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ISOLATION, IDENTIFICATION, TOXIN PROFILE AND ANTIBIOGRAM OF *ESCHERICHIA COLI* ISOLATED FROM BROILERS AND LAYERS IN MYMENSINGH DISTRICT OF BANGLADESH

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ABSTRACT

The study was conducted to isolate and identify *E. coli* from apparently healthy broilers and layers from different poultry farms adjacent to the Bangladesh Agricultural University, Mymensingh, Bangladesh, during the period of January to May 2006 and characterize their ability to produce enterotoxin and also the antibiogram of the isolates. A total of 110 fecal samples were collected from broiler (n=55) and layer (n=55) chickens. *E. coli* were isolated and identified by cultural, biochemical, motility test and the heat-stable toxins were determined by Infant Mouse Assay (IMA). In case of broilers, 35 (63.6%) samples were found positive while 31 (56.4%) from layers. The overall prevalence of *E. coli* was 60%. Among the isolates of *E. coli*, 22.86% isolates from broiler and 38.71% isolates from layer were found positive for their ability to produce enterotoxin based on mice inoculation test. The antibiotic sensitivity pattern showed that the isolates were highly sensitive to chloramphenicol, ciprofloxacin, kenamycin and cephalexin and an increasing trend of resistance was recorded in both broiler and layer isolates. It may be concluded from the results of this study that the high resistance of *E. coli* to antibiotics constitutes a threat to poultry industry in Bangladesh.

Key words: *E. coli*, toxin, antibiogram

INTRODUCTION

Major species of *E. coli* encounter in the lower portion of the intestine of human, warm blooded animals and birds, where they are mostly responsible for gastroenteritis (Pelczar *et al.,* 1986). *E. coli* produces two distinct enterotoxins: a high-molecular weight, immunogenic, heat labile toxin (LT) and/or a low-molecular weight, non-immunogenic, heat stable toxin (ST) (Greenberg and Guerrant, 1986; Robertson *et al.,* 1986). The LTs of *E. coli* from human and porcine origin have been shown to share a common structure that activates adenylate cyclase and cross reacts immunologically with the heat-labile enterotoxin of *Vibrio cholerae*. These enterotoxins have been serogrouped as LT-1 (Pickett *et al.,* 1986). LT-11 a variant of LT-1 has recently been isolated from some isolates of *E. coli*. The LT-11 has characteristics that are similar to those of LT-1 but that are different in their antigenic specificity (Holmes *et al.,* 1996). Two types of heat stable enterotoxins (STs) have been described, based on their methanol solubilities: a methanol-soluble molecule with biologic activity in suckling mice, rats and piglets (referred to as STa) and a methanol-insoluble molecule with biological activity in piglets (referred to as STb) (Greenberg and Guerrant, 1986).

In Bangladesh, for many years, antibiotic is randomly used for treatment purpose. There is clear evidence of abuse of antibiotics, for which emergence of multi-drug resistant *E. coli* are continuously increasing (Hussain *et al.,* 1982; Nazir *et al.,* 2005). This leads to indiscriminate use of antimicrobial drugs in poultry industry without prior testing that might have resulted antibiotic resistance causing a serious problem because it limits the therapeutic possibilities in the treatment of bacterial disease. So, the research work was undertaken to isolating and identify *E. coli* from chicken, their toxin profile analysis and antibiogram nature.

MATERIALS AND METHODS

The whole study was performed in three steps in the bacteriology laboratory of Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh, during the period of January to May 2006. The first step includes characterization of the *E. coli.* The second step leads to toxin profile analysis and the third step includes the antibiogram.

Isolation of E. coli

A total of 110 fecal samples from healthy broiler (n = 55) and layer (n = 55) chickens were collected from different poultry farms adjacent to BAU, with the help of sterile cotton buds and transferring the buds immediately to sterile nutrient broth. The samples were wrapped with ice, kept in box, and transferred within 30 minutes.

Identification of the isolates

The isolates of *E. coli* were identified by observing gross colony morphology using Eosin Methylene Blue (EMB) agar, McConkey Agar, Salmonella-Shigella (SS) agar, Grams staining properties and motility as described by Merchant and Packer (1967). The isolates were subjected to different biochemical tests such as sugar fermentation test, Indole production test, Methyl-Red and Voges-Proskauer (MR-VP) test, following the standard methods described by Cowan (1985). Pure culture of *E. coli* was isolated using EMB agar.

Maintenance of stock culture

Nutrient agar slants were used to maintain the stock culture for each of the *E. coli* isolate. The *E. coli* were inoculated in the slant by streaking and were incubated at 37^0C for 24 hours. Finally, glycerol was overlaid and the culture was kept at room temperature.

Toxin profile

Overnight broth cultures were centrifuged at 1300 rpm for 15-20 minutes and supernatants were collected and transferred into new vials, then gentamycin was added @ 5µg/ml and kept at room temperature for overnight, following the method described by Gianella (1976). The purity of the toxins were tested by streaking the supernatants on EMB agar and incubated at 37^0C for 24 hours. If no colony was formed, then the supernatant was used for detection of heat-stable (ST) toxin by Infant Mouse Assay (IMA).

Enterotoxigenic effect in suckling mice and determination of ST toxin by IMA

Sixty Swiss Albino suckling mice of 1-4 days old were separated from their mother immediately before used and divided into two groups A and B consisting of three mice in each group. An amount of 2.5 µl crude culture supernatant containing suspected enterotoxin were administered to the mice of group A through oral route with the help of micropipette. Mice of group B were kept as control. Mice were incubated at 37^0C for 24 hours to observe toxic effects.

IMA was used for the detection of heat-stable toxin. Day old Swiss Albino suckling mice were used for the test which were inoculated with 0.1 ml of crude culture supernatant and kept at room temperature for 4 hours, then sacrificed by cervical dislocation. The abdomen was opened and the entire intestine was removed. The weight of the gut and the remaining carcass were taken and the ratio was calculated for each mouse. The average ratio of less than 0.070 was considered negative while 0.070~0.085 was considered positive for ST toxin (Gianella, 1976).

Antibiotic sensitivity test

Antibiotic sensitivity test of isolated *E. coli* was performed with the standardized commercial sensitivity discs (Mast Diagnostics, Mast group Ltd., Merseyside, UK) following Disc Diffusion Method (Bauer *et al*., 1966). Sensitivity to antibiotic was studied on blood agar plates with ampicillin (10 µg/disc), cephalexin (30 µg/disc) chloramphenicol (30 µg/disc), ciprofloxacin (5 µg/disc), erythromycin (15 µg/disc), kanamycin (30 µg/disc) and nalidixic acid (30 µg/disc). An amount of 0.5 freshly grown pure culture of *E. coli* was poured on blood agar plates and allowed to spread gently over the entire surface with a glass rod spreader. After 1 to 2 minutes, the discs were placed at a distance of about 1 cm apart and incubated at 37^0C for overnight. On the basis of the diameter of zones of inhibition produced around the antibiotic discs the inhibitory effect of the antibiotic to the growth of the culture was recorded as resistance, less sensitive (1.0-1.5 mm), moderately sensitive (3.0-3.5 mm) and highly sensitive (6.0-6.5 mm).

RESULTS AND DISCUSSION

Isolation and identification of E. coli

The results of gross colony morphology on EMB agar, McConkey agar and SS agar, Grams staining and motility test are summarized in Table 1.

Table 1. Identifying characteristics of *E. coli*

Source (n = 55)	Motility	Colony characteristics			Morphology	Staining properties
		EMB agar	McConkey agar	SS agar		
Broiler feces	+	Yellow green metallic sheen	Bright pink or red colonies	Pinkish colony	Short rod, single, pair or in short chain	Gram negative
Layer feces	+	Yellow green metallic sheen	Bright pink or red colonies	Pinkish colony	Short rod, single, pair or in short chain	Gram negative

n = Number of *E. coli* isolates.

For biochemical characterization, a series of biochemical tests selective for *E. coli* were performed with the suspected Gram-negative rod shaped bacteria. All the isolates fermented the five basic sugars producing acid and gas. All the isolates were Methyl Red positive, Voges-Proskauer test negative and Indole test positive. Out of 110 samples, 66 samples were found to be positive for *E. coli* isolates. The prevalence of *E. coli* in the faecal sample was 60.0% (Table 2). Bhattacharjee *et al.* (1996) reported 40.82% prevalence of *E. coli* in chicken from Bangladesh but Nazir (2004) stated the over all prevalence was 62.5% from chicken, which is closed to the present findings.

Table 2. Prevalence of *E. coli* in broiler and layer chickens

Sources of samples	Total samples examined	Samples positive	Prevalence (%)
Broiler	55	35	63.6
Layer	55	31	56.4
Total	110	66	60.0

Toxin profile

Enterotoxigenic *E. coli* (ETEC) were detected based on mortality and survivability of 1-4 day old mice (60) within 24 hours of postinoculation. Out of 35 broiler isolates, 8 (22.86%) *E. coli* were found positive for ETEC. Out of 31 layer isolates, 12 (38.71%) were found positive for their enterotoxigenicity. It can be speculated that the toxic effects could be due to heat-labile (LT) toxin (Yamamoto and Yokota, 1983). Positive results of ETEC denoted in the following (Table 3).

Table 3. Prevalence of ETEC

Source	Crude culture supernatant (toxin)			Positive effect	No. of isolates tested	Positive for ETEC	
	Quantity	Route	Incubation			Number	%
Broiler	25 µl	Oral	24 hours	Death (3/3)	35	8	22.86
Layer	25 µl	Oral	24 hours	Death (3/3)	31	12	38.71
Total					66	20	30.3

The results of IMA showed that all the *E. coli* isolates, tested in IMA, were found negative for ST (Table 4). In case of broiler isolates the obtained value (gut weight and carcass weight ratio) ranged from 0.092 to 0.103 and in case of layer isolates 0.095 to 0.114.

Table 4. Determination of heat-stable (ST) toxin by IMA

Source	Crude culture supernatant (toxin)			Gut weight and carcass weight ratio		Results
	Quantity	Route	Incubation	Ranges of standard value	Ranges of obtained value	
Broiler	0.1 ml	Oral	4 hours	0.070 to 0.085	0.092 to 0.103	ST-Negative
Layer	0.1 ml	Oral	4 hours	0.070 to 0.085	0.095 to 0.114	ST-Negative

Antibiogram nature of E. coli

Among the *E. coli* isolated from broilers, 100% were resistant to nalidixic acid, 97.14% to cloxacillin, 91.42% to erythromycin and 62.85% to ampicillin and these findings were almost similar to the reports of Nazir *et al*. (2005). Though, Prescott and Baggot (1993) reported good activity of erythromycin against some gram negative bacteria. About 91.43% broiler isolates were moderately sensitive to cephalexin, 77.74% to ciprofloxacin and 85.71% to kanamycin while 54.28% isolates were highly sensitive to chloramphenicol and 45.71% were moderately sensitive to the same antibiotic. On the other hand, 14.29% and 22.86% isolates were highly sensitive to kanamycin and ciprofloxacin respectively (Table 5), whereas Al-Ghamdi *et al.* (2001) found 34.7% resistant to ciprofloxacin. Fasihuddin and Khatoon (1994) also the resistancy of *E. coli* isolates against common antibiotics.

Among the *E. coli* isolated from layers, 100% were resistant to cloxacillin and nalidixic acid and 93.55% isolates were resistant to erythromycin which are similar to the findings of Al-Ghamdi *et al.* (2001). A total of 32.26% layer isolates were found resistant to Ciprofloxacin and 25.81% to Ampicillin (Table 5). Nazir *et al.* (2005) also recorded the same findings. Islam *et al.* (2004) recorded an increasing trend of resistance in broilers than in ducks due to indiscriminate use of antibiotics in Bangladesh (Hussain *et al.*, 1982).

Table 5. Antibiotic sensitivity and resistance pattern of *E. coli* isolates

Source of *E. coli*	Resistance		Less sensitive		Moderately sensitive		Highly sensitive	
	Antibiotic	%	Antibiotic	%	Antibiotic	%	Antibiotic	%
Broiler	OB	97.14	AP	37.15	K	85.71	C	54.28
	NA	100	E	8.58	CL	91.43	CIP	22.86
	AP	62.85	OB	2.86	CIP	77.74	K	14.29
	E	91.42	-	-	C	45.71	CL	8.57
Layer	OB	100	AP	75.19	C	50	K	32.26
	NA	100	E	6.45	CL	64.52	C	25
	CIP	32.26	CIP	58.06	K	48.39	–	–
	E	93.55	K	19.39	CIP	9.68	–	–
	AP	25.81	CL	35.48	–	–	–	–
	–	–	C	25	–	–	–	–

AP= Ampicillin, C = Chloramphenicol, CL = Cephalexin, CIP = Ciprofloxacin, OB = Cloxacillin, E = Erythromycin, K = Kanamycin and NA = Nalidixic acid.

Among the *E. coli* isolated from layer, 32.26% were highly sensitive and 64.52% moderately sensitive to kanamycin and cephalexin respectively. A total of 25% and 19.39% layer isolates were less sensitive to chloramphenicol and kanamycin respectively. However 25% isolates were found highly sensitive to chloramphenicol (Table 5). These findings are similar to the reports of Prasad *et al.* (1997). So, it may be concluded that the prevalence of *E. coli* in fecal samples from broilers and layer chickens and that of ETEC among the isolates remain worth of taking serious note of it and the antibiogram nature of the isolates is quite significant in respect of indiscriminate use of antibacterial drugs.

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