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Occurrence and characterization of *Escherichia coli* O157:H7 and other serotypes in goat and sheep meat in India

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ABSTRACT

In the present study 307 goat and sheep meat samples were tested for *Escherichia coli* serotypes, which resulted in 4 Enterohaemorrhagic *Escherichia coli* (EHEC) from goat meat. All the four isolates produced verotoxin as evident from the *in-vitro* vero cell assay and the multiplex PCR. Serotyping of these isolates indicated that one isolate belonged to serotype O157 (M2), 2 to serotype 071 (23 and C3) and 1 to 060 (M). Molecular typing also confirmed that, isolate M2 belonged to 0157 serotype with positive amplification for *eae A* and *hly A* primers. PCR with H7 gene specific primers, also gave positive amplification with isolate M2, indicating that this isolate belonged to 0157:H7 serotype. Further studies for antibiotic resistance indicated that the *E. coli* 0157HH7 isolate from goat meat had multiple antibiotic resistance.

Key words: *E. coli* 0157:H7, Goats, Molecular characterization, Mutton, Serotyping

Enterohaemorrhagic *Escherichia coli* (EHEC) has been globally recognized as an important food-borne pathogen which often goes undetected. Consumption of contaminated meat is one of the most important sources of infection to humans. Enterohaemorrhagic *Escherichia coli* O157:H7 is a major cause of bloody diarrhoea in humans and is associated with haemorrhagic colitis and haemolytic uremic syndrome (Karmali *et al.* 1985). *E coli* O157:H7 found in the digestive tract of cattle and sheep and their feces are the potential primary source of *E. coli* O157:H7 causing contamination of numerous food products. The bacterium is transmitted to food products primarily through the fecal-oral route or by cross contamination. EHEC produce cytotoxins namely shigatoxins (stxs), which is classified into 2 major classes; stx 1 and stx 2 (Boerlin *et al.* 1999). Apart from stxs, there are other virulence factors which contribute to the pathogenicity of EHEC (Kim *et al.* 2005). The *eae* gene that codes for Intimin is a 94 to 97 KDa outer membrane protein produced by all attaching and effacing (A/E) enteric pathogens. Another putative virulence factor is haemolysin coded by the haemolysin operons.

Human *E.coli* O157:H7 outbreaks have been associated with the consumption of undercooked meat or unpasteurized milk (Whipp *et al.* 1994). Though *E. coli* O157:H7 has been isolated from retail lamb, chicken, and pork, the source of contamination in these products could well have been from beef. Several reports are available on the isolation and identification of *E. coli* O157: H7 from beef (Kim and Doyle 1992, Radu *et al.* 1998, Dutta *et al.* 2000). In India, *E. coli* O157 was isolated from beef (Dhanashree and Mallya 2008, Dutta *et al.* 2000), dairy products (Singh *et al.* 2009), and seafood (Sehgal *et al.* 2008). However, not much information is available on the isolation of *E. coli* O157:H7 from goat and sheep meat in India. Therefore, in this study the presence of *E. coli* O157:H7 and other serotypes in sheep and goat meat was investigated.

MATERIALS AND METHODS

Samples: Sheep and goat meat samples (307) were collected from slaughterhouses and retail outlets and homogenized in sterile phosphate buffered saline. The samples were enriched in buffered peptone water and incubated for 6 h at 37°C (Kim *et al.* 2005).

Conventional culture method: The enriched samples were streaked into Eosin Methylene blue (EMB) agar for the isolation of haemorrhagic *E. coli.* Single colonies exhibiting metallic sheen were selected and streaked onto SMAC agar supplemented with ceffixime (0.05 mg/L) and tellurite (2.5 mg/l). CT-SMAC plates were incubated at 37°C for 18–24 h. The sorbital non-fermenting colonies were inoculated into tryptic soy broth and the overnight cultures were tested by

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standard biochemical tests for confirmation of *E. coli*. The isolates were tested for antibiotic sensitivity using standard protocols.

Rapid latex agglutination: The non-sorbitol fermenting colourless colonies on CT-SMAC agar plates were subjected to *E. coli* O157:H7 rapid latex agglutination test (*E. coli* LAT kit) and motility tests. For assessing the motility, overnight cultures were inoculated into motility test medium and incubated at 37°C for 24 h.

Isolation of genomic DNA: Genomic DNA was isolated from *E. coli* cultures as described earlier (Radu *et al.* 2001). Briefly, 1 ml of broth cultures were centrifuged at 12 000 rpm for 5 min and the cell pellet was suspended in 1 ml of distilled water. Cells were boiled for 20 min, centrifuged for 5 min and the supernatant was used as template in PCR for assessing the presence of several virulence factors like stx/ (Beebakhee *et al.* 1986) and *hlyA.*

Multiplex PCR and flagellar H 7 PCR: Multiplex PCR for the detection of stx 1, stx 2, eaeA and hlyA was performed in a Gene AMPPCR thermal cycler and the oligonucleotide primers used are given in Table 1 (Kim *et al.* 2005). PCR assays were carried out in a 50 µl volume containing 4 µl of template DNA from the isolate and reference strain. The multiplex PCR-I had primers for vt1/stx1, vt2/stx2 and aeaA genes and multiplex PCR-II had primers for eaeA gene and hlyA gene. The cycle conditions for multiplex PCR-I consisted of an initial denaturation at 94°C for 2 min, followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 62°C for 1.5 min and 72°C for 2 min with a final extension at 72°C for 5 min. For multiplex PCR-II, the cycle conditions consisted of an initial denaturation at 94°C for 3 min, followed by 34 cycles of 94°C for 20 sec, 58°C for 40 sec and 72°C for 90 sec with a final extension at 72°C for 5 min. The amplified PCR products were analyzed in 2% agarose gels. Another PCR was carried out for the confirmation of H7 flagellar gene. The primer sequences for H7 flagellar gene are given in Table 1. PCR was carried out in 25 µl volume containing 12.5 µl of master mix, 20 pmol each of forward and reverse primers and 2 µl of template. The cycle conditions were 94°C for 1 min, 65°C for 2 min and 72°C for 2 min with a final extension at 72°C for 5 min. PCR products were analyzed in 1.5% agarose gels.

Table 1. Details of primers used in multiplex and flagellar H7 PCR

Primer	Sequence	Amplicon size
Multiplex PCR-1		
Vt1/stx1-F	cgctctgcaataggtactcc	256 bp
Vt1/stx1-R	cgctgrtgracctggaaagg	
Vt2/stx2-F	tccatgacaacggacagcag	186 bp
Vt2/stx2-R	gcttctgctgtgacagtgac	
eaeA-F	gcttagtgctggtttagtattg	620 bp
eaeA-R	ccagtgaactaccgccaaag	
Multiplex PCR-II		
eaeA-F	gtggcgaatactggcgagact	890 bp
eaeA-R	ccgcattctttttcaccgtcg	
hlyA-F	aggatgtggtttattctgga	165 bp
hlyA-R	cttcacgtgaccatacatant	
H7-F	gcgctgtcgattctatcgagc	625 bp
HR-R	caacggtgactttatcgccattcc	

Vero cell cytotoxicity assay: Vero cell cytotoxic assay was carried out with the isolates which were confirmed by multiplex and flagellar H7 PCR. Vero cells were cultured in Eagles Minimum essential medium supplemented with 10% fetal bovine serum. *E. coli* culture supernatants filtered through 0.2 µm filter were used to inoculate confluent monolayers of vero cells. The cytopathogenic effects were examined at 12 h interval.

RESULTS AND DISCUSSION

Out of the 307 meat samples tested, 101 samples produced colonies with metallic sheen of EMB agar indicating that they are *E. coli* isolates. Four of these isolates recovered from goat meat produced white colonies on CT-SMAC agar plates suggesting that these could be of *E. coli* O157 serotype. These 4 isolates when subjected to LAT, 1 isolate produced strong agglutination and the other 3 resulted in partial agglutination. Serotyping of these isolates indicated that only 1 isolated belonged to serotype O157 (M2). Two belonged to serotype 071 (23 and C3) and 1 belonged to 060 (M). All EHEC produce one or more antigenic types of verotoxin also known as shiga-like toxin (Gannon *et al*. 1997), which is an important marker. In our study all the 4 isolates produced verotoxin, which resulted in rounding of cells in 24 h and sloughing of cells in 48 h. This was further confirmed in multiplex PCR with all the four isolates resulting in positive amplification for the VT2 toxin.

After identification by cultural characters, motility and serotyping, all the 4 isolates were subjected to multiplex PCR for *stx1*, *stx2*, *eaeA* and *hlyA* genes and flagellar H7 PCR. Of the 4 isolates tested, isolate M2 gave positive amplification with *eae A, stx1* and *stx2* primers, with 620, 256 and 186 bp amplicons. The other 3 isolates were positive only for *stx2* primers though with weak amplification. In the second multiplex PCR also, isolate M2 alone gave positive amplification with *eae A* and *hly A* primers. The data available on the prevalence of *E. coli* 0157:H7 in India is limited especially in goat meat. These results have confirmed that all the 4 isolates obtained from goat meat belong to EHEC. However, our serotyping results indicated that only 1 of these 4 isolates (M2) belonged to 0.157. Molecular typing also confirmed that, isolate M2 belonged to 0157 with positive amplification for *eae A* and *hyl A* primers. Nevertheless, we did a PCR with H7 gene specific primers, which gave positive amplification only with isolate M2, indicating that M2 belong to 0157:H7 serotype. Further, isolate M2 gave positive amplification with H7 gene specific primers.

Isolate M2 was sensitive to the antibiotics amoxicilin,

clavunic acid, ciprofloxacillin, ceftriasone and tazobactum, and resistant to chloramphenicol and amikacin. Although the *E. coli* O157:H7 isolate obtained in this study was sensitive to many antibiotics, it was resistant to chloramphenicol and amicacin. Earlier studies with Indian isolates of *E. coli* O157: H7 from non-goat origin indicated that they were resistant to ampicillin, tetracycline, streptomycin and nalidixic acid but not to chloramphnicol and amikacin (Khan *et al.* 2002).

Presence of *E. coli* O157: H7 in goat meat raises important public health issues. Goat meat is very popular in southern parts of India and many prefer goat meat to beef. Considering the popularity of goat meat and the close proximity of goats with humans, a substantial amount of human population may be at risk for exposure to shiga toxin producing *E. coli* O157: H7 in this part of the globe. Nevertheless, the food and cooking habits under Indian conditions may help to avoid such infections for humans at least to some extent. Though prevalence of *E. coli* O157: H7 was not as high as that of other countries, more careful investigation programmes are needed in the future involving herds, slaughterhouses and meat packaging units. It is concluded that our study showed the occurrence of *E. coli* O157: H7 in goat meat with multiple antibiotic resistance.

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