Journal of Applied Veterinary Sciences, 8 (2): 44 – 54 (April, 2023)
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

Evaluation of Antibiotic Resistance Pattern of *Escherichia Coli* and *Salmonella* Species Isolated from Cloaca of Indigenous Chickens in Live-bird Markets in Marodi Jeh Region, Somalia

Hibaq Yusuf Cige[1], Oladipo Olufemi Omotosho[2*] and Abdalla Mohamed Ibrahim[3]
[1]Pan African University, Institute of Life and Earth sciences including Agriculture and health, Nigeria
[2]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria
[3]Abrar University, Mogadishu, Somalia

***Corresponding Author:** Oladipo Olufemi Omotosh, **E-Mail:** oo.omotosho@gmail.com

ABSTRACT

Poultry is an important source of protein globally, today, but *Escherichia coli* (*E. coli*) and *Salmonella* species continue to be food-borne pathogens and contribute to the growing resistance to the antimicrobial agents. There is limited information on these pathogens and their antimicrobial susceptibility pattern in Maroodi Jeh region, Somaliland. Therefore, this study was designed to isolate, characterize and evaluate the antimicrobial susceptibility of *E. coli* and *Salmonella* species from indigenous chickens in live-bird markets in the Marodi Jeh region, Somaliland. In a cross-sectional study, a total of 384 cloaca swab samples were collected from Chickens of both sexes, from two live-bird markets (Waheen and Xisbi), and were grouped into growers and adults. The samples were screened using cultural, biochemical, and Gram staining techniques to isolate and identify *E. coli* and *Salmonella* species. The antimicrobial sensitivity testing was conducted on all the positive isolates using disk diffusion method. Data were analyzed using descriptive statistics and Chi-square at $p< 0.05$. The prevalence of *E. coli* was 19.5%, while that of *Salmonella* species was 8.9%. *E. coli* and *Salmonella* species were more frequently isolated from adults (68%, 70.6%), females (72%, 58.8%), and from the Waheen market (70.7%, 94.1%). Totally, 98.6% of the isolates showed resistance to different combinations of antibiotics. The highest resistance was recorded against chloramphenicol (66.6%), tetracycline (45.3%), gentamycin (20%), and ampicillin (17.3%). *E. coli* isolates were sensitive to one antibiotic (44%) or between two to four antibiotics (54.7%), while *Salmonella* isolates, were sensitive to one antibiotic (35.3%) or between two to four antibiotics (64.7%). In conclusion, the present study showed a higher colonization rate of *E. coli* than *Salmonella* species in the cloaca of chickens with most of the isolates being resistant to multiple antibiotics.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2023.179843.1200
Received : 10 December, 2022.
Accepted :14 March, 2023.
Published in April, 2023.

Keywords: Antimicrobial resistance, Chickens, *Escherichia coli*, Evaluation, Indigenous, *Salmonella*.

J. Appl. Vet. Sci., 8(2): 44-54.

INTRODUCTION

Production of poultry has substantial positive influence on the economy and it is crucial to family nutrition in emerging nations **(Mujyambere *et al*., 2022).** In rural smallholder farming families in Africa, poultry holds a unique position as it contributes to the supply of high-quality protein. To meet human needs, eggs and poultry meat both enrich and contribute to a diet that is well-balanced. Village poultry is especially crucial for enhancing young children's diets in Sub-Saharan Africa **(Dana *et al*., 2010).**

Native or indigenous breeds of chicken play a significant role in the rural economy. They are common sources of secondary income for the support of the rural portion of the population, who are mostly underprivileged and marginalized. They are sources of wholesome chicken eggs and meat but have relatively low yields. Improvement of the performance of native poultry can be achieved through better husbandry, feed, and health care. However, genetic improvement can be achieved through selection and crossbreeding, or by combining both of them. Although improvement through selection may take some time, it will be permanent. Improvement may occur more quickly through crossbreeding, but efforts must focus on

producing native-type birds with greater production potential **(Ferreira *et al*., 2009; Hafez, 2005).**

Escherichia coli constitute a part of the microbiota in the intestine of birds, although some strains are regularly associated with a number of clinical signs in domestic animals **(Al-Sabawi and Jwher, 2022)**. They may have a lower colonization rate than other major commensals but are considered as an important cause of disease in poultry which may be due to the several virulence factors they possess **(Stecher and Hardt, 2008; Eman *et al*., 2021)**.

Some pathogens, such as those belonging to the Enterobacteriaceae family, which are abundant in vegetables, cereals, soil, fruits, flowers, water, animals, and trees are easily transmitted in poultry flocks due to the high density of the birds being raised **(Holt *et al*., 1994; Vandemaele *et al*., 2003; Hafez, 2005 and Ferreira *et al*., 2009)**. One of the most prevalent enteric pathogen linked to foodborne illnesses worldwide is Salmonella, which can cause significant monetary loss. Globally, Salmonella is responsible for about 93.8 million illnesses contracted through food with an estimated 155,000 deaths **(Eng *et al*., 2015)**.

Scientific data suggests that using antibiotics in animals raised for human consumption is likely the main factor causing the formation and dissemination of resistant strains. Resistance to antibiotics is an existing critical issue of clinical and public health concern, globally **(Angulo *et al*., 2000, Leoni *et al*., 2012)**.

To promote growth and treat infectious diseases, antimicrobials are frequently administered in livestock production. Because antibiotics are routinely used in chicken production to promote growth, both pathogenic microorganisms and the normal flora are becoming more resistant. The administration of antimicrobials through feed has potential to change gut flora and may have an impact on the environment, and food chain, by putting a pressure for selection on populations of resistant bacteria **(Furtula *et al*., 2010)**. One of the bacteria that commonly becomes resistant to antibiotics is *E. coli*, because of its abundance in both animals and humans, and its role as a commensal and pathogenic organism **(Zhao *et al*., 2012)**.

In developing countries, the risk of zoonotic transmission of antibiotic resistant bacteria is higher because of the close contact with livestock, unhygienic living conditions, sharing of personal items and the uncontrolled use of antibiotics in food animals. Poultry are an important source for the dissemination of antimicrobial resistant *Salmonella* species to other animals and humans **(Darwish *et al*., 2013)**. Although, limited data on these problems are available, Somalia; much like other African nations; has a significant risk of zoonotic transmission of antibiotic resistant *E. coli* and *Salmonella*.

MATERIALS AND METHODS

Study Area

The location of this study was Marodi Jeh, Somaliland. This region is located in the northwest zone of Somalia, between the GPS coordinates of 6°6′47″N and 47°59′17″E (Longitude and Latitude). This region is where the capital city of Somaliland, Hargeisa is located. It shares borders with Awdal region to the north, Sahil region to the southeast, and south to the border of Ethiopia and northeast to red sea (Fig. 1). A population of 12,693,796 people lives in Marodi Jeh region, the largest population among the six regions of Somaliland. The Marodi Jeh region consists of six districts namely: Gabiley, Baligubadle, Salahley, Alaybaday, Arabsiyo, and Darasalam **(Amare, 1994; The World Factbook, 2023).**

The majority of Marodi Jeh's yearly precipitation, at just under 400 millimeters (16 in), falls between April and September. The average monthly temperature ranges from 18°C (64°F) in December and January to 24°C (75°F) in June.

Fig.1: Map of Somaliland region; Source **(The World Factbook, 2023)**.

Ethical Approval

In accordance with the International Guidelines for Animal Welfare, sampling from local chickens was done in accordance with experimental procedure and standard approved by the Animal Welfare, Research and Ethics committee of Somaliland veterinary board at Hargeisa, Somaliland.

Study Design

To ascertain the antibiotic resistance profile of *E. coli* and *Salmonella* from Chicken in the Marodi Jeh region of Somaliland, a cross-sectional investigation was undertaken from March to August

2022 to assess the occurrence of *E. coli* and *Salmonella* species, and also evaluate their antibiotic resistance profile.

Study Population

The group under investigation was indigenous chickens sourced from Marodi Jeh region, Somaliland purposively selected, based on the number of chickens available in the two selected markets (Xisbi and Waheen). The chickens were categorized into two age groups: growers (≤ 6 Months) and adults (>6 months).

Sample Size Determination

There was no previous record on the prevalence and antibiotic resistance pattern of *Escherichia coli* and *Salmonella* species isolated from indigenous birds in the Marodi Jeh region, Somaliland. As a result, the sample size was determined to be 384 using Thrusfield's calculation, at 50% predicted prevalence, 5% required absolute precision, and 95% confidence interval **(Thrusfield, 2005)**.

$$SN = z^2 p (1-p),$$

$$N = \frac{1.96^2 Pexp (1-Pexp)}{d2}$$

Where, N = sample size
D = required absolute precision
Z = statistic for a level of confidence
P = expected prevalence

Sample Collection

The cloaca swab samples (384) were collected using sterile swabs wet with buffered peptone water, and placed in sterile vial tubes holding 8 mL of the solution in order to avoid the swabs from drying out. The swabs were obtained from Waheen (n = 193) and Xisbi (n = 191) marketplaces. They were shipped to the Ministry of Livestock and Fisheries Development's Central Veterinary Laboratory (CVL) on icepacks and kept at 4°C while the scientific investigation was being done. Owner's consent was obtained before the collection of samples.

Isolation and characterization of *E. coli*

Conventional bacteriological techniques were utilized to isolate *E. coli* (**Iso-6579, 2012**). MacConkey Agar was streaked with a loopful of overnight culture suspension made in peptone water, incubated aerobically for 24 hours at 37°C. They were subcultured on Eosin Methylene Blue (EMB) agar. Gram's stain was then, used to characterize the metallic sheen green colonies under a microscope.

After that, potential *E. coli* colonies were moved to nutrient agar for additional identification with the aid of differential screening media. Triple sugar iron (TSI) agar was used for additional characterization, and was assumed to be a probable *E. coli* isolate according to observations of yellow slant, yellow butt, the appearance of gas bubbles, and the lack of black precipitate in the butt. According to **Quinn *et al*. (2011)**, various biochemical assays then performed on the isolates. Including tests for motility, methyl-red, the Voges-Proskauer (MR-VP) test, citrate utilization (IMViC), and indole synthesis (**Quinn *et al*. 2011**).

Standard bacteriological techniques for detection of Salmonella were used (**ISO, 2007**). The swab were pre-enriched in buffered peptone water (BPW), then cultured for 24 hours at 37°C (**Kim *et al*., 2014**). Pre-enrichment media samples were added to Rappaport-Vasiliadis medium which was then cultured for 24 hours at 37°C. Bacteria grown on an enriched medium were spread onto Salmonella-Shigella (SS) agar and incubated at 37°C for 18- 24 hours. The XLD agar and BGA medium were used for subculture the probable *Salmonella* colonies.

By microscopic analysis of stained smears from typical colonies, Salmonella was identified. All likely non-lactose fermenting colonies of Salmonella were chosen from the nutrient agar in accordance with **Quinn *et al*. (2011**)'s guidelines, and then they were inoculated into the Simmon's citrate, urea broth, triple sugar iron (TSI), tryptone water, methylene red, sulfur indole motility test (SIM media), and Voges-Proskauer (MR-VP) broths. If acid butt (yellow) and alkaline slant (red) on TSI with production of hydrogen sulfide, positive for lysine (purple), negative for urea hydrolysis (red), and positive for tryptophan, the colony was then confirmed to be *Salmonella* species.

***Salmonella* and *E. coli* Antimicrobial Susceptibility Testing**

The agar disk diffusion method was employed to investigate the in-vitro antibiotic susceptibility of *Salmonella* and *E. coli* isolates (**Bauer *et al*., 1966**). The antibiogram contained the following drugs: gentamycin (GEN-10µg), ampicillin (AMP-10 µg), tetracycline (TE-30 µg), ciprofloxacin (CIP-5 µg), streptomycin (SRP-10 µg), cefoxitin (FOX-30 µg), chloramphenicol (CHL-30 µg), and ceftriaxone (CTR-30 µg).

Adjusted 0.5 McFarland turbidity was prepared from each isolate. The surface of the Mueller-Hinton agar plate was then evenly covered with the swab to produce homogeneous inoculums. Then, sterile discs impregnated with antibiotics and foetal bovine serum were placed on top of the plates. The inoculated plates were then covered with antibiotic-impregnated discs using sterile forceps. After 15 minutes of placing the discs, the plates were turned over and left at 37°C for incubation.

The plates were checked after 18- 24 hours of incubation, and the diameters of the entire inhibition zones were measured to the nearest whole millimeter using a digital caliper. Using the Clinical and Laboratory Standard Institute's (Scientific, 2011) interpretation table, the diameter of the clear for various antimicrobials, was then classified as Sensitive (S), Intermediate (I), and Resistant (R) (Table 1).

Table 1: Interpretive standard chart for Zone Diameter for Enterobacteriaceae **(CLSI, 2013)**

Antimicrobial Agent	Disc potency (μg)	Zone diameter, nearest whole mm		
		Resistance	Intermediate	Susceptible
Chloramphenicol	30	≤12	13-17	≥18
Tetracycline(TE)	30	≤11	12-14	≥15
Ampicillin	10	≤13	14-16	≥17
Cefoxitin	30	≤14	15-17	≥18
Ciprofloxacin	5	≤14	15-17	≥21
Gentamycin	10	≤12	13-14	≥15
Streptomycin	10	≤11	12-14	≥15
Ceftriaxone	30	≤19	20-22	≥23

Data Analysis

After entering into an MS Excel Spreadsheet, descriptive statistics were used to summarize the data on the Statistical Package for Social Sciences. Age, sex, and source were used to categorize prevalence and computed for *Salmonella* and *E. coli*. Using Chi-square analysis, the relationships between the investigated factors and the result variables were examined. The odds ratio was used to compare the strength of the relationship, and the difference was considered significant when the P-value was less than 0.05. The antimicrobial resistance (AMR) percentages were calculated and categorized into susceptible, intermediate, and resistant classes.

RESULTS

Prevalence and risk factors of *E. coli* and *Salmonella* species infection in indigenous chickens from two markets in Somalia

Out of the 384 samples examined by Gram staining and biochemical tests, 75 (19.5%) were positive for *E. coli* as presented in (Table, 2). Based on age, out of the indigenous chicken positive for *E. coli* infection (n=75), 24 (32%) were growers and 51 (68%) were adults. Based on sex, 21 (28%) were males and 54 (72%) were females. In addition, based on source, 22 (29.3%) obtained from Xisbi market were positive while the remaining 53 (70.6%) were from Waheen market. The prevalence of *E. coli* infection was significantly higher ($p<0.05$) in Waheen market higher than Xisbi Market (Table 2).

Table 2: Prevalence and risk factors of Escherichia coli infection in Indigenous chickens

Variables	Factors	No sample	Prevalence (%) *E. coli*	OR (95% CL)		P-Value
Age	Grower	75	24 (32.0%)	1.668	(0.958-	0.050
	Adult		51(68.0%)	2.905)		
Source	Xisbi Market	75	22 (29.3%)	0.344	(0.199-	0.000
	Waheen Market		53 (70.7%)	0.593)		
Sex	Male	75	21 (28.0%)	0.825	(0.472-	0.298
	Female		54 (72.0%)	1.441)		
Total			75/384			

(19.5%)

Furthermore, 34 (8.9%) out of the 384 samples were positive for *Salmonella* species infection. Based on age, 10 (29.4%) from+ growers and 24 (70.6%) from adult chickens were positive. Based on sex, males 14 (41.2%) and females 20 (58.8%) were positive. In addition, based on source, samples from Xisbi market 2 (5.9%) and Waheen market 32 (94.1%) were positive. The prevalence was significantly higher ($p<0.05$) in Waheen market higher than Xisbi Market as similar to *E. coli* and there was no significance relationship to age and sex (Table 3).

Table 3: Prevalence and risk factors of Salmonella species infection in Indigenous chickens

Variables	Factors	No sample	Prevalence (%) *E. coli*	OR (95% CL)	P-Value
Age	Grower	34	10 (29.4%)	1.362 (0.625-2.905)	0.278
	Adult		24 (70.6%)		
Source	Xisbi Market	34	2 (5.9%)	0.052 (0.012-0.220)	0.000
	Waheen Market		32 (94.1%)		
Sex	Male	34	14 (41.2%)	1.548 (0754-3.178)	0.157
	Female		20 (58.8%)		
Total			34/384 (8.9%)		

Antibiotic Resistance Pattern

In total, 75 *E. coli* isolates were found in indigenous chickens from the live bird market in Marodi Jeh region, Somaliland. They were tested against eight antibiotics for sensitivity or resistance. The results reveal that 12 (16.0%), 13 (17.3%) and 50 (66.7%) isolates being sensitive (S), intermediate (I) and resistant (R) to chloramphenicol. In the same order, 27 (36%), 14 (19%), and 34 (45%) to tetracycline, 37 (49%), 25 (33%) and 13 (17%) to ampicillin, 43 (57%), 22 (29%), and 10 (13%) to Cefoxitin, 50 (67%), 24 (32%), and 1 (1%) to Ceprofloxin, 36 (48%), 24 (3,67%), and 15 (26.67%) to gentamycin, 42 (56%), 33 (44%) and 0 (0%) to Streptomycin and 35 (47%), 39(52%) and 1 (1%) to ceftriaxone. (Table 4).

A total of 34 Salmonella isolates were screened for antibiotic resistance. Nine (26%), 10 (30%) and 15 (44%) isolates were sensitive (S), intermediate (I) and resistant (R) to chloramphenicol. In the same order, 19 (56%), 4 (12%) and 11 (32%) to tetracycline, 8 (24%), 15 (44%), and 11 (32%) to ampicillin, 16 (47%), 9 (26%), and 9 (27%) to Cefoxitin, 23 (67%), 4 (12%), and 7 (21%) to Ciprofloxacin, 13 (38%), 15 (44%), and 6 (18%) to gentamycin, 22 (65%), 11 (32%), and 1 (3%) to streptomycin and 17 (50%), 9 (26%), and 8 (24%) to ceftriaxone (Table 4).

Table 4: Frequency of occurrence and antibiotics resistance profile of Salmonella and Escherichia coli isolates from indigenous chickens

Antibiotics	Disc potency (µg)	Frequency of antimicrobial resistance (%)					
		Salmonella isolates (n=34)			*Escherichia coli* (n=75)		
		Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Chloramphenicol	30	9	10	15	12	13	50
Tetracycline	30	19	4	11	27	14	34
Ampicillin	10	8	15	11	37	25	13
Cefoxitin	30	16	9	9	43	22	10
Ciprofloxacin	5	23	4	7	50	24	1
Gentamycin	10	13	15	6	36	24	15
Streptomycin	10	22	11	1	42	33	0
Ceftriaxone	30	17	9	8	35	39	1

The pattern of resistance to antibiotics reveal that 34 *Escherichia coli* isolates were resistant to one antibiotic i.e. either of gentamicin, tetracycline, cefoxitin, ampicillin and streptomycin, 41 isolates were resistant to between 2 and 4 antibiotics having several combinations (Table, 5). Most single drug resistance was against gentamicin i.e. 12 out of 34 isolates. For the multiple drug resistant groups of isolates, the most frequent combination was chloramphenicol and ampicillin and similar tetracycline and cefoxitin involving 9 out of 41 *E. coli* O157:H7 isolates (12%). Also, eight isolates were resistant to ampicillin and ceftriaxone (11%) and six isolates were resistant to chloramphenicol, ceftriaxone and amoxicillin combination (8%), and five isolates were resistant to cefoxitin, ceftriaxone and tetracycline combination (7%).

In contrast, the pattern of resistance to antibiotics for *Salmonella* reveal that 12 isolates exhibited resistance to one antibiotic i.e. either of gentamicin, tetracycline, and streptomycin, 22 isolates were resistant to between 2 and 4 antibiotics having several combinations (Table, 5). Most single drug resistance was against tetracycline i.e. 7 out of 12 isolates. For the multiple drug resistant groups of isolates, the most frequent combination was chloramphenicol, streptomycin and gentamicin involving 6 out of 22 *Salmonella* isolates (18%). Also, five isolates were resistant to gentamicin and streptomycin (15%), and four isolates were resistant to tetracycline and streptomycin combination (12%). on the other hand, three isolates were resistant to cefoxitin, streptomycin, chloramphenicol and tetracycline combination (9%).

Table 5: Overall distribution pattern of antimicrobial resistance to Escherichia coli and Salmonella isolates

Frequency of antimicrobial pattern (%)					
Antibiotics	*Salmonella* isolates (n=34)		Antibiotics	*Escherichia coli* (n=75)	
	Resistance			Resistance	
	Frequency	Percentage		Frequency	Percentage
GEN	3	8.8%	GEN	12	16%
GEN, S	5	14.7%	CHL, AMP	9	12%
S, TE, CIP	4	11.7%	AMP, TE	9	12%
TE	7	20.6%	TE	3	3.4%
S	2	5.8%	FOX	7	9.4%
GEN, S, CHL	6	17.7%	FOX, AMP, CHL	6	8%
CTR, S, TE	4	11.8%	CTR, FOX, TE	5	6.7%
GEN, S, TE, CHL	3	8.9%	CTR, GEN, TE, CHL	4	5.4%
			AMP	11	14.6%
Nil Resistance			AMP, CTR	8	10.6%
	0	0%	S, CIP	1	1.3%
Total	34	100%	75		100%

GEN: Gentamycin, S: Streptomycin, TE: Tetracycline, CIP: Ciprofloxacin, CHL: Chloramphenicol, AMP: Ampicillin, FOX: Cefoxitin, CTR: Ceftriaxone

The isolates displayed various patterns of resistance. They showed resistance to a range of one to four antibiotics. Antibiotic mono-resistance was seen in 44% and 35% of *E. coli* and *Salmonella* species, respectively. In 36% of *E. coli*, and 15% of *Salmonella* species isolates, divalent resistance to antibiotics was found. In 15% of *E. coli* and 41% of *Salmonella* species trivalent resistance was found. In 5% of *E. coli* and 9% *Salmonella* species tetravalent resistance was seen (Table 6).

The single resistance to GEN and AMP was the most prevalent antimicrobial resistance pattern in *E. coli* and resistance to TE and GEN in Salmonella species. Multiple resistances were defined as antimicrobial resistance to two or more antibiotics. CHL AMP, FOX TE, AMP CTR, GEN S CHL, FOX AMP CHL, CTR FOX TE, CTR GEN TE were found to exhibit multi-resistance.

Table 6: Resistance pattern of *E. coli* and *Salmonella* species isolates

Antibiotic	No. of *E. coli* isolates	No. *Salmonella* isolates	Resistance pattern
One	12	3	GEN
	3	7	TE
	0	2	S
	7	0	FOX
	11	0	AMP
Total	33 (44%)	12 (35%)	
Two	0	5	GEN, S
	1	0	S, CIP
	9	0	CHL, AMP
	9	0	FOX, TE
	8	0	AMP, CTR
Total	27 (36%)	5 (15%)	
Three	0	6	GEN, S, CHL
	0	4	S, TE, CIP
	0	4	CTR, S, TE
	6	0	FOX, AMP, CHL
	5	0	CTR, FOX, TE
Total	11 (15%)	14 (41%)	
Four	0	3	GEN, S, TE, CHL
	4	0	CTR, GEN, TE, CHL
Total	4 (5%)	3 (9%)	
Grand total	74 (99%)	34 (100%)	

DISCUSSION

This study provides evidence of the continuing colonization of the cloaca of indigenous chickens in the Marodi Jeh region of Somalia by *Salmonella* and *E. coli*. Both bacteria displayed several patterns and varying degrees of antibiotic resistance. The prevalence of *E. coli* detected in this study (19.5%) is lower than earlier reports of 50% in local chickens in Dar es Salaam, Tanzania **(Mwambete and Stephen 2015)** and 82% in Kigali city, Rwanda **(Manishimwe *et al.*, 2017)**. The differences in environmental factors and geographic location may account for the lower prevalence we obtained.

In the present study, adult chickens had a higher (68.0%) infection rate compared to growers (32.0%), although this difference was not statistically significant. The current findings are in line with the conclusions of **Pitcovsk *et al*,. (1987), Sarba *et al.*, (2019)** who reported that *E. coli* isolation rate was higher in adult chickens (37.8%) than in young chicks (23.6%), while the reverse was the observation of **Zhao and Maurer (2005),** who found that young chicks had more *E. coli* isolation rate than adults. They alluded that the higher colonization in chicks may be due to the lack of full immuno-competence and the wearing off of the protection afforded by maternal antibodies.

In comparison to male chickens (28.0%), female chickens (72.0%) had a larger prevalence of *E. coli*. This is at variance with the results of this study of **Zanella *et al.*, (2000)**, who found that isolation rates for male and female hens were similar, 31.4% and 33.1%, respectively. The fact that more females were sampled in this study than males may be the cause of the higher infection rates in females. Additionally, inadequate shelter, inadequate nutrition, and high levels of stress during female birds' egg production may all contribute to possible immunosuppression leading to the high incidence.

Escherichia coli prevalence varied in the current study according to sample location; as a result, Waheen live bird market had a higher prevalence rate (70.7%) than those at Xisbi live bird market (29.3%). The current study supports the findings of **Mali (2019**), who found that *E. coli* was isolated in Kenya,

Kawangware (80%), Mathare (60%), and Kibera (48%). This variation in prevalence may be due to regionally unique factors like climatic shocks. Additionally, poor hygiene, absence of a dedicated entrance and exit door that runs counter to general biosecurity recommendations, a lack of a facility for isolation, open access to the public, absence of a fence that facilitates the spread of disease among humans and animals, and formites may be contributing factors.

Each sample was tested for colonization of *Salmonella* in a manner similar to *E. coli* testing. 34 (8.9%) of 384 cloaca samples tested positive. Our findings corroborated those of **Mali (2019)** who noted 12% *Salmonella* infection. Previous research found that Tanzania and Nigeria had greater rates of *Salmonella* infection, with prevalence rates of 22%, and 31% of *Salmonella* species in chicken droppings, respectively **(Agada *et al.*, 2014; Mwambete and Stephen, 2015)**. *Salmonella* species was found in 55% of chicken feces samples according to a Burkina Faso investigation **(Kagambèga *et al.*, 2013)**. The *Salmonella* colonization rate was found to be lower in developed nations than it is now. There have been reports of flock totals as low as 1.57%. In Poland, broiler chicken prevalence decreased from 2.19% in 2014, to 1.22% in 2016. In 2018, Denmark reported that flock prevalence was 2.6%. **(Jibril *et al.*, 2020)**.

According to the sampling site, Waheen Live Bird Market had a greater prevalence rate of 32 (94.1%) than Xisbi Live Bird Market 2 (5.9%). The current observation is consistent with earlier research by **Mali (2019)**, which found that Mathare had a greater prevalence of *Salmonella* (20%). While, Kibera had a lower number (4%). On the other hand, Waheen and Xisbi live bird markets showed a significant correlation between prevalence and location. This is in line with the research of **Mali, (2019)**, who found a considerable variation in the prevalence of *Salmonella* species in the various localities. However, geographic-specific factors like climate shocks may be responsible for the disparity in prevalence between the marketplaces.

Additionally, poor hygiene, a lack of a dedicated entrance and exit door against general biosecurity recommendations, a lack of isolation facility, open access to the public, lack of a fence that facilitates the spread of disease between humans and animals, and formites, such as chicken baskets, are risk factors for *Salmonella* infection that occur to varying degrees in the two markets and may be a factor in the markets' significantly different infection rates **(Zanella *et al.*, 2000)**. Resistance to antimicrobials is currently a significant problem worldwide **(Miles *et al.*, 2006)**. The indiscriminate use of antimicrobials in animals, humans, and crops is believed to be the primary factor contributing to the development, selection, and propagation of antimicrobial resistant bacteria, in both human and veterinary medicine **(Simonsen *et al.*, 2004)**.

Susceptibility to antimicrobials by *E. coli* isolates obtained in this study was ascertained using eight different antibiotics. 74 isolates (98.6%) showed antibiotic resistance to different antibiotic combinations. The three drugs to which isolates showed the most resistance were gentamycin 15 (20.0%), chloramphenicol (66.7%), and tetracycline (45.3%). This is consistent with what **Akond *et al.*, (2009)** discovered. They previously reported that 52% of *E. coli* isolates were resistant to tetracycline, and **Apun *et al.*, (2008)** stated that tetracycline was the antibacterial agent to which *E. coli* isolates were most frequently resistant. Additionally, **Kang *et al.*, (2005)** revealed that the tetracycline-resistant *E. coli* isolates from the chicken farm was extremely resistant even at 150 g/ml. In addition, a high resistance rate to tetracycline and chloramphenicol was also noted in isolates from live bird markets in Benue State, Nigeria.

The abuse and repetitive use of antibiotics for promoting the growth in the poultry industry may be the cause of the antibiotic resistance seen in this study. In *E. coli* isolates from a poultry farm in Bangladesh's Chittagong District, they showed 96.6% Tetracycline resistance. Only 71% of *E. coli* isolates from Turkey, according to **Schroeder *et al.*, (2000)** exhibited tetracycline resistance.

To understand the susceptibility of the 34 *Salmonella* isolates, eight different antibiotics were used. *Salmonella* isolates with the highest levels of resistance to antibiotics included chloramphenicol 15 (44%), tetracycline 11 (32%) and amoxicillin 11 (32%). The current investigation supports the findings of **Hassan *et al.*, (2014)** who found chloramphenicol and tetracycline to be (100%) resistant. **Siemon *et al.* (2007)** reported a reduced resistance to amoxicillin isolated from conventionally raised poultry (62%), whereas **Ahaduzzaman *et al.*, (2014)** found a comparable result in effluents from the environment.

Nevertheless, *Salmonella* exhibited resistance to ciprofloxacin (87.5%), according to **Musgrove *et al.*, (2006)** no *Salmonella* resistance to ciprofloxacin was discovered in isolates derived from commercial chicken. In France, 0.1% of the isolates from humans were resistant, according to **Gay *et al.*, (2006)**. According to the data, all *Salmonella* isolates were susceptible to gentamicin and neomycin. In the current investigation, it was shown that of the *Salmonella* isolates, 23 (67%) were sensitive to ciprofloxacin, and 22 (65%) to streptomycin.

Tetracycline (19%), ampicillin (8-24%), and chloramphenicol (9-26%) were the antibiotics with the lowest number of susceptible isolates. The current findings support the findings of **Musgrove *et al.* (2006**) who reported 63.4%, and **Zhao *et al.*, (2008)** who reported 39.9% and **Hassan *et al.*, (2014)** who found no isolate of *Salmonella* was sensitive to tetracycline.

The prolonged, extensive, and widespread usage of these medications on chicken farms and other food producing animals may be the cause of the drugs' resistance patterns (**Taye *et al.*, 2013; Omotosho *et al.*, 2016)**. The resistance will develop when these antimicrobial substances are used on bacteria whether it's for prophylaxis, treatment, or to boost livestock development. Bacteria that have had their genetic makeup altered by the genes that prevent them from replacing a target receptor (modified with mediated plasmid). The resistance could result from this via conjugation or transformation **(Miles *et al.*, 2006)**. Additionally, the issue is probably related to the frequent use of these antibiotics in both humans and animals to treat enteric infections.

The isolates displayed various patterns of resistance. They showed one to four antibiotic resistance. For *E. coli* and *Salmonella* species, single antibiotic resistance was found in 44% and 35%, respectively. Divalent antibiotic resistance was found in 27% of *Salmonella* species Isolates, and 35% of *E. coli* isolates. 25% of *Salmonella* species Isolates, and 15% of *E. coli* isolates both exhibited trivalent resistance. In 5% of *E. coli* isolates, and 9% of *Salmonella* species tetravalent resistance was found. Chloramphenicol, tetracycline, and gentamycin were the most common combination while chloramphenicol was the most common antibiotic for which resistance was observed.

CONCLUSION

Escherichia coli and *Salmonella* were isolated from samples collected from the cloaca of indigenous chickens in two live bird markets in the Marodi Jeh district of Somaliland with a much higher prevalence in Waheen market than in Xisbi market. The antimicrobial-resistant isolates of *Salmonella* and *E. coli* was highly resistant to tetracycline and chloramphenicol. The majority of the *Salmonella* and *E. coli* isolates exhibited multidrug resistance for two to four antibiotics. These germs have the potential of being transferred to humans through the food chain, this could have a serious impact on public health. Consequently, improved hygiene in handling indigenous chickens and additional research is therefore recommended.

ACKNOWLEDGMENT

We thank the African Union for providing the lead author with a scholarship for her master's degree at Pan African University's Institute of Life and Earth Sciences, including Agriculture and Health, Nigeria, so she could do the research.

Conflict interest

The authors reports that there are no conflicts of interest.

REFERENCES

AHADUZZAMAN M., HASSAN M.M., ALAM M., ISLAM S., and UDDIN I., 2014. Antimicrobial resistance pattern against Staphylococcus aureus in environmental effluents. Research Journal for Veterinary Practitioners. 2:13–16. http://dx.doi.org/10.14737/journal.rjvp/2014/2.1.13.16

AGADA, G.O.A., ABDULLAHI, I.O., AMINU, M., ODUGBO, M., CHOLLOM, S.C., OKEKE, L.A., and OKWORI, A.E.J., 2014. Risk factors associated with Salmonella sp contamination of Commercial poultry farms in Jos, Plateau State, Nigeria. International Journal of Current Research 6(84): 6292 – 6301

AKOND, M.A., ALAM, S., HASSAN, S.M.R., and SHIRIN, M., 2009. Antibiotic resistance of *Escherichia coli* isolated from poultry and poultry environment of Bangladesh. Internet Journal of Food Safety, 11, 19-23 DOI: https://doi.org/10.3844/ajessp.2009.47.52

AL-SABAWI, A. H., and JWHER, D. M., 2022. Isolation and characterization of stx1 and stx2 toxin-producing *Escherichia coli* in neonatal lambs with diarrhea in Nineveh governorate, Iraq. Journal of Applied Veterinary Sciences, 7 (4): 23– 27. DOI: https://dx.doi.org/10.21608/javs.2022.147953.1159

AMARE TEKLE, 1994. Eritrea and Ethiopia: From Conflict to Cooperation. ISBN 9780932415974.

ANGULO, F. J., JOHNSON, K. R., TAUXE, R. V., and COHEN M. L., 2000. Microbial Drug Resistance. Significance and Sources of Antimicrobial-Resistant Nontyphoidal Salmonella Infections in the United States, 6(1)(123;45), 77–83. DOI: 10.1089/mdr.2000.6.77

APUN, K., CHONG, Y. L., ABDULLAH, M.T., and MICKY. V., 2008. Antimicrobial susceptibilities of *Escherichia coli* Isolates from Food Animals and Wildlife Animals in Sarawak, East Malaysia. *Asian Journal of animal and veterinary advance,* 3 (**6**):409-416. DOI: 10.3923/ajava.2008.409.416.

BAUER, A.W., KIRBY, W.M.M., SHERRIES, J.C., and TURCK, M., 1966. Antibiotic Susceptibility Testing by a Standard Agada, G. O. A, Abdullahi, I. O., Aminu, M., Odugbo, M, Chollom, S. C.,

OKEKE, L.A., and OKWORI, A.E.J., 2014. Risk factors associated with *Salmonella* sp. contamination of Commercial poultry farms in Jos, Plateau State, Nigeria. International Journal of Current Research 6(84): 6292 – 6301

CLSI, 2013. Clinical and Laboratory Standards Institute (CLSI) Guidelines—Performance Standards for Antimicrobial Susceptibility Testing. CLSI Document M100.

DANA, N., VAN DER WAAIJ, L.H., DESSIE, T., and ARENDONK J. A., 2010. Production objectives and trait preferences of village poultry producers of Ethiopia: implications for designing breeding schemes utilizing indigenous chicken genetic resources. Tropical Animal Health and Production 42, 1519–1529 . https://doi.org/10.1007/s11250-010-9602-6

DARWISH, W. S., ELDALY, E. A., EL-ABBASY, M. T., IKENAKA, Y., NAKAYAMA, S., and ISHIZUKA, M., 2013. Antibiotic residues in food: the African scenario. Jpn J Vet Res. 61 Suppl:S13-22. PMID: 23631148.

VANDEMAELE F., VANDEKERCHOVE D., VEREECKEN M., DERIJCKE J., DHO-MOULIN M., and GODDEERIS B. M., 2003. Sequence analysis demonstrates the conservation of fimH and variability of *fim*A throughout avian pathogenic *Escherichia coli* (APEC). Vet. Res. 34:153–163. DOI: 10.1051/vetres:2002062

E**MAN, M. D., HEBA, N. D., and JAKEEN, K. E., 2021.** Isolation and Identification of the most common Bacteria Isolated from Intestine of Broiler Chickens in Egypt. Journal of Applied Veterinary Sciences, 6 (4): 23 – 27. DOI: https://dx.doi.org/10.21608/javs.2021.87134.1092

E**NG, S. K., PUSPARAJAH, P., MUTALIB, N.-S.A., SER, H., CHAN, K., and LEE, L.-H. 2015.** *Salmonella*: a review on pathogenesis, epidemiology and antibiotic resistance. Front Life Sci. 8:284–293.

FERREIRA, T., SILVA, E. N., FÁBIO, J., SESTI, L., and ZUANAZE, M.A.F. (Eds). 2009. Doenças das aves. 2.ed. Campinas: Fundação APINCO de Ciência e Tecnologia Avícolas, pp.457-471. https://doi.org/10.1080/21553769.2015.1051243

FURTULA, V., FARRELL, E. G., DIARRASSOUBA, F., REMPEL, H. PRITCHARD, J., DIARRA, M., S. *et al*., 2010. Veterinary pharmaceuticals and antibiotic resistance of *Escherichia Coli* isolates in poultry litter from commercial farms and controlled feeding trials. Poultry Science, 89; pp. 180-188. DOI: 10.3382/ps.2009-00198

GAY, K., ROBICSEK, A., STRAHILEVITZ, J., PARK, C. H., JACOBY, G., BARRETT, T. J., MEDALLA, F., CHILLER, T.M., and HOOPER, D.C., 2006. Plasmid–Mediated Quinolone Resistance in Non–Typhi Serotypes of *Salmonella enterica*. Clin. Infect. Dis. 43:297–304. DOI: 10.1086/505397

HAFEZ, H. M. 2005. Governmental regulations and concept behind eradication and control of some important poultry diseases. World's Poultry Science Journal. Volume 61,- Issue 4. https://doi.org/10.1079/WPS200571

HASSAN M., AMIN K. B., AHADUZZAMAN M., ALAM M., FARUK M.S., and UDDIN I., 2014. Antimicrobial resistance pattern against E. coli and Salmonella in layer poultry. Research Journal for Veterinary Practitioners. 2:30–35.

HOLT, J. G. KNR, SNEATH, and Whiteman. B. W. 1994. Bergey's Manual of Determinative Bacteriology. Wiley & Sons, Inc, Online ISBN: 9781118960608DOI: 10.1002/9781118960608.

I**SO-6579, 2002.** Microbiology of food and animal feeding stuffs-Horizontal method for the detection of *Salmonella* spp. International Standard Organisation.

ISO, 2007. ISO/TS 21872 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of potentially enteropathogenic Vibrio spp.

KAGAMBÈGA, A., LIENEMANN, T., AULU, L., TRAORÉ, A. S., BARRO, N., SIITONEN, A., and HAUKKA, K., 2013. Prevalence and characterization of *Salmonella enterica* from the feces of cattle, poultry, swine and hedgehogs in Burkina Faso and their comparison to human *Salmonella* isolates. BMC Microbiology. doi: 10.1186/1471-2180-13-253.

KANG, H. Y., JEONG. Y. S., OH, J. Y., TAE, S. H., CHOI, C. H., MOON, D. C., LEE, W. K., LEE, Y. C., SEOL, S. Y., CHO, D. T., and LEE, J. C., 2005. Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. Journal Antimicrobiology Chemistry, **55**:639-644. https://doi.org/10.1093/jac/dki076

KIM, H. B., YOON, M., LEE, S. J., JANG, Y. H., and CHO, N. H., 2014. Prevalence and antibiotic resistance characteristics of *Salmonella* spp. isolated from food-producing animals and meat products in Korea. Prev Vet Med 38, 85-93. doi: 10.1292/jvms.13-0093.

LEONI, K. R., RENATA, O. A., LUCIANA, K. O., EDNA, T. L., JOVANIR, I. M. F., and LISIANE, A. M., 2012. em frangos de corte criados em galpões climatizados de uma integração na região Oeste do Paraná. Semina. : Pesquisa de *Salmonella* Spp, 33(6)(78;98), 2327–2336.OIE Lmfbast, Terrestrial Manual: Pp.113.

MALI LANGATA, L. 2019. Prevalence of Antimicrobial Resistant Genes in *Escherichia coli* and Salmonella species from Chicken droppings in selected locations in Nairobi County, Kenya. A Ph.D Thesis submitted to the School of Pure and Applied Sciences of Kenyatta University.

MANISHIMWE, R., BUHIRE, M., UYISUNZE., TURIKUMWENAYO, J., and TUKEI, M., 2017. Characterization of antibiotic resistant *Escherichia coli* in different poultry farming systems in the Eastern Province and Kigali City of Rwanda. Revue Elevage Medicine Veterinaire Pays Tropicaux, 70 (1), 13-19. DOI: https://doi.org/10.19182/remvt.31392

MILES, T D. MCLAUGHLIN, W., and BROWN P.D., 2006. Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans, BMC Veterinary Research, 2 (**1**):1-9. doi: 10.1186/1746-6148-2-7

MUJYAMBERE, V., ADOMAKO, K., OLYMPIO, S. O., NTAWUBIZI, M., NYINAWAMWIZA, L., and MAHORO, J., 2022. Local chickens in east african region: Their production and potential. Poultry Science 101 (1), 101547. doi:10.1016/j.psj.2021.101547

MUSGROVE, M. T., JONES, D. R., NORTHCUTT, J. K., COX, N. A., HARRISON, M. A., FEDORKA–CRAY, P. J., and LADELY, S. R., 2006. Antimicrobial Resistance in Salmonella and Escherichia coli Isolated from Commercial Shell Eggs. Poultry. Sci. 85:1665–1669. http://dx.doi.org/10.1093/ps/85.9.1665

MWAMBETE, K. D., and STEPHEN, W. S., 2015. Antimicrobial resistance profiles of bacteria isolated from chicken droppings in Dar es Salaam. International Journal of Pharmacy and Pharmaceutical Sciences, 7(9), 268-271. Corpus ID: 56342825

OMOTOSHO, O. O., OLAOGUN, S. C., and ODUKAYE, A. O., 2016. Occurrence of Skin Abscess and Sensitivity Pattern of Associated Bacterial Organisms in Pigs on Ifelodun Farm Settlement, Ogun State, Nigeria. Alexandria Journal of Veterinary Sciences 51(1): 10-16 www.alexjvs.com 2047,-1110 ISSN 10.5455/ajvs.227437. doi: 10.5455/ajvs.227437

PITCOVSKI, J., HELLER, D. E., CAHANER, A., and PELEG, B. A., 1987. Selection for early responsiveness of chicks to Escherichia coli and Newcastle disease virus. Poultry Science 66:1276-1282.

QUINN, P.J., MARKEY, B.K.F., LEONARD, C., HARTIGAN, P., FANNING, S., and FITZPATRICK, E.S., 2011. A Text book of Veterinary Microbiology and Microbial Disease. Wiley-Blackwell Publishers. pp. 123-146

SCHROEDER, C. M., NAUGLE, A. L., SCHLOSSER, W. D., HOGUE, A. T., ANGULO, F. J., ROSE, J.S., EBEL, E. D., DISNEY, W. T., HOLT, K. G., and GOLDMAN, D. P., 2000. Estimate of Illnesses from *Salmonella* Enteritidis in Eggs, United States, Emerg. Infect. Dis. 11, 113-5. doi: 10.3201/eid1101.040401.

SIEMON, C. E., BAHNSON P. B., and GEBREYES W. A., 2007. Comparative Investigation of Prevalence and Antimicrobial Resistance of Salmonella between Pasture and Conventionally Reared Poultry. Avian. Dis. 51(1): 112–117.

SIMONSEN, J. W. TAPSALL, B. ALLEGRANZI, E. A. TALBOT, A., and LAZZARI, S., 2004. The antimicrobial resistance containment and sur_veillance approach—a public health tool,‖ Bulletin of the World Health Organization,**12:** 21-28. PMID: 15654407 PMCID: PMC2623104.

SCIENTIFIC, C. 2011. Review of Staphylococcal Food Poisoning in Hong Kong. Scientific Committee on Enteric Infections and Foodborne Diseases, Department of Heal. Hong Kong. International Journal of Food Microbiology, **1***:* 1-1.

STECHER B., and HARDT W. D., 2008. The role of microbiota in infectious disease. Trends in Microbiology 16 (3):107-14.

THE WORLD FACTBOOK, 2023. Available at https://www.cia.gov/the-world-factbook/countries/somalia/ accessed on March 8, 2023

THRUSFIELD, M. 2005. *V*eterinary Epidemiology. 3rded. *Blackwell science Ltd, London, **Pp***. 228246.

YOUCEF M., MARIE-PIERRE L., MARIE-LOU G., YOUNES C., GAYATRI S., TAREK R., SATINDER K. B., CAROLINE C., ANTONIO A. R., and STÉPHANE G., 2018. Use of antibiotics in broiler production: Global impacts and alternatives, Animal Nutrition, Volume 4, Issue 2, Pages 170-178, https://doi.org/10.1016/j.aninu.2018.03.002. doi: 10.1016/j.aninu.2018.03.002.

ZANELLA, A., ALBORALI, G. L., BARDOTTI, M., CANDOTTI, P., GUADAGNINI, P. F., ALLEN, M., and PARK, S. M., 2000. Severe *Escherichia coli* O111 septicaemia and polyserositis in hens at the start of lay. Avian Pathology, 29:311–317 https://doi.org/10.1080/03079450050118430

ZHAO, J. J., and MAURER, S.H., 2005. Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. *Veterinary Microbiology*, 107(**3-4**): 215-224 https://doi.org/10.1016/j.vetmic.2005.01.021

ZHAO, K. BLICKENSTAFF, S. BODEIS-JONES, S. A. GAINES, E., and TONG, P. F., 2012. Comparison of the prevalences and antimicrobial resistances of *Escherichia coli* isolates from different retail meats in the United States, 2002 to 2008 Applied and Environmental Microbiology, Avian Pathology, **21***:*11–17. doi: 10.1128/AEM.07522-11

ZHAO, S., WHITE, D. G., FRIEDMAN, S. L., GLENN, A., BLICKENSTAFF, K., AYERS, S. L, ABBOTT J. W., HALL–ROBINSON E., and MCDERMOTT, P. F., 2008. Antimicrobial resistance in *Salmonella enterica* serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. Appl. Environment. Microbiol. 74(21): 6656–62. https://doi.org/10.1128/aem.01249-08

How to cite this article:

Hibaq Yusuf Cige, Oladipo Olufemi Omotosho and Abdalla Mohamed Ibrahim, 2023. Evaluation of Antibiotic Resistance Pattern of *Escherichia Coli* and *Salmonella* Species Isolated from Cloaca of Indigenous Chickens in Live-bird Markets in Marodi Jeh Region, Somalia. Journal of Applied Veterinary Sciences, 8 (2): 44 - 54.

DOI:**https://dx.doi.org/10.21608/javs.2023.179843.1200**