Small Ruminant Research 161 (2018) 7–12

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

# Detection and molecular characterization of sorbitol fermenting non-O157 Escherichia coli from goats

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ARTICLE INFO

*Keywords:*
Non-O157 *E.coli*
Goats
Multiplex PCR
PFGE

ABSTRACT

Shiga toxigenic *Escherichia coli* (STEC) O157 and several other serogroups of non-O157 STEC strains present as commensalism bacteria in small ruminants that possess a high potential of attaining pathogenic virulent genetic elements. The pathogenicity of non-O157 strains is emerging progressively in animals as well as in humans especially in the rural areas as they get transmitted through unsanitary practices of living, consumption of uncooked meat and milk, human-livestock close proximity as well as within livestock pathogenic bacterial transmission. The present study was carried out to determine the prevalence of non-O157 *E.coli* isolates and characterize based on clinical history, antibiotic sensitivity testing, multiplex PCR (mPCR) detection of virulence genes, genotype identification using pulse field gel electrophoresis (PFGE) and polyacrylamide gel electrophoretic separation of antigenic proteins to determine their prevalence, virulence and genetic comparison between host and environment for epidemiological significance. A total of 300 *E.coli* isolates were recovered from rectal swabs of goats and their surrounding environment over a period of one year (2016–2017) by selective isolation. Among which 50 isolates were confirmed to be from the non-O157 *E.coli* family. The mPCR analysis of these 50 isolates revealed the presence of two or more virulent genes, viz., *hylA* (90%), *fliC* (74%), *eaeA* (56%), *stx1* (48%) and *stx2* (22%).Four isolates exhibited multidrug-resistance to amoxiclav, doxycycline, ciprofloxacin and ceftriazone. The PFGE fingerprint profile showed six major clusters at 100% similarity from the 50 isolates. The major antigenic proteins identified from the isolates were *stx1*A, *stx2*B and *fliC*. This study has significant implications for understanding the molecular diversity of emerging pathotypes of non-O157 in young goats in terms of virulence and epidemiological aspects.

## 1. Introduction

Ruminants have been implicated as the principal reservoirs of pathogenic *Escherichia coli* which are a major source of foodborne outbreaks affecting human health (Ferens and Hovde, 2011; Swift et al., 2017). As long as these bacteria do not attain genetic elements and virulence factors they remain as benign commensalism bacteria within the gut (Osman et al., 2013). Several strains of pathogenic *E.coli* cause diarrhea in goats, sheep and cattle affecting mostly young ones leading to heavy mortality and morbidity (Etcheverría and Padola, 2013). Younger goats have a higher susceptibility rate (65%) of developing an infection to pathogenic *E.coli* as compared to adults (37.1%) (Islam et al., 2016). Numerous pathogenic *E.coli* expressing various combinations of virulence factors like shiga toxins and enteropathogenic toxins were recovered from clinical and non-clinical cases in calves, goats and sheep (Wani et al., 2006; Osman et al., 2013). Non-O157 *E.coli*serogroups are emerging to cause serious illness in humans and ruminants (Hussein, 2007). Therefore, the similarities in phylogenies of O157 *E.coli* and non-O157 *E.coli* suggest an ongoing micro evolutionary process in which the shiga toxin gene (*stx*) is transferred between the two groups causing an array of diverse strains (Eichhorn et al., 2015).

The west coast of south India is hot and humid with high rainfall and goat farming in this region is in the form of small holdings. The majority of goat farms are stall feeding reared on raised platforms. The incidence of diarrhoeagenic pathogens particularly *E.coli* is persistently prevalent during all the seasons more frequently in young goats due to poor housing and management conditions in this region. The most prominent genes in *E.coli* that correspond to the virulence factor of the bacteria are *stx1*, *stx2*, *eaeA*, *hylA* and *fliC* (Pan et al., 2002; Bai et al., 2010; Osman et al., 2012). The current outlook on virulence studies

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https://doi.org/10.1016/j.smallrumres.2018.02.008
Received 13 September 2017; Received in revised form 5 February 2018; Accepted 16 February 2018
Available online 19 February 2018
0921-4488/ 

**Table 1**
PCR conditions of target gene along with their primer details.

| Target Gene | Nucleotide Sequence (5′-3′) | Amplicon Size (bp) | PCR conditions | | | Reference |
|---|---|---|---|---|---|---|
| | | | Denaturation | Annealing Temp | Extension | |
| ***Stx1*** | TGTCGCATAGTGGAACCTCA<br>TGCGCACTGAGAAGAAGAGA | 655 | 95 °C 3 min | 56° C 30sec | 72 °C 2 min | (Paton and Paton, 1998) |
| ***Stx2*** | CCATGACAACGGACAGCAGTT<br>TGTCGCCAGTTATCTGACATTC | 477 | 95 °C 3 min | 56° C 30sec | 72 °C 2 min | (Paton and Paton, 1998) |
| ***EaeA*** | GACCCGGCACAAGCATAAGC<br>GACCCGGCACAAGCATAAGC | 384 | 95 °C 3 min | 56° C 30sec | 72 °C 2 min | (Paton and Paton, 1998) |
| ***hlyA*** | GCATCATCAAGCGTACGTTCC<br>GACCCGGCACAAGCATAAGC | 534 | 95 °C 3 min | 56° C 30sec | 72 °C 2 min | (Paton and Paton, 1998) |
| ***FLIC*** | AGCTGCAACGGTAAGTGATTT<br>GGCAGCAAGCGGGTTGGTC | 949 | 95 °C 3 min | 55° C 30sec | 72 °C 2 min | (Bai, et al. 2010) |
| ***Rfb*** | GGCAGCAAGCGGGTTGGTC<br>TTAGAATTGAGACCATCCAATAAG | 296 | 95 °C 3 min | 55° C 30sec | 72 °C 2 min | (Bai, et al. 2010) |

indicates that *E.coli* of any serotype can acquire the toxin gene (*stx1*, *stx2*) but the ability of an organism to produce these toxins alone is insufficient for that organism to cause disease. Therefore, the *E.coli* strain would have to possess multifactorial virulence genes to induce pathogenicity (Law, 2000; Zschock et al., 2000). Additionally, in a study conducted by Jacob et al. (2013), non- O157 serotype of *E.coli* isolates from goats indicating the presence of two or more virulence genes (14.5%; 43/296) which confirms the bacterial pathogenic factor. In India, there is not enough information on the isolation and characterization of non-O157*E.coli* strains from livestock animals reported. Few studies have indicated the prevalence of shiga toxin-producing *Escherichia coli*(STEC) from non-diarrheic calves, lambs, chicken and feral pigeons in Kolkata andenteropathogenic E. coli (EPEC) strains from healthy goats in Jammu and Kashmir. (Wani et al., 2004, 2006)

In the present study, non-O157 *E.coli* strains were characterized from goat rectal swabs based on clinical history, antibiotic sensitivity testing and multiplex PCR (mPCR) detection of virulence genes. Furthermore, genotype identification was carried out using PFGE and PAGE separation of antigenic proteins to determine a comparison between host and environment for epidemiological significance.

## 2. Materials and methods

Sample collection: A total of 300 (250 rectal swabs and 50 environmental) samples were collected from multiple farms all over the region, where goats were reared under a free-range as well as stall feeding system in the west coastal state of India—Goa, over a period of one year (2016–2017). The coastal climate is hot and humid, which allows very few breeds of goats that are suitable to be reared. The rectal swabs were obtained from diarrhoeic and non-diarrhoeic goats cases along with their corresponding environmental surroundings. The majority of samples were collected from adults and young goats which were kept together with adults (80%) as younger goats are more susceptible to infections and thus were more liable to have pathogenic bacteria.

Selective isolation and screening: The rectal swabs were enriched with brain heart infusion (BHI) broth (HiMedia, Mumbai, India) at 37° C for 24 h and streaked on eosin-methylene blue agar (HiMedia, Mumbai, India). After incubation, colonies with cultural characteristics similar to *E.coli* were streaked onto selective *E.coli* O157 MUG (4-methylumbelliferyl-beta-D-glucuronide) identification agar plates (HiMedia, Mumbai, India) which were screened for O157 and non-O157 *E.coli* strains.All the multiple strains from a single sample were considered and subjected to phenol base broth biochemical assay and to antibiotic sensitivity assays.

Biochemical and antibiotic-sensitivity assays: The pure cultures were screened for sorbitol fermenting ability to distinguish between *E.coli* O157: H7 and non-O157: H7 *E.coli* using the phenol red sorbitol test. The selected isolates were then subjected to antibiotic agents by disk diffusion test on Muller-Hilton agar and data were classified as susceptible or resistant according to the specifications of Clinical and Laboratory Standards Institute, 2012. The antibiotic compounds used were amoxiclav (AMC), doxycycline (DO), ciprofloxacin (CIP) and ceftriaxone (CTR) as these are the most commonly used antibiotics for respiratory and enteric bacterial infections in India (Rasheed et al., 2014; Alves et al., 2017).

Genomic DNA isolation and multiplex PCR assay for virulence genes: One mLof overnight grown BHI broth culture was centrifuged at 7500 rpm for 5 min and the pellet was suspended in 180 µl of buffer (QIAamp, DNA mini kit). Proteinase K (QIAGEN, Germany) was added and the suspension was incubated at 56 °C for 2–3 h for complete lysis of bacterial cell wall. The lysate was washed, centrifuged and the supernatant containing DNA was quantified using a Nanodrop®N-1000 spectrophotometer (ThermoScientific, USA) for using as a template in PCR. All PCR reactions were performed using the gradient thermocycler (Eppendorf pro, Germany). Published primer sequences (Paton and Paton, 1998; Bai et al., 2010) were used (Table 1) and thermocycling conditions were standardized by gradient PCR. The following optimal conditions for the mPCR assay was established: reaction volume of 25 µl which consisted of 2X Gotaq green master mix (400 µM of each dNTPs, GoTaq® DNA polymerase, 2X Green GoTaq® Reaction Buffer (pH 8.5) and 3 mM MgCl2) (Promega, USA), 1.0 µM each of forward and reverse primers, 100 ng of the template DNA and nuclease-free water to make up remaining volume. The reaction was performed at 95 °C for 3 min of initial denaturation, 95 °C for 30 s of denaturation, annealing temperature for reaction set (*stx1*, *stx2, eaeAand hylA*) was at 56 °C and for reaction set (*fliC* and *rfbr*) was at 55° C for 2 mins, extension at 72 °C for 2 min and 72 °C for 10 min of final extension. The amplified DNA along with the positive strain control ATCC 25922 and a negative control (PCR mixture without template) was separated using gel electrophoresis at 1.2% agarose, stained with ethidium bromide and visualized using GelDocAplhaimager fluorescent imaging system (AlphInnotech, USA).

Pulse Field Gel Electrophoresis: Fifty isolates of non-O157 *E.coli* colonies from the overnight cultures were suspended in cell suspension buffer (100 mM Tris; 100 mM EDTA, pH 8) and cell concentration was adjusted to an optical density (OD) of 0.8–1 at 610 nm. Chromosomal bacterial DNA was prepared in 1% agarose gel plugs by mixing cell suspension buffer with an equal amount of low melting point agarose (Bio-Rad, USA) and Proteinase K (20 mg/mL) which was lysed in lysis buffer for four hours at 56 °C in the water bath. The plugs were washed three times with Milli-Q water, which was kept at 56 °C prior to the

washing and thrice with TE buffer at 56 °C (50 mM Tris; 50 mM EDTA; pH 8.0). Agarose plugs (2 mm size) was then subjected to restriction enzyme (RE) digestion with *XbaI* for three hours. The assay was performed according to the PulseNet Gel protocol (Ribot et al., 2001) in CHEF DR II system (Biorad, USA) using 1% pulse field certified agarose (Biorad, USA). The electrophoresis conditions used for non-O157 *E.coli* were as follows: Initial time- 6.76s; Final time-35.38s; Voltage–6 V; run time–20 h. A molecular standard Lambda phage marker (Promega, USA) was processed at each run along with *E.coli* isolates. Images were obtained using the GelDocAplhaimager fluorescent imaging system (AlphInnotech, USA) and the PFGE band patterns were normalized. A dendrogram was generated and evaluated using BioNumerics software (Applied Maths, Belgium).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE): Total protein was isolated from the overnight grown bacterial cultures by using Trizol® method (Life Technologies, Invitrogen). The lysate was resuspended in lysis buffer along with protein loading dye and loaded onto the gel (15 µl). SDS-PAGE gel electrophoresis was carried out in a discontinuous system of Laemmli using 10% separating gel and 5% stacking gel. A marker of known molecular weight (Sigma-Aldrich, USA; MW- 36,000- 200,000 Da) was used along with the samples. The molecular weight of proteins was obtained with the help of standard graphs, by taking the distance of marker travelled (in mm) along x-axis and MW of proteins along the y-axis. A cumulative percentage of positives were calculated.

## 3. Results

Isolation and characterization: A total of 300 *E.coli* were obtained and characterized from goats (83%) as well as from surrounding environment(16%). 50 isolates among them were identified as either O157 or non-O157 *E.coli* strains by selective streaking on MUG O157 identification agar. All the 50isolates showed sorbitol fermentation which confirmed the cultures as non-O157 *E.coli* strains (Table 2).

Multiplex PCR of virulence genes: The mPCR profile of the 50 non-O157 *E.coli* isolates was screened for six different virulence genes namely *stx1*, *stx2*,*eaeA*,*hylA* together and *fliC* and *rfbr* together (Fig. 1). Approximately 40% of the isolates showed to possess at least three virulent genes whilst 26% of the isolates were positive for four virulent genes (Table 2)

Molecular subtyping of isolates using PFGE: PFGE pulsotype analysis resulted in 41 banding patterns, from which 39 isolates were grouped into 11 clusters consisting of isolates from rectal swabs and their surrounding environment with similar fingerprint profiles using the *Xbal* restriction enzyme (Fig. 2).Genetic similarity of 70% was observed in the isolates within 7 clusters from goats and their environment (wall, soil, water and feed) which expressed *fliC*, *hylA* and *eaeA* simultaneously. At 100% genetic similarity, six clusters were observed which comprised of 13 isolates obtained from 7 young goats and remaining six from adults. All the isolates obtained from young goats (kids) showed a presence of at least three or more virulent genes in comparison to adults. The combination of *eaeA*, *fliC*, *and hylA* and*stx1* appeared with the highest frequency rate in comparison to other combinations and was predominantly found in young goats.

Analysis of protein profile using SDS-PAGE: The banding pattern showed that the proteins from the isolates had molecular sizes similar to those of stx1a, stx1b, stx2a, eaeA, hylA and fliC which were obtained using a universal protein resource (UniProt) (Table 3). The protein fliC showed the highest presence in 47 isolates, followed by stx1A (25/50), hylA (23/50), stx1B and stx2A (22/50) and lastly eaeA (18/50).

## 4. Discussion

In this study, the prevalence of virulence genes and the genetic diversity of sorbitol fermenting non-O157 *E.coli* strains in clinical and healthy goats in the western coastal region of India were investigated. Although O157: H7 *E.coli* were known to have potent toxins and are predominantly found in the gut of small ruminants whereas non-O157 *E.coli* also are considered to be of highest clinical importance (Wang et al., 2002; Karmali et al., 2010; Wisener et al., 2015). Three hundred isolates were obtained from clinical diarrhoeic young as well as adult goats and from their respective environment (feed, soil water, and wall swabs), screened further on MUG O157 *E.coli* agar and sorbitol biochemical assays to obtain 50 isolates that had the cultural characteristic of non-O157 *E.coli*. Furthermore, 80% of the 50 isolates were obtained from young goats which support the fact that young goats are more susceptible to infection than adults (Islam et al., 2016). Multiplex PCR, SDS-PAGE and pulse field gel electrophoresis aided in the determination of the virulence genes as well as the phylogenetic relationships between these isolates respectively.

Resistance to one or more antibiotic agents was found in 70.8% isolates of *E.coli* and four isolates were resistant to all antibiotics tested from the total of 50 samples. In the present study, multidrug resistance profiles included classes of antimicrobials commonly used in India, such as amoxiclav, ciprofloxacin, ceftriaxone, and doxycycline hydrochloride. (Rasheed et al., 2014; Alves et al., 2017). The dominant type of resistance was to amoxiclav which was detected in 19 isolates (37.5%) similarly in others studies non-O157 isolates showed resistance in 20 isolates (13.3%) (Rasheed et al., 2014). whilst the maximum percentage of sensitivity (83.3%) was observed to ciprofloxacin (Table 2).

Despite the dissimilar phylogenies between O157: H7 and non-O157 *E.coli*, both these groups of bacteria have astonishing similarities considering their whole gene repertoire, these genes include not only *stx1* and *stx2* but also many other virulent genes (Ogura et al., 2009; Horcajo et al., 2013). The standardized six gene mPCR assay employed to detect multiple virulence genes was proven effective as it could detect at least two genes in each isolate. Four genes set combination viz., *stx1*, *hylA*, *fliC* and *eaeA* were present in 25% of isolates (Table 2). Similar findings were reported in goats indicating an average of 35.4% of gene set combination viz., *stx1*, *stx2*, *fliC*, and *hyla* (Yakubu et al., 2011).This study showed that the genes *hylA* and *fliC* were predominantly found with a prevalence of 91.6% and 58.3% respectively whilst fourteen isolates (58%) showed presence of*stx1*and 33% had the *stx2*indicating a higher frequency of *stx1* gene in comparison to *stx2*. Similar studies were reported which indicated the highest gene in *E.coli* to be *hylA* that showed 5.9% occurrence in goats whilst *stx1* gene prevalence was 79.5% and *stx2* was 35% in non −O157 *E.coli* isolates (Osman et al., 2013; Parsons et al., 2016). The frequency of *eaeA* was lower (41.6%) in comparison which was supported by similar findings (Oporto et al., 2008; Pinaka et al., 2013).

The isolates revealed stx1a, stx1b, stx2a, eaeA, hylA and fliC protein profiles in an electrophoretic separation. Eighteen isolates showed the presence of eaeA protein in the range of 90–101 kDa, as reported by earlier findings (Jerse and Kaper, 1991) that indicated eaeA gene encoded a 94-kDa membrane protein. Stx1B and stx2A were present in 22 isolates with a molecular weight corresponding to > 35 kDa. The present study suggested that similar to O157: H7 isolates, the non-O157*E.coli*also could release enterotoxins that can cause severe damage to the intestinal wall in goats.

The correlation between virulence genes, antibiotic sensitivity and PFGE genotype profile showed high diversity in the isolates which indicated that there were numerous clones of non-O157 *E.coli* circulating among goats in the coastal regions of Goa, India. The isolates showed a 100% genetic similarity in six clusters that possessed three or more virulence factors prevalent particularly in young goats than in adults suggesting the age factor influencing the host-pathogen interactions and causing severe disease in young animals. As suggested in previous studies, age could be a major contributing factor to the genetic diversity and might participate in the anatomic and functional development of the rumen in goats (Jiao et al., 2015).The six clusters with 100% genetic similarity showed predominant presence of virulent factors viz.,

**Table 2**
Details of Clinical history, source of samples of 50 sorbitol fermenting non-O157 *E.coli* strains. An antibiotic sensitivity assay was performed using AMC-Amoxiclav- Ceftriaxone-Ciprofloxacin and DO-Dooxycycline hydrochloride. Multiplex PCR targeting six major virulence genes stx1, stx2, eaeA, hylA, fliC, rfbr was also shown.

| Isolate ID | Age of Goats | Environment | District in Goa | Antibiotic sensitivity test | | | | Virulence genes | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | AMC | CTR | CIP | DO | *Stx1* | *Stx2* | *eaeA* | *hylA* | *fliC* | *rfbr* |
| **1** | Kid | | North | − | − | − | − | + | − | + | + | + | − |
| **2** | Kid | | North | − | − | − | + | + | − | + | + | + | − |
| **3** | Adult | | North | − | − | + | + | − | − | + | + | − | − |
| **4** | Kid | | South | − | − | + | + | + | + | − | + | + | − |
| **5** | Kid | | South | − | − | − | − | + | + | − | + | + | − |
| **6** | Adult | | North | − | − | − | − | − | + | − | − | + | − |
| **7** | Adult | | North | − | − | + | + | − | + | − | + | − | − |
| **8** | Adult | | North | − | − | + | + | − | − | + | + | − | − |
| **9** | Kid | | North | − | − | + | − | + | + | − | + | − | − |
| **10** | Adult | | North | − | + | + | − | + | − | − | + | − | − |
| **11** | Adult | | North | − | + | + | + | − | − | + | + | − | − |
| **12** | Kid | | North | + | + | + | − | − | − | + | + | − | − |
| **13** | | Feed | North | + | + | + | + | + | − | − | + | + | − |
| **14** | | Wall | South | + | + | + | + | + | − | − | + | + | − |
| **15** | | Wall | South | + | + | + | + | + | + | − | + | − | − |
| **16** | | Water | South | + | + | + | − | − | + | − | − | − | − |
| **17** | | Feed | South | + | + | + | − | + | − | − | + | + | − |
| **18** | | Soil | North | + | + | + | + | + | − | − | + | + | − |
| **19** | | Feed | North | + | + | + | + | − | − | + | + | + | − |
| **20** | | Soil | North | − | + | + | + | + | + | − | + | + | − |
| **21** | | Wall | South | − | + | + | + | − | − | + | + | + | − |
| **22** | | Water | South | + | + | + | + | − | − | + | + | − | − |
| **23** | Kid | | South | + | + | + | + | + | − | − | + | + | − |
| **24** | Kid | | South | − | + | + | − | + | − | + | + | + | − |
| **25** | Kid | | South | − | + | + | − | − | − | + | + | + | − |
| **26** | Kid | | North | − | + | + | − | − | − | + | + | + | − |
| **27** | Kid | | North | − | + | + | − | − | − | + | + | + | − |
| **28** | Kid | | North | + | + | + | + | − | − | + | + | − | − |
| **29** | Kid | | South | + | + | + | − | − | − | + | + | + | − |
| **30** | Kid | | South | − | − | + | + | + | − | + | + | + | − |
| **31** | Kid | | South | + | + | + | + | + | − | + | + | + | − |
| **32** | Kid | | South | + | + | + | + | + | − | + | + | + | − |
| **33** | Kid | | South | + | + | + | − | − | − | + | + | + | − |
| **34** | Adult | | North | + | + | + | + | − | − | + | + | − | − |
| **35** | Adult | | North | − | + | + | + | − | + | − | + | + | − |
| **36** | Adult | | North | + | − | + | + | − | − | + | − | + | − |
| **37** | Kid | | North | − | + | + | + | + | − | − | − | + | − |
| **38** | Kid | | North | + | + | + | + | + | − | + | + | + | − |
| **39** | Kid | | North | + | + | + | + | − | − | + | + | + | − |
| **40** | Adult | | South | + | + | + | + | + | − | − | − | + | − |
| **41** | Kid | | South | − | + | + | | − | − | + | + | + | − |
| **42** | Kid | | South | − | + | + | | + | − | − | + | + | − |
| **43** | Kid | | North | + | + | + | | + | − | + | + | + | − |
| **44** | Adult | | North | − | + | + | | − | + | − | + | − | − |
| **45** | Adult | | North | − | + | + | | − | + | − | + | − | − |
| **46** | Kid | | North | − | + | + | | + | − | + | + | + | − |
| **47** | Kid | | North | + | + | + | | − | − | − | + | + | − |
| **48** | Adult | | South | + | + | + | | + | − | − | + | + | − |
| **49** | Kid | | South | − | − | + | | − | − | + | + | + | − |
| **50** | Adult | | South | + | + | + | + | − | − | + | + | + | − |

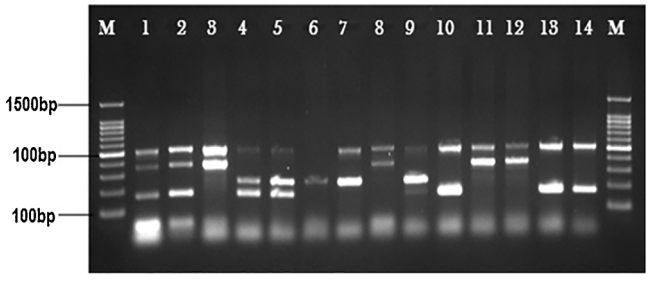


**Fig. 1.** Multiplex PCR for *E.coli* virulence genes; *STX1, STX2, eaeA and phylA*. Lane M: 100bp plus DNA marker, Lane 1–14 *E.coli* isolates. Expected amplicon sizes for genes are as follows; *STX1*- 180bp; *STX2*- 225bp; *eaeA* 384bp; *hylA*- 534bp.

*eaeA*, *hylA*, *fliC*, and *stx1*whereas, two clusters with 70% genetic similarity obtained from the environment (feed and house) showed similar virulence factors. Therefore, the isolates obtained from host and environment could possibly suggest that the environment could be a reservoir of non-O157 *E.coli* and could play a significant role in the faeco-oral transmission due to poor manure management practices (Lupindu et al., 2014; Söderlund et al., 2014). The genetic diversity among *E.coli* isolates from host and environment could be caused due to the determinants of virulence and antimicrobial resistance of gut flora of goats via horizontal gene transfer (Gyles and Boerlin, 2014; Jiao et al., 2015).This suggests that the normal flora of goats could undergo genetic diversity and might contribute to pathogen evolution which can be a significant reservoir of diversity in these isolates (Stecher et al., 2013; Shin et al., 2014). Further, genetic diversity in commensalism *E.coli* depends on the age group as younger goats showed the highest diversity.

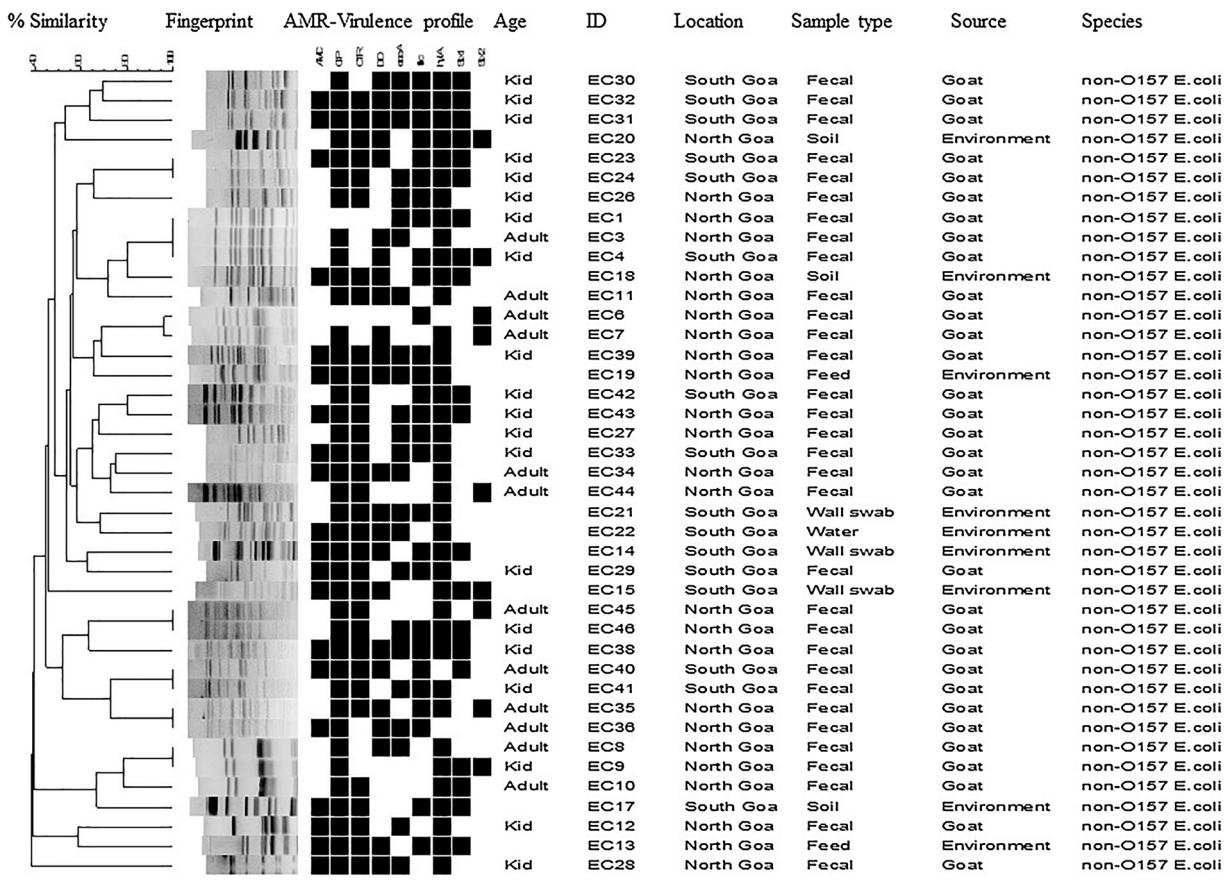


**Fig. 2.** XbaI-PFGE dendrogram showing the prevalence of the 49 non-O157 *E.coli* isolates from goats and.

**Table 3**
SDS-PAGE separation of the major proteins from 50 isolates.

| PROTEIN | PROTEIN MASS (Da) from UniProt | POSITIVE PERCENTAGE (N = 50 ISOLATES) |
|---|---|---|
| ***STX*1 A** | 34,824 Da | 25/50 = 50% |
| ***STX*1B** | 35,624 Da | 22/50 = 44% |
| ***STX*2 A** | 35,714 Da | 22/50 = 44% |
| ***eaeA*** | 101,836 Da | 18/50 = 36% |
| **Hila** | 99,000 Da | 23/50 = 46% |
| ***fliC*** | 51,295 Da | 47/50 = 94% |

## 5. Conclusion

The present study showed prevalence of virulent genes and multi-drug resistant non-O157 *E.coli* from goats in coastal India. The isolates with more than three virulence factors were predominantly present in young goats, suggesting age as a predisposing factor for genetic diversity. The non-O157 *E.coli* strains from host and environment showed a genetic similarity suggests the environment as a possible reservoir for establishing infection.

## Conflict of interest

None.

## Acknowledgement

The authors would like to thank the Director, ICAR-Central Coastal Agricultural Research Institute, Goa for providing necessary financial support for carrying out the research work.

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