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Salmonella: Isolation and antimicrobial susceptibility tests on isolates collected from poultry farms in and around Modjo, Central Oromia, and Ethiopia

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Abstract

A cross-sectional study was conducted during period of February, 2015 to May, 2015 with the objective of Salmonella isolation from poultry farms in and around Modjo town and to determine antimicrobial susceptibility profiles of the isolates. Accordingly, at total of 205 samples in which 100 cloacal swabs, 75 fresh feces, 10 litter samples, 8 chicken feed samples, 8 poultry drinking water and 4 chicken handlers' hand swab samples were collected.31(15.12%) isolates were detected from 205 collected samples. The studied poultry farms had different prevalence rates but not statistically significant. The lowest prevalence was 5(10.64%) whereas the highest was 10 (20.00%). These isolates 11(11.00%), 14(18.67%), 4 (40.00%) and 2 (25.00%) were recovered from cloacal swabs, fresh feces, litter and poultry drinking water samples respectively. Of the 31 isolates, 21 (67.74%) were motile (contributes to zoonoses) while 10(32.26%) were non-motile. Thirty of 31 isolates were resistant to one or more of antibiotics. Of 30, 19 were multidrug resistant while 11 isolates were only resistant to tetracycline. One isolate was resistant to tetracycline and Kanamycin. Furthermore, 2, 5, 4, and 7 isolates were tetra-, penta-, hexa-, and hepta-resistant, respectively. All the 31 isolates were susceptible to Ciprofloxacin and Gentamycin. 18 (94.73%) of multi-drug resistant (MDR) isolates were found resistant to five to seven different antimicrobials. According to this finding, Salmonella was isolated from different sample type, poultry growth stage, and breeds indicating its wider distribution. The detection of multi drug resistant 61.29% (19/31) isolates and 67.74% with likely of zoonotic potential indicated the salmonellosis could be an emerging poultry and public health problem. Therefore, further research is needed on major risk factors and molecular characterization for serotyping and genomic studies to have an idea about genes responsible for pathogenecity and drug resistance of the isolates of *Salmonella*.

Key words: Isolates, Multidrug resistant, Motility, Poultry breeds, Poultry farms, Poultry growth stage, Sample type

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Introduction

Food borne disease (FBD) has emerged as an important issue of growing public health and economic problem in many countries. The ultimate goal of all food safety programs is to stop contaminated food products from reaching the consumer. Surveillance for food borne diseases is conducted to delineate the occurrence and burden of important public health concern (Olasunmbo et al., 2014). Salmonellosis is one of the major food borne diseases in the world and it is estimated that 93.8 million cases of gastroenteritis due to *Salmonella* species occur globally each year, with 155,000 deaths (Majowicz et al., 2010).

Salmonella are short bacilli, 0.7-1.5 x 2.5 m, Gram-negative, aerobic or facultative anaerobic, oxidase negative, catalase positive, indole and VogesProskauer (VP) negative, methyl red and Simmons citrate positive, H_2S producing and urea negative. They ferment sugars with gas production, non sporogenic, and are normally motile with peritricheal flagella, except for *Salmonellapullorum* and *Salmonella gallinarum*, which are non motile (Forshell and Wierup, 2006). Optimal pH for multiplication is around 7.0; pH values above 9.0 or below 4.0 are bactericidal. Ideal temperature is between 35 to 37°C, with minimum of 5°C and maximum of 47°C. As for salt concentration, *Salmonella* do not survive concentrations over 9% (Franco and Landgraf, 1996).

Foods of animal origin, especially poultry and poultry products, are often involved in sporadic cases and outbreaks of human salmonellosis (Sanchez-Vargas et al., 2011). Prior to this Saif (2008) also quoted that poultry and poultry products are a common food borne illness vector and consistently among the leading animal sources of *Salmonella* that enter the human food supply. He also added that humans encountered this problem by consuming raw or undercooked food especially of poultry and egg products. The routine practice of using antimicrobial agents to livestock to prevent and treat disease is an important factor in the emergence of antibiotic resistant bacteria that are subsequently transferred to humans through the food chain (Tollefson, 1997; Witte, 1998). Most infections caused by antimicrobial resistant *Salmonella* are acquired by contaminated foods of animal origin (Angulo et al., 2000 ; Fey et al., 2000). Gyles (2008) indicated that the use of antibiotics for growth promotion is banned in European Union (EU) but permitted in USA and Canada and much of the rest of the world. Studies from different countries reveal that *Salmonella* serotypes isolated from foods of animal origin have multidrug resistance profiles (Holt et al., 2007 ; Prats et al., 2000). The role of meat and poultry products in the dissemination of antimicrobial-resistant zoonotic bacterial pathogens also is well documented (Logue et al. (2003); White et al. (2001); Witte (2000) ; Zhao et al. (2006)).

The poultry sector in Ethiopia can be characterized into three major production systems based on some selected parameters such as breed, flock size, housing, feed, health, technology, and bio-security: village or backyard poultry production system (up to 40 birds with local breeds), small scale poultry production system (50 to 500 flock size exotic breeds kept for operating on a more commercial basis) and commercial poultry production system (greater or equal to 10,000 of exotic birds) (Alemu et al., 2008).

In Ethiopia during a past decade, there has been gradual increase of commercial small and medium scale, market oriented flock production over the dominating traditional domestic poultry production. This reflects the efforts of the Government of Ethiopia to boost the productive basis of domestic birds within a genetic improvement programme by introducing and distributing exotic breeds, provide improved extension advice and services and to generally exploit the capacity of the sector to boost rural productivity (with the implications therein for raising incomes, providing employment and alleviating poverty). These programmes have been introduced courtesy of poultry multiplication and distribution centres (PMDC) (Gezahegn and Rich, 2010).

However, a report by Pagani and Wossene (2008) described the poultry multiplication and distribution centres as an unqualified success, and there is evidence that they have helped chicken production in urban and peri-urban areas to become a profitable venture over the last 15–20 years, with more families keeping small to medium-size flocks (approximately 50–1000 birds) under semi-intensive management (FAO, 2008). Although these PMDC have been set up in order to meet the increased demand for poultry products with small and middle class urban sector, most Ethiopians poultry producers have still poor knowledge on importance of many infectious diseases on production and public health (Sambo, 2014).

Several studies have been conducted on the prevalence and antimicrobial resistance of *Salmonella* in processed poultry, poultry products, and poultry processing plants in other countries and few examples are Agada et al. (2014) ; Urji et al. (2005) from Nigeria, Kagambega et al. (2013) from Burkina Faso, Al-Abadi and Al-Mayah (2012) in Iraq, Khan et al. (2014) from Pakistan, Hassanain et al. (2012) from Egypt, Jahan et al. (2012) from Bangladesh, Chen et al. (2006), Logue et al. (2003), Nayak and Kenney (2002) and Sanchez et al. (2002) from USA and White et al. (2001), Witte (2000) and Zhao et al. (2006) from England. Despite it, also there are more published and unpublished papers of study on *Salmonella* from dairy cattle and abattoir of large and small ruminants and other feed items in Ethiopia. These include the works of Teklu (2008), Molla (2004), Zewudu (2004), Addis (2011), Tadesse and Dabassa (2012) and unpublished: Bedashu (2014), Tadesse (2014), Yebeltie (2014) and Shibbiru (2015). However, little informations are also available about the isolation and antimicrobial resistance of *Salmonella* from chicken eggs and meats in Ethiopia. There were little informations on *Salmonella* associated with poultry farms (Kindu and Addis (2013); Kassaye et al. (2010) ; Ashwani et al. (2014)) on poultry related sources and mostly on *Gallinarum* and *S.pullorum*.

Thus, there is need for increased and sustainable surveillance of the most risk factors and antimicrobial resistant phenotypes of *Salmonella* isolates from poultry and poultry products. Therefore, the present study was initiated to isolate *Salmonella* from small scale poultry farms in and around Modjo town and to determine magnitude of antimicrobial susceptibility for the isolates.

Materials and Methods

Study area

Study was conducted on poultry farms in and around Modjo, Central Oromia, Ethiopia. Modjo is located 72km at south east of Addis Ababa with geographical location of 8.3ºN and 39º E at altitude of 1774 meters above sea levels. The area gain rainfall twice a year those known as long and short season rainy season. The main rainy season extends from June to September. The average annual rainfall, temperature, and mean relative humidity are 776mm, 19.4 Cº and 59.9% respectively.

Study Design and Study Population

A cross sectional study was conducted from February 2015 to May 2015 to isolate *Salmonella* from poultry farms and to determine antimicrobial susceptibility for the isolates. The sample were collected systematically from selected four small scale poultry farms and three of them are found in Modjo town and one was found in TaddeKebele around Modjo town. Prior sample collection cooperation letter were sent to each poultry farm. The sampling days were randomly assigned to each selected poultry farms and during the study period, each poultry farms were visited once a month. In the study, different sample types have been collected, namely, sample from poultry, poultry house, their feed and drinking water as well as pooled sample from hands of farm attendants for *Salmonella* isolation from poultry farms.All of the farms were kept their poultry on litter bedded houses. Houses' walls were made of wooden stable and mud which has many of wire meshed windows on the most top of the walls and roofs are also made of pieces of iron sheets. In the houses there are feeding toughs, hanged watering pools and light ampoules. They all have given concentrate feed that is commercially prepared and provided by local animal feed suppliers. All of them were kept layers for eggs and some also kept pullets and cockerels along with layers. They sell their products to the local shopper, restaurants and hotels.

Sample collection

Cloacal swab from the chicken, litter sample from poultry house, and pooled hand swab from the personnel working in the houses, feed sample from chicken feed and water sample from chicken drinking water were sampled aseptically. Each day of sampling 3-5 fecal samples, 4-5 cloacal swab samples and one sample on alternative day of sampling for litter, feed, and water samples and totally four handlers' hand swab samples were collected. All farms were visited 4 times for sample collection. Each sample was collected with sterile cotton swab then placed in rubber capped test tube containing 10ml of buffered peptone water. Approximately one gram of feaces was dispatched by sterile cotton swab and taken into the same test tube above containing 10ml of buffered peptone water. The samples were transported in portable coolers (ice box) immediately to the Microbiology Laboratory of the College of Veterinary Medicine and Agriculture, Addis

Ababa University and incubated at 37ºC for 24 hours. Then further processes were followed after samples have incubated for 24 hours.

Isolation of *Salmonella*

The isolation of *Salmonella* was done according to the technique recommended by International Organization for Standardization (ISO-6579, 2002), Global *Salmonella* Survey of WHO guidelines (Global Salm-Surv, 2003) and Quinn et al., (2004). According to this principle detection of *Salmonella* requires four successive stages: pre-enrichment in non-selective liquid media, enrichment in selective liquid media, selective plating on selective solid agar presumptive suspected isolates were identified and confirmed through screening against 6 biochemical tests. Subsequently, motility test was also done for positive isolates to determine motile from non-motile *Salmonella*. Motility test was conducted to differentiate those poultry adapted like *Gallinarum*and S.*pullorum* which are non-motile from those *Salmonella* species which are circulate between poultry and human (zoonotic) like S.*Enteritidis*and S.*typhmurium.* All media and reagents being used during study period were from three companies (OXOID in UK, HEMEDIA of India and CONDA of China) and were bought from country local suppliers. All this media and reagents were used according to the instructions of the manufacturers' .

Biochemical confirmation of *Salmonella* isolates

All suspected *Salmonella* isolates were subjected to the following biochemical tests for confirmation: Triple Sugar Iron (TSI) test, Indole test, Citrate utilization test, Methyl red test, vogues Proskauer (VP) test, and urease test. Colonies producing red slant (alkaline), with yellow butt (acid) on TSIA with blackening due to hydrogen sulphide (H_2S) production and e (gas production) in butt, negative for Indole test, positive for Methyl red test (red broth culture), negative for urea hydrolysis (yellow), positive for citrate utilization (deep blue slant), and negative for Voges-Proskauer (VP) test were considered to be *Salmonella* positive (ISO 6579, 2002; Quinn et al., 2004). Presumptive *Salmonella* isolates that were found fulfilled the *Salmonella* characteristics on all biochemical tests indicated above were transferred and cultured on Nutrient Agar (NA) for antimicrobial sensitivity and motility tests**.**

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the isolates was performed with Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute of U.S.A (CLSI, USA) and Kirby-Bauer Disk Diffusion Susceptibility Test Protocol (Jan, 2013) on Muller Hinton agar medium. From each biochemically confirmed isolate, loopful of well grown colonies on nutrient agar were transferred with sterile loop into sterile tubes containing 2ml of normal saline solution (0.85%NaCl). The inoculated colonies mixed well with saline solution by vortex until smooth suspension was formed. Saline solution (if suspension more turbid) or colonies (if suspension less turgid) were added to the suspension until it achieved to the 0.5

McFarland turbidity standards. Then sterile cotton swab were dipped into the suspension and the bacteria were swabbed uniformly over the entire surface of Muller Hilton Agar plate.

The plates were being held at room temperature for 3 minutes in biosafety cabinet to allow drying. Ten antimicrobial disks with known concentration of antimicrobial were placed on the Muller Hinton Agar plate; nine of them in circular pattern and one at the center and the plates were incubated for 22 hrs at 37ºC. All these ten antimicrobial discs were OXOID company's products and includes: Tetracycline (TE) (30µg), Ampicillin (AMP) (10µg), Cefoxitin (FOX) (30µg), Chloramphenicol (C) (30µg), Ciprofloxacin (CIP) (5µg), Gentamycin (CN) (10µg), Kanamycin (K) (30µg), Nalidixic acid (NA) (30µg), Streptomycin (S) (10µg) and Sulphamethoxazole-trimethoprim (SXT) (1.25/23.75µg). The diameters of clear zone of inhibition produced by diffused antimicrobial on lawn inoculated bacterial colonies were measured to the nearest mm using caliper. All ten zone of inhibition against ten antimicrobial agents for each isolate were recorded and compared with standards and interpreted as resistant, intermediate, or susceptible according to published interpretive chart (CLSI, 2013).

Data Management and Analysis

The raw data generated from the study were arranged, organized, coded and entered to Excel spread sheet (Microsoft® office excel 2007). Then the data was analyzed using SPSS version 20 through descriptive analysis with chi-square statistics. The results of analyses were mostly described in proportion. Proportion were estimated as the numbers of samples detected positive to *Salmonella* from the total sample tested as well as the numbers of antimicrobial resistant isolate to the detected positive isolate.

Results

Distribution of *Salmonella* isolation

Out of total 205 samples collected from the four poultry farms include cloacal swab, fresh feces, pooled litter, drinking water, feeding, and personnel hand swab 31(15.12%) [95% CI: 14.59-15.65] *Salmonella* isolates were detected. From all 5(10.64%), 7 (12.73%), 9 (16.98%) and 10 (20.00%) were *Salmonella* isolated from sample collected from Farm one, Farm two, Farm three and Farm four, respectively. No statistically significant differences were there between all four farms [X^2=2.052, df =3, *P-value=0.562*].Of 31, the contribution of samples for *Salmonella* positivity was 25(80.65%) from live chicken while 6(19.35%) from litter and water. There were no statistically significance differences in *Salmonella* recovery between samples collected from live poultry and other sample types [X^2=0.652, df=1, *P-value=0.420*]. From samples from live chicken 11(11.00%) and 14(18.67%) *Salmonella* isolates were obtained from cloacal swab and fresh feces samples, respectively. Six isolates were gained from samples other than live chicken; 4 (40.00%) from litter samples and 2 (25.00%) from poultry drinking water. No positive isolates were obtained from poultry feed and farm attendant's hand swab samples. Statistically, no significance differences were seen in recovery of *Salmonella* from different samples types [X^2= 9.626, df=5 , *P-value = 0.087*]. Statistically

distributions of isolates within samples from different poultry breeds were not significantly different as of 8 (9.30%) from Bovans brown, 13(17.81%) from ISA brown, and 4(25.00%) from White leghorn [X^2=3.984, df=2, *P-value=0.364*].

Also no significance difference were detected in distribution of isolates in different poultry growth stage as 4(25.00%), 17(14.17%), and 4(10.26%) isolated were obtained from cockerels, layers and pullets, respectively [X^2=2.018, df=2, *P-value=0.364*]. Detailed results were shown in Table 1.

Frequency results of motility tests

All31 (15.12%) positive isolates were screened for motility test. Twenty-one (67.74%) isolates were found motile while 10 (32.26%) were not. Among 21 motile isolates 9 (42.86%) were those isolate obtained from fresh feces samples, while 8 (25.81%), 3 (14.29%), and 1(4.76%) were those isolated from cloacal swab, litter and poultry drinking water, respectively, as shown in Table 2.

Table 1. Distribution of *Salmonella* isolates from different poultry farms, breeds, poultry, sample sources and growth stage.

Variables		Positive	Total	Prevalence (%)	95% LL&UL CI	X^2	*P*-value
Farms	Farm one	5	47	10.64	9.71, 11.57		
	Farm two	7	55	12.73	11.78, 11.67		
	Farm three	9	53	16.98	15.87, 18.09	2.052	0.562
	Farm four	10	50	20.00	18.76, 21.24		
Breed	Bovans brown	8	86	9.30	8.66, 9.95		
	ISA brown	13	73	17.81	16.84, 18.78	3.984	0.139
	White leg horn	4	16	25.00	22.55, 27.45		
Poultry	Cockerels	4	16	25.00	22.55, 27.45		
growth	Layers	17	120	14.17	13.49, 14.48	2.018	0.364
stage	Pullets	4	39	10.26	9.25, 11.26		
Sample	Cloacal swab	11	100	11.00	10.35, 11.65		
type	Feed	0	8	0.00	-		
	Fresh feces	14	75	18.67	17.69, 19.64	9.626	0.087
	Litter	4	10	40.00	36.08, 43.92		
	Attendants hand swab	0	4	0.00	-		
	Drinking water	2	8	25.00	21.54, 28.46		
Sample	Poultry	25	175	14.29	13.73, 14.85	0.652	0.420
source	Poultry environment	6	30	20.00	18.40, 21.60		
	Total	31	205	15.12	14.59, 15.65		

X^2= symbol of Chi-square
LL CI= Lower limit of 95% confidence interval
UL CI= Upper limit of 95% confidence interval

Table 2. Motility test for positive isolates and its distribution in different farms and sample types

Motility test	Farm and samples for which test was conducted				Total and %age
	Farm one	Farm two	Farm three	Farm four	
			4 CS	4 CS	CS 8 (25.81%)
Motile	2 Ff	4 Ff	2 Ff	1 Ff	Ff 9 (42.86%)
	1 L	1L	1L		L 3 (14.29%)
			1W		W 1(4.76%)
					Tt 21(67.74%)
		1CS	1CS	1CS	CS3 (30.00%)
Non-motile	1 Ff		1Ff	3 Ff	Ff 5 (50.00%)
	1 l				L 1 (10.00%)
		1 W			W 1 (10.00%)
					Tt 10(32.26%)

CS= cloacal swab, Ff= fresh feces, L= litter, W= water, Tt= total

Table 3. Frequency of antimicrobial resistant and susceptibility

Antimicrobials	Levels susceptibility associated with numbers of isolates			Total
	Resistant	Intermediate	Susceptible	
CN	0 (0.00%)	0 (0.00%)	31 (100%) (11 CS, 14 Ff, 4 L, 2 W)	31
TE	23 (74.2%) (8 CS,12 Ff, 2 l, 1 w)	4 (12.9%) (2 CS, 2 Ff)	5 (16.1%) (1CS, 1 Ff, 2 L,1W)	31
AMP	17 (54.5%) (6 CS, 9 Ff, 2 W)	1 (3.2%) (1 CS)	13 (41.9%) (4 CS, 5 Ff, 4 L)	31
NA	18 (58.1%) (7 CS, 9 Ff, 2W)	5 (16.1%) (3 CS, 1 Ff,1 L)	8 (25.8%) (1 CS, 4 Ff,3 L)	31
FOX	18 (58.1%) (7 CS, 9 Ff,2 W)	0 (0.00%)	13 (41.9%) (4 CS, 5 Ff, 4 L)	31
S	6(19.4%) (2CS, 3 Ff, 1 W)	11(35.5%) (4 CS, 6 Ff, 1W)	13 (41.9%) (4 CS, 5 Ff, 4 L)	31
CIP	0 (0.00%)	10 (32.3%) (4 CS, 4 Ff, 1 L, 1W)	21 (67.7%) (7 CS, 10 ff, 3 l, 1w)	31
K	16 (51.6%) (6 CS, 8 Ff, 2 W)	4 (12.9%) (1 CS, 2 Ff,1 L)	11 (35.5%) (4 CS, 4 Ff, 3 L)	31
SXT	17 (54.5%) (6 CS, 9 Ff, 2 W)	0 (0.00%)	14 (45.2%) (5 CS, 5 Ff, 4 L)	31
C	6 (19.4%) (3 CS, 3 Ff)	12 (38.7%) (4 CS, 6 Ff, 2 W)	13 (41.9%) (4 CS, 5 Ff, 4 L)	31

CS= cloacal swab, Ff= fresh feces, L= litter, W= water

Frequency of Mono antimicrobial resistance distribution

The 31 positive *Salmonella* isolates were screened for antimicrobial susceptibility test against ten antimicrobials. Thirty (96.77%) were resistant to one or more of antimicrobials. All isolates were susceptible to Gentamycin. Although all isolates were supposedly susceptible to Ciprofloxacin, 10 (32.3%) isolates were intermediately susceptible. Eleven (36.67%) of 30 resistant isolates were only resistant to Tetracycline; the rest 19 isolates were resistant to two or more antimicrobials. More than half of cloacal swab sample isolates were resistant to Tetracycline, Ampicillin, Nalidixic acid, Kanamycin, and Sulphamethoxasole-Trimethoprim (Table 3).

Frequency of resistance based on motility

From this study it was found that most of the resistant isolates were found motile.

Multi-drug resistance frequency distribution

Among 30 resistant isolates, 19 (63.33%) were resistant to two or more antimicrobials (multi-drug resistance (MDR)). The large proportion of multi-drug resistant isolates 17 (89.47%) were resistant to four to seven different antimicrobials while the other one resistant isolates was resistant to two different antimicrobials. 2 (6.67%), 5 (16.67%), 4 (13.33%), and 7 (23.33%) were tetra-resistant, penta-resistant, hexa-resistant, hepta-resistant, respectively with 11different resistance patterns. Among 19 MDR isolates, 9 (47.37%) from fresh feces, 8(42.11%) cloacal swab, and 2 (10.53%) poultry drinking water samples isolates. Among four isolated *Salmonella* from litter samples no one was resistant to more than one drug.

Table 4. Multi drug resistance and patterns

Number of antimicrobials	Types of Antimicrobials resisted and number of isolates	Number of resistant isolates (%)
Two	TE K (1 Ff)	1 (3.33%)
Four	AMP NA FOX SXT (2 Ff)	2 (6.67%)
Five	AMP NA FOX K SXT(1 CS, 1 Ff, 1 W)	5 (16.67%)
	TE NA FOX S K (1 CS)	
	TE NA FOX AMP SXT (1 CS)	
Six	TE AMP NA FOX K SXT (1 Ff)	4 (13.33%)
	AMP NA FOX S K SXT (1 CS)	
	TE AMP NA FOX S SXT (1 Ff)	
	TE AMP NA S K SXT (1 CS)	
Seven	TE AMP NA FOX K SXT C(2 CS, 2 Ff)	7 (23.33%)
	AMP NA FOX S K SXT C (1 CS)	
	TE AMP NA FOX S K SXT (1 W,1 Ff)	
Total	8 CS, 9 Ff, 2 W	19 (61.29%)

TE= Tetracycline, NA= Nalidixic acid, S= Streptomycin, FOX= Cefoxitin, AMP= Ampicillin, SXT= Sulphamethoxazole-Trimethoprim, C=Chloramphenicol, K= Kanamycin

The resistance patterns of some isolates were overlaps (the same resistance to different antimicrobials); for instance, two different isolates were the same hepta-resistance (Table 4).

Discussion

Frequency of *Salmonella* Isolation

The results of present study on *Salmonella* isolation indicates that 31(15.12%) isolates were obtained from 205 samples collected from cloacal swabs, fresh feces, pooled litter, drinking water, feeding, and personnel hand swabs. The intention of the current study was not to deal with prevalence as of sufficient sample size was not collected, rather to isolate *Salmonella* and to determine the response of isolates to antimicrobials.

From this preliminary study the rate of *Salmonella* isolation is comparable with prevalence reported in Ethiopia and in other countries. As example, 11.5% (Aseffa et al., 2011) from chicken table eggs by bacteriological methods in Ethiopia, 11.4% (Hassanain et al., 2012) in Egypt, 19.71% (Ashwani et al., 2014) by serology in Ethiopia, 12.5% (Urji et al., 2005) in Nigeria by bacteriological methods.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, and 45% (Jahan et al., 2012) in Bangladesh. 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 and 45% (Jahan et al., 2012) in Bangladesh.These differences above (higher or lower prevalence) from present finding might be resulted from the difference in study design, isolation technique, different in sample type and amount and difference in geographical location, breeds of birds and types of chicken and difference in quality of works.

Although large numbers of isolates were obtained due to large numbers of sample collected from layers, no statistically significance difference were detected in distribution of isolates in different poultry growth stage as of 4(25.00%), 17(14.17%), and 4(10.26%) isolated were obtained from cockerels, layers and pullets respectively in present study. In contrast to these, Kindu and Addis (2013) from Jimma were proved that layers and cocks were to be highly infected with *Salmonella* (46.2%) followed by broilers (41.3%) respectively. These differences are likely due to differences in proportions and types of representative samples and study design.In this study, all *Salmonella* isolates were isolated from live poultry, litter and drinking water samples. 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. However, in this study feed and human hand swab didn't give positive isolation which supports the report of Davies and Hinton (2000) "Even though feed is widely accepted as a source of possible contamination, the incidence of outbreaks being attributed to feed is very low".

The present numbers isolates from fresh fecal samples 46.67% (14/31) was not in agreement with findings of Urji et al. (2005) in Nigeria (12.5%) and Kagambega et al. (2013) 55% in Burkina Faso. The 36.67% (11/31) of cloacal swab isolate was in line to that of Al-Abadi and Al-Mayah (2012) who found that the frequencies of *Salmonella* isolates found by cloacal swabs samples in Iraq. Isolate from water samples 2/31(6.45%) were also less agreed with findings of Jahan et al. (2012) 60% in Bangladesh same type samples. In the current study, from litter bedding 4 of the 31 isolates were detected. No isolate were identified from the feed samples which in concord with finding of Al-Abadi and Al-Mayah (2012) 0% from ration samples.

Frequency of motile isolates

Salmonella in poultry are commonly classified into two groups on the basis of the diseases caused. The first group which consists of the poultry host-adapted, pathogenic, non-motile *Salmonellae*, *S. pullorum*causes Pullorum disease in chickens, and *S. gallinarum*is responsible for Fowl typhoid (Kwon et al., 2000). The second groups of *Salmonellae* are known as the paratyphoid *Salmonellae* and, they contain the two motile leading serotypes that are responsible for human infection, *S. typhimurium,* and *S. enteritidis* (Gast, 2003). The serotypes, *S. typhimurium*, and *S. enteritidis*, which produces illness in humans, usually remain sub-clinical in layer birds (Quinn et al., 2002). Accordingly, most of non host specific, motile *Salmonella* in poultry are probably zoonotic which cause disease in humans through food chains. With this view and understanding that motility tests were conducted for all 31 *Salmonella* isolates identified by biochemical tests.

Accordingly, 21(67.74%) were motile while 10(32.26%) were found non- motile. This result is in line with Jahan et al. (2012) finding of motility tests (59.26% were motile while 40.74% were non-motile) in Bangladesh.The motile isolates were suspected to be zoonotic serovars like *S. typhimurium,* and *S. enteritidis* while non motile once suspected as poultry adapted Salmonellosis (*S. pullorum* and *S. gallinarum*).

Distribution of the motile isolates in recovered sample types was traced back and revealed that 8 (25.81%) were cloacal swab isolates, 9 (42.86%) were fresh feces isolates, 3 (14.29%) were litter isolates and 11(4.76%) is isolate from water sample. Likewise, distributions of non-motile isolates were traced and found as 3 (30.00%), 5 (50.00%), 1(10.00%), 1(10.00%) isolates was identified from cloacal swab, fresh feces, litter, water samples respectively.

Frequency of mono resistant isolates

Of all 31 *Salmonella* isolates screened for antimicrobial susceptibility test against ten antimicrobials. All the isolates were susceptible to Gentamycin and Ciprofloxacilin, nevertheless 10 (32.3%) intermediately susceptible to Ciprofloxacin. This finding is similar finding of Begum et al. (2010) on *Salmonella* isolates from chicken eggs, intestines and environmental samples. For the rest 8 different drugs, 30 (96.77%) 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, 23 (74.2%), 17 (54.5%), 18(58.1%), 18(58.1%), 7 (22.6%), 16 (51.6%), 17 (54.5%), and 6 (19.4%) were resistant to Tetracycline, Ampicillin, Nalidixic acid, Cefoxitin, Streptomycin, Kanamycin, Sulphamethoxasole-Trimethoprim, and Chloramphenicol respectively High resistant to Tetracycline, Ampicillin, Nalidixic acid, Cefoxitin, Kanamycin, Sulphamethoxasole-Trimethoprim 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, betalactams, Trimethoprim and Chloramphenicol. Of 30 resistant isolates to anyone of the 8 drugs, 11 isolates were only resistant to Tetracycline while the rest 19 isolates were resistant to at least for two of the 8 different drugs. Consequently, (23/31) isolates were resistance to Tetracycline. Thus, Tetracycline was the most common single resistance (76.67%). 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.

Multi-drug resistance

Nineteen of 30 (63.33%) resistant isolates were multi-drug resistance (MDR) (resistant to two or more antimicrobials). This was concord with the findings of Payne et al. (2006) on broiler farms in which 96% of the isolates were resistant to greater than one antimicrobial agent (s) and Silvia et al. (2005) all strains isolated from poultry related samples were resistant to at least one antimicrobial agent.

All except one (18/19) multi-drug resistant isolates were resistant to four to seven different antimicrobials. Only one isolate was resistant to two different antimicrobials. Two isolates (6.67%), 5 (16.67%), 4 (13.33%), and 7 (23.33%) resistant isolates were shows tetra-, penta-, hexa-, and hepta-resistance, respectively, with different resistance patterns. This finding support the one that Sangeeta et al. (2010) reported on resistant isolated from chicken eggs poultry farms and from markets in that two isolates were resistant to as many as 10 antibiotics whereas, 2 isolates were resistant to 9 antibiotics, 2 to 8 and 5 to 7 antibiotics. It also seems consort with that of Jahan et al. (2012) in which out of 27 multi-resistant isolates, five isolates were resist to five different antimicrobials, 6 to 8, 7 to7, and 7 to 8 different antimicrobials with different resistance patterns. These all multi drug *Salmonella* isolates were confirms what Poppe et al. (1995 and 2002) reported as saying *Salmonella*e are among those most known to carry plasmids, which encode for drug resistance R (resistance) plasmids. This implies that widespread use of antimicrobials in animals or humans may cause an increase in the frequency of occurrence of bacteria resistant to other antimicrobials as the R plasmid may encode resistance to additional antimicrobials.

In conclusion, 15.12% *Salmonella* were isolated in Modjo. Distribution of *Salmonella* is not limited by sample type, poultry breeds, age and chicken production stage indicating its widespread and ubiquitous nature. Most of isolates were motile that reflects the majority of isolates have probability of zoonotic potential. Alarmingly, majority of the isolates have developed multi-drug resistance endangering poultry production and public health as these drugs are used widely for treatment and prophylaxis in animals and humans. Therefore, future research should be focused on molecular characterization for serotyping and *salmonella* population structure genetic studies along with genes responsible for pathogenecity and drug resistance of the isolates of *Salmonella*.

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