Prevalence, organ distribution and antimicrobial susceptibility profile of *Salmonella* isolated from chickens purchased from markets in selected districts of West Shoa, Ethiopia

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Abstract

Salmonella is one of the major causes of heavy losses in chicken and foodborne diseases worldwide. The current study was conducted from November 2015 to May 2016 to estimate the prevalence of *Salmonella* and determine the antimicrobial susceptibility of isolates in chickens. Chickens (n=205) were purchased from local markets of five selected districts of West Shoa Zone, Central Ethiopia. Following clinical examination, chicken were euthanized and 2-3 ml of blood sample was collected immediately. Then after postmortem examination, samples were collected from the liver, kidney, ovary, and spleen. The slide agglutination test was used to assess the seroprevalence of *Salmonella* antibodies. Isolation of *Salmonella* was performed according to the ISO-6579 procedure. The isolates were subjected to antimicrobial susceptibility testing (using 13 antimicrobial drugs) following the Kirby-Bauer disc diffusion method. The seroprevalence of *Salmonella* antibodies was 63.5% (95% CI: 55.9-70.5). The isolation rate of *Salmonella* was 19.0% (95% CI: 13.9-20.1) at the chicken level and 7.3% (95% CI: 5.5-9.4) at the organ level. The detection rate was 11.2%, 7.0%, 6.1%, and 4.4% for spleen, liver, ovary, and kidney, respectively. The majority of the *Salmonella* isolates were susceptible to norfloxacin (97.4%) and chloramphenicol (92.3%). All the 39 isolates were resistant to amoxicillin, tetracycline, and nitrofurantoin. Three multidrug resistance patterns to six antimicrobial classes were observed. Four isolates were resistant to five antimicrobial classes. Therefore, regular surveillance of *Salmonella* and its antimi-

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crobial resistance is needed for a better understanding of the epidemiological dynamics. Awareness creation for chicken farmers about improving farming practices and the risks of antimicrobial resistance warrants special attention.

Keywords: Antimicrobial susceptibility; Chicken; Prevalence; *Salmonella*; Ethiopia

Introduction

Salmonellae are Gram-negative bacteria that are facultative intracellular human and animal pathogens and members of the family *Enterobacteriaceae*. *Salmonella* Gallinarum and *S*. Pullorum are the highly host-adapted serovars affecting birds.*S*. Enteritidis and *S*. Typhimurium are the common serotypes causing disease in humans and animals (Gal-Mor, 2019). Salmonellosis is an infection ranging from subclinical to acute fatal septicemia or chronic diarrhea and death (Quinn *et al.*, 2011). In Ethiopia, salmonellosis is one of the important diseases of poultry reported from local and exotic chickens (Aragaw *et al.*, 2010; Chaka *et al.*, 2012; Kindu, and Addis, 2013). Fowl typhoid, which is caused by *S*. Gallinarum is a per-acute, acute, or chronic disease affecting mostly adult chickens, whereas pullorum disease, caused by *S*. Pullorum, affects young chickens of mostly 2−3 weeks of age (Parveen *et al.*, 2007).

In Ethiopia, fowl typhoid and pullorum diseases are mentioned to cause heavy economic losses through mortality, morbidity, and reduced productivity (Chanie *et al.*, 2009). Poultry salmonellosis has the ability of both vertical and horizontal transmission, which complicates the spread and control of the disease. As a result, layer chickens can become sub-clinically infected carriers and pass the infection to their embryo in the egg (Muktaruzzaman *et al.*, 2010). Other *Salmonella* serovars such as *S*. Typhimurium might also colonize the gastrointestinal tract of adult poultry asymptomatically (Gal-Mor, 2019). There are several reports on the detection of non-typhoidal *Salmonella* serovars from chicken samples (Molla *et al.*, 2003; Eguale, 2018), as poultry is the major source of foodborne salmonellosis for humans (Sanchez *et al.*, 2002).

On the other hand, *Salmonella* species could acquire antimicrobial resistance (AMR). The resistant strains are non-treatable with currently available antimicrobials, thus are serious global public health concerns both in humans and food animals (Parveen *et al.*, 2007). In Ethiopia, the veterinary drug regulation and guidelines are not well developed and there is no enforcing the standard to

practice responsible and prudent use of antimicrobials. As a result, indiscriminate use of antimicrobials, the sale of counterfeit drugs, the involvement of untrained personnel, and illegal drug market are big challenges (DACA, 2009). Moreover, as the veterinary service is not well developed, chicken owners have a practice of treating sick chickens using antimicrobials either from veterinary or medical pharmacies (Sambo *et al.*, 2015; Tufa *et al.*, 2018).

Culture-based and serological studies detected *Salmonella* Gallinarum, *S*. Pullorum, and other serovars from chicken in Ethiopia previously (Aragaw *et al.*, 2010; Berhe *et al.*, 2012; Dagnew *et al.*, 2020). There is also a report on the existence of multidrug-resistant *Salmonella* strains in chicken farms (Bekele and Ashenafi, 2010). However, there is little information about the prevalence and antimicrobial resistance of *Salmonella* species from chicken in Ethiopia (Molla *et al.*, 2003, Eguale, 2018) and no data is available in the study area. Thus, for a better understanding of the situation, and to make an evidence-based decision, investigation in this aspect will have a significant contribution. Therefore, the objectives of the present study were to estimate the prevalence of *Salmonella* and to determine antimicrobial susceptibility of the isolates in selected districts of West Shoa, Central Ethiopia.

Materials and methods

Description of the Study Area

Chickens were purchased from local markets of Ambo, Holeta, Guder, Ijaji, and Dire Inchini districts of the West Shoa Zone, Oromia, Ethiopia. Ambo is an administrative center of the Ambo district and center of West Shoa Zone located 114 Km West of Addis Ababa. It has a midland altitude. Holeta is located 40 Km west of Addis Ababa and Dire Inchini is located 70 Km southwest of Ambo, both are in highland altitude range. Guder and Ijaji are located 15 Km and 80 Km West of Ambo, respectively, and both are in the tropical climate. All the study districts experience a bimodal rainfall pattern. The chicken population of each district is approximately between 350, 000 to 500,000 (Etefa and Dibaba, 2011).

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Study animals

Chickens of both sexes and local and hybrid breeds managed under the backyard or small-scale management system were included. The chickens in this system, scavenge their feed with a limited supplement and often share the same house with humans or other livestock. Chickens were categorized as young (≤6 months) and adult (>6 months) based on their age (Calnek *et al.*, 1991). Chickens with clinical signs of diseases such as decreased appetite, depression, weight loss, ruffled feathers, and watery to mucoid diarrhea, dehydration, and anemia were categorized as clinically sick and otherwise apparently healthy.

Study design and sample size determination

A cross-sectional study was conducted from November 2015 to May 2016. The sample size was determined using Thrusfield (2007) formula with an expected prevalence of 8% (Aragaw *et al.*, 2010) and with 0.05 precision. Accordingly, the calculated sample size was 113 chickens, but 205 chickens were considered for this study. Eighty-four clinically sick were purchased purposely and 121 apparently healthy chickens were randomly selected and purchased from local markets. Sick and healthy chickens were separately transported in a cage with adequate space and ventilation. Chickens were slaughtered immediately upon arrival, but whenever there is a delay they were kept for a maximum of one day in Ambo University in a room with adequate living space, ventilation, and feed and clean water in ad libitum.

Postmortem examination

Post mortem examination was performed according to procedures by Chauhan and Roy (2007). The cervical dislocation was used to euthanize chickens. Following slaughtering, the gross lesions on the visceral organs were recorded.

Sample collection

Approximately 2-3 ml of the blood sample was collected immediately following euthanasia from each chicken in a new plain vacutainer tube. The blood samples were kept in a slant position at room temperature overnight. After 24 hrs. of clotting, the sera were subjected to centrifugation at 1000 RPM for 10 minutes and sera were collected using sterile Pasteur pipettes in cryovial tubes, labeled and stored frozen at –20°C until tested. A total of 741 samples comprising liver (n=205),

spleen (n=205), kidney (n=205) and ovaries (n=131) was sampled. About 25g of each organ was collected from the internal portion aseptically in a sterile polyethylene zipped plastic bag (Falconpack, UAE). The samples were kept at +4°C for a maximum of 24 hrs until culturing.

Serological examination

A serum slide agglutination test was performed following the test procedure described by OIE (2012). The sera and the reagent (crystal violet stained *Salmonella* Gallinarum antigens) were brought to room temperature (22 ± 5 °C) before use. Thirty microliters of the reagent and equal volume of the serum were gently mixed on a sterile slide by rocking and rotating for about 2-3 minutes. Any degree of agglutination was taken as evidence of seropositivity. The serological test was performed at the National Veterinary Institute, Bishoftu, Ethiopia.

Isolation and identification of *Salmonella*

Isolation of *Salmonella* was performed as recommended by ISO 6579 (2002) Amendl: 2007. Tissue samples were crushed by gentle maceration in polyethylene zipper bag mixed with buffered peptone water (BPW) in a ratio of 1: 9 and is incubated at 37 ℃ for 24 hrs. Then, 0.1 ml aliquots were inoculated into tubes containing 10 ml Rappaport Vassiliadis (RV) broth (HiMedia, Pvt. Ltd., India) and are incubated for 24 hrs at 42 ℃. Xylose Lysine Deoxycholate Agar (XLD) and Brilliant Green Agar (BGA) (HiMedia, Pvt. Ltd., India) plates were inoculated in parallel from the RV broths and incubated for 24-48 h at 37 ˚C. Up to five suspect colonies with typical *Salmonella* morphology on XLD (red colonies with/without black spots) and BGA (pink colonies) were streaked onto nutrient agar plates and incubated at 37 ℃ for 24-48 hrs. Finally, colonies from nutrient agar were confirmed biochemically by inoculating into Lysine Iron Agar (LIA), Triple Sugar Iron (TSI) Agar, Simmon Citrate (SC) agar Urea agar test, and motility test.

Antimicrobial susceptibility testing

All *Salmonella* isolates were tested for susceptibility to 13 different antimicrobial drugs using Kirby Bauer disc diffusion method as described by CLSI (2014). The antimicrobial impregnated discs (Oxoid Ltd, Hampshire, England) used were streptomycin, amikacin, gentamicin, amoxicillin, cefotaxime, ceftazidime, cefuroxime, chloramphenicol, ciprofloxacin, nitrofurantoin, tetra-

cycline, trimethoprim-sulfamethoxazole and norfloxacin with appropriate concentration. Two to three pure colonies were picked up and emulsified in 3 to 4 ml of sterile normal saline. Dilution of the suspended colonies was performed until the turbidity matches the 0.5 McFarland Standards and transferred to Mueller-Hinton agar (HiMedia, Pvt. Ltd., India) using a sterile cotton swab. Following air drying the plates were incubated aerobically at 37 °C for 18 to 24 hrs. The diameters of the zone of inhibitions were measured with a caliper and the susceptibility of the isolates was recorded as susceptible, intermediate, and resistant. *Escherichia coli* ATCC 35218 was used as quality control.

Data analysis

Data were entered into an MS Excel Spreadsheet and descriptive statistics were used to summarize the data. SATA version 11 (Stata Corp, 2009) for Windows **was used for analysis.** Prevalence was sorted by district, sex, age, and season, health status, diarrhea status, and organ. The association of the studied variables with the outcome variables was analyzed using logistic regression. Variables that were non-collinear and with P-value less than 0.25 during the univariable analysis were selected for multivariable logistic regression analysis. The odds ratio was used to compare the degree of association and the difference was considered significant when the P-value was less than 0.05. The percentages of antimicrobial resistance (AMR) were calculated as susceptible, intermediate, and resistant.

Ethical consideration

Permission for this study was obtained from the Ambo University Animal Research Ethics Review Committee (ARERC) and conducted under the approved protocol Ref. No. RD/AREC/003/2015.

Results

Clinical and postmortem findings

The clinical signs recorded in sick chickens in this study were decreased appetite, depression, dehydration, weight loss, ruffled feathers, and watery to mucoid diarrhea, anemia with pale, and shrunken combs. Accordingly, out of the 205 chickens, 84 (44.12%) and 121 (55.88%) were clinically sick and apparently healthy, respectively. *Salmonella* culture positive spleen, liver, and kidney showed one or more of the pathological signs such as enlargement,

congestion, white necrotic foci, and mottling. Ovarian follicles were regressed, misshapen, and discolored (Fig. 1). Out of 40 lesions, almost all were recorded from adult chickens.
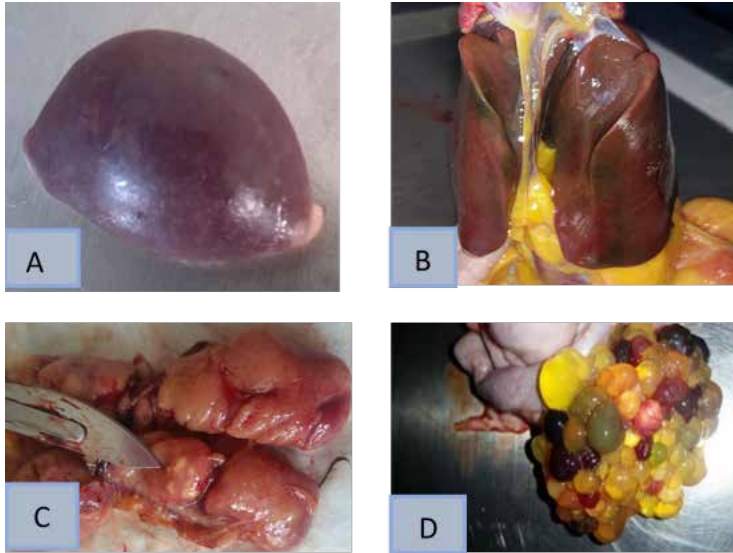
Figure 1. Gross lesions on *Salmonella* positive organs: enlarged and mottled spleen (A), discoloration and enlargement of the liver (B), enlargement and white spot appearance of kidney (C) discoloration and misshaping of ovarian follicles (D)

Serological result

Seroprevalence of *S.* Gallinarum was 63.5% (95% CI: 55.9-70.5). According to the multivariable logistic regression analysis, the study site showed significant variation in seroprevalence (p<0.05), whereas other variables did not show significant association (p>0.05). Hence, chickens from the Guder area were with greater Odds of seropositivity (OR=3.76, 95% CI: 1.09,12.98, p= 0.036) as compared to those from Dire Inchini (Table 1).

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Table 1. Logistic regression analysis of factors for seroprevalence of *S.* Gallinarum in poultry

Factors	Category	No. positive (%)	Univariable		Multivariable	
			OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Breed	Local	95 (62.9)	1			
	Cross	18 (66.7)	1.18 (0.50,2.80)	0.709		
Age	Young	60.6 (60.6)	1			
	Adult	70 (65.4)	1.23 (0.66,2.29)	0.510		
Sex	Male	38 (60.3)	1			
	Female	75 (65.2)	1.23 (0.65,2.32)	0.516		
Study site	Dire Inchini	17 (41.5)	1		-	-
	Ijiaji	11 (44.0)	1.11 (0.41,3.03)	0.840	0.84 (0.29,2.45)	0.754
	Ambo	34 (66.7)	2.82 (1.20,6.61)	0.017	1.82 (0.59,5.60)	0.295
	Guder	33 (82.5)	6.65 (2.39,18.55)	0.000	3.76 (1.09,12.98)	0.036
	Holeta	18(85.7)	8.47 (2.15,33.37)	0.002	4.82 (0.88,26.28)	0.069
Season	Wet	38 (46.3)	1		-	-
	Dry	75 (78.1)	4.13 (2.16,7.92)	0.000	2.15 (0.91,5.14)	0.082
Health status	App. Healthy	59 (57.8)	1		-	-
	Sick	54 (71.0)	1.79 (0.95,3.367)	0.070	0.64 (.20,2.02)	0.448
Diarrhea	Absent	73 (58.9)	1		-	-
	Present	40 (74.1)	2.00 (0.98,4.04)	0.055	2.00 (0.62,6.48)	0.247
Total		113(63.5%)				

CI= Confidence interval

Culture-based prevalence

From 205 chickens examined, 19.0% (95% CI: 13.9-20.1) were positive for *Salmonella* spp. About 38.5% (15/36) were non-motile, while 61.5% (24/39) of the isolates were motile. Of the 741 organs sampled, 7.3% (95% CI: 5.5-9.4) were *Salmonella* positive. The isolation rates from the spleen, liver, ovaries, and kidneys were 11.2%, 7.0%, 6.1%, and 4.4%, respectively (Table 2). Not all the studied variables showed a significant difference (*p*> 0.05). However, a rel-

atively higher isolation rate was observed in chickens of the Guder district (29.0%) as compared to Ijaji (8.0%), and adults (25%) as compared to young chickens (23.1%)(Table 3).

Table 2. The isolation rate of *Salmonella* from chicken organs

Organ	No. of organs	No. positive	Isolation rate (%)
Spleen	205	23	11.2
Liver	200	14	7.0
Ovary	131	8	6.1
Kidney	205	9	4.4
Total	741	54	7.3

Chi-square value =3.47, *p*-value >0.05

Table 3. Prevalence of *Salmonella* compared with the breed, sex, age, study sites, season, health and status of diarrhea

Factors	Category	No. of chicken	No. positive (%)	Odds ratio	95% CI	*p*-value
Breed	Cross	30	4 (13.3)			
	Local	175	35 (20.0)	1.62	0.53, 4.96	0.390
Sex	Female	131	22 (16.8)			
	Male	74	17 (22.9)	1.47	0.73, 3.00	0.281
Age	Young	82	21 (17.1)			
	Adult	123	18 (21.9)	1.37	0.68, 2.76	0.384
Site	Ijaji	25	2 (8.0)			
	Dire Inchini	41	4 (9.8)	1.24	0.21, 7.34	0.810
	Ambo	68	14 (20.6)	2.98	0.63, 14.18	0.170
	Holeta	40	10 (25.0)	3.83	0.76, 19.22	0.102
	Guder	31	9 (29.0)	4.70	0.91, 24.24	0.120
Season	Wet	98	15 (15.3)			
	Dry	107	24 (22.4)	1.60	0.78, 3.26	0.197
Health status	Healthy	121	21 (17.4)			
	Sick	84	18 (21.4)	1.30	0.64, 2.62	0.466
Diarrheal status	Diarrheic	57	9 (15.8)			
	Non-diarrheic	148	30 (20.3)	1.35	0.60, 3.07	0.465
Total		205	39 (19.0)			

CI= Confidence interval

Antimicrobial susceptibility

Salmonella isolates showed higher susceptibility to norfloxacin (97.4%), chloramphenicol (92.3%), trimethoprim-sulfamethoxazole (89.7%) and streptomycin (84.6%). All the isolates were resistant to amoxicillin, tetracycline, and nitrofurantoin, thus 100% multidrug-resistant (MDR) to these three antimicrobial classes (Table 4). Besides, three different MDR patterns to six antimicrobial classes were observed. Four isolates were multidrug-resistant to five antimicrobial classes (Table 5).

Table 4. Antimicrobial susceptibility test of *Salmonella* isolates

Antimicrobial class	Antimicrobial Agent	Disc Code	Potency (µg)	No. (%) resistant	No. (%) intermediate	No. (%) susceptible
Aminoglycosides	Streptomycin	S	10	2 (5.1)	4 (10.3)	33 (84.6)
	Amikacin	AMK	30	0	10 (25.6)	29 (74.4)
	Gentamycin	GEN	10	0	15 (38.5)	24 (61.5)
ß-lactams	Amoxicillin	AMX	25	39 (100)	0	0
Cephems	Cefotaxime	CTX	30	0	10 (25.6)	29 (74.3)
	Ceftazidime	CAZ	30	5 (12.8)	5 (12.8)	29 (74.4)
	Cefuroxime	CRX	30	3 (7.7)	21 (53.8)	15 (38.5)
Phenicols	Chloramphenicol	CHL	30	3 (7.7)	0	36 (92.3)
Quinolones	Ciprofloxacin	CPR	5	0	20 (51.3)	19 (48,7)
Nitrofurans	Nitrofurantoin	NIT	300	39 (100)	0	0
Tetracyclines	Tetracycline	TET	30	39 (100)	0	0
Folate pathway inhibitors	Trimethoprim-sulfamethoxazole	SXT	23.75	0	4 (10.3)	35 (89.7)
Fluoroquinolones	Norfloxacin	NOR	10 µg	0	1(2.6)	38 (97.4)

Table 5. Resistance patterns in *Salmonella* isolated from chicken (N=39)

Number	Antimicrobial resistance pattern	No. of resistant isolates (%)
Three	AMX TET NIT	39 (100)
Four	AMX TET NIT CRX	20 (51.3)
Five	AMX TET NIT SXT CHL	4 (10.3)

AMX Amoxicillin, TET Tetracycline, NIT Nitrofurantoin, CRX Cefuroxime, SXT Trimethoprim-sulfamethoxazole, CHL Chloramphenicol.

Discussion

Salmonella in poultry causes heavy losses through mortality and reduced production (Jordan and Pattison, 1996). The current study revealed 63.5% seropositivity to chicken salmonellosis, which is in agreement with 61.7% report of Sundar *et al.* (2007) and 64.2% Ashenafi *et al.* (2003). However, the present finding was higher than the 35.9% reported by Afera *et al.* (2012) from Ethiopia and 45.9% seroprevalence reported by Ahmed *et al.* (2008) from Bangladesh. The possible causes of the high prevalence recorded in this study could be the reason that salmonellosis in chicken is complicated by vertical and horizontal transmission. Subclinical carrier layer chickens might pass the infections to their embryos in the egg and to the rest of the flock, as there is no practice of culling carrier chickens in most of the chicken keeping communities in Ethiopia. The higher odds of seropositivity in Guder as compared to chickens from Dire Inchini could be due to possible contamination of chicken in the nearby village chickens from sick chicken brought to the big livestock market at Guder.

The 19.0% culture-based prevalence of *Salmonella* in the current study was comparable to a 16.7% reported by Abdi *et al.* (2017) but much higher than the 8% reported by Aragaw *et al.* (2010) using direct cloacal swab plating technique. This high prevalence in the present study could be related to the higher probability of *Salmonella* detection from spleen, liver, ovary, and kidney of carrier chickens. In this study, 38.5% of the isolates were non-motile and are either *S.* Gallinarum or *S.* Pullorum which are responsible for active clinical cases or chronic carrier state.

The organ level isolation rate was 7.3%, which is lower than 19.7% in China (Li *et al.*, 2013). Higher prevalence such as 47% (Uyttendaele *et al.*, 1999), and 23% were also reported(Alali *et al.*, 2012). In these studies, *Salmonella* contamination from the surface of the retail chicken carcass was the target, which could result in a higher prevalence rate compared to chicken organs that are free of any external contamination. The higher detection rate of *Salmonella* spp. in the spleen (11.2%) and liver (7.0%) compared to ovaries (4.4%) in the current study is nearly in line with the report of Rahimi (2012) from Iran who reported 14% (spleen), 6% (liver), and 4% (ovaries). Nazir *et al.* (2014) reported that the liver and spleen were the primary target organs involved in *S.* Gallinarum infection, irrespective of the route of infection. The infected liver and spleen were characterized by lesions such as hepatomegaly, discoloration of

liver, splenomegaly, congestion, and necrotic foci (Nazir *et al.*, 2012). This is in line with the current finding of lesions in the positive liver and spleen.

The lower detection rate of *Salmonella* spp. in young (17.1%) than adult (21.9%) chickens in the present study are in agreement with the reports of Temelli *et al.* (2012). According to Zewdu and Cornelius (2009), the prevalence of *Salmonella* increased with the increasing age of chickens. Generally, *Salmonella* in adult chickens might be attributed to the physiological stress of layers during egg-laying and molting, which depress their immune response and increase the susceptibility as compared to young chickens (Landers *et al.*, 2005). In this study, there was a lower detection rate of *salmonella* from exotic than local chickens. This could be because most of the exotic chickens sampled in the present study were growers recently distributed to farmers from the growing center, which are not expected to be the carrier. Moreover, the sample size of exotic chickens is small. The absence of a significant association of *Salmonella* detection rate and seropositivity with sick chicken could be because the clinical signs considered were not specific and some of the isolates might not be pathogenic to chicken.

Antimicrobial resistance in *Salmonella* spp. has been a global problem, with the rates increased to as high as 70% in some countries (Su *et al.*, 2004). In the present study, 100% resistance to amoxicillin, tetracycline, and nitrofurantoin are in accordance with the 100% resistance to amoxicillin and tetracycline (Hassan *et al.*, 2014), 100% to nitrofurantoin (Rampling *et al.*, 1990), and 97.8% to tetracycline (Abdi *et al.*, 2017). In contrast to the present finding, a lower resistance rate of *Salmonella* from poultry farms was reported to the aforementioned antimicrobials (Eguale, 2018; Dagnew *et al.*, 2020). This variation could be due to the different samples considered which in turn might also be associated with different serovars.The other probable reason could be that most chickens in this study were obtained from local chicken traders who use different antimicrobials unprofessionally for prophylaxis in healthy and treatmentof sick chickens, that might result in possible contamination with resistant strains. The MDR at least to three antimicrobials used which is similar to the pevious report (Yildirim *et al.*, 2011). The absence of resistance to norfloxacin indicates that the drug is a reserve drug as reported by Lee *et al.* (2005). These reports indicate that the utilization of antimicrobials is different from region to region and between antimicrobials themselves.

The finding of MDR to six antimicrobial classes in the present study supports the report of Abdi *et al.* (2017) who reported resistance isolates from chicken to 8 antimicrobials. The current finding supports the idea that *Salmonellae* are among the most known bacteria to carry plasmids, which encode for drug resistance (Poppe *et al.*, 2002). The widespread use of antimicrobials in animals/humans might cause an increase in the frequency of bacteria resistant to other antimicrobials as the R plasmid may encode resistance to additional antimicrobials.

Generally, poor hygiene and biosecurity, undeveloped diagnostic capabilities, lack a strong system for notification of pathogens, and antimicrobial resistance could contribute to higher disease burden in Ethiopia (Pagani and Wossene, 2008). However, the limitation of genotype data and the inability to get enough information from chicken owners in the present study made it difficult to analyze their role on pathogenesis and risk factor for drug resistance, respectively. According to ISO 2002, parallel selective enrichment- RP and Tetrathionate are recommended, but as the latter doesn't support the growth of *S.* Gallinarum and *S.* Pullorum we didn't use it, which might have affected the isolation rate of another *Salmonella* serotypes.

Conclusion

The present study revealed *Salmonella* is prevalent in chickens in the study areas. A higher isolation rate of *Salmonella* was recorded in the spleen and liver. A considerable number of *Salmonella* isolates from chickens were multi-drug-resistant to antimicrobial drugs commonly used for animals and humans. Therefore, there is a need for regular surveillance and monitoring of salmonellosis and antimicrobial resistance. Awareness creation for chicken farmers about improving farming practices and the risks of antimicrobial resistance warrants special attention.

Acknowledgments

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