Shobuj Bangladesh Publications House
International Journal of Natural and Social Sciences
ISSN: 2313-4461

Frequency of drug resistant *Escherichia coli* isolated from commercial broiler chicken in Bangladesh

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ABSTRACT

The study was conducted to isolate and identify *Escherichia coli* from naturally healthy looking broiler chicken sold at local market in Sylhet region of Bangladesh. The study was also focused on the investigation of drug sensitivity and resistance pattern of the isolated organisms. A total of 100 samples comprising of 80 cloacal swabs and 20 liver samples were collected from apparently healthy broiler. Of these 35 (43.75%) cloacal swabs and 7 (35%) liver samples were found positive for *E. coli*. All isolates revealed same morphological, cultural and biochemical characteristics. All isolates possessed *E. coli* specific 16s rRNA gene detected by PCR using the primers ECO-1 and ECO-2. The antibiotic sensitivity test demonstrated that the isolates were multidrug resistant against Gentamycin, Erythromycin, Penicillin, Cephalexine, Amoxicillin, Nalidixic Acid while sensitive to Ceftriaxon. The high level of antibiotic resistance in Broiler chicken of Bangladesh indicates that widespread use of antibiotics as feed additives for growth promotion and disease prevention could have negative implications for human and animal health and the environment.

Key words: Broiler, *E. coli*, liver sample, antibiotic resistance.

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INTRODUCTION

Escherichia coli (*E. coli*) is a Gram-negative, facultative anaerobic, rod-shaped bacterium, commonly found in the lower intestine of warm-blooded animals, causes serious food poisoning in their hosts. (CDC, 2012). *E. coli* is the causal agent of diarhoeal diseases, as well as other systemic diseases in animals and human (Buxton and Fraser, 1978). Some serotypes of *E. coli* can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination (CDC, 2012; Vogt and Dippold, 2005). Pathogenic *E. coli* are usually identified by detection of a specific virulence factor or of a serotype associated with a virulence factor (Hasina, 2006). In Bangladesh, improper use of antibiotic may develop resistance to *E. coli*. New generation of bacterial strains are being developed and new types of antibiotics are required for the prevention and control of such type of diseases (Nazir, 2004). There are reports of resistance of *E. coli* to antibiotics with associated treatment failure (Talan et al., 2004; Blondaeu, 2004). Included in the list of affected antimicrobials are penicillin, cephalosporin, sulpha drugs (Flutt et al., 2000; Sahm et al., 2001) and fluoroquinolones (Goettsch et al., 2000). Fluoroquinolone resistant *E. coli* strains often show resistance to other drugs such as ampicilin, tetracycline, chloramphenicol, trimethoprin, sulphamethoxazole and Gentamycin (Garau et al.,

How to cite this article: MN Khatun, ATM Mahbub-E-Elahi, S Ahmed, MS Parvej, S Akhter, WK Ansari and MS Ali (2015). Frequency of drug resistant *Escherichia coli* isolated from commercial broiler chicken in Bangladesh. International Journal of Natural and Social Sciences, 2(4): 01-05.

1999; Komp et al., 2003).Various types of molecular works on *E. coli* have been done throughout the world with antibiotic sensitivity pattern of isolates (Alexa et al., 2011; Cookson et al., 2006; Fratamico et al., 2004). In Bangladesh, there are many reports on isolation and characterization of *E. coli* from foods, vegetables, rectum of different animals (Singh et al., 2012, Zinnah et al., 2007). In these cases, pathogenic *E. coli* was isolated from fruits, vegetables, diseased animals or human being. However, drug resistant *E. coli* is the major problem in poultry industry of Bangladesh. Indiscrimination use of the antibacterial drugs may lead to serious hazards of drug resistance by entering the resistant organisms in human food chain through chicken meat or chicken by products. There is growing concern over the transmission of resistant bacteria through the food chain and to that effect, the W.H.O. has recognised that antibiotic use in animals affects antibiotic resistance in human (Anonymous, 2000). The present study was designed to provide information on the present status of antibiotic resistance patterns in *E. coli* in apparently healthy broiler birds sold in Bangladesh.

MATERIALS AND METHODS

Collection of samples

Samples were aseptically collected from apparently healthy birds following a convenience sampling method without repetition of any birds. Sterile cotton buds were used for the collection of swab samples, and the swab was transferred to nutrient broth instantly. The liver samples were collected after slaughtering of birds. The samples were transported to the Bacteriology Laboratory at the Department of Microbiology and Hygiene, Sylhet Agricultural University.

Cultural and biochemical examination

The nutrient broth containing swab samples were incubated for 6 hours at 370 C for bacterial multiplication. After incubation samples from the nutrient broth were cultured on to EMB agar, MacConkey agar, Salmonella –Shigella agar (SS agar), Brilliant green agar (Nazir et al., 2005). Isolated organisms with specific growth characteristics of *E. coli* in different culture media were subjected to sugar (dextrose, fructose, maltose, lactose and sucrose) fermentation, MR-VP and indole production test following the procedure mentioned by Buxton and Fraser (1978).

DNA Extraction

Bacterial DNA was extracted from the isolates using the method described by Queipo-Ortun et al. (2008) with little modification. Briefly, the organisms were cultured onto nutrient broth. After overnight incubation at 37^0C 1 ml of broth culture were taken in a ependrof tube, centrifuged at 10000 rpm for 2 minutes, discarded supernatant and the pellet cells were mixed with 200µl of deionized water. The mixture was then heated in boiling water for 10 minutes followed by dipping into ice for 10 minutes and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and used as template DNA in PCR.

PCR for the confirmation of *E. coli*

The *E. coli* were confirmed by PCR using primers specific to *E. coli* 16S rRNA gene). ECO-1 (5´-GACCTCGGTTTAGTTCACAGA-3´) and ECO-2 (5´-CACACGCTGACGCTGACCA-3´) were used as forward and reverse primer, respectively for amplification target sequence of 585bp. PCR reaction was performed following the procedure described by Schippa et al. (2010) with slight modification. A 25µl reaction mixture was prepared that containing 12.5 µl of 2 X PCR master mixes (Promega, USA), 1 µl of each primer (10 pmol/ µl), 2 µl of template DNA and 8.5 µl of nuclease free water. After initial incubation at 95^oC for 3 min, a 30-cycle amplification protocol was followed as 94^oC for 45 s, 58^oC for 45 s and 72^oC for 60 s, and a final extension step of 72^oC for 3 min. The PCR products was resolved in 1.5% agarose gel. After electrophoresis the gel was stained for 10 minutes in ethidium bromide and distained with water for visualization.

Antibiotic sensitivity test

Antibiotic sensitivity test of isolated *E. coli* was performed with the standardized commercial antibiotic discs (Oxoid, UK) following Disc

Diffusion Method (Bauer et al., 1966). Sensitivity to antibiotic was studied on Muller Hinton agar plates (Himedia, India) with Ceftriaxone, Gentamycin, Erythromycin, Penicillin, Cephalexine, Amoxicillin, Nalidixic Acid. An amount of 0.1 mL freshly grown pure culture of *E. coli* was poured on agar plates and allowed to spread gently over the entire surface with a glass rod spreader. After 5 minutes, the discs were placed at a distance of about 1 cm apart and incubated at 370C 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 and analyzed according to CLSI (2012).

RESULTS AND DISCUSSION

Prevalence of *E. coli*

In the present study, 43.75% (35) cloacal swabs and 35% (7) liver samples were found positive for *E. coli* following cultural, morphological, biochemical and molecular methods. The overall prevalence of *E. coli* in broiler birds was 42% in study area. Himi et al. (2015) reported 54% *E. coli* in layer chicken from Bangladesh, Nazir (2004) stated the overall prevalence was 62.5% from chicken but Bhattacharjee et al. (1996) reported 40.82% prevalence of *E. coli* in chicken from Bangladesh which is closed to the present findings.

Cultural, morphological and molecular tests

All the isolates upon overnight incubation at 37° C produced purple black colored colonies with characteristic metallic sheen on EMB agar (Figure 1), large bright pink colored colonies with lactose fermentation on MacConkey agar (Figure 2), slight pinkish colonies on SS agar (Figure 3) (Kalin et al., 2012, Hasina, 2006; Derakhshantar and Ghanbarpour, 2002). In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative short rod arranged in single or paired. All the isolates fermented 5 basic sugars with the production of both acid and gas. The isolates were positive to MR and indole production but negative to VP test. All isolates possessed *E. coli* specific 16S rRNA gene were amplified at 585 bp in PCR using ECO-1 and ECO-2 primers (Hassan et al., 2014).

Figure 1*: E. coli* produced with metallic sheen in EMB agar.

Figure2*: E. coli* produced bright pink colored colonies in MacConkey agar.

Figure-3: On Salmonella-Shigella agar the *E. coli* produced slight pinkish colonies.

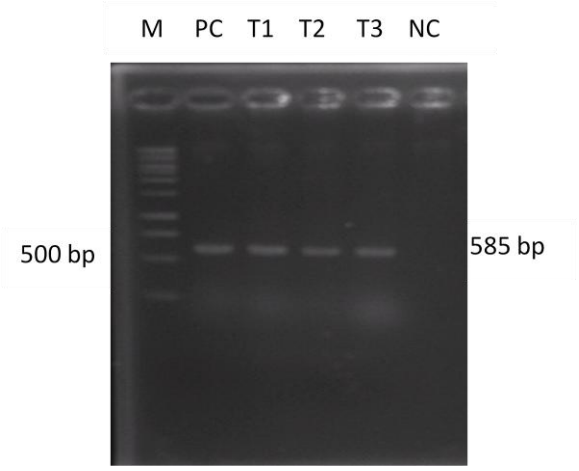

Figure-4: Representative PCR image of *E. coli* targeting *16S rRNA* gene. M = 1kb DNA Ladder, PC = Positive control, T1-T3 = Tested samples, NC = Negative control.

Antimicrobial resistance has grown to become a global problem (Gunner et al., 2004). Inappropriate use in terms of overuse and misuse of antibiotics both in humans, veterinary use and agriculture is considered the most important factor promoting the emergence, selection and dissemination of antibiotic resistant micro organisms in both veterinary and human medicine (Neu, 1992; Witte. 1998; Gunner et al., 2004). Large proportions of antibiotics (50%) of the total global consumption are administered to food producing animals in growth promotion purposes, but 80% of such total administration is unnecessary (Harrison and Lederberg, 1998). It is also known that resistant faecal *E. coli* from poultry can infect humans both directly and through food, by colonizing the human intestinal tract and contributing resistant genes to human's endogenous flora (Van Den Bogaard et al., 2001). In the present study, 100% isolated *E. coli* were found sensitive to Ceftriaxone and most of the isolates were resistant to Gentamycin, Erythromycin, Penicillin, Cephalexine, Amoxicillin, Nalidixic Acid. Hossain et al. (2008) isolated *E. coli* from broilers and reported that 100% were resistant to nalidixic acid, 97.14% to cloxacillin, 91.42% to erythromycin and 62.85% to ampicillin. Nazir et al. (2005) isolated *E. coli* from poultry and found resistant to nalidixic acid, cloxacillin, erythromycin and ampicillin. Though, Prescott and Baggot (1993) reported good activity of erythromycin against some gram negative bacteria. We found about 100% resistant against Gentamycin, Erythromycin, Penicillin, Cephalexine, Amoxicillin and Nalidixic Acid whereas Al-Ghamdi et al. (2001) found 34.7% resistant to ciprofloxacin. Based on present study, it may be concluded that, Ceftriaxone is the first choice of treatment against E.coli infection in poultry farm at Sylhet region.

The practice of indiscriminate use of antibiotics in food producing animals has undesirable consequences on human health because of the presence of drug residue in foods, thus jeopardizing the effectiveness of the treatment of bacterial, fungal and parasitic infections worldwide (Weber, 1979; Cosgrove and Carmeh, 2003). It may be noted that the drug sensitivity may be valuable as background information for future therapy for the effective control of this bacteria in poultry and human food chain. This study presents a good example of typical use of antimicrobial agents by most poultry farmers in Bangladesh.

CONCLUSION

Pathogenic *E. coli* may produce disease in poultry if the immunity of poultry is reduced due to various reasons. The environmental condition of the farm may be a reason for the highest prevalence of the bacterium in poultry, which have been transmitted to human via meat. The environment inside and outside of the farm should be kept clean and the feed supply should be carefully monitored. Ceftriaxon would be the choice of drug against *E. coli*. However, the findings from this work support the need for a critical review of the usage of antimicrobial agents in livestock in Bangladesh and the importance of taking concrete steps in terms of policy to curtail the indiscriminate use of antimicrobial agents in a bid to prevent the possible adverse consequences in animal production, as well as in humans. There is an urgent need to formulate a policy and put the necessary plan in place to execute a policy targeted at the promotion of rational use of antimicrobial agents, as an important element in antimicrobial resistant containment and the dose of antibiotic to poultry should be carefully controlled

as the intermediate sensitive bacterium can gain resistance to antibiotics.

REFERENCES

Alexa PL, Konstantinova and Sramkova ZZ (2011). Faecal shedding of verotoxigenic *E. coli* in cattle in the Czech Republic. Veterinary Medicine, 56: 149-155

Buxton A, and Fraser G (1978). Animal Microbiology. Vol. 1. *E. coli*, Balckwell Scientific Publications, Oxford, London, Edinburg, Melbourne. pp: 94-102

CDC (2012). *Escherichiau coli*. CDC National Center for Emerging and Zoonotic Infectious Diseases. Accessed on 2012-10-02.

Cookson AL, Taylor SC and Attwood GT (2006). The prevalence of Shiga toxin-producing *E. coli* in cattle and sheep in the lower North Island, New Zealand. Newzealand Veterinary Journal, 54:28-33.

Clinical and Laboratory Standards Institute. January 2012. Vol. 32 No. 3.

Fratamico PM, Bagi LK, Bush EJ and Solow BT (2004). Prevalence and characterization of Shiga Toxin-Producing *E. coli* in Swine feces recovered in the National Animal Health Monitoring System's Swine 2000 Study. Applied Environmental Microbiology, 70:7173–7178.

Hasina B (2006). Enteropathotypic characterization of *E. coli* isolated from diarrhoeic calves and their antibiogram study. M.S. Thesis, Department of Microbiology and Hygiene, BAU, Mymensingh, p. 68.

Nazir KHMNH (2004). Molecular base of diversified *E. coli* isolates potentiating antibiotic resistant pattern and compromising epidemiology. MS Thesis submitted to the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh.

Nazir KHMNH, Rahman MB, Nasiruddin KM, Akhtar F, Khan MFR and Islam MS (2005). Antibiotic sensitivity of *E. coli* isolated from water and its relation with plasmid profile analysis. Pakistan Journal of Biological Science, 8:1610-1613.

Queipo-Ortun MI, Colmenero JDD, Macias M, Bravo MJ and Morata P (2008). Preparation of Bacterial DNA Template by Boiling and Effect of Immunoglobulin G as an Inhibitor in Real-Time PCR for Serum Samples from Patients with Brucellosis. Clinical Vaccine Immunology, 15: 293-296.

Schippa S, Iebba V, Barbato M, Nardo GD, Totino V, Checchi MP, Longhi C, Maiella G, Cucchiara S and Conte MP (2010). A distinctive 'Microbial signature' in celiac pediatric patients. BMC Microbiology, 10: 175.

Singh A, Khan MSR, Saha S, Hassan J and Roy U (2012). Isolation and Detection of Antibiotic Sensitivity Pattern of *E. coli* from Ducks in Bangladesh and Nepal. Microbes and Health, 1: 6-8.

Zinnah MA, Bari MR, Islam MT, Hossain MT, Rahman MT, Haque MH, Babu SAM, Ruma RP and Islam MA (2007). Characterization of *E. coli* isolated from samples of different biological and environmental sources. Bangladesh Journal of Veterinary Medicine, 5:25-32.