

# Molecular characterization of shigatoxin producing *Escherichia coli* isolated from camels and human (awners)

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## Abstract

This study is designed to evaluate molecular detection of virulent genes (sxt-1, sxt-2 and eae) in shiga – toxin producing *Escherichia coli*. The results showed that 20 out of 200 samples (10%) collected from camels and 5 out of 50 samples (10%) collected from awners (30 children and 20 adults) producing STEC 0157: H7 and give positive genes (stx-1, stx-2 and eae) from both STEC 0157: H7 isolated from cattle and human (awners)

**Keywords:** STEC 0157: H7 stx1,2, eae genes, camel and human

## 1. Introduction

Shiga toxin producing *Escherichia coli* ( STEC ) are described by their ability of producing one or more shiga like toxins. Shiga toxin were firstly described in *shigella dysenteriae*, the organism cause bloody diarrhea (1). at the end of seventh decade of twentieth century it was found that the culture filterates of some *E. coli* strains might be neutralized via antisera to shiga toxin of *shigella dysenteriae* (2) this observation indicated that a pathogenic strains of *E. coli* produce shiga like toxins (3) resemble shiga toxin produced by *shigella dysenteriae* and the genes for shiga toxin are located within DNA of *E. coli* when infected by bacteriophage, The DNA become inserted into the genome of the host bacterium allowing the production of shiga like toxin ( stx ) by *E. coli* (4). and STX family cover two immunologically non – cross – reactive groups titled stxI and stx II coded via stx1 and stxII genes both stx1 and Stx11 are able to induce toxic effect to vero cell (5) shiga toxin 1 may be divided into three subclasses stx Ia, stx Ic (6) and stx Id (7) its protein is extremely preserved and has a homology of more than 98% to stx of *shigella dysenteriae* ( type 1) (8) while stx2 is divided into eight subclasses on root of change in the amount of amino acid (9) These are stx 2a (10), stx 3c (11). stx2d (12), stx2dictivable, stx 2e(13) the extremely divergent stx 2f(11) and stx2g (13), shiga toxin 2 had 55% homology with stx 1(9), because of large variant amino acid arrangement of B subunit- stx bind to the Gb3 receptor by B subunit, Gb3 receptor is found in several organs such as kidney, liver, brain, and causing severe diseases (14) whereas, A subunit at by constraining protein synthesis (3,9), so lead to opoptosis due to signing by sxt or ribocytotoxic strain response.

It is found through epidemiological studies that the toxicity of stx-2 was more higher than stx-1 and the strains holding stx-2 are more dangerous than that hold ( stx-1 ( STEC) and associated with ( HUS) hemolytic uremic syndrome (15) so the present study aimed to identify and detecting the stx1, stx2 and

intimin in isolated strains of STEC 0157 from camels faeces and their awners ( adult and children ) fecal samples.

## 2. Materials and methods

Molecular characterization of *E. coli* 0157: H7

### Extraction of DNA

Genomic DNA of 0157 STEC separate was extracted by utilizing (pressio TM Minig DNA Bacteria Kits Geneaid U.S.A). it provides an efficient method for purifying total DNA cultured bacterial cells. This test was done according to the manufactures company.

### Measuring of pureness and concentrations of DNA

Purity and concentration of extracted DNA were measured by utilize Nano drop spectrophotometers.

### Primers

In this study five primers were obtained from Bioneer, Korea. Two of these primers were used to detect somatic Ags (0157 and H7) These primers were organized agreeing to data of the corporation STX / and Stx 2 genes primers that designed by (16) while intimin ( eae A) gene primer was designed by (17)

### PCR master mix preparation

All necessary chemicals were completely thawed and were stored into the ice. The substance was mixed thoroughly via inversion and spin them down previously to pipetting PCR mater mix response was prepared by utilizing PCR PreMix, Bioneer ( Korea ) according to corporation instructions.

### Detection of wzx (O-antigen – flippase) genes by using PCR

In order to detect O-serogroup associated with O157 STEC, an O serogroup targeting the wzx ( O-antigen ) gene was used according to (18) ( 1.5  $\mu$ l of equally forward and reversed ) were mixed with master mix

and 5  $\mu\text{l}$  of template DNA and 12  $\mu\text{l}$  of nuclease free water for completion the amplification mix to about 20  $\mu\text{l}$  according to Bioneer, Korea. The pipes containing an amplification mix were transported to thermocycler and programs started as follow. The amplification was conducted by original denaturation at 95C<sup>0</sup> for about 15 min. followed by thirty series of denaturations at 94C<sup>0</sup> for 30 sec. Primer annealing at 57 C<sup>0</sup> for about 1.5 min. followed by extension at 72 C<sup>0</sup> for about 1.5min and a final extension for 10 min at 72 C<sup>0</sup>. Amplified DNA was electrophoresed in an agarose gel.

### Detections of stx 1, stx2 and eaeA by using PCR

Detecting of stx1, stx2 and eaeA gene via PCR was done according to (19) The PCR amplification mixture was done according to Bioneer, Korea which comprises master mix, 5  $\mu\text{l}$  of the model DNA, 1.5  $\mu\text{l}$  of both forward and reversed primers and 12  $\mu\text{l}$  of nuclease free water for completion amplification mix to 20  $\mu\text{l}$  then transported to thermocyclers and programs stated as follow. Temperatures circumstances contained of an initial 95C<sup>0</sup> denaturations steps for about 3 minutes shadowed in 35 series of 95C<sup>0</sup> for about 20 sec. 58 C<sup>0</sup> for about 40 sec. and 72C<sup>0</sup> for about 90 sec. Last series was shadowed via 72C<sup>0</sup> gestation to about 5 min. Enlarged DNA trashes were resolute by gels electrophoresis

### PCR produce investigation (agarose gels electrophoresis):

It is an central for completion of PCR assay, that is used to analyze the PCR products via agarose gels electrophoresis as follows:

1. Two percent of agaroses gels were prepared 1X TBE cushion and heated by hot magnetic stirrer till each crystal was disappeared in the agarose.
2. Next chilling 3  $\mu\text{l}$  of ethidium bromide for each 100 ml gel solutions were added.

## 3. Results and Discussion

### polymerase reaction results:

#### Extraction of genome DNA of STEC 0157:

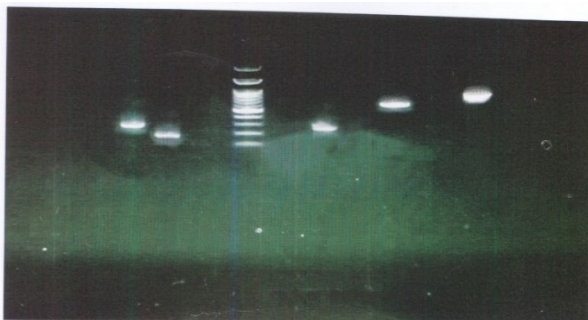


Figure 1: Agarose gel electrophoresis image showing the PCR product analysis of rfb 0157, fliCH7(625bp), Stx 1(180bp), stx 2(225bp) and eae-y1( 820bp) genes in STEC isolate from camel, Where M: marker (2000-100bp).

The DNA of isolated strains was extracted:

concentration and purity of the extracted DNA were measured by Nano drop spectrophotometer and the concentration of DNA ranged from 25- 189.9 ng/ml.

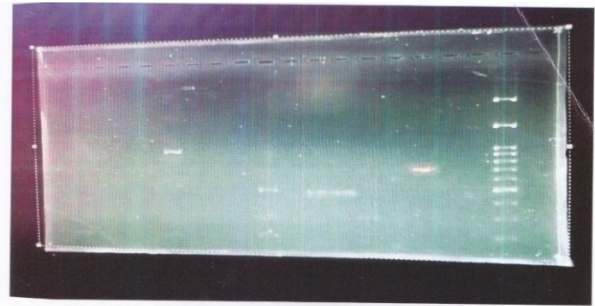


Fig -2: Agarose gel electrophoresis image showing the PCR product analysis of rfb0157 (259bp), fliCH7(625bp), Stx 1(180bp), Stx 2(225bp) and eae-y1(820bp) genes in STEC isolate from children. Where M: marker (2000-100bp).

The prevalence of virulence genes 0157 STEC have shown that 0157 STEC strains may carry the stx 1,2 and eae genes in both children and camels: these results are in agreement with (20), who reported that ( 60.0%) of STEC strains carried the stx1 gene, carried both stx1 and stx2 genes also this agreed with (21) Shiga toxin I gene was also higher in a study carried out by (22), in the study of (23), among STEC strains, (50%) isolates carried stx1, (16.67%) stx2 and 8(33.33%) carried both. (24) reported that stx2 gene was more prevalent than stx1 in STEC strains. Our study indicated positive result to eaeA gene which agreed (25,26). The majority of STEC strains pathogenic to human beings were eaeA-positive and eaeA has been identified as a risk factor for HUS development (27) reported that the diarrhetic calves and cattle represent an important reservoirs of eaeA positive. The association of eaeA-positive clinical STEC isolates and severe diarrhea and HUS has been documented (28) However outbreaks and cases associated with eaeA negative STEC strains were also reported (29)

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