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# Detection of Shiga-Toxigenic *Escherichia Coli* in Milk Samples of Cattle by PCR

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***Abstract:*** *The purpose of this study was to determine the virulence genes and serotype of Shiga -toxin producing Escherichia coli (STEC) strains isolated from milk samples of cattle. A total of 51 milk samples collected from different unorganized farms in an around Guwahati city were screened for E. coli and out of these 51 samples 30 (58.82%) yielded E. coli. All the 30 E. coli isolates were subjected to screening for different virulent factors like stx1, stx2 and eae by PCR amplification using specific oligonucleotide primer. Seven (23.33%) of the 30 E. coli isolates carried at least one gene sequence among these groups: three isolate carried stx1 gene sequence and four carried eae gene sequence, no isolate carried the stx2 gene sequence. The stx1 positive serogroups were belonged to serogroups O118 (one isolate) and O153 (two isolates), and eae positive serogroups were belonged to O2 (four isolates) serogroups.*
***Keywords:*** STEC, *stx*1, *stx*2, *eae*

## I. Introduction

Shiga-Toxigenic *Escherichia coli* (STEC) is considered to be most common food-borne zoonotic pathogen causing various disease conditions in both animals and humans [1]. Ruminants are considered as important source of STEC with cattle being regarded as the primary reservoir [2, 3]. Humans may acquire STEC infections primarily from consumption of undercooked beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water contaminated with feces of animal [4,5,6]. The virulent strains of STEC are associated with one or more types of Shiga toxin (Stx1, Stx2 or Stx2 variants) as well as the property of producing intimin required for attachment- effacement lesions encoded by *eae* gene [2, 6,7].

There are at least 200 serotypes of *E. coli* that are capable of producing Shiga toxins [8,9,10]. However, of these serotypes *E. coli* O157:H7 is the most well-known to both microbiologists and the general public but several non-O157 STEC strains are also associated with production of shiga-toxin [1,2,8,11,12]. Isolation of O157:H7, O157: H- or the other STEC serotype from dairy cattle emphasize the role of raw milk as an important vehicle of transmission [3, 13]. Considering the public health importance of STEC, a study was undertaken to isolate the STEC from milk samples of cattle and molecular detection of certain virulence genes in the isolates.

## II. Materials and methods

**2.1. Isolation and Identification of *E. coli*:** A total of 51 milk samples were collected from different unorganized farm in and around Guwahati city. All samples were immediately streaked onto MacConkey's Lactose Agar (MLA) plates for primary isolation of *E. coli* as the method described by Collis and Lyne [14] and incubated aerobically at 37°C for 24 hours. Lactose fermenting colonies suggestive of *E.coli* were sub-cultivated on Eosin Methylene Blue (EMB) agar medium for purification and differentiation *E.coli* from other lactose fermenter. Colonies showing characteristic metallic sheen on EMB agar were identified as *E. coli* on the basis of staining characteristics as well as biochemical characteristics, *viz*. Indole, Methyl red, Voges proskaeur and citrate utilization (IMViC) as well as tests *viz.* sugar fermentation, urea hydrolysis, production of $H_2S$ [15].

**2.2. Serotyping of *E.coli*:** The isolated *E. coli* strains were serotyped on the basis of somatic (O) and flagellar (H) antigens in the National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli (Himachal Pradesh) as per Edwards and Ewing [15].

**2.3. Extraction of DNA:** The template DNA was obtained from each *E. coli* isolate as per the method of Titball *et al*. [16]. Briefly, 16-18 hours growth of *E.coli* were obtained in 2 ml Luria Bertoni (LB) broth. Broth cultures were centrifuged at 12000 rpm for 10 minutes at 4°C. The pellets were

suspended in 75 µl of Tris EDTA buffer and heated in boiling water bath at 100°C for 20 minutes, followed by snap chilling in ice for 20 minutes and finally centrifuged at 12000 rpm for 20 minutes at 4°C. The supernatant was collected without disturbing the sediment and directly used as template DNA for PCR for screening of virulence genes.

**2.4. Polymerase chain Reaction:** Extracted DNA were subjected to PCR for screening of *stx*1, *stx*2 and *eae* genes in the *E. coli* isolates by method recommended by Vidal *et al.* [17] using primers reported by various previous workers (Table 1).

PCR was carried out with a 25.0 µl final reaction volume in 2x Dream Taq Green PCR Master mix (Fermentas, Life Sciences), comprising of 4mM $MgCl_2$, 0.4mM of each dNTPs, 0.05units/ml of *Taq* DNA polymerase, 150 mM tris-HCL PCR buffer, 0.5µl(10 pmole) each of primer for *stx*1 and *eae* gene and 1.0µl (20pmole) of primer for *stx*1 gene, in 0.2 ml thin wall PCR tube. The whole reaction was carried out with a PCR conditions, 96°C for 4 min, 35 cycles of 95°C for 20 sec., 57°C for 20 sec., 72 °C for 1 min and followed by 72°C for 7 min in a Gradient thermal cycler (Techne, Germany).

The PCR amplified products were separated by electrophoresis with 1.8% (w/v) agarose gel containing ethidium bromide (0.5 µg/ ml) 1x TBE along with 100 bp ladder as a molecular weight marker. Electrophoresis was carried out at 85V for 1 hour. The amplified product was visualized as a single compact band of expected size under UV light (Fig.1) and documented by gel documentation system (Kodak Germany).

## III. Result and discussion

Out of total 51 milk samples tested, 30 (58.82%) yielded *E. coli*. Seven (23.33%) of the 30 *E. coli* isolates carried *stx1* and *eae* gene sequence among the group *stx1, stx2* and *eae*. Detection of shiga-toxigenic *E.coli* in the raw milk samples of apparently healthy cows in agreement with the findings of Kiranmayi *et al.*[18] who also detected STEC in 20 per cent raw cow's milk. Similar observation was also reported by Gaulin *et al.* [19]. Three isolates carried *stx1* gene sequence and four carried *eae* gene sequence, no isolate carried the *stx*2 gene sequence. The *stx1* positive serogroups were belonged to serogroups O118 (one isolate) and O153 (two isolates), and *eae* positive serogroups were belonged to O2 (four isolates) serogroups. The *stx*1, *stx*2 and *eae* genes encode for Shiga toxin 1, Shiga toxin 2 and Intimin respectively, which are the characteristics cytotoxins released by Shiga-toxigenic *Escherichia coli* (STEC).

## IV. Figures and tables

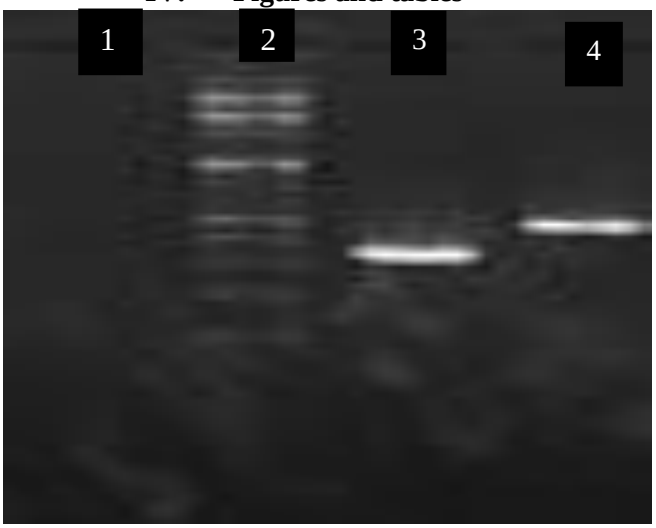


**Fig. 1. Gel Electrophoresis of STEC**
**Lane 1= Negative Control, Lane 2= 100 bp DNA ladder**
**Lane3= Sample +ve for *stx1* gene (348 bp, Lane4= Sample +ve for *eae* gene (482 bp)**

**Table 1. Details of primers used for PCR reaction**

| Primers | Sequence (5´- 3´) | Target gene | Amplicon (bp) | Reference |
|---|---|---|---|---|
| *stx*1(F)<br>*stx*1(R) | CAG TTA ATG TGG TGG CGA AGG<br>CAC CAG ACA ATG TAA CCG CTG | *stx*1 | 348 | Cebula *et al*,.1995 [20] |
| *stx*2(F)<br>*stx*2(R) | ATC CTA TTC CCG GGA GTT TAC G<br>GCG TCA TCG TAT ACA CAG GAG C | *stx*2 | 584 | Cebula *et al.*,1995 [20] |
| *eae*(F)<br>*eae*(R) | TCA ATG CAG TTC CGT TAT CAG TT<br>GTA AAG TCC GTT ACC CCA ACC TG | *eae* | 482 | Vidal *et al.*,2005 [17] |

## V. Conclusion

The findings of present studies assumed a special significance in terms of potencial public health hazards as these may be cross transferred to man via food chain. STEC strains are associated with haemorrhagic colitis (HC) and Haemolytic uraemic syndrome (HUS) in human and also in oedema disease in pigs. Raw milk may be easily contaminated with animal faeces and becomes a good source of infection for human if the milk is consumed without proper pasteurization and it is important from zoonotic point of view.

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