

# Virulence and Antibiogram Study of *Escherichia coli* Isolated from Faecal Sample of Buffalo

Raghavendra Prasad Mishra<sup>1\*</sup>, Udit Jain<sup>1</sup>, Janardan K Yadav<sup>1</sup>, Barkha Sharma<sup>2</sup>, Saraswati Ojha<sup>3</sup> and Mohd Saif<sup>4</sup>

<sup>1</sup>Department of Veterinary Public Health, DUVASU, Mathura, Uttar Pradesh, INDIA <sup>2</sup>Department of Veterinary Epidemiology and Preventive Medicine, DUVASU, Mathura, Uttar Pradesh, INDIA <sup>3</sup>Department of Livestock Products Technology; (DUVASU), Mathura, Uttar Pradesh, INDIA <sup>4</sup>Department of Pharmacology and Toxicology; (DUVASU), Mathura, Uttar Pradesh, INDIA

\*Corresponding author: RP Mishra; Email: rmishra523@rediffmail.com

**Received:** 23 Nov., 2017

Revised: 05 May, 2018

Accepted: 15 May, 2018

#### ABSTRACT

The study was undertaken for detection of virulence gene using polymerase chain reaction (PCR) and investigate their antibiotic sensitivity pattern in fecal sample of buffalo. In present study, a total of 180 samples were processed for isolation of verocytotoxic *E. coli* (VTEC). Out of 180 samples, 19 samples were found positive for VTEC. All VTEC strains were subjected to antibiotic sensitivity test using 16 antibiotic discs. Of these, ciprofoxacin and imipenem were found highly sensitive (100%) against VTEC strains followed by ceftriaxone (89.47%), gentamicin (84.21%), amikacin (84.21%) and chloramphenicol (78.94%). Least sensitivity was recorded for enrofloxacin (78.94%), followed by cefixime (63.15%), erythromycin (57.84%) and tetracycline (52.63%).

Keywords: antibiotic sensitivity test, Vercytotoxic E. coli. faecal sample, PCR

Diarrhea is one of the most common and multifactorial disease of man and animals mainly caused by E. coli (Kumar et al., 2013). E. coli is the most commonly observed gastrointestinal flora of animals and environmental contaminant considered as important food borne pathogen causing serious complications in man and animals (Malik et al., 2013; Dhama et al., 2013; Ankita et al., 2014). Verocytotoxic E. coli (VTEC) was first identified as a distinct group of E. coli, which had the ability to produce toxins with profound and irreversible effect on vero cells. VTEC is also termed as shiga-like toxin producing E. coli (SLTEC) or shiga toxin producing E. coli or STEC. Acronym STEC is derived from the fact that the toxins are shiga like that is similar to those produced by Shigella dysenteriae type1 (Brien et al., 1987). The EHEC belong to the Verocytotoxin producing E. coli (VTEC). VTEC always do not induce clinical signs and are not enterohaemorrhagic until addition virulence factor are present like enterohemolysin and adherence factors

(intimin). The adherence factor(s) enables the organism to attach to and colonize intestinal mucosal cells (Hiruta *et al.*, 2001). Among VTEC, serotype O157:H7 has been closely associated with the sporadic and clinical outbreaks of hemorrhagic colitis (HC), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in human beings (Croxen and Finlay, 2010; Gyles and Fairbrother, 2010; Sanchez *et al.*, 2013). Healthy domestic ruminants are recognised as the main natural reservoir of STEC and large game animal maybe healthy carriers of STEC (Diaz *et al.*, 2011; Sanchez *et al.*, 2010).

Antimicrobial drug resistance in bacterial isolates that have potential to enter our food supply is a growing public health concern. Antibiotics are being frequently incorporated as sub therapeutic and animal food supplements to cure and prevent disease in animals. This non judicious use of antibiotics generates a selective pressure that has led to the emergence of antibiotic resistance in the microbes



including *E.coli* (Ajayi *et al.*, 2011). Considering the impact of pathogenic *E.coli* on humans, animals and its considerable public health significance, the present study was conducted to discern the virulence factors and antibiotic susceptibility profile of *E.coli* isolated from faeces of apparently healthy cattle as well as environment (soil) from dairy farms of Uttar Pradesh. Keeping in view the importance of this organism, the present study was planned to reveal the genotypic study of VTEC in faecal samples of domestic and wild ruminants.

# MATERIALS AND METHODS

### Sampling and isolation of E. coli

A total of 180 buffalo fecal samples were collected from Mathura district. The samples were collected aseptically in UV sterile polythene bags and immediately transported to the laboratory under chilled conditions for microbiological analysis. For primary isolation of E. coli (VTEC), 10gm of faecal sample were enriched in 90 ml modified trypticase soya broth (mTSB) containing acriflavine (10 mg/l) to reduce the growth of gram positive organism. The method used for collection of materials, and isolation and identification techniques were made as per the lines suggested by World organization for Animal Health (OIE, 2014). These samples were incubated at 37°C for 6 h. MacConkey's Agar (MCA) was used as differential media, while Eosin methylene blue (EMB) agar (Hi-Media, India) was used as selective media. Suspected E. coli strains were subjected to morphological, cultural and biochemical characterization as per the standard method (Ewing, 1986).

#### Molecular characterization

Multiplex Polymerase chain reaction (PCR) was used for detection of virulent genes ( $stx_p$ ,  $stx_2$ , eaeA and hlyA) of VTEC. All the *E. coli* isolates were subjected to genomic DNA isolation. The bacterial growth in TSB broth (HiMedia) was centrifuged at 3000 rpm for 15 min to make the pellet of bacterial cells. These cells were washed twice with PBS (pH 7.4) to remove any impurity of broth media. Bacterial DNA was extracted by using DNA extraction kit (Genei, Bangalore) as per the manufacturer's protocol. For the PCR reaction, PCR Master Mix solution (Genei, Bangalore) was used. To amplify DNA targeted to virulent genes ( $stx_p, stx_2$ , eaeA and hlyA) of VTEC by using primers on 3µl of DNA template in 25µl reaction mixture (Paton and Paton, 1998). After an initial denaturation step at 95°C for 4 min, 30 amplification cycles were performed, each consisting of 94 °C for 2 min., 65 °C for 2 min. and 72°C for 1.5 min and followed by a final extension step at 72°C for 2.5 min. After the amplification, amplicons were separated in 1.5% gel in tris acetate EDTA (TAE) buffer at 60 volt for 80 min, stained with 0.5% ethidium bromide solution and visualized under ultraviolet light.

## Antimicrobial sensitivity of E.coli

Antibiogram of all isolates of VTEC were performed by the disc diffusion method (Fig. 2) of (Bauer et al. 1966) using 16 commonly used antibiotics viz. Amikacin (30µg), Ampicillin (10µg), Amoxycillin (30µg), Cefixime (5 µg), Streptomycin (10µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Enrofloxacin (10µg), Erythromycin (10µg), Gentamicin (10µg), Tetracyclin  $(10 \mu g),$ Norfloxacin (10µg), Ofloxacin  $(10 \mu g),$ Ceftriaxone (30µg), Imipenem (10µg). Levofloxacin (5µg). The sensitivity was determined on the basis of zone of inhibition around the discs as per the chart supplied by the firm (Hi-Media).

#### **RESULTS AND DISCUSSION**

Out of 180 faecal sample, a total of 180 *E. coli* strains were obtained. All the strains of *E. coli* were screened to detect the presence of VTEC genes using multiplex PCR (Fig. 1). An overall prevalence of VTEC in Buffalo faecal sample was found to be 10.56% (19/180). Out of 19 VTEC, 6 VTEC was found to be positive for  $stx_1$  gene (180 bp) and 7 VTEC for  $stx_1$  and  $stx_2$  (180 bp and 255 bp), 2 VTEC were found positive for  $stx_1$  with *eaeA* (384 bp) gene and 4 were positive for  $stx_n$ , stx, and *eaeA* gene.

In the present study as given in Table 1. Ciprofoxacin and Imipenem was found to be cent percent sensitive. Ceftriaxon (89.47%), Gentamicin (84.21%), Amikacin (84.21%), Chloramphenicol (78.94%) and Norfloxacin (73.68%) were found sensitive against VTEC strains. Antibiotics Enrofloxacin (78.94%) showed highest resistance pattern followed by Cefixime (63.15%), Erythromycin (57.84%) and Tetracycline (52.63%).

Sl. No.	Antimicrobial Disc	Sensitive (n = 19)	Intermediate (n = 19)	Resistant (n = 19)
1	Ampicillin	12 (63.15%)	1 (05.26%)	6 (31.57%)
2	Chloramphenicol	15 (78.94%)	2 (10.52%)	2 (10.52%)
3	Ciprofloxacin	19 (100%)		
4	Erythromycin	6 (31.57%)	2 (10.52%)	11 (57.84%)
5	Gentamicin	16 (84.21%)	2 (10.52%)	1 (05.26%)
6	Norfloxacin	14 (73.68%)	3 (15.78%)	2 (10.52%)
7	Ofloxacin	12 (63.15%)	3 (15.78%)	4 (21.05%)
8	Imipenem	19 (100%)		
9	Tetracycline	5 (26.31%)	4 (21.05%)	10 (52.63%)
10	Streptomycin	7 (36.84%)	3 (15.78%)	9 (47.36%)
11	Amikacin	16 (84.21%)	1 (05.26%)	2 (10.52%)
12	Amoxicillin	11 (57.84%)	2 (10.52%)	6 (31.57%)
13	Levofloxacin	13 (68.42%)	2 (10.52%)	4 (21.05%)
14	Cefixime	2 (10.52%)	5 (26.31%)	12 (63.15%)
15	Ceftriaxon	17 (89.47%)	1 (05.26%)	1 (05.26%)
16	Enrofloxacin	3 (13.51%)	1 (05.42%)	15 (78.94%)

Table 1: Antibiogram of the Verocytotoxic E.coli strains isolated from fecal sample of Buffalo



**Fig. 1:** Agarose gel showing PCR amplified product for VTEC genes isolates from Buffalo faecal sample

Lane 1: 100bp DNA Ladder; Lane 2, 13:  $stx_1$ ,  $stx_2$ ; Lane 3,4,5,6,7:  $stx_1$ ; Lane 9:  $stx_1$  and *eaeA*; Lane 11:  $Stx_1$ ,  $Stx_2$  and *eaeA* 

In previous study, the prevalence of VTEC in Buffalo were reported as 8.9% (Eriksson *et al.*, 2005). However, prevalence of VTEC in higher level was reported by previous workers as prevalence of VTEC was 16.66% (Parul *et al.*, 2014), 18% (Rogerie *et al.*, 2001) and 18.47% (Rabin, 1994). In contrast, investigations have shown a higher detection rate of 46% (Kobayashi *et al.*, 2001) in fecal samples of Buffalo. Lower isolation rate i.e.



Fig. 2: In vitro Antimicrobial drug sensitivity test for VTEC strains isolated from Buffalo fecal sample.

LE – Levofloxacin; IE – Imepenam; NX – Norfloxacin; C – Ciprofloxacin; AK - Amikacin.

9% (Balanco *et al.*, 1993). prevalence rate as low as 1.0% has also been reported (Khurana and Kumar, 2005).

High level of antimicrobial sensitivity was reported to Ciprofloxacin, Imipenem, Amikacin, Ceftriaxone and Gentamicin this was in accordance to the finding of (Zinnah, 2008; Bradford *et al.*, 1999) and (Chattopadhyay *et al.* 2003) also reported high sensitivity against ciprofloxacin



which show similar finding as in present study. Some drugs as Table 1, shows sensitivity pattern similar to (Sharma, 2004). Present study shows high resistance to Ampicillin, Amoxicillin, Streptomycin and Tetracycline. The findings of present study are in agreement with earlier reports (Ajayi, 2011; Abdulla et al., 2013; Lim et al., 2007). Finding of drug resistance of drugs shows in Table 1, was also found similar as given (Muffling et al., 2007). However Enrofloxacin resistant was reported by (Shah, 1989; Khan et al., 2002). This increasing resistance pattern of E.coli may be attributed to the over use and non judicious use of various antimicrobials. Commensal E.coli face various selective pressures in the environments of intestine which further favours the development, persistence and dissemination of robust strains that may be resistant to antimicrobial agent.

# **ACKNOWLEDGEMENTS**

The authors are highly thankful to Indian Council of Agricultural Research, New Delhi and Dean, College of Veterinary Science and Animal Husbandry, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigvan Vishvidhvalava Ewam Go-Anusandhan Sansthan (DUVASU), Mathura, U.P., India for providing necessary funds and facilities to carry out the investigations.

#### REFERENCES

- Abdullah, M., Akter, M.R., Kabir, S.M.L., Khan, M.A.S. and Aziz, M.S.I. 2013. Characterization of bacterial pathogens isolated from calf diarrhoea in Panchagarh District of Bangladesh. J. Agric. Food. Tech., 3(6): 8-13.
- Anita, Kumar, A., Verma, A.K., Gupta, M.K. and Rahal, A. 2014. Multi drug resistant pathogenic Escherichia coli in water sources and Yamuna river in and around Mathura, India. Pak. J. Biol. Sci., 17(4): 540-544.
- Ajayi, A.O., Oluyege, A.O., Olowe, O.A and Famurewa, O. 2011. Antibiotic resistance among commensals Escherichia coli isolated from feces of cattle in Ado- Ekiti, Nigeria. J. Ani. Vet. Adv., 10(2): 174-179.
- Bardiau, M., Gregoire, F., Muylaert, A., Nahayo, A. Duprez, J N., Mainil J. and Linden, A. 2010. Enteropathogenic (EPEC), enterohaemorragic (EHEC) and verotoxigenic (VTEC) Escherichia coli in wild cervids. J. Appl. Microbiol., 109(6): 2214-22
- Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Path., 45: 493-496.

- Blanco, M., Blanco, J., Blanco, J.E. and Ramos, J. 1993. Enterotoxigenic, verotoxigenic and necrotoxigenic Escherichia coli isolated from cattle in Spain. Am. J. Vet. Res., 54: 1446-1451.
- Brien, O.A.D. and Holmes, R.K. 1987. Shiga and Shiga-like toxins. Microbiol. Rev., 51: 206-220.
- Bradford, P.A., Petersen, P.J., Fingerman, I.M. and White, D.G. 1999. Characterization of expanded-spectrum cephalosporin resistance in E. coli isolates associated with bovine calf diarrheal disease. J. Antimicrob. Chemother., 44: 607-610.
- Chattopadhyay, U.K., Gupta, S. and Dutta, S. 2003. Search for shiga toxin producing Escherichia coli (STEC) including O157:H7 strains in and around Kolkata. Indian J. Med. Microbiol., 21: 17-20.
- Croxen, M.A. and Finlay, B.B. 2010. Molecular mechanisms of Escherichia coli pathogenicity Nat. Rev. Microbiol., 8: 26-38.
- Dhama, K., Rajagunalan, S., Chakraborty, S., Verma, A.K., Kumar, A., Tiwari, R. and Kapoor, S. 2013. Food-borne pathogens of Animal origin diagnosis, prevention and control and their zoonotic significance- A review. Pak. J. Biol. Sci., **16**(20): 1076-1085.
- Diaz, S., Vidal, D., Herrera-Leon, S. and Sanchez, S. 2011. Sorbitol fermenting, b-glucuronidase-positive, Shiga toxin negative Escherichia coli O157:H7 in free-ranging red deer in South-Central Spain. Foodborne Pathog. Dis., 8: 1313-1315
- Eriksson, E., Aspon, A., Gunnarsson, and Vagsholm, I. 2005. Prevalence of verotoxin producing Escherichia coli (VTEC) O157 in Swedish dairy herds. Epidemiol. Infect., 133: 349-358
- Ewing, W.H. 1986. The genus Escherichia, In: P. R. Edwards and W. H Ewing (ed), Edwards and Ewing's identification of enterobacteriaceae, 4 ed. Elsevier Science Publishing Co Inc., New York, pp. 93-122.
- Gyles, C.M. and Fairbrother, J.M., 2010. Escherichia coli In: Gyles, C.L., Prescott, J.F., Thoen, C.O. (Eds.), Pathogenesis of bacterial infections in animals. Blackwell Publishing, pp. 267-308.
- Hiruta, N., Murase, T., Okamura, N., 2001. An outbreak of diarrhea due to multiple antimicrobial resistant shiga toxin producine E.coli O26:h7 In a nursery. Epidemiol. Infect., **127**: 221-227.
- Hiroshi, A., Sou-ichi, M., Toshikazu, S., Teizo, T., Hisao, K., Tetsuya, I. and Kouich T. 2013. Detection and genetical characterization of shiga toxin-producing Escherichia coli from Wild Deer. Microbiol. Immunol., 42(12): 815-822.
- Khan, A., Yamasaki, S., Sato, T., Ramamurthy, T., Pal, A., Datta, S., Chowdhary, N.R., Das, S.C., Sikdar, A., Tsukamoto,

Journal of Animal Research: v.8 n.3, June 2018

T., Bhattacharya, S.K., Takeda, Y. and Nair, G.B. 2002. Prevalence and genetic profiling of virulence determinants of non O157 Shiga toxin-producing *Escherichia coli* isolated from cattle, beef and humans, Calcutta, India. *Emerg. Infect. Dis.*, **8**: 54-62.

- Khurana, P. and Kumar, A. 2005. Occurance of verotoxic *E. coli* in faeces and milk of Cattle. *Harayana Vet.*, **44**: 83-85.
- Kobayashi, H., Shimada, J., Nakazawa, M., Morozumi, T., Pohjanvirta, T., Pelkonen, S. and Yamamoto, K. 2001. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* from healthy cattle in Japan. *Appl. Environ. Microbiol.*, 67: 484-489.
- Kumar, A., Verma, A.K., Sharma, A.K. and Rahal, A. 2013. Presence of extended spectrum Beta-lactamases producing alpha hemolytic *Escherichia coli* in yellow-wattled Lapwing (*Vanellus malabaricus*). Asian J. Anim. Sci., 7(2): 64-69.
- Lim, S.K., Lee, H.S., Nam, H.M., Cho, Y.S., Kim, J.M., Song, S.W., Park Y.H. and Jung, S.C. 2007. Antimicrobial resistance observed in *Escherichia coli* strains isolated from fecal samples of cattle and pigs in Korea during 2003–2004. *Int. J. Food Microbiol.*, **116**: 283–286.
- Mailk, S., Kumar, A., Verma, A.K., Gupta, M.K., Sharma, S.D., Sharma, A.K. and Rahal, A. 2013. Incidence and drug resistance pattern of colibacillosis in cattle and buffalo Calves in Western Uttar Pradesh in India. *J. Anim. Health Prod.*, 1(1): 15-19.
- Muffling, V.T., Smaijlovic, M., Nowak, B., Sammet, K. Bulte, M. and Klein, G. 2007. Preliminary study of certain serotypes, genetic and antimicrobial resistance profiles of verotoxigenic *Escherichia coli* (VTEC) isolated in Bosnia and Germany from cattle or pigs and their products. *Int. J. Food Microbiol.*, 117(2): 185-91.
- OIE, 2004. Verocytotoxigenic *Escherichia coli. In:* Word Organization for Animal Health, Pairs manual of diagnostic tests and vaccines for terrestrial animals, 5<sup>th</sup> edition.

- Parul, Bist, B. Sharma, B. and Manjula. 2014. Prevalence of verotoxin producing Escherichia coli non O157 in diarrhoeic and healthy calves. One Health: Harnesting biotechnology for addressing veterinary and biomedical concerns on food safety, zoonoses and environment sustainability. 12<sup>th</sup> annual conference of IAVPHS, Guwahati, India. 245-46
- Paton, A.W. and Paton. 1998. Detection and Characterization of Shiga Toxigenic *Escherichia coli* by using multiplex PCR Assays for *stx*1, *stx*2, *eaeA*, enterohemorrhagic *E. coli hlyA*, *rfb* O111, and *rfb* O157 J. C. microbial, pp. 598–602.
- Rabin, B. 1994. Prevalence of verotoxic *Escherichia coli* in man animals, food and public health significance. M.V.Sc thesis, Deemed University, IVRI, Izatnagar.
- Rogerie, F., Marecat, A., Gambade, S., Dupond, F., Beaubois P. and Lange M. 2001. Characterization of Shiga toxin producing *E. coli* and O157 serotype isolated in France from healthy domestic cattle. *Int. J. Food Microbiol.*, 63: 217–223.
- Sanchez, S., Martínez, R., Rey, J., García, A., Blanco, J., Blanco, M., Blanco, J.E., Mora, A., Herrera-León, S., Echeita, A., Alonso, J.M., 2010. Pheno-genotypic characterisation of *Escherichia coli* O157:H7 isolates from domestic and wild ruminants. *Vet. Microbiol.*, **142**(3): 445-9.
- Sanchez, S., Sanchez, D.S., Martinez, R., Llorente, M.T., Herrera-Leon, S., Vidal, D., 2013. The new allelic variant of the subtilase cytotoxin (sub AB<sub>2</sub>) is common among Shiga toxin producing *Escherichia coli* strains from large animals and their meat and meat products. *Vet. Microbiol.*, **166**: 645-649.
- Sharma, D.K., Soni, S.S., Kashyap, S.K. and Shringi, B.N. 2004. Seroprevalence, antibiotic sensitivity pattern and transfer of plasmid coded characters of *E. coli. Indian Vet. J.*, 81: 6-8.
- Zinnah, M.A., Haque, M.H., Islam, M.T., Hossain, Bari, M.R., Babu, S.A.M., Rahman, M.T. and Islam, M.A. 2008. Drug sensitivity pattern of *Escherichia coli* isolated from samples of different biological and environmental sources. *Bangl. J. Vet. Med.*, 6(1): 13-18.