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ORIGINAL RESEARCH

Prevalence and Antimicrobial-Susceptibility Profiles of Salmonella in Smallhold Broiler Supply Chains in Central Ethiopia

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Purpose: Salmonellosis is a foodborne zoonoses found worldwide. The main purpose of this study was to isolate and identify Salmonella and assess their antimicrobial susceptibility profiles from smallhold broilers supply chains and slaughterhouses in Bishoftu and Modjo, central Ethiopia.

Methods: Four smallhold broiler farms under the auspices of Chico Meat were selected randomly. Feed, water, and water- and feed-trough samples were collected from broiler farms, while cecal contents were collected from slaughtered chicken at Chico Meat slaughterhouse. Conventional bacteriological techniques were used to isolate and identify Salmonella from the samples. Kirby-Bauer disk diffusion was employed to assess the antimicrobial susceptibility of the isolates.

Results: Salmonella was isolated from 131 (24.3%, 95% CI 20.74–28.15) of the 539 samples tested. Salmonella was found in 43 of the 250 samples collected from Bishoftu (22%, 95% CI 17.02%–27.65%) and 76 of the 289 samples collected from Modjo (26.29%, 95% CI 21.32%-31.77%). Salmonella was isolated from 26.46% of the cloacal samples, 21% of the cecal contents, 30.77% of the feed samples, 25% of the water samples, 22.22% of samples from feed troughs, and 20% of samples from water troughs. The highest level of resistance (80.81%) was observed against tetracycline, followed by kanamycin (71.72%), chloramphenicol and amoxicillin (67.68%), sulfamethazole-trimethoprim (61.62%), naldixic acid (63.64%), and streptomycin (59.60%), whereas most of the isolates were susceptible to gentamicin (69.70%). Resistance to more than two drugs was also observed.

Conclusion: Salmonella was found in high prevalence in broilers, their feed, and their environment. Moreover, a majority of the isolates were resistant to most antimicrobials used in medical and poultry practices. This has significant implications for public health and antimicrobial resistance.

Keywords: antimicrobials, broilers, central Ethiopia, resistance, Salmonella, smallhold

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Introduction

The broiler industry has huge potential to circumvent food and nutrition insecurity in developing countries. Salmonella has the capacity to adapt to changing environments and can develop resistance against routine sanitary practices, chemical disinfectants, and antibacterial drugs. The newly growing broiler sector in Ethiopia is confronting various infectious diseases, including Salmonella infection, ^{2,3} which is transmitted vertically and results in decreased production.

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Common serotypes of *Salmonella* isolated from broilers are *S. pullorum*, *S. gallinarum*, *S. infantis*, *S. typhimurium*, and *S. enteritidis*. In broilers, *Salmonella* infection mostly results in pullorum disease or bacillary white diarrhea in chicks 2–3 weeks old, causing high mortality. Broiler chickens can acquire *Salmonella* from parent stock, feed, water, or the environment and serve as a reservoir for humans. 5

Salmonellosis remains one of the most frequent foodborne zoonoses, constituting a worldwide major public health concern, mainly due to consumption of poultry products, such as broiler meat.^{5,6} Although different serotypes have been associated with salmonellosis, *S. enteritidis* is the most frequent in the EU and US, followed by *S. typhimurium*. *S. enteritidis* is commonly associated with poultry and poultry products, while *S. typhimurium* is less commonly associated with poultry. Foods of animal origin, in particular contaminated poultry products (eggs and meat) are considered the main vehicles of *Salmonella* infection and are clearly associated with the worldwide epidemic of *S. enteritidis*. Diverse epidemiological studies have also supported a considerable contribution of poultry foodstuffs to the salmonellosis burden.⁶

Antimicrobials have been utilized for a long time in broiler practices, both for growth promotion and control of infections. However, such use has been associated with the development of resistance by bacteria. Resistance to various antimicrobials by Salmonella has been reported in Pakistan, 7 Ghana, 8 and Ethiopia. 9 The misuse of antimicrobials in humans and animals has led to an increase in the number of multidrug-resistant bacterial strains, which has been identified by WHO and health authorities as a global public health and veterinary concern. 10 The number of antimicrobial-resistant Salmonella strains isolated from human cases has been linked to the widespread use of antimicrobial agents in food-animal practices. 11 The occurrence of antimicrobial-resistant widespread Salmonella in increased morbidity and mortality and costs of treatment. This has social and economic consequences, necessitating the need for ongoing monitoring of the emergence of antimicrobial resistance in Salmonella spp. 11

In Ethiopia during the last few years, there has been a gradual increase in poultry multiplication and distribution centers, along with smallhold broiler farms. ¹² Studies have revealed widespread occurrence of *Salmonella* in chicken, cattle, camels, sheep, goats, humans, foodstuffs, and the environment. ^{13,14} Resistance to various

antimicrobials has been reported in Salmonella isolated from beef¹⁵ and milk. ¹⁶ Since broiler meat is the secondmost popular meat in the world, accounting for around 36% of total meat production, 17 there is a dire need to develop better understanding and assist poultry stakeholders to reduce Salmonella and delay development of resistance to antimicrobials. Routine bacteriological monitoring of feed, water, and broilers and their products is needed to safeguard public health and improve meat production. It is also important to regularly monitor the development of resistance to antimicrobials by Salmonella in order to efficiently treat Salmonella infections in humans. This study was conducted with the objectives of isolation and identification of Salmonella from smallhold broilers chickens, their inputs and environs, and assessment of the antimicrobial profiles of isolates in the towns of Bishoftu and Modjo in central Ethiopia.

Methods

Study Areas

Bishoftu is located in Oromia Regional State, Ethiopia, approximately 47 km southeast of Addis Ababa (Figure 1). Its topography is undulating, with flatland to the north and east of the town, which is surrounded by many lakes, and hills to the south. The total land area of the town is about 15,273 ha, and it lies at an altitude of 1,900–1,995 m above sea level. It has well-developed poultry and dairy enterprises. Modjo (also spelled Mojo) is in central Ethiopia, and takes its name from the nearby river. Located in East Shewa Zone of Oromia, its elevation is 1,788–1,825 m above sea level, and it is about 64 km southeast of Addis Ababa. There are several smallhold and a few commercial poultry farms in the town.

Description of Farms and Chico Meat Slaughterhouse

The samples (cloacal swabs, samples from water and feed troughs, feed and water samples) considered for isolation of *Salmonella* were collected from smallhold broiler farms located in Bishoftu and Modjo, and cecum contents were collected from chicken slaughtered at Chico Meat slaughterhouse in Bishoftu. Established by Jacobs's Integrated Farm, Chico Meat distributes day-old broiler chickens to smallhold farmers in Bishoftu, Modjo, and Adama. These smallhold farms serve as out-growers and supply the broilers to the slaughterhouse in Bishoftu. The farmers are provided with day-old chickens, feed, and other inputs,

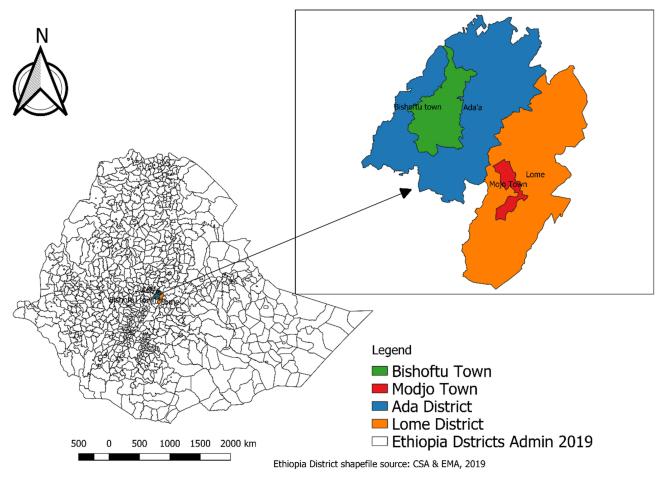


Figure 1 Map of Ethiopia depicting the study areas.

Notes: This map was developed from Ethiopian district administrative shape files 2019 using QGIS version 3.2.1, 2020.

which are discounted when the chickens are provided to the Chico Meat slaughterhouse. The farmers use management practices directed by the Chico Meat team, and all of them provide the finished chicken to the slaughterhouse. Chico Meat is a modern slaughterhouse with a processing capacity of 22,500 kg a day or 15,000 chickens a week. It has established links with local restaurants, hotels, supermarkets, resorts, and other retailers in all parts of Ethiopia. Chico Meat distributes Ross 308, Hubbard classic, and Cobb 500 broiler chickens imported from Belgium and Germany to out-growers. The smallhold farms or outgrowers keep broiler chickens numbering 500–2,500 chickens for 35–45 days.

Study Design and Samples

This was a cross-sectional study. Bishoftu and Modjo were selected because the out-growers supplied with day-old chickens were mostly situated in these two towns, where are ten out-grower or smallhold broiler farms under the

auspices of Chico Meat. From those farms, four were randomly selected using a lottery system. Two farms, designated B1 and B2, were selected randomly from Bishoftu, and two, M1 and M2, from Modjo. A total of 539 samples comprising 189 cloacal samples, 52 feed samples, 48 water samples, 60 samples from water troughs, and 90 samples from feed troughs were collected. In addition, 100 cecal contents were collected from 100 chickens slaughtered at Chico Meat. The chickens sampled at slaughter house were randomly selected from those supplied to the slaughterhouse. From each of the farms selected from Bishoftu, cloacal swab samples were collected from 50 chickens. From farm B1, 14 feed samples of about 5 g each and eleven water samples were collected. From farm B2, 16 feed samples and nine water samples were collected. In addition, ten and 40 samples were collected from water and feed troughs, respectively. From the two farms selected from Modjo, 89 cloacal samples (44 from farm M1 and 45 from farm M2) were

collected. Eleven feed samples and 14 water samples were collected from each of the selected farms. Fifty samples were collected from each of water and feed troughs. Also, 25 chickens were randomly selected at Chico Meat from each of the four farms studied, and their cecal contents were collected for isolation and identification of *Salmonella*.

Sampling Method

Both wings of the broiler chickens were held with one hand, keeping the animal's heads down so as to expose the caudal parts of the chicken. Before collection of samples, the surface of the cloaca was disinfected using 70% alcohol for 2 minutes. A sterile cotton swab was moistened with buffered peptone water, inserted into the cloacae, and rolled inside several times. The swabs were immediately transferred to universal bottles containing 10 mL buffered peptone water and kept in an icebox. Approximately 10-15 g feed was collected into a universal tube. Feed samples were collected before being provided to the chickens. These samples were collected into sterile plastic bags and kept in an icebox. Similarly, 5 mL water was collected from each source (tap water versus tank water) into sterile bottles and kept in an icebox. Swab samples were also collected from feed and water troughs before any feed and water were blended. In the slaughter house, about 1-1.5 g cecal contents were squeezed into sterile universal bottles, each containing 10 mL buffered peptone water, ¹⁸ and placed in an icebox. All samples were individually labeled, placed in separate plastic bags, transferred into a sterile icebox and transported to the Microbiology Laboratory, College of Veterinary Medicine and Agriculture, Addis Ababa University for isolation of Salmonella.

Isolation of Salmonella

Isolation of *Salmonella* was conducted using the bacteriological methods described for detection and isolation of *Salmonella* in ISO guidelines. ¹⁹ Briefly, samples were incubated at 37°C for 18–24 hours in buffered peptone water and nutrient broth to allow suscitation of *Salmonella* prior to transferral into selective enrichment media. ²⁰ Rappaport–Vassiliadis medium with soya broth was inoculated with samples from pre-enrichment medium and incubated at 37.5°±1°C for 24±3 hours. ²¹ Bacteria that grew on enriched media were plated onto *Salmonella–Shigella* (SS) agar. First, samples from enriched medium were plated onto SS agar, incubated at 37°C±1°C for 18–24 hours, and examined for the presence of suspected *Salmonella*

colonies. Pure *Salmonella* colonies from SS agar were subcultured onto XLD agar and BGA.

Identification of Salmonella

Identification of *Salmonella* was done by both microscopic examination of stained smears from typical colonies²² and standard biochemical tests, such as triple sugar iron agar slants (Oxoid, Basingstoke, UK),²³ lysine decarboxylase test using lysine decarboxylase broth, methyl red Voges–Proskauer broth, Simmons citrate agar, sulfide indole motility medium (SIM), and indole and urease tests using urea broth (HiMedia, Mumbai, India) according to the procedures describe by Quinn et al.²²

Antimicrobial-Sensitivity Tests

Antimicrobial susceptibility of the Salmonella isolates was conducted using standard Kirby-Bauer disk diffusion according to the guidelines of the Clinical Laboratory Standards Institute (CLSI).²⁴ A bacterial suspension was prepared from a freshly grown colony in sterile nutrient broth, turbidity adjusted to 0.5 McFarland standard, and the suspension spread carefully over a Müller-Hinton agar plate using a sterile cotton swab. Eleven antimicrobials commonly used in poultry practices and treatment of human cases of salmonellosis were used for this assay (Table 1). The medium containing the bacterial suspension and the antimicrobial disks were incubated aerobically at 37°C for 18–24 hours. The zone of inhibition around each antimicrobial disk was measured using a transparent ruler. The results were interpreted as sensitive, intermediate, or resistant based on the diameter of zone of inhibition as described by the CLSI²⁴ and are presented in Table 1.

Data Management and Analysis

Descriptive statistics are used to describe the prevalence of *Salmonella*. The effects of various risk factors on the isolation of *Salmonella* were analyzed by logistic regression in R software and the strength of associations expressed using ORs and 95% CIs. *P*<0.05 was used to identify significant statistical associations.

Results

Prevalence of Salmonella

Salmonella was isolated from 131 (24.30%, 95% CI 20.74%–28.15%) of the 539 samples tested. Salmonella was found in 51 of the 250 samples collected from Bishoftu (20.40%, 95% CI 15.58%–25.93%) and 76 of the 289 samples collected from Modjo (26.29%, 95% CI 21.32%–31.77%).

Table I Antimicrobials used for assessment of sensitivity profiles of Salmonella isolates and classification of sensitivity patterns

	Resistance (mm)	Intermediate (mm)	Susceptible (mm)
Gentamicin (10 μg)	≤12	12–15	≥15
Erythromycin (15 μg)	≤13	13–18	≥18
Streptomycin (10 µg)	≤II	11–15	≥15
Chloramphenicol (30 μg)	≤12	12–18	≥18
Sulfamethazole–trimethoprim (25 μg)	≤10	10–16	≥16
Tetracycline (10 μg)	≤14	14–19	≥19
Ampicillin (10 μg)	≤13	13–17	≥17
Cloxaciline (5 μg)	≤10	11–12	≥13
Naldixic acid (30 μg)	≤13	14–18	≥19
Amoxicillin (25 μg)	<u>≤</u> 13	14–17	≥17
Kanamycin (30 μg)	≤13	14–17	≥18

Salmonella spp. were isolated from 26.46% of the cloacal samples, 21.00% of the cecal contents, 30.77% of the feed samples, 25.00% of the water samples, 22.22% of samples from feed troughs, and 20.00% of samples from water troughs. At the farm level, 19.20% (95% CI 12.71%–27.21%), 24.80% (95% CI 17.51%–33.32%), 27.08% (95% CI 20.02%–35.11%), and 25.52% (95% CI 18.65%–33.42%) of the samples collected from B1, B2, M1, and M2, respectively, gave positive results for Salmonella (Table 2).

Salmonella spp. were isolated from a higher proportion of samples collected from chickens aged 31–40 days (28.47%) than samples collected from younger chickens (20.00%) and those older than 40 days. Higher proportions of samples collected from Cobb 500 (28.57%) and Hubbard classic (28.47%) breeds demonstrated positive results for Salmonella than those collected from Ross 308 (13.33%) chickens. The prevalence of Salmonella isolation

was higher in tank-water samples (27.27%) than samples of tape water (21.92%). However, none of the variables considered was statistically significantly associated with prevalence of *Salmonella* spp. Details of the distribution of *Salmonella* spp. in the samples are given in Table 3.

Antimicrobial-Susceptibility Profiles

A total of 99 Salmonella isolates were randomly selected and tested for their antimicrobial susceptibility. The highest level of resistance (80.81%) was observed against tetracycline, followed by kanamycin (71.72%), chloramphenicol and amoxicillin (67.68%), sulfamethazole—trimethoprim (61.62%), naldixic acid (63.64%), and streptomycin (59.60%), and most of the isolates were susceptible to gentamicin (69.70%, Table 4). From the 99 Salmonella isolates tested, 55 were resistant to two to five antimicrobials, 14 resistant to six to seven, and three

Table 2 Prevalence of Salmonella isolated from each sample collected from selected study farms

Sample type	Tested, n	Salmonella ⁺	Prevalence	95% CI
Cloacal swab	189	50	26.46%	20.32%–33.35%
Cecum content	100	21	21.00%	13.49%-30.29%
Feed	52	16	30.77%	18.72%-45.10%
Water	48	12	25.00%	13.64%-39.59%
Water trough	60	12	20.00%	10.78%-32.33%
Feed trough	90	20	22.22%	14.13%-32.21%
Farm			•	
ВІ	125	24	19.20%	12.71%–27.21%
B2	125	31	24.80%	17.51%–33.32%
MI	144	39	27.08%	20.02%–35.11%
M2	145	37	25.52%	18.65%-33.42%
Total	539	131	24.30%	20.74%–28.15%

Table 3 Proportion of samples yielding positive results for Salmonella by risk factor

		Tested, n	Salmonella ⁺	Prevalence	OR	95% CI	P
Age (days)	21-30 31-40 41-45	95 144 50	19 41 11	20.00% 28.47% 22.00%	0.565	0.14-3.33	0.429
Breed	Ross 308 Hubbard classic Cobb 500	75 144 70	10 41 20	13.33% 28.47% 28.57%	0.903	0.24–3.48	0.881
Source of water	Tap Tank	146 143	32 39	21.92% 27.27%	1.472	0.65–3.39	0.358
Type of house	Hoop Cement block	194 95	45 26	23.20% 27.37%	0.375	0.03–3.84	0.412
Farm location	Residential compound Separate	145 144	41 30	28.28% 20.83%	0.650	0.23–0.39	0.264
Type of sample	Cloacal swab Cecum content Feed Water	189 100 52 48	50 21 16 12	26.46% 21.00% 30.77% 25.00%	3.268	0.41-29.10	0.272

resistant to eight to ten antimicrobials. Intermediate resistance was observed against all antimicrobials tested, ranging from 16.16% for tetracycline to 35.35% for erythromycin.

Discussion

Broiler-meat production remains an important economic activity with huge potential for achieving food and nutrition security. However, in the absence of regular monitoring and surveillance, it can be a source of zoonotic pathogens. This study revealed the widespread occurrence of *Salmonella* in broilers, their inputs, and environs in smallhold farms in Bishoftu and Modjo. This indicates

widespread occurrence of *Salmonella* along the broiler value chain. Chico Meat is distributing broiler meat to various parts of Ethiopia. Without appropriate supervision and intervention, this has important implications for public health. The widespread occurrence of *Salmonella* in all samples tested (water, feed, cloacal swabs, water and feed troughs, and cecal contents) coupled with the lack of knowledge about the role of biosecurity could have contributed to the transmission of the bacteria among flocks and probably among the farms. This is evident by the fact that the smallhold broiler farmers included in this study share farm implements and other inputs among themselves. The hygienic status of the farms studied was

Table 4 Antimicrobial-sensitivity profiles of Salmonella isolated

	Resistance (%)	Intermediate (%)	Susceptible (%)
Gentamicin (10 µg)	10 (10.10)	20 (20.20)	69 (69.70)
Erythromycin (15 μg)	24 (24.24)	35 (35.35)	40 (40.41)
Streptomycin (10 µg)	59 (59.60)	24 (24.24)	16 (16.16)
Chloramphenicol (30 µg)	67 (67.68)	17 (17.17)	15 (15.15)
Sulfamethazole-trimethoprim (25 µg)	61 (61.62)	22 (22.22)	16 (16.16)
Tetracycline (10 μg)	80 (80.81)	16 (16.16)	3 (3.03)
Ampicillin (10 μg)	54 (54.55)	23 (23.23)	22 (22.22)
Cloxacilline (5 µg)	29 (29.29)	27 (27.27)	43 (43.44)
Naldixic acid (30 μg)	63 (63.64)	23 (23.23)	13 (13.13)
Amoxicillin (25 μg)	67 (67.68)	19 (19.19)	13 (13.13)
Kanamycin (30 μg)	71 (71.72)	18 (18.18)	10 (18.18)

also poor and could be one of the factors that contributed to increased risk of contamination by *Salmonella*. The use of unclean feeding materials and unhygienic houses have been shown to be sources of contamination by *Salmonella* in poultry.²⁵

The overall proportion of samples yielding Salmonella in this study is comparable to reports of 23.2% in cloacal swabs from Asossa and Bambasi²⁶ and the 28% in fecal samples collected from broilers from Senegal.²⁷ However, our observation is higher than most previous studies conducted in Ethiopia, including Ali et al⁹ from Bishoftu and Modjo, Abunna et al²⁸ from Modjo, Aragaw et al²⁹ from Hawassa, Abdi et al³⁰ from central and southern Ethiopia, and Bekele and Ashenafi³¹ from Addis Ababa. Our findings are lower than the results of some studies carried out in Ethiopia and elsewhere in the world. For instance, higher proportions than ours were reported in chicken from Jimma, western Ethiopia.³² Similarly, higher proportions of Salmonella-positive samples were reported by van den Gissen et al³³ in the Netherlands, Khan et al⁴ in Trindad, and Ishihara et al³⁴ in Japan. The differences observed in the proportion of samples yielding Salmonella between our study and others could be due to differences in the isolation techniques used, geographical locations, biosecurity measures adopted, breeds studied, and husbandry practices used.

A relatively higher proportion of samples from Modjo were positive for *Salmonella* than Bishoftu, although the difference was not statistically significant. This could be due to differences in poultry management among owners from Modjo and Bishoftu. Broiler farmers in Bishoftu have training and experience from other poultry farmers, since commercial poultry farms have long been established in the town, while most smallhold broiler farmers in Modjo have taken up the business recently and lack experience and training. Previous studies have also revealed that poor and faulty management, particularly poor biosecurity practices, are associated with occurrence of *Salmonella* on broiler farms. 35,36

According to the results of this study, a higher proportion of older broiler chickens carry *Salmonella* than chicks, suggesting the chance of getting *Salmonella* from the environment increases with age, sonsistent with earlier studies,³⁷ but contradicting Ansari-Lari et al,³⁸ who reported higher prevalence of *Salmonella* in young chicks. A difference in the proportion of samples yielding positive results was observed between housing types for the broilers, though not statistically significant. It was higher in

cement ones than those constructed from round wire. This could be due to the possibility of accumulation of waste and leftover feed and water on the floor. Since the cement blocks do not allow circulation of sufficient air, water and waste materials excreted from the chickens create wetness on the floor, which is conducive for the multiplication and survival of *Salmonella*. In contrast, the wire mesh allows the circulation of air, reducing humidity and the risk of *Salmonella*. The effects of housing type on the occurrence of *Salmonella* in poultry have been documented elsewhere. ³⁹

Sensitivity profiles of eleven antimicrobials were tested. We suggest gentamicin is the most promising drug to treat infections with Salmonella. Most of the other antimicrobials were losing efficacy due to emergence of resistance, while some were found to be in a transitional phase. The Salmonella isolates tested were resistant to the commonly used antimicrobials in poultry practices (tetracycline, kanamycin, amoxicillin, chloramphenicol, nalidixic acid, and sulfadimethoxine-trimethoprim). The antimicrobials tested are found at all veterinary-drug vendors and on all farms. Their use has not been based on the prescription of veterinarians. They are used indiscriminately by farm owners and attendants whenever any sign of illness is detected. In addition, feed processors include some antimicrobials as growth promoters. The indiscriminate use of these drugs could have contributed to the emergence of resistance in the Salmonella isolates. These drugs are also used widely in medical practices to treat infections with Salmonella. The occurrence of resistance against these drugs has important public health implications. Resistance against kanamycin, sulfadimethoxine-trimethoprim, tetracycline, nalidixic acid, chloramphenicol reported in this study is consistent with others. 40,41 Antimicrobial resistance in Salmonella isolated from various livestock and products have been reported in Ethiopia^{15,16,42,43} and elsewhere in the world,⁴⁴ suggesting the global occurrence of resistance. Continued use of the same antimicrobials could exert selection pressure on resistant bacteria, favoring their global distribution. The presence of widespread antimicrobial resistance can adversely affect public health, as treatment of illness caused by Salmonella becomes difficult.

In conclusion, this study revealed that *Salmonella* occurs widely in broilers, inputs, and the environment on smallhold farms. The *Salmonella* isolates obtained in this study were resistant to tetracycline, kanamycin, chloramphenicol, amoxicillin, sulfamethazole–trimethoprim,

naldixic acid, and streptomycin. The emergence of resistance to various antibiotics commonly used in medical and poultry practices have important implications for public health.

Data Sharing Statement

The data collected and used to support this article can be requested from the first or corresponding author.

Ethics Approval and Consent to Participate

Ethics approval was obtained from the Ethics Review Committee of the College of Veterinary Medicine and Agriculture, Addis Ababa University (VM/ERC/16/05/13/2021). The Chico Meat abattoir and the owners of the smallhold broiler farms that were included in this study were informed about the purpose of the study, and the consent of each was obtained.

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Author Contributions

All authors made a significant contribution to the activities accomplished for the publication of this article and contributed to study conception, design, and execution, collection of samples, laboratory analysis and interpretation, drafting and editing of the paper, agreed on the journal to which the article was submitted, gave final approval to the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest for this work.

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