



Research Article

Isolation, Identification and Antimicrobial Sensitivity Profiling of *Escherichia coli* and *Salmonella* Spp. from Dead Broiler in Local Market of Dhaka City

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Abstract

Background and Objective: Broiler is an important meat type chicken in Bangladesh. Different bacterial infections affect broiler chicken farming and farmers use antibiotics to protect the birds, as a result antimicrobial resistance in broiler chicken increased day by day. This study was conducted to isolate *E. coli* and *Salmonella* spp. strains from broiler for assessing their susceptibility and resistance patterns to some selected antimicrobials. **Materials and Methods:** This study was carried out in Sher-e-Bangla Agricultural University, Dhaka for isolation, identification and antibiotic sensitivity profiling of *Escherichia coli* and *Salmonella* spp. from dead broiler in local market of Dhaka city. A total of 150 liver and heart samples were collected from dead broilers and cultured in different media for isolation of *Escherichia coli* and *Salmonella* spp. Confirmatory diagnosis is done by biochemical tests. **Results:** The prevalence of *E. coli* and *Salmonella* spp. in liver samples was 45.33% and 40% and in heart samples it was 44% and 38.67% respectively. The overall prevalence of *E. coli* and *Salmonella* spp. in broilers was 44.67% and 39.33%. Antibiotic sensitivity profiling of the isolated *E. coli* and *Salmonella* spp. was performed by the disc diffusion method against 10 commonly used antibiotics. The highest rate of sensitivity against *E. coli* was found with Ceftriaxone (52.24%) followed by Gentamicin (49.25%) and Streptomycin (44.78%). The highest rate of resistance was recorded in Tetracycline (52.24%) followed by Ampicillin (50.75%), Cefuroxime (47.76%), Amoxicillin (46.27%) and Co-Trimoxazole (46.27%). The highest rate of antibiotic sensitivity against *Salmonella* spp. was found with Ceftriaxone (52.54%) followed by Gentamicin (50.85%) and Streptomycin (47.46%). The highest rate of resistance of *Salmonella* was recorded in Cefuroxime (54.24%) followed by Ampicillin (52.54%), Cefixime (50.85%), Tetracycline (49.15%), Amoxicillin (47.46%) and Co-Trimoxazole (45.76%). **Conclusion:** Based on the present study, antimicrobial resistance against *E. coli* and *Salmonella* spp. increased day by day. From this study, it may be concluded that Ceftriaxone is highly sensitive to *E. coli* and *Salmonella* spp. which showed the best result followed by Gentamicin and Streptomycin in the present study.

Key words: Antimicrobial resistance, antimicrobial sensitivity, *E. coli*, poultry production, *Salmonella* spp.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In Bangladesh, the poultry industry contributes significantly to economic growth and creates numerous employment opportunities for the rural community. Bangladesh is one of the key players in the south Asian livestock industry, especially in poultry industry¹. According to BBS-2023², the average daily poultry meat consumption per capita household was 40 g. Broiler is an important poultry species within the poultry industry and their population currently stands at 525 million birds. It is the most widely produced and consumed poultry variety³. Poultry farming in Bangladesh plays a significant role in improving agricultural growth and reducing malnutrition⁴. Approximately 20% of the protein consumed in developing countries comes from poultry meat and eggs⁵. Livestock and poultry is an integral part of farming system in Bangladesh and has created direct, indirect employment opportunity. More than 10 million people of Bangladesh directly depend on these sectors for their livelihoods⁶. But it is true to say that this profitable sub-sector is seriously interrupted by a number of infectious and contagious diseases such as Newcastle Disease (ND), Infectious Bursal Disease (IBD), Salmonellosis, Fowl cholera, Infectious Coryza, Chronic respiratory disease, Aspergillosis, Coccidiosis, Helminthiasis etc. Poultry sector in Bangladesh is now facing a major challenge due to these harmful diseases⁷. Diseases can be categorized by common causes, such as genetic, mechanical, toxic and nutritional. Infectious diseases are caused by viruses, bacteria and fungi. Parasitic diseases are caused by protozoa, worms and external parasites such as mites and lice⁸. Important bacterial diseases of poultry in Bangladesh are pullorum disease, colibacillosis and fowl cholera⁹ which are responsible for a high percentage of morbidity and mortality. *Escherichia coli* are Gram-negative bacteria, normal inhabitants of the intestinal tract of birds. Pathogenic strains can cause diseases such as air sac disease, salpingitis, omphalitis etc. alone or in combination with other pathogenic agents (viruses, Mycoplasma)^{10,11}. Salmonellosis is one of the most important bacterial diseases in poultry causing heavy economic losses through mortality and reduced meat and egg production¹². There are mainly two types of non-motile avian *Salmonella* spp. namely, *Salmonella gallinarum* and *Salmonella pullorum*, which are responsible for Fowl Typhoid (FT) and Pullorum Disease (PD) of poultry respectively. Antibiotics have been used successfully in poultry for different purposes such as growth promotion, prophylaxis, or therapeutics¹³. However, their use in animal production and human therapy has resulted in an increase in bacterial resistance¹⁴. The presence of multidrug resistance to antimicrobial agents complicates the management of intrainestinal and extraintestinal infections caused by *E. coli*

and *Salmonella* spp. which are major causes of illness, death and increased healthcare costs¹⁵. There are several reports on resistance of *E. coli* to several antibiotics such as tetracycline, nalidixic acid, cefuroxime, chloramphenicol, gentamicin, ampicillin, kanamycin, trimethoprim/sulfamethoxazole, etc.¹⁶⁻¹⁸. Several studies have reported the prevalence of multi-drug resistance genes in different serotypes of *Salmonella*^{19,20}. Since bacteria acquire most resistance genes through horizontal transfer, conjugative genetic elements such as plasmids and transposons are common vectors for the dissemination of antimicrobial resistance genes to the diverse microorganisms. Therefore, the present study was designed to isolate *E. coli* and *Salmonella* spp. strains from broiler for assessing their susceptibility and resistance patterns to some commonly used antimicrobials.

MATERIALS AND METHODS

Experimental design: The entire study was divided into two major steps: The first step included a selection of sources, collection of samples, isolation, identification and characterization of microorganisms based on their colony morphology, staining properties, motility and biochemical characteristics and molecular identification. After this, 150 sample (liver-75 and heart-75) were collected from 75 dead broiler. Then 126 bacterial sample were identified by their cultural characteristics and biochemical properties. In the second step, 126 isolates of microorganisms isolated from broilers were tested for drug sensitivity and resistance.

Study area and duration: The study was conducted on the dead broiler from July to December 2020 in Dhaka city, Bangladesh which is one of the most concentrated poultry areas of Bangladesh.

Study population: Postmortems were performed on 150 dead samples collected from different local markets in Dhaka at the lab of Medicine and Public Health, SAU and Dhaka.

Sample collection and preservation: A total number of 75 dead broilers (5 from each area) were collected from 15 local markets of (Kawran Bazar, Shaymoli, Adabor, Mohammadpur Town Hall, Dhanmondi, Agargaon, Mirpur-1, Mirpur-10, Gabtali, Jatrabari, Sadarghat, Hemayetpur, Amin Bazar, Savar, Ashulia) Dhaka city, Bangladesh.

Post-mortem examination and sample collection for microbiological test: The post-mortem examinations were performed using the standard operation procedure described by the Atlas of Avian Necropsy²¹. The post mortem sign of airsacculitis, fibrinous coat was the sign of *E. coli* infection

where pin point hemorrhage, necrotic foci, pericarditis, perihepatitis was the sign of *Salmonella* infection. According to this sign liver and heart samples were collected aseptically and used for microbiological tests. A total of 150 samples (75 liver and 75 heart) were collected from dead broiler and were transferred to the Medicine and Public Health Laboratory, SAU via a cool-chain maintained in a cool box and stored at -20°C for further analysis.

Isolation and identification of collected samples: Isolation and identification of bacteria were done by using cultural and biochemical properties described by Perrin *et al.*²². Primary culture of bacteria was done in nutrient broth. After primary culture, bacterial inoculums from nutrient broth were streaked on the MacConkey agar to observe the colony morphology of the isolates. Then, *E. coli* suspected samples were sub-cultured on EMB agar and *Salmonella* suspected samples were sub-cultured on *Salmonella*-Shigella agar. Several biochemical tests were performed for confirmation of *E. coli* and *Salmonella* isolates. *E. coli* fermented the carbohydrate, the medium changes from red-orange to yellow. While *Salmonella* spp. change color from pink to yellow and in both cases gas bubbles will appear in the tubes. In Catalase test both bacteria produced foam. In methyl red test, *E. coli* produced red color where in *Salmonella* broth turned to bright red color. Both bacteria formed a red ring on the surface of the tube in the Indole test. In the Voges-Proskauer test, *E. coli* showed a positive reaction, while *Salmonella* showed a negative reaction.

Antimicrobial discs: Commercially available antimicrobial discs (OXOID Limited, Canada) were used to test the drug sensitivity and resistance pattern and to interpret their disease potential. The antimicrobial agents GEN-10 (Gentamicin), S-10 (Streptomycin), TE-30 (Tetracycline), CXM-30 (Cefuroxime), CFM-5 (Cefixime), CTR-30 (Ceftriaxone), CL-10 (Colistin), AMX-30 (Amoxicillin), AMP-25 (Ampicillin), COT-25 (Co-Trimoxazole) were used to test the sensitivity and resistance pattern of the selected *E. coli* and *Salmonella* spp. isolated from liver and heart of broiler broiler following standard AST protocol¹⁸. Enterobacteriaceae drugs and their Zone Diameter Interpretive Standard (mm) were counted through The Clinical and Laboratory Standards Institute (CLSI)²³ (Table 1).

RESULTS

Identification of *E. coli* and *Salmonella* spp. on Post-Mortem (PM) findings: According to post-mortem results, 67 samples were confirmed as *E. coli* positive and the prevalence rate was 44.67% (Table 2). Among them 44 (34.33%) samples were identified by observing the sign of airsacculitis whereas

23 (44.07%) samples were identified by observing the sign of fibrinous coat which were significant at 1% level. According to post-mortem results, 59 samples were confirmed as *Salmonella* positive and the prevalence rate was 39.33% (Table 2). Among them 26 (44.07%) samples were identified by observing the sign of pinpoint hemorrhage, 14 (23.73%) samples were identified by observing the sign of necrotic foci, 11 (18.64%) samples were identified by observing the sign of pericarditis and 8 (13.56%) samples were identified by observing the sign of peri-hepatitis which were also significant at 1% level (Fig. 1).

Identification of microorganism by cultural test: There were 150 broiler samples, the isolates of *E. coli* and *Salmonella* were determined by isolating liver samples from 75 broiler samples, the isolates of *E. coli* were 34 (45.33%) and the isolates of *Salmonella* were 30 (40%) (Table 3). Similarly, from 150 broiler samples, the isolates of *E. coli* and *Salmonella* were determined by isolating heart samples from 75 broiler samples, where the isolates of *E. coli* were 33 (44%) and isolates of *Salmonella* were 29 (38.67%) (Table 3).

Prevalence of antimicrobial resistance patterns against *E. coli* isolates: A large number of *E. coli* isolates from broiler samples were found to be sensitive to ceftriaxone (52.24%), gentamicin (49.25%) and streptomycin (44.78%) (Fig. 3). A little number was sensitive to cefuroxime, amoxicillin (20.90%), cefixime, co-trimoxazole (17.91%) and ampicillin (14.93%). The highest resistance of *E. coli* was against tetracycline (50.75%). They showed comparatively higher resistance against ampicillin (50.75%), cefuroxime (47.76%), amoxicillin and co-trimoxazole (46.27%). The lowest resistance of *E. coli* was against ceftriaxone (17.91%). They showed lower resistance against gentamicin (19.40%), streptomycin (25.37%) and colistin (22.39%). Most of the isolates showed intermediate sensitivity against colistin (47.76%). Against gentamicin and cefuroxime, they showed equal intermediate sensitivity (31.34%) and streptomycin, tetracycline, cefixime and ceftriaxone (29.85%) also showed equal intermediate sensitivity which was lowest (Fig. 2,3).

Prevalence of antimicrobial resistance pattern against *Salmonella* isolates: A total of 59 different *Salmonella* isolates were collected from 150 dead broiler (liver and heart). A large number of *Salmonella* isolates from broiler samples were found sensitive to ceftriaxone (52.54%), gentamicin (50.85%) and streptomycin (47.46%) (Fig. 4). A small number of *Salmonella* isolates were sensitive to cefuroxime (20.34%). The highest resistance was against cefuroxime (54.24%) followed by ampicillin (52.54%), cefixime (50.85%), tetracycline (49.15%), amoxicillin (47.46%) and co-trimoxazole (45.76%). Comparatively lower resistances were found against streptomycin (25.42%), colistin (25.42%), gentamicin (23.73%)

Table 1: Drugs with their disc concentration for the Enterobacteriaceae family

Antibiotics	Disc concentration (µg/disc)	Zone Diameter Interpretive Standard (mm)		
		Resistance (%)	Intermediate (%)	Sensitive (%)
GEN-10	10	≤12	13-14	≥15
S-10	10	≤11	12-14	≥15
TE-30	30	≤11	12-14	≥15
CXM-30	30	≤14	15-17	≥18
CFM-5	5	≤14	15-17	≥18
CTR-30	30	≤19	20-22	≥23
CL-10	10	≤11	12-14	≥15
AMX-30	30	≤13	14-17	≥18
AMP-25	25	≤13	14-16	≥17
COT-25	25	≤10	11-15	≥16

Source: CLSI= The Clinical and Laboratory Standards Institute²³

Table 2: Identification of microorganisms based on post-mortem (PM) results

Name	PM findings	Number	Percent (%)	χ ² value	p-value
<i>E. coli</i>	Airsacculitis	44	34.33	11.90	0.001*
	Fibrinous coat	23	44.07		
	Overall prevalence of <i>E. coli</i>	67	44.67		
<i>Salmonella Spp.</i>	Pinpoint hemorrhage	26	44.07	15.76	0.001*
	Necrotic foci	14	23.73		
	Pericarditis	11	18.64		
	Perihepatitis	08	13.56		
Overall prevalence of <i>Salmonella spp.</i>		59	39.33		

p<0.01 indicates significant at 1% level

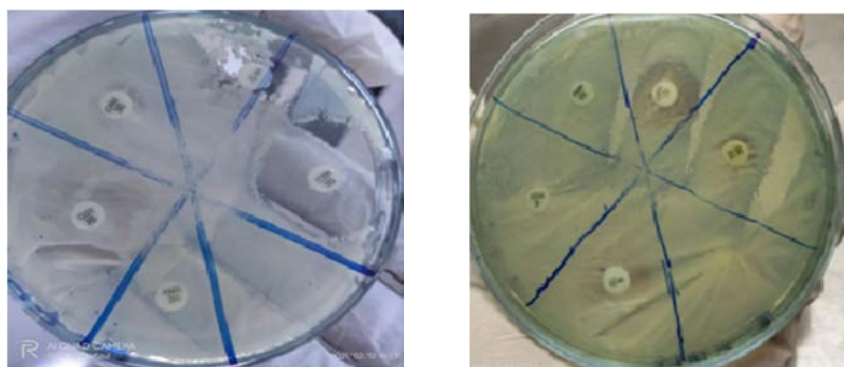


Fig. 1: Antibiotic sensitivity test for *E. coli* organism

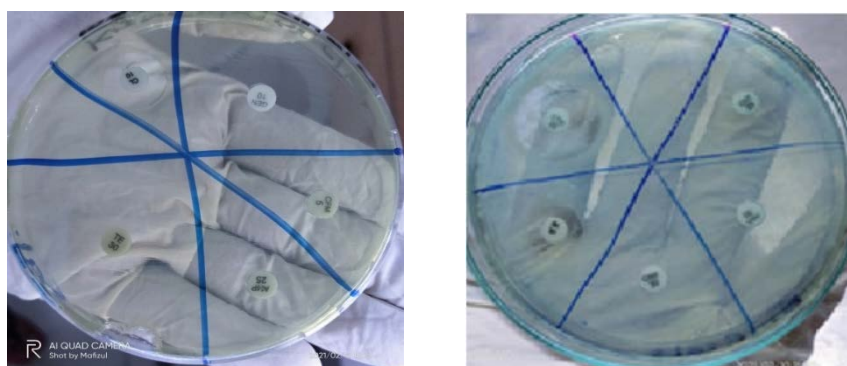


Fig. 2: Antibiotic sensitivity test for *Salmonella* organism

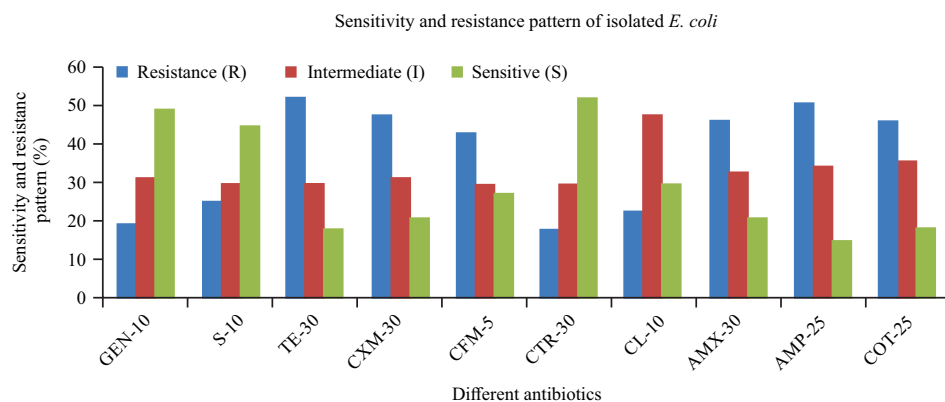
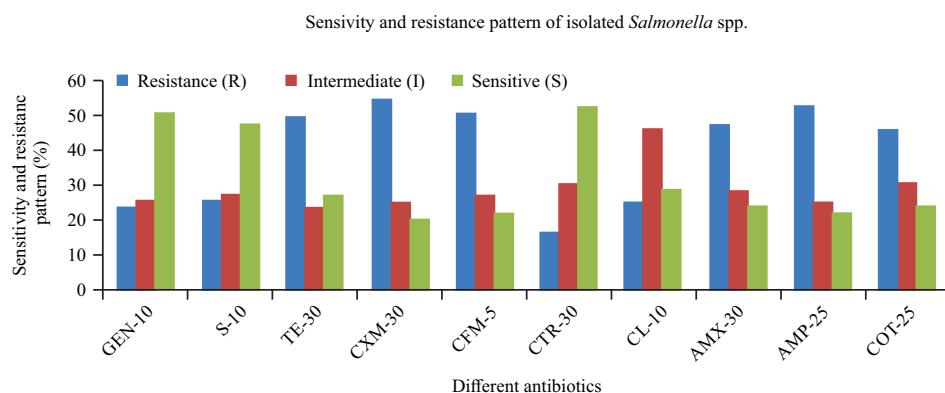
and ceftriaxone (16.95%). Most of the isolates showed intermediate sensitivity against colistin (45.76%) followed by ceftriaxone (30.51%) and co-trimoxazole (30.51%). An

equal intermediate sensitivity (27.12%) was found against streptomycin and cefixime. An equal intermediate sensitivity (25.42%) was also found against gentamicin, cefuroxime

Table 3: Prevalence percentage of *E. coli* and *Salmonella* spp. in liver and heart samples

Total samples	Isolated organism	No. of positive cases	Prevalence (%)	χ^2 value	p-value
Liver sample-75	<i>E. coli</i>	34	45.33	0.44	0.509
	<i>Salmonella</i>	30	40.00		
Heart sample-75	<i>E. coli</i>	33	44.00	0.44	0.507
	<i>Salmonella</i>	29	38.67		

p<0.05 indicates significant at 5% level, p>0.05 indicates the non-significant data

Fig. 3: Prevalence of antimicrobial resistance pattern against *E. coli* isolatesFig. 4: Prevalence of antimicrobial resistance pattern against *Salmonella* isolates

and ampicillin and this was the lowest intermediate sensitivity against *Salmonella* spp. (Fig. 4).

DISCUSSION

The present study was conducted primarily for the isolation and identification of *E. coli* and *Salmonella* spp. isolated from liver and heart samples of dead broilers in Dhaka and also to determine the current status of drug sensitivity and resistance pattern of the isolates to determine the drug of choice for therapeutic use against infection caused by these organisms. Colony characteristics of *E. coli* in five different agar media and fermentation ability with five basic sugars were similar with a bit of exception. Interesting findings of the colony characteristics of the isolates were also observed. All the *E. coli* isolates were able to produce greenish metallic sheen colonies on the EMB agar, bright pink colonies on

MacConkey agar and yellowish green colonies surrounded by an intense yellow-green zone on BG agar. All the *Salmonella* isolates were able to produce grey color colonies in EMB agar, red to pink-white colonies surrounded by brilliant red zones in MacConkey agar, pink color colonies in BG agar, Colonies with black centers in SS agar and whitish color colonies in Nutrient agar.

A red colour indication in methylene red test was similar to the findings of Mousum *et al.*²⁴. Another fundamental element in identifying *E. coli* and *Salmonella* organisms was the ability or inability of five basic sugars to ferment with acid and gas production. However, differentiation of *Salmonella* species was difficult based on their sugar fermentation pattern. In this study, all the *Salmonella* isolates fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment sucrose and lactose. This result agrees with the findings of Han *et al.*²⁵. In the present study, the

isolated *E. coli* organisms fermented dextrose, maltose, lactose, sucrose and mannitol with the production of both acid and gas. *E. coli* produced less acid and gas during sucrose fermentation. Among 150 samples, 67 samples were infected with *E. coli*, 59 samples were infected with *Salmonella* and rest 24 samples were unidentified. The infection with *E. coli* was 44.67%. Infection with *E. coli* for liver and heart sample was respectively 45.33% and 44%. Similar results were found by Hossain *et al.*²⁶ who reported that the prevalence was 63.6%, whereas Jakaria *et al.*²⁷ found 82% prevalence and Bashar *et al.*²⁸ found 100% prevalence of *E. coli* in poultry. The prevalence of *Salmonella* in broiler was 39.33% and Infection with *Salmonella* for liver and heart sample was respectively 40% and 38%. This finding is supported by Cardinale *et al.*,²⁹ who observed that the prevalence of *Salmonella* was 30% in broiler. Generally low prevalence of *Salmonella* was found in poultry as reported by Nhung *et al.*³⁰, Jajere *et al.*³¹ and Geidam *et al.*³². In order to Investigate Antimicrobial Resistance (AMR), 67 *E. coli* isolates were tested for antimicrobial sensitivity in 150 broiler (liver+heart) samples using disc diffusion method. The sensitivity test revealed that most of the *E. coli* isolates, from broiler liver and heart samples were sensitive to ceftriaxone followed by gentamicin and streptomycin. In terms of resistance, most of the isolates were resistant to tetracycline and ampicillin followed by cefuroxime, amoxicillin, co-trimoxazole and cefixime. In the present study a high percentage of *E. coli* isolates, were sensitive to ceftriaxone (52.24%) and gentamicin (49.25%) followed by streptomycin showing 44.78% sensitivity. On the other hand, 52.24% of the isolates were resistant to tetracycline and 50.75% to ampicillin followed by cefuroxime (47.76%), amoxicillin (46.27%), co-trimoxazole (46.27%) and cefixime (43.28%). A similar result was reported by Azad *et al.*³³, who observed 100% resistance in *E. coli* isolates to ampicillin, tetracycline and trimethoprim-sulfamethoxazole isolated from broiler colocal swab samples in Rajshahi area, Bangladesh. Sarker *et al.*³⁴ revealed that 56.76% were sensitive to ceftriaxone and gentamicin and 43.24% were sensitive to erythromycin, while Azad *et al.*³³ reported that 36% were sensitive to gentamicin and 100% were sensitive to erythromycin. *Escherichia coli* was sensitive to amoxicillin (36.4%), ampicillin (36.4%), tetracycline (54.6%), streptomycin and co-trimoxazole (81.8%), gentamicin and ceftriaxone (90.9%), cefuroxime and cefixime (100%) in response to dairy cow³⁶. In contrast to this study, Zhang *et al.*,³⁵ reported high rate of resistance toward gentamicin (95%), amikacin (46%). In this study a number of isolates also showed intermediate reaction to colistin (47.76%) followed by co-trimoxazole (35.82%), ampicillin (34.33%), amoxicillin (32.84%), gentamicin and cefuroxime (31.34%), streptomycin, tetracycline, cefixime and ceftriaxone (29.85%). Intermediate Sensitivity drugs could not be compared due to lack of relevant literature.

From 150 broiler (liver+heart) samples, 59 *Salmonella* isolates were subjected to antimicrobial sensitivity test which was carried out by disc diffusion method. Ten different drugs were used for this study. The sensitivity test revealed that most of the *Salmonella* isolates, from broiler liver and heart samples were sensitive to ceftriaxone followed by gentamicin and streptomycin. In terms of resistance, most of the isolates were resistant to cefuroxime, ampicillin and cefixime followed by tetracycline, amoxicillin and co-trimoxazole. In the present study a high percentage of *Salmonella* isolates, from broiler samples were sensitive to ceftriaxone (52.54%) followed by gentamicin and streptomycin showing 50.85% and 47.46% sensitivity respectively. On the other hand, 54.24, 52.54 and 50.85% of the isolates were resistant to cefuroxime, ampicillin and cefixime, respectively followed by tetracycline (49.15%), amoxicillin (47.46%) and co-trimoxazole (45.76%). In a previous study, Saifullah *et al.*³⁶ revealed multi-drug resistance of *Salmonella* where the highest resistance was found against Ampicillin (88.23%) followed by Cephalexin (82.35%). The rate of sensitivity of the isolates was the highest to Ciprofloxacin (100%) followed by Azithromycin (82.35%) Gentamicin (76.47%) and Nalidixic acid (76.47%). In this section, the variation was found in the sensitivity pattern of *Salmonella* isolates against cefixime, gentamicin and streptomycin. A number of isolates also showed intermediate reaction to colistin (45.76%), ceftriaxone and co-trimoxazole (30.51%), amoxicillin (28.81%), streptomycin and cefixime (27.12%), gentamicin, cefuroxime and ampicillin (25.42%) and tetracycline (23.73%). Arora *et al.*,³⁷ reported similar results in India and observed 18% resistance against amoxicillin-clavulanic acid, 18% was found against ampicillin, 20% was found against cefotaxime. *Salmonella* spp. is resistant to antimicrobial drugs differently depending on their usage in animal production and humans, as well as on ecological differences in the epidemiology of *Salmonella* spp. infections³⁸. The significance of occurrence of antibiotic resistance in food-borne pathogens have been increased sharply and probably linked with the extensive use of antimicrobial agents in veterinary medicine and human³⁹. Several species of *Salmonella* are known to carry multi drug resistant genes⁴⁰ which have been a matter of concern.

CONCLUSION

A high percentage of *E. coli* isolates from the broiler were sensitive to ceftriaxone, gentamicin and streptomycin followed by colistin and cefixime while most of the *E. coli* isolates were resistant to tetracycline, ampicillin, ceftriaxone, amoxicillin and co-trimoxazole. In the case of *Salmonella* isolates, good sensitivity was found against ceftriaxone followed by gentamicin and streptomycin while most of the *Salmonella* spp. isolates were resistant to ceftriaxone,

ampicillin, cefixime, tetracycline and amoxicillin. It is assumed that one or more drug-resistant clones have gradually acquired resistance to other drugs by conjugation with multi-drug-resistant strains. Based on the present study, it may be concluded that ceftriaxone, gentamicin and streptomycin will be the first drugs of choice. Colistin and cefixime will be the second drugs of choice to resist the infections caused by *E. coli* and *Salmonella* in broiler and as well as human, cattle, sheep, goat, chicken and duck. Based on the results of the present study, it is recommended that indiscriminate use of antimicrobial agents should be avoided to eliminate health hazards in man and animals caused by *E. coli* and *Salmonella* spp. through preventing the development of multi-drug-resistant mutants in nature.

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