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# Serogroup detection, antibiotic resistance patterns and virulence gene profile of *Escherichia coli* isolated from diarrheic sheep

SABAHAT GAZAL[1], A K TAKU[2], M A BHAT[3], BABLU KUMAR[4] and G A BADROO[5]

*Sher-e- Kashmir University of Agricultural Sciences and Technology, R.S. Pura, Jammu, Jammu and Kashmir181102 India*

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ABSTRACT

*Escherichia coli* isolates (115) were obtained from faecal samples collected from diarrheic sheep in Jammu, J&K. All the isolates were subjected to virulence characterization by multiplex PCR using primers for *stx1, stx2, eae* and *ehxA* genes. A total of 19 (16.5%) isolates were found to harbour toxic genes, out of which 12 isolates carried *stx1*, 3 carried *eae* whereas 3 were detected positive for both $stx_1$ and *exhA genes*. Only 1 of these 19 isolates was found to carry 3 genes ($stx_1$, $stx_2$ and *eae*). Upon serogrouping of all the isolates O69 was found to be the predominant serogroup (present in 30 of 115 isolates). In this study 12 serogroups were found. *In vitro* sensitivity test was also carried out to determine antibiotic resistance patterns of the isolates to various drugs. Maximum resistance was noted against antibiotics like polymixin and nitrofurantoin (38.46%) whereas maximum sensitivity was noted to chloramphenicol, ciprofloxacin and gentamicin (92%). Thus, these drugs can be recommended to treat diarrhoea in ovines.

**Key words**: Antibiogram, *Escherichia coli,* mPCR, Serogrouping, Sheep

*Escherichia coli* are a part of normal enteric microflora in most healthy individuals, yet are capable of causing serious diarrhoeal diseases, and other systemic diseases in animals and human (Buxton and Fraser 1977). Among virulence genes associated with *E. coli,* shiga toxins ($stx_1$ and $stx_2$), also called verotoxins (VT1 and VT2), are potent phage encoded toxins (Paton and Paton 1998) associated with food poisoning and potentially fatal illnesses in animal species and humans. Virulence - associated factor, intimin, is responsible for intimate attachment of the bacteria to intestinal epithelial cells, causing attaching-and-effacing lesions in intestinal mucosa (Jerse *et al*. 1990). It is encoded by the chromosomal gene *eae*, which is part of a pathogenicity island termed the locus for enterocyte effacement. Another factor enterohemolysin (Ehly) or enterohemorrhagic *E. coli* hemolysin (EHECHlyA) is encoded by the *ehxA* gene (Schmidt *et al*. 1995) and may also affect the virulence.

Continuous feeding of antibiotics to livestock for growth promotion has led to loss of their efficacy against various bacteria (Smith and Halls 1966). The resistance is especially important in *Enterobacteriaceae* due to the presence of transferable plasmids encoding multidrug resistance, whose dissemination among different enterobacterial species can pose a critical public health threat (Blake 2003, Carattoli 2009). Even though several studies have been done on cattle but there is scarcity of data regarding characterization of *E. coli* isolates from diarrheic sheep, an important animal. The aim of this study was to characterize the *E.coli* present in diarrheic sheep in terms of presence of one or many virulence associated genes, serogroups and their antibiotic resistance patterns in Jammu, J&K. Molecular characterization and serogrouping of the isolates were conducted to determine the possibility of sheep being a source of pathogenic *E. coli* strains circulating in human population.

Present address: [1,5] Ph.D. Scholar (gazalsabahat@gmail.com, gulzardr@ymail.com), [2]Professor and Head (aniltaku @hotmail.com),[3] Associate Professor, Division of Veterinary Microbiology and Immunology.[4] Assistant Professor, Department of Veterinary Microbiology, Bihar Veterinary College, Patna (babbacteriol@gmail.com).

## MATERIALS AND METHODS

*Sample:* Rectal swab samples from 115 diarrheic sheep were collected from various sheep farms in and around Jammu, J&K, India. The swabs were immediately transported to laboratory, streaked on MacConkey's Lactose Agar and incubated aerobically at 37°C for 24h. Well isolated pink colonies (1 from each plate) were picked and subjected to biochemical tests for *E. coli* (Carter *et al*. 1994).

*Serogrouping:* Confirmed *E. coli* isolates were sent to National Salmonella and Escherichia Centre, Central Research Institute, Kasauli (HP) for 'O' serogrouping.

*Molecular characterization of* E. coli *isolates:* All the *E. coli* isolates were subjected to multiplex PCR for $stx_1$, $stx_2$,

*eae* and *ehxA* genes using already established primers (Paton and Paton 1998). DNA was extracted from the isolates by suspending a loopful of confluent bacterial growth in 1 ml of sterile distilled water followed by boiling for 10 min. The DNA was used as template for multiplex PCR. PCR amplifications were performed in 0.2 ml thin walled PCR tubes. The PCR mixture contained a final concentration of 10 mM Tris–HCl, pH 9.0, 50 mM KCl, 3.5 mM $MgCl_2$, 1.0 µM concentration of each primer, 0.2 mM concentrations of each 2¹-deoxynucleoside 5¹-triphosphate and 1.0 U of Taq DNA polymerase. Oligonucleotide primers were procured commercially. Samples were subjected to 2 regimes of amplification. First regime consisted of 15 cycles. Each cycle consisted of 1 min of denaturation at 95 °C, 2 min of annealing at 65 °C and 1.5 min of extension at 72 °C. The second regime consisted of 20 cycles of 1 min of denaturation at 95 °C, 2 min of annealing at 60 °C and 2 min of extension at 72 °C. This was followed by final extension of 5 min at 72°C. Amplified PCR products were analysed by gel electrophoresis in 2% agarose containing ethidium bromide (0.5µg/mL) (Sambrook and Russell 2001). The products were visualised with UV illumination and imaged with gel documentation system.

In vitro *antibiotic sensitivity assay:* All the isolates were subjected to *in vitro* antibiotic sensitivity test as per Bauer *et al*. (1966). The commonly used antimicrobials like gentamicin, polymixin, nitrofurantoin, ampicillin, amoxyclav (amoxycillin + clavulanic acid), ciprofloxacin, ceftriaxone and chloramphenicol were tested for their *in vitro* efficacy against *E. coli* isolates from diarrheic sheep.

## RESULTS AND DISCUSSION

Out of 115 *E. coli* isolates subjected to molecular characterization by multiplex PCR, only 19 were found to carry the toxic genes. Thus the present study revealed the prevalence of virulence gene in *E. coli* isolates from diarrheic sheep in J&K, India to be 16.5%. However, a higher prevalence of 36% was observed by Blanco *et al*. (2003) in Spain. Out of these 19 isolates, 12 (63.15%) produced an amplicon of 180bp for $stx_1$, 3 (15.78%) isolates produced 384bp amplicon of *eae* whereas 3 (15.78%) produced amplicons of both 180bp and 534bp for $stx_1$ and *ehxA* gene respectively. Only 1 of these 19 isolates (5.26%) was found to carry 3 genes viz. $stx_1$, $stx_2$ (255bp amplicon) and *eae*. The virulence genes associated with ovine *E.coli* isolates are shown in Fig.1. Thus the major toxic gene $stx_1$ was present in 16 of 19 (84.2%) toxic gene positive *E. coli* isolates.

Table 1. List of primer sequences and predicted length of the PCR amplification products used for characterization of *E. coli* isolates

| Primer | Sequence | Amplicon size |
|---|---|---|
| *stx*1F | ATAAATCGCCATTCGTTGACTAC | 180bp |
| *stx*1R | AGAACGCCCACTGAGATCATC | |
| *stx*2F | GGCACTGTCTGAAACTGCTCC | 255bp |
| *stx*2R | TCGCCAGTTATCTGACATTCTG | |
| *eae*AF | GACCCGGCACAAGCATAAGC | 384bp |
| *eae*AR | CCACCTGCAGCAACAAGAGG | |
| *ehxAF* | GCATCATCAAGCGTACGTTCC | 534bp |
| *exhA*R | AATGAGCCAAGCTGGTTAAGCT | |

Table 2. Serogroups of *E.coli* isolates obtained in the study.

| *E.coli* serogroup | No. of isolates | Percentage (%) |
|---|---|---|
| O69 | 30 | 26.08 |
| O158 | 10 | 8.69 |
| O141 | 8 | 6.95 |
| O139 | 6 | 5.21 |
| O101 | 5 | 4.35 |
| O171 | 3 | 2.6 |
| O14 | 3 | 2.6 |
| O90 | 2 | 1.73 |
| O103 | 2 | 1.73 |
| O4 | 1 | 0.86 |
| O43 | 1 | 0.86 |
| O60 | 1 | 0.86 |
| UT (Untypeable) | 25 | 21.74 |
| Rough | 18 | 15.65 |

In diarrheic sheep 12 different serogroups were detected, of which O69 was the predominant serogroup present in 30 isolates followed by O158, O141and O139. Serogroups O101, O171, O14, O90, O103, O4, O43, O60 were also detected in the present study. Twenty-five isolates were untypeable whereas 18 were rough. The serogroups obtained in the study are presented in Table 2. Among the 12 different serogroups found in diarrheic sheep, O158 has been associated with infantile enteritis in London (Rowe *et al*. 1974), O103 has also been frequently reported from human

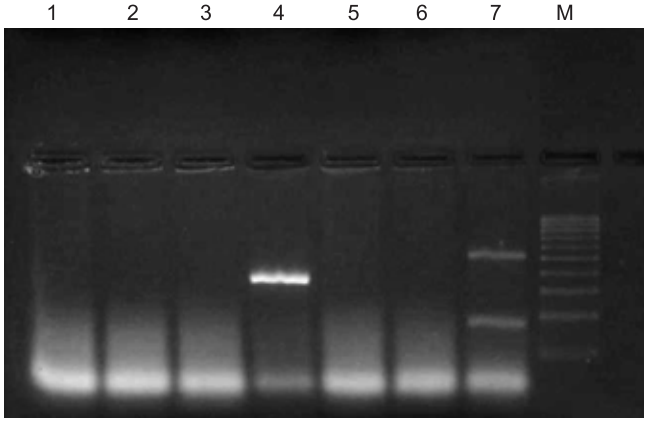


Fig. 1. Multiplex PCR for determination of virulence genes of *E.coli* isolates. Lane 4 carries *eae* (384bp) positive sample whereas lane7 carries $stx_1$ (180bp) and *ehxA* genes (534bp). Lane M depicts 100bp molecular weight marker.

patients and food in Germany (Werber*et al*. 2008) while O4 has been associated with human prostatitis, pyelonephritis and cystitis cases (Andreu *et al*. 1997). The presence of these serogroup in sheep indicated that these may have zoonotic importance and as a result sheep may transmit the serogroups and serve as a source of infection for humans.

The *in vitro* sensitivity assay carried out to determine the antibiotic resistance profile of ovine *E. coli* isolates revealed that the isolates were sensitive to a range of antimicrobials like chloramphenicol, ciprofloxacin and gentamicin (92% sensitive), followed by ceftriaxone and amoxyclav (84.5% isolates sensitive). However, resistance was found against antibiotics like polymixin and nitrofurantoin (38.46%). Similar results were reported by Purkayastha *et al*. (2010) who reported sheep *E. coli* isolates to be sensitive to ciprofloxacin, nalidixic acid, co-trimoxazole and chloramphenicol. Although the resistance could not be associated with toxic genes, the use of antibiotics for the treatment of STEC infections may be contraindicated because certain antibiotics induce the release and dispersion of shiga-toxin encoding bacteriophages (Zhang *et al.* 2000), which can complicate clinical situation of patients (Wong *et al.* 2000).The genetic changes and emergence of *E. coli* with multiple drug resistance are the major obstacles for the treatment of *E. coli* infection in different hosts, although the genetic bases for emergence of new strains with multiple drug resistance remain mostly unknown. Further studies targeting the prevalence of O157 (a well-known human pathogen) in sheep can be carried out.

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