RESEARCH ARTICLE

Detection of Shiga Toxin Producing *Escherichia coli* from Human Faecal Samples by Multiplex PCR

Ramya Putturu[1]*, Shashi Kumar Manyam[1], Jagadeesh Babu Angalakudithi[2], Sujatha Singh[3], Gopala Reddy Alla[4]

ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) infection occurs via the faecal-oral route and results in symptoms ranging from mild uncomplicated diarrhoea to severe bloody diarrhoea. It's complications can be fatal especially among patients with severe renal and neurological disorders. Resistance against different antibiotics by STEC is also a big concern now a days. Hence, present study was planned with a total of 220 human faecal samples (farm workers: cattle- 30, buffalo- 30, sheep- 20, goat-20, pig- 20, poultry- 50, and diarrheic patients attended to the hospital- 50) of different ages collected from different livestock farms in Proddatur, Andhra Pradesh and surrounding villages. Samples were subjected to isolation and identification of *E. coli*. The *E. coli* isolated were characterized by PCR as STEC using primers for two genes, *i.e., stx1* and *stx2*. All the STEC isolates were subjected to ABST by using ten different antibiotic discs. Out of 220 human faecal samples, 216 (98.18%) were positive for *E. coli* by cultural method and 57.8% for STEC by PCR. On Muller-Hinton agar *STEC* isolates were highly resistant to ampicillin, cepahlothin (100% each) followed by tetracycline (98.4%), streptomycin (95.2%), sulphonamides (91.2%), trimethoprim (84.8%), kanamycin (36%), while highly sensitive to gentamicin (84.8%), followed by colistin (82.4%), chlorophenicol (62.4%), kanamycin (51.2%). The high prevalence of *E. coli* among farm animal workers further suggests the significant role of faeco-oral transmission as animals are the reservoir for this organism. By taking proper personal hygienic measures faeco-oral transmission can be minimized.

Key words: *Escherichia coli*, PCR, Shiga toxin, STEC.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.22.2.04

INTRODUCTION

Escherichia coli is one of the most important pathogenic bacteria which is a normal inhabitant of large intestine in human and warm-blooded animals (Lara *et al.,* 2016). Therefore *E. coli* can be transmitted to raw milk and milk products by fecal contamination during milking process along with poor hygienic practices (Garbaj *et al.,* 2016). It is transmitted to humans primarily through consumption of contaminated foods such as raw/undercooked ground meat products, raw milk contaminated vegetables and sprouts. Now a days, this pathogen has raisen as a major concern of food safety in many countries (Johnson *et al.,* 1996). Many amongst the enteric infections caused by *E. coli* are transmitted by inter-human contacts such as those caused by Entero-invasive *Escherichia coli* (EIEC) Enteropathogenic *Escherichia coli* (EPEC) or Enteroaggregative *Escherichia coli* (EAggEC), while those ascribed to Enterotoxigenic *Escherichia coli* (ETEC) or Shiga toxin-producing *Escherichia coli* (STEC) are primarily transmitted to humans through the consumption of contaminated water or food (Piazza *et al.,* 2020). STEC infection occurs via the faecal-oral route and results in symptoms ranging from mild uncomplicated diarrhoea to severe bloody diarrhoea. Complications including haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) can occur in some cases, both of which can result in fatality especially among patients with severe renal and neurological disorders (Borgattaa *et al.,* 2012).

The first recognized report of food borne infection with *Escherichia coli* O157:H7 in humans was the haemorrhagic colitis outbreak caused by hamburger consumption. Ground

[1] Department of Livestock Products Technology, College of Veterinary Science, Rajendranagar, Hyderabad-500030, PVNR Telangana Veterinary University, Telangana, India

[2] Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Tirupati-517501, SVVU, Andhra Pradesh, India

[3] Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Korutla-505326, PVNR Telangana Veterinary University, Telangana, India

[4] Deparment of Veterinary Pharmacology & Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad-500030, PVNR Telangana Veterinary University, Telangana, India

Corresponding Author: Dr. P. Ramya, Assistant Professor, Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Proddutur, Kadapa District-516360, SVVU, Andhra Pradesh, India. e-mail: puttururamya@gmailcom

How to cite this article: Ramya, P., Shashi Kumar, M., Jagadeesh Babu, A., Singh, S., Gopala Reddy, A. (2026). Detection of Shiga Toxin Producing *Escherichia coli* from Human Faecal Samples by Multiplex PCR. Ind J Vet Sci and Biotech, 22(2), 18-23.

Source of support: Nil

Conflict of interest: None

Submitted 13/01/2026 **Accepted** 29/01/2026 **Published** 10/03/2026

---

beef is responsible for more human outbreaks of *Escherichia coli* O157:H7 than any other source (Ashgan *et al.,* 2015). Griffin (1995) have considered contaminated under cooked ground beef and hamburgers as the major vehicles for STEC. EHEC produces toxins known as vero-toxins whose coding genes are conveyed by temperate bacteriophages or Shiga-like toxins because of their similarity to the toxins produced by *Shigella dysenteriae. Shiga toxins* inhibit the protein synthesis of host cells leading to cell death and also causes vascular injury of the intestinal epithelium. The STEC strains that carry the *stx2* gene produce more virulent disease symptoms than those which carry *stx1* gene and considered epidemiologically more important (Wei *et al.,* 2020).

Multiplex PCR was developed to detect several genes of many organisms simultaneously within a single reaction (Malorny *et al.,* 2003). Same technique can be employed to detect various toxins produced by a single organism targeting various virulence genes. Multiplex PCR has proved to be more rapid sensitive and specific to detect *stxs* genes as compared to DNA colony hybridization tests (Day *et al*., 2017). With this procedure DNA is amplified to increase the level of target DNA when STEC are present in very low number. Hence, keeping in view of the public health significance of STEC, the present study was designed to detect the prevalence and antimicrobial resistance patterns of STEC isolates from faecal samples of workers in different livestock farms in and around Proddatur, Andhra Pradesh.

MATERIALS AND METHODS

A total of 220 human faecal samples (Cattle farm workers-30, Buffalo farm workers-30, Sheep farm workers-20, Goat farm workers-20, Pig farm workers-20, poultry farm workers-50 and Diarrheic patients attended to the hospital-50) were collected from different livestock farms in and around Proddatur, Andhra Pradesh (India) and analysed for the presence of *E. coli* by cultural method and STEC by PCR. All the samples were collected aseptically and pre-enriched in buffered peptone water incubated at 37°C for 24 h. After pre-enrichment, 1 mL of each inoculum was transferred into selective broth (Himedia), *i.e.,* Tryptic soy broth and incubated at 37°C for 24 h. All the enriched samples were subjected to selective isolation by using MacConkey agar and Eosine Methylene Blue (EMB) agar and confirmed by using biochemical tests.

The biochemically confirmed *E. coli* isolates were subjected to PCR for the confirmation of STEC by using primers specific to *stx1* and *stx2* (Manna *et al.,* 2006) synthesised from Merck GeNei^{TM} (Table 1). PCR includes DNA extraction by Boiling and Snap chilling method (Arora *et al.,* 2006) from the selective broth cultures and then the DNA template was added to PCR reaction mixture and subjected to PCR assay consists of a 5 min initial denaturation at 94˚C followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at 94˚C for 1 min, and extension at 72˚C for 1 min with a 10 min final extension at 72˚C followed by maintenance at 4˚C. The amplified product was tested for the presence of desired gene by using Agarose Gel Electrophoresis. The samples that showed the presence of anyone of *stx1* (614 bp) or *stx2* (779 bp) genes were considered as positive for STEC (Fig. 1, 2) and the samples without any genes were considered as non-STEC isolates.

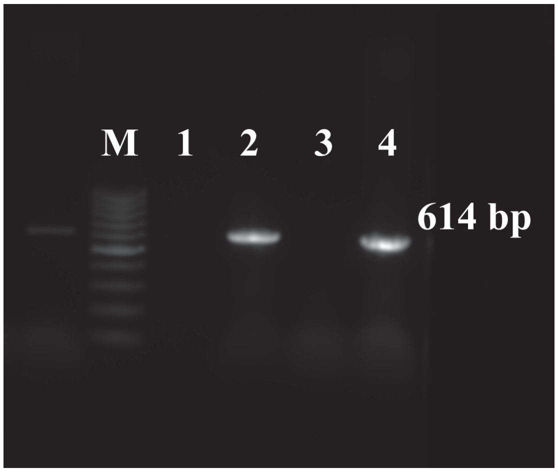

Fig. 1: Results of human faecal samples for *stx1.* Lane M: 100bp DNA Ladder, Lane 2, 4: Positive human faecal samples for *stx1*

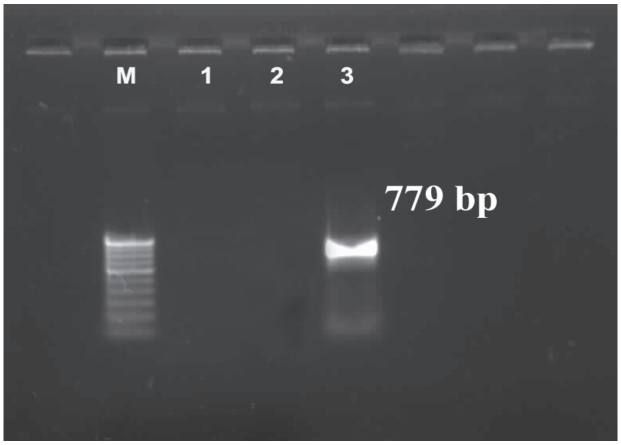

Fig. 2: Results of human faecal samples for stx2. Lane M: 100bp DNA Ladder, Lane 3: Positive human faecal samples for stx2

Table 1: Sequence of the oligonucleotide primers used for identification of *STEC*

Primer	Target gene	Primer sequence (5^1-3^1)	Expected amplicon size	Reference
stx 1: F	*stx1*	ATAAATCGCCATTCGTTGACTAC	614 bp	Manna *et al.* (2006)
stx 1: R		AGAACGCCCACTGAGATCATC		
stx 2: F	*stx2*	GGCACTGTCTGAAACTGCTCC	779 bp	
stx 2: R		TCGCCAGTTATCTTGACATTCTG		

All the positive STEC isolates by PCR from faecal samples were subjected to antibiotic sensitivity test by using disc diffusion method against 10 different antibiotics using the standard disc diffusion technique.

RESULTS AND DISCUSSION

The prevalence of *E. coli* in cattle, buffalo, sheep and pig farm workers was 100% followed by 98% in poultry farm workers, 96% in patients attended to hospital and 95% in goat farm workers, with overall prevalence of 98.18% (Table 2). Boonyasiri *et al.* (2014) reported a slight lower overall prevalence (90%) of *E. coli* in animal farm workers, whereas Hanson *et al.* (2002) reported still lower prevalence (51-53%) in pig and poultry farm workers. A prevalence of 96% observed in diarrheic patients was very high when compared to the prevalence (8.6-30.2%) reported by Khan *et al.* (2002) and Rao *et al.* (2014), whereas a moderate prevalence (64.8%) in human faecal samples was reported by Rao (2015).

Table 2: Prevalence of *E. coli* in faecal samples of Human beings working in different farms and also diarroeic patients from hospitals

S. No	Source	No. of samples	Positive samples (%)
1	Cattle farm workers	30	30 (100.0)
2	Buffaloes	30	30 (100.0)
3	Sheep	20	20 (100.0)
4	Goat	20	19 (95.0)
5	Pig	20	20 (100.0)
6	Poultry	50	49 (98.0)
7	Diarrhheic patients	50	48 (96.0)
	Total	**220**	**216 (98.18)**

Out of 216 *Escherichia coli* isolates from human faecal samples, the overall prevalence of *STEC* was 57.8%, with 11.2% (14/125) carried only *stx1*, 57.6% (72/125) carried only *stx2* and 31.2% (39/125) carried both *stx1 and stx2* (Table 3). Out of 125 *STEC* positive isolates, the highest prevalence was noticed from pig farm workers, *i.e.,* 75% followed by 65% in sheep farm workers, 64.5% in human diarrheic patients, 56.6% in cattle farm workers, 53.3% in buffalo farm workers, 52.6% in goat farm workers and least 46.9% in poultry farm workers. Bose *et al.* (2025) submitted first confirmed risk assessment report of Shiga toxin-producing *E. coli* (STEC) and its impact on public health from the area of West Bengal, India.

Lower prevalence of 49.1% and 23.6% in diarrheic patients was reported by Jomezadeh *et al.* (2009) and Hamed *et al.* (2017), respectively, whereas very low prevalence of 0.5% to 1.8% was reported by others (Khan *et al.*, 2002; Palanisamy *et al.*, 2017) in diarrheic patients, compared to the prevalence of 64.5% in the present study. However, relatively higher prevalence of 71% and 86.1% was also reported by Ludwig *et al.* (2001) and Olowe *et al.* (2014), respectively.

In cattle farm workers 11.7%, 58.8% and 29.4% of *STEC* positive isolates harboured *stx1*, *stx2* and both *stx1 and stx2* genes, respectively, whereas in buffalo farm workers 12.5%, 56.2% and 25% of *STEC* positive isolates harboured *stx1*, *stx2* and both *stx1 and stx2* genes, respectively. In sheep farm workers 7.6%, 53.8% and 38.4% of *STEC* positive isolates harboured *stx1*, *stx2* and both *stx1 and stx2* genes, respectively, whereas in goats 10%, 60% and 30% of *STEC* positive isolates harboured *stx1*, *stx2* and both *stx1 and stx2* genes, respectively.

In pig farm workers 13.3%, 60% and 26.6% of *STEC* positive isolates harboured *stx1*, *stx2* and both *stx1 and stx2* genes, respectively. In poultry farm workers 8.6%, 60.8% and 34.7% of *STEC* positive isolates harboured *stx1*, *stx2* and both *stx1 and stx2* genes, respectively. Very low prevalence of *stx1* (3.8%) and *stx2* (0.5%) was reported by Trung *et al.* (2016) in poultry farm workers. In diarrheic patients, 12.9%, 54.8% and 32.2% harboured *stx1*, *stx2* and both *stx1 and stx2* genes, respectively. Similar to the higher prevalence of *stx2* in the present study, Karmali *et al.* (2003) in a study at USA, observed very high prevalence of *stx2* (93.1%), wherein none carried *stx1* gene. Scheutz *et al.,* (2025) reported that *STEC* with *stx2* are more important than *stx1* in the development of HUS and that, strains that express *stx2* alone are more likely to be associated with the progression to HUS than strains that produce both *stx1 and stx2*. Ludwig *et al.* (2001) reported that 71% of children with HUS were due to *stx2* producing *STEC*.

Antibiotic Resistance for *STEC* Isolates

The positive *STEC* isolates by PCR from human faecal samples were found to be highly resistant to ampicillin, cepahlothin (100% each) followed by tetracycline (98.4%), streptomycin (95.2%), *sul*phonamides (91.2%), trimethoprim (84.8%), kanamycin (36%), chloromphenicol (19.2%), colistin (4.8%) and least resistant to gentamicin (4%). They were highly

Table 3: Prevalence of STEC from faecal samples of human beings working in different farms and also diarrhoeic patients from hospitals

S. No	Source	No. positive for *E.coli*	STEC by PCR (%)	Presence of *stx* among SETC		
				stx1	*stx2*	Both *stx1* & *stx2*
1	Cattle	30	17 (56.66)	02	10	05
2	Buffaloes	30	16 (53.33)	02	09	04
3	Sheep	20	13 (65.00)	01	07	05
4	Goat	19	10 (52.63)	01	06	03
5	Pig	20	15 (75.00)	02	09	04
6	Poultry	49	23 (46.93)	02	14	08
7	Human	48	31 (64.58)	04	17	10
	Total	**216**	**125 (57.8%)**	**14 (11.2)**	**72 (57.6)**	**39 (31.2)**

Table 4: Antibiotic resistance of STEC isolated from human faecal (n=125) samples

S. No.	Antibiotic	No. of isolates Sensitive (%)	No. of isolates Intermediate (%)	No. of isolates Resistant (%)
1	Ampicillin (10 μg)	0	0	125 (100%)
2	Cephalothin (30 μg)	0	0	125 (100%)
3	Chloramphenicol (30 μg)	78 (62.4)	23 (18.4)	24 (19.2)
4	Colistin (10 μg)	103 (82.4)	16 (12.8)	06 (4.8)
5	Gentamycin (10 μg)	106 (84.8)	14 (11.2)	05 (4.0)
6	Kanamycin (30 μg)	64 (51.2)	16 (12.8)	45 (36.0)
7	Sulphonamides (300 μg)	03 (2.4)	08 (6.4)	114 (91.2)
8	Streptomycin (10 μg)	01 (0.8)	05 (4.0)	119 (95.2)
9	Tetracycline (30 μg)	01 (0.8)	01 (0.8)	123 (98.4)
10	Trimethoprim (5 μg)	08 (6.4)	11 (8.8)	106 (84.8)

sensitive to gentamicin (84.8%), followed by colistin (82.4%), chloromphenicol (62.4%), kanamycin (51.2%), trimethoprim (6.4%), sulphonamides (2.4%), streptomycin and tetracyclin (0.8% each) (Table 4).

The current resistance of *STEC* isolates from human faecal samples to ampicillin (100%) was similar to reports of Naik and Desai (2012) and Arabi and Banazadehi (2013), whereas Nsofor and Iroegbu (2013) reported slightly less resistance of 85.7%. A resistance of 50%, 27.2% and 7.4% against ampicillin was reported by Schroder *et al.* (2002), Ji-Yeon *et al.,* (2006), and by Mukharjee *et al.* (2017), respectively.

Schroeder *et al.* (2002) and Hameed *et al.* (2017) observed a resistance of 50% and 76.4%, respectively, against cephalothin for the isolates from human faecal samples, which is less than 100% resistance observed in the present study. The chloromphenicol resistance reported by Schroder *et al.,* (2002) for the isolates from human faecal samples was 25.1%, while slightly lower resistance (19.2%) was observed in the present study. High resistance of 68.75%% and 67.4% against chloromphenicol was reported by Naik and Desai (2012) and Nsofor and Iroegbu (2013), respectively. A sensitivity of 62.4% observed in the present study was lower than 100% reported by Hameed *et al.* (2017).

Very low resistance (4.8%) against colistin for the isolates from human faecal samples was observed in the present study compared to higher resistance (9.9%) observed by Rao *et al.* (2011). Compared to very high resistance (71.4%) reported by Danielle *et al.* (2018) high sensitivity (82.4%) was observed against colistin in the present study.

A sensitivity of 84.8% against gentamicin for the *STEC* isolates from human faecal samples was observed in the present study, which was less than 100% observed by Hameed *et al.* (2017). Very low resistance (4%) against gentamicin for the *STEC* isolates from human faecal samples was observed in the present study compared to the resistance of 6.7% to 62.9%, reported by others (Naik and Desai, 2012; Nsofor and Iroegbu, 2013; Olowe *et al.*, 2014).

Rao *et al.* (2011) reported higher resistance (85.7%) against kanamycin for *STEC* isolates from human faecal samples than the 36% resistance observed in the present study. The resistance of *STEC* isolates from human faecal samples against sulphonamides was 91.2%, which was higher than the resistance of 25% and 65.5% reported by Schroder *et al.* (2002), and Fazeli and Salehi (2007), respectively.

Cent percent resistance of the *STEC* isolates from human faecal samples against streptomycin was reported by Rao *et al.* (2011), which was nearer to the resistance (95.2%) observed in the present study, but was higher than 50.0% to 78.7%, reported by Schroder *et al.* (2006), Nsofor and Iroegbu (2013), whereas zero resistance was reported by Ji-Yeon *et al.* (2006).

The current 98.4% resistance of *STEC* isolates from human faecal samples against tetracycline was comparable to resistance of 93.75% to 100% resistance reported by Rao *et al.* (2011) and Naik and Desai (2012), while lower resistance from 50% to 79.8% was reported by others (Schroeder *et al.,* 2006; Fazeli and Salehi, 2007; Nsofor and Iroegbu, 2013), and only 18.2% by Ji-Yeona *et al.* (2006). The *STEC* isolates from human faecal samples in the present study had higher resistance (84.8%) against trimethoprim than the resistance of 71.4%, 65.5%, 25% and 4% reported by Rao *et al.* (2011), Fazeli and Salehi (2007), Schroder *et al.* (2002) and Mukharjee *et al.* (2017), respectively.

CONCLUSION

The present study indicates a high prevalence of STEC *i.e.,* 57.8% among farm animal workers which further suggests the significant role of faeco-oral transmission as animals are the reservoir for this organism. By taking proper personal hygienic measures faeco-oral transmission can be avoided / minimized.

ACKNOWLEDGEMENT

The authors are grateful to the PVNRTVU, Hyderabad, and SVVU, Tirupathi for providing facilities to carry out the work.

REFERENCES

Arabi, F.M.Z., & Banazadehi, A. (2013). Prevalence and antimicrobial susceptibility patterns of uropathogens among patients referring to valieasr laboratory in Najafabad Isfahan Iran Middle-East. *Journal of Science and Research 13*(1), 85-90.

Arora, S., Agarwal, R.K., & Bist, B. (2006). Comparision of ELISA and PCR vis-a-vis cultural methods from detecting *Aeromonas* spp. In foods of animal origin. *International Journal of Food Microbiology*, *1*06(2), 177-183.

Ashgan, M.H., Abdullah, A.A., Adel, M.Z., El-Jakee, J.K., Al-Zogibi, O.Z., Hemeg, H.A., & Ihab, M.I. (2015). Molecular characterization of *Escherichia coli* O157:H7 recovered from meat and meat products relevant to human health in Riyadh Saudi Arabia. *Saudi Journal of Biological Sciences, 22*(6), 725-729.

Boonyasiri, A., Tangkoskul, T., Seenama, C., Saiyarin, J., Tiengrim, S., & Thamlikitkul, V. (2014). Prevalence of antibiotic resistant bacteria in healthy adults foods food animals and the environment in selected areas in Thailand. *Pathogens and Global Health*, *108*(5), 235-245.

Borgattaa, B.N., Kmet-Lunacekb., & Relloc, J. (2012). *E coli O104: H4* outbreak and haemolytic uraemic syndrome. *Med Intensiva.* Jan 11 Doi 101016/jmedin201111022.

Bose, D., Mondal, K.K., & Chatterjee, P.R. (2025). Shiga toxin-producing *Escherichia coli* (STEC) strains: Its impact on public health in and around Murarai, West Bengal, India. *The Microbe,* 7, 100379.

Danielle, J.I., Myron, M.L., Karen. L.K., Holt. K.E., & Roy, M.R. (2018). Dynamics of antimicrobial resistance in intestinal *Escherichia coli* from children in community settings in South Asia and sub-Saharan Africa. *Nature Microbiology*, *3,* 1063-1073.

Day, M.D., Jenkins, C., Dallman, T.J., Hopkins, K.L., Elson, R., Godbole, G., & Woodford, N. (2017). Antimicrobial resistance in Shiga toxin-producing *Escherichia coli* serogroups O157 and O26 isolated from human cases of diarrhoeal disease in England. *Journal of Antimicrobial Chemotheropy, 72,* 145-152.

Fazeli, H., & Salehi, R. (2007). Antibiotic resistance pattern in shiga toxin producing Escherichia coli isolated from diarrheal patients in Al-Zahra Hospital Isfahan Iran. Research in Pharmaceutical Science, 2(1), 29-33.

Garbaj, A.M., Awad, E.M., Azwai, S.M., Abolghait, S.K., Naas, H.T., Moawad, A.A., Gammoudi, F.T., Barbieri, I., & Eldaghayes, I.M. (2016). Enterohemorrhagic *Escherichia coli O157* in milk and dairy products from Libya: Isolation and molecular identification by partial sequencing of 16S rDNA. *Veterinary World, 9*(11), 1184-1189.

Griffin, P.M. (1995). *Escherichia coli* O157:H7 and other enterohaemorrhagic *Escherichia coli* In: Blaser MJ Smith JI Ravdin HB Greenberg HB and Guerrant RL (eds) *Infections of the Gastrointestinal Tract*. Raven Press, New York, pp. 121-145.

Hamed, O.M., Sabry, M.A., Hassanain, N.A., Hamza, E., Hegazi, A.G., & Salman, M.B. (2017). Occurrence of virulent and antibiotic-resistant Shiga toxin-producing *Escherichia coli* in some food products and human stool in Egypt. *Veterinary World*, *10*(10), 1233-1240.

Hanson, R., Kaneene, J.B., Padungtod, P., Hirokawa, K., & Zeno, C. (2002). Prevalence of *Salmonella* and *E. coli* and their resistance to antimicrobial agents in farming communities in Northern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*, *33*(3), 120-126.

Ji-Yeon Kim, So-Hyun Kim, Nam-Hoon Kwon, Won-Ki Bae, Ji-Youn Lim Hye-Cheong, Koo Jun-Man Kim, Kyoung-Min, Noh Woo-Kyung, Jung Kun-Taek Park, & Yong-Ho Park. (2006). Isolation and identification of *Escherichia coli O157:H7* using different detection methods and molecular determination by multiplex PCR and RAPD. *Journal of Veterinary Science*, *7*(1), 7.

Johnson, R.P., Clarke, R.C., Wilson, J.P., Read, S.C., Rahn, K., Renwick, S.A., Sandhu, K.A., Alves, D., Karmali, M.A., Lior, H., McEwen, S.A., Spika, J.S., & Gyles, C.L. (1996). Growing concerns and recent outbreaks involving non-O157:H7 serotypes of verotoxigenicE coli. *Journal of Food Protection, 59,* 1112-1122.

Jomezadeh, N., Sheikh, F., Khosravi, A.D., & Amin, M. (2009). Detection of shiga toxin producing *E. coli* strains isolated from stool samples of patients with diarrhea in Abadan hospitals. *Iranian Journal of Biological Sciences, 9,* 820-824.

Karmali, M.A., Mascarenhas, M., Ziebell, S.S., & ReidSmith, J.S. (2003). Association of genomic O island 122 of Ecoli EDL 933 with verocytotoxin producing *E. coli* seropathotypes that are linked to epidemic/serious disease. *Journal of Clinical Microbiology*, *41,* 4930-4940.

Khan, A., Das, S.C., Ramamurthy, T., Sikdar, A., Khanam, J., Yamasaki, E., Takeda, Y., & Balakrish Nair, G. (2002). Antibiotic resistance virulence gene and molecular profiles of shiga toxin-producing *Escherichia coli* isolates from diverse sources in Calcutta, India. *Journal of Clinical Microbiology 40*(6), 2009-2015.

Lara, V.M., Carregaro, A.B., Santurio, D.F., de Sa MF., Santurio, J.M., & Alves, S.H. (2016). Antimicrobial susceptibility of *Escherichia coli* strains isolated from *Alouatta* spp feces to essential oils Evid Base. *Complementary and Alternative Medicine*, *1643762*(10), 30.

Ludwig, K., Karmali, M.A., Sarkim, V., Bobrowski, C., Petric, M., & Karch, H. (2001) Antibody response to shiga toxins *Stx2* and *Stx1* in children with enteropathic hemolytic-uremic syndrome. *Journal of Clinical Microbiology*, *39,* 2272-2279.

Malorny, B., Hoorfar, J., Bunge, C., & Helmuth, R. (2003). Multicenter validation of the analytic accuracy of salmonella pcr: toward an international standard. *Applied Environmental Microbiology, 69*(1), 290-296.

Manna, S.K., Brahmane, M.P., Manna, C., Batabyal1, K., & Das, R. (2006). Occurrence, virulence characteristics and antimicrobial resistance of *Escherichia coli* O157 in slaughtered cattle and diarrhoeic calves in West Bengal, India. *Letters in Applied Microbiology, 43,* 405-409.

Mukharjee, S., Mosci, R.E., Anderson, C.M., Snyder, B.A., Collins, J., Rudrik, T., & Manning, S.D. (2017). Antimicrobial drug-resistant shiga toxin producing *Escherichia coli* infections Michigan USA. *Emerging Infectious Diseases*, *23*(9), 1609-1611.

Naik, J., & Desai, P. (2012). Detection of enterohaemorrhagic *Escherichia coli* (*E coli* O157:H7) and its drug resistance pattern. *Journal of Environmental Research and Development, 7*(1), 51-55.

Nsofor, C.A., & Iroegbu, C.U. (2013). Antibiotic resistance profile of Escherichia coli isolated from five major geopolitical zones of Nigeria. *African Journal of Bacteriology Research*, *5*(3), 29-34.

Olowe, O.A., Bukola, W., Idris, O.O., Mabayoje, V.O., Opaleye, O.O., Catherine, O.A., Olowe, R., Paul, A.A., & Ojurongbe, O. (2014). Genotypes and phenotypes of shiga toxin-producing *Escherichia coli* (sTec) in abeokuta southwestern Nigeria. *Infection and Drug Resistance*, *7,* 253-259.

Palanisamy, P., Chang, Y.C., Scaria, J., Filho, RACP., Peters, K.E., Doiphode, S.H., Sultan, A., & Mohammed, H.O. (2017). Genetic relatedness among shiga toxin–producing *Escherichia coli* isolated along the animal food supply chain and in gastroenteritis cases in Qatar using multilocus sequence typing. *Foodborne Pathogens and Disease*, *14*(6), 318-325.

Piazza, I., Beaton, N., Bruderer, R., Knobloch, T., Barbisan, C., Handat, L., Sudau, A., Siepe, I., Rinner, O., de Souza, N., Picotti, P., & Reiter. L. (2020).A machine learning-based chemoproteomic approach to identify drug targets and binding sites in complex proteomes. *Nature Communications*, *11,* 4200.

Rao, K.V. (2015). Studies on the incidence of shiga toxin - producing Escherichia coli (STEC) in dairy farm animals. *M.V.Sc. Thesis*. Sri PV Narasimha Rao Telangana Veterinary University, Hyderabad, India.

Rao, L., Lv, L., Zeng, Z., Chen, S., He, D., & Chen, X. et al (2014). Increasing prevalence of extended-spectrum cephalosporin-resistant Escherichia coli in food animals and the diversity of CTX-M genotypes during 2003-2012. *Veterinary Microbiology, 172*, 534-541.

Rao, T.S., Gill, J.P.S., Ravikumar, G.V.V.P.S., & Ghatak, S. (2011). Multi drug resistance patterns of Shiga toxin – producing *Escherichia coli* (STEC) and non – STEC isolates from meats RTE meat foods drinking water and human diarrhoeic samples of Punjab India. *Archives of Clinical Microbiology*, *2*(2-3), 1-12.

Scheutz, F., Joensen, K.G., Schjørring, S., Olesen, S., Engberg, G., Holt, H.M., *et al.* (2025). Shiga toxin-producing *E. coli* (STEC) from Danish patients, 1997-2023: Diagnostic trends and bacteriological findings. *Microorganisms, 13*(10), 2342.

Schroeder, C.M., Meng, J.M., Zhao, S., DebRoy, C., Torcolini, J., Zhao, C., McDermott, P.F., Wagner, D.D., Walker, R.D., & White, D.G. (2002). Antimicrobial resistance of *Escherichia coli* O26 O103 O111 O128 and O145 from animals and humans. *Emerging Infectious Diseases*, *8*(12), 1410-1414.

Trung, N.V., Nhung, H.N., Carrique-Mas, J.J., Mai, H.H., Tuyen, H.T., Campbell, J., Nhung, N.T., Minh, P.V., Wagenaar, J.A., Mai, N.T.N., Hieu, T.Q., Schultsz, C., & Hoa, N.T. (2016). Colonization of Enteroaggregative *Escherichia coli* and Shiga toxin-producing *Escherichia coli* in chickens and humans in southern Vietnam. *BMC Microbiology 16*(208), 1-6.

Wei, Q., Bhasme, P., Wang, Z., Wang, L., Wang, S., Zeng, Y., & Li, Y. (2020). Chinese medicinal herb extract inhibits PQS-mediated quorum sensing system in *Pseudomonas aeruginosa*. *Journal of Ethnopharmacology*, *248*, 1-7.