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Research Article

Antimicrobial Resistance Pattern against *E. coli* and *Salmonella* in Layer Poultry

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ABSTRACT

E. coli and *Salmonella* are leading cause of illness in layer farms. The emergence of antimicrobial–resistant *E. coli* and *Salmonella* are associated with the indiscriminate use of antibiotics in poultry farming. The present study aimed at determination of antimicrobial resistance pattern of *E. coli* and *Salmonella* strains isolated from commercial layer from different layer farms under Chittagong district of Bangladesh, during the period of September to December, 2012. Isolation and identification of *E. coli* and *Salmonella* were done by using standard methods. A total of 13 isolates of *E. coli* and 8 isolates of *Salmonella* were studied. Isolated *E. coli* and *Salmonella* were tested for resistance to 10 different antimicrobial agents, using disc diffusion method. The *E. coli* were found 100% resistant to Tetracycline, Ciprofloxacin, Enrofloxacin and Pefloxacin followed by Amoxicillin (84.62%), Kanamycin (69.24%), Colistin (63.75%), Doxycycline (53.75%) and Neomycin (23.08%). Conversely, *E. coli* isolates show high sensitivity to Gentamicin (100%) and Neomycin (76.92%). Among the *Salmonella* isolates, 100% were found resistant to Amoxicillin and Tetracycline followed by Enrofloxacin (87.5%), Ciprofloxacin (87.5%), Pefloxacin (87.5%), Doxycycline (50%), Colistin (50%) and Kanamycin (50%). *Salmonella* isolates showed high sensitivity (100%) to Gentamicin and Neomycin. All of the isolates showed multiple antimicrobial resistances. Rational use of antibiotics need to be adopt in commercial poultry farming system of Bangladesh to prevent the emergence of drug resistance *E. coli* and *Salmonella*. Moreover, Gentamicin might be the drug of choice for both avian colibacillosis and salmonellosis.

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INTRODUCTION

Poultry farming is recognized profitable business in Bangladesh and getting popularity as employment opportunities. Over the 80% of the country's people live in the rural sector and highly dependent on agricultural system (BBS, 2000). This reflection has got in the recent years due to the raising of commercial poultry farms to meet the demand of poultry meat and egg resulted from the establishment poultry belt in Dhaka, Chittagong, Gazipur, and Narshingdi district. The poultry farming has dramatically increased in recent years in Bangladesh but disease is one of the main constrains for their development.

Avian Colibacillosis and Salmonellosis has been found to be major infectious diseases of all ages of birds. *E. coli* are one of the common microbial floras of gastrointestinal tract of poultry and human being (Jawetz et al., 1984). Although most isolates of *E. coli* are nonpathogenic but they are considered as indicator of fecal contamination in food and about 10 to 15% of intestinal coliforms are opportunistic and pathogenic serotypes (Barnes et al., 1997) and cause a variety of lesions in immune–compromised hosts as well as in poultry. Infection with bacteria genus *Salmonella* are responsible for a variety of acute and chronic disease in poultry reported in Bangladesh (Bhattacharjee et al., 1996).

Escherichia coli are the primary causative agent of cellulitis, septicemia, and airsacculitis in poultry and *Salmonella* are the causative agent of pullorum disease, fowl typhoid and fowl paratyphoid (Gomis et al., 1997). Therefore, these are the most significant poultry bacterial pathogen. Antimicrobial resistance is a global problem, and emerging antimicrobial resistance has become a public health fact worldwide (Kaye et al., 2004). A variety of foods

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and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food–animal production (Schroeder et al., 2004). Though many bacteria recovered from poultry or poultry–related samples have been monitored, few published studies have reported on antimicrobial resistance in bacteria, particularly *Salmonella* and *E. coli* (Antunes et al., 2003).

About 65 years ago, from the time when antibiotics became widely available, they have been acclaimed as miracle drugs talented to destroy disease–causing bacteria. But with each transitory decade, bacteria that resist not only single, but multiple, antibiotics making some diseases particularly troublesome to control have become progressively more prevalent. Antimicrobial resistance take place when bacteria adjust or adapt in a way that permits them to stay alive in the presence of antibiotics designed to kill them, bacteria evolve resistance to these drugs, typically by acquiring chromosomal mutations and multidrug resistant plasmid (Finch et al., 2003; Nichol et al., 2003).

Antibiotics are extensively used as growth promoters in poultry production or to control infectious disease and abuse are considered to be the most vital selecting force to antimicrobial resistance of bacteria (Moreno et al., 2000). Due to enormous use of antibiotics in the field of veterinary medicine, an increased number of resistant bacterial strains were developed in recent years. In different parts of the world, multi drug resistant strains of *E. coli* are ubiquitous in both human and animal isolates (Amara et al., 1995) and multiple drug resistant, nonpathogenic *E. coli* found in the intestine are probably an important reservoir of resistance genes (Osterblad et al., 2000) and momentarily drug–resistant *E. coli* of animal origin may colonize the human intestine (Marshall et al., 1990).

Acquired multi drug resistance to antimicrobial agents creates an extensive trouble in case of the management of intra and extra intestinal infections caused by *E. coli*, which is a major source of illness, death, and increased healthcare costs (Gupta et al., 2001). There only little scattered work on antimicrobial sensitivity testing was performed in Chittagong region. Therefore, the present study was designed to detect the antimicrobials that are no longer active against avian colibacillosis and salmonellosis in Chittagong, Bangladesh. The present study was targeted to isolate the *E. coli* and *Salmonella* strain from poultry sample and determine the antibiotic resistance patterns against *E. coli* and *Salmonella.*

MATERIALS AND METHODS

Study Area and Duration

The study was conducted on layer poultry at Chittagong District, which is one of the most concentrated poultry areas of Bangladesh, during the period of September to December, 2012. A total of 30 dead birds from different layer farms of Chittagong were subjected to postmortem during the study period at PRTC laboratory, Chittagong.

Diagnosis of Disease

Diagnosis of disease was made on the basis of post mortem examination and standard microbiological examination, using standard methods for bacterial isolation and identification described by OIE (2000).

Isolation and Identification

The liver and spleen sample was collected aseptically and used for microbiological test. Isolation and identification of bacteria were done by using the method described by Collins and Lyne (1976). Culturing on various selective media, examination of colony characteristics, observation of the organisms under microscope and various biochemical tests were done to isolation and identification of *E. coli* and *Salmonella* organisms.

Culturing on Agar Media

For Suspected cases of Colibacillosis, after collections of samples were inoculated into peptone broth for primary enrichment, then incubate the broth 24 hours at 37^0C and from broth streaked on MacConkey Agar and Eosin Methylene Blue (EMB) agar plate. The plate was incubated at 37^0C examined after 24 hours for growth and change in the color of the medium. After overnight incubation the bacterial growth was observed as large pink colonies at MacConkey and mid night blue metallic sheen colonies at EMB agar. Both lactose fermenting and non lactose fermenting colonies were found. *Salmonella pullorum* and *Salmonella gallinarum* both the organisms will grow on differential plating media such as MacConkey and SS Agar. It has been shown that *Salmonella pullorum* occasionally fails to grow on certain selective media such as Briliant Green agar or *Salmonella*–Shigella agar but grows satisfactorily on Bismuth Sulfite and McConkey agars (Carlson et al., 1974). Confirmation of *Salmonella* was done by culturing on selective media such as **Xylose lysine deoxycholate** (XLD) Agar and Brilliant Green Agar (BGA) Agar and observation of colony characteristics such as black centered pale pink colony and red–pink–white opaque colored colonies surrounded by brilliant red zones, respectively.

Biochemical Tests

For confirmation of *E. coli* and *Salmonella* various biochemical tests were done for confirmation of the isolates as described by Cruickshank et al. (1995).

Antibiotic Sensitivity

The antibiotic sensitivity of the isolated strain at different concentration was performed by using standard paper disc diffusion method described by NCCLS, (2009). The following antibiotics and disc potencies were used: GEN: Gentamicin (10µg), DO: Doxycycline (30µg), CIP: Ciprofloxacin (5µg), ENR: Enrofloxacin (5µg), AMC: Amoxicillin (10µg), N: Norfloxacin (10µg), CL: Colistin (10µg), TE: Tetracycline (30µg), Pf: Pefloxacin (10µg), K: Kanamycin (30µg) from HIMEDIA Ltd (Mombai, India).

Data Analysis

Data obtained was imported to the Microsoft Office Excel–2007 and transferred to the software STATA/IC–11 for analysis. Descriptive statistics was done by using the STATA/IC–11 software and expressed as percentages of different variables like resistance, intermediate and sensitivity pattern of antimicrobials.

RESULTS

A total of 13 individual colonies of *E. coli* and 8 individual colonies of *Salmonella* were isolated from poultry liver samples through different test. Table 1 presented antimicrobial resistant pattern against *E. coli*. Among the 13 isolates, all were sensitive to Gentamicin and all were resistant to Ciprofloxacin, Enrofloxacin, Pefloxacin and

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Tetracycline. In case of Colistin and Doxycycline 7 isolates were resistant and 6 were sensitive. Antimicrobial resistant pattern in Norfloxacin showed 3 isolates were resistant and 10 were sensitive. Seven isolates were resistant, 2 were sensitive and 4 were intermediate sensitive to kanamycin. In case of Amoxicillin 11 isolates were resistant, 1 was sensitive and 1 was intermediate sensitive.

Table 1: Antimicrobial resistance pattern against *E. coli*

Sample	Antibiotic Disc									
	GEN	CL	CIP	PF	DO	N	TE	K	ENR	AMX
1	S	R	R	R	S	S	R	R	R	R
2	S	S	R	R	S	S	R	R	R	R
3	S	R	R	R	S	S	R	R	R	R
4	S	R	R	R	R	R	R	R	R	I
5	S	S	R	R	S	R	R	R	R	R
6	S	S	R	R	S	S	R	S	R	R
7	S	R	R	R	S	R	R	S	R	R
8	S	R	R	R	R	S	R	I	R	R
9	S	R	R	R	R	S	R	I	R	R
10	S	R	R	R	R	S	R	R	R	R
11	S	S	R	R	R	S	R	R	R	R
12	S	S	R	R	R	S	R	I	R	S
13	S	S	R	R	R	S	R	I	R	R

R= Resistance; I= Intermediate; S= Sensitive; GEN= Gentamicin; CL= Colistin; CIP= Ciprofloxacin; PF= Pefloxacin; DO= Doxycycline; N=Neomycin; TE= Tetracycline; K= Kanamycin; ENR= Enrofloxacin; AMX=Amoxicillin

Table 2: Prevalence of antimicrobial resistance pattern against *E. coli* isolates

Antibiotics	Isolates	Pattern		
		Resistance (%)	Intermediate (%)	Sensitive (%)
Ciprofloxacin	13	100	0	0
Enrofloxacin	13	100	0	0
Pefloxacin	13	100	0	0
Tetracycline	13	100	0	0
Amoxicillin	13	84.62	7.69	7.69
Kanamycin	13	69.24	15.38	15.38
Colistin	13	53.75	0	46.15
Doxycycline	13	53.75	0	46.15
Neomycin	13	23.08	0	76.92
Gentamicin	13	0	0	100

Antibiotic susceptibility pattern and prevalence of antimicrobial resistance of *E. coli* isolates from samples of layer farms has been outlined in table 1 and table 2, respectively. Resistance spectrum of *E. coli* for 10 antibiotics tested in descending order were respectively, Ciprofloxacin (100%), Enrofloxacin (100%), Pefloxacin (100%), Tetracycline (100%), Amoxicillin (84.62%), Kanamycin (69.24%), Colistin (53.75%), Doxycycline (53.75%), Neomycin (23.08%) and Gentamicin (0%). In this study is revealed that no isolate were found sensitive to Ciprofloxacin, Enrofloxacin, Pefloxacin and Tetracycline. On the other hand no isolate were found resistant to Gentamicin. Intermediate sensitivity was only found to two antibiotics (Amoxicillin and Kanamycin). All the isolates of *E. coli* showed multiple drug resistance (up to against 9 antibiotics out of 10 used in the test).

Antimicrobial resistant pattern of *Salmonella* isolates were shown in table 3. Among the 8 isolates, all were sensitive to Gentamicin and Neomycin and all were resistant to Tetracycline and Amoxicillin. In case of Ciprofloxacin, Enrofloxacin and Pefloxacin 7 isolates were resistant and 1 isolate was sensitive. Antimicrobial resistant pattern in Kanamycin showed 4 isolates were resistant, 3 were sensitive and 1 was intermediate sensitive. In case of Colistin 4 isolates were resistant and 4 isolates were sensitive. Four isolates were resistant, 2 were sensitive and 2 were intermediate sensitive to Doxycycline.

Table 3: Antimicrobial resistance pattern against *Salmonella* isolates

Sample	Antibiotic Disc									
	GEN	CL	CIP	PF	DO	N	TE	K	ENR	AMX
1	S	R	R	S	R	S	R	S	R	R
2	S	S	S	R	S	S	R	R	R	R
3	S	R	R	R	S	S	R	S	R	R
4	S	R	R	R	R	S	R	R	R	R
5	S	S	R	R	R	S	R	R	S	R
6	S	S	R	R	R	S	R	S	R	R
7	S	R	R	R	R	S	R	R	R	R
8	S	S	R	R	R	S	R	I.S	R	R

R= Resistance; I= Intermediate; S= Sensitive; GEN= Gentamicin; CL= Colistin; CIP= Ciprofloxacin; PF= Pefloxacin; DO= Doxicycline; N=Neomycin; TE= Tetracycline; K= Kanamycin; ENR= Enrofloxacin; AMX=Amoxicillin

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Table 4: Prevalence of antimicrobial resistance pattern of *Salmonella* isolates

Antibiotics	Isolates	Pattern		
		Resistant (%)	Intermediate (%)	Sensitive (%)
Tetracycline	08	100	0	0
Amoxicillin	08	100	0	0
Ciprofloxacin	08	87.5	0	12.5
Enrofloxacin	08	87.5	0	12.5
Pefloxacin	08	87.5	0	12.5
Kanamycin	08	50	12.5	37.5
Colistin	08	50	0	50
Doxycycline	08	50	25	25
Neomycin	08	0	0	100
Gentamicin	08	0	0	100

Antibiotic susceptibility pattern and prevalence of antimicrobial resistance of *Salmonella* isolates from samples of layer farms has been outlined in Table 3 and Table 4, respectively. Resistance spectrum of *Salmonella* for 10 antibiotics tested in descending order were respectively Tetracycline (100%), Amoxicillin (100%), Ciprofloxacin (87.5%), Enrofloxacin (87.5%), Pefloxacin (87.5%), Kanamycin (50%), Colistin (50%), Doxycycline (50%), Neomycin (0%) and Gentamicin (0%).

In this study it was revealed that no isolate were found sensitive to Tetracycline and Amoxicillin. On the other hand no isolate were found resistant to Neomycin and Gentamicin. Intermediate sensitivity was only found to two antibiotics (Kanamycin and Doxycycline). All the isolates of *Salmonella* showed multiple drug resistance (up to against 8 antibiotics out of 10 used in the test).

DISCUSSION

Organisms were isolated based on colony characteristics and biochemical tests. The present study revealed that all of the isolates of *E. coli* from commercial chicken were resistance to multiple antibiotics (› =4) which coincided with the findings of Zhao et al. (2005), Guerra et al. (2003) and Islam et al. (2008). Multiple antimicrobial resistance might happened due to indiscriminate use of antibiotics, chemotherapeutics and or disperse of drug resistant microorganism in the environment (Van de Boogard and Stobberingh, 2000). All *E. coli* isolates were found resistant (100%) to Ciprofloxacin which is higher than the earlier report (Saenz, et al., 2001; Kang et al., 2005). In present study there were found no isolate of *E. coli* was sensitive to Enrofloxacin, this finding agree with Cooke et al. (2002) who reported Enrofloxacin resistance in *E. coli* isolated from dogs with urinary tract infections. Resistances that observed against Tetracycline is more or less similar with Islam et al. (2008), they showed 96.6% resistance to Tetracycline of *E. coli* isolated from poultry farm at Chittagong District in Bangladesh. Schroeder et al. (2001) stated comparatively lower resistance (71%) to Tetracycline of *E. coli* isolated from turkey. In present study it was revealed higher value of resistance (84.62%) of *E. coli* to Amoxicillin than reported by Schroeder *et al*. 2001 (28%) where *E. coli* are isolated from turkey. Resistance that was observed to Kanamycin (69.24%) is more or less agree with Akond et al. (2009) in a study on chicken collected from different poultry markets of Dhaka, Bangladesh (76%). It was revealed that 53.75% sensitive isolates of *E. coli* to Colistin and this finding have similarity with Catchpole et al. (1997) who observed Colistin is active against most strains of *E. coli* in a study on reassessment of the in–vitro activity of Colistin sulphate sodium. The resistance of *E. coli* against Doxycycline was 53.75% isolates which agree with Raum et al. (2008) who stated 29–58% resistance of *E. coli* to Doxycycline isolated from stool sample in a study in Germany. *E. coli* showed resistance against Neomycin (23.08%), Stephan and Schumacher (2001) observed O100: H–STEC strains isolated from healthy slaughter pigs were resistant to neomycin. In this study it was observed that all the isolated *E. coli* were sensitive to gentamicin and this finding is in agreement with Alam et al. (2006) who reported that most of the environmental strains were (97%) sensitive to Gentamicin. However, Schroeder et al. (2001) and Saenz et al. (2001) showed 24% resistance in turkey isolates and 38% resistance in broiler isolates of *E. coli* to Gentamicin.

Salmonella were found resistant to multiple antibiotics (›4) which is coincided with the findings of Weill et al. (2006) who reported 67% of *Salmonella enterica* serotype Typhimurium isolates of humans in France. There were no isolate of *Salmonella* found sensitive to Tetracycline which corroborate with the findings of Musgrove et al. (2006) who stated 63.4% and Zhao et al. (2008) who reported 39.9%, respectively. Resistance of *Salmonella* to Amoxicillin that revealed in this study (100%) is higher than reported by Siemon et al. (2007) isolated from conventionally reared poultry (62%) but similar finding was observed by Ahaduzzaman et al. (2014) in environmental effluents. *Salmonella* showed resistance against Ciprofloxacin (87.5%), however, Musgrove et al. (2006) found no resistance of *Salmonella* against Ciprofloxacin in isolates obtained from commercial chicken and Gay et al. (2006) also showed 0.1% resistant isolates from human. In this study 87.5% *Salmonella* isolates showed resistance against Enrofloxacin and this finding is higher than the findings of Antunes et al. (2003) who reported 50%. Resistance of *Salmonella* to Pefloxacin was almost 88% in the current study which does not correlate with the findings of Ajayi et al. (2011) who found 20% resistant *Salmonella* in cattle fecal isolates. Nearly 50% isolates of *Salmonella* were resistant to Kanamycin, Colistin and Doxycycline which are supported by the findings of Musgrove et al. (2006) and Murugkar et al. (2005). In this study it was observed that all the isolated *Salmonella* were sensitive to Neomycin and Gentamicin. However, Carmrainana et al. (2004) reported 53.4% resistant isolates of *Salmonella* to Neomycin in a findings where organisms were isolated from a poultry slaughterhouse in Spain. Our

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findings demonstrate that multidrug–resistant strains of *E. coli* and *Salmonella* isolates were frequently present in layer poultry farm of Chittagong District. The high prevalence of multidrug–resistant *E. coli* and *Salmonella* in layer poultry reflects a reservoir of resistance in birds that can be transmitted to humans. If these resistance organisms to antimicrobial persist, there will be a great problem of antimicrobial choice in near future. Proper efforts should be needed to reduce the prevalence of resistant *E. coli* and *Salmonella* in layer farms, including the adoption of guidelines for the prudent use of antimicrobial agents in animals used for food.

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COMPETING INTERESTS

Authors declare that they have no competing interests.

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