Haryana Vet. (October, 2022) **61(SI-2),** 113-117 **Research Article**

DETECTION OF *ESCHERICHIA COLI* O157 FROM BOVINE FAECES IN AND AROUND HISAR

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ABSTRACT

The present investigation was undertaken to isolate and molecularly characterize *Escherichia coli* O157 from bovine faeces in and around Hisar. A total of 163 cattle faecal samples were collected randomly. All the samples were screened by using standard microbiological methods for the detection of *E. coli* O157. The identified isolates were then confirmed as *E. coli* O157 using latex agglutination test and polymerase chain reaction-based assays along with nucleotide sequencing. A total of 23 presumptive *E. coli* O157 isolates were recognized by culture based assays. Further, 17 isolates were confirmed as *E. coli* O157 by latex agglutination test whereas, 21 isolates turned to be positive by PCR. On nucleotide sequencing analysis, the isolates exhibited similarity with other published sequences which further confirmed that the recovered isolates of present study were of O157 serotypes. The PCR appeared to be more sensitive and reliable test for confirmation of *E. coli* O157 in comparison to Latex agglutination test. In antimicrobial susceptibility testing, maximum resistance was witnessed for metronidazole and bacitracin followed by ceftazidime/clavulanic acid, polymyxin-B, ampicillin/cloxacillin and amoxycillin.

Keywords: *E. coli* O157, Cultural isolation, Latex agglutination test, PCR, Nucleotide sequencing.

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The Enterohemorrhagic *E. coli* (EHEC) is a group of intestinal *E. coli* that produce a potent toxin, i.e. Shiga like toxin (SLT) or Verocytotoxin (VT) and is responsible for causing diseases ranging from mild diarrhoea, fatal haemorrhagic colitis (HC) and haemorrhagic uremic syndrome (HUS) in humans and the most frequently isolated serotype is *E. coli* O157:H7. Cattle as a natural reservoir of *E. coli* O157 is known to excrete this organism along with faeces and thus pose the risk for transmission to humans. Contamination of food, water and other environmental niches with bovine faeces has emerged as an important public health concern, especially with regard to *E. coli* O157:H7 infections. Detection of *E. coli* O157 by direct culture is an expensive, labour-intensive and time-consuming process. Additionally, high concentrations of competing, “background” microorganisms increase the difficulty of successful isolation of *E. coli* O157.

Lack of information on prevalence of *E. coli* O157 in cattle faeces prompted us to undertake the isolation and molecular characterization of *E. coli* O157 serotype from faecal samples. Additionally, a comparative evaluation of bead based assay(s) and molecular assay(s) for the detection *E. coli* O157 was also carried out along with antimicrobial susceptibility testing of the field isolates obtained in the study.

MATERIALS AND METHODS

Sample collection

In the present study, cattle faecal samples (n=163) collected in sterile containers which comprised of freshly voided faeces of adult cattle (n=117) and recto-anal swabs of calves (n=46) from bovine dairy establishments, gaushalas, TVCC, dairy samples and from stray cattle in and around Hisar during the period from March 2021 to August 2021 were analysed. Briefly, the upper layer of freshly voided faeces was removed with a sterile cotton stick and about 10g of faeces from the middle portion of the pat was then collected in a sterile container. All the collected samples were kept on ice, transported to the laboratory and processed on same day.

Cultural isolation of *E. coli* O157 from faecal samples

One gram of each faecal sample was inoculated into 9 ml (1 in 9 dilution) of HiCrome enrichment broth base for *E. coli* O157:H7 supplemented with novobiocin and potassium tellurite and was incubated at 37°C for 18-24 hrs. A loopful of inoculums from the enrichment medium was inoculated onto HiCrome *E. coli* O157:H7 selective agar base supplemented with novobiocin and potassium tellurite. The agar plate was incubated for 24 h at 37°C. Growth of typical dark purple to magenta-coloured colonies (Sorbitol negative) indicated presumptive *E. coli* O157. The presumptive colonies were further confirmed by inoculation onto selective isolation medium, CT-SMAC (Cefixime-tellurite Sorbitol MacConkey’s agar) and incubating for 24 h at 37°C. Growth of typical straw

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coloured to colourless colonies (Sorbitol negative) were considered to support the presumptive *E. coli* O157 of the previous step.

Confirmative identification of *E. coli* O157 isolates by latex agglutination test

The samples exhibiting presumptive *E. coli* O157 by cultural isolation were further confirmed by using latex agglutination test for the presence of O157 antigens using Hi *E. coli* TM Latex test kit (HiMedia).

Molecular Confirmation of *E. coli* O157 by PCR

All presumptive *E. coli* O157 isolates based on cultural isolation were examined for the existence of *E. coli* O157 specific genes, i.e., rfb O157, eaeA O157 and fliCh7. The oligonucleotide primers used for PCR amplification of rfb O157, eaeA O157 and fliCh7 genes (Gannon *et al.*, 1997; Wang *et al.*, 2014) along with their PCR conditions have been presented in table 1, table 2 and table 3. The DNA of ATCC O157 strain procured from department was used as a positive control.

The rfb O157 amplifications of two field isolates were sequenced in order to prove that the amplicon matched to the rfb O157 sequences. The gained sequences were aligned with the data sequences in NCBI.

Antibiotic sensitivity testing

The *in vitro* drug sensitivity testing of *E. coli* O157 isolates was determined by following the CLSI guidelines and the method of Bauer-Kirby (Bauer *et al.*, 1966). The assay was performed by using commercially prepared disc (HiMedia, India) with known concentration of antibiotics as per manufacturers' recommendations. The antibiotic discs used in the assay were amoxycillin (AMX 30), ampicillin/cloxacillin (AX 10), chloramphenicol (C30), ciprofloxacin (CIP 10), co-trimoxazole (COT 25), gentamicin (GEN 10), metronidazole (MT 5), norfloxacin (NX10), tetracycline (TE 30), bacitracin (B8), nalidixic acid (NA 30), polymyxin-B (PB 300), cefoxitin (CX 30), kanamycin (K 30) and ceftazidime/clavulanic acid (CAC 30/10).

RESULTS AND DISCUSSION

In the present study, faecal samples were inoculated onto HiCrome enrichment broth base for *E. coli* O157:H7 supplemented with novobiocin and potassium tellurite. Prabhakar *et al.* (2017) also employed HiCrome enrichment broth for *E. coli* O157:H7 for selective enrichment of seafood samples for isolation of *E. coli* O157:H7. However, several researchers from India and other countries have used tryptone soya broth supplemented with novobiocin as enrichment medium for *E. coli* O157:H7 (Bindu and Krishnaiah, 2010). Inoculum from the enrichment medium was inoculated onto HiCrome *E. coli* O157:H7 selective agar base supplemented with novobiocin and potassium tellurite. A total of 32 isolates showing typical purple to magenta colored moiety were considered as presumptive *E. coli* O157 (Fig. 1).

In the current study typical purple to magenta-colored colonies on HiCrome *E. coli* O157:H7 selective agar which were considered presumptive *E. coli* O157 isolates were inoculated CT-SMAC. CT-SMAC has been recommended by the Centers for Disease Control and Prevention as a selective medium for *E. coli* O157:H7. Various studies have reported a successful isolation of *E. coli* from meat samples, human faeces, small intestine of chicken, cattle faeces, raw milk and ground beef using CT-SMAC agar (Bindu and Krishnaiah, 2010).

A total of 23 isolates (14.11%) were suspected as *E. coli* O157 on the basis of their straw coloured to colourless colony morphology on CT-SMAC (Fig. 2). These isolates were then subjected to Latex agglutination test and PCR for detection of *E. coli* O157. A total of 17 isolates were detected by Latex agglutination test as *E. coli* O157 whereas 21 isolates (12.88%) were detected by PCR as *E. coli* O157 including four isolates that were not detected by latex agglutination test. The results of cultural isolation along with the results of latex agglutination test and PCR are presented in table 4. The results of agarose gel electrophoresis for PCR products of rfbO157, eaeAO157 and fliCh7 are presented in fig. 3, fig. 4 and fig. 5. The lower detection of *E. coli* O157 isolates by latex agglutination test may be due to prozone effect as agglutination reactions may get inhibited if the number of antibodies is higher than the number of antigens (Parija, 2012). Similar results were reported by Hu *et al.* (1999) who conducted a study on *E. coli* O157 isolates from bovine faeces and reported that out of six samples, three were found to be positive with the multiplex PCR assay, and two were positive with the conventional culture method and latex agglutination test for *E. coli* O157.

Out of the 21 detected isolates, 16 (9.81%) isolates were obtained from freshly voided faecal samples of adult cattle and 5(3.06%) isolates were obtained from recto- anal mucosal swabs of calves. Nearly similar results were reported by Chapman *et al.* (1994) where *E. coli* O157 was isolated at a rate of 8.2% from faeces of adult cattle and 3.2% from calves, respectively. Overall *E. coli* O157

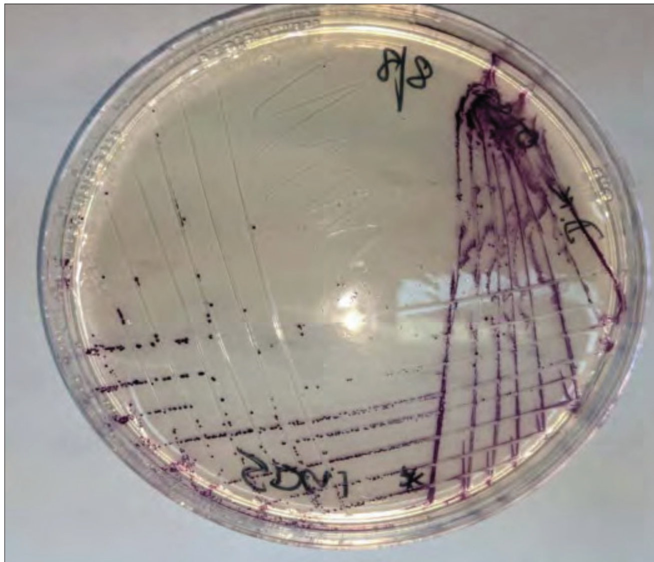

Fig. 1. Purple colonies of *E. coli* O157 on HiCrome *E. coli* O157:H7 selective agar base supplemented with novobiocin and potassium tellurite. Sample ID: SDG1

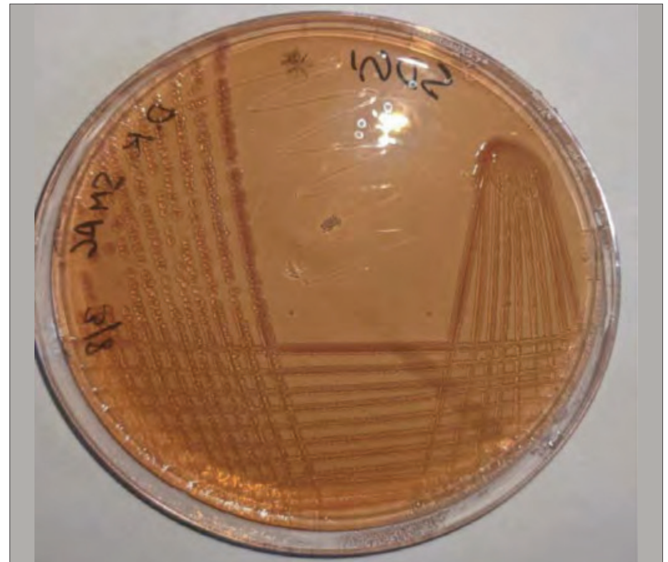

Fig. 2. Colourless colonies of *E. coli* O157 on Cefixime-tellurite Sorbitol MacConkey's agar. Sample ID: SDG1

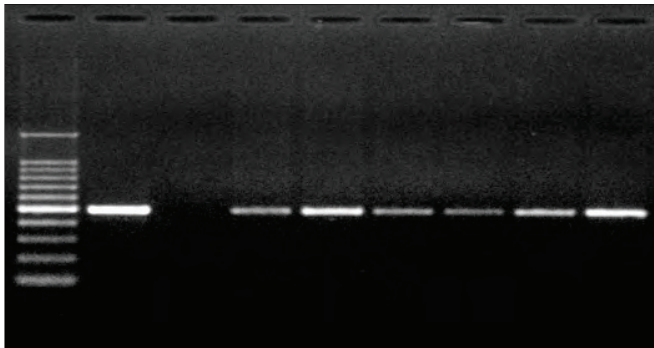

Fig. 3. Agarose gel electrophoresis showing amplified PCR product of rfbO157 gene; M = DNA Ladder 100 bp, Lane 1=Positive control, Lane-2=Negative control, Lane 3-8 = *E. coli* O157 positive amplicon

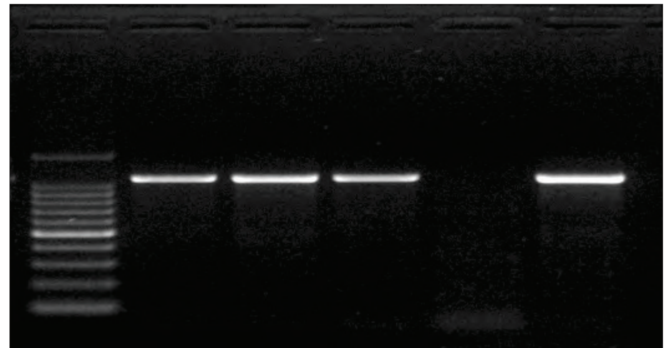

Fig. 4. Agarose gel electrophoresis showing amplified PCR product of eaeAO157 gene; M = DNA Ladder 100 bp, Lane 1- 3 = *E. coli* O157 positive amplicon, Lane 4= Negative control, Lane 5 = Positive control

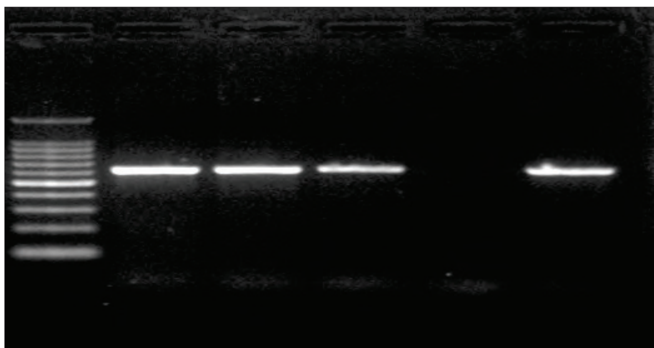

Fig. 5. Agarose gel electrophoresis showing amplified PCR product of fliCh7 gene; M = DNA Ladder 100 bp, Lane 1- 3 = *E. coli* O157 positive amplicon, Lane 4= Negative control, Lane 5 = Positive control

detection rate in the present study was 12.88% (21/163) on the basis of PCR. An Indian study conducted by Kumar, (2016) reported *E. coli* O157 from cattle faeces with an overall occurrence of 12.10% which is similar with findings of current study. However, studies conducted by Hancock *et al.* (1997) reported lesser prevalence (1%) of *E. coli* O157 from cattle faeces.

In the present study, presence of *E. coli* O157 was confirmed by PCR amplification of rfbO157, eaeAO157 and fliCh7genes and subsequent nucleotide sequencing of two isolates *viz.*, sample no. 34 (Sample id: H-I 036932) and sample no. 35 (Sample id: S2G2)) was carried for rfbO157 gene. The homology of sequence results of rfbO157 gene were compared with available nucleotide in the GenBank database (Fig. 6). The sample 34, 35 sequences were in same clade of O157:H7 serotype along with other isolates of USA, Japan, Canada, Bangladesh, United Kingdom, Germany and UAE, whereas, sequence from USA was in different clade forming part of same node indicating genetic differences with isolates under study and other isolates of this clade. The sequences from Japan and USA were present in a different clade representing *E. coli* O157:H16 and H45 serotypes. The absence of

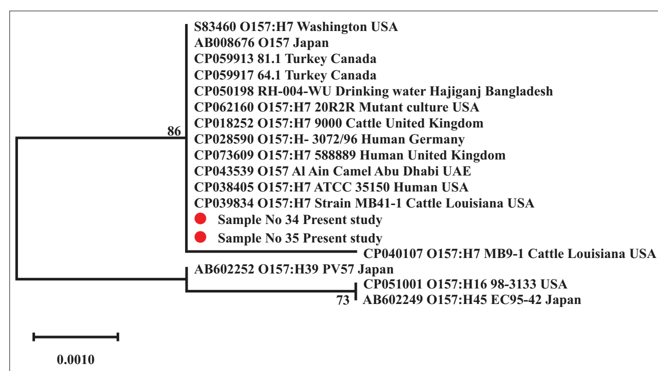

Fig. 6. Unrooted phylogenetic tree based on rfbO157 gene sequences constructed using MEGA-X.

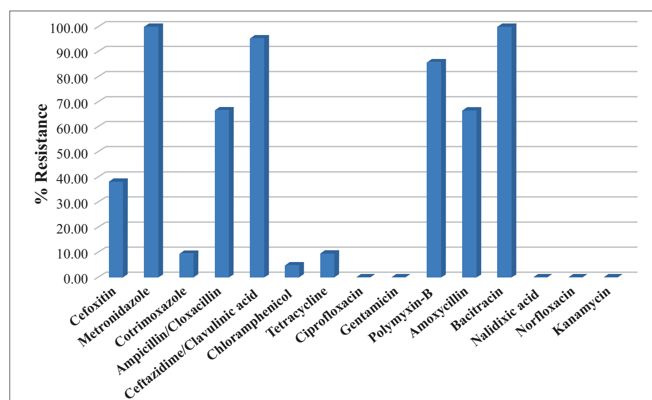

Fig. 7. Antibiotic resistance pattern of *E. coli* O157 isolates

Table 1. Oligonucleotides used for the characterization of *E. coli* O157

Sr. No.	Target gene	Literature	Primer	Sequence	Amplicon Size (bp)
1	*rfb*O157	Wang *et al.*, 2014	F	AAGATTGCGCTGAAGCCTTTG	497
			R	CATTGGCATCGTGTGGACAG	
2	*eaeA*O157	Gannon *et al.*,1997	F	CAGGTCGTCGTGTCTGCTAAA	1087
			R	TCAGCGTGGTTGGATCAACCT	
3	*fli*C_{h7}	Gannon *et al.*,1997	F	GCGCTGTCGAGTTCTATCGAGC	625
			R	CAACGGTGACTTTATCGCCATTCC	

Table 2. Cycling conditions for PCR of *rfb*O157 and *eaeA*O157 gene

S. No.	Step	Temperature/Time	No. of cycles
1.	Initial denaturation	95 ºC/5min	
2.	Final denaturation	95 ºC/45 secs	35 X
3.	Annealing	58 ºC/45 secs	
4.	Initial extension	72 ºC/45 secs	
5.	Final extension	72 ºC/8 mins	
6.	Hold	4 ºC	

Table 3. Cycling conditions for PCR of *fli*C_{h7} gene

S. No.	Step	Temperature/Time	No. of cycles
1	Initial denaturation	94 ºC/5min	
2	Final denaturation	94 ºC/30 secs	35 X
3	Annealing	65 ºC/45 secs	
4	Initial extension	72 ºC/1 min	
5	Final extension	72 ºC/8 mins	
6	Hold	4 ºC	

divergence or presence of little divergence (0-0.4%) was observed between studied and analysed isolates. Strachan *et al.*, 2015 by sequence analysis found geographic variation of *E. coli* O157 genotypes by comparing *E. coli* O157 isolate sequences from cattle and sheep of Scotland and New Zealand, Netherlands, Canada and the USA. Comparisons between cattle sources and humans within countries (Scotland, USA, Netherlands and Canada) showed significant differences. The percent identity matrix revealed that the nucleotide sequences of sample number 34 and 35 shared 100 percent identity between themselves and shared similar identity with the respective 12 sequences obtained from GenBank database using BLAST analysis. Abrar *et al.*, 2019 found that one regional *E. coli* O157 isolate exhibited homology of 100% to 16 different strains of *E. coli* O157:H7 sequence in the GenBank.

In the present study, the *E. coli* O157 isolates confirmed by PCR were subjected for antibiotic sensitivity test using Muller Hinton agar for the routinely used antibiotics. A total of 21 *E. coli* O157 isolates were tested for antimicrobial susceptibility against 15 antibiotics using commercially prepared disc (HiMedia, India) with known concentration of antibiotics as per CLSI guidelines. The results found that maximum resistance was present for metronidazole (100%) and bacitracin (100%) followed by ceftazidime/clavulanic acid (95.24%), polymyxin-B (85.71%), ampicillin/cloxacillin (66.67%) and amoxycillin (66.67%). The resistance observed for other antimicrobials was 38.10% for cefoxitin, 9.52% for both cotrimoxazole and tetracycline and 4.76% for chloramphenicol. No resistance was observed against five antibiotics *viz.* ciprofloxacin, gentamicin, nalidixic acid, norfloxacin and kanamycin (Fig. 7). The variation in the antimicrobial resistance among *E. coli* O157 isolates in different locations and studies have been previously reported in various studies (Tadesse *et al.*, 2018). The possible reason for variation may be use of different antimicrobials at different

Table 4. Comparative evaluation of bead-based assay(s) and molecular assay(s) for *E. coli* O157 detection

Sr. No.	Sample ID	HiCrome *E. coli* O157:H7 selective agar	CT-SMAC	Latex agglutination test	PCR	Samples chosen for nucleotide sequencing
1	S365	Purple	Straw	POSITIVE	POSITIVE	
2	S403	Magenta	Straw	POSITIVE	POSITIVE	
3	BN-C1	Purple	Colourless	POSITIVE	POSITIVE	
4	S7G1	Purple	Straw	POSITIVE	POSITIVE	
5	S201	Magenta	Straw	NEGATIVE	POSITIVE	
6	H1-036932	Purple	Colourless	POSITIVE	POSITIVE	Sample-34
7	S341	Purple	Straw	POSITIVE	POSITIVE	
8	SDG1	Purple	Colourless	POSITIVE	POSITIVE	
9	S7SM	Purple	Straw	POSITIVE	POSITIVE	
10	109L	Purple	Straw	NEGATIVE	POSITIVE	
11	L215	Magenta	Straw	POSITIVE	POSITIVE	
12	SB2	Purple	Colourless	POSITIVE	POSITIVE	
13	S8SM	Magenta	Straw	NEGATIVE	POSITIVE	
14	S2G2	Purple	Straw	NEGATIVE	POSITIVE	Sample-35
15	Sec-14SE	Purple	Colourless	POSITIVE	POSITIVE	
16	Sec-14S3	Purple	Straw	POSITIVE	POSITIVE	
17	H-II 341694	Purple	Straw	POSITIVE	POSITIVE	
18	H-II C3	Purple	Colourless	POSITIVE	POSITIVE	
19	H-1 775061	Magenta	Straw	POSITIVE	POSITIVE	
20	H-1 C1	Purple	colourless	POSITIVE	POSITIVE	
21	Sec-14 S1	Purple	Straw	POSITIVE	POSITIVE	
22	H-1 746527	Magenta	Straw	NEGATIVE	NEGATIVE	
23	H-1 036649	Magenta	Straw	NEGATIVE	NEGATIVE	
	Total	23	23	17	21	

farms and countries, dose of drug used, type of samples, number of isolate and genetic variation of the isolate among different geographical areas.

It can be concluded that cattle in and around Hisar are harbouring *E. coli* O157 and shedding them in faeces. Additionally, the nucleic acid based tests like PCR appears to be better in the detection of *E. coli* O157 as compared to cultural isolation and latex agglutination test.

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