*Biological Forum – An International Journal* **15(5a): 640-646(2023)**

**ISSN No. (Print): 0975-1130**
**ISSN No. (Online): 2249-3239**

# Detection of Shiga Toxin Producing *Escherichia coli* (STEC) O157-A Food and Waterborne Zoonotic Pathogens as Implications of One Health Perspective

***Darshan D. Patel[1*], Murtaza A. Hajoori[2] and Jignaben P. Naik[3]***

*[1]Bhagwan Mahavir Center for Advance Research, Bhagwan Mahavir University (BMU), BMEF Campus, Nr. Aakash E-space, Bharthana, Vesu, Surat (Gujarat), India.*

*[2]Bhagwan Mahavir College of Applied Science (BMCAS), Bhagwan Mahavir University (BMU), BMEF Campus, Nr. Aakash E-space, Bharthana, Vesu, Surat, (Gujarat), India*

*[3]Department of Microbiology & Medical Laboratory Technology, Shri JSB & Shri KMB Arts, Shri ANS Science & Shri NFS Commerce College, Affiliated VNSGU, Kholwad, Kamrej Char Rasta, Surat (Gujarat), India.*

*(Corresponding authors: Darshan D. Patel[*])*

*(Received: 01 May 2023; Revised: 08 May 2023; Accepted: 10 May 2023; Published: 15 May 2023)*
*(Published by Research Trend)*

**ABSTRACT: Specific *Escherichia coli* strain producing Shiga toxin (STEC) O157, is a food and waterborne pathogen with severe public health implications. From a zoonotic standpoint, STEC is the only pathogenicity group of chief interest, able to cause severe disease in humans when being transmitted through the food chain from their animal reservoirs. Shiga toxin-producing microbes are perceptible, plentiful and can exist in diverse environmental conditions. The diversity of microorganisms presents in environmental samples and the limitations of protein detection in the samples make it challenging to identify environmental STEC directly and specifically. We report the first-time isolation of this pathogen in the South Gujarat of India through standardized culture procedures and polymerase chain reaction (PCR). Environmental samples- food, water and animal excreta were cultured and STEC were selectively isolated and identified by sorbitol fermentability and β-glucuronidase activity. The latex agglutination test was used to detect O157 antigens, and multiplex PCR was carried out to detect virulence-related genes. A total of 386 *E. coli* isolates, 36 were Sorbitol non-fermenting (SNF), 30 were negative for β-glucuronidase activity. Among SNF isolates, 20 were confirmed STEC O157 by latex test. STEC that carried *eaeA* (10), *hlyA* (7), *stx1*(3) and *stx2*(5) genes were detected. Hence, this pathogen is a probable cause of food-waterborne disease in the study area, its investigation in both livestock and their products should be improved to characterize the impact of its zoonotic transmission and important for implications of one health.**

**Keywords:** Zoonotic *Escherichia coli*, STEC O157, Sorbitol non-fermenting, β-glucuronidase activity.

## INTRODUCTION

Zoonotic *Escherichia coli* (*E. coli*) represent a hazardous public health problem worldwide ever since identified as a food borne pathogen in 1982. STEC a specific *E. coli* strain producing Shiga toxin strains are the only pathogenic group of *E. coli* that has a definite zoonotic origin, with ruminants and, in particular, cattle being recognized as the major reservoir for human infections (Ferens & Hovde 2011). All STEC strains are pathogenic in humans, capable of causing at least diarrhoea. Among the STEC serotypes, *E. coli* O157 is one of the most important food and water-borne pathogens globally. STEC O157 infection in humans largely occurs through consuming contaminated food causing various human gastrointestinal tract diseases, including watery or bloody diarrhoea and might develop a life-threatening disease, such as haemorrhagic colitis, Thrombotic Thrombocytopenic Purpura and Haemolytic Uraemic Syndrome. Individual cases and outbreaks have also been associated with direct animal contact (e. g. farm visits), environmental contamination and human-to-human transfer via the faecal-oral route. The outcome of infection is dependent on several strain and host factors. In its report on the global burden of foodborne disease, WHO estimated that in 2010 food borne STEC caused more than 1.2 million illnesses, 128 deaths and nearly 13 000 Disability Adjusted Life Years (DALYs) (Kirk *et al.*, 2015; WHO, 2015, 2019). STEC public health microbiology extents the fields of medical, veterinary, food, water and environmental microbiology, requiring a "One Health" perspective and laboratory scientists with the ability to work effectively across disciplines (Caprioli *et al.*, 2015).

STEC infections are characterized by the production of Shiga toxins (Stx), so called because of their similarity with the toxin produced by *Shigella dysenteriae* serotype 1. These are also referred to as verocytotoxins-producing *E. coli* (VTEC) because of their cytotoxicity for Vero cells and enterohemorrhagic *E. coli* (EHEC) as infection resulting in bloody dysentery.

The highly virulent diarrheagenic STEC/EHEC/VTEC O157 serotype is distinguished from non-O157 *E. coli* (typical *E. coli*) by its inability to ferment sorbitol (SNF); thus, growing as colourless colonies on supplemented sorbitol MacConkey agar (SMAC). There are 2 major types Shiga toxin (Stx1 and Stx2) and the stx toxin genes are carried by lambdoid bacteriophages integrated into the *E. coli* chromosome. Many STEC are attaching and effacing (A/E) bacteria, which carry the *eaeA* gene on the locus of enterocyte effacement (LEE) and form distinctive lesions on the surfaces of intestinal epithelial cells. Virulence genes that have been associated with STEC include: the Shiga toxin genes ($stx_1$ and $stx_2$), the intimin (*eaeA*) gene, as well as the enterohemolysin (*hlyA*) gene (Paton & Paton 2002).

Regions with limited sanitation facilities have higher rates of infections with various enteric pathogens (Meena *et al.*, 2023). It is therefore important to identify different hosts and their relative contribution to pathogen shedding into the environment, and to assess the subsequent health risks to humans. To date, the potential of contaminated food, animal excreta and various water sources as reservoirs of SNF *E. coli* O157 has not been investigated in Surat, a highly industrialized area of South Gujarat region (Western India), where childhood diarrheal disease is common, coupled with close association of humans and food animals, and poor sanitation facilities, there is a need to identify reservoirs of STEC/SNF *E. coli/E. coli* O157.STEC-related diseases have been linked to consumption of contaminated food or water, direct contact with infected animals or environments. Monitoring is critical in order to devise mitigation strategies and empiric treatment of infections associated with the organisms and to indicate the threat of these organisms to public health in the study area this study was aimed.

## MATERIALS AND METHODS

*A. Study area and Sampling*

The present study was conducted in and around the city of Surat, a highly industrialized city in state of Gujarat, Western India, because of the public health importance of these infections. Human/animal faecal, soiled waste, food and water samples were collected over 15 months (January 2022 to April 2023). Samples were collected from food, water and animal excreta from different sources as per guidelines (WHO, 2003).

***Food:*** All vegetable and fruit samples were randomly collected in sterile plastic bags or container, from the local market of Surat city which were properly labeled and transported to the laboratory. Vegetables including green coriander, spinach, potatoes, green onion, green garlic, bottle gourd, brinjal, fenugreek, ginger, carrot, cucumber, cabbage and fruits including banana, mango, chikoo, papaya, watermelon and apple were collected. The meat includes chicken and goat meat and fish sample. Milk sample included in this study were cow and buffalo milk collected from dairy and cow farm.

***Water:*** Potable water samples like drinking water, lake water, well water, river water collected from different area of Surat city.

***Animal excreta and soiled waste:*** Fresh fecal sample was collected aseptically from caw, buffalo, sheep, goats, and their handlers into sterile container. Non potable water, sewage sample, soiled waste includes garbage, sludge from water supply or waste treatment plants, and other discarded materials obtained from industrial, commercial, mining, or agricultural operations, and from household and community activities were collected in sterile container.

*B. Transportation of sample*

After collection, all the samples were well labelled and transported to the laboratory immediately in an insulating foam box with ice or ice packs (4°C) to Microbiology laboratory, for bacteriological analysis.

*C. Sample processing*

Bacteriological analysis of samples was proceeded on the same day. Sample processing was carried out by following standard microbiological procedure for STEC detection.

***Enrichment:*** This selective enrichment procedure was to help in increasing the number of organisms & allowed the selective growth of *E. coli* from samples.1g of each solid sample was weighed out/1ml of each liquid sample was placed and homogenized into 9 ml of sterile modified EC broth supplemented with novobiocin (HiMedia, Mumbai, India), selective enrichment medium for *E. coli*. Inoculated EC broth was incubated at 37ºC for 18-24 hours followed by selective plating.

***Isolation by Plating on selective media:*** A loopful inoculum from EC broth was streaked onto MacConkey's agar and incubated at 37ºC for 24 hours for the characteristic pink colonies. Usually, STEC do not possess phenotypic characteristics that are distinguishable from those of other *E. coli*. However, an important exception to this is that STEC O157 are usually both unable to ferment sorbitol within 24 h of incubation and lack β-D-glucuronidase activity (March & Ratnam 1986). These characteristics are utilized in the routine selective isolation of STEC O157. The most widely used solid medium for the isolation of non-sorbitol-fermenting STEC O157 is sorbitol MacConkey (SMAC) agar. This medium contains 1% sorbitol in place of lactose in the standard MacConkey agar. Because of the inability to ferment sorbitol, STEC O157 grow after overnight incubation as colorless colonies (SNF) and can be distinguished from most of the remaining intestinal *E. coli* strains (SF) (around 75 to 94%) that ferment sorbitol and grow as pink colonies (March & Ratnam 1986). Inoculums from selective media MacConkey agar (MAC) were sub-cultured onto sorbitol MacConkey agar (SMAC)(HiMedia, Mumbai, India) by the use of replica plating techniques. These agar plates were incubated at 37°C for 18-24 hours.

***Presumptive Identification of E. coli:*** After incubation, a loopful of each colourless colony (SNF, presumptive *E. coli* O157)/pink colony (SF,

presumptive non-O157 *E. coli*) were subjected to identification as per procedure described by Abbott *et al*. (1994); Cheesbrough (1993, 2005). Presumptive *E. coli* colonies were inoculated onto Eosin Methylene Blue (EMB) agar (HiMedia, Mumbai, India) and plates were incubated at 37ºC for 18-24 hours for the characteristic metallic sheen specific of *E. coli*. The well separated pure colonies were picked up and used for standard morphological and biochemical tests (Vijayan *et al.*, 2017; Sinha *et al.*, 2015; Rasheed *et al.*, 2014; Cheesbrough 1993). Primary identification tests like Gram's staining and Motility test were performed. Secondary identification tests like Indole production (I), Methyl Red (MR) reaction, Voges Proskauer (VP) reaction, Citrate utilization test(C), fermentation of lactose and glucose using Triple Sugar Iron agar were proceeded (Fig. 1). All the *E. coli* isolates were stocked on nutrient agar stabs and stored at 4°C for further studies (Sharma *et al.*, 2019).

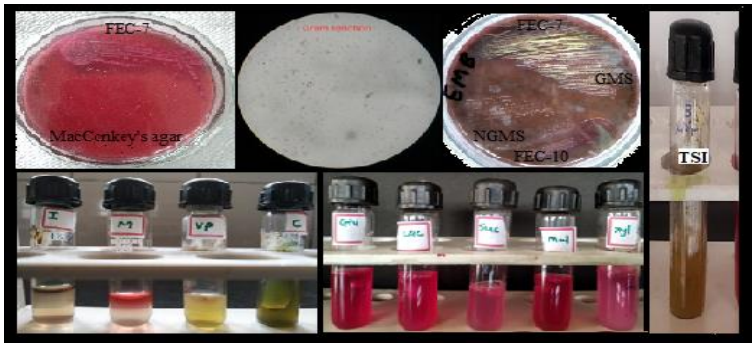


**Fig. 1.** Performed standard microbiological procedure for identification of *E. coli*. Colonies of pure cultures of the isolates on MacConkey agar- Lactose-fermenting (pink colonies); Gram negative; Isolates with characteristic greenish metallic sheen appearance on EMB agar; Confirmation of the isolates using biochemical tests including TSI (Triple Sugar Iron agar), and IMViC[indole(I) positive, methyl-red (MR) positive, VP negative, Citrate negative, fermentation of sugars produced both acid and gas], which are indicative of the identification of *E. coli.*

***Phenotypic Identification of STEC O157***: The confirmed SNF *E. coli* was tested for the presence of β-D-glucuronidase activity by using 4-methylumbelliferyl-beta-D-glucuronide containing EC broth (MUG-EC broth) as well as MUG-SMAC agar (HiMedia, Mumbai, India). SNF*E. coli* inoculated MUG-EC broth tube and sticked MUG-SMAC agarplat were incubated at 37° C for 24-48 hours and after incubation, examined under UV light. Absence of fluorescence in the tube indicated no activity of β-glucuronidase and was MUG test negative (Vijayan *et al.*, 2017) (Fig. 2).


**Fig. 2.** Performed microbiological test cultural response on SMAC for Sorbitol fermentability, MUG test for β-glucuronidase activity and combined MUG-SMAC test.

*D. Serological identification of E. coli O157 and non-O157 E. coli*

Phenotypical STEC O157 isolates (that phenotypically confirmed *E. coli*, being both sorbitol-nonfermenting and β-glucuronidase-negative) were tested for the presence of O157 antigens by latex agglutination with Hi *E. coli* O157™ Latex Test Kit (HiMedia, Mumbai, India) as shown in Fig. 3 (Vinay *et al.*, 2018). Presumptive STEC O157 colony was picked and emulsified in a drop of phosphate buffered saline (PBS) on the small circle at the base of the test ring reaction area. The latex suspension was well mixed using a loop and placed onto the circle on the appropriate test card. The test card was gently hand rocked and observed for agglutination within 1-2 mins. Agglutination indicated positive reaction and identified the *E. coli* serotypes.

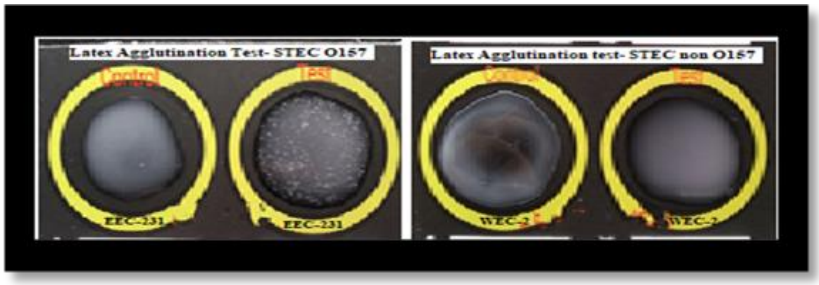


**Fig. 3.** Serological identification of STEC O157 by Latex agglutination test.

*E. Molecular confirmation of the isolates*

Finally, the isolates were confirmed with a number of PCR tests specific for identification of several characteristics of STEC O157, including the presence of *stx* genes (*stx*$_1$and *stx*$_2$), the intimin gene(*eaeA)*, the enterohemolysin gene(*hlyA*) (Vijayan *et al.,* 2017). *E. coli* isolates were processed for further confirmation by detection of genus specific gene & virulence genes by Polymerase Chain Reaction (PCR) (Prajapati *et al.,* 2015).

***Extraction of genomic DNA****:* DNA was extracted from overnight enriched cultures using HiPurA® Genomic DNA Purification Kits (HiMedia, Mumbai, India) by following manufacturers protocols. As per Sensitivity mentioned in insert yield of approximately up to 20 µg of pure DNA was extracted.

***Detection of virulence genes by multiplex PCR(mPCR)****:* Phenotypically confirmed *E. coli* isolates were genotypically confirmed *E. coli* by PCR with primers specific to *E. coli16SrRNA* gene. Isolates that found positive for *E. coli 16SrRNA* genes were further screened by mPCR targeting four virulence genes(*stx1, stx2, eaeA* and *hlyA*) with primers (Table 1), protocol and conditions (Table 2) as per the method described by Paton and Paton (1998) with slight alteration. The multiplex PCR is standardized using reference *E. coli* strain DNA obtained from ICMR-NICED, Kolkata. The mPCR was carried out in a final reaction volume of 25µl using 0.2ml thin wall sterile and nuclease free PCR tubes. (BIO-RAD, T100$^{TM}$Thermal Cycler, USA)

**Table 1: List of primers (5´-3´) used in multiplex PCR.**

| Target gene | Primer | Primer sequence (5'-3') | Size(bp) | Reference |
|---|---|---|---|---|
| *EC16SrRNA* | *EC16SrRNA* (F) | GACCTCGGTTTAGTTCACAGA | 585 | (Schippa *et al.,* 2010) |
| | *EC16SrRNA* (R) | CACACGCTGACGCTGACCA | | |
| *stx1* | *stx1* (F) | ATAAATCGCCATTCGTTGACTAC | 180 | (Paton & Paton 1998) |
| | *stx1* (R) | AGAACGCCCACTGAGATCATC | | |
| *stx2* | *stx2* (F) | GGCACTGTCTGAAACTGCTCC | 255 | |
| | *stx2* (R) | TCGCCAGTTATCTGACATTCTG | | |
| *eaeA* | *eaeA*(F) | GACCCGGCACAAGCATAAGC | 384 | |
| | *eaeA*(R) | CCACCTGCAGCAACAAGAGG | | |
| *hlyA* | *hlyA*(F) | GCATCATCAAGCGTACGTTCC | 534 | |
| | *hlyA*(R) | AATGAGCCAAGCTGGTTAAGCT | | |

**Table 2: Multiplex PCR (mPCR) protocol Steps and Cyclic conditions.**

| mPCR Protocol | | | mPCR Cyclic Condition | |
|---|---|---|---|---|
| Reaction mixtures | | Volume (µl) | PCR Steps | |
| Master mix | | 12.5 | Initial denaturation | 95°C – 10min |
| Primers (each) | Forward | 1.0 | Denaturation | 94°C – 45sec |
| | Reverse | 1.0 | Annealing | 60°C – 30sec |
| Template DNA | | 1.0 | Extension | 72°C –30sec |
| Nuclease free water | | Up to 25 | No. of cycles | 30 |
| Total volume | | 25 | Final extension | 72°C – 10 min |

On completion of the reaction the amplified products were analysed on agarose gel electrophoresis through 2% agarose gel stained with 5 µg/ml of ethidium bromide with a 50 bp DNA ladder as molecular size marker, visualized under UV light and results were noted (Sinha *et al.,* 2015). The PCR product was photographed by the gel documentation system (Infinity-STS-1150/26 MX Xpress, Eppendorf India Ltd.).

***Standard organisms for quality control****:* To monitor the performance of the laboratory test and ensure accurate test results, the standard strains of *E. coli* ATCC 25922 obtained from Department of Microbiology, Surat Municipal Institute of Medical Education & Research (SMIMER), Surat were used as control strains.

*F. Data management and analysis*

All raw data collected from the study were summarized, compiled, entered and coded in Microsoft Excel 2007 spreadsheet and analysed to present the different data types collected from the current study.

## RESULTS AND DISCUSSION

Initially, the *E. coli* was screened on the basis of characteristics colony morphologies on MacConkey agar. Out of the total of ≈800 examined samples, total 386 phenotypical *E. coli* were isolated and 36 isolates were identified STEC phenotypically (Table 3).

**Table 3: Categories, environmental source that yielded *E. coli* Isolates.**

| *E. coli* Categories | Environmental source yielded total no. of isolates | | | |
|---|---|---|---|---|
| | Food | Water & Waste | Animal Excreta | Total |
| **Presumptive *E. coli*** | 152 | 108 | 126 | 386 |
| **Phenotypical STEC** | 20 | 09 | 07 | 36 |
| **Serotype O157** | 10 | 04 | 06 | 20 |

**Table 4: Sample source type wise yielded *E. coli* isolates, Phenotypical STEC and Serotype O157.**

| Sample Type (n= 800) | Presumptive *E. coli* | Phenotypical STEC | Serotype O157 |
|---|---|---|---|
| **Food from plant sources (n=57)** | | | |
| Vegetables | 20 | 07 | 03 |
| Fruits | 20 | 04 | 01 |
| Other Raw food | 17 | 01 | 00 |
| **Food from animal sources (n=95)** | | | |
| Milk & milk product | 20 | 06 | 01 |
| Poultry | 24 | 01 | 01 |
| Meat | 23 | 01 | 04 |
| Fish | 28 | 00 | 00 |
| **Water & Waste (n=108)** | | | |
| Potable / Underground | 35 | 03 | 00 |
| Irrigation/ lake, pond, river | 26 | 02 | 02 |
| Drain/ Sewage | 35 | 04 | 02 |
| Soiled waste | 12 | 00 | 00 |
| **Animal excreta (n=126)** | | | |
| Cattle | 73 | 04 | 04 |
| Goat | 10 | 00 | 00 |
| Poultry | 43 | 03 | 02 |
| **Total** | **386** | **36** | **20** |

Detailed sample type wise to *E. coli* categories wise distribution were as shown in Table 4.

This study revealed the prevalence of *E. coli* was 48.25 %. Out of the total of 386 presumptive *E. coli* examined, 36Phenotypical STEC (9.32 %) and 20 (5.5%) were serotype *E. coli* O157, respectively. The sample type-based prevalence of *E. coli*, STEC and STEC O157 were as shown in table 4. Among the examined sample types, the highest STEC 12 out of 57 foods from plant source. Lowest prevalence rates of STEC were recorded from animal excreta (Goat) and soiled waste, respectively.

Potential virulence gene profile of environmental total *E. coli* isolates was shown in Table 5.

**Table 5: Distribution of Virulence genes- Shiga toxin producing genes (*stx*$_1$ and *stx*$_2$) and two other intimin gene (*eaeA)* and hemolysin gene (*hlyA*) in *E. coli* isolates (n=358 *16srRNA* positive).**

| Presence of Virulence gene in *E. coli* isolates | Sources of *E. coli* isolates | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Food (n=134) | | Water & waste (n=99) | | Animal excreta (n=125) | | Total (and/or all) (n=358) | |
| | No. | (%) | No. | (%) | No. | (%) | No. | (%) |
| *stx*$_1$ only | 0 | 0.00 | 0 | 0.00 | 3 | 2.40 | **3** | **0.84** |
| *stx*$_2$ only | 0 | 0.00 | 1 | 1.01 | 4 | 3.20 | **5** | **1.40** |
| *eaeA* only | 4 | 2.99 | 2 | 2.02 | 4 | 3.20 | **10** | **2.79** |
| *hlyA* only | 1 | 0.75 | 2 | 2.02 | 4 | 3.20 | **7** | **1.96** |
| *stx*$_1$ and *stx*$_2$ | 0 | 0.00 | 0 | 0.00 | 1 | 0.80 | **1** | **0.28** |
| *stx*$_1$ and *eaeA* | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | **0** | **0.00** |
| *stx*$_1$ and *hlyA* | 0 | 0.00 | 0 | 0.00 | 2 | 1.60 | **2** | **0.56** |
| *stx*$_2$ and *eaeA* | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | **0** | **0.00** |
| *stx*$_2$ and *hlyA* | 0 | 0.00 | 0 | 0.00 | 1 | 0.80 | **1** | **0.28** |
| *eaeA* and *hlyA* | 1 | 0.75 | 0 | 0.00 | 0 | 0.00 | **1** | **0.28** |
| *stx*$_1$, *stx*$_2$ and *eaeA* | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | **0** | **0.00** |
| *stx*$_1$, *stx*$_2$ and *hlyA* | 0 | 0.00 | 0 | 0.00 | 3 | 0.80 | **3** | **0.84** |
| *stx*$_1$, *stx*$_2$, *eaeA* and *hlyA* | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | **0** | **0.00** |
| **Total (and/or all)** | **6** | **4.48** | **5** | **5.05** | **22** | **17.60** | **33** | **9.22** |

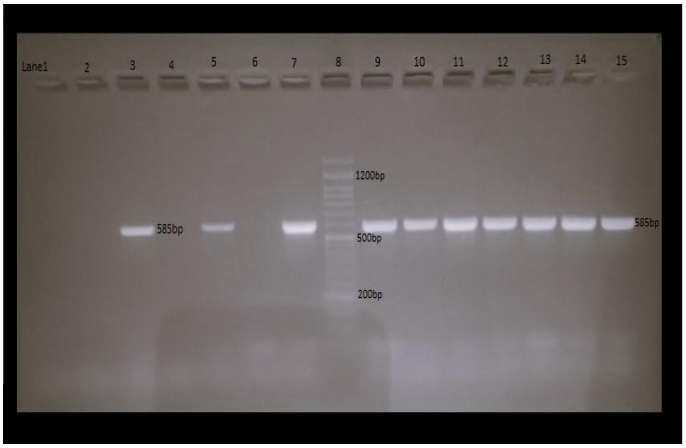


**Fig. 5**. Results of polymerase chain reaction assays displaying amplicons of the Species specific *16srRNA* gene for *E. coli* isolates. L1, 2, 3: reagent control, negative control, positive control, respectively; L5,7, 9 to15: isolates positive for *16srRNA* gene; L4, 6: isolates negative for*16srRNA* gene; L8: DNA ladder (50 bp).

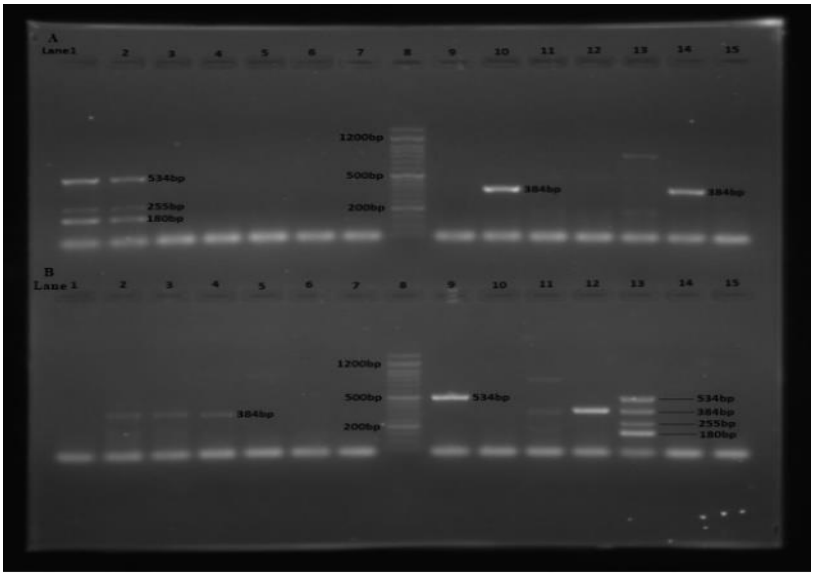


**Fig. 6**. Results of polymerase chain reaction assays displaying amplicons of the specific virulence producing *stx*$_1$, *stx*$_2$, *eaeA* and *hlyA* genes for *E. coli* isolates. L(A)1,2: isolates positive for *stx*$_1$, *stx*$_2$, *hlyA* genes; L(A)10, 14, L(B)2, 3, 4, 11,12: isolates positive for *eaeA* gene; L(B)9: isolate positive for *hlyA*; L(A)3 to 7, 9, 11 to 13, 15, L(B)1, 5 to 7, 10: isolates negative for all the genes, L(B)13, 14, 15: positive control, negative control and reagent controls, respectively; L(A&B)8: DNA ladder (50 bp)

All the isolates of *E. coli* which were presumptively identified on the basis of cultural, Gram's staining and biochemical tests were confirmed by Polymerase chain reaction using genus specific 16SrRNA primers (Fig. 5). Total 358 isolates were confirmed as *E. coli* by amplifying genus specific 16SrRNA primers. Distribution of Shiga toxin producing genes, *stx*$_1$ and *stx*$_2$, and two other virulent genes, *eaeA* and *hlyA* in the SF and SNF strains of *E. coli* isolates (n=358 16srRNA positive) are depicted in Table 5. Fig. 6 shown PCR amplifying product for virulence gene profile. Out of 358 isolates of *E. coli* three sample was found positive for all *stx*$_1$, *stx*$_2$ and *hlyA* virulence gene (Table 5, Fig. 6). Among the isolates recovered, the frequency of detection of *eaeA* was higher followed by *hlyA*, *stx*$_2$ and *stx*$_1$ gene.

## DISCUSSION AND CONCLUSION

We studied potential risk factors for STEC O157 infection. In this study, a survey of environmental samples (Food, Water and Animal excreta) revealed the widespread distribution of *E. coli* across diverse sources. Outbreaks involving EHEC can be spread through infected food, water, direct contact with infected humans or animals or exposure to infected environments (Ota *et al.,* 2019). In the report of WHO (2019) a total of 957 STEC outbreaks from 27 different countries were included in the analysis. Overall, outbreak data identified that 16% (95% Uncertainty interval-UI, 2-17%) of outbreaks were attributed to beef, 15% (95% UI, 2-15%) to produce (fruits and vegetables) and 6% (95% UI, 1-6%) to dairy products. The food sources involved in 57% of all outbreaks could not be identified. The attribution proportions were calculated by WHO region and the attribution of specific food commodities varied between geographic regions. In the European and American sub-regions of the WHO, the primary sources of outbreaks were beef and produce (fruits and vegetables). In contrast, produce (fruits and vegetables) and dairy were identified as the primary sources of STEC outbreaks in the WHO Western Pacific sub-region (WHO, 2019).

The utility of testing for STEC presence/absence as part of monitoring programs for food safety assurance in processing is limited by the typically low levels and prevalence of STEC in food. Process performance monitoring may be accomplished more effectively and efficiently by quantitatively monitoring sanitary and hygiene indicator organisms. These indicator organisms do not indicate pathogen presence; instead, they provide a quantitative measure of the control of microbial contamination in the product and processing environment. Periodic testing for high risk STEC can also be conducted for verification of process performance.

With regard to isolates of STEC, a basic amount of laboratory information needs to be collected in order to maximize the isolates usefulness for surveillance purposes. This information includes confirmation of STX production or presence of *stx* genes; O:H serotype; antimicrobial resistance pattern; characterization of other known virulence genes; biochemical confirmation as an *E. coli*; as well as any further typing information available. The significance of the detection of a STEC strain in a food should be considered on a case-by-case basis taking into account the potential health risk associated with the STEC strain and the food profile. Although STEC chiefly O157 are an important cause of HUS and diarrhoea, infections are markedly under-recognized because clinicians do not request testing of stools for STEC and very few laboratories screen stools for O157 STEC.

Public health authorities may conduct tests for the purposes of source identification, surveillance or diagnosis. Diagnostic testing is used to determine the pathogen responsible for the illness presenting in a

patient for ensuring appropriate medical intervention; it may also be used to determine whether an individual is a carrier of the pathogen and constitutes a transmission risk. Source Identification is conducted for identifying the source of the pathogen and may involve testing food, clinical or environmental samples. Surveillance testing may also involve food, clinical or environmental samples, but is conducted to determine the exposure risk associated with potential sources.

## FUTURE SCOPE

Our study highlights the possible health risk allied with the presence of Shiga toxigenic *E. coli* (STEC) in environmental samples. STEC can cause very serious human illness. Numbers of reported cases are usually low but, because of the serious nature of the illness, individual cases constitute a considerable health burden. The findings of this study suggest the need for a thorough risk assessment on virulence factors of *E. coli* (STEC) particularly isolated from commonly food practices, food production, distribution and consumption patterns; different potable water and environmental sources to control cross contamination risks. The combination of phenotypic and genotypic approaches should be used in routine screening for the reliable identification of typical and emerging STEC pathotypes. Moreover, the surveillance system should be established to monitor STEC-associated outbreak.

**Acknowledgement.** I spread my sincere thanks to the Education Department, Government of Gujarat for supporting the research financially by awarding the research Scholarship SHODH-ScHeme of Developing high quality (Ref No: 202110746, KCG/SHODH/2022-23).

**Conflict of Interest.** None.

## REFERENCES

Abbott, S. L., Hanson, D. F., Felland, T. D., Connell, S., Shum, A. H., & Janda, J. M. (1994). *Escherichia coli* O157: H7 generates a unique biochemical profile on Micro Scan conventional gram-negative identification panels. *Journal of clinical microbiology*, *32*(3), 823-824.

Caprioli, A., Scavia, G., & Morabito, S. (2015). Public health microbiology of Shiga toxin-producing *Escherichia coli*. Enterohemorrhagic *Escherichia coli* and other Shiga Toxin-Producing *E. coli*, 245-259.

Cheesbrough, M. (1993). Medical Laboratory Manual for Tropical Countries. Vol. II. Microbiology, ELBS. Butterworth- Heinemann Ltd., Oxford.

Cheesbrough, M. (2005). District laboratory practice in tropical countries, part 2. Cambridge university press.

Ferens, W. A., & Hovde, C. J. (2011). *Escherichia coli* O157: H7: animal reservoir and sources of human infection. *Foodborne pathogens and disease*, *8*(4), 465-487.

Kirk, M. D., Pires, S. M., Black, R. E., Caipo, M., Crump, J. A., Devleesschauwer, B., & Angulo, F. J. (2015). World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal, and viral diseases, 2010: a data synthesis. *PLoS medicine*, *12*(12), e1001921.

March, S. B., & Ratnam, S. A. M. U. E. L. (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* O157: H7 associated with hemorrhagic colitis. *Journal of clinical microbiology*, *23*(5), 869-872.

Meena, S., Meena, A. R., Chobhe, K. A., Behera, C., & Saini, R. (2023). Potential Health Risk Associated with Application of Organic Amendments. *Biological Forum – An International Journal*, *15*(2), 852-860.

Ota, M., Kamigaki, T., Mimura, S., Nakashima, K., & Ogami, T. (2019). An enterohaemorrhagic *Escherichia coli* outbreak spread through the environment at an institute for people with intellectual disabilities in Japan in 2005. *Western Pacific surveillance and response journal: WPSAR*, *10*(2), 14.

Paton, A. W., & Paton, J. C. (2002). Direct detection and characterization of Shiga toxigenic *Escherichia coli* by multiplex PCR for *stx*$_1$, *stx*$_2$, *eaeA*, *hlyA*, and *saa*. *Journal of Clinical Microbiology*, *40*(1), 271-274.

Paton, J. C., & Paton, A. W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clinical microbiology reviews*, *11*(3), 450-479.

Prajapati, B. I., Gelani, R. N., Patel, A. C., & Savalia, C. V. (2015). Molecular characterization and antimicrobial resistance of *E. coli* isolated from fresh water market fish. *Journal of Pure and Applied Microbiology*, *9*(4), 3071-3077.

Rasheed, M. U., Jamil, K., Thajuddin, N., Pasupuleti, M., Ahamed, P., & Muthukumaresan, K. P. (2014). Distribution of the *stx*$_1$, *stx*$_2$ and *hlyA* genes: Antibiotic profiling in Shiga-toxigenic *E. coli* strains isolated from food sources. *Int. J. Curr. Microbiol. App. Sci.*, *3*(5), 348-361.

Schippa, S., Iebba, V., Barbato, M., Di Nardo, G., Totino, V., Checchi, M. P., Longhi, C., Maiella, G., Cucchiara, S. & Conte, M. P. (2010). A distinctive 'microbial signature' in celiac paediatric patients. *BMC microbiology*, *10*, 1-10.

Sharma, I., Henbi, L. N., & Yaiphathoi, S. (2019). Detection of virulence properties in *E. coli* isolated from packed food products from North East India. *Journal of Biotech Research*, *10*, 255-261.

Sinha, N., Kshirsagar, D. P., Brahmbhatt, M. N., & Nayak, J. B. (2015). Detection and virulence gene characterization of Shiga toxigenic *E. coli* from buffalo meat samples. *Indian Journal of Animal Research*, *49*(5), 694-698.

Vandepitte, J. (2003). Basic laboratory procedures in clinical bacteriology. World Health Organization (WHO).

Vijayan, C., Ajaykumar, V. J., Bhattacharya, A., & Bhanurekka, V. (2017). Detection of enterohaemorrhagic *E. coli* O157: H7 from beef and chevon sold in and around Puducherry. *Journal of Entomology and Zoology Studies*, *5*(6), 1395-1403.

Vinay, P. T., Mallinath, K. C., Kharate, A., Suryakanth, P., & Revappayya, M. (2018). Occurrence of *Escherichia coli* O157: H7 in faecal sample of sheep and goats in North East Karnataka. *Int. J. Curr. Microbiol. App. Sci.*, *7*, 242-250.

World Health Organization (2015). WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. World Health Organization.

World Health Organization (2019). Attributing illness caused by Shiga toxin-producing Escherichia coli (STEC) to specific foods: report.

**How to cite this article:** Darshan D. Patel, Murtaza A. Hajoori and Jignaben P. Naik (2023). Detection of Shiga Toxin Producing *Escherichia coli* (STEC) O157-A Food and Waterborne Zoonotic Pathogens as Implications of One Health Perspective. *Biological Forum – An International Journal, 15*(5a): 640-646.