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PREVALENCE AND CHARACTERIZATION OF SHIGATOXIGENIC *ESCHERICHIA COLI* IN BROILER BIRDS IN MYMENSINGH

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ABSTRACT

Shigatoxigenic *Escherichia coli* (STEC) are major food-borne pathogens. They transmit to human through contaminated meat and meat products of animals and poultry, and frequently associated with various types of human illness including haemolytic uremic syndrome. This preliminary study showed the prevalence of STEC in 60 cloacal swab samples of live healthy broiler chickens collected randomly when sold at a wholesale market in Mymensingh district of Bangladesh. Isolation and identification of *E. coli* was carried out using Eosin Methylene Blue (EMB) agar media and 16S rRNA gene specific polymerase chain reaction (PCR). Among the 60 samples, 49 (81.67%) were found positive to *E. coli.* These *E. coli* isolates were screened for the detection of STEC by PCR using *stx*1 and *stx*2 gene specific primers. Among the 49 positive samples, 5 (10.20%) were found positive for *stx*1 gene, and 26 (53.06%) were positive for *stx2* gene. In addition, 6 (12.24%) isolates were found positive to both *stx*1 and *stx*2 genes, and the remaining 12 (22.46%) were negative. The high prevalence of STEC in the broiler chicken alarms the public health impact as the people are always in close contact with these live broiler chickens in the open market as well as processing of meat at home before cooking. However, further studies are required to uncover the major source(s) for the transmission of STEC to human in rural Bangladesh.

Keywords: Shigatoxigenic *E. coli*, prevalence, characterization, cloacal swab, broiler

INTRODUCTION

In Bangledesh, due to almost assured and quick return in a relatively short period of time, poultry rearing is considered superior to the other sectors in agriculture. The poultry industry comprising of commercial poultry with broiler and layers plays an important role in the development of economy in Bangladesh. In Bangladesh, the total chicken population is about 259.79 million (Bangladesh Economic Review, 2014). The sector has already proved itself as a potential income generation and poverty alleviation as well as improving human nutrition through the supply of meat and eggs to their daily life and also contributes 2.79% GDP (BBS, 2008).

Poultry sectors across the globe are affected by various bacterial pathogens including avian pathogenic *Escherichia coli* (APEC) (Bélanger *et al*., 2011). Among these APEC, some produce shiga toxin-producing *E. coli* (STEC) and also known as verotoxin producing *E. coli* (VTEC) causing disease in humans and animals. The common feature of STEC is the production of shiga toxins (Stx) that are considered to be the major virulence factors.

There are two major groups of shiga toxins: Stx1 and Stx2, expressed by *stx*1 and *stx*2 located in the genome of lambdoidprophages (Friedman and Court, 2001). The most common sources for shiga toxin are the bacteria *Shigella dysenteriae* and the STEC (Beutin *et al*., 2006; Spears *et al*., 2006). STEC are becoming an ever increasing problem as an etiological agent of food-borne gastrointestinal disease. Stx is characterized by the cytotoxicity due to disruption of protein synthesis within host cells. STEC strains are capable of causing human illness, especially haemorrhagic colitis, bloody diarrhoea and haemolytic uraemic syndrome (HUS). Although most sporadic cases and outbreaks have been reported from developed countries, human infections also have been described in Latin America, India and other developing countries (Kaddu-Mulindw *et al*., 2001**;** Leelaporn *et al.*, 2003). STEC may be transmitted through faecal material contaminated water or food, or through direct contact with animals or persons (Paton and Paton, 2002). Several works have been done in Bangladesh regarding the isolation and molecular characterization of shiga toxin producing *E. coli* from the intestinal contents and meat of cattle, diarrheic human patients and from the intestinal contents and meat of cattle diarrheic human patients,

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the environment by several researchers (Hossain *et al.*, 2011; Islam *et al.*, 2008; Alam *et al.*, 2006). To the best of our knowledge, no work has yet conducted for the isolation and molecular characterization of STEC from cloacal swab sample of broiler birds in Bangladesh. This study was undertaken to determine the occurrence of STEC in cloacal swab samples of healthy broiler chickens sold in rural markets, and evaluation of their public health significance.

MATERIALS AND METHODS

Sample collection

The swab samples from cloacae (n=60) were collected randomly from different local live broiler bird markets of Mymensingh district, Bangladesh, following a convenient sampling method. Properly sterile cotton buds were used for the collection of swab samples. After collection, the swab was instantly transferred to nutrient broth and kept in ice box. The swab samples were transported to the Bacteriology Laboratory at the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh.

Cultural and biochemical examination

Overnight incubation of the nutrient broth containing swab samples was done at 37°C. Then samples from the nutrient broth were cultured on Eosin Methylene Blue (EMB) agar medium (Zadik *et al*., 1993). One colony from each EMB plate that showed metallic sheen on EMB agar was subjected to sugar (dextrose, maltose, lactose, sucrose, and mannitol) fermentation, Methyl Red-Voges Proskauer (MR-VP) and indole production tests following the procedures described by Nazir *et al*. (2005) and Elafify *et al*. (2016).

DNA Extraction and molecular identification

DNA was obtained from the isolates using boiling method (Queipo-Ortun *et al.*, 2008). In brief, 100 µl de-ionized water was taken into an eppendrof tube. A pure bacterial colony from overnight culture on 37°C of EMB agar was properly mixed with the de-ionized water. The tube was then transferred to boiling water and boiled for 10 minutes then immediately kept on ice for 10 minutes and then centrifuged at 10,000 rpm for 10 minutes. Supernatant was collected and used as DNA template. The DNA sample was kept in -20°C until used.

The isolated organisms that were suspected to be *E. coli* by their cultural and biochemical characteristics were confirmed as *E. coli* by PCR with primers specific to *E. coli* 16S rRNA gene (Table 1). DNA extracted from known *E. coli* was sued as PCR positive control while water was used as the negative control. PCR was performed following the procedure described by Hassan *et al.* (2014). A 25 µl reaction contained 1x *Taq* Polymerase PCR master mix and 0.4 µM final concentration of each primer. Electrophoresis of the PCR products was done using 2% agarose gel. After electrophoresis, the gel was stained for 10 minutes in ethidium bromide for visualization in UV Tran-illuminator.

Isolates that were found positive for *E. coli* 16SrRNA genes were further screened for *stx*1 and *stx*2 by PCR with primers (Table 1) specific for *E. coli stx*1 and *stx*2 genes (Talukdar *et al*., 2013) with slight modification. There was 25 µl reaction containing 1x *Taq* Polymerase PCR master mix and 0.4 µM final concentration of the primers (*stx*1 or *stx*2). Thermal profile for the detection of *stx*1 and *stx*2 consisted of an initial denaturation at 94^{o}C for 5 min, followed by denaturation at 94^{o}C for 1 min, annealing for 1 min (61^{o}C for *stx*1, 59^{o}C for *stx*2) and extension at 72^{o}C for 1 min (total 30 cycles), with a final extension at 72^{o}C for 5 min. PCR amplification was performed using a thermo cycler (Eppendorf Personal, Germany). Appropriate PCR positive (DNA from STEC) control and non-template negative control were also used in each PCR reactions.

Table 1. List of primers used for the detection of *E. coli* 16S rRNA gene, *stx*1 and *stx*2 genes

Primer Name	Sequence (5' - 3')	Amplicon Size (bp)	Target gene
ECO-1	GACCTCGGTTTAGTTCACAGA	585	16S rRNA
ECO-2	CACACGCTGACGCTGACCA		
stx 1F	CAC AAT CAG GCG TCG CCA GCG CAC TTG CT	606	*stx*1
stx 1R	TGT TGC AGG GAT CAG TCG TAC GGG GAT GC		
stx 2F	CCA CAT CGG TGT CTG TTA TTA ACC ACA CC	372	*stx*2
stx 2R	GCA GAA CTG CTC TGG ATG CAT CTC TGG TC		

RESULTS AND DISCUSSION

This study revealed the presence of *E. coli* in 81.67% (49/60) cloacal swabs collected from healthy broiler birds (Table 2). The overall prevalence of this study supported the findings of Jakaria *et al*. (2012), who described a prevalence of 78.86% in the apparently healthy chicken in Myemnsingh district. In another study, Roy *et al*. (2012) isolated *E. coli* from 52% samples comprising internal organs of broilers.

These 49 *E. coli* (one isolate per sample) were examined further for the presence of *stx*1 and *stx*2 genes by PCR to identify STEC. Among them, 5 (10.20%) isolates were found positive for *stx*1 alone (Figure 1), while 26 (53.06%) for *stx*2 alone (Table 2 and Figure 2). In addition, 6 (12.24%) were found positive for both *stx*1 and *stx*2. On the other hand, the remaining 12 (24.28%) isolates were found as non-STEC, since they were found negative for both *stx1* and *stx*2 genes (Table 2). Supporting our present findings, low prevalence of shiga-toxin was also reported by Rehman *et al*. (2014).

Contaminated meat is considered as the major source for the outbreak of STEC in human. The occurrence of STEC in the broiler chicken sold in retail market is alarming to the rural public health of Bangladesh as they are always in close contact with these broiler chickens in the wholesale market and /or domestic meat processing. So far, this is the first report on the occurrence of STEC in broiler chicken in Bangladesh, and further studies are required to uncover the major source(s) for the transmission of STEC to human in Bangladesh.

Table 2. Prevalence of shigatoxigenic *E. coli* in cloacal samples

Total number of samples	16S rRNA gene	Number of PCR positive samples (N=49)			
		*stx*1	*stx*2	Both *stx*1 and *stx*2	Non-STEC
60	49 (81.67%)	5 (10.20%)	26 (53.06%)	6 (12.24%)	12 (24.28%)

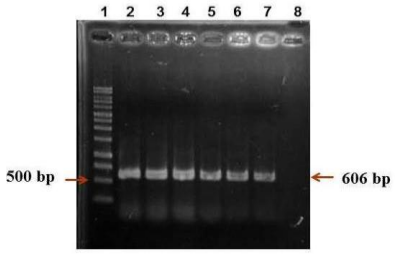

Figure 1. PCR based detection of *stx1* gene. Lane 1: 1kb DNA marker; lane 2 to 6: *E. coli* isolates; Lane 7: Positive control, Lane 8: Negative control

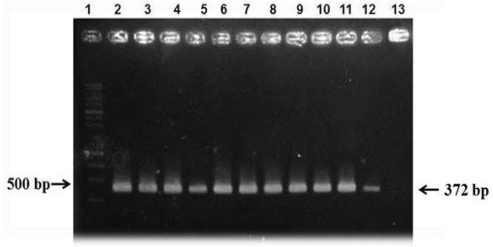

Figure 2. PCR based detection of *stx*2 gene. Lane 1: 1kb size DNA marker; lane 2 to 11: E. coli isolates; Lane 12: Positive control, Lane l3: Negative control

CONCLUSIONS

The STEC has been successfully detected by PCR from apparently healthy broiler chicken sold in retail market in Mymensingh, Bangladesh. The high prevalence of STEC in the broiler chicken has great public health significance since these birds can be a potential source for human STEC infection.

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