and environmental samples**.** For the rest 7 different drugs, 43 (97.82%) were resistant to one or more of antimicrobials. This finding was in agreement with a numbers finding on *Salmonella* antibiogram tests, for isolates from poultry and poultry products samples like Maria (2010) from America, Jahan *et al*. (2012) in Bangladesh, Tabo *et al*. (2013) in Chad, Carraminana *et al*. (2004) from Spain. However, the current finding is not in agreement with results of Singh *et al*. (2013) from India, and Antunes *et al*. (2003) from Portugal, but different with resistant patterns. Disagreement may be due to different strains of isolates and/or difference in levels of strains' resistivity.

Accordingly, 73.07%, 65.38%, 30.76 %, 26.92%, 15.38%, and 11.53% of the isolates were resistant to Tetracycline, Streptomycin, Trimthoprim, Chloramphenicol, Gentamycin, and ciprofloxacillin respectively.
A High resistant to Tetracycline and Streptomycin were in agreement with what Maria, (2010) and Jahan *et al*. (2012) found on poultry related resistant isolates. And also this finding goes with what Davies (1996) found that most of the *Enterobacteriaceae* family including *Salmonella* is resistant to the drugs including Aminoglycosides, Beta lactams, Trimethoprim and Chloramphenicol. Comparable result was reported by Beshatu F. (2014) in Dire Dawa municipal abattoir, who showed, highest level of resistance for tetracycline (100%), nitrofurans (100%), streptomycin (81.8%) and kanamycin (79.5%).

Of 26 resistant isolates to anyone of the six drugs, 19 isolates were only resistant to Tetracycline while the rest isolates were resistant to at least for two of the 5 different drugs. Thus, Tetracycline was the most common single resistance (73.07%). These may be due to wider use of Tetracycline and its affordable nature from local pharmacy and most frequently utilized and exposed antimicrobials from among all veterinary drugs in Ethiopia. Similarly, a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. Hence, tetracycline are the only most commonly used antimicrobials for the treatment of other infections as well as salmonellosis in veterinary practice in Ethiopia, as the result, there was spread of drug resistance reported by many researchers which was in line with the recent findings.

7.CONCLUSION AND RECOMMENDATIONS

9.48% of salmonella prevalence was investigated in the study area of the poultry species. 5.47% of salmonella in Sasu breed and 14.06% of the Bovans breed salmonella were reported. *Study origin*, age categories, flock size, and sample types were non- significants whereas the breed, management, previous treatment history, vaccination satus, disease history, housing system, body condition, and sanitary/management condition were potential risk factors and which were statistically significant value for salmonella infection ($p<0.05$). 73.07%, 65.38%, 30.76 %, 26.92%, 15.38%, and 11.53% of the isolates were resistant to Tetracycline, Streptomycin, Trimthoprim, Chloramphenicol, Gentamycine, and Ciprofloxacillin respectively. However, the majority of the isolates were susceptible to Ciprofloxacin, Gentamycin, Chloramphenicol followed by Trimthoprim.

Based on the above conclusion, the following recommendations are forwarded:-

- Poultry farms are a potential source of *Salmonella* infection with antimicrobial resistance, and significant threat to public health particularly to those who have direct or indirect contact to poultry and poultry products so, hygienic management of poultry products,
- Identified potential risk factors should be managed properly in order to minimize the transmission of salmonella species,
- Biosecurity measures should be strictly applied in poultry farms where cross contamination was high,
- Chickens should be checked for healthyness and adaptation of the environment for that particular area before rearing was planned to design and precondition, predisposing factors should be assessed before production was conducted in farms so as to reduce or eradicated salmonellosis which was carrier once infect the chickens.
- Attention should be taken in selecting antimicrobials in treating *Salmonella* infection both in animals and human being based on antimicrobial sensitivity test.

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