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Prevalence, Characteristics and Antibiogram Profiles of *Escherichia coli* Isolated from Apparently Healthy Chickens in Mymensingh, Bangladesh

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A B S T R A C T

Escherichia coli known to cause food-borne illnesses worldwide that are closely associated with the consumption of contaminated poultry and egg products. This study was undertaken for cultural, biochemical and antibiotic sensitivity analyses of *E. coli* recovered from apparently healthy chickens. Cloacal samples (n=350) were aseptically collected from layers (n=150), broilers (n=150) and indigenous chickens (n=50). The samples were enriched in nutrient broth and streaked onto eosin methylene blue (EMB) agar, MacConkey (MC) agar, blood agar, salmonella-shigella (SS) agar and brilliant green agar (BGA) for cultural characterization of the *E. coli* isolates. Culture-positive samples yielded characteristic colonies of *E. coli* with metallic sheen on EMB agar, bright pink or red colonies on MC agar, hemolysis on blood agar, slight pink smooth colonies on SS agar and green color colonies on BGA media. The *E. coli* isolates produced acid and gas by fermenting sugars (dextrose, sucrose, lactose, maltose and mannitol) and gave positive reaction to indole, methyl red (MR) and catalase tests, but were negative to Voges-Proskauer (VP) test. The prevalence of *E. coli* in layers, broilers and indigenous chickens were 78.67, 82 and 70%, respectively. The antibiotic sensitivity pattern demonstrated that *E. coli* isolates were mostly sensitive to ciprofloxacin, gentamicin and cephalexin, and resistant to streptomycin, tetracycline, amoxicillin and nalidixic acid. Data of this study suggested that intestine of chicken could be a major reservoir of antibiotic resistant *E. coli.*

Keywords: *Escherichia coli*, Prevalence, Characteristics, Chickens, Antibiogram profiles

Introduction

Escherichia coli are Gram negative bacteria under the family Enterobacteriaceae. *E. coli* are widely distributed in nature, being present in soil and surface water and in animal and human feces. The most important reservoir of *E. coli* is the intestinal tract of animals and poultry (Bélanger *et al.*, 2011). In addition, it is present on the bird's skin and feathers. These bacteria cause colibacillosis in chickens which is characterized by air sacculitis, cellulitis, omphalitis, peritonitis, salpingitis, synovitis and coligranuloma. This disease has an important economic impact on poultry production worldwide. The majority of economic losses results from mortality and loss of productivity of the affected birds (Otaki *et al.*, 1995).

E. coli strains always belong to both pathogenic and non-pathogenic types (Pupo *et al.*, 1997). In the caecal flora of healthy chickens, 10-15% of the *E. coli* strains may belong to an O-serotype. As soon as the first hour after hatching, the birds start building up their *E. coli* flora. The bacteria drastically increase their numbers in the gut. Birds may be infected with *E. coli* from contaminated environment, direct contact with sick birds, feces, water and feed (Dho-Moulin *et al.*, 1999). Rodents may act as a carrier of avian pathogenic *E. coli* (APEC) and hence a source of contamination for the birds (Barnes *et al.*, 1997).

Most of the strains of *E. coli* are harmless but some strains can cause food poisoning. *E. coli* food poisoning is usually caused by: enteropathogenic *E. coli* (EPEC) enterotoxigenic *E. coli* (ETEC); enterinvasive (EIEC) and enterohemorrhagic *E. coli* (EHEC) (Mead *et al*., 1999). *E. coli* enters the body through the consumption of food containing bacteria. Eating of inadequately cooked meat is the most common way that causes *E. coli* food poisoning. Raw poultry meat may contain *E. coli* that can cause food poisoning (Gormley *et al.*, 2011). When a chicken is eviscerated, the healthy parts can be easily contaminated. Food animal such as cattle, pig and chickens appear to be reservoir of this organism (Geser *et al.*, 2012). Multidrug resistance *E. coli* are produced due to use of antibiotics in animal's feeds as well as incomplete course of treatment against *E. coli* infection of humans and animals (Marshall *et al.*, 1990). The present study was undertaken to determine the prevalence and characteristics of *E. coli* in apparently healthy chickens at farms, live bird markets and villages of Mymensingh area in Bangladesh.

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Materials and Methods

Sample collection

Swab samples were aseptically collected from apparently healthy layers of Bangladesh Agricultural University (BAU) Poultry Farm (n=100) and Boira Poultry Farm (n=50), broilers at Kamal Ranjit (KR) market (n=50), BAU Sesh-more market (n=50), and Kewatkhali market (n=50) and indigenous chickens at Boira village (n=50), Mymensingh. Samples were obtained from 16-18 weeks old laying hens, 10-12 weeks old broilers and 12-16 weeks old indigenous chickens.

Enrichment

Immediately after collection, samples were separately enriched in Nutrient Broth (NB) by incubating at 37^0C for overnight.

Isolation of *E. coli*

After enrichment in NB, a small amount of inoculum from NB was streaked duplicate onto eosin methylene blue (EMB) agar media and incubated at 37^0C for overnight (Cheesbrough, 1985).

Characterization of *E. coli*

Characterization of the *E. coli* was performed on the basis of cultural characteristics, Gram staining, motility test and antibiogram profiles. Colonial morphology of *E. coli* such as- size, margin, elevation and color were recorded on EMB agar, nutrient agar (NA), brilliant green agar (BGA), blood agar, salmonella-shigella (SS) agar, triple sugar iron (TSI) agar and MacConkey (MC) agar media to study cultural characteristics (Cheesebourgh,1985). Biochemical characterization of *E. coli* was performed by sugar fermentation reactions. Also, methyl red-Voges Proskauer (MR-VP), catalase and indole tests were done according to the method described by Cowan (1985).

Antibiogram study

Ten *E. coli* isolates were randomly selected for antibiotic sensitivity assay. A previously described modified disc diffusion or Kirby-Bauer method (Bauer *et al.*, 1966) was used to determine the susceptibility of *E. coli* isolates against antibiotic agents. In brief, the procedure involved measuring the diameter of the zone of inhibition that results from diffusion of the antimicrobial agents into the medium surrounding the disc. The reactions of test organisms to each antibiotic were classified as sensitive, intermediate and resistant according to the diameter of zone of inhibition recommended by NCCLS (2003). Ten commercially available antimicrobial discs (Oxoid Ltd, Baringstoke, Hampshire, England) were used in this study, which are mentioned with corresponding standard disc concentration in Table 1.

Table 1. Antibiotic concentration of discs used in antibiotic sensitivity assay for *Ê. coli*

Name of antibiotic	Concentration per disc (µg)
Chloramphenicol	30
Ciprofloxacin	5
Gentamicin	10
Cephalexin	30
Kanamycin	30
Cephradine	30
Amoxicillin	10
Streptomycin	10
Tetracycline	30
Nalidixic acid	30

Results

Prevalence of *E. coli*

The overall prevalence was 78.86%. The prevalence of *E. coli* in different chicken lines from all sources in this study are presented in Table 2.

Cultural, staining and motility characteristics

E. coli on EMB agar produced greenish black colonies with metallic sheen. On MC agar, the colonies were bright pink in color, transparent, smooth and raised. Hemolysis was produced in blood agar media. Slight pink smooth colonies were seen on SS agar and green color colonies were found on BGA media. The organisms appeared Gram negative, small rod shaped and arranged in single, pair or short chain. All the isolates were found to be motile.

Biochemical tests

The five basic sugars *viz*. dextrose, sucrose, lactose, maltose and mannitol were fermented by all the isolates with the production of acid and gas. Acid production was indicated by the color change from reddish to yellow and the gas production was seen by the appearance of gas bubbles in the inverted Durham's tubes. All *E. coli* isolates were catalase, indole and MR positive but VP negative.

Antibiogram profiles

Antibiotic sensitivity tests of chicken *E. coli* isolates revealed as sensitive, intermediate and resistant profiles which are shown in Table 3.

Table 2. Prevalence of *E. coli* in layers, broilers and indigenous chickens

Chicken lines (n)	*E. coli* positive samples (n)	Prevalence (%)	Overall prevalence (%)
Layers (150)	118	78.67	
Broilers (150)	123	82.00	78.86
Indigenous (50)	35	70.00	

Table 3. Antibiotic sensitivity pattern of the *E. coli* isolates from healthy chickens

Name of antibiotics	Antibiotic sensitivity profiles		
	Sensitive	Intermediate	Resistant
Ciprofloxacin	+	-	-
Gentamycin	+	-	-
Chloramphenicol	+	-	-
Cephalexin	+	-	-
Kanamycin	-	+	-
Cephradine	-	+	-
Streptomycin	-	-	+
Tetracycline	-	-	+
Amoxicillin	-	-	+
Nalidixic acid	-	-	+

+ = yes; - = No

Discussion

In this study, the overall prevalence of *E. coli* in chickens was recorded as 78.86%. Hossain *et al.* (2008) reported 60% prevalence, whereas Nazir (2004) observed 62.5% prevalence of *E. coli* in chickens. In another study, 66.67% prevalence of *E. coli* was noticed in duck (Avishek, 2010).

Antibiotic resistance of *E. coli* isolates of chickens recorded in this experiment might be due to indiscriminate use of antibiotics (Bonnet *et al.*, 2009). These drug resistant *E. coli* can be spread in the environment where humans and

animals acquire infections resulting difficulties in treating these cases (Alexander *et al.*, 2009). The prevalence of antibiotic resistant *E. coli* observed in this study appeared to be similar to several other studies (Khan *et al.*, 2005; Nazir *et al.*, 2005). Chicken intestine can serve as a reservoir for *E. coli* strains capable of causing extra-intestinal infection in avian and mammalian hosts (Ewers *et al.*, 2009). Clinically, healthy chickens probably have zoonotic potential since transmission between birds and humans via physical contact, contaminated dust and egg is possible. Feces tends to be leaked from the carcass when the evisceration stage itself gives an opportunity for the interior carcass to receive intestinal bacteria (Gormley *et al.*, 2011). Contaminated meat and other foods may play a role in the local spread of *E. coli* strains (Vincent *et al.*, 2010). The use of antimicrobial agents in food animal production may cause emergence of antimicrobial drug-resistant strain of *E. coli.* Antimicrobial drug-resistant *E. coli* was reported in humans due to the consumption of contaminated chicken (Manges *et al.*, 2007).

Conclusion

Data in this study suggested that chickens from farms and live bird markets may act as major reservoir of *E. coli* in Mymensingh region of Bangladesh. Harmful isolates of *E. coli* might cause food-borne illness and may spread resistance strain in humans following consumption of contaminated poultry and poultry by-products.

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