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Determination of Virulence Factors and Antibiotic Resistance with Special Emphasis on Extended Spectrum β Lactamase Producing *Escherichia coli* from Sheep and Goats in Jammu, India

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ABSTRACT

The present study was aimed at investigating the pathogenic strains of *Escherichia coli* in sheep and goats and their antibiotic resistance patterns with special emphasis on extended spectrum beta lactamases producing *E. coli*. A total of 200 presumptive *E. coli* isolates were obtained from 120 faecal samples of sheep and goats. Out of these 200 isolates, 62 isolates showed the presence of at least one virulence gene studied. Among these, 10 isolates carried *eaeA* gene either alone or in combination with *ehxA* and were categorized as EPEC. 44 isolates carried *stx* genes and were categorized as STEC, 08 isolates carried *ehxA* genes and were designated as EHEC. All 62 isolates showing the presence of at least one virulence gene were further screened for ESBL production by using primers specific for bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV} and bla_{OXA} genes. Out of 62 isolates, only 22 were tested positive for presence of ESBL genes. Further, the prevalence of bla_{TEM}, $bla_{CTX\text{-}M}$ and bla_{SHV} genes was found to be 54.54%, 27.27% and 36.36% respectively. Antibiotic sensitivity profiling of the isolates positive for virulence genes revealed that all isolates were resistant to cefotaxime.

HIGHLIGHTS

- The most abundant ESBLs type in Sheep and Goat of Jammu region is bla_{TEM} gene.
- Antibiotic sensitivity profiling shows highest resistance against cefotaxime.

Keywords: *E. coli*, ESBL, multiplex PCR, Virulence genes

Escherichia coli is a part of normal enteric microflora in most healthy individuals, yet are capable of causing serious diarrheal diseases, and other systemic diseases in animals and human. The diarrhoeagenic *E. coli* have been categorized into six pathotypes based on mechanism of pathogenesis and the illness caused. These pathotypes include shigatoxigenic *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffusely adherent *E. coli* (DAEC) and enteroaggregative *E. coli* (EAggEC)

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(Ghilardi *et al*. 2001). Human disorders viz. hemorrhagic colitis and hemolytic uremic syndrome are caused by Shiga toxin-producing *Escherichia coli* (STEC) (Nataro and Kaper 1998). STEC isolates that causes hemolytic uremic syndrome are known as enterohemorrhagic *E. coli* (EHEC) (Mainil 1999). Although healthy cattle are the primary STEC reservoir, some research suggest that sheep also have a role in the spread of these organisms leading to the risk of human infection (Ferens and Hovde 2011). Shiga toxins 1 and 2, which are encoded by the stx_1 and stx_2 genes, respectively, are the primary virulence factors of STEC that impede protein synthesis in eukaryotic cells (Gyles *et al*. 2010). According to epidemiological research, stx_2 is more frequently linked to serious disorders and the development of HUS than stx_1 (Nishikawa *et al*. 2008). Although STEC's main virulence factor is the synthesis of stx_1 and/or stx_2 and variants, the capacity to adhere to the intestinal epithelium is the predominant pathogenicity factor. STEC strains carry genes encoding Shiga toxins and may possess other virulence genes for intimin and enterohemolysin. EPEC isolates are defined as intimin-containing diarrhoeagenic *E. coli* isolates that possess the ability to form attaching and effacing (AE) lesions on intestinal cells and do not possess genes coding for Shiga toxin. Another important adhesin in STEC that does not encode the location of enterocyte effacement is STEC auto-agglutinating adhesin (SAA). It is responsible for adhesion and colonization of the bovine and ovine intestinal epithelium (Zweifel *et al*. 2005; Kumar *et al*. 2012).

Antimicrobial resistance (AMR) is a growing problem in veterinary medicine because it involves many different species of animals and microorganisms, as well as different animal rearing environments and resistance mechanisms. Some of the most common pathogenic bacteria, such as *E. coli* and *Staphylococcus*, are becoming increasingly resistant to first-line antibiotics (Lewis *et al*. 2007, Pop and D'Agata 2005). Extended spectrum beta-lactamases are the enzymes that mediate resistance to extended–spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem). The majority are derivatives of the TEM and SHV β-lactamase families, while others, including CTX-M, OXA and KPC β-lactamases, have only recently been discovered. The main mechanism underlying the rise of antibiotic resistance is horizontal gene transfer by mobile genetics elements like plasmids and integrons (Correa *et al*. 2014).

Therefore, the impact of healthy farm animals as a possible reservoir for ESBL producing Enterobacteriaceae on the food processing chain has to be assessed. Even though several studies have been conducted in cattle, but there is scarcity of data regarding characterization of *E. coli* isolates from sheep and goats. The aim of this study was to screen *E. coli* isolates from sheep and goats for presence of one or more virulence associated genes, presence of genes that lead to production of Extended spectrum beta lactamases (ESBLs) and determine the antibiotic resistance patterns of *E. coli* isolates from Jammu, J&K, India.

MATERIALS AND METHODS

Sample collection and Phenotypic tests for the detection of *E. coli*

A total of 120 fecal samples collected aseptically from apparently healthy sheep and goats and were inoculated on MacConkey's agar plates followed by incubation at 37°C for 24 hours. Two well-isolated pink colonies from each plate were picked at random and sub-cultured on Eosin Methylene Blue (EMB) agar and incubated at 37°C. The colonies exhibiting distinctive green metallic sheen were presumptively identified as *E. coli*. These colonies were further streaked to obtain pure culture and subjected to various biochemical tests like Indole, MR, VP, Citrate utilization test (IMViC). All the isolates exhibiting characteristic IMViC pattern of ++-- were considered to be *E. coli* and further subjected to PCR for virulence determination as well as for production of ESBLs.

Multiplex PCR for detection of virulence genes viz. stx_1, stx_2, *eaeA* and *ehxA* genes

DNA was extracted from the isolates by suspending a loop full of confluent bacterial growth in 500 µL sterile distilled water followed by boiling for 10 mins, immediate cooling on ice for 10 minutes and centrifugation at 10,000g for 5min. The supernatant (containing DNA) was used as template for multiplex PCR reaction. All the confirmed *E. coli* isolates were subjected to molecular screening by

multiplex PCR (m- PCR) for stx_1, stx_2, *eaeA* and *ehxA* genes using specific primers (Paton and Paton 1998).

Electrophoresis

About 10 microlitres of amplified PCR products were analyzed by gel electrophoresis using 1% agarose containing ethidium bromide (0.5ug/mL). The products were visualized with UV illumination and imaged with gel documentation system.

Detection of ESBL producing *E. coli* strains

Molecular detection of ESBL genes

All the *E. coli* isolates that carried virulence genes were further tested for the presence of bla_{TEM}, bla_{SHV}, bla_{OXA} and $bla_{CTX\text{-}M}$ genes by multiplex PCR assay (Fang *et al*. 2004). About 10 microlitres of PCR product was electrophoresed in a 1% (w/v) agarose gel for 1 hr at 5 V/cm with a Standard molecular weight marker. The products were visualized with UV illumination and imaged with gel documentation system.

Antimicrobial susceptibility testing

In vitro antibiotic sensitivity assay was used for phenotypic detection of isolates positive for virulence genes producing *E. coli* as per Bauer *et al*. (1966). The commonly used antimicrobials like, Amoxy-clav (Amoxycillin + Clavulanic acid), Cefipime, Chloramphenicol, Cefotaxime, Cepfoperazone, Ceftazidime, Ciprofloxacin, Doxycycline hydrochloride, Kanamycin and Nalidixic acid were tested for their in vitro efficacy against *E. coli* isolates from sheep and goats.

RESULTS AND DISCUSSION

Isolation of Presumptive *Escherichia coli*

Out of 120 samples, a total of 200 isolates presumptively identified as *E. coli* were obtained. All the isolates produced pink colonies on MacConkey agar and exhibited metallic sheen on EMB agar. The isolates were further subjected to a range of biochemical tests and exhibited a positive catalase and negative oxidase reaction in addition to IMViC pattern of ++-- which was used to confirm the identity of selected isolates.

Detection of Virulence genes by PCR

All the 200 *E. coli* isolates were screened for the presence of virulence genes viz. stx_1, stx_2, *eaeA*, and *ehxA* genes by PCR. The stx_1 gene produced an amplicon of 180bp while stx_2, *eaeA* and *ehxA* genes produced an amplicon of 255 bp, 384 bp and 534 bp respectively (Fig. 1). All isolates that showed the presence of *eaeA* gene but no stx_1 or stx_2 gene were classified as Enteropathogenic *E. coli* (EPEC),

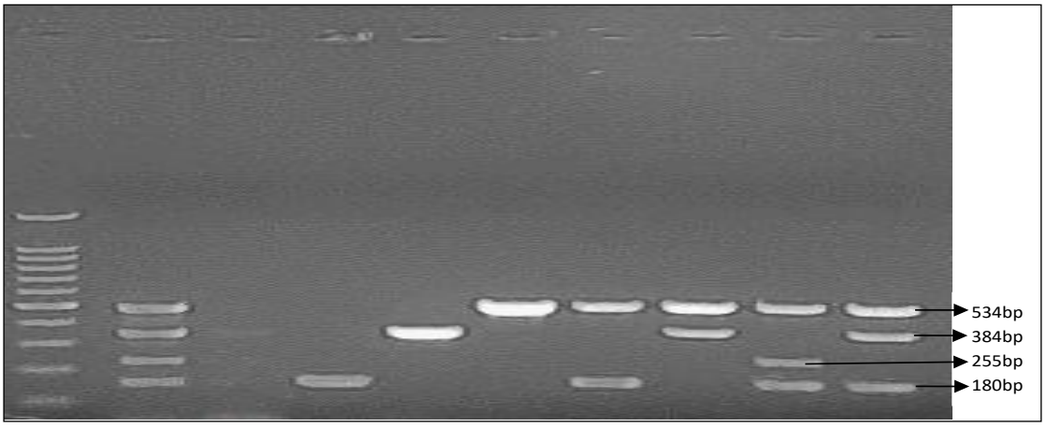

Fig. 1: Representative *stx1*, *stx2*, *eaeA* and *ehxA* genes profile of *E. coli* isolates using multiplex PCR. Lane M depicts 100 bp DNA ladder while Lane 1 contains positive control for *stx1* (180 bp b), *stx2* (255 bp), *eaeA* (384 bp) and *ehxA* (534 bp) genes. Lane 2 is negative control, Lane 3 is *stx1* positive, Lane 4 is *eaeA* positive, Lane 5 is *ehxA* positive, Lane 6 is *stx1*, *ehxA* positive, Lane 7 is positive for both *eaeA*, *ehxA* genes, Lane 8 is positive for *stx1*, *stx2*, *ehxA* genes while Lane 9 is positive for *stx1*, *eaeA*, *ehxA* genes.

whereas isolates with stx_1, stx_2, or both genes but no *eaeA* gene were classified as Shiga toxigenic *E. coli* (STEC). The isolates having only *ehxA* gene were classified as Enterohaemorrhagic *E. coli* (EHEC).

In the current study the virulence gene profile of *E. coli* isolates from sheep and goats was determined. A total of 76.47% (26/34) sheep isolates harboured *stx*1 gene while 70.58% (24/34) harboured *ehxA*, 23.52% (08/34), *eaeA* and 5.88% isolates harboured (02/34) *stx*2 genes. Among goat isolates, 64.28% (18/28) harboured *stx*1 gene, 7.14% (02/28) had *stx*2, 21.42% (06/28) had *eaeA* while 71.42% (20/28) harboured *ehxA* genes. Similar results have been obtained by Cobeljic *et al*. (2004) who found STEC in 66.8% of sheep and 73.8% of goats in Serbia. However, the present study is in contrast to Orden *et al*. (2003), who found prevalence rates of VTEC in 24.4% and 16.2% in healthy sheep and goats respectively in Spain. Zaheri *et al*. (2020) found STEC prevalence rates of 61.6% in sheep and 23.9% in goats. In contrast, Zschock *et al*. (2000) found a high prevalence rate of STEC in healthy goats in Germany (75.3%). These changes could be due to the fact that STEC shedding patterns are influenced by a variety of factors including food, age, environmental circumstances, and seasonal fluctuations (Kudva *et al*. 1997). STEC was found in just 32.3% of sheep by Adesiyun *et al*. (1992). In contrast, Fegan and Desmarchelier (1999) found STEC in 45% of sheep. Wray *et al*. (1997) on the other hand, reported a lower proportion of healthy sheep infected with VTEC (6.1%). The frequency found in this study is equivalent to the 78.3% STEC prevalence seen in healthy sheep in Brazil (Ferreira *et al*. 2015). In the current investigation, the prevalence of STEC strains in sheep was found higher than in goats, which is in equivalence to studies conducted by Beutin *et al*. (1993), who recovered STEC from sheep (66.6%) and goats (56.1%), respectively. The prevalence of STEC was 63% in sheep and 45% in goats, whereas a study conducted by (Zschock *et al*. 2000) found STEC in 32.1% sheep and 75.3% goats in Germany. Wani *et al*. (2009) isolated Shiga toxin-producing *E. coli* (STEC) from 24.1% of lambs without diarrhoea in Kashmir, which is lower than the current study. Fegan and Desmarchelier (1999) found *stx* in 88% of stool samples from sheep grazing on pasture in Australia while STEC was discovered in 66% of the sheep (Beutin *et al*. 1993).

Detection of Virulence genes by PCR

All the 62 isolates carrying virulence genes were tested for the presence of bla_{TEM}, bla_{SHV}, bla_{OXA}, and bla_{CTX-M} genes by multiplex PCR. The amplicon of 445 bp, 237 bp and 593 bp, for bla_{TEM}, bla_{SHV}, bla_{CTX-M} and were detected in 54.54% (12/22), 36.36% (08/22) and 27.27% (06/22) isolates respectively (Fig. 2). Among sheep isolates bla_{CTX-M}, bla_{SHV} and bla_{TEM} genes were detected in 20.0% (02/10), 50.0% (05/10) and 40.0% (04/10) of the isolates, respectively. In contrast, 25.0% (03/12) isolates from

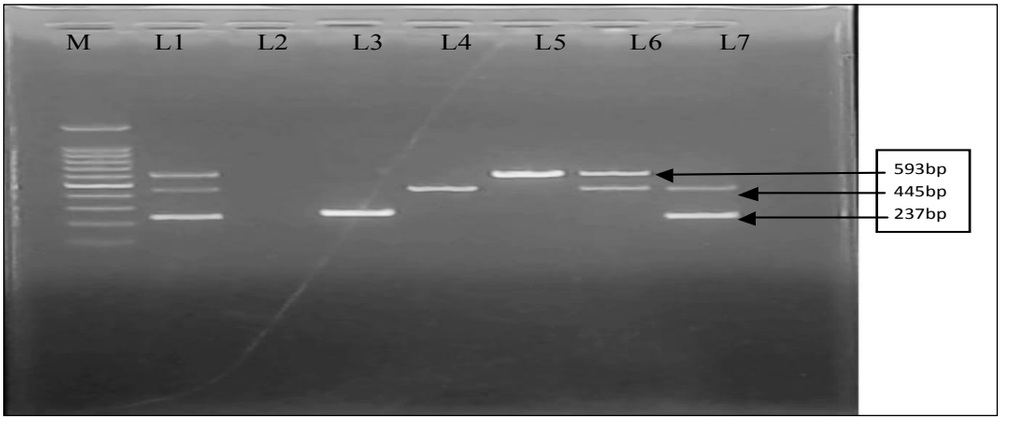

Fig. 2: Detection of bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes of virulent *E. coli* isolates using multiplex PCR. Lane M depicts 100 bp DNA Ladder. Lane 1 shows positive control for bla_{SHV} (237 bp band), bla_{TEM} (445 bp) and bla_{CTX-M} (593 bp) genes; Lane 2 shows Negative control; Lane 3 is positive for bla_{SHV}; Lane 4 for bla_{TEM}; Lane 5 for bla_{CTX-M}; Lane 6 is positive for both bla_{TEM} and bla_{CTX-M} and Lane 7 is positive for bla_{SHV} and bla_{TEM}.

goats harboured bla_{TEM} genes, 25.0%, (3/12) had bla_{SHV} while 33.33% (04/12) harboured bla_{CTX-M}.

Overall, in the current study the prevalence of ESBL producing *E. coli* in the caprine and ovine population of Jammu region was found to be 35.48%. These values are higher than those found in Switzerland where 8.6% of *E. coli* isolates from sheep were found to carry ESBLs (Geser *et al*. 2011). In contrast, ESBL producing *E. coli* isolates from sheep feces and goat feces were found to be 22.5% and 10% respectively, in Tunisia (Sghaier *et al*. 2019).

Antimicrobial susceptibility testing

In vitro antibiotic sensitivity assay of ESBL producing *E. coli* isolates against ten antibiotics was conducted and the results were interpreted as resistant and sensitive based on the guidelines given by Clinical Laboratory Standard Institute (CLSI, 2010). Among the ESBL producing *E. coli* isolates from goats, resistance was found against Cefotaxime (100%), Ceftazidime (92.85), Cefoperazone (85.71%) and Cefipime (89.28%). These isolates were however sensitive to Amoxicillin/ Clavulanic acid (89.28%), Chloramphenicol (100%), Doxycycline hydrochloride (92.85%) and Nalidixic acid (78.57%). The resistance rates among ESBL-producing *E. coli* isolates in the sheep was highest against Cefotaxime (100%), Ceftazidime (97.05%), Cefoperazone (87.87%) and Cefipime (76.47%) while the isolates were sensitive to Chloramphenicol (100%), Amoxicillin/ Clavulanic acid (88.23%) and Doxycycline hydrochloride (91.17%). The sensitivity pattern of ESBL producing *E. coli* isolates from sheep and goats to 10 antibiotics has been presented in Table 1.

Our findings are in contrast to work done by Sharaf *et al*. (2016) who found chloramphenicol resistance in 30% of *E. coli* isolates. The percentage of isolates resistant to ceftazidime, ceftriaxone, aztreonam, and cefotaxime in the study by Sharaf *et al.* (2016) was 11.5%, 11.5%, 7.6% and 5.7% respectively, which is quite low in comparison to the current study findings. Ghanbarpour and Kiani (2013) discovered that 192 ovine STEC isolates were resistant to at least one of eight antibiotics tested; resistance to penicillin (98.4%), cephalexin (94.8%), and tetracycline (91.2%) was highest, while resistance to sulfamethoxazole + trimethoprim (51%) and ciprofloxacin (51%) was lowest (62%) sulfamethoxazole + trimethoprim (51%) and ciprofloxacin (51%) was lowest as compare to our findings. Okpara *et al*. (2017) conducted a study that all ESBL-producing *E. coli* isolates were tetracycline resistant in addition to being resistant to β-lactam antibiotics (ampicillin, cefotaxime, and ceftazidime).

Table 1: Sensitivity pattern of ESBL producing *E. coli* isolates from sheep and goats

Antimicrobials	Sheep		Goat	
	S	R	S	R
Amoxicillin/ Clavulanic acid	30	04	25	03
Cefipime	08	26	03	25
Chloramphenicol	34	00	28	00
Cefotaxime	00	34	00	28
Cefoperazone	05	29	04	24
Ceftazidime	01	33	02	26
Ciprofloxacin	13	20	06	20
Doxycycline hydrochloride	31	03	26	02
Kanamycin	12	22	10	18
Nalidixic acid	25	09	22	06

They were also resistant to streptomycin (73.6%), nalidixic acid (84.9%), sulfamethoxazole/trimethoprim (81.1%), compound sulfonamides (81.1%), trimethoprim (83.0%), gentamicin (35.8%), chloramphenicol (37.7%), ciprofloxacin (28.3%), kanamycin (20.8%), and amikacin (9.4%). The resistance to kanamycin, nalidixic acid, ciprofloxacin in present study is 64.5%, 24.2% and 69.3% respectively. This may be due to indiscriminate use of cephalosporins at field level and the antibiotics such as chloramphenicol are not used so frequently.

CONCLUSION

Sheep and goats in Jammu region are an important carrier of STEC and EPEC. The effectiveness of cephalosporins which are the antimicrobials used for treating serious infections is being compromised by the presence of ESBL producing bacteria. This study emphasizes the need for extensive study in this direction to ascertain the immensity of the issue of antibiotic resistance occurring among animals and humans. Transferable plasmids play an important role in the horizontal transfer of antimicrobial resistance genes and their capability to spread between

bacterial cells by means of conjugation greatly enhances the dissemination of the *bla* genes. The presence of β-lactam resistant commensal/pathogenic *E. coli* in animals in this region may pose a serious public health threat, since these bacteria may act as a reservoir of resistance genes, which can be disseminated to the normal flora of the man and animals. There is a dire need to increase the awareness among veterinarians and physicians on the challenges deriving from antibiotic resistance.

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REFERENCES

Adesiyun, A.A., Kaminjolo, J.S., Loregnard, R. and Kitson-Piggott, W. 1992. Campylobacter infections in calves, piglets, lambs and kids in Trinidad. *Br. Vet. J*., **148**: 547-556.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.,* **45**: 493-496.

Beutin, L., Steinruck, H., Zimmermann, S. and Scheutz, F. 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J. Clin. Microbiol*., **31**: 2483-2488.

Clinical and Laboratory Standard Institute (CLSI) (2010) Performance Standards for Antimicrobial Susceptibility Testing; 20th Informational Supplement. CLSI document M100-S20. CLSI, Wayne, PA: Clinical and Laboratory Standard Institute.

Cobeljic, M., Dimic, B., Opacic, D., Lepsanovic, Z., Stojanovic, V. and Lazic, S. 2004. The prevalence of Shiga toxin-producing *Escherichia coli* in domestic animals and food in Serbia. *Epidemiol. Infect*., **133**: 359-366.

Correa, F.E., Dantas, F.G., Grisolia, A.B., Crispim, B.A. and Oliveira, K.M. 2014. Identification of class 1 and 2 integrons from clinical and environmental Salmonella isolates. *J. Infect. Dev. Ctries*., **8**: 1518-1524.

Fang, H., Lundberg, C., Olsson-Liljequist, B., Hedin, G., Lindback, E., Rosenberg, A. and Struwe, J. 2004. Molecular epidemiological analysis of *Escherichia coli* isolates producing extended-spectrum beta-lactamases for identification of nosocomial outbreaks in Stockholm, Sweden. *J. Clin. Microbiol*., **42**: 5917-5920.

Fegan, N. and Desmarchelier, P. 1999. Shiga toxin-producing Escherichia coli in sheep and pre-slaughter lambs in eastern Australia. *Lett. Appl. Microbiol*., **28**: 335-339.

Ferens, W.A. and Hovde, C.J. 2011. Escherichia coli O157:H7: animal reservoir and sources of human infection. *Foodborne Pathog. Dis.,* **8**(4): 465-87.

Ferreira, M.R.A., Silva, T.S., Stella, A.E., Conceição, F.R., Reis, E.F. and Moreira, C.N. 2015. Detection of virulence factors and antimicrobial resistance patterns in Shiga toxin-producing *Escherichia coli* isolates from sheep. *Pesqui. Vet. Bras*., **35**(9): 775-780.

Geser, N., Stephan, R., Kuhnert, P., Zbinden, R., Kaeppeli, U., Cernela, N. and Haechler., H. 2011. Faecal carriage of Extended- spectrum beta Lactamase producing *Enterobacteriaceae* in Swine and Cattle at Slaughter in Switzerland. *J. Food Prot.,* **74**(3): 446-449.

Ghanbarpour, R. and Kiani, M. 2013. Characterization of non-O157 Shiga toxin-producing Escherichia coli isolates from healthy fat-tailed sheep in southeastern of Iran. *Trop. Anim. Health Prod.,* **45**: 641-648.

Ghilardi, A.C.R., Gomes, T.A.T. and Trabulsi, L.R. 2001. Production of cytolethal distending toxin and other virulence characteristics of *Escherichia col*i strains of serogroup O86. *Mem. Inst. Oswaldo Cruz*., **96**(5):703-708.

Gyles, C.L. and Fairbrother, J.M. 2010. *Escherichia coli*. In: Gyles CL, editor. *Pathogenesis of bacterial infections in Animal,* **(4)**: 267-308.

Kudva, I.T., Hatreld, P.G. and Hovde, C.J. 1997. Characterisation of E. coli O157:H7 and other Shiga toxin-producing E. coli serotypes isolated from sheep. *J. Clin. Microbiol*., **35**: 892-899.

Kumar, A., Taneja, N., Kumar, Y. and Sharma, M. 2012. Detection of Shiga toxin variants among Shiga toxin– forming Escherichia coli isolates from animal stool, meat and human stool samples in India. *J. Appl. Microbiol.,* **113**: 1208-1216.

Lewis, J.S., Herrera, M., Wickes, B., Patterson, J.E. and Jorgensen, J.H. 2007. First report of the emergence of CTX-M-type extended-spectrum β-lactamase (ESBLs) as the predominant ESBL isolated in a U.S. health care system. *Antimicrob. Agents Chemother.,* **51**: 4015-4021.

Mainil, J. 1999. Shiga/verocytotoxins and Shiga/verotoxigenic *Escherichia coli* in animals. *Vet. Res.,* **30**: 235-257.

Nataro, J.P. and Kaper, J.B. 1998. Diarrheagenic Escherichia coli. *Clin. Microb. Rev.,* **11**: 142-201.

Nishikawa, Y., Zhou, Z., Hase, A., Ogasawara, J., Kitase, T., Abe, N., Nakamura, H., Wada, T., Ishii, E. and Haruki, K. 2008. Diarrhoeagenic *Escherichia coli* isolated from stools of sporadic cases of diarrhoeal illness in Osaka city, Japan

between 1997 and 2000: Prevalence of enteroaggregative *E. coli* heat-stable enterotoxin 1 gene-possessing *E. coli*. *Jpn. J. Infect. Dis.,* **55**: 182-190.

Okparaa, E.O., Ojoa, O.E., Awoyomic, O.J., Dipeoluc, M. A., Oyekunlea, M.A. and Schwarz, S. 2017. Antimicrobial usage and presence of extended-spectrum β-lactamase producing *Enterobacteriaceae* in animal-rearing households of selected rural and peri-urban communities. *Vet. Microbiol.,* **218**: 31-39.

Orden, J.A., Blanco, M., Blanco, A.J.E., Mora, A., Cid, D., Gonzalez, E.A., Blanco, J. and Fuente, R. 2003. Prevalence and characterization of Vero cytotoxin-producing *Escherichia coli* isolated from diarrhoeic and healthy sheep and goats. *Epidemiol. Infect*., **130**: 313-32.

Paton, A.W. and Paton, J.C. 1998. Detection and Characterization of Shiga toxigenic Escherichia coli by Using Multiplex PCR assays for *stx1, stx2, eaeA,* Enterohemorrhagic *E. coli hlyA, rfbO111*, and *rfbO157*. *J. Clin. Microbiol.,* **36**(2): 598-602.

Pop, V.A.E. and D'Agata, E.M. 2005. The rising influx of multidrug-resistant Gram-negative bacilli into a tertiary care hospital. *Clin. Infect. Dis.,* **40**: 1792-1798.

Sghaier, S., Abbassi, M.S., Pascual, A., Serrano, L., Alba, P.D., Saida, M.B., Hassen, B., Ibrahim. C., Hassen, A. and Cerero, L.L. 2019. Extended-spectrum β-lactamase-producing *Enterobacteriaceae* from animal origin and wastewater in Tunisia: first detection of O25b-B23-CTX-M-27-ST131 *Escherichia coli* and CTX-M-15/OXA-204-producing *Citrobacter freundii* from wastewater. *J. Glob. Antimicrob. Resist.,* **17**: 189-194.

Sharaf, E.F. and Shabana, I.I. 2016. Prevalence and molecular characterization of Shiga toxin-producing *Escherichia coli* isolates from human and sheep in Al-Madinah Al-Munawarah. *Rev. Infect.,* **2**(1): 81-87.

Wani, S. A., Hussain, I., Fayaz, I., Mir, M. A. and 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.

Wray, C. and Woodward, M.J. 1997. Escherichia coli infections in farm animals. *In:* Sussman, M. (Ed.), *Escherichia coli*–Mechanisms of Virulence, pp. 49–84. Cambridge University Press, Cambridge, U.K.

Zaheri, H., Ghanbarpour, R., Jajarmi, M. Bagheri, M. Ghanadian, A. and Badouei, M.A. 2020. Public health aspects of Shiga toxin-producing *Escherichia coli* (STEC) strains in sheep and goats of Bakhtiari pastoral tribe, Iran. *Trop. Anim. Health Prod.,* **52**(5): 2721-2724.

Zschock, M., Hamann, H.P., Kloppert, B. and Wolter, W. 2000. Shiga-toxin-producing *Escherichia coli* in faeces of healthy dairy cows, sheep and goats: prevalence and virulence properties. *Lett. Appl. Microbiol*., **31**: 203-208.

Zweifel, C., Schumacher, S., Blanco, M., Blanco, J.E., Tasara, T., Blanco, J. and Stephan, R. 2005. Phenotypic and genotypic characteristics of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) from Swiss cattle. *Vet. Microbiol*., **105**: 37-45.