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Isolation, identification antimicrobial susceptibility and associated risk factors of Salmonella in semi-intensive poultry farms of Kafa zone, Southwest Ethiopia

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ABSTRACT

Salmonellosis is one of the major causes of poultry disease. The study aimed to isolate, identify, determine susceptibility and associated risk factors of salmonella specious in semi-intensive poultry farms of Kafa zone, southwest Ethiopia. A cross-sectional study was conducted on four purposively selected districts. Three farms were randomly selected per district and fecal samples were taken from a total of 302 chickens. Questionnaire was administered to farm owners and data was analyzed using STATA statistical software package. The overall prevalence of *Salmonella enterica* species in Kafa zone was 9.27% with Gimbo district 10.39%, Bita district 10.66%, Shishoende district 12% and Chena district 4%. Source of chickens, farm types and breed risk factors showed significant association ($P < 0.05$) with the disease prevalence. Having diarrhea and continuous farm systems significantly associated ($P < 0.05$). All isolates were 100% resistant to Oxytetracycline and Ampicillin. Among 28 isolated *Salmonella enterica* species, 92.85% ($n = 26$) of them were showed multidrug resistance while 2 (7.14%) of them showed extensively drug resistance. Half of multidrug-resistant isolates were resistant to 5–6 antimicrobials, while 7.14% of isolates showed resistance to 7 antimicrobials. This study shows prevalence of *Salmonella* and its association with the breed, farm type, source of chicken and presence of diarrhea. A high antimicrobial resistance observed shows presence of concerns due to the emergence of Antimicrobial Resistance (AMR) in the poultry farms. Therefore, awareness should be created to the farmers on measures to avoid the risk factors of poultry disease and the occurrence of antimicrobials resistance in poultry farms.

1. Introduction

Ethiopia has an estimated total population of 54,495,026 poultry; among them 90.85% indigenous, 4.39% exotic and 4.76% hybrid breeds (CSA, 2017). Currently semi-intensive chicken production system is widely expanding in urban, pei-urban and rural areas of Ethiopia. This production system is characterized as flock of 50–200 improved breeds per household kept in small nighttime house with laying nest, feeder, vaccine and feed supplements (Afras, 2018). More than half percent of households in Ethiopia hold a varying collection of poultry flocks size (FAO, 2019) and it stimulated local economic development of peri-urban centers (Mutami, 2015).

However, chicken production is constrained by various factors, among which are low genetic potential of the indigenous breeds, high prevalence of infectious diseases and traditional feeding practice (Abda, Mamo, Worku & G., 2015; Dinka, Chala, Dawo, Bekana & Leta., 2010). In village chicken as well as poultry farms, infectious diseases, like Salmonellosis, Newcastle disease, Fowl cholera, Infectious Bursal Disease, mark's disease, fowl pox, and coccidiosis are remained to be responsible for morbidity and mortality (Habte et al., 2017).

Salmonellosis is a major cause of bacterial enteric illness in both humans and animals. *Salmonella* nomenclature is complex, and scientists use different systems to refer to and communicate about this genus (Brenner, Villar, Angulo, Tauxe & Swaminathan, 2000). The seven subgroups of genus *Salmonella* are subgroup I (enteric); subgroup II (salamae); subgroup IIIa (arizonae); subgroup IIIb (diarizonae); subgroup IV (houtenae); subgroup V (bongori); and subgroup VI (indica). Subgroup I contains most of the salmonellae organisms that are significant animal pathogens (Quinn, Carter, Markey & Carter, 1994).

Contaminated soil, vegetation, water, and components of animal-

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containing animal products, and the feces of infected individuals are the sources of infection (Scott, Kennedy & Chengappa, 2013). *Salmonella* is primarily transmitted by the fecal-oral route, often through ingestion of contaminated feed and water for chicken (Dwight & Yuan, 1999). It is known to spread from one country to another or within the country via live animal trade as well as by humans due to food-borne infections (F & Wierup, 2006). *Salmonella enterica* is the third top cause of foodborne illnesses with 78 million worldwide annual morbidities and 59 thousand mortality (Havelaar et al., 2015). The distribution of the pathogenic strains *Salmonella* were also causing a considerable loss in poultry farms but, also it is the main concern of human beings due to the transmission of *Salmonella* to human beings through consumption of chicken origin foods, contact with infected chicken and products (Gantois et al., 2009). As a result of the continuous use of some antibiotics for the treatment of *Salmonella* pathogens antibiotic resistance has been a common problem that blocks or limits the effective control of salmonellosis. (Schlundt, Toyofuku, Jansen & Herbst, 2004).

In Ethiopia, the prevalence of *Salmonella* serotypes affecting poultry industries varies. Some research reports showed as high as 15.12% (Abunna et al., 2017), while others reported 0.80% and 16.13% *S.* Gallinarum/ *S.* Pullorum serotypes in cloacal swab samples and post-mortem tissue samples respectively (Abdi et al., 2017). Although the above pilot studies conducted on the prevalence of *Salmonella* conducted in modern/intensive poultry farms in different urban areas of Ethiopia, there is no published study on isolation, identification and antimicrobial susceptibility pattern of *Salmonella* in semi-intensive chicken farms in rural and peri-urban districts of Kafa zone. Therefore, this current study was aimed to isolate, identify *Salmonella* and to determine its antimicrobial susceptibility pattern of isolates from chickens as well as to determine the factors associated with the prevalence of the disease.

2. Methods

2.1. Study area and study population

The study was conducted in four selected districts namely Chena, Bita, Gimbo and ShishoendeinKafa zone of South Nations Nationalities and Peoples Regional State of Ethiopia. Kafa is located in the South-western part of Ethiopia 470 Km from Addis Ababa. The total animal population of Kafa zone is cattle (3,243,380), sheep (1,821,415), goats (869,579), chicken (4,611,641), horse (317,418), mule (96,377), and donkey (28,782) (KZLFR, 2019).The study population was exotic poultry breed found in semi-intensive farms of selected districts of Kafa zone. All age groups and both sex were included in the study without exclusion criteria.

2.2. Study design

A cross-sectional study was conducted from November 2019 to August 2020 in semi-intensive poultry farms found in selected districts to isolate, identify salmonella species and to determine its antimicrobial susceptibility pattern of isolates from chickens as well as to determine the factors associated with the prevalence of the disease. Districts were purposively according to the potential of poultry production/availability of poultry farms. Within each district there are several poultry farms out of which three semi-intensive farms were randomly selected. From a total of 302 chickens required for the study, the number of chickens selected per selected farm was kept to be proportional to the chicken population of the farms (Table 1). Simple random sampling was used to select chickens and selected chickens were separated in another class or cage to minimize bias during sample collection. Age, sex, breed type, feeding types, water source, districts, house type, clinical status, the purpose of production and total flock population was recorded in the data collection sheet. A structured questionnaire was administered for farm owners to obtain additional information on the management system. Questions were related to farm type, size, biosecurity management, type of housing, cleaning of housing, sanitation of breeders, feeding management, common clinical signs occurring, availability of veterinary services, isolation of sick/dead chickens and use of antimicrobial drugs for therapeutic, or prophylactic purposes.

Table 1
Prevalence of *Salmonella enterica* species in district and farm level.

Districts	Farms	Number of examined	Total population of poultry	Positive (%)
Gimbo	Farm 1	30	291	0 (0)
	Farm 2	25	238	5 (20)
	Farm 3	22	107	3 (13.63)
Bita	Farm 4	15	115	0 (0)
	Farm 5	32	402	6 (18.75)
	Farm 6	28	340	2 (7.14)
Shishoende	Farm 7	26	311	3 (11.53)
	Farm 8	24	225	4 (16.66)
	Farm 9	25	276	2 (8)
Chena	Farm 10	20	244	0 (0)
	Farm 11	28	400	1 (3.57)
	Farm 12	27	350	2 (7.40)
Total		302		28 (9.27)

2.3. Sample size and sampling procedure

The sample size was calculated by using the formula given by Thrusfield, (Thrusfield, 2005) by using 5% accepted level of precision and expected prevalence of 27% in Bonga poultry multiplication center (Abdi et al., 2017).

$$N = Z^2 \cdot \frac{p_{exp}(1 - p_{exp})}{d^2} = 1.96^2 \cdot \frac{0.27(1 - 0.27)}{0.05^2} = 302.87$$

Based on the above formula total of 302 chickens were sampled and equal numbers of chickens per district were allocated. The number of chickens selected per farm within the district was kept proportional to the chicken population of the farms. Cloacal swab samples were collected from randomly selected live chickens using sterile cotton swab pre-moistened in buffered peptone water (BPW) (HiMedia, India) in the ratio of one to nine (i.e. 1 g swab sample ratio 9 ml BPW) (OIE, 2012), keeping aseptic procedure according to the method described in ISO-6579 (2002). All collected samples were placed in icebox and transported to the Mizan Regional Veterinary Laboratory Center within 2–4 h after sampling.

2.4. Laboratory isolation and identification

2.4.1. Non-selective and selective enrichments of samples

The pre-enriched BPW samples were incubated at 37°C for 18–24 h. Then 100μl mixtures was transferred to 10 ml Rappaport-Vassiliadis (HiMedia, India) medium and incubated at higher temperature 41.5°C for 18–24 h, to provide *Salmonella* with an advantage over most competitive organisms. Additionally; 1 ml of the pre-enrichment broth was transferred into a tube containing 10 ml of Muller-Kauffmann tetrathionate broth (HiMedia, India) and were incubated at 37 °C for 24 h (ISO-6579, 2002).

2.4.2. Isolation of salmonella

After 24 h of selective enrichment a loopful of cultures from both was streaked onto selective media Xylose Lysine Deoxycholate (XLD) agar (HiMedia, India) and Brilliant Green (BG) agar, modified (HiMedia, India) and incubated at 37°C for 24–72 h. Plates were observed just after 24 h of incubation and Gram staining was conducted and *Salmonella* suspected colonies were maintained on Tripticase Soy agar (Oxoid) at 4°C temperature for further characterization.

2.4.3. Biochemical identification of salmonella isolates

The presumptive *Salmonella* colonies from tripticase soya agar slants was streaked in nutrient agar in petridish for biochemical confirmation and only pure cultures taken from non-selective media were used. Five typical or suspect colonies were chosen and subjected to the different biochemical tests used for *Salmonella* identification (ISO-6579, 2002; Quinn, Carter, Markey & Carter, 2004). The identified *Salmonella* isolates was maintained on nutrient agar for antibiotic sensitivity test.

2.5. Antimicrobial sensitivity test

The antimicrobial susceptibility testing of the isolates was performed with Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute of USA (CSLI, 2018) and Kirby-Bauer Disk Diffusion Susceptibility Test Protocol (Jan, 2016) on Mueller Hinton agar medium (HiMedia, India). Totally 14 antimicrobial discs with a known concentration of antimicrobials were used. The name, code and disk content is indicated in Table 5. The diameters of clear zone of inhibition produced bacterial colonies were measured to the nearest millimeter using a digital caliper and compared with standards and interpreted as resistant, intermediate, or susceptible according to published zone size interpretive chart (CSLI, 2018).

2.6. Data management and analysis

Data was collected in data collection sheet, entered into a database designed using Microsoft Excel spreadsheet and analyzed using STATA statistical software package (version 13). Proportions for categorical variables were compared using the Pearson chi-square (χ^2) test for the association of all possible risk factors to the prevalence of the disease. In all cases $P < 0.05$was taken as a statistically significant association with the occurrence of disease while $P > 0.05$ was considered statistically not significant. The strength of association of the disease occurrence with risk factors was analyzed using multivariable logistic regression with $P < 0.05$ was considered as a significant association.

3. Results

3.1. Prevalence and risk factors of salmonella species

The overall prevalence of *Salmonella enterica* species in the Kafa zone was 9.27% ($n = 28$). The highest 20% prevalence was found in Farm 2 while, the lowest 0% prevalence was in three farms (farm 1, 4 and 10) within the respective district (Table 1).

The risk factors significantly associated with the prevalence of *Salmonella enterica* species were breed, source of chicken, health status and farm type (Table 2). For instance the 17.02% prevalence of *Salmonella enterica* species in white leghorn breeds was higher than in Sasso breeds with a statistically significant difference ($P < 0.05$). The Source of chickens also showed a statistically significant difference ($P < 0.05$) in that, the 17.02% prevalence of *Salmonella enterica* species in chickens from Bishoftu is higher than those from Gubire. Based on physical examination, apparently healthy compared to diarrheic chickens showed a statistically significant difference ($P < 0.05$) with the prevalence of 7.91% and 25% respectively. There was also a statistically significant difference ($P < 0.05$) in the prevalence of farms with all-in all-out and continuous farming systems with 6.21% and 13.6% respectively (Table 2).

Multiple logistic regressions were used to measure the strength of association of risk factors (odds ratio) with the prevalence of salmonella species. The forward stepwise analysis was used to exclude risk factors with no statistically significant difference and only risk factors with significance difference were included below (Table 3). Based on forwarding stepwise analysis clinically diarrheic and continuous farm type were showed statistically significant difference ($P < 0.05$) while, the rest risk factors were not showed significant difference (Table 3).

All of the poultry farms were using litter (wooden shave) in the floor and providing well/stream water sources for the drinking of chickens (Table 4). Depending on the previous history of diarrhea occurrence, in farms where the whitish, bloody and mixed form of diarrhea occurred 100% positivity was showed ($P > 0.05$) (Table 4). And, 57.14% ($n = 16$) of isolates were isolated from farms were the bloody type of diarrhea. From 9 poultry farms that uses Oxytetracycline antibiotics 7 of them (77.77%) were positive for *Salmonella* (Table 4). In a farm where poor treatment response reported 100% ($n = 8$) farms were positive and 82.14% of *Salmonella* was recovered from them ($P < 0.05$) (Table 4).

3.2. Antimicrobial susceptibility test of isolates

The antimicrobial susceptibility test result revealed that all *Salmonella* isolates developed resistance to Oxytetracycline and Ampicillin antimicrobials. The rest antimicrobial resistance pattern was Kanamycin 71.42%, Nalidixic acid 57.14%, Sulphamethaxazole 46.42%, Ciprofloxacin 32.14%, Streptomycin 32.14%, Neomycin 3.57% and Chloramphinicol 3.57%. The antimicrobial resistance pattern was never showed 0% for Gentamicin, Cefotaxime, Ceftriaxone and Norfloxacin

Table 2
Risk factors for prevalence of *Salmonella enterica* species.

Risk factors	Categories	Number of examined	Positive (%)	Chi-square (χ^2)	P-value
Districts	Gimbo	77	8 (10.39)	3.4294	0.330
	Bita	75	8 (10.66)		
	Shishoende	75	9 (12)		
	Chena	75	3 (4)		
Breed	White leghorn	47	8 (17.02)	3.9742	0.046
	Sasso	255	20 (7.84)		
Age	<1 months	52	4 (7.69)	0.7822	0.676
	1–2 months	183	16 (8.74)		
	Above 2 months	67	8 (11.94)		
Sex	Male	121	11 (9.09)	0.0078	0.929
	Female	181	17 (9.39)		
Source of chickens	Gubire	255	20 (7.84)	3.9742	0.046
	Bishoftu	47	8 (17.02)		
Health status	Normal	278	22 (7.91)	7.6675	0.006
	Diarrheic	24	6 (25)		
Purpose of production	Dual	236	21 (8.89)	0.1788	0.672
	Layers	66	7 (10.6)		
Farm types	All in-all out	177	11 (6.21)	4.7503	0.029
	Continuous	125	17 (13.6)		
Feed source	Commercially formulated	190	18 (9.47)	0.0249	0.875
	Locally formulated	112	10 (8.92)		

Table 3
Odds ratio of statistically significant risk factors in logistic regression.

Salmonella positive	Odds Ratio	Std. Err.	z	P>|z|	[95% Conf. Interval]	
Health status (Diarrheic)	4.360533	2.418561	2.66	0.008	1.470355	12.93174
Farm types (Continuous)	3.63791	2.017634	2.33	0.020	1.226772	10.78797

Table 4
Categorized risk factors for the prevalence in farms level.

Risk factors	Categories	Number of farms observed	Number of positive farms (%)	Number of isolates (%)	Chi-square (χ^2)	*P*-value
House type	Litter	12	9 (75)	28(100)	–	–
Water source	Well/stream	12	9 (75)	28(100)	–	–
Previous diarrhea occurrence history	Whitish diarrhea + other signs	2	2 (100)	5(17.85)	8.4444	0.077
	Bloody diarrhea + other signs	5	5 (100)	16(57.14)		
	Yellowish diarrhea + other signs	1	0 (0)	0(0)		
	Mixed + other signs	1	1 (100)	5(17.85)		
	No signs	3	1 (33.33)	2(7.14)		
Drugs used to treat sick chickens	Amprolium only	2	1 (50)	5(17.85)	1.7143	0.634
	Oxytetracycline only	2	2 (100)	6(21.42)		
	Both drugs (Amprolium and Oxytertacycline)	7	5 (71.42)	15(53.57)		
	No drugs	1	1 (100)	2(7.14)		
The response of diseased chickens for treatment	Good	4	1 (25)	5(17.85)	8.0000	0.005
	Poor	8	8 (100)	23(82.14)		

antimicrobials (i.e. between susceptible and intermediate pattern (Table 5). And, the rest antimicrobials showed different percentages of susceptibility, intermediate and resistance patterns.

Among 28 isolated *Salmonella enterica* species, 92.85% ($n = 26$) of them were showed multidrug resistance while 7.14% ($n = 2$) of them showed extensively drug resistance (Supplementary Table 1). 50% of multidrug-resistant isolates were resistant to 5–6 antimicrobials, while 7.14% of isolates showed maximum resistance pattern to 7 antimicrobials. The highest antimicrobial resistance pattern 35.71% ($n = 10$) was shown for five antimicrobials pattern categories next to four categories 28.57% ($n = 8$) and the least was for two, three and seven with 7.14% ($n = 2$) antimicrobials pattern category (Supplementary Table 2). The antimicrobial susceptibility result for each isolate was recorded in Supplementary Table 3.

4. Discussion

The overall prevalence of *Salmonella enterica* species in Kafa zone was 9.27%. This finding is greater than previous reports (Bayu, Asrade, Kebede, Sisay & Bayu, 2013; Eguale, 2018; Taddese et al., 2019) whom reported 2.98%, 4.7% and 4.69% in Jimma, central Ethiopia and Addis Ababa respectively. The reason for higher prevalence in the present study could be difference in the source of the breeding stock as well as difference in the farm biosecurity. However, it is lower than that of (Abunna et al., 2017; Alebachew & Mekonnen, 2013; Langata, Maingi, Musonye, Kiiru & Nyamache, 2019; Makaya, Matope & Pfukenyi, 2012; Rezaul et al., 2017) who reported 15.12%, 41.9%, 10%, 12% and 32% in Modjo (Ethiopia), Jimma (Ethiopia), Zimbabwe, Kenya and Dhaka (Bangladesh) respectively. The poultry production system may also play role in the distribution of pathogens, by shedding of the pathogen to the environment. Besides, direct transmission of the bacteria from humans as well as the difference in contamination levels of poultry feeds may be the factor for the prevalence of salmonella species.

Prevalence of *Salmonella enterica* species in white Leghorn breeds 17.02% was found to be higher than Sasso breeds 7.84% with a statistically significant difference ($P < 0.05$). A similar report was reported in Abunna et al. (Abunna et al., 2017) with the highest infection rate 25% for white Leghorn than bovans and ISA browns. In this study, only exotic poultry breeds were incorporated and thus comparison was done between two exotic breeds. In fact, comparing local breeds with exotic it is clear that local breeds are more adapted to their environment, more resistant to disease than exotic breeds (FAO, 2019). The factor for variation of infection in both breeds in this study might be related to the genetic variation to disease resistance, variation in the farm

Table 5
Antimicrobial susceptibility test pattern.

Antimicrobial disks	Disk codes	Disk potency	Number of susceptible isolates (%)	Number of intermediate isolates (%)	Number of resistance isolates (%)
Amoxyclav (Amoxicillin /Clavulanic acid)	AMC	30 μg	13(46.42%)	10(35.71%)	5(17.85%)
Streptomycin	STR	10 μg	4(14.28%)	15(53.57%)	9(32.14%)
Oxtytetracycline	OX	30 μg	0(0%)	0(0%)	28(100%)
Ampicillin	AMP	2 μg	0(0%)	0(0%)	28(100%)
Gentamicin	GEN	10 μg	27(96.42%)	1(3.57%)	0(0%)
Kanamycin	K	5 μg	0(0%)	8(25.57%)	20(71.42%)
Neomycin	N	30 μg	18(64.28%)	9(32.14)	1(3.57%)
Chloramphinicol	CHL	30 μg	9(32.14%)	18(64.28%)	1(3.57%)
Cefotaxime	CTX	30 μg	28(100%)	0(0%)	0(0%)
Ceftriaxone	CRO	30 μg	26(92.85%)	2(7.14%)	0(0%)
Sulphamethaxazole/trimethoprim	SXT	25 μg	11(39.28%)	4(14.28%)	13(46.42%)
Ciprofloxacin	CIP	5 μg	5(17.85%)	14(50%)	9(32.14%)
Norfloxacin	NOR	10 μg	22(78.57%)	6(21.42%)	0(0%)
Nalidixic acid	NA	30 μg	5(17.85%)	7(25%)	16(57.14%)

management system, duration of time that they spent in the farm.

Prevalence in the case of a source of chickens revealed that Gubire 7.84% and Bishoftu 17.02% with a statistically significant difference ($P < 0.05$). Chickens of day-old age brought from different commercial (intensive) poultry farms where their parent's adapted good biosecurity, disease control and prevention practices regularly. But in the semi-intensive farming system, the change in the management, biosecurity and disease control practices are very different. Such conditions might increase the disease prevalence in the poultry farming system. The infected bird, such as carriers, not only spread the infection to their own generation but also succeeding generations, through egg transmission (Shivaprasad, 2000; Wray & Davies, 1999). On top of this, there was no chickens vaccinated against *Salmonella* in all farms and also, no data obtained about the vaccination history of ancestor chickens in commercial farms.

Apparently healthy chickens infected with *Salmonella* less than clinically sick (diarrheic) chickens with the prevalence of 7.91% and 25% respectively. Positivity of chickens with the diarrheic sign was related with *Salmonella* infection ($P < 0.05$). The presence *Salmonella* positive chickens with and without a diarrheic sign indicate that several Salmonella carrier chickens exist in the farm. Previously sick but recovered chickens may still continue to shed the microorganisms and animals with chronic salmonellosis infection may also shed the microorganism (Radke, McFall & Radostits, 2002). As table eggs and poultry meat can transmit to humans, it poses a risk factor for public health (Hugas & Beloeil, 2014). Carrier chickens may get sick if body resistance is lowered by environmental stress or inter-current infection. The study showed that it was 4.3 times more likely to recover *Salmonella* in diarrheic chickens than apparently healthy chickens ($P < 0.05$). Chickens are frequently colonized with *Salmonella* by horizontal and vertical transmission at the primary production level without detectable symptoms (Barrow, Jones, Smith & Wigley, 2012; Cosby et al., 2015).

Farm types of all-in all-out and continuous were revealed 6.21% and 13.6% respectively with a significant difference among the farm type ($P < 0.05$). *Salmonella* has been reported to survive in poultry houses for at least 53 weeks in dust and up to 26 months in thin layers of litter, dried feces and feed (Davies & Breslin, 2003) following depopulation of a flock. As it was stated in Habte et al., (Habte et al., 2017), Wray and Davies (Wray & Davies, 1999) all-in all-out management styles allow simultaneous depopulation of facilities between flocks and allow time for periodic clean-up and disinfection to break the cycle of disease. In contrast with Habte et al., (Habte et al., 2017); Wray and Davies (Wray & Davies, 1999) report, the prevalence of *Salmonellosis* in all-in all-out management types in the present study was less than that of the continuous management system. This indicates that sanitation and disinfection of houses during all-in all-out time were poor to break the cycle of the pathogen in all-in all-out management styles. Continuous types of farms are 3.6 times more likelihood of being positive for *Salmonella* than all-in all-out types of farms ($P < 0.05$).

Depending on the previous history of diarrheal occurrence in the farm *Salmonella* positivity was revealed, in farms where the whitish, bloody and mixed type of diarrhea was seen. And, 57.14% ($n = 16$) of isolates were isolated from farms were the bloody type of diarrhea showed. Different journal articles reported that abnormal feces color (white, green, yellow) poultry infected with salmonellosis. While farms showed yellowish diarrhea were found to be negative for salmonellosis. This indicating that yellowish-colored diarrheal clinical signs are probably not related to the salmonellosis infection but, maybe due to other infections. In contrast, the report of Nazir et al. (Nazir et al., 2012) stated that watery yellow diarrhea was characteristic signs for acute Salmonellosis cases.

All *Salmonella* isolates were found to have developed resistance to Oxytetracycline and ampicillin antimicrobials. This result was in agreement with Abdi et al., (Abdi et al., 2017) resistance report to ampicillin, and Tesema et al., (Tessema, Bedu, Ejo & Hiko., 2017) report to tetracycline. Sannat et al., (Sannat et al., 2017) reported that *Salmonella* Gallinarum isolates were resistant to Chloramphenicol, Ampicillin, Ceftazidime, Cefexime, Cefepime, Azithromycin, Nalidixin, Tetracycline, Oxytetracycline and streptomycin. Since resistance to older antibiotics (e.g. Ampicillin, Chloramphenicol and Trimethoprim-Sulfamethoxazole) has been increasing for many years, recommended treatment options for salmonellosis included fluoroquinolones (Ciprofloxacin) and extended-spectrum cephalosporins (Chen, Wang, Su & Chiu, 2013; Parry & Threlfall, 2008). The problem is probably associated with the indiscriminate use of antimicrobial agents in feed to enhance growth promotion and prevent disease outbreaks that may serve as a selective pressure for killing the sensitive strains and may ultimately replace the drug sensitive microorganisms to be eliminated and favor the wide spread of drug resistance strains in the animals and humans (Muhammad, A, Hassan & Momena., 2009). Among the 9(75%) poultry farms using Oxytetracycline antibiotics for treatment of poultry disease and 7(58.33%) of them were positive for *Salmonella* and 82.14% of *Salmonella* isolates was recovered from them. Furthermore, however, the excess/overuse or underdose of antimicrobials can generate genomic selective pressures to enable microbes to adapt and acquire resistance (Assefa & Girma, 2019). Due to the limited access and relatively high price drugs, the reports of the prevalence of antimicrobial-resistant *Salmonella* to relatively low-priced and regularly available antibiotics are alarming for a low-income society.

In the current study, 92.85% ($n = 26$) of the isolate were showed multidrug resistance. This could be due to improper use of antibiotics without veterinary prescription. Similar results were reported (Abunna et al., 2017; Mthembu, Zishiri & El Zowalaty, 2019). As described in an experimental study (Kohanski, DePristo & Collins, 2010), sub-lethal bactericidal antibiotic therapy (underdosing) induces a heterologous increase in resistance for a range of other antibiotics. Those resisted antimicrobials drugs should be not used for treatment drugs in the farms. As a limitation to this study; serotyping was not done to identifying isolates into serotype level. Furthermore, detection of the resistant gene from antimicrobial-resistant isolates was not done due to a lack of diagnostic kits.

5. Conclusion

The present study showed that the prevalence of *Salmonella* has a significant association with the breed, farm type and source of chicken. The finding showed concerns about the emergence of AMR, especially a multi-drug resistance pattern. Based on the present finding we recommend that awareness creation for poultry breeders on the importance of good poultry management, disease prevention/control. Chicks should be obtained from *Salmonella*-free breeding flocks. The use of chemical disinfectants, regular hand washing, and wearing personal protective should be routinely practiced. Drug choice should be applied to clear resistant pathogen from the farms. To avoid the spreading of antimicrobial resisting *Salmonella* pathogen in the farm's selection of effective drugs should be needed during treatment, of sick chickens.

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Availability of data and materials

The data supporting the findings are presented in the manuscript. The corresponding author can also be reached for any data inquiry.

Ethics approval and consent to participate

The study was approved by the research proposal review committee

of the faculty of veterinary medicine, Hawassa University. Written informed consent was obtained for both questionnaires interview and sample collection to keep the confidentiality of specific poultry farm/farms at the time of sample collection. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Not applicable.

Informed consent information sheet

Purpose of the Study: The study aim at isolating, identifying and determining the antimicrobial susceptibility pattern of *Salmonella* from chickens thereby to determine the factors associated with the prevalence of the disease.

Description of the Research: Farm owners will be asked for short questionnaire survey to gather information about the current poultry farm management status and risk factors associated with the disease. Fecal samples will be collected from selected chicken in the farm for laboratory isolation and identification of the bacteria.

Potential Risks and Benefit: The study will be conducted through interviews and you are being asked for a little of your time about your farm. The fecal sample collection procedure has no risk. The finding of this research help you to know the current status of the disease and drug resistance pattern as you can get feedback recommendations that benefits the farmer.

Rights of the participant: Participating and not participating is the full right and participants can stop from participation in the study at any time. And also the participant can skip question which does not want to respond. Participants can ask any questions which is not clear for understanding.

Confidentiality: Any information you tell us will not be disclosed to third party. Farm name will not be specified. Result from the laboratory test will be kept confidential and you can be informed of the results upon its completion.

Address of the field investigator:

- **Tamirat Haile**: Mizan Regional Veterinary Laboratory Center, Mizan-aman, P.O.Box 254, Ethiopia, Cell phone: +25,120,110,182, e-mail: tamevet55@gmail.com

Farm owners consent form

In undersigning this document, I am giving my consent to participate in the study entitled as "**Isolation, identification and antimicrobial susceptiblity of *Salmonella* in semi-intensive poultry farms of Kafa zone, Southwest Ethiopia**" I have been informed that the purpose of this study is to assess the prevalence, antimicrobial sensitivity and risk factor associated with *Salmonella* in semi-intensive poultry farms. I have understood that participation in this study is entirely voluntarily. I have been told that my answers to the questions will not be given to anyone else and no reports of this study ever specify me and my farm. I have also been informed that my participation or non-participation or my refusal to answer questions will have no effect on me. I understood that participation in this study does not involve risks. I understood that the principal investigators are the contact person if I have questions about the study or about my rights as a study participant. I voluntarily agree to participate in this research.

CRediT authorship contribution statement

Sultan Abda: Conceptualization, Writing – review & editing. **Tamirat Haile:** Data curation, Formal analysis. **Mesele Abera:** Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2021.100206.

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