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Isolation, Identificatcion and Antibiogram Profile of *Salmonella pathogen* from Free Ranglng Chicken Cloacal Swabs in Konta-Zone South West Ethiopia

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ABSTRACT

Salmonellosis is the major food born disease in the world with series public health problem. A cross sectional study was conducted from march to June 2024 to determine the prevalence of *Salmonella* in the study area to isolate, identify, and antimicrobial resistance profile of *Salmonella* species in chicken cloacal swabs from the Konta Zone of Southwest Ethiopia. A total of 384 cloacal swab samples from backyard poultry farms raising exotic, local, and hybrid breeds. The descriptive statistic method was used for analysis to determine the significance of difference or variation of prevalence. The study was conducted utilizing the conventional methods and the biochemical tests were done for the detection of *Salmonella* spp**.** The overall prevalence of *Salmonella* species was 31(8.06%), with significant (p>0.05) variations across districts and poultry breeds. The antimicrobial susceptibility tests were done by the agar disk diffusion method. The result of 31 (100%) resistance rates was observed for erythromycin, while tetracycline showed 25(80.6%) resistance rate. Moderate resistance levels were noted for Kanamycin, spectinomycin, and amoxicillin 12 (20-40%). Gentamicin and ceftriaxone antibiotics showed susceptible, suggesting they may still be effective treatment options. Exotic breeds demonstrated a higher prevalence 21 (16.15%) compared to local breeds 2 (1.12%). Female chicken exhibited a higher prevalence 24(11.48%) than male chicken 7 (4.00%). Chida 2 district exhibited the highest prevalence, necessitating targeted interventions. Continuous surveillance and research are crucial to understand *Salmonella* dynamics in the region. The isolated Salmonella strains, revealing alarming levels of antimicrobial resistance. Implementing effective control measures, and public health interventions, is essential to reduce the incidence of *Salmonella* -related foodborne illnesses and protect public health. In general, implement Community based Participatory disease surveillance system would be conducted to assess and prioritize the major chicken diseases in study area.

HIGHLIGHTS

- Climatic conditions have been shown to affect Salmonella survival and proliferation.
- Exotic and commercial poultry breeds may be more susceptible to Salmonella infections.

Keywords: Antimicrobial Profile, Cloacal Swabs, Poultry, *Salmonella*, Konta

Ethiopia is home to approximately 56.87 million chickens, (95%) of them raised in low-input, low-output village production system (Tesafa, 2018). Recent years have seen a significant increase in *Salmonella* outbreaks among humans in the country, highlighting a growing public health concern. The scope of foodborne illnesses, particularly those caused by *Salmonella,* is well-documented, revealing both acute and long-term health consequences (Huang, 2024). Poultry meat, eggs, and their products are vital components of the human diet due to their nutritional value; however, they are perishable and can pose health risks when improperly handled, leading to illnesses such as salmonellosis (Aworh, 2021) .

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The burden of pathogens in poultry multiplication centers remains poorly understood in Ethiopia, raising concerns about the potential distribution of infected chickens. *Salmonella* is a major pathogen affecting the poultry industry, causing severe economic losses characterized by reduced production rates, 100% morbidity, and up to 20% mortality in affected flocks. The pathogen's ability to spread through both vertical and horizontal transmission complicates control efforts on farms. Foodborne diseases are increasingly recognized as significant global public health challenges, with *Salmonella* being a leading cause of foodborne illness worldwide, resulting in millions of cases of gastroenteritis and substantial mortality (Gourama, 2020) *Salmonella* contamination can occur through two primary routes: vertical transmission (trans-ovarian) and horizontal transmission (trans-shell) (Awny *et al.,* 2018).

Vertical transmission occurs when bacteria infect the reproductive organs of hens, leading to contaminated egg production. Conversely, horizontal transmission often results from fecal contamination on eggshells or through environmental vectors such as farmers and rodents (Pesavento *et al.,* 2017). A study conducted in Ethiopia found that out of 366 examined chicken eggs, 27 (7.4%) tested positive for *Salmonella* , with varying rates of contamination between eggshells and contents (Hailu *et al.*, 2020). The widespread use of antimicrobials in poultry farming has contributed to increased antibiotic resistance among *Salmonella* strains. Many farmers administer antibiotics without proper guidance or understanding of their implications for human health (Gole *et al*., 2014). This resistance poses significant risks, especially in developing countries where close contact between humans and animals is common, exacerbating public health challenges related to food safety (Duchenne-Moutien and Neetoo, 2021).

Despite these concerns, there is limited research on the epidemiology of salmonellosis in Ethiopia due to inadequate surveillance systems and underreporting. This study aims to address these gaps by investigating the antimicrobial susceptibility patterns of *Salmonella* isolates from chicken cloacal swabs in Konta Zone. It will explore the perceptions of farmers and consumers regarding antimicrobial resistance and identify effective strategies for managing foodborne disease related to *Salmonella* (Duchenne-Moutien and Neetoo, 2021).

Statement of the Problem

In Ethiopia as in other developing countries, it is difficult to evaluate the burden of salmonellosis because of the limited scope of studies and lack of coordinated epidemiological disease surveillance systems. In addition, under-reporting of cases and presence of other diseases considered to be of high priority may have overshadowed the problem of salmonellosis. Salmonellosis is endemic in the country and there is a desire to strengthen the monitoring and surveillance of salmonellosis using suitable examining tools so as to prevent and control its occurrence.

In addition to this, the extent of *Salmonella* contamination of eggs sold on open market, household chicken handling, fecal contact and antimicrobial profile of the *Salmonella* isolates has not been adequately studied and very limited information exists in Ethiopia and the same is true in Konta zone (Abera and Tesema, 2019).

The presence of resistant organisms in the poultry and poultry products for consumption is a safety concern to the population (Bortolaia *et al.,* 2016) and treatment concern for the physicians, resistant organisms might pose prolonged treatment in cases of outbreaks, delayed recovery or treatment failure. There is a scarcity of knowledge concerning poultry farm development associated with antimicrobial resistance and foodborne bacteria. Information on the antimicrobial resistance pattern of the *Salmonella isolates* from chicken cloacal swab could be useful for successful treatment, as well as planning strategic use of drugs to minimize resistance in the future (Hossain *et al.,* 2021).

Insufficient information at hand in this regard is the main factor that hindered efforts to prevent and control the disease. Based on the transmutation nature of *Salmonella and* previous disease surveillance made in Ethiopia, it was hypothesized that eggs sold in local markets, direct fecal contact and poultry farm may serve as a source of *Salmonella species* particularly among those raw egg consumers and considerable proportion of them might have developed resistance to antimicrobials that are commonly used both in the veterinary and public health sectors. So having these understanding, this study is designed to fill the above gap in the study area with respect to antimicrobial susceptibility pattern and public health importance of *Salmonella*, response strategies and try to answer the following questions (Caudell *et al.,* 2020).

To what extent do farmers and consumers perceive that antimicrobial susceptibility pattern and associated risk factors of *Salmonella* enhanced the adaptive capacity of people in the study area? Which strategies are more capable in tackling the problem of food borne illness related to *Salmonella* and which are not. What determines farmers and consumers perception and practices towards *Salmonella related* food borne illness in prevention and control strategies (Ehuwa, *et al.*, 2021).

MATERIALS AND METHODS

The study area

The study was conducted in Konta zone which is located in the, between 6º, 46'-7', 27'' North latitude and 36º, 32'-36', 87'' East longitude at south western part of Ethiopia. Ameya the capital of Konta zone is found at a distance of 465 kms away from the capital city of Ethiopia (Addis Ababa), 84 Km from the regional capital town Bonga, 199Km from Mizan Veterinary regional laboratory and 104 kms away from Jimma town. Agro ecologically Konta zone consists of 40%, lowland (790-1500 m.a.s.l), 54% midland (1500-2300 m.a.s.l) and 6% highland (>2300 m.a.s.l). The mean annual temperature and rainfall varies from 15.1°C-27.5°C and 1200-2290 mm respectively (KZAO, 2022). The district comprises the population of 398,666 poultry and poultry related diseases(KZAO, 2023). As a result, this study site is selected purposively because of first road accessibility and availability of poultry population second, the severity of disease problem observed in the area (KZHDO, 2023).

Study animal

The study consisted of Chicken which scavenging natural on day time was managed under the backyard rearing system. The chicken under study comprised of the exotic, local and hybrid. Chicken of both sex and old group (cockerels and layers) were included in the study.

Study design

Cross-sectional study was conducted on Chicken cloacal swabs directly from household taking 3 sample from household that who have above 8 chicken were collected

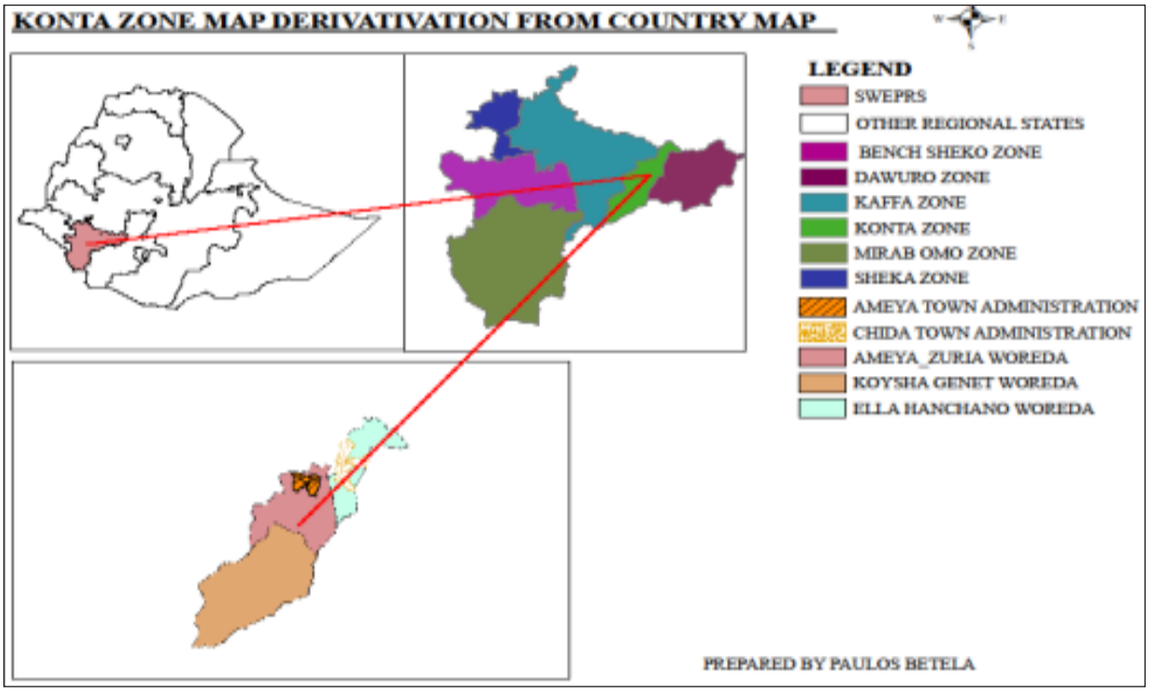

Fig. 1: Geographic map of the study area. (KZAO, 2022)

by using a simple random sampling technique from March, 2024 to June, 2024.

Sample size and sampling technique

The sample size required for this study was determined there is no previous study in the area and by considering 50% expected prevalence using the following formula according to (Thrusfield, 2005).

$$N = \frac{(Z_\alpha)^2 P(1-P)}{d^2}$$

Where:

N = number of sample size.

P = prevalence (50%) = 0.5

d = marginal error between the samples and population (5%) = 0.05

Z_α = the standard normal deviation corresponding 95% of confidence level = 1.96

$$N = \frac{(1.96)^2 (0.5)(1-0.5)}{(0.05)^2} = 384$$

Total of 384 cloacal swab samples from village chicken production systems household in Konta-Zone Ameya and chida district directly from the farmers was collected. Chickens of various breeds local, exotic and cross breed) and old age (cockerels and layers) was selected and sampled. The sample taken by proportional sampling method from a total of 128 households who have above 8 chickens purposely selected and chicken in a selected household are considered as one flock. The number of flocks selected range from each of those households, 30% of the single flock of population was sampled.

Samples of 93/2,410/ cloacal samples from Chida 1, 106/2,621/ cloacal samples from Chida 2, 98 /2,518/ cloacal samples from Ameya 01 and 87/2,112/(KZAO, 2024) cloacal samples from Ameya 3 district household was collected using a simple random sampling technique. From flocks, by using simple random sampling technique 384 chickens were successfully sampled.

Sample collection and transportation

A cloacal sample was collected using sterile commercial swab sticks and an aseptic procedure is strictly adopted during collection. A sample from the cloacae was collected by using sterile commercial swab sticks by inserting the swab stick into the cloaca of the chicken for about 20 seconds, after which the swab stick was removed and immediately placed in universal bottles containing 10ml Selenite F broth and then labeled. And the sample placed in the ice box and was transported to the Mizan Veterinary regional laboratory for bacteriological culture and biochemical screening (JAHAN, 2017).

Laboratory analyses

The methods used in the isolation of *Salmonella* were according to the techniques recommended by the International Organization for Standardization (Temelli *et al.,* 2012). The isolation involves three steps: pre-enrichment in pre-enrichment broth media, enrichment in selective media, plating on selective media and biochemical confirmation of suspected colonies from selective agar media (Teske *et al.,* 2007).

Pre-enrichment in non-selective broth medium

All samples were pre-enriched separately with an appropriate amount of BPW (CONDA) (1:9) and were incubated for 18–24 h at 37 ± 1°C. After mixing thoroughly, the samples were incubated at 34°C - 38°C for 16-18hrs.

The swabs was directly inoculated into 10 ml BPW in screw capped bottles and incubated at 34°C- 38°C for 16-18 hrs.

Enrichment in selective broth media

Tetrathionate broth base (Titan Biotech Ltd.) and Rappaport Vassiliadis *Salmonella* enrichment broth (Himedia MH1491) were used for selective enrichment of all samples (Mohammed and Dubie, 2022). A portion (1 ml) of the pre-enriched culture was aseptically transferred to 10 ml of tetrathionate broth base containing test tube, and another 0.1 ml pre-enriched culture was aseptically transferred to test tubes containing 10 ml of Rappaport

Vassiliadis *Salmonella* enrichment broth and incubated at 37 ± 1°C and 41.5 ± 0.5°C for 24 h, respectively (Hammack *et al*., 2008)

Salmonella pathogen isolation and colony identification procedures

The study was conducted utilizing the conventional methods for the detection of *Salmonella* spp. following the standard guidelines. There were four definite sequential steps: (1) Nonselective pre-enrichment, (2) Selective enrichment, (3) Plating out and identification, and (4) Confirmation by using Triple Sugar Iron (TSI) agar.

Buffered peptone water was used as non-selective pre-enrichment broth, which was prepared according to the instruction of the manufacturer (Oxoid). In brief, 13 gram of powder was completely dissolved per 1000 ml of distilled water (Tofazzal 2014). An amount of cloacal swab of a sample was placed into 200 ml of buffered peptone water (BPW); the mixture was shaken approximately for 2 minutes and incubated at 37°C ± 1°C for 18 ± 2 hours. Xylose Lysine Deoxycholate (XLD) was used as selective enrichment broth and it was prepared according to the instruction of the manufacturer. After incubation a loop full of broth was streaked on Xylose Lysine Deoxycholate (XLD) medium and incubated at 37°C for 24 hours. Colonies with black centers were considered presumptive *Salmonella* spp., for plating out and identification Salmonella-Shigella (SS) agar was used (Hossain 2018). SS agar (Difco) were prepared according to the instruction of the manufacturer and then autoclaved. After autoclaving the media at 121°C for 15 minutes, it was cooled to 50°C and approximately 30 ml to 50 ml was poured into the 15 × 150 mm Petri dishes. The depth of the agar in the Petridishes was maintained approximately at 4 mm. The freshly prepared plates were used on the same day. The pH of the medium was regularly tested for its consistency. After incubation for 48 ± 3 hours, a loop-full black colony was transferred from the XLD and streaked onto the surface of SS agar. The plates were incubated at 37°C± 1°C for 24 ± 3 hours. The plates were incubated in an inverted position and after incubation. The plates were checked for the development of typical Salmonella colonies. Typical colonies of Salmonella on SS agar were blackish due to the production of hydrogen sulfide. Triple Sugar Iron agar (TSI agar) was used to see the typical colonies and biochemical reactions for the identification of *Salmonella pathogen*, typical colonies grown on the SS agar plates were transferred and inoculated in triple sugar iron agar (TSI) slant and incubated at 37°C ± 1°C for 24 ± 3 hours. The TSI agar slant surface was streaked and the butt was stabbed and incubated at 37°C ±1°C for 24 ± 3 hours (Bahjat, Al-Taee *et al.* 2019).

Plating out and isolation

Xylose Lysine Deoxycholate (XLD) agar (Oxoid CM0469) and brilliant green agar (BGA) base modified (Himedia M016) plates were used for plating out and isolation purpose. A loop full of inoculums from RV broth and tetrathionate broth was transferred and streaked onto the surface of XLD agar and BGA base modified separately (Mohammed and Dubie, 2022). The plates were incubated at 37 ± 1°C for 18–24 h. After incubation, the plates were examined for typical and atypical colonies of *Salmonella*. On XLD agar, typical colonies can be colorless, very light, slightly shiny and transparent (color of the medium) with a dark tinted center, surrounded by a light red area and yellow edge, or of pink to red color, with a black center or without a black center. Hydrogen sulphide (H_2S) positive colonies are colorless or light pink with darker center, and lactose positive colonies are yellow or without the characteristic blackening; whereas on BGA, typical colonies are transparent, colorless or light pink, and the color around colonies changes from pink to light red. For confirmation, presumptive *Salmonella* colonies were selected from every selective plating media and sub cultured on nutrient agar (Oxoid CM0003) in a manner that allow isolated colonies to develop and incubate at 37°C for 18–24 h for further confirmation by biochemical tests (Soria and Bueno, 2016).

Biochemical Test

Triple sugar iron (TSI) agar

The Triple sugar iron agar slants was prepared with a thick butt. A loop full 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. Typical Salmonella cultures show alkaline (red) slants and acid (yellow) butts with gas production (bubbles) and formation of hydrogen sulfide (blackening of the agar) (Ahmed, 2018).

Urea agar test

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 (Krieg and Padgett, 2011). The basal medium was sterilized by autoclaving at 121°C for 15 minutes. When it has cooled to about 50°C, 100 ml of a 20 percent 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 tube was incubated at 37°C for up to 96 hours (Khan, 2018). The observations were made at an interval of 4, 24, 48 and 96 hours. Urease positive cultures changed the color of the indicator to red (Annex 4).

Citrate utilization test

Simmons's citrate agar was sterilized by autoclaving at 121°C for 15 minutes at 15 lb. pressure and cooled for slant formation. The strains was cultured on the prepared Simmons's citrate agar medium, incubated at 37°C for 48 hours and observations was recorded. Opacity and change in color of bromothymol from green to blue indicated a positive reaction (Mohamed, 2017).

Xylose lysine deoxycholate

Lysine decarboxylation broth was inoculated with the loop full culture of the test organism and one was kept un inoculated control. Both tubes was incubated for 24 hours at 37°C. Turbidity and a purple color after incubation indicate a positive reaction. A yellow color indicates a negative reaction (Alemu, *et al,*. 2020).

Indole test

Indole is a nitrogen-containing compound that can be formed from the degradation of the amino acid tryptophan by certain bacteria. Trytone was used as a substrate because it contains much tryptophan. The indole reacts with aldehyde compound of Kovac's reagent and forms red cultured compound that is more soluble in alcohol. For indole test peptone water was prepared and the ingredients was dissolved in distilled water, dispensed in test tubes and sterilized by autoclaving at 121°C for 15 minutes. The tubes of the medium was 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 was indicated by the development of red color in the alcoholic layer of the reagent and no color in the control tube (Alemu *et al*., 2020).

Biochemical confirmations

The biochemical indication of the organism was done by performing the biochemical tests. The biochemical tests were done as stated on Bergey's *Manual of Determinative Bacteriology* (Chowdhury 2018). Each isolated colony with typical *Salmonella* morphology was characterized biochemically by triple sugar iron (TSI) agar (Oxoid CM0277), Urease (Himedia M111A), Simmons' citrate agar (Himedia M099, India), Indole (Oxoid CM0129), lysine iron agar (LIA; Oxoid CM0579), methyl red (MR) and Vogues–Proskauer (VP) (Himedia M070) tests. Colonies producing red slant (alkaline), yellow butt (acidic) on TSI agar with H_2S production and bubbles formation/cracking at the butt (gas production), negative urea utilization (yellow), positive citrate utilization (deep blue slant), negative for indole production from tryptophan, positive LIA agar (alkaline slant/ alkaline butt), positive for MR test and negative for VP test were considered *Salmonella* positive (ISO 6579, 2002). LIA (Oxoid CM0579) was usedto demonstrate hydrogen sulfide production and the decarboxylation or deamination olysine. These *Salmonella* positive samples showed alkaline slant/alkaline butt. Isolates presumptive of *Salmonella* for all tests were cultured on nutrient agar (Oxoid CM0003) for antimicrobial susceptibility testing (Abunna, *et al*., 2017).

Antimicrobial susceptibility test of *Salmonella* isolates

The antimicrobial susceptibility testing for *Salmonella* isolate was worked by following the Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Oxoid CM0337) as described in the Clinical and Laboratory Standards Institute (CLSI) guidelines (Shah, Nadeem *et al.,* 2020) . From each isolate, biochemically confirmed well isolated colonies grown on nutrient agar (Oxoid) were transferred into sterilized tubes containing 5 ml of tryptone

soya broth (Oxoid). The broth culture was incubated at 37°C for 4 h until it achieved the 0.5 McFarland turbidity standard. A sterilized cotton swab was dipped into the suspension, and the bacteria were swabbed uniformly on the entire surface of Muller–Hinton agar plate. The plates were held at room temperature for 30 min to allow drying. Antibiotic discs with known concentrations of antimicrobial were applied aseptically onto the surface of the plates at an appropriate special arrangement with the help of a sterile pair of forceps on Mueller–Hinton agar plates. The plate was then inverted and incubated at 37°C for 24 h (Gebreyohannes, Moges *et al.* 2013). The antibiotic discs (Oxoid) The used disks are: Amoxicillin 25µg (AMX), Erythromycin 15µg (ERY), Gentamycin 10µg (GEN), Ceftriaxone 30µg(CFR), Clindamycin 2µg (CLN), Naldixicacid 30µg (NA), Spectinomycin 100µg (SPC), Tetracycline 10µg (TET). After incubation, the diameter of clear zones produced by antimicrobial inhibition of bacterial colony growths were measured to the nearest mm using a transparent straight line ruler and classified as susceptible, intermediate and resistant categories according to CLSI guidelines (Antimicrobial and Sandeep, 2016) .

Questionnaire Survey

A total of 64 households are randomly by using semi-structured questionnaire from the selected district and, all chicken in a selected household are considered as one flock. A questionnaire survey was conducted randomly from 50% household consumers to identify conditions of handling practices, transportation, waste material disposal system, preparation and utilization patterns chicken in the study area by interviewing type (Annex 13).

Data management and analysis

The data were entered into Microsoft excel 2010 and analyzed using the SPSS statistical software package version 20 (IBM SPSS statistics). Descriptive statistics were used to compute proportions and frequency distributions of the rate of *Salmonella* isolation among various districts, breeds and sex. Pearson›s Chi-square (χ^2) test was used to assess the association in the positivity of *Salmonella* isolates from samples originating from chicken cloaca. *Salmonella* isolates were further screened for susceptibility to 8 different drugs and classified as susceptible, intermediate and resistant using frequency and proportions. A difference was taken as significant at $P < 0.05$ at 95% confidence interval for the variables.

RESULTS

Prevalence of *Salmonella pathogen*

A total of 384 cloacal swab samples were collected from 4 different districts in Konta zone of Southwest Ethiopia (Table 1) and processed for the presence of Salmonella. Out of 384 samples, 31 were positive for Salmonella, resulting in an overall prevalence of approximately 31 (8.07%.). The results indicated a highly significant difference between each district, with Chida 2 district showing a significantly ($p<0.001$) higher incidence compared to the other districts.

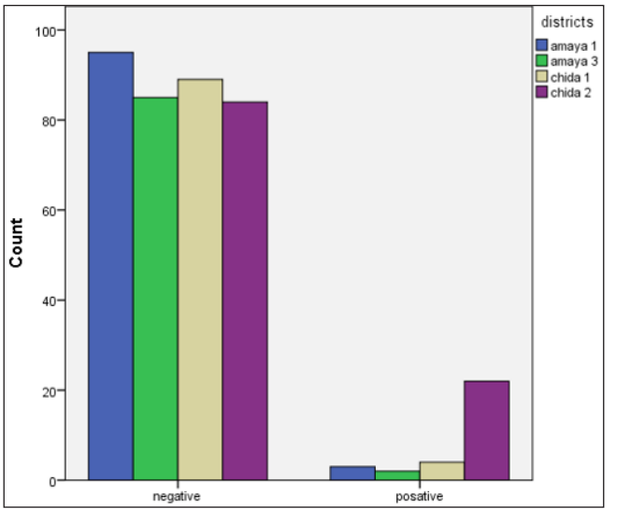

Fig. 2: Prevalence of *Salmonella pathogen* from different districts

Prevalence of *Salmonella pathogen* from Different Breeds

The analysis of Salmonella isolation from different breeds of poultry revealed significant ($p<0.01$) differences in prevalence among local, crossbred, and exotic chicken. Out of 384 samples, 31 were positive for Salmonella, with varying rates of positivity across the breeds. The result shows that exotic breeds exhibited the highest prevalence of Salmonella, with a positivity rate of approximately

16.15% (21 positive out of 130 totals). In contrast, local breeds had a markedly lower positivity rate of just 1.12% (2 positive out of 179 totals), while crossbred poultry had a positivity rate of about 10.67% (8 positive out of 75 total).

Table 1: Risk factors for Salmonella in the study area

Variables	No of examined	No of Positive	No of Negative	$\chi 2$	p-value
Districts					
Amaya 1	98	3	95		
Amaya 3	87	2	85		
Chida 1	93	4	89	31.98	0.00
Chida 2	106	22	84		
Total	384	31	353		
Breeds					
Local	179	2	177	23.788	0.00
Cross	75	8	67		
Exotic	130	21	109		
Total	384	31	353		
Sex					
Male	175	7	168	7.187	0.007
Female	209	24	185		
Total	384	31	353		

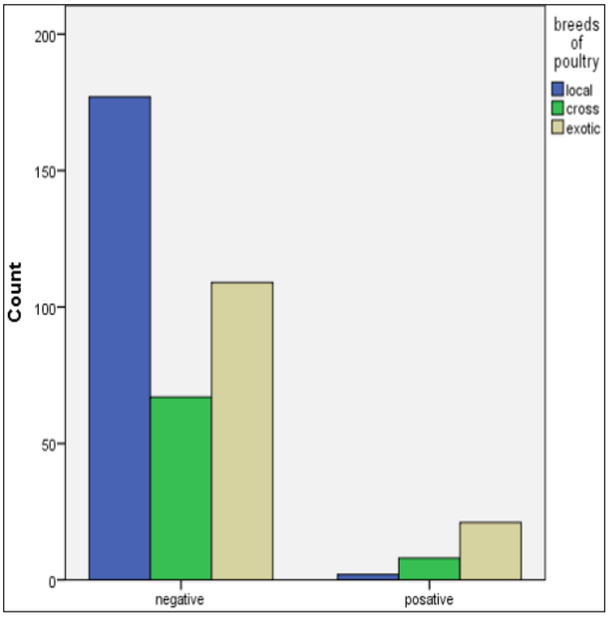

Fig. 3: Prevalence of *Salmonella pathogen* from different breeds

Prevalence of *Salmonella pathogen*

Based on the Sex of Chicken

The analysis of Salmonella isolation based on the sex of poultry revealed significant differences in prevalence between male and female chicken. The data indicates that female chicken had a higher prevalence of Salmonella (11.48%) compared to males (4.00%).

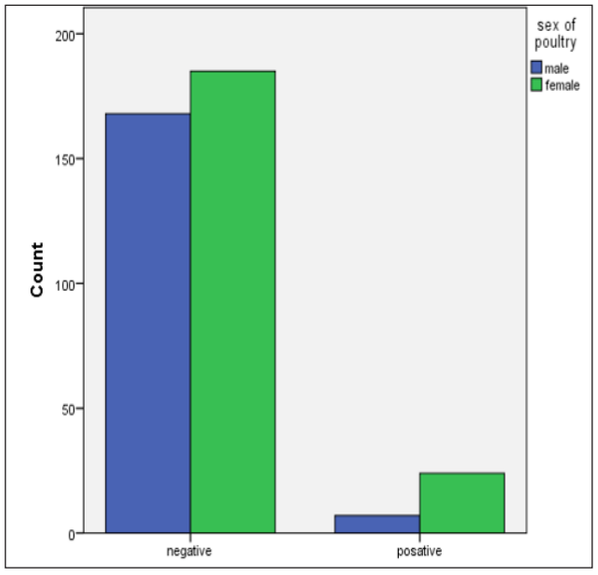

Fig. 4: Prevalence of *Salmonella pathogen* from different sex

Antibiogram profile of *Salmonella pathogen*

All the isolated thirty one *positive salmonella* were tested for susceptibility to selected antibiotics. Antibiograms used in this study were, amoxicillin, Kanamycin, tetracycline, Spectinomycin, cefoxtin, Erythromycin, gentamycin, and Clindamycin. The highest resistance was observed against Erythromycin (100%) and Tetracycline (80.6%), indicating these antibiotics are ineffective for treating infections caused by *Salmonella* spp. in the study area. On the other hand, Sensitive to Ceftriaxone and Gentamicin suggesting they may still be effective treatment options.

Table 2: Antimicrobial resistance of *Salmonella* isolates from chicken

Antibiotics	Drug potency	Resistance(%)	Intermediate(%)	Sensitive(%)
Ceftriaxone	100 µg	6(19.35)	3(14.28)	22(70.97)
Spectinomycin	30µg	12(38.70)	2(6.77)	17(54.81)
Gentamicin	10 µg	6(19.35)	3(9.7)	22(70.9)
Amoxicillin	30 µg	12(38.7)	3(9.7)	16(51.6)
Kanamycin	30 µg	18(58)	3(9.7)	9(29)
Clindamycin	2µg	18(58)	6(19.35)	7(22.58)
Erythromycin	15 µg	100	0	0
Tetracycline	30 µg	25(80.6)	3(9.7)	3(9.7)

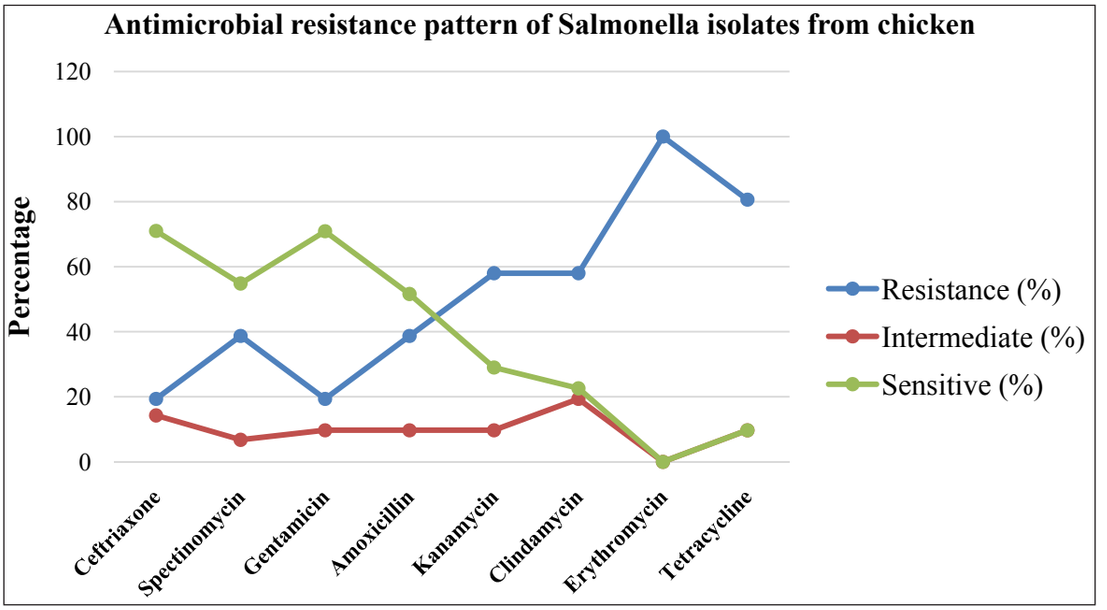

Fig. 5: Antimicrobial resistance of *Salmonella* isolates from chicken

DISCUSSION

The present study showed a 31(8.07%) overall prevalence of Salmonella in this study area, corroborates with previous reports by Kindu and Addis (2013) and Bayu *et al.* (2013) who reported values of 4.69 and 13.88%, respectively. Differences in prevalence across studies may be due to geographic and seasonal variation, animal management practices, and hygienic conditions. The significant association between district and Salmonella prevalence aligns with existing literature that highlights regional variations in Salmonella occurrence(McLure, Shadbolt *et al*., 2022).

In Ethiopia, similar studies have documented varying prevalence of Salmonella. For example, a study conducted in Southern Ethiopia reported a prevalence of 16.7% among poultry and environmental samples (Tadesse, 2018). Another study indicated a prevalence of 14.6% in poultry farms in central Ethiopia, with significant resistance to multiple antibiotics observed among the isolates (Kebede *et al*., 2022). These findings highlight the pressing public health concern posed by Salmonella in poultry production systems within the country.

For instance, a study conducted in Egypt reported varying isolation rates of Salmonella from poultry farms,

emphasizing that environmental conditions and farming practices play critical roles in pathogen transmission (Ismail *et al.,* 2022). Similarly, research from Hong Kong identified that food sourced from wet markets was significantly associated with non-typhoidal Salmonella gastroenteritis, indicating how local food handling practices can influence infection rates (Barac *et al.,* 2024).

Furthermore, climatic conditions have been shown to affect Salmonella survival and proliferation. The variation in Salmonella prevalence across districts underscores the importance of management practices and biosecurity measures in poultry production. Poor biosecurity protocols are often linked to higher rates of contamination. The high prevalence observed in Chida 2 necessitates targeted interventions to mitigate risks associated with poultry consumption. This discrepancy could be attributed to differences in management practices among households or environmental factors affecting Salmonella transmission. A study by Tadesse *et al.* (2020) further supports this notion, reporting higher infection rates in exotic poultry breeds compared to local varieties. In Ethiopia, studies have corroborated these findings regarding the susceptibility of exotic breeds (Derbie, 2020). For example, Tadesse *et al.* (2020) reported an overall prevalence of Salmonella at 14.6% in exotic chicken populations, highlighting significant resistance to multiple antibiotics among the isolates. This suggests that the intensive management practices associated with commercial poultry production may contribute to increased Salmonella prevalence and antimicrobial resistance. These findings underscore the importance of stringent biosecurity measures in intensive poultry farming to mitigate the risk of Salmonella outbreaks (Derbie, 2020).

Exotic and commercial poultry breeds may be more susceptible to Salmonella infections due to a combination of genetic predisposition, management practices, and environmental factors. For instance, a study conducted in Nigeria by Akinwunmi *et al.* (2020) revealed that commercial poultry had higher of Salmonella infection compared to local breeds. This difference was attributed to the intensive farming practices associated with commercial poultry production. Similarly, the higher prevalence of Salmonella in exotic breeds could be linked to various factors, including their inherent susceptibility to diseases, intensive rearing practices, and potential exposure to contaminated feed or water sources (Lamichhane, Mawad *et al.,* 2024). Furthermore, another study conducted in Southern Ethiopia found a prevalence of 16.7% among poultry and environmental samples (BMC Vet Res, 2018), reinforcing the notion that exotic breeds are particularly vulnerable in intensive farming systems.

In contrast, local breeds may possess better resistance to certain pathogens due to their adaptation to local environmental conditions and traditional management practices (Hassan *et al.,* 2020). The crossbreed chickens showed moderate levels of Salmonella positivity, suggesting that hybrid vigor might provide some level of resistance, but not enough to match the resilience observed in local breeds. This finding is consistent with research indicating that crossbreeds can exhibit varying levels of disease resistance based on their genetic background and management practices (Abebe *et al.,* 2019).

The low overall prevalence rate of 21(8.07%) in this study contrasts with findings from other regions where higher rates have been reported. For instance, studies conducted in urban settings have shown prevalence rates exceeding 20%, possibly due to different management systems and environmental factors (Mekonnen *et al.,* 2021). The lower rate observed in this study may reflect effective local management practices or environmental conditions that limit Salmonella transmission.

Female chickens may be more susceptible to Salmonella infections due to physiological factors such as reproductive stress or differences in immune response (Hassan *et al.,* 2020). Females are often housed in closer quarters during breeding, which could facilitate the spread of pathogens. Female birds undergo hormonal changes related to egg production, which can influence their immune response (Wickramasuriy Park *et al.,* 2022). Research has shown that stress associated with egg-laying can compromise immune function, making hens more susceptible to infections (Davis *et al*., 2019). Female birds may engage in nesting behaviors that increase their exposure to contaminated environments. Studies have indicated that hens often spend more time on the ground, which can be a source of Salmonella contamination from feces or contaminated feed (Holt *et al.,* 2020).

CONCLUSION AND RECOMMENDATION

Serovars of *Salmonella* have been recognized as important zoonotic pathogens in not only humans, but also animals.

Free scavenging chickens also known as local or village chickens are the most essential of the poultry species in terms of number and development. The prevalence although variations highlight the influence of geographic, seasonal, and management factors on *Salmonella* transmission. Notably, the Chida 2 district exhibited a significantly higher incidence of *Salmonella,* emphasizing the need for targeted interventions to mitigate public health risks associated with poultry consumption. The analysis also indicated that exotic poultry breeds had a higher prevalence compared to local breeds, suggesting that local breeds may possess better resistance to certain pathogens due to their adaptation to local conditions.

Based on the findings of this study, the following recommendations are forwarded:

- ❒ Focus interventions in districts with higher *Salmonella* prevalence, particularly in Chida 2 district. Strategies could include educational outreach for farmers about best practices in chicken management and hygiene.
- ❒ Encourage the use of local and crossbreed poultry breeds that demonstrate better resistance to *Salmonella.* Farmers should be educated about the benefits of local breeds over exotic ones in terms of disease resistance and overall resilience.
- ❒ Promote responsible use of antibiotics in poultry production to mitigate the development of antimicrobial resistance. This includes avoiding unnecessary antibiotic treatments and adhering to veterinary guidelines for antibiotic use.
- ❒ Establish continuous surveillance systems to monitor *Salmonella* dynamics and antimicrobial resistance patterns in poultry populations.
- ❒ Implement public health campaigns aimed at educating consumers about safe food handling practices, including proper cooking methods and hygiene measures to reduce the risk of foodborne illnesses associated with *Salmonella.*
- ❒ In general, implement Community based Participatory disease surveillance system would be conducted to assess and prioritize the major chicken diseases in study area.

GENERATIVE AI DECLARATION

The author(s) confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI. The authors developed and verified all scientific content, data analysis, interpretation of results, and conclusions. The authors take full responsibility for the accuracy, integrity, and originality of the work presented, and no AI tool was listed as an author.

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