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Isolation, Identification and Antimicrobial Susceptibility Test of *Salmonella* in Semi-Intensive Poultry Farms of Hawassa City, Sidama Regional State, Ethiopia

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Abstract

Objectives: Poultry salmonellosis is considered as one of the devastating disease affecting the poultry industry. Cross-sectional study was conducted in semi-intensive poultry farms at Hawassa city, with the objectives of isolation,identification and antimicrobial susceptibility test of *Salmonella*.

Methods: A total of 122 cloaca and internal organs swab samples were collected from 10 poultry farms and subjected to various culture and biochemical examinations. Bacteriological test was conducted using different selective indicatory, differential and biochemical test media. The antimicrobial susceptibility test of the isolates was performed on Mulluer-Hinton agar medium for 9 selected antimicrobials disks.

Results: The overall distribution of salmonellosis in the Hawassa city was 11.47% (n=14). Management status, and health status were factors showed statistically significant association ($P<0.05$) with the disease occurrence. Out of 14 isolates 14.3% were motile while, 85.7% were non-motile. The antimicrobial susceptibility test result revealed that all isolates were 100% to susceptible to Ciprofloxacin, Chloramphenicol, Nalidixin acid, and Tetracycline while, all isolates were 100% resistant to Erythromycin, Gentamycin, Streptomycin, Kanamycin and Amoxillin. Among 14 isolates, 71.43 % (n=10) showed multidrug resistance. From the isolates 10 isolates to 3 drugs, 3 isolates to 4 drugs, and 1 isolate to 5 drugs have developed resistance.

Conclusion: The study demonstrates the public health and economic implications of Salmonellosis in Hawassa poultry systems, underlining the need for improved management, biosecurity, and rational antibiotic use to mitigate disease transmission and growing antimicrobial resistance. Hence: further serotyping and molecular study for identification of common serotypes circulating in the area and drug resistance strain isolate need to be conducted.

Keywords: Antimicrobial Susceptibility; Hawassa Identification; Isolation; Poultry Farm; Salmonella

Introduction

Poultry production is a key agricultural activity in Ethiopia, with an estimated population of 56.53 million birds raised under traditional, small-scale intensive, and large-scale commercial systems [17]. The sector provides important economic, social, and cultural benefits and contributes significantly to household nutrition in developing countries. Poultry is particularly valuable because it is affordable, easy to manage, and grows faster than other livestock species, making it an important source of animal protein. In addition, poultry production supports local economic growth by fostering micro-enterprises involved in input supply, processing, packaging, marketing, and service delivery [45].

Despite its substantial contribution, poultry productivity in Ethiopia is generally low. This poor performance is mainly associated with frequent disease outbreaks, limited availability of improved breeds, and inadequate feeding and management practices. Among the infectious diseases affecting poultry, salmonellosis is one of the most economically significant, causing considerable direct and indirect losses throughout the production and marketing chain [8].

Salmonellosis is a major zoonotic disease caused by bacteria of the genus Salmonella and represents a serious public health challenge with substantial economic implications worldwide [43]. These bacteria are widely distributed in the environment and are capable of causing disease in humans, animals, and birds. Poultry meat, eggs, and related products are the most common sources of human infection [26, 1]. Globally, millions of cases are reported each year, resulting in thousands of deaths [47], particularly in tropical regions where scavenging-based poultry production systems play a major role in household food security [42].

The genus Salmonella consists of two species, Salmonella enterica and Salmonella bongori, with more than 2,500 serovars of S. enterica identified worldwide [40]. While most Salmonella serovars are motile, Salmonella gallinarum and Salmonella Pullorum are non-motile and are specifically linked to clinical disease in poultry. These pathogens are responsible for substantial economic losses due to flock replacement and treatment costs, particularly in developing countries, including Ethiopia [61].

Globally, salmonellosis is a significant disease of chickens, with host-adapted serovars such as Salmonella gallinarum and Salmonella pullorum causing fowl typhoid and pullorum disease, respectively [50]. These infections lead to enteritis and high mortality, mainly affecting adult birds and growing chicks. The causative agents, Salmonella enterica serovar Gallinarum biovar Gallinarum and Salmonella enterica serovar Pullorum, are highly adapted to poultry and rarely cause serious disease in species other than chickens, turkeys, and pheasants [22].

Salmonellosis remains one of the most prevalent foodborne diseases worldwide, although its occurrence differs among countries [56]. Most infections are zoonotic in origin, arising from animal reservoirs, whereas certain serotypes such as Salmonella Typhi and Paratyphi are restricted to humans [23, 9]. Typical clinical signs include diarrhea, fever, and abdominal pain, appearing 12–72 hours after exposure and usually lasting 4-7 days; however, severe illness is more likely in infants, elderly individuals, and those with compromised immune systems [9].

All avian species are susceptible to Salmonella infection, although disease severity depends on factors such as age, host susceptibility, and bacterial virulence. Affected birds may show reduced feed intake, depression, dehydration, weight loss, ruffled feathers, and pale combs, and the pathogen can be transmitted both vertically and horizontally, making control difficult [36]. The widespread use of antimicrobials in food animals has contributed to the emergence of antimicrobial-resistant Salmonella, posing a serious risk to public health [24, 60, 38, 52]. In Ethiopia, increasing resistance to commonly used antimicrobials has been reported [10, 53, 1], raising food safety concerns and complicating disease management, particularly in intensive production systems and concentrated animal feeding operations [55].

There are a lot of research reports regarding presence of poultry diseases in different poultry farms of Ethiopia. Despite those facts, insufficient studies have been conducted on isolation, identification and antimicrobial susceptibility testing of *Salmonella* particularly in poultry farms of Hawassa city. It is known that and antimicrobial susceptibility testing is paramount for proper treatment and prevention of chickens. Therefore, the objective of this study was to isolate, identify and antimicrobial testing of *Salmonella* and to assess risk factors associated with its occurrence in chickens in Hawassa City poultry farms.

Materials and Methods

Description of the Study Area

The study was conducted on clinically sick and freshly died chickens selected from ten different poultry farms located in Hawassa city, Sidama Regional State. Hawassa city is located 275 km south of Addis Ababa. The annual rainfall and temperature of the area varies from 800-1000 mm and 20.1-25^{O}C respectively [15]. The average annual temperature of the area varies from 10 to 25 ^{O}C while rainfall varies between 500 and 1000 mm. The city is situated at an elevation of 1708 meters above sea level and located at 7^{0} 3'north latitude and 38^{0} 29' east longitudes (Figure 1). There are no large commercial poultry farms in Hawassa city, there are semi-intensive farms operated by private owners, almost all are mainly for the dual purpose. These farms hold exotic breed of chickens some are on cage and most of them are deep littler floor. Nevertheless many households in the town and its locality keep local chickens in backyards with extensive management (scavenging) system, clinically sick and freshly died (within 12hours) chickens were selected from private poultry farm outskirt of Hawassa University poultry farm, Dato poultry farm, Tabor poultry farm, Cheffe poultry farm, Hayikdar poultry farm, Tula poultry farm, Bahiladerash poultry farm, Maradona poultry farm, Millinem poultry farm, and Piassa poultry farm.

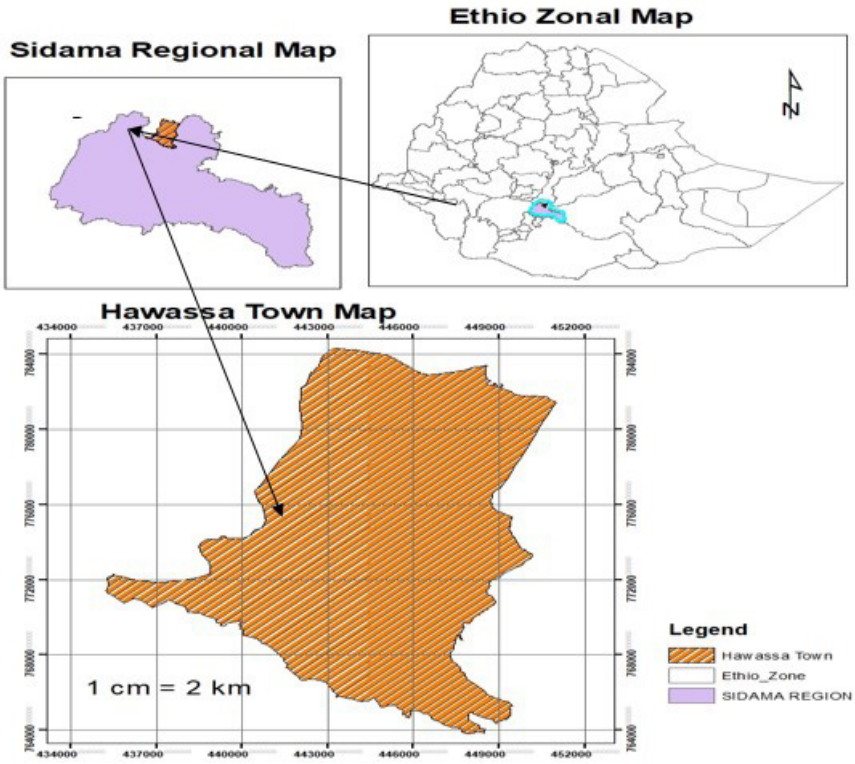

Figure 1: Map of the Study Area

Study Animals

The study involved a total of 122 sick and freshly dead chickens. Swab samples were collected from 111 clinically sick chickens, while 33 internal organ swab samples:- (liver, spleen and cecum) were collected from 11 freshly died chickens found in Hawassa poultry farms, Chickens managed under semi-intensive system of exotic breed (white leghorn and bovans) and all age groups and both sex were included in the study.

Study Design

A cross - sectional study was carried out in poultry farms found in Hawassa city, with the objectives of isolation, identification and antimicrobial susceptibility test of *Salmonella* isolates from semi-intensive managed poultry farms in Hawassa city. Before sample collection, letter was distributed to each poultry farm and communicated with owners by introducing the objectives of study and sample type. After an agreement was made with each selected poultry farms, sampling days were assigned. Cloacae and internal organs samples were collected from ten (10) selected semi-intensive poultry farms.

Study Method

Purposive sampling method was applied for selection of study sites by considering the availability of chickens, accessibility and absence or presence of poultry diseases in selected farms. The study involved bacteriological investigation of samples collected from sick and dead chickens for isolation, identification and antimicrobial susceptibility test of the *Salmonella* pathogen responsible to cause diseases in chickens. Therefore, cloacal swab samples were collected from sick chickens, while internal organs swab samples were collected from freshly dead chickens.

Sample Collection and Transportation

Claocal swab samples were collected from live sick chickens by using saline-moistened sterile cotton tipped; this was done by holding the chickens with its head down and its posterior end facing up for easy location of the anal opening. With right hand, a saline-moistened sterile cotton swab was inserted into the anal opening in a rotational manner to avoid hurting the chickens. Samples were collected by gently inserting and rotating sterile cotton tipped, then the swab sample swabs kept in properly sterile test tubes, that containing buffered peptone water (BPW), and labeling with name of farms, day of sampling, age, breeds, then placed into sterile ice box and transported to the Microbiology Laboratory of Veterinary Hawassa University for bacteriological analysis.

For aseptic collection of internal organs (liver, spleen, lung and caecum) swab samples from freshly dead (death within 12hrs), the carcass was placed on its back on a tray and legs are drawn outwards away from the body while the skin between the legs and the abdomen was incised. After opening the abdomen liver, spleen, lung and cecum sample were taken aseptically, the samples were placed on separate sterile petri-dishes. The surfaces of liver, lung and spleen obtained during postmortem were decontaminated by searching with hot spatula before they were cut with sterile scalpel blade for collection of samples from their interior using sterile cotton tipped swabs. The cecum was cut with sterile blade and the internal wall was swabbed on sterile cotton swabs, then after collection of the samples were labeled and placed in $4^{O}C$ at Hawassa University Veterinary Microbiology Laboratory, then samples cultured on bacteriological media and incubated at 37°C for 24 hrs for microbiological examination. The isolation was conducted utilizing the conventional methods for the detection of *Salmonella* species following the standard guidelines from (ISO- 6579, 49).

Laboratory Isolation and Identification of *Salmonella*

Pre-Enrichment in Non - Selective Liquid Media

Salmonella was isolated and identified according to the technique by which the international organization for standardization (ISO-6579, 49). Swabs samples that were collected from cloaca and organs were pre-enriched into buffered peptone water at ratio of 1:9 of the sample was inoculated into 10ml of buffered peptone water (BPW) and incubate at 37 °C for 24hrs. Pre- enrichment allows the recovery and multiplication of sub-lethally damaged *Salmonella* cells [14].

Enrichment in Selective Liquid Media

The swab samples were pre-enriched in appropriate amount of buffered peptone water in (1: 9) ratio and incubated at 37 °C for 24 hrs. Rappaport-Vassiliadis medium (RV) broth was used for selective enrichment of the samples, 0.1 ml of the pre-enriched sample was transferred into a tube containing 10 ml of RV broth and incubated at 42°C for 24 hours [14].

Plating out and Isolation of *Salmonella*

A loop full of inoculums from each RV cultures were plated onto XLD and incubated at 37°C for 24-72 hours. After incubation, the plates were examined for the presence of typical and suspect colonies. Typical colonies of *Salmonella* grown on XLD-agar have red colonies with or without black center with change of the color of media to pink (ISO 6579, 2002), while H_2S negative variants grown on XLD agar are pink with a darker pink center. Typical or suspected colonies were selected from the selective plating media, streaked onto the surface of pre dried nutrient agar plates and incubated at 37°c for 24 hours.

These pure *Salmonella* colonies/ isolates/ were characterized morphologically using Gram's stain according to the method described by [39]. Briefly, a small colony was picked up from nutrient agar plates and confirmation was done by using biochemical test (ISO- 6579, 2002).

Confirmation of Salmonella by Biochemical Tests

Isolated organisms with supporting growth characteristics of *Salmonella* were subjected to different biochemical tests such as: Triple sugar iron agar (TSI), Urease test in Christensen's Urea agar slope, agar, Simmon's citrate, Lysine iron agar, MR-VP reaction, indole reaction and motility test. For the confirmation of the typical *Salmonella* colonies using biochemical tests, typical and suspected colonies of *Salmonella* were selected from the selective plating media, streaked onto the surface of nutrient agar plates and incubate at 37°c for 24 hours according to ISO-6579(2002), all suspected non-lactose fermenting *Salmonella* colonies were picked from the nutrient agar and inoculate into following biochemical tests and interpret the results.

Triple Sugar Iron (TSI) Agar

On the Triple sugar iron agar slants were prepared with a thick butt. A loopful culture of pure growth from nutrient agar was stabbed into the butt and streaked on the slant and was incubated for 24 hours at 37°C. This typical *Salmonella* cultures show alkaline (red) slants and acid (yellow) butts with gas production (bubbles) of some samples and formation of hydrogen sulfide (blackening of the agar (ISO 2002; 49).

Urea Agar

The hydrolysis of urea releases ammonia and production of ammonia increases the pH of the medium that change color of phenol red (pH indicator) to rose pink, and later to moderate red. The basal medium was sterilized by autoclaving at 121 °C for 15 minutes. When it has cooled to about 50°C, a 40% solution of pure urea previously sterilized by filtration was added and

poured into test tubes. The isolates were inoculated into the urea to determine urease production. The inoculated tubes can be incubated at 37°C for up to 96 hours. The observations may be made at an interval of 4, 24, 48 and 96 hours. Urease positive cultures changed the color of the indicator to red (ISO- 2002; 48).

Simmon's Citrate Utilization Test

Simmon's citrate agar was sterilized by autoclaving at 121°C for 15 minutes and cooled for slant formation. The strains were cultured on the prepared Simmon's citrate agar medium, incubated at 37°C for 48 hours and observations were recorded. Opacity and change in color of bromothymol from green to blue indicated a positive reaction [49].

L-Lysine Decarboxylation Medium/Lysine Iron Agar

In the Lysine- decarboxylation broth was inoculated with the loop full culture of the test organism and one was kept un inoculated control. Both tubes were incubated for 24 hours at 37°C. Turbidity and after incubation indicate purple color slant with H_2s production shown a positive reaction. A yellow color indicates a negative reaction (ISO6579:2002).

Indole Test

SIM media prepared and dispensed in test tubes and sterilized by autoclaving at 121°C for 15 minutes. The tubes of the medium were inoculated with test isolates using sterile Platinum loop and incubated at 37°C aerobically for up to 96 hours. Finally, 0.5 ml of kovac's reagent was added to each of the inoculated and un inoculated controls. The tubes were shaken gently and the results were recorded. Positive results were indicated by the development of red color ring in the alcoholic layer of the reagent but in this case no color change or red ring formation [49].

Methyl-Red (MR) Test

Inoculate MR/VP broth with a pure culture of the organism and incubate at 37°c for minimum 48 hrs in ambient air, then by adding 5dropmethyl red reagent per 5ml of broth. And was observed red color (positive test).

Voges-Proskauer (VP) Test

Inoculate a tube of MR/VP broth with a pure culture of the test organism and incubate aerobically at 35°c, at the end of this, aliquat 1ml of broth to clean test tube then add 0.6ml of 5% alpha-naphthol, followed by 0.2ml of 40% KOH and after10-15minute deep rose color (negative test recorded).

Motility Test

The motility test was performed to differentiate motile *Salmonella* from non-motile one. This test was performed SIM medium, where a sterile straight wire used to inoculate 5 ml of sterile SIM medium taken earlier in a screw caped test tube with a smooth pure colony of that test organism. When inoculating the SIM medium, a stab was made with a sterile straight wire and stoppered the tube followed by incubation at 35-37°c for overnight. Motility is shown by a spreading turbidity from the stab line or turbidity throughout the medium compared with an un inoculated tube). In general, the studied tests results show: Indole, MR, VP and citrate, negative, positive, negative and positive respectively.

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 (CLSI, 2018), and Kirby-Bauer Disk Diffusion Susceptibility Test Protocol (Jan, 2016) on Muller Hinton agar medium (Hi Media, India). The Muller Hinton agar was prepared as per the instructions

provided by the manufacturer.

Approximately 3-5 colonies isolated from pure culture was transferred into a test tube of 5 ml nutrient broth. The broth culture was incubated at 37°c for 4 hours, there was prepared saline solution (0.85% Nacl), the turbidity of the suspension was adjusted by saline solution (if suspension more turbid) or colonies (if suspension less turbid) was added to the suspension until it achieved to the 0.5 McFarland turbidity standards. The 0.5McFarland turbidity standard was prepared as per the standard guidelines described by the Clinical and Laboratory Standards Institute (Jan, 2016). A 0.5 McFarland standard is equivalent to a bacterial suspension containing between $1x10^8$ and $2x10^8$CFU/ml of Escherichia col. Before each use, the standard was shaken well, mixing the fine white precipitate of barium sulphate in the tube. Density of the suspension of bacteria cells was compared to the McFarland turbidity standard (0.5 McFarland turbidity standards for antimicrobial susceptibility testing purpose) by holding the suspension and McFarland turbidity standard in front of alight against a white back ground with contracting black lines.

Muller-Hinton Agar plate was prepared and a sterile cotton swab was dipped into the suspension and swabbed on the surfaces of Muller-Hinton Agar plate. Then, the antibiotic discs was placed on the agar plate using sterile forceps and pressed gently to ensure the complete contact with the agar surface. The plates were read after 24 hours of incubation at 37^0C under aerobic condition. The isolates was classified in accordance with the guideline of the Clinical Laboratory Standards (CLSI, 2018) as susceptible, intermediate or resistance for each antibiotic tested according to the manufacturer's instructions by measuring, the diameter of the zone of inhibition around the antibiotic disc. This method allowed for the rapid determination of the efficacy of the drugs. Intermediate results were considered as resistant [27]. Multiple antibiotic resistant (MAR) phenotypes was recorded for isolates showing resistance to three and more antibiotics [51].

Totally, 9 antimicrobial discs with known concentration of antimicrobial like: Ciprofloxacin (CIP), (5µg) Chloramphenicol (CHL), (30µg). Gentamicin (GEN), (10µg). Tetracycline (TET). Erythromycin (ERT), (15µg). Nalidixinacid (NA), 30µg). Kanamycin (K), (5µg). Amoxicillin (AMC), (30µg) Streptomycin (STR), (10µg) were used. In Muller Hinton agar plate maximum of six antimicrobial disc was aseptically placed on the 24mm apart on centers; five of them in circular pattern and one at the center and plates were incubated for18-24hours at 37°c. The diameters of clear zone of inhibition produced by diffused antimicrobial on lawn inoculated bacterial colonies were measured by using digital caliper to the nearest millimeter. Zone of inhibition in each isolates for all nine antimicrobial discs was recorded and compared with standards and interpreted as: resistant, intermediate, and susceptible according to published zone size interpretive chart (CLSI,2018), according to CLSI, 4drugs are susceptible (zero resistant):-(CIP,TET,NA,CHL,) and 5 drugs are resistant:- (ERY, GEN, STR, K and AMC).

Data Management and Analysis

The raw data and laboratory results generated from the study were arranged, organized, coded and entered to MS Excel spread sheet, then the data was analyzed using STATA software version (13) through descriptive analysis with chi-square statistics. The results of analysis were mostly described in proportion. The proportions were estimated as the numbers of the samples detected positive from the total sample tested.

Ethical Clearance

Farm owners were ethical cleared orally for the cloacae and organs swab samples collection, personal privacy of the persons interviewed for the questioners was fully protected, for the confidentiality case.

Results

From the total 122 samples collected 14 (11.47%) of the samples were identified as positive for *Salmonella* species. The highest *Salmonella* distribution were observed in cheffe (50%) while the lowest isolate was recorded in hayik dare poultry farm (12.5%) as shown in (Table 1).

Table1: Origin of Isolate Based on Bacteriological Culture and Biochemical Tests.

Farms	No. of chickens examined	Positive in percent (%)	X^2	p-value
Bahiladerash	10	-		
Maradona	10	-		
H.U.P.F (cage)	18	-		
Millinem	12	-		
Piassa (cage)	14	-	28.07	
Tabor (cage)	10	2(20)		0.001
Dato (cage)	11	2(18.18)		
Cheffe	12	6 (50)		
Hayikdare	16	2(12.5)		
Tula	9	2(22.23)		
Total	122	14(11.47)		

In this study the occurrence of *Salmonella* related to the specific risk factors were determined as the proportion of affected chickens out of the total examined. As indicated in (Table 2) observation data result shows management status and health status are amongst the potential risk factors, which are significantly associated ($p<0.05$) with *Salmonella* disease in poultry farms. However, breed, sex, age, and housing have no significant difference with *Salmonella* ($p>0.05$) as indicated in (Table 2).

Table 2: Risk Factors Associated with Occurrences of Salmonellosis

Variable	Category	No. of examined	Positive (%)	X^2	p-value
Health status	Clinically sick	111	11(9.9)	17.47	0
	Freshly dead	11	3(27.28)		
Breed	Bovans brown	73	8(10.96)	0.048	0.827
	White leghorn	49	6(12.24)		
Sex	Male	30	3(10)	0.0852	0.77
	Female	92	11(11.95)		
Age	Young	34	4(11.76)	0.0039	0.95
	Adult	88	10(12.5)		
Sample type	Cloaca swab	111	11(9.9)	2.97	0.085
	Organ swab	11	3(27.26)		
Management status	Poor	67	12(17.91)	6.06	0.014
	Good	55	2(3.64)		

Housing system	Cage	53	4(7.52)	1.423	0.233
	Floor	69	10(14.49)		

Salmonella isolate from three organ samples in 11 chickens from different farm during study period, one female adult chicken infected two organ (liver and cecum), and also other adult female chicken from other farm, infected one organ (spleen) as shown (Table 3).

Table 3: Distribution of Salmonellosis from Examined Organ Samples

Sample type	No of organs examined	Frequency (%)
Liver swab	11	1 (33.4)
Cecum swab	11	1 (33.4)
Spleen swab	11	1 (33.4)
Total	33	3 (100)

Factors Related for the Positivity of Salmonellosis in Farm Level

Among the poultry farms which applying dipping chemically always at the entrance of the house were 33.33% (n=1) positive for salmonellosis as farms which not always applying chemicals were 100% (n=4) with statistically significant difference with (x^2=87.32, p=0.000) (Table 4). The poultry farms which had an opportunity of contact with other or wild birds /animals had showed 100% (n=1) positive and while farms with no opportunity contacting were 44.5% (n=4) positive for the bacteria with significant statistically difference (x^2=10.37, p=0.001) (Table 4).

Table 4: Factors Related for the Positivity of Salmonellosis in Farm Level

Questions	**Response**	**No. of farms observed**	**No. of Positive Farms (%)**	**X^2**	**p-value**
Did you clean feeding/watering regularly?	Yes	10	5(50)		
	No	0	0(0)	-	-
Did you isolate sick chickens from healthy one/s?	Yes	9	4(44.5)		
	No	1	1(100)	10.38	0.001
Is there dipping chemical always at the entrance of your farm?	Yes	6	1(33.33)		
	No	4	4(100)	87.32	0
Did you wash your hand always before and after handling of chickens?	Yes	7	3(42.85)		
	No	3	2(66.66)	0.57	0.45
Is there an opportunity of accessing other or wild birds/animals?	Yes	1	1(100)		
	No	9	4(44.5)	10.37	0.001

Cultural and Morphological Characterization *Salmonella*.

From the total 122 samples were cultured on different selective medium and 14 culture of them were showing growth of *Salmonella* colonies on XLD (Figure 2), as well as its growth on *Salmonella-shigella* agar (Figure 3), And the grown isolate were

subjected to sub culturing for pure colony appreciation, Then single bacteria colony streak on the surface of nutrient agar plates to obtain pure culture and *Salmonella* colonies was picked from the nutrient agar (figure 4), after bacteria colony characterized, identification of *Salmonella* through biochemical tests was conducted.

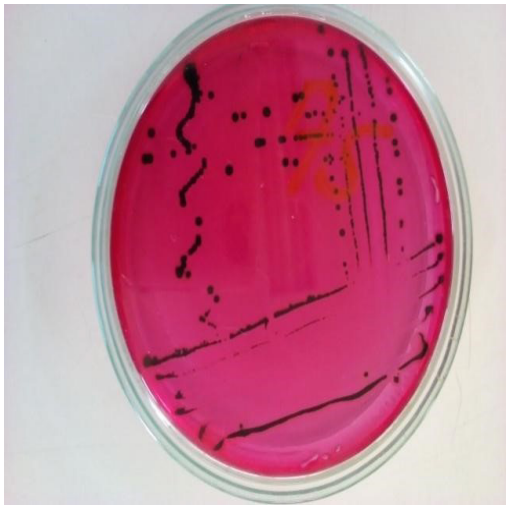

Figure 2: Growth of *Salmonella* Colony On XLD Agar.

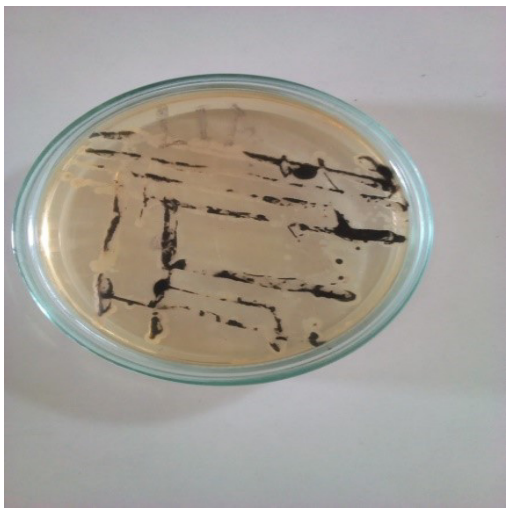

Figure 3: *Salmonella* Colony on S-S Agar

Figure 4: Suspected *Salmonella* Colony on Nutrient Agar

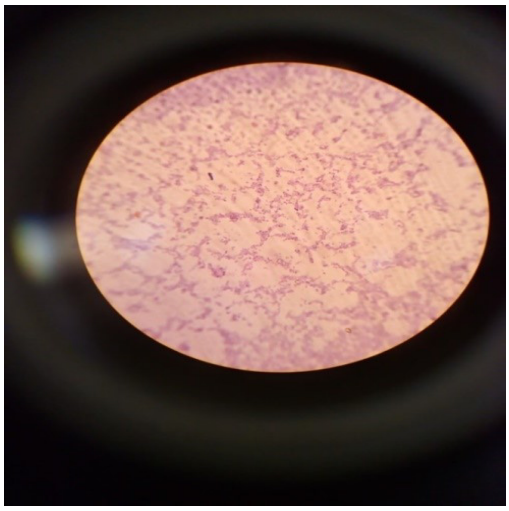

Figure 5: Gram Stain of S*almonella,* Gram-negative, pink colored, rod shaped,

Biochemical Characteristics of Bacteria Isolates

Presumptive *Salmonella* colonies were picked from the nutrient agar and inoculated into several biochemical tests and incubated for 24 to 48 hours at 37°c for identification of bacteria isolates. The results of reaction: colonies producing an alkaline slant with acid (yellow color) butt on TSI with hydrogen sulphide production, positive for Lysine (purple color slant) and purple butt with hydrogen sulphide, negative for urea hydrolysis(red color),negative for tryptophan utilization (indole tests) (yellow-brown ring), negative for voges-proskauer, positive for methyl red tests, and positive for citrate utilization. *Salmonella* fermented dextrose, maltose and glucose with acid and gas production. Catalase positive and oxidase negative were considered to be *Salmonella* positive (ISO, 6579; 49).

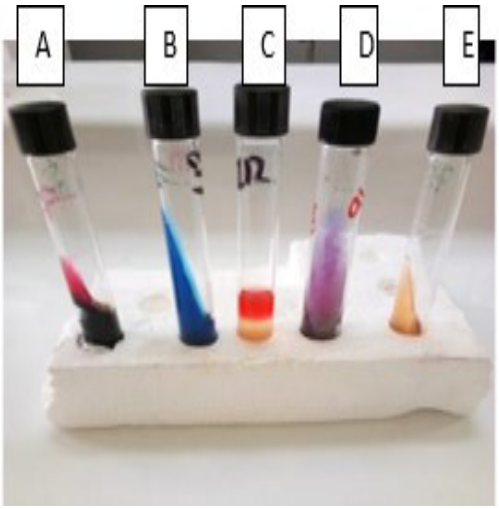

Figure 6: Biochemical Reaction of *Salmonella* In Different Media**.** A) TSI: Red Slant, Yellow Butt, Hydrogen Sulphide Production With Gas Formation, (B) Citrate Test: Blue Slant, Green Butt (Positive), (C) MR Test (Positive), (D) LIA Test Result (Positive), (E) Urease Test Result Negative, As Shown Figure Above.

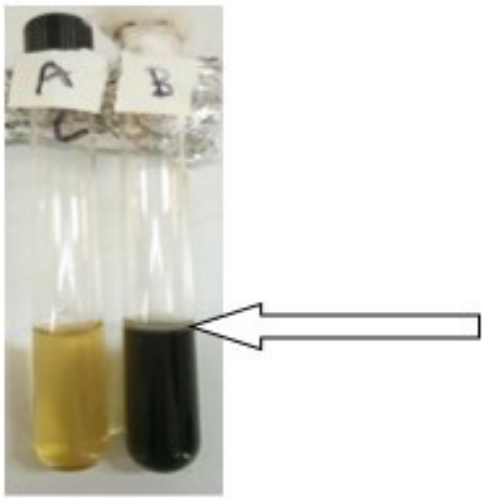

Figure7: Indole Indicating Reaction no Ring Formation on SIM Medium. A) Control, (B) Positive For H_2S, Negative For Indle Reaction as Figure shown above.

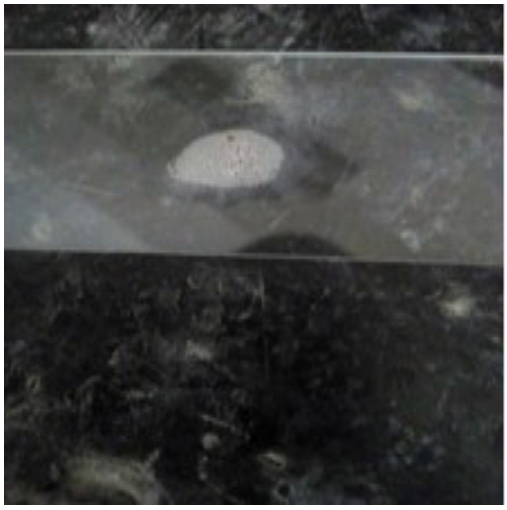

Figure 8: Positive Catalase Test of Isolates. Catalase Test Positive, There Is Bubble Formation.

Figure 9: Negative Oxidase Test of Isolates.

Negative For Oxidase Test as shown above

All of the isolate were MR and Citrate positive but VP and Indole were negative, while Catalase positive and oxidase negative as shown (Table 5). All isolates fermented glucose and mannitol but did not ferment lactose and sucrose, from total isolate 3 were fermented glucose and maltose and produced both acid and gas non- lactose fermented, and suspected as *Salmonella pullorum*, while 9 isolates were fermented glucose and maltose acid production without gas, non- lactose fermented. This isolates considered as *Salmonella gallinarium* and 2 isolates are suspected as *Salmonella typhimurium*, it is motile on SIM medium.

Table 5: Results shown in Biochemical Tests and Its Characteristic of *Salmonella* Isolates

Test types	Results	Characteristics shown from *Salmonella* isolates
XLD	+	pink colonies with or without black center, H_2s produced
S-SA	+	Black smooth colonies
NA	+	White, moist,circular disc with smooth colonies
TSI	+	Red slant with production of H_2s and yellow butt
LIA	+	Purple slant and purple butt with H_2s
Citrate utilization	+	Blue slant with green butt
Indole	-	No color change
MR	+	Diffused red color
VP	-	No color change
Urease	-	No color change(no hydrlzed)
Motility in SIM	+and/or -	Diffused or grow only stab line
Catalase	+	Bubble produced
Oxidase	-	Color was not changed

XLA=Xylose-Lysine Deoxycholate, S-SA=Salmonella Shigella agar, NA=Nutrient agar, TSI=Triple Sugar iron, LIA=Lysine Iron agar, MR=Methyl red, VP=Vogues Proskuaers, SIM=Sulphide Indole Motility, +=positive, -=negative.

Motility Test Characteristics of *Salmonella* Isolates

Among 14 positive isolates, 12 isolates were found to be non-motile characterized by forming the stab line without producing turbidity in the SIM medium and 2 isolates were found motile characterized by changing of the color of SIM medium. All 14 (11.47%) positive isolate were screened for motility test 12 (85.7%) isolates were found non-motile while 2 (14.3%) were motiles which were isolated from cloacae swab and internal organs swab as shown (Table 6).

Table 6: Motility Test Results for *Salmonella* Isolates.

Salmonella species	**Individual isolates**	**Proportion (%)**
None-motile	12	12(85.7)
Motile	2	2(14.3)
Total	**14**	**14(100)**

Antimicrobial Susceptibility Test

The antimicrobial susceptibility tests were performed on overall isolates of *Salmonella* species. Commercially available antibiotics discs like; Ciprofloxacin-5µg (CIP), Chloramphenicol-30µg (CHL), Gentamicin-10µg (GEN), Tetracycline-30µg (TET), Erythromycin-15µg (ERT), Nalidixin acid-30µg (NA), Kanamycin-5µg (K), Amoxillin-30µg (Amc), Streptomycin-10µg (STR).

All isolates were resistant to three or more of the tested antimicrobials. And the size of zone of inhibition of every antibiotics disc was measured in millimeter and while those zone of inhibition compared with zone of diameter interpretive standards from CLSI (2018) as indicate below in Figure 10.

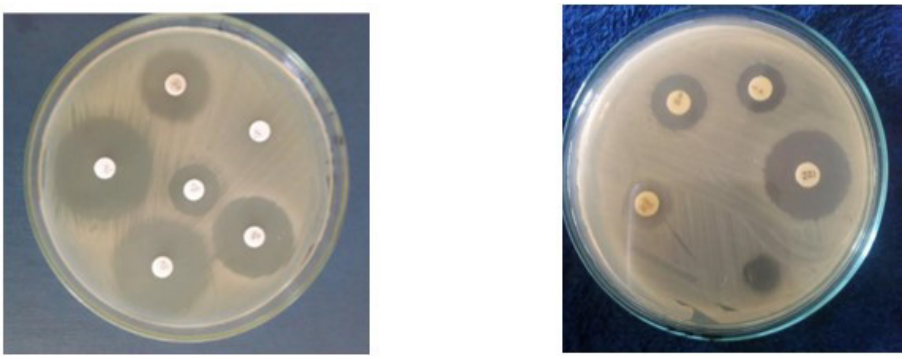

Figure 10: Antimicrobial Drug Sensitivity Test on Isolates From Hawassa City Poultry Farms, CIP, CHL, GEN, TET, ERT, NA, K, Amc, STR

All the *Salmonella* isolates exhibited zero resistance (100% susceptible) to, Ciprofloxacin-5µg, chloramphenicol-30µg, Tetracycline-30µg and Nalidixin acid-30µg. Some isolates were the most frequent multi drug resistant (MDR) patterns to 4 drugs, which were Erythromycin, Gentamicin, Streptomycin, and Kanamycin respectively, as shown (Table 7). The antibiotic susceptibility testing revealed that the *Salmonella* isolates exhibited the highest resistance against, Erythromycin-15µg (92.86%), and the least resistance against Amoxillin-30µg (64.4%), However, GEN, STR and K exhibited 85.7%, 78.6%, and 71.5%, respectively.

Table 7: Antimicrobial Susceptibility Test Result for *Salmonella* Isolates (n=14).

Antimicrobial agent	Disk content (µg)	No. of isolate	Susceptible (%)	Intermediate (%)	Resistance (%)
Ciprofloxacin (CIP)	5µg	14	31(100)	-	-
Chloramphenicol(CHL)	30µg	14	20(100)	-	-
Gentamicin(GEN)	10µg	14	-	2(14.3)	12(85.7)
Tetracycline(TET)	30µg	14	17(100)	-	-
Erythromycin(ERT)	15µg	14	-	1(7.14)	13(92.86)
Nalidixin acid(NA)	30µg	14	21(100)	-	-
Kanamycin(K)	10µg	14	-	4(28.57)	10(71.5)
Amoxillin (Amc)	30µg	14	-	5(35.71)	9(64.4)
Streptomycin(STR)	10µg	14	-	3(21.43)	11(78.6)

Key: S-susceptible, I-intermediate, R-resistance of Salmonella isolated from cloacal and organ swab of chickens.

Out of 14 isolates 10 (71.43%) had multidrug resistance indicating one isolate was resistance to three or more drugs, in this regard 10 isolates to 3 drugs, 3 isolate to 4 drugs, and 1 isolates to 5 drugs, have developed resistance. From Total *Salmonella* 10 (71.43%), 3 (21.43%), and 1 (7.14%) of the isolates were resistant for 3-5 drugs respectively, as shown below (Table 8).

Table 8: Antimicrobials Resistance Pattern for The *Salmonella* Isolates

No. of antimicrobials	Antimicrobials resistance Pattern (No. of isolates)	No. of isolates (%)
Three	ERY, K, AMC (10)	10 (71.43)
Four	ERY, K, AMC, and STR (3)	3 (21.43)
Five	ERY, K, AMC, GET and STR (1)	1 (7.14)
Total		14(100%)

Key: GEN=Gentamicin; K=Kanamycin; AMC=Amoxillin; ERT=Erythromycin; STR=Streptomycin

Discussion

The present study exhibited the overall 14 (11.47%) isolates of *Salmonella* from cloacae and internal organs swab samples collected from chickens. The present finding was similar with [59, 11, 25] reported 12.5%, 11.5% and 11.4% in Nigeria, Ethiopia and Egypt, respectively. This similarity could be due to the geographical location, breed, sample type and age of the chickens.

The present finding were higher than previous studies in Ethiopia and other countries, thus 1.6% [37] in Addis ababa, 2.98% (Taddese *et al*., 2009) in Jimma, 4.7% [18] in central Ethiopia, 8% [32] in Hawassa, *S. gallinaruim* and *S. pullorum* from cloacal and organs swab by bacteriological methods, 10.9% [4] in Nigeria, and 9.2 % [6] in Iraq in culture techniques in cloacal swab samples. The higher occurrence might be due to the difference in geographical location, breed of chickens, management status.

Likewise, the present study was lower than previous reports of 15.12% [2] in Modjo, Central Oromia, of *Salmonella* infection in poultry, 41.9% [7] in Jimma, 14% (Endrias, 2004) in Addis Ababa supermarkets, 18% [13] in Dire Dawa municipal abattoir, by [41] who detected 21.1% of *Salmonella* in chickens carcass in Ethiopia, 30.4% [31] in DAZARC poultry farm, at Bishoftu, central Oromia, 41.9% [34] from fecal sample by bacteriological method. 56.6% [33] in Pakistan, and 45% [29] in Bangladesh in cloacal poultry samples. The difference may be due to the Environmental persistence of *Salmonella* affects its epidemiology in poultry by creating opportunities for the horizontal transmission of *Salmonella* among chickens with in the flocks and managements status.

Effects of Potential Risk Factors on the Occurrence of Salmonellosis

Based on management status occurrence of *Salmonella* was significantly associated with hygienic practice. poultry at farms with poor management status are severely affected than those with good management practice (17.91%) in higher occurrence of *Salmonella* infection was recorded in poor management status, whereas (3.64%) lower infection was investigated in good management status which was significantly associated with infection (X^2=6.06, p=0.014). This might be due to absence of good management practice, floor bedding of poultry house, feed and watering.

Based on sex, this finding was exhibited male 10% and female 11.95% with non-significant difference (p>0.05). This study was in agreement with the report of [57], with nearly similar indication for sex with male 31.65% and female 32%. The difference with the report of [7], with male 39% and female 43%, in this finding increase in female may be due to the sequestered nature of localized infection of the ovarian follicles and during egg production and molting immune response might be depressed, increase chance to attack *Salmonella* infection.

In present finding age difference showed that increasing the infection of *Salmonella* with increasing age of chickens, age category in this study depending on questionnaire from each farm respondents from one day-old to five month category as young

and above five months as adults' chickens. In this finding exhibited *Salmonella* infection in young chickens (1-5month) 10.53% and in adult chickens (above 5 months) 11.9% with p>0.05. This finding was agreement with [54]. Highest infection was found to be in adult age 30.76% and lowest infection was found to be in young age 13.33%. This study was disagreement with [12], report with the susceptibility of chickens to infection with *Salmonella* tends to decrease with age, in this study might be attributed with the reality that layers are physiologically stressed during egg production and molting which significantly depress the immune response of layers and increase the susceptibility to *Salmonella* infection and adult chickens might be due to attributed to stay longer duration of in the farms than young chickens and by this reason adult chickens have enhances more chance to infected with *Salmonella* infection than young chickens.

Salmonella occurrence in white leghorn breeds 12.24% was found to be higher than bovans breed 10.96% with statistically (p>0.05). In fact compering local breed with exotic, obvious local breed are more resistant to disease than exotic breeds (FAO, 2018), but only exotic poultry breeds were incorporated in this study, so that comparison done between two exotic breeds and case of exotic breed no report was found clearly, which breed were more susceptible and resistant for *Salmonella* infection, but [58], was report that in Haramaya poultry farm, who indicated, the occurrence of *Salmonella* in eggs on the base of chicken breed sources was 2% for white leghorn and 2.9% for bovans. The factor for the variation of infection rate in both breed in present study might be to the unequal exposure to the risk factor, different in management system in farm, and genetic variation to resistant disease.

In the present finding based on housing system of (12.9%), high amount of indication of *Salmonella* in farms with floors bedding was diagnosed, whereas 6.89% lower infection was recorded in cage types, which influence the occurrence of *Salmonella*, and was statistically non-significant (p>0.05), this finding was similar to the study reported [20] that indicated chickens kept in floor bedding 11.2% than kept in cage system 8.6% This finding disagree with the study [3] in French who stated that, the apparent distribution was significantly higher in caged flocks than in on-floor flocks. This variation may be due to poor sanitation, and poor management status in farm.

Salmonella in poultry are commonly classified into two groups on the basis of the diseases caused. The first group which consists of the poultry hosts adapted, pathogenic, non-motile *Salmonella*, *S. pullorum* causes pullorum disease in chickens, and *S. gallinarum* is responsible for Fowl typhoid [35]. The second groups of *Salmonella* are known as the paratyphoid *Salmonella* and, they contain the two motile leading serotypes that are responsible for human infection, *S. typhimurium,* and *S. enteritidis* [21]. The serotypes, *S. typhimurium*, and *S. enteritidis*, which produces illness in humans, usually remain sub-clinical in layer birds [49]. Accordingly, most of non-host specific, motile *Salmonella* in poultry are probably zoonotic which cause disease in humans through food chains. Depending on of above views and understanding that, motility tests were conducted for 2 (14.3%) *Salmonella* isolates identified by secondary biochemical tests, while 12 (85.7%) were found non-motile. The motile isolates were suspected to be zoonotic serovars like *S. typhimurium*, and while non-motile once suspected as poultry adapted salmonellosis (*S. pullorum* and *S. gallinarum*).

Antimicrobial Susceptibility Pattern

Among 14 Salmonella isolates screened for antimicrobial susceptibility test against nine antimicrobials. All the isolates were susceptible to Ciprofloxacin, Chloramphenicol, Tetracycline and Nalidixin acid. The reason why these antimicrobials were zero resistant/susceptible/ in this study might be due to that they are not used in the study area in veterinary clinics or services except Tetracycline and nature of the drugs. This finding agrees with the work carried out [44] in Nigeria, however, contradicted by the work carried out in Cameroon [5]. Such variation and similarity could might be result from the difference in the geographical location, different strains of isolates and/or difference in levels of strain's resistivity.

The study exhibited high level of resistance observed for Streptomycin (78.6%) and Kanamycin (71.5%), which agree with previ-

ously study reported by [13] in Dire Dawa maniacs abattoir, Streptomycin (81.1%) and Kanamycin (79.5%), respectively. The antibiotic susceptibility testing revealed that the Salmonella isolates exhibited the highest resistance against, Erythromycin-15µg (92.86%), and the least resistance against Amoxicillin-30µg (64.4). In this study out of 14 isolates, 10 (71.43%), were multi-drug resistant (MDR) to different combination of three or more tested antimicrobials, All multi-drug resistant isolates were resistant to three to five (3-5) different antimicrobials, 10 isolates resistant to 3 drugs, 3 isolate resistant to 4 drugs, and1 isolates resistant to 5 drugs.

According to the study, 13 (92.86%), 12 (85.7%), 11 (78.6%), 10 (71.5%) and 9 (64.4%), were resistant to Erythromycin, Gentamycin, Streptomycin, Kanamycin and Amoxillin respectively. The high level of Salmonella resistance to antibiotic may be due to the fact that, in many of the sub-Saharan African countries antimicrobials can be purchased without any prescription and indiscriminate use of antimicrobial agents by unskilled practitioners both in the veterinary and public health sectors is common [46].

Conclusion and Recommendations

Salmonella infection remains a one of major poultry pathogen. The result of present study *Salmonella* in semi-intensive poultry farm in area exhibited of 14 (11.47%) isolates, among them 12 (85.7%) isolates were found to be non- motile, while 2 (14.3%) were motile. In this study management status and health status potential risk factors, which were statistically significant value for *Salmonella* infection ($p<0.05$) whereas breed, age and sex factors were not significant ($p>0.05$). In this study 9 antimicrobial discs that tested by disc diffusion method. Out of 14 isolates 10 (71.43%) had multidrug resistance indicating one isolate was resistance to three or more drugs, in this regard 10 isolates to 3 drugs, 3 isolate to 4 drugs, and 1 isolates to 5 drugs, have developed resistance, However, all isolates were susceptible (zero resistance) for 4 antimicrobials agents. Based on the above conclusion, the following recommendations were forwarded:-

Awareness creation for poultry breeders' rational use of all management practice in and around poultry farms, antimicrobial agent should not be used without veterinarian prescription and in the absence of the disease,

Chickens should be vaccinated according to vaccine calendar, to against occurrence of salmonellosis.

Identified the potential risk factors and properly managed in order to reduce transmission of *Salmonella* and Separate of chickens that having different age groups, infected and health on.

Further study, on molecular characterization of serotyping and genetic studies for pathogenicity and drug resistance isolates of *Salmonella.*

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