Frontiers in Environmental Microbiology
2018; 4(1): 16-21
http://www.sciencepublishinggroup.com/j/fem
doi: 10.11648/j.fem.20180401.13
ISSN: 2469-7869(Print); ISSN: 2469-8067(Online)

Detection of Multidrug Resistant and Shiga Toxin Producing *Escherichia coli* (STEC) From Apparently Healthy Broilers in Jessore, Bangladesh

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To cite this article:
Jasmin Akter Runa, Mohammed Bakhtiar Lijon, Mohammed Abdur Rahman. Detection of Multidrug Resistant and Shiga Toxin Producing *Escherichia coli* (STEC) From Apparently Healthy Broilers in Jessore, Bangladesh. *Frontiers in Environmental Microbiology*. Vol. 4, No. 1, 2018, pp. 16-21. doi: 10.11648/j.fem.20180401.13

Received: December 26, 2017; **Accepted**: January 19, 2018; **Published**: February 2, 2018

Abstract: The research work was undertaken for detection and determination of antibiogram profile of *Escherichia coli* (*E. coli*) that produce Shiga toxin from apparently healthy broilers (n=8) from different commercial farms of Jessore, Bangladesh. Broiler cloacal swabs (n=8) were collected by inserting the sterile swab stick in the cloacae of broiler and inoculated into peptone water for enrichment for 24 hours at 37°C. Enriched culture was streaked onto Eosin Methylene Blue (EMB) agar for isolation of pure bacterial culture. Then pure bacterial culture was streaked onto Mac-Conkey (MC) agar to identify Gram negative bacteria. Cultural method, Gram staining, biochemical reaction and polymerase chain reaction technique were used to identify the bacteria. The antibiogram profiles of bacteria were investigated against 6 commonly used antibiotics (Ciprofloxacin, Ampicillin, Colistinsulphate, Erythromycin, Neomycin and Penicillin) by disc diffusion method. Five *E. coli* isolates were identified and Shiga toxin producing *E. coli* (STEC) was detected by amplifying 372-bp fragment of Stx2 gene in polymerase chain reaction (PCR) assay. The prevalence of the Shiga toxin generating *E. coli* (STEC) in broiler cloacal swab was 62.5%. All isolates (100%) were resistant to Ampicillin, Colistinsulphate, Erythromycin, Neomycin and Penicillin and sensitive to Ciprofloxacin. The findings of this research strongly imply that broiler harbor multidrug resistant and Shiga toxin producing *E. coli* (STEC) which may cause public health problem if enter into human food chain.

Keywords: Antibiogram, *Escherichia coli*, PCR Assay, Prevalence, Shiga Toxin

1. Introduction

E. coli is a gram-negative, rod-shaped and facultative anaerobic bacterium belongs to *Enterobacteriaceae* family [1] commonly found in gastrointestinal tract of human and animals including poultry [2]. It is pathogenic to both human and poultry [3] to some extent although it helps to hinder colonization of pathogenic bacteria in the intestine [4], aids in digestion and can benefit their hosts by producing small amounts of vitamins B_{12} and K_2. Most isolates of *E. coli* are nonpathogenic but existence of this bacterium provides evidence of faecal contamination of food. Coliforms are pathogenic and opportunistic serotypes that found in intestine around 10-15%. It causes a variety of lesions in immune compromised hosts along with poultry [5]. Some serogroups of *E. coli* generally classified into six subgroups including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli*, enterhemorrhagic *E. coli* (EHEC), enteroadherent *E. coli* and diffusely adherent *E. coli* [6]cause disease and food poisonings. The EHEC strains are one of subset of Shiga toxin (Stx) producing *E. coli* (STEC) strains which is isolated from patients [7]. STEC has recently been found from broiler in Bangladesh [8]. Food contaminated with

STEC strains is the main cause of human infections [9]. STEC are responsible for severe clinical symptoms such as hemorrhagic colitis (HC) and the potentially lethal hemolytic uremic syndrome (HUS) [10]. *E. coli* having Shiga toxin is distinguished by the production of Cytotoxin that break up protein synthesis with in host cells. For their activity on Vero cells these toxins are called Verocytotoxins. In Bangladesh misused of antibiotic has been extensively increased either to promote the growth or to control infectious disease of poultry. Emergences of multi-drug resistant *E. coli* are continuously increasing because of the greater abuse of antibiotic [11]. Antibiotic may be the guide to the appearance and transmission of resistant *E. coli* that can pass into people through food or direct contact with infected animals. These resistant organisms may play a vital role in the dissemination of antimicrobial resistance to human pathogens [12]. Aims of the research work were (i) isolation, identification and detection of Shiga toxin generating *E. coli* (STEC) from broiler and (ii) determination of antibiogram profile of Shiga toxin generating *E. coli* (STEC) against six commonly used antibiotics.

2. Materials and Methods

2.1. Collection of Sample

A total of 8broiler samples were collected from different commercial farms at Jessore, Bangladesh in Period of January to June, 2013. The samples were transported to Department of Microbiology, Jessore Science and Technology University (JSTU) for bacteriological study.

2.2. Processing and Enrichment of Samples

The sterile swab sticks was inserted into the cloacae of the broilers and placed in 5 ml peptone water. Samples were mixed well by vortex mixer machine separately and resulting solution was then incubated at 37°C overnight for enrichment.

2.3. Isolation of Bacteria

One loopfull of enrichment culture of cloacal swab was separately streaked duplicate onto selective media for *E. coli* such as Eosin Methylene Blue (EMB) agar that was incubated aerobically at 37° C for 24 hours. When all plate shown monotypeof colony distinctly, it was considered as pure. To purify the isolates repeated streaking onto EMB agar plates were carried out. Pure bacterial culture streaked onto differential media such as Mac-Conkey agar (MC) to differentiate between gram negative and gram positive bacteria. Finally 5 well-spaced colonies were selected for further study according to their cultural and morphological characteristics.

2.4. Identification of Bacteria

On the basis of cultural characteristics and colony morphology on the EMB agar and MC agar bacteria were identified. Gram's staining method [13], Motility test [14], Sugar fermentation test [15] and biochemical tests such as: Oxidase test [16], Catalase test [17], Citrate utilization test [16], Indole test [15], Voges-Proskauer (VP) test [18] and Methyl red reaction [18] were carried out for bacterial identification.

2.5. Molecular Detection of Bacteria

A genus specific PCR method was conducted to identify Shiga toxin generating *E. coli* (STEC) by amplifying 372-bp fragment of *Stx2* gene using previously published primers [19].

2.6. Antibiotic Sensitivity Test

Five isolates were tested for antimicrobial drug susceptibility against six different antibiotics such as: Ciprofloxacin, Ampicillin, Colistinsulphate, Erythromycin, Penicillinand Neomycin (Himedia, India). The antibiotics sensitivity test was performed according to instructions of the Clinical and Laboratory Standard Institute (CLSI) [20].

3. Results and Discussion

3.1. Isolation of E. coli

Colony characteristics of *E. coli* observed in EMB and MC agar and found green metallic sheen colony in EMB agar and rose pink lactose fermented colony in MC agar (Figure 1). Similar Colonial feature were reported by [21, 22]. In Gram's staining isolated bacteria were found Gram negative short rod in single or paired arranged (Figure 2) which was supported by several authors [21-23].

Table 1. *Prevalence of E. coli in broiler.*

Name of Samples	Number of samples tested	Number of culture positive samples	Number of PCR positive samples	Prevalence (%) of *E. coli* of the study
Broiler cloacal swabs	8	5	5	62.5

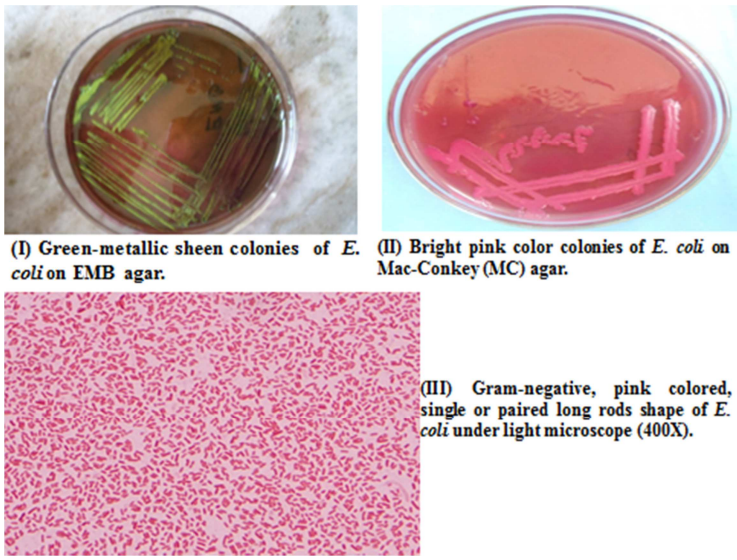

Figure 1. *Cultural properties of E. coli on EMB (I) and MC (II) agar and Gram's staining characteristics of E. coli under light microscope (III).*

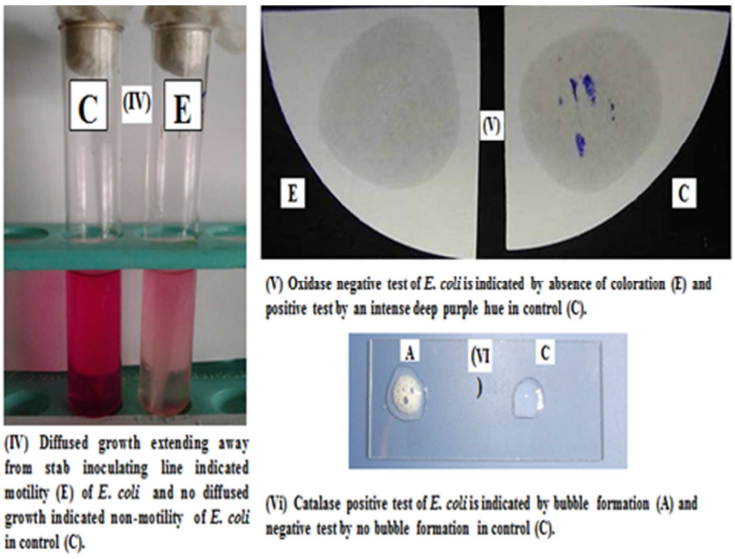

Figure 2. *Motility (IV), Oxidase (V) and Catalase (VI) test results of E. coli.*

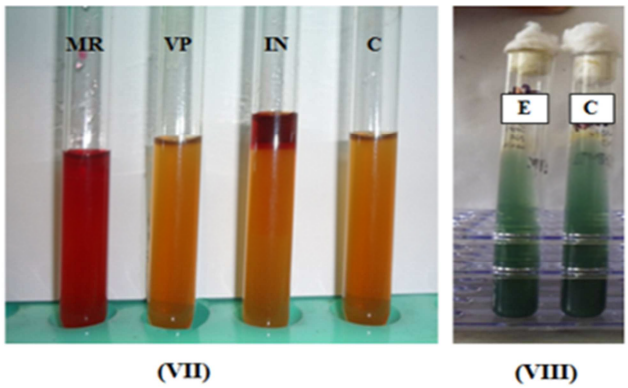

Figure 3. *Results of IMViC tests of E. coli. (VII) Development of red color of the broth indicates the positive Methyl red test (MR), No change of color of the broth indicates negative Voges-Proskauer test (VP), red color band at the junction of broth indicates the positive Indole test (IN), No change of color of the control broth (C). (VIII) No change of green color of the citrate medium to blue color indicates negative Citrate test (E) and no change of color of medium in control (C).*

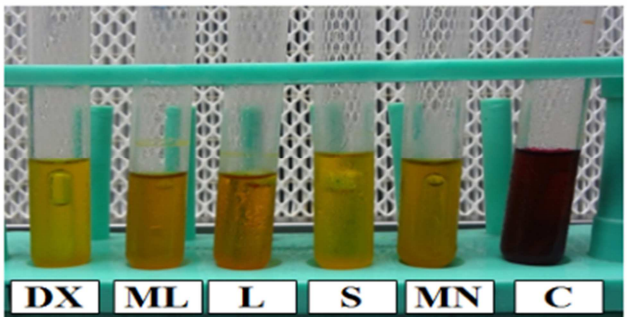

Figure 4. *Results of sugar fermentation tests of E. coli. E. coli fermented dextrose (DX), maltose (ML), lactose (L), sucrose (S) and mannitol (MN) with the production of both acid and gas. No change of color of sugar broth in control (C).*

3.2. Identification of E. coli

All the isolates of *E. coli* were exhibited the motile feature in MIU media (Figure 2). Motile feature of *E. coli* in MIU

media wasdescribed by the authors [24]. The isolates revealed negative reaction on Oxidase test and positive reaction on Catalase test (Figure 2). Negative Oxidase test and positive Catalase testresults were also reported by other authors [23, 25] in their research work. The isolates exhibited positive reaction in Indole test, Methyl Red test and Citrate test but negative reaction in Voges-Proskauer test (Figure 3) which were reported by many authors[21-23, 25]. In this study, the isolates of *E. coli* were found to ferment the five basic sugars with the production of both acid and gas (Figure 4). Similar sugar fermentation test results were reported by several authors [21, 22, 25]. Results of sugar fermentation, motility and biochemical testsare summarizes in the Table (2).

Table 2. *Summary of sugar fermentation, motility and biochemical test results for E. coli.*

Name of tests	Results of this study	Results of Bergey's Manual**	Results of other investigators		Interpretation
			Results	References	
1. Sugar fermentation tests profiles using					
Dextrose (DX)	AG	AG	AG	[22]	
Sucrose (S)	AG	AG	AG	[22]	
Lactose (L)	AG	AG	AG	[23]	
Maltose (ML)	GA	AG	AG	[22]	
Mannitol (MN)	AG	AG	AG	[22]	
2. Motility test using MIU media	Motile	Motile	Motile	[24]	
3. Biochemical test					*Escherichia coli*
Oxidase	+	+	+	[23]	
Catalase	+	+	+	[23]	
Citrate	−	−	−	[23]	
Indole	+	+	+	[23]	
MR	+	+	+	[23]	
VP	−	−	−	[23]	

Legend: DX= Dextrose, ML= Maltose, L= Lactose, S= Sucrose, MN= Mannitol; AG=Acid and Gas, + = Positive, − = Negative, **= Bergey's Manual of Determinative Bacteriology [25]

3.3. Detection of Shiga Toxin Producing E. coli (STEC)

A genus specific PCR method was carried out to screen Shiga toxin generating *E. coli* from broiler. DNA extracted from green metallic sheen colony grown on EMB agar successfully amplified 372-bp fragment of Stx2 gene confirmed broiler bacterial isolates are *E. coli* and produced Shiga toxin (Figure 5). Our result of PCR was coincided with the findings of other authors [26, 27]. The amplification of Stx2 gene from broiler represents the pathogenic from of *E. coli* that have public health importance where threat like bloody diarrhea, life-threatening hemolytic-uremic syndrome and hemorrhagic colitis. This evidence supported by [28, 29].

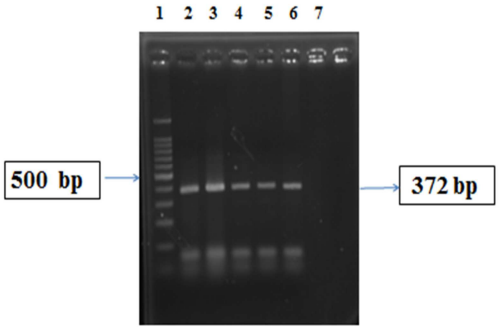

Figure 5. *PCR of Stx2 gene of E. coli. Lane 1: 100 bp DNA ladder, Lane 2, 3, 4, 5 and 6: Tested Sample, Positive for Stx2 gene, lane 7: Negative Control.*

3.4. Prevalence of Shiga Toxin Producing E. coli

Five (n=5) *E. coli* were isolated, identified and detected from the broiler cloacal swab. The prevalence of *E. coli* in cloacal swab of broiler was 62.5% (Table 1)which is similar to the findings of the author [30]. 66% prevalence of *E. coli*was reported by the author [23] in cloacal swab of broiler which is closed to the present findings. Another researcher [22] also reported 63.6% prevalence of *E. coli* in broiler.

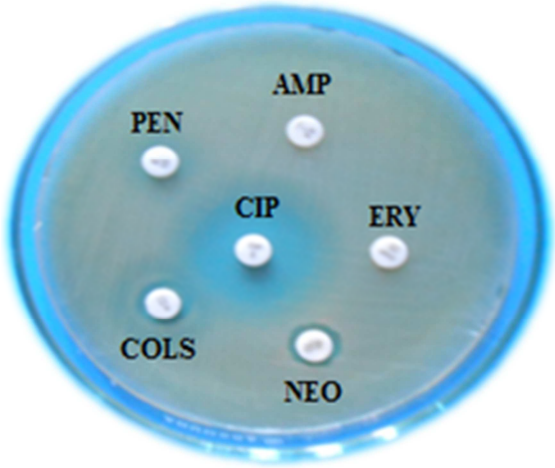

Figure 6. *Antibiogram profiles of E. coli against ampicillin (AMP), penicillin (PEN), ciprofloxacin (CIP), erythromycin (ERY), colistinsulphate (COLS) and neomycin (NEO). The isolates were found resistant to ampicillin, penicillin, erythromycin, colistinsulphate and neomycin and sensitive to ciprofloxacin.*

3.5. Antibiogram profile of Shiga Toxin Producing E. coli (STEC)

On the basis of zone of inhibition *E. coli* isolates were found sensitive against Ciprofloxacin (CIP) (Figure 6). This finding coincides with the previous result of [23, 31].

Resistance of *E. coli* was observed against Erythromycin (ERY), Ampicillin (AMP), Neomycin (NEO), Penicillin(PEN) and Colistinsulphate (COLS) and found the entire isolates of broiler to shown resistance properties (Figure 6). The result was supported by several authors[23, 31, 32]. Reckless use of antibiotics made the organisms resistant against several antibiotics. Antibiogram profiles of STEC shown in Table (3).

Table 3. *Summary of antibiogram profiles of E. coli against six commonly used antibiotics.*

No. of isolates tested	Antibiogram profiles of *E. coli*																	
	AMP			PEN			CIP			ERY			COLS			NEO		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
5	5	0	0	5	0	0	0	0	5	5	0	0	5	0	0	5	0	0

Legend: AMP: Ampicillin, PEN: Penicillin, CIP: Ciprofloxacin, ERY: Erythromycin, COLS: Colistinsulphate, NEO: Neomycin

4. Conclusion

Outcome of this research work indicated that broiler harbors Shiga toxin generating *E. coli* (STEC). Current study suggest that multidrug resistant *E. coli* is prevalent in broiler of the study area which may cause public health hazard if enter into the food chain. The occurrence of the bacteria should strongly beadvised as deleterious for health and recommended the preventing risk factors. Ciprofloxacin was proved to be the best antibiotics to treat *E. coli* infection as it was highly effective.

Conflict of Interest

The authors declare that they have no competing interests.

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