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Prevalence and Antimicrobial Resistance Profile of *E. coli* and *Salmonella* spp. from Liver and Heart of Chickens

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ABSTRACT

E. coli and *Salmonella* spp. are responsible for causing colibacillosis and salmonellosis in chickens respectively. This research work was undertaken to study the prevalence of colibacillosis and salmonellosis in commercial chickens of Chattogram, and to know the antibiogram profiles of the isolated bacteria. A well-structured questionnaire was used to know the prevalence of colibacillosis and salmonellosis. Previously collected liver and heart samples through postmortem of a total of 100 dead and sick chickens were used. MacConkey agar, EMB agar, and XLD agar were used to isolate, and identify *E. coli* and *Salmonella* spp. Finally, Gram's staining and different biochemical tests were performed to identify these two bacterial isolates. 14 different commercially available antimicrobial discs like ciprofloxacin, enrofloxacin, streptomycin, colistin sulphate, neomycin, cefoxitin, amoxycillin, tetracycline, gentamicin, norfloxacin, azithromycin, doxycycline, cloxacillin, and erythromycin were used. Data were analyzed with *p*-value by using Graph Pad Software. 48 samples were recorded as positive for *E. coli* and 5 for *Salmonella* spp. The prevalence of *E. coli* and *Salmonella* spp. were varied depending on different parameters like age, bird rearing system, farm size, source of water, source of food, medication and vaccination. Form antibiogram study it was revealed that *E. coli* was highly sensitive to colistin sulphate and ciprofloxacin; intermediate to gentamicin followed by cefoxitin and resistant to other 10 antimicrobials. In case of *Salmonella* spp., it was recorded as sensitive to colistin sulphate, and cefoxitin; intermediate to ciprofloxacin, and resistant to other 11 antimicrobials. The findings of this research work would certainly help the poultry farmers to select proper antibiotics against colibacillosis and salmonellosis in chickens of Bangladesh and to overcome the multi-drug resistant problem of the bacteria.

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Introduction

During the last two decades in Bangladesh, poultry farming is emerging as a strong agro-based industry from the backyard poultry rearing system to commercial intensive rearing systems (Uddin et al., 2010). Poultry population in Bangladesh is estimated about 3658.52 lakh where chickens, and ducks are about 3041.06 lakh, and 617.46 lakh respectively (DLS, 2021). The requirement of meat and eggs of the country is although not being met but the contribution of poultry meat to the total meat products is 37% (DLS, 2021) and egg production is 63.65% (Hamid et al., 2017) of the national need. The poultry sector contributes 22-27% to the total animal protein supply, and 1.4% to the country's GDP (DLS, 2021).

In Bangladesh, development of poultry sector is being hampered by a number of factors like diseases, poor husbandry, low productivity and shortage of feed. Among these factors, diseases caused by bacteria, virus, protozoa, fungus, mycoplasma, chlamydia and rickettsia are considered as the major factor causing 30% mortality of chicken per year (Das et al., 2005). In Bangladesh, pullorum disease (PD), fowl cholera (FC) and colibacillosis are important bacterial diseases which are responsible for causing high percentage of morbidity and mortality in poultry (Samad et al., 2000).

In birds, *E. coli* is responsible for causing colibacillosis which is considered as one of the principal causes of

morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various disease conditions either as primary pathogen or as a secondary pathogen (Kabir et al., 2010). It is a gram negative, rod shaped, motile, capsulated, flagellated, oxidase negative, lactose fermenter, non-acid fast, non-spore forming facultative anaerobe (Sohidullah et al., 2016; Begum et al., 2016). Young chickens up to three weeks of age are highly susceptible to this disease, but chickens of four weeks and older are considered quite resistant to primary colibacillosis (Goren et al., 1978). It may cause about 28% death in Sonali birds of Bangladesh (Biswas et al., 2006).

Salmonellosis is one of the most important bacterial diseases in poultry responsible for causing heavy economic losses through mortality and it can also reduce meat and egg production (Haider et al., 2004). Avian salmonella infection occurs in poultry either as acute or chronic form by one or more member(s) of the genus *Salmonella*, under the family enterobacteriaceae (Hofstad et al., 1984). There are mainly two types of non-motile avian *Salmonella* spp. namely *Salmonella gallinarum* and *Salmonella pullorum* which are short non-flagellated, non-spore forming, non-capsulated, gram negative plump rods (Sohidullah et al., 2016) are responsible for causing fowl typhoid (FT) and pullorum disease (PD) in poultry respectively. Shivaprasad et al. (2000) reported 0-100% mortality in chickens by PD and Christensen *et al*. (1994) reported 10-93% mortality by FT in chickens. Rahman et al. (2004) reported highest age wise prevalence of avian salmonellosis in adult layers (53.25%) in comparison to brooding (14.55%), growing (16.10%) and pullet (16.10%) chickens.

Antibiotic treatment can play a vital role for controlling infectious diseases of poultry. But antimicrobial resistance of bacteria occurs due to bad exercise or especially abuse of antimicrobial agents (Moreno et al., 2000). At the field level in our country, most of the poultry diseases are diagnosed on the basis of symptoms and gross lesions but for confirmation of the diseases, laboratory diagnosis is necessary. Considering the above facts, the current study was undertaken to study the prevalence of common bacterial diseases in commercial chickens of Chattogram districts and to know the antibiogram profiles of the isolated bacteria which will be helpful towards the management of poultry diseases in Bangladesh.

Materials and Methods

Study Period, Place and Sample

The study was conducted from February to July 2018 at the Department of Pathology and Parasitology, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram. Liver and heart samples were used that we previously collected (Halder et al., 2021) through postmortem of a total of 100 dead and sick chickens of various farms of Chattogram, Bangladesh.

Questionnaire for Overall Assessment

To know the prevalence of colibacillosis and salmonellosis on the basis of different factors, we collected related information by using our previously designed well-structured questionnaire (Halder et al., 2021).

Isolation and identification of E. coli, and Salmonella spp.

Sterilized cotton swab was inserted into the liver and heart after shearing. Then it was inoculated into falcon tube containing nutrient broth and selenite broth for *E. coli* and *Salmonella* spp. respectively, and incubated at 37°C temperature for 24 hours. Then the broth samples were sub-cultured on MacConkey agar and Xylose Lysine Deoxycholate (XLD) agar for isolation of *E. coli* and *Salmonella* spp. respectively, and incubated for another 24 hours at 37°C. After then the culture positive samples were again sub-cultured on Eosin Methylene Blue (EMB) agar for *E. coli*, and incubated for 24 hours at 37°C. All these things were done according to the method described by Sohidullah et al. (2016) and Begum et al. (2016), and the media were brought from HiMedia Laboratory Pvt. Limited, Mumbai-4000, 86, India. The representative bacterial colonies were characterized morphologically by using Gram's stain according to the method described by Sohidullah et al. (2016) and Begum et al. (2016). All of the reagents like crystal violet, Gram's iodine, safranin, acetone alcohol, immersion oil was brought from the German company, Merck. These two isolates were characterized biochemically by indole test, and Triple Sugar Iron (TSI) agar test for *E. coli* and *Salmonella* spp. respectively, and tests were performed according to the method described by Cheesbrough, 1985.

Antimicrobial Susceptibility

Antibiogram susceptibility test was performed by employing the Kirby-Bauer disc diffusion method (Bauer et al., 1966) using 14 different commercially available antimicrobial discs (HiMedia, India and Oxoid Ltd., England) on Mueller Hinton agar (HiMedia, India) to assess the susceptibility and resistance pattern of the isolates. The selected antimicrobials used were Ciprofloxacin (CIP) (5 µg/disc), Enrofloxacin (ENR) (5 µg/disc), Streptomycin (S) (10 µg/disc), Colistin Sulphate (CT) (10 µg/disc), Neomycin (N) (10 µg/disc), Cefoxitin (CX) (30 µg/disc), Amoxycillin (AML) (10 µg/disc), Tetracycline (TE) (30 µg/disc), Gentamicin (CN) (10 µg/disc), Norfloxacin (NOR) (10 µg/disc), Azithromycin (AZM) (15 µg/disc), Doxycycline (DO) (30 µg/disc), Cloxacillin (OB) (5 µg/disc), and Erythromycin (E) (15 µg/disc). The interpretation on susceptibility was done according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2012).

Statistical Analysis

Data were entered into a database (Spreadsheet of Microsoft Excel) and then those were analyzed with *p*-value by using Graph Pad Software.

Results and Discussion

The large pink colored colonies were suspected for *E. coli* on MacConkey agar. Then after sub-culturing, the growth having characteristic metallic sheen on EMB agar was considered as *E. coli* positive. Black centered colonies on XLD agar were considered as positive for *Salmonella* spp. In case of Gram's staining, *E. coli* showed gram negative, pink colored, short plump rod-shaped appearance arranged as single, paired or short chain under microscope where *Salmonella* spp. revealed gram negative, pink

colored, very short plump rod-shaped appearance arranged as single or paired under microscope. In case of indole test for identifying *E. coli*, production of red color in the reagent layer indicated *E. coli* positive. In case of TSI agar test for the identification of *Salmonella* spp., alkaline reaction with red color of the medium was found in the slant, but acidic reaction with yellowing of the medium was found in the butt. In that case, gas was found. During culture, we recorded 48% *E. coli* (Table 1) which was higher than the findings of Hasan et al. (2010) who found 32% colibacillosis in commercial chickens. On the basis of cultural properties, we recorded 5% prevalence of salmonellosis (Table 2) that was 3 times lower than the result of the Hasan et al. (2010) who found 13.64% positive in their study.

The prevalence percentages of colibacillosis, and salmonellosis were varied proportionally according to age, bird rearing system, farm size, source of water, source of food, medication and vaccination (Tables 1 and 2). Out of 100 chickens, colibacillosis was found in 48 birds (Table 1), and salmonellosis was found in 5 birds (Table 2). We failed to compare our findings regarding colibacillosis, and salmonellosis, with other works of similar nature due to lack of research articles related with some of the risk factors like production type, litter type, age, farm size, source of water and type of feed and vaccination (Table 1, and 2).

Table 1. Association of different variables with samples that were positive in culture media (*E. coli*):

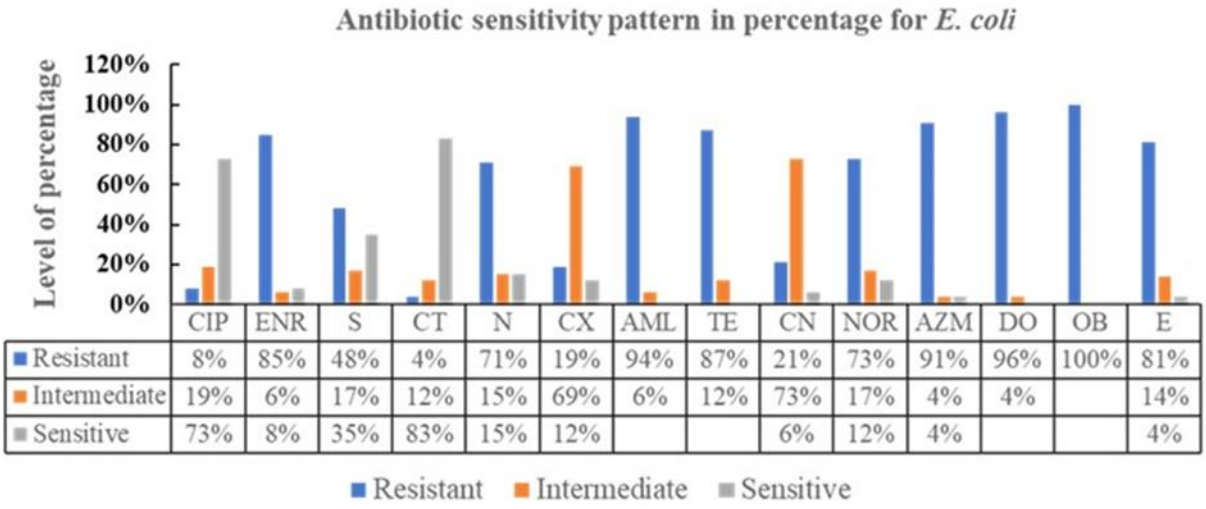
Variables	Category/level	Number of observation	Number of positive	Percentage (%)	P-value (Chi-square test)
Production type	Broiler	67	32	47	0.87
	Layer	26	12	46	
	Sonali	7	4	57	
Age (week)	0 - ≤ 2	5	3	60	0.04**
	>2 - ≤ 4	58	29	50	
	>4	37	16	43	
Farm size	small	22	8	36	0.69
	medium	41	16	39	
	large	37	24	64	
Bird rearing system	Cage	20	8	40	0.47
	Litter	80	40	50	
Vaccination	Yes	84	41	48	0.39
	No	16	7	43	
Type of feed	Handmade	13	5	38	0.46
	Readymade	87	43	49	
Source of water	Tube well	72	29	40	0.56
	WASA	28	19	68	
Previous treatment	Yes	83	40	48	0.62
	No	17	8	47	

Note: *, **, and *** represents 1% (P<0.01), 5% (P<0.05), and 10% (P<0.1) levels of significance; Data were collected through questionnaire

Table 2. Association of different variables with samples that were positive in XLD agar (*Salmonella* spp.):

Variables	Category/level	Number of observation	Number of positive	Percentage (%)	P-value (Chi-square test)
Production type	Layer	26	3	11	0.001***
	Broiler	67	2	3	
	Sonali	7	0	0	
Age (week)	0 - ≤ 2	5	0	0	0.19
	>2 - ≤ 4	58	1	2	
	>4	37	4	11	
Farm size	small	22	0	0	0.05**
	medium	41	2	5	
	large	37	3	8	
Bird rearing system	Cage	20	2	2	0.60
	Litter	80	3	4	
Type of feed	Handmade	13	1	8	0.04**
	Readymade	87	4	5	
Source of water	Tube well	72	1	1	0.54
	WASA	28	4	14	
Previous treatment	Yes	83	5	6	0.07*
	No	17	0	0	
Vaccination	Yes	84	5	6	0.09*
	No	16	0	0	

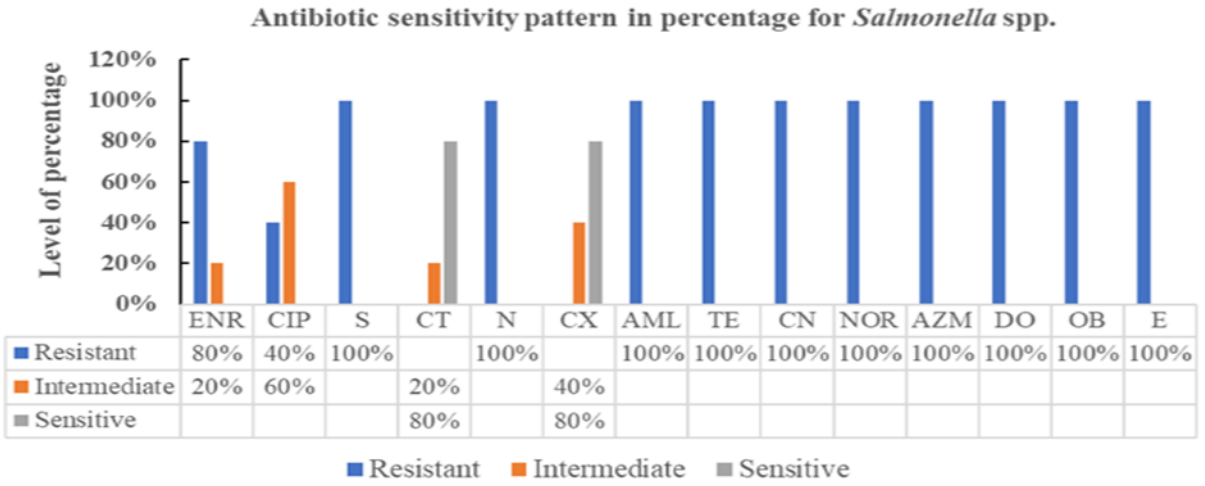
Note: *, **, and *** represents 1% (P<0.01), 5% (P<0.05), and 10% (P<0.1) levels of significance; Data were collected through questionnaire



	CIP	ENR	S	CT	N	CX	AML	TE	CN	NOR	AZM	DO	OB	E
Resistant	8%	85%	48%	4%	71%	19%	94%	87%	21%	73%	91%	96%	100%	81%
Intermediate	19%	6%	17%	12%	15%	69%	6%	12%	73%	17%	4%	4%		14%
Sensitive	73%	8%	35%	83%	15%	12%			6%	12%	4%			4%

Figure 1. Antibiotic sensitivity pattern in percentage for *E. coli*

Ciprofloxacin (CIP), Enrofloxacin (ENR), Streptomycin (S), Colistin Sulphate (CT), Neomycin (N), Cefoxitin (CX), Amoxycillin (AML), Tetracycline (TE), Gentamicin (CN), Norfloxacin (NOR), Azithromycin (AZM), Doxycycline (DO), Cloxacillin (OB), and Erythromycin (E)



	ENR	CIP	S	CT	N	CX	AML	TE	CN	NOR	AZM	DO	OB	E
Resistant	80%	40%	100%		100%		100%	100%	100%	100%	100%	100%	100%	100%
Intermediate	20%	60%		20%		40%								
Sensitive				80%		80%								

Figure 2. Antibiotic sensitivity pattern in percentage for *Salmonella* spp.

Ciprofloxacin (CIP), Enrofloxacin (ENR), Streptomycin (S), Colistin Sulphate (CT), Neomycin (N), Cefoxitin (CX), Amoxycillin (AML), Tetracycline (TE), Gentamicin (CN), Norfloxacin (NOR), Azithromycin (AZM), Doxycycline (DO), Cloxacillin (OB), and Erythromycin (E)

In case of production type, prevalence of colibacillosis was higher in sonali (57%) birds than commercial layer (46%) and commercial broiler (47%) (Table 1). However, Islam et al. (2012) did not find any significant difference in the occurrence of colibacillosis in broiler (28.6%) and layer (27.6%) birds. The highest prevalence of colibacillosis (60%) was found in 0 - ≤ 2 weeks of age groups with the lowest (43%) in >4 weeks of age group of poultry (Table 1). Islam et al. (2012) also reported the prevalence of colibacillosis where the highest number (42.60%), and the lowest number (1.03%) were recorded in the age group of 8-21 days, and 36-60 days of poultry respectively.

The highest prevalence of salmonellosis (11%) was recorded in >4 weeks of age group and the lowest prevalence (0%) was recorded in age group of 0-≤2 weeks of poultry (Table 2). These findings were correlated with the findings of Mahmud et al. (2011), who reported salmonellosis to occur in 30-35 weeks onward in chicken, and also related with the findings of Hossain et al. (2010), where salmonellosis was reported to occur as the highest level in adult compared to young chicken. The prevalence of salmonellosis was recorded as the highest level (8%) in case of handmade feed supply, and the lowest level (5%) in case of readymade feed supply (Table 2), and the relationship was statistically significant (P<0.05).

A total number of 53 samples were subjected to sensitivity testing to a panel of 14 antimicrobials by Kirby-Bauer disk diffusion method (Figure 3 and 4). The activity of antimicrobials was expressed as "sensitive", "intermediate" and "resistant". During this study, 48 positive samples for *E. coli* and 5 positive samples for *Salmonella* spp. were subjected to antibiotic sensitivity test, and the results are shown in Figure 1, and Figure 2.

In antibiotic sensitivity test, *E. coli* was found as 83% sensitive to colistin sulphate (CT) and 73% sensitive to ciprofloxacin (CIP) (Figure 1). These findings were almost similar to the reports of the KHMNH et al. (2005). *E. coli* was highly (87%) resistant to tetracycline (TE) (Figure 1). This finding was supported by Rahman et al. (2004). For ciprofloxacin, the *E. coli* isolates showed 73% sensitivity (Figure 1). However, Rahman et al. (2004) reported *E. coli* to be highly sensitive to ciprofloxacin. Sohidullah et al. (2016), and Begum et al. (2016) also reported *E.* coli to be sensitive to ciprofloxacin.

Salmonella spp. were found 80% sensitive to colistin sulphate (Figure 2). This finding is correlated with the findings of Nagappa et al. (2007) who reported Polymyxin (colistin) to become sensitive within several years of the approval of this class of drugs for use in poultry. However, Suresh et al. (2006) reported *Salmonella* spp. to be highly resistant to polymyxin group of drugs. *Salmonella* organisms were completely resistant to azithromycin and tetracycline (Figure 2). Suresh et al. (2006) and Sohidullah et al. (2016) got similar results who reported *Salmonella* spp. to be highly resistant to azithromycin and tetracycline. Suresh et al. (2006) reported *Salmonella* spp. to be highly resistant to neomycin which also similar in our study. For amoxycillin the *Salmonella* spp. strains showed 100% resistance (Figure 2) which is similar to the study of Nagappa et al. (2007). *Salmonella* spp. was also showed 100% resistance to the streptomycin (Figure 2) as like as the findings of Johnson et al. (2005). In the present study, *Salmonella* spp. was found intermediately sensitive to ciprofloxacin (60%) (Figure 2). However, Begum et al. (2010) found sensitivity against ciprofloxacin to the organisms and Suresh et al. (2006) reported 8.9% resistance to the ciprofloxacin.

In conclusion, it would be said that the findings of this research work would certainly help the veterinarians to select the proper antibiotics against colibacillosis, and salmonellosis in poultry of Bangladesh, and overcome the multi-drug resistant problem of *E. coli* and *Salmonella* spp.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This study did not require any ethical approval since the involvements of dead and sick chickens with their internal organs like liver and heart were ensured following local legislation and institutional concern.

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