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Original article

Escherichia coli in diarrhoeic lambs: Prevalence, virulence and antibiotic resistance

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Abstract

The present study aimed to detect the prevalence, virulence and antimicrobial resistance genes profile of *Escherichia coli* isolated from diarrhoeic lambs. A total of 61 faecal samples were collected from diarrhoeic lambs. The presence of various virulence and antimicrobial resistance genes in *E. coli* isolates was determined by the use of PCR. In total, 46 *E. coli* isolates were recovered from 61 rectal swabs of diarrhoeic lambs. Out of these 46 isolates, PCR showed that seven isolates (15.22%) carried the *stx1* or *stx2* gene and were found positive for Shiga-toxin-producing *E. coli* (STEC). Four isolates (8.70%) were found to be Enteropathogenic *E. coli* (EPEC) and all these EPEC isolates were atypical EPEC pathotypes. STEC and intimin-positive isolates were recovered only from one isolate, hence, out of 46 isolates, only one isolate (2.17%) was confirmed as Enterohaemorrhagic *E. coli*. The *lt* and *st* genes were not detected in any of the *E. coli* isolates recovered from field samples. Therefore, all the isolates were confirmed as non-Enterotoxigenic *E. coli*. Further, thirty-five isolates (76.09%) were found to be Entero--aggregative *E. coli* pathotypes. All the *E. coli* isolates were also tested for antimicrobial resistance against 15 different antibiotics. All the *E. coli* isolates were found to be resistant to penicillin-G, cephalothins, and azithromycin and the majority of isolates of *E. coli* were sensitive to chloramphenicol, ofloxacin, and sulfafurazole. Two antibiotic resistance genes *i.e. tetA* and *blaTEM* were detected in 10.87% (n=5/46) and 28.26% (n=13/46) of *E. coli* isolates, respectively.

Keywords: diarrhoeic lambs, *Escherichia coli*, virulence genes

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Introduction

Lamb diarrhoea is one of the most commonly reported diseases in lambs up to 3 months of age (Ghanbarpour et al. 2017). It causes economic losses, especially in neonatal animals resulting in weight loss, morbidity, and mortality (Ghanbarpour et al. 2017). Among the causative agents of diarrhea, *Escherichia coli* is the most commonly encountered pathogen (Yimer et al. 2015). Pathogenic *E. coli* is classified into intestinal pathogenic *E. coli* (IPEC) and extraintestinal pathogenic *E. coli* (ExPEC) according to the production of virulence factors (Habouria et al. 2019). Based on the type of virulence factor and host clinical symptoms, intestinal pathogenic *E. coli* strains associated with diarrhoea (IPEC) are further categorized into six pathotypes: Shiga-toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Entero-invasive *E. coli* (EIEC), Enterotoxigenic *E. coli* (ETEC), Entero-aggregative *E. coli* (EAEC), and Diffusely adherent *E. coli* (DAEC) (Mishra et al. 2019).

STEC isolates carry genes encoding Shiga toxins 1 and/or 2 (*stx1, stx2*) while EHEC produces *stx1*/or *stx2* and intimin which is encoded by the *eaeA* gene (Askari Badouei et al. 2014). EPEC isolates are intimin-containing diarrhoeagenic *E. coli* isolates that can form attaching and effacing (AE) lesions on intestinal cells but do not possess genes coding for Shiga toxins (Gomes et al. 2016). EPEC strains may be either typical, producing bundle-forming pili (encoded by the *bfpA* gene), or atypical, which are negative for these appendages (Gomes et al. 2016). The EIEC strains have a plasmid that carries the *ial* gene which helps to invade the colon epithelial cells (Mishra et al. 2019). ETEC isolates are the major cause of diarrhoea in newly born farm animals causing a major loss to the livestock industry (Cho and Yoon 2014). Enterotoxins and fimbrial antigens are the major virulence factors of ETEC (Duan et al. 2012). Fimbrial adhesins mediate the attachment of bacteria to epithelial surfaces in the small intestine and thereby facilitating bacterial colonization with both heat-labile (LT) and heat-stable (ST) enterotoxins. These toxins induce the secretion of fluids and electrolytes from the intestinal epithelial cells, which ultimately leads to diarrhoea (Wang et al. 2019). The EAEC isolates are characterized by the presence of aggregative adherence factors (AAFs). EAEC-associated components of the ETT2 (*E. coli* type III secretion system 2) are encoded by the *etrA* gene (Zhao et al. 2021). The most suitable methods for the differentiation of diarrhoeagenic and non-pathogenic strains of *E. coli* are DNA-based methods (Türkyilmaz et al. 2013).

Bacteria develop resistance to the antibiotics which are frequently used for treatment. Therefore, continuous monitoring at regional and local levels in terms of their characterization, including antibiotic sensitivity patterns, is essential to devise a successful treatment strategy. Looking towards the importance of virulence and antimicrobial resistance genes of *E. coli* associated with lamb diarrhoea, this study was planned to detect the virulence and antimicrobial resistance genes of *E. coli* strains isolated from diarrhoeic lambs.

Materials and Methods

Ethics statement

The faecal samples used in the study were collected from diarrhoeic lambs. The history of animals with the symptoms of diarrhoea was recorded at the time of sampling. The study was approved by Institutional Animal Ethics Committee (Protocol No. /IAEC/RES/02/03).

Study area and sample collection

The study was conducted at the College of Veterinary and Animal Science, Navania, Udaipur, Rajasthan, India. A total of 61 faecal samples were collected from 0 to 4 months-old diarrhoeic lambs from nine sheep farms, *i.e.* one institutional sheep farm of Mega Sheep Seed Project (MSSP), ICAR in College of Veterinary and Animal Science, Navania, and eight sheep farms (farmer's flock) in nearby eight villages viz. Udakhera, Laxmanpura, Jaspura, Asawara, Bhatewar, Sawaniya, Khemli, and Dabok from June 2022 to November 2022. These farms and villages were randomly selected based on the availability of sheep population. Faecal samples were collected directly from the rectum of diarrhoeic lambs using sterile cotton swabs (HiMedia Laboratories Pvt. Ltd., Mumbai, India). The collected swabs were kept on the ice and immediately brought to the Department of Veterinary Microbiology, CVAS, Navania, Udaipur. Demographic data of the lambs such as age and sex were recorded.

Bacteriological examination

Faecal sample swabs were subjected to bacteriological examination. All the samples were subjected to isolation of bacteria and biochemical characterization of bacterial isolates as per the standard techniques (Markey et al. 2013). In the biochemical characterization were performed by Gram staining, catalase test, oxidase test, motility test, O-F test, Indole test, methyl red test, Voges Proskaurer test, citrate utilization test, triple sugar iron agar test, urease test and haemolysis test (Markey et al. 2013). Further, the pathogenicity (invasiveness) of the *E. coli* isolates was determined

Table 1. Oligonucleotide sequences used for the detection of *Escherichia coli,* various virulence and antimicrobial resistance genes.

S. No.	Name of Genes	Primer	Sequence (5' – 3')	Annealing (°C)	Amplicon size (bp)	Reference
1.	*uspA*	*uspA*-F	5'-CCGATACGCTGCCAATCAGT-3'	49°C	884	Mishra et al. 2019
		uspA-R	5-ACGCAGACCGTAGGCCAGAT-3'			
2.	*uidA*	*uidA*-F	5'-GCGTCTGTTGACTGGCAGGTGGTGG-3'	67°C	510	Johnson et al. 2017
		uidA-R	5'-GTTGCCCGCTTCGAAACCAATGCCT-3'			
3.	*stx1*	*stx1*-F	5'-AAATCGCCATTCGTTGACTACTTCT-3'	55.2°C	366	Momtaz et al. 2013
		stx1-R	5'-TGCCATTCTGGCAACTCGCGATGCA-3'			
4.	*stx2*	*stx2*-F	5'-CGATCGTCACTCACTGGTTTCATCA-3'	57°C	282	Momtaz et al. 2013
		stx2-R	5-GGATATTCTCCCCACTCTGACACC-3'			
5.	*eaeA*	*eaeA*-F	5'-GACCCGGCACAAGCATAAGC-3'	55°C	384	Wani et al. 2003
		eaeA-R	5-CCACCTGCAGCAACAAGAGG-3'			
6.	*bfpA*	*bfpA*-F	5'- ATGGTGCTTGCGCTTGCTGC-3'	52°C	158	Mishra et al. 2019
		bfpA-R	5'-AATCCACTATAACTGGTCTGC-3'			
7.	*lt*	*lt* -F	5'-GGC GAC AGA TTA TAC CGT GC-3'	51.4°C	450	Mishra et al. 2019
		lt -R	5'-CGG TCT CTA TAT TCC CTG TT-3'			
8.	*st*	*st*-F	5'- ATT TTT CTT TCT GTA TTG TCT T-3'	45.5°C	190	Mishra et al. 2019
		st-R	5'-CAC CCG GTA CAA GCA GGA TT-3'			
9.	*etrA*	*etrA*-F	5'-CTTCTTCCTAACGAAACTATCATTA-3'	56.5°C	913	Zhao et al. 2021
		etrA-R	5'-TGACATATCAACTTTCTCTTACGC-3'			
10.	*tetA*	*tetA*-F	5'- GGTTCACTCGAACGACGTCA-3'	53°C	577	Momtaz et al. 2013
		tetA-R	5'-CTGTCCGACAAGTTGCATGA-3'			
11.	*blaTEM*	*blaTEM*-F	5'- ATCAGCAATAAACCAGC-3'	51°C	857	Maynard et al. 2003
		blaTEM-R	5'-CCCCAAGAACGTTTTC-3'			

as per the technique recommended by standard techniques (Mishra et al. 2019).

Extraction of bacterial DNA

The isolation of bacterial genomic DNA from *E. coli* isolates was carried out by heat treatment method (Li et al. 2017). The concentration and purity of DNA were estimated in a UV absorbance bio-spectrophotometer (Eppendorf, Hamburg, Germany).

Identification of *E. coli* by Polymerase Chain Reaction (PCR)

The molecular confirmation of *E. coli* was done by PCR amplification of the universal stress protein A (*usp*A) gene and *uidA* gene. The oligonucleotide sequences along with their amplicon sizes are listed in Table 1. A total of 25 µl volume was used for the PCR amplification which contained 3 µl DNA, 12.5 µl 2 × master mix (Genetix Biotech Asia Pvt. Ltd., New Delhi, India), 0.75 µl (25 pmol) of each primer (forward and reverse), and then nuclease-free water (NFW) was added to make a final volume of 25 µl. Then, the PCR reaction mixtures were set into an automated thermal cycler (Biorad Pvt. Ltd., California, USA). The amplified PCR products were subjected to electrophoresis and it was allowed to run for 1 hr. of 70 V in agarose gel (1.5%) containing ethidium bromide (0.5 µg/ml). Following electrophoresis, DNA bands were visualized and compared with the DNA ladder, and the images were captured by using the gel documentation system (Biogen Scientific, Cambridge, USA).

PCR assay for detection of virulence genes

The presence of seven virulence genes *viz stx1, stx2, eaeA, bfpA, lt, st* and *etrA* encoding various virulence factors was screened in all the *E. coli* isolates by PCR. The oligonucleotide sequences along with their

amplicon sizes are listed in Table 1. The PCR reaction mixtures were prepared and set into an automated thermal cycler. Amplified PCR products were analyzed by gel electrophoresis in 1.5% agarose containing ethidium bromide. Following electrophoresis, DNA bands were visualized and compared with the DNA ladder and images were captured by using the gel documentation system.

Antimicrobial susceptibility testing

All the *E. coli* isolates were subjected to *in vitro* antimicrobial susceptibility testing using 15 antimicrobial agents *viz.* penicillin-G, ampicillin, cephalothin, ceftriaxone, cefixime, ofloxacin, ciprofloxacin, gentamicin, tetracycline, sulfafurazole, co-trimoxazole, trimethoprim, chloramphenicol, polymyxin-B and azithromycin by Kirby-Bauer disc diffusion method (Bauer et al. 1966) according to the Clinical and Laboratory Standards Institute's guidelines (CLSI, 2020).

PCR assay for detection of antimicrobial resistance genes

Molecular detection of antibiotic resistance genes *viz*. *tet(A)* and *blaTEM* genes was done by using PCR. The oligonucleotide sequences along with their amplicon sizes are listed in Table1. The PCR reaction mixtures were prepared and set into an automated thermal cycler. Amplified PCR products were analyzed by gel electrophoresis in 1.5% agarose containing ethidium bromide. Following electrophoresis, DNA bands were visualized and compared with the DNA ladder and images were captured by using the gel documentation system.

Results

Prevalence of *E. coli*

Out of 61 faecal samples obtained from diarrhoeic lambs, forty-six isolates were identified as *E. coli* based on morphological, biochemical, and molecular confirmation. Results revealed that the overall prevalence of *E. coli* was 75.41% (n=46/61).

Distribution of virulence genes

All these 46 isolates of *E. coli* were further subjected to PCR for the detection of virulence genes *viz*. *stx1, stx2, eaeA, bfpA, lt*, *st*, and *etrA*. The prevalence of STEC in *E. coli* isolates was 15.22% (n=7/46) as seven isolates contained at least one virulence gene for STEC *i.e. stx1* or *stx2.* Among the virulence genes of STEC, 13.04% (n=6/46) *E. coli* isolates positive for the *stx1* gene, 6.52% (n=3/46) of isolates positive for the *stx2* gene, and 4.35% (n=2/46) isolates produced positive amplicon for both *stx1* and *stx2*. The *stx1* gene was more prevalent than the *stx2* gene. EPEC (*eaeA*-positive non-STEC) was detected in 8.70% of *E. coli* isolates in diarrhoeic lambs. The *eaeA* gene was detected in 16.67% of STEC isolates from diarrhoeic lambs and classified as EHEC. All 46 isolates were non-ETEC as they were negative for both *lt* and *st* genes. EAEC encoding gene *etrA* was detected in 76.09% (n=35/46) of *E. coli* isolated and was found the most prevalent pathotype.

Antimicrobial susceptibility testing

In all *E. coli* isolates resistant to antimicrobial agents in decreasing frequency order: azithromycin (100%), cephalothin (100%), penicillin-G (100%), polymixin-B (91.30%), cefixime (43.48%), gentamicin (41.30%), ampicillin (28.26%), ceftriaxone (21.74%), tetracycline (21.74%), trimethoprim (17.39%), co-trimoxazole (13.04%), ciprofloxacin (6.52%), sulfafurazole (6.52%), ofloxacin (4.35%) and chloramphenicol (0%).

Distribution of antimicrobial resistance genes

In the present study, the *tetA* gene was reported in 10.87% (n=5/46) of *E. coli* isolates. In the current study, the *blaTEM* gene was found in 28.26% (n=13/46) of *E. coli* isolates.

Discussion

In the present study, the prevalence of STEC in *E. coli* isolates was 15.22%. The present findings are in agreement with other reports (Bhat et al. 2008, Türkyilmaz et al. 2013) reported almost similar prevalence for STEC in diarrhoeic lambs as 17.5% and 17.76%, respectively. In contrast to the present findings, a higher prevalence of STEC was detected by other studies (Bandyopadhyay et al. 2011, Ghanbarpour et al. 2017, Sujatha 2018) as 32%, 40.34%, 87.05%, and 32.05%, respectively. However, a lower prevalence of STEC in diarrhoeic lambs was reported by other scientists (Wani et al. 2003, Wani et al. 2009) as 6.66% and 9.6%, respectively. The occurrence of STEC may vary with different geographical locations and seasons (Bandyopadhyay et al. 2011), the difference in animal husbandry practices, breeds, and age of the animal, and agro-climatic variation (Gupta et al. 2022). Among the virulence genes of STEC, the *stx1* gene was more prevalent than the *stx2* gene. The results

of the present study are in accordance with other scientific studies (Bhat et al. 2008, Sujatha 2018) that reported a higher prevalence of the *stx1* gene than the *stx2* gene in diarrhoeic lambs. In contrast, studies by other authors (Wani et al. 2003, Wani et al. 2009, Bandyopadhyay et al. 2011, Türkyilmaz et al. 2013, Ghanbarpour et al. 2017) reported a higher prevalence of *stx2* gene than *stx1* gene in diarrhoeic lambs. The detection of the *stx2* gene in the present study is a matter of concern for animal handlers because the *E. coli* isolates harbouring the *stx2* virulence gene have been implicated in dreadful human infections such as haemolytic colitis and haemorrhagic uremic syndrome and those carrying *stx1* may trigger diarrhoea in immune-compromised individuals (Ghanbarpour et al. 2017).

EPEC was detected in 8.70% of *E. coli* isolates in diarrhoeic lambs. The present study findings are in agreement with the findings of (Bhat et al. 2008) who reported almost similar prevalence (12.5%) of EPEC in diarrhoea lambs. However, a higher prevalence of EPEC was detected by some authors (Wani et al. 2003, Bandyopadhyay et al. 2011, Türkyilmaz et al. 2013) as 26.67%, 18%, 20.6%, and 67.94% respectively, while others (Wani et al. 2009, Ghanbarpour et al. 2017) reported a lower prevalence of EPEC as 6.1% and 4.48% respectively. The variation in the prevalence of EPEC may be due to geographical, climatic, or seasonal variations. All EPEC isolates in the present study were found to be atypical EPEC (aEPEC) as the *bfpA* gene was not detected in these isolates. These observations are in agreement with the findings of (Ghanbarpour et al. 2017) who reported that the majority of the EPEC isolates were aEPEC. This is also in concur with the findings of some authors (Chandran and Mazumder 2013) who revealed that humans are the only reservoir of typical EPEC (tEPEC) except for a few isolates from dogs, whereas, others (Wani et al. 2009, Türkyilmaz et al. 2013) reported tEPEC among EPEC isolates in diarrhoeic lambs as they detected the *bfpA* gene. The tEPEC is a leading cause of infant diarrhoea in developing countries, whereas aEPEC is found frequently (Trabulsi et al. 2002). In the present study, the *eaeA* gene was detected in 16.67% of STEC isolates from diarrhoeic lambs and classified as EHEC. The results of the present study are in agreement with findings of some authors (Bandyopadhyay et al. 2011, Sujatha 2018) who detected the *eaeA* gene in 18% and 16.22% of STEC isolates, respectively. This is in contrast to the findings of others (Bhat et al. 2008, Türkyilmaz et al. 2013) who found the *eaeA* gene in 2.85% and 10.2% of STEC isolates, respectively, whereas, others (Wani et al. 2009, Ghanbarpour et al. 2017) reported the *eaeA* gene in 46.2% and 48.72% of STEC isolates, respectively. The existence of the *eaeA* gene in STEC isolates showed that the *eaeA*-positive isolates could be more dangerous for human being than *eaeA*-negative isolates because the *eaeA* gene may be needed for the expression of full virulence of STEC for humans (Ramamurthy 2008).

In the present study, all 46 isolates were non-ETEC. The findings of the present investigation were in agreement with Sujatha (2018), who also observed that none of the isolates belonged to ETEC in diarrhoeic lambs. However, some authors (Bandyopadhyay et al. 2011, Türkyilmaz et al. 2013, Ghanbarpour et al. 2017) detected ETEC isolates in diarrhoeic lambs. The difference in the prevalence of *st* and *lt* genes may be associated with different factors such as geographical locations (Ghanbarpour et al. 2017), season, environment, and hygienic conditions in the farm (Sujatha 2018). The absence of enterotoxin genes in the present study indicated that *E. coli* in this geographical area may carry other genes, which, we have not tested in this study or ETEC may not play an important role in the frequent occurrence of lamb diarrhoea in this region of Rajasthan. In the present study, EAEC was found the most prevalent pathotype. The present study findings are in agreement with the observations of Zhao et al. (2021) who reported almost similar prevalence (80.2%) of EAEC in diarrhoeic sheep. In the current study, *etrA* (76.09%) gene was the most prevalent among all virulence genes.

The presence of virulent isolates of *E. coli* in the environment may be a potential source of contamination of food and water. Moreover, these isolates also contain a potential reservoir of virulence genes acquired from different sources. *E. coli* is a very dynamic organism with the capacity for horizontal gene transfer which increases its genetic diversity and sometimes this can lead to the emergence of new pathogenic isolates (Sujatha 2018).

An increase in the resistance of *E. coli* isolates towards some antibiotics may be attributed to the indiscriminate use of antibiotics and due to under-dosing and irrational therapy, particularly for the treatment of diarrhoea (Tarunpreet et al. 2019). Similar findings were reported by Momtaz et al. (2013), who revealed that the majority of the *E. coli* isolates were resistant to penicillin. Almost similar antibiotic resistance patterns of *E. coli* isolates were detected by Pachaury and Kataria (2012) in diarrhoeic lambs, kids, and calves. Similarly, Kumar et al. (2022) also observed that the majority of *E. coli* isolates from diarrhoeic sheep were resistant to penicillin. Similarly, Tarunpreet et al. (2019) reported high sensitivity to chloramphenicol and low sensitivity to cefixime. On the contrary, Kumar et al. (2022) showed resistance to antimicrobial agents in decreasing order was ampicillin (93.3%), tetracycline

(68.8%), amoxicillin, chloramphenicol, cotrimoxazole and ofloxacin (31.3%) each and ceftriaxone (18.8%) in *E. coli* isolates from diarrhoeic lambs.

Antimicrobial agents play a significant role in the treatment of animal and human diseases around the world. But, indiscriminate use of antimicrobial agents in milk and meat-producing animals has always resulted in the development of antimicrobial-resistant bacteria (Kumar et al. 2022). The distribution of antimicrobial resistance genes found in the present study was very low. This may be due to the lower use of antimicrobial agents in this area of Rajasthan by the local veterinarians. In the present study, the *tetA* gene was reported in 10.87% of *E. coli* isolates. On the contrary, Kumar et al. (2022) reported a higher prevalence (47.5%) of the *tetA* gene in diarrhoeic sheep and goats. In the current study, the *blaTEM* gene was found in 28.26% of *E. coli* isolates. This result concurs with the findings of Kumar et al. (2022) who reported almost similar prevalence (30%) of *blaTEM* in sheep and goats. In contrast to the present study, a higher prevalence of *blaTEM* (91.21%) was reported (Sujatha 2018). The differences in the distribution of antimicrobial resistance genes in bacteria may be due to differences in antimicrobial use and different geographical zone.

In conclusion, in this study, *E. coli* is highly prevalent in diarrhoeic lambs in the Udaipur region of Rajasthan, India. Isolation of STEC and EHEC is a serious public health concern in this study area, where the sheep handlers live in close contact with sheep. Little concern for hygiene may be a source of an important reservoir of STEC and EPEC infection in humans. The prevailing *E. coli* in diarrhoeic lambs in the Udaipur region were found completely resistant to azithromycin, cephalothin, and penicillin-G. Chloramphenicol, ofloxacin, and sulfafurazole against which minimal resistance was observed, may be used to treat *E. coli*-associated diarrhoea in the Udaipur region of Rajasthan.

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References

Askari Badouei M, Lotfollahzadeh S, Arman M, Haddadi M **(2014)** Prevalence and resistance profiles of enteropathogenic and shiga Toxin Producing *Escherichia coli* in diarrheic calves in Mashhad and Garmsar districts, Iran. Avicenna J Clin Microbiol Infect 1: 22802.

Bandyopadhyay S, Mahanti A, Samanta I, Dutta TK, Ghosh MK, Bera AK, Bandyopadhyay S, Bhattacharya D **(2011)** Virulence repertoire of Shiga toxin-producing Escherichia coli (STEC) and enterotoxigenic Escherichia coli (ETEC) from diarrhoeic lambs of Arunachal Pradesh, India. Trop Anim Health Prod 43: 705-710.

Bauer AW, Kirby WM, Sherris JC, Turck M **(1966)** Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45: 493-496.

Bhat MA, Nishikawa Y, Wani SA **(2008)** Prevalence and virulence gene profiles of Shiga toxin-producing Escherichia coli and enteropathogenic Escherichia coli from diarrhoeic and healthy lambs in India. Small Rumin Res 75: 65-70.

Chandran A, Mazumder A **(2013)** Prevalence of diarrhea-associated virulence genes and genetic diversity in Escherichia coli isolates from fecal material of various animal hosts. Appl Environ Microbiol 79: 7371-7380.

Cho YI, Yoon KJ **(2014)** An overview of calf diarrhea – infectious etiology, diagnosis, and intervention. J Vet Sci 15: 1-17.

Clinical and Laboratory Standards Institute **(2020).** Performance standards for antimicrobial disk susceptibility test. M02 standard, 13th ed., Clinical and Laboratory Standards Institute, Wayne. PA.

Duan Q, Yao F, Zhu G **(2012)** Major virulence factors of enterotoxigenic Escherichia coli in pigs. Ann Microbiol 62: 7-14.

Ghanbarpour R, Askari N, Ghorbanpour M, Tahamtan Y, Mashayekhi K, Afsharipour N, Darijani N **(2017)** Genotypic analysis of virulence genes and antimicrobial profile of diarrheagenic Escherichia coli isolated from diseased lambs in Iran. Trop Anim Health Prod 49: 591-597.

Gomes TA, Elias WP, Scaletsky IC, Guth BE, Rodrigues JF, Piazza RM, Ferreira LC, Martinez MB **(2016)** Diarrheagenic Escherichia coli. Braz J Microbiol 47 (Suppl1): 3-30.

Gupta MD, Sen A, Shaha M, Dutta A, Das A **(2022)** Occurrence of Shiga toxin-producing Escherichia coli carrying antimicrobial resistance genes in sheep on smallholdings in Bangladesh. Vet Med Sci 8: 2616-2622.

Habouria H, Pokharel P, Maris S, Garénaux A, Bessaiah H, Houle S, Veyrier FJ, Guyomard-Rabenirina S, Talarmin A, Dozois CM **(2019)** Three new serine-protease autotransporters of Enterobacteriaceae (SPATEs) from extra-intestinal pathogenic Escherichia coli and combined role of SPATEs for cytotoxicity and colonization of the mouse kidney. Virulence 10: 568-587.

Johnson JR, Johnston BD, Gordon DM **(2017)** Rapid and specific detection of the Escherichia coli sequence type 648 complex within phylogroup F. J Clin Microbiol 55: 1116-1121.

Kumar K, Sharma NS, Kaur P, Arora AK **(2022)** Molecular detection of antimicrobial resistance genes and virulence genes in E. coli isolated from sheep and goat faecal samples. Indian J Anim Res 56: 208-214.

Li F, Li F, Chen B, Zhou B, Yu P, Yu S, Lai W, Xu H **(2017)** Sextuplex PCR combined with immunomagnetic separation and PMA treatment for rapid detection and specific identification of viable Salmonella spp., Salmonella enterica serovars Paratyphi B, Salmonella Typhimurium, and Salmonella enteritidis in raw meat. Food Control 73: 587-594.

Markey B, Leonard F, Archambault M, Cullinane A, Maguire D **(2013)** Clinical Veterinary Microbiology, Second Edition, Elsevier Health Sciences, Mosby Ltd.

Maynard C, Fairbrother JM, Bekal S, Sanschagrin F, Levesque RC, Brousseau R, Masson L, Larivière S, Harel J **(2003)** Antimicrobial resistance genes in enterotoxigenic Escherichia coli O149:K91 isolates obtained over a 23-year period from pigs. Antimicrob Agents Chemother 47: 3214-3221.

Mishra AK, Singh DD, Gururaj K, Kumar A, Dixit A, Shrama N, Gupta G, Yadav S **(2019)** Molecular characterization of diarrhoegenic Escherichia coli isolated from neonatal goat-kids. J Anim Res 9: 51-59.

Momtaz H, Dehkordi FS, Rahimi E, Ezadi H, Arab R **(2013)** Incidence of Shiga toxin-producing Escherichia coli serogroups in ruminant's meat. Meat Sci 95: 381-388.

Pachaury R, Kataria AK **(2012)** Evaluation of antimicrobial efficacy against E. coli isolates of different serogroups obtained from diarrhoeic neonates of calves, kids, and lambs. Media Peternakan 35: 175-178.

Ramamurthy T **(2008)** Shiga toxin-producing Escherichia coli (STEC): the bug in our backyard. Indian J Med Res 128: 233-236.

Sujatha T **(2018)** Studies on antimicrobial resistance of E. coli isolates from lamb diarrhoea cases [M.V.Sc. thesis] submitted to Sri Venkateswara Veterinary University: Tirupati. https://krishikosh.egranth.ac.in/items/a3b07b32-3ca7-4d38-8fba-8ebd7e9ae987

Tarunpreet, Sharma SK, Singh AP, Goklaney, Deepika **(2019)** Prevalence of colibacillosis disease and clinico-haemato biochemical changes in lambs in southern part of rajasthan. Vet Pract 20: 95.

Trabulsi LR, Keller R, Tardelli Gomes TA **(2002)** Typical and atypical enteropathogenic Escherichia coli. Emerg Infect Dis 8: 508-513.

Türkyilmaz S, Eskiizmirliler S, Tunaligil S, Bozdogan B **(2013)** Identification, characterization and molecular epidemiology of Escherichia coli isolated from lamb and goat kids with diarrhoea. Acta Vet Brno 82: 357-362.

Wang H, Zhong Z, Luo Y, Cox E, Devriendt B **(2019)** Heat--Stable Enterotoxins of enterotoxigenic Escherichia coli and Their Impact on Host Immunity. Toxins (Basel)11: 24.

Wani SA, Bhat MA, Samanta I, Nishikawa Y, Buchh AS **(2003)** Isolation and characterization of Shiga toxin-producing Escherichia coli (STEC) and enteropathogenic Escherichia coli (EPEC) from calves and lambs with diarrhoea in India. Lett Appl Microbiol 37: 121-126.

Wani SA, Hussain I, Fayaz I, Mir MA, Nishikawa Y **(2009)** Subtype analysis of stx1, stx2 and eae genes in Shiga toxin-producing Escherichia coli (STEC) and typical and atypical enteropathogenic E. coli (EPEC) from lambs in India. Vet J 182: 489-490.

Yimer M, Gezhagne M, Biruk T, Dinaol B **(2015)** A review on major bacterial causes of calf diarrhea and its diagnostic method. J Vet Med Anim Health 7: 173-185.

Zhao X, Lv Y, Adam FE, Xie Q, Wang B, Bai X, Wang X, Shan H, Wang X, Liu H, Dang R, Wang J, Yang Z **(2021)** Comparison of antimicrobial resistance, virulence genes, phylogroups, and biofilm formation of Escherichia coli isolated from intensive farming and free-range sheep. Front Microbiol 12: 699927.