Molecular detection of Shiga toxin-producing *Escherichia coli* (STEC) O157 in sheep, goats, cows and buffaloes

Asim Shahzad1 · Fahim Ullah1 · Hamid Irshad2 · Shehzad Ahmed1 · Qismat Shakeela3 · Abrar Hussain Mian1

Received: 17 April 2021 / Accepted: 5 August 2021 / Published online: 10 August 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

**Abstract**

**Background** Shiga toxin-producing *E. coli* (STEC) are important foodborne pathogens that causing serious public health consequences worldwide. The present study aimed to estimate the prevalence ratio and to identify the zoonotic potential of *E. coli* O157 isolates in slaughtered adult sheep, goats, cows and buffaloes.

**Materials and methods** A total of 400 Recto-anal samples were collected from two targeted sites Rawalpindi and Islamabad. Among them, 200 samples were collected from the slaughterhouse of Rawalpindi included sheep (n = 75) and goats (n = 125). While, 200 samples were collected from the slaughterhouse of Islamabad included cows (n = 120) and buffalos (n = 80). All samples were initially processed in buffered peptone water and then amplified by conventional PCR. Samples positive for *E. coli* O157 were then streaked onto SMAC media plates. From each positive sample, six different Sorbitol fermented pink-colored colonies were isolated and analyzed again via conventional PCR to confirm the presence of *rfbE* O157 gene. Isolates positive for *rfbE* O157 gene were then further analyzed by multiplex PCR for the presence of STEC other virulent genes (*sxt1*, *stx2*, *eae* and *ehlyA*) simultaneously.

**Results** Of 400 RAJ samples only 2 (0.5%) showed positive results for *E. coli* O157 gene, included sheep 1/75 (1.33%) and buffalo 1/80 (1.25%). However, goats (n = 125) and cows (n = 120) found negative for *E. coli* O157. Only 2 isolates from each positive sample of sheep (1/6) and buffalo (1/6) harbored *rfbE* O157 genes, while five isolates could not. The *rfbE* O157 isolate (01) of sheep sample did not carry any of STEC genes, while the *rfbE* O157 isolate (01) of buffalo sample carried *sxt1*, *stx2*, *eae* and *ehlyA* genes simultaneously.

**Conclusion** It was concluded that healthy adult sheep and buffalo are possibly essential carriers of STEC O157. However, *rfbE* O157 isolate of buffalo RAJ sample carried 4 STEC virulent genes, hence considered an important source of STEC infection to humans and environment which should need to devise proper control systems.

**Keywords** *Escherichia coli* (STEC) O157 · RAJ · Conventional PCR · Multiplex PCR

**Introduction**

Shiga toxin-producing *E. coli* (STEC) are considered important foodborne pathogens of zoonotic importance which causing mild to severe bloody diarrhea with the emergence of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) which is a life-threatening disease [1, 2]. There are nearly more than 200 serotypes of STEC are recognized and the most frequent outbreaks of STEC are documented to be related to serotype O157: H7 strain throughout the globe [3]. *E. coli* O157: H7 serotype is the most important strain in hundreds of the other *E. coli* serogroups which live inside healthy humans and animal’s digestive organs it delivers an intense toxin that can cause serious public complications [4]. The toxin produced by STEC is in similarity with *Shigella*...
dysentery producing toxin is also called Shiga-like toxins, or verotoxins [5]. In 1982 the pathogen STEC O157: H7 was recognized for the first time during an outbreak in the United States (US) [6, 7]. Since then, for public health importance nowadays E. coli O157: H7 is widely recognized as a food-borne pathogen [8, 9]. STEC primary transmission occurs through fecal–oral route by either indirectly use of a broad preparation of unhygienic foods, through contaminated water ingestion or directly through animals contact and their condition as well as from individual to individual straightforwardly [5, 10]. Ruminant animals especially cattle, goats and sheep serve as a natural reservoir for STEC, which exist in the guts of these animals and appear to be the supportive hosts for STEC O157: H7. Thus, when animals are butch-ered, bacteria from animal intestines may contaminate their meat [11, 12]. In most human cases cattle have been considered the suspected domestic ruminant of the source of infection [13]. Sheep have also been suggested as a source of human infection and a major cause of contamination to the food industry [14–16]. Like cattle and sheep, goats have also been considered the sub-clinical carrier of STEC O157, as they are the asymptomatic shedder of these bacterial pathogens [17]. In 2010, there are 1.78 STEC infection cases for each 1 lac populace are reported in the United States (US) [18]. In the European Union, STEC infection rate in 2011, is 1.93 cases per 1 lac populace [19]. Similarly, in New Zealand in 2011, the documented rate of STEC is 3.5 cases for each 1 lac populace (154 cases) [20]. In Argentina, between 2002 and 2015 only 4 cases of HUS were reported which are each 1 lac populace (154 cases) [20]. In the studied animals, the output variable was the status of Escherichia coli O157. Slaughterhouses were visited seven and six times respectively, during the hot months from May to July 2017, because STEC O157 can easily survive in warm temperatures. On each visit, Twenty-five samples were randomly collected from both regions which included adult sheep, goats, cows and buffaloes. These RAJ samples were placed into modified Stuart’s transport medium (Bacti Swab NPB, Thermo Scientific, Lenexa, KS), and maintained approximately at 4 °C until processed in the Laboratory.

RAJ swab samples processing

RAJ swab samples were initially processed in 20 ml of buffered peptone water (BPW) taken in the sterilized universal bottle. Each RAJ swab sample was enriched in the enrichment broth (BPW) by cutting the swab sample inside each universal bottle using a sterilized scissor and incubated for 24 h at 37 °C.

DNA extraction from enriched broth (BPW)

After enrichment, DNA extraction was carried out from enriched broth (BPW; Difco™, Becton, USA) by boil cell lysate method [31, 32]. A 1-ml aliquot of enriched broth was taken and centrifuged at 13,000 rpm for 3 min. The supernatant was discarded after centrifugation and the pellet was re-suspended in 500 µl double distilled water (ddH2O). Vertexing was done at high speed for 10 s. The aliquot was heated at 95 °C for 10 min. Suspension of the lysed bacterial cell was then cooled at 4 °C for 5 min and was re-centrifuged again at 13,000 rpm for 3 min. The supernatant containing the DNA was then collected and transferred to another eppendorf tube. It was then subjected towards conventional PCR to detect rfbE O157 gene [33].
Initial screening for rfbE O157 serotype by conventional PCR

The rfbE gene is responsible for the production of the lipopolysaccharide (LPS) O side chain of the STEC O157: H7 cell surface and is a highly preserved gene specific to the serotype E. coli O157: H7 [34]. Conventional PCR was performed in the Gene Amp PCR system 9700 (Applied Biosystems, Melbourne, Australia). Already standardized Oligonucleotide specific sequence of rfbE O157 primers along with the amplified product size were specifically used for the synthesis of the rfbE O157 gene (Table 1). Chemical components contained buffer 2.5 µl (Invitrogen, NZ), each primer 0.5 µl, dNTP 0.6 µl (Fermentas), 2.5 µl of MgCl2 (Invitrogen, NZ), 0.3 µl unit of Taq DNA polymerase (Invitrogen, NZ), 16.1 µl of nuclease-free water completed to a final volume of 25 µl of extracted DNA. Thermocycling conditions were programmed for 7 min at 95 °C, followed by 35 cycles for 24 s at 95 °C, 45 s for 60 °C, 45 s at 72 °C, with final extension for 8 min at 72 °C, followed by maintenance at 4 °C.

Table 1 Primers used for amplification of E. coli O157 antigen-specific gene in conventional PCR

| Target gene (serogroup) | Primer sequence (5’–3’) | GC% | Amplicon size (bp) | GenBank accession number/reference |
|-------------------------|-------------------------|-----|--------------------|-----------------------------------|
| rfbE (O157)             | F-5’TTTCAC ACTTAT TGGGATGT GTCCTCA A’3 | 36% | 88 AF163329         | [35]                              |
|                         | R-5’CGATGTT TATCTG CAAGG CATG’3         | 41.7%|                    |                                   |

E. coli O157 isolation and confirmation by conventional PCR

Agar media is one of the important sources for desire colonies isolation and for the use of further confirmation purposes [36]. STEC O157 can typically and also be effectively recognized by its capability to ferment Sorbitol in 24 h on Sorbitol MacConkey agar media as compared to other E. coli strains. The RAJ swab samples suspected positive for E. coli O157 gene by conventional PCR were then streaked onto Sorbitol MacConkey Agar media plates (SMAC) and incubated at 37 °C for 24 h. After the incubation period, only Sorbitol fermented pink-colored colonies were grown on SMAC media plates. About 6 different isolated colonies were selected from each plate for further analysis. DNA extraction was carried out from each colony using the simple boiling method [31, 32]. The extracted DNA was then analyzed via conventional PCR under similar conditions to conform the presence of rfbE (O157) gene.

Multiplex PCR for STEC virulent genes (stx1, stx2, eae and hlyA)

Isolates positive for rfbE O157 serogroup by conventional PCR were then briefly subjected towards multiplex PCR (Gene Amp PCR system 9700; Applied Biosystems, Melbourne, Australia) to detect the presence of STEC other virulent genes (stx1, stx2, eae and hlyA) simultaneously. Already standardized (Oligonucleotide) specific sequence of primers and its desirable base-pair sizes were used by multiplex PCR assay for the amplification of STEC virulent genes (Table 2). Chemical components contained 2.5 µl buffer (Invitrogen, NZ), 0.5 µl each of the 8 primers (4 primer pairs) stx1, stx2, eae and hlyA, 0.6 µl of each dNTP (Fermentas), 2.5 µl MgCl2 (Invitrogen, NZ), 0.3 µl unit of Taq DNA Polymerase (Invitrogen, NZ) and 13.1 µl of Nuclease-free water completed to final volume 25 µl along with 2 µl of extracted isolate DNA. Thermocycling conditions were programmed for 7 min at 95 °C, followed by 40 cycles for 45 s at 95 °C, 45 s for 60 °C, 45 s at 72 °C, with final extension

Table 2 Primers used for amplification of STEC (stx1, stx2, eae and hlyA) specific genes in multiplex PCR

| Target gene | Primer sequence (5’–3’) | GC% | Amplicon size (bp) | References |
|-------------|-------------------------|-----|--------------------|------------|
| stx1        | F-5’GAC TGC AAA GAC GTA TGT AGA TTC G’3 | 44  | 150                | [37]       |
|             | R-5’ATC TAT CCC TCT GAC ATC AAC TGC’3 | 45.8|                    |            |
| stx2        | F-5’ATT AAC CAC ACC CCA CCG’3 | 55.6| 200                | [37]       |
|             | R-5’GTC ATG GAA ACC GTT GTC AC’3 | 50  |                    |            |
| eae         | F-5’GTA AGT TAC ACT ATA AAA GCA CCG TCG’3 | 40.7| 106                | [37]       |
|             | R-5’TCT GTG TGG ATG GTA ATA AAT TTT TG’3 | 30.8|                    |            |
| hlyA        | F-5’GACATCATCAAGCGTGACTGTTCC’3 | 52.4| 534                | [38]       |
|             | R-5’AATGAGGCAAGCTGGTTAAGCT’3 | 45.5|                    |            |
for 8 min at 72 °C, followed by maintenance at 4 °C, after which the PCR products were electrophoresed through an agarose (2% w/v) gel (Invitrogen, NZ) and visualized using ethidium bromide under Gel documentation system. The isolates were then sub-cultured onto the SMAC media plates to confirm pure growth and stored at − 80 °C in nutrient broth containing 15% (v/v) glycerol.

Results and discussion

In the present study, of 400 RAJ swab samples, only 2 (0.5%) showed positive results for *E. coli* O157 gene, included sheep 1/75 (1.33%) and buffalo 1/80 (1.25%). However, goats (n = 125) and cows (n = 120) showed negative results for *E. coli* O157 (Table 3). From each positive sample (sheep and buffalo), 6 different Sorbitol fermented pink-colored colonies were isolated onto two SMAC agar media plates (Fig. 1). DNA was extracted from each colony using simple boil cell lysate method and analyzed via conventional PCR to confirm the presence of *rfbE* O157 gene. Results indicated that only 2 isolates from each positive sample of sheep (1/6) and buffalo (1/6) harbored *rfbE* O157 genes (Figs. 2 and 3), while, rest of the five isolates showed negative results.

In comparison with a recent study, a high prevalence ratio 10/320 (6.3%) of STEC O157 was reported in cattle samples collected from the fecal rectum (n = 160) and hide brisket area (n = 160) at the abattoir in Northern Italy [39]. This is much higher than the prevalence rate of 2/400 (0.5%) observed in our study. Similarly, a total of 12/1200 (1.0%) of STEC O157 strains were isolated from bovine 8/620 (1.3%), caprine 1/130 (0.8%) and ovine 3/230 (13%) [40]. Followed by another study, in which a total of 8 (0.8%) STEC O157: H7 isolates were recovered from fecal samples of sheep 7/361 (1.9%) and goats 1/178 (0.6%) in Central Greece [41]. Whereas in our findings the prevalence rate of STEC O157 reported in RAJ sample of sheep was 1/75 (1.33%), while no STEC O157 was detected in goat samples. In contrast to our study, a higher *E. coli* O157 was reported in hides and fecal samples of cattle (49.4%), sheep (6.3%) and goats (2.5%), respectively [42]. Similarly, out of 210 samples of beef, buffalo and lamb meat the prevalence rate of *E. coli*

| Species of animals | Total RAJ samples | *E. coli* O157 positive sample | STEC virulent genes detected | Overall prevalence % |
|-------------------|------------------|-------------------------------|----------------------------|----------------------|
| Sheep             | 75               | 1                             | Not detected               | 1.33                 |
| Goats             | 125              | Negative                      | Not detected               | 0                    |
| Cows              | 120              | Negative                      | Not detected               | 0                    |
| Buffalos          | 80               | 1                             | *stx1, stx2, eae, and hlyA* | 1.25                 |
| Total             | 400              | 2                             |                            | 0.5                  |

Fig1 Isolated colonies from *E. coli* O157 positive RAJ sample of sheep (a) and buffalo (b) for *rfbE* O157 gene confirmation
O157: H7 was reported as (2.8%) in beef and (1.4%) in buffalo. However, lamb meat samples showed a negative result for this serogroup [43]. In addition, the prevalence rate of E. coli O157 was also recovered from fecal samples of camel (4.3%), goats (2%) and cattle (1.46%). However, none of the E. coli O157 was recovered from sheep fecal samples [44].

To concern the observed variation of our findings with these studies could be attributed to the differences in a wide range of sample collection, culture and molecular-based methods being applied for screening, detection and characterization of STEC. In a recent study, samples collected from ovine and bovine hosts via fecal palpitation and recto-anal junction swabs were reported most appropriate for the identification of STEC [45, 46]. For E. coli O 157 colonization the RAJ site is a good indicator to collect a sample [47].

According to these reports, we acquired the same appropriate methodology for a sample collection from the studied animals.

During the enrichment process, the recovery of high STEC and other E. coli O157 strains may be difficult to isolate because of the presence of other competing flora in the medium. Hence, this could be one of the obvious reasons behind the recovery of a low number of STEC while testing samples [48, 49]. Additionally, the temperature required for STEC detection during the enrichment process may be preferred to particular serotypes to show enough growth [50, 51].

Similarly, culture-based methods (selective and differential media) is almost difficult to differentiate STEC from other E. coli strains, as STEC can only be recognized by its capability to produce Shiga toxins, however, it cannot be used as a phenotypic marker for the identification of STEC when there is an availability of mixed culture [52]. Besides, there is no assurance for the accuracy, specificity and safe use of these cultural methodologies [53]. As in the O serogroup of STEC a huge variability has also been observed [54, 55]. As compare to immunomagnetic separation techniques, Sorbitol MacConkey agar has also been recommended for direct STEC O157: H7 isolation. However, its less sensitive factor was confirmed [56].

While some appropriate molecular techniques have been applied in recent studies for the quantification of STEC in bovine feces [36 , 57, 58], in ovine feces [59] and in agricultural food matrices [60]. However, these molecular techniques are more costly to apply in less facilitative areas as compare to culture-based methods. The misidentification of a culture-positive sample, giving false-negative and false-positive results, the targeted genes need to analyze may be present in different viable cells and the detection of a gene does not indicate if it may be expressed or not are the certain limitations and apparently main reasons behind the detection of a limited number of targeted samples [52]. It is also stated that polymerase chain reaction (PCR) may sometimes incapable to differentiate between live and dead cells, as the amplified DNA from dead STEC cells and the presence of background flora in the sample sometimes makes the PCR more vulnerable to give the exact prevalence ratio of STEC [61].

The DNA extracted from each single rfbE O157 isolate of sheep and buffalo RAJ samples were then briefly subjected towards multiplex PCR to detect the presence of STEC other virulent genes (sxt1, stx2, eae and ebhA) simultaneously (Fig. 4). Results revealed that the single rfbE O157 isolate (01) of sheep sample did not carry any of STEC genes (Fig. 5). It only harbored rfbE O157 gene and thus possessed rear chances of dissemination in the region of Rawalpindi. On the other hand, the single rfbE O157 isolate (01) of buffalo sample carried four STEC clinical virulent genes (sxt1,
stx2, eae and ehlyA) at the same time (Fig. 5), thus it possessed high zoonotic potential of transferring to human and environment in the region of Islamabad.

In consistence with our findings, a total of 6 (4%) E. coli O157; H7 was separated from fecal samples and all isolates were found that contained ehxA, stx2c and eaeA-γ1. The non-O157 STEC observed in 2 (1.5%) fecal samples contain one isolate which carried ehxA, stx2c; stx2α and stx2γ and the other isolates containing stx2α only [62]. STEC O157 was also reported as (81–87%) in Irish cattle and beef-derived isolates. Among them, the most predominant virulent strain was stx2 and eae [63, 64]. Followed by similar results were noted among E. coli O157 isolates from cattle in France [65]. Likewise, a total of 55/1317 (4.18%) STEC O157 was also recovered from RAMS samples of cattle in Ireland. Amongst 50/55 E. coli O157 isolates harbored stx2 genes and all were eae positive [66].

In the current study, none of the E. coli O157; H7 was detected in RAJ samples of goats and cows. The reason may be due to very fewer chances of E. coli O157 colonization in intestinal hosts of these animals. As goat cannot be colonized exclusively with E. coli O157 and they have been considered the sub-clinical carrier of STEC [67, 68].

The present study revealed a very low prevalence ratio of rfbE O157 recovered from healthy slaughtered adult sheep and buffalo in the region of Rawalpindi and Islamabad, Punjab Pakistan. One of the possible reasons for the low prevalence rate 2 (0.5%) reported in our study is the inclusion of healthy adult ruminant animals (sheep, goats, cows and buffaloes). The animals bring to the slaughterhouses for buttering in these regions are majority of the adult age.

Studies indicated that when sheep and cattle get older so changes in the composition of gut microbiota (gastrointestinal tract and recto-anal site) of these animals occur consequently the prevalence rate of STEC decreases [69, 70]. In the United States, STEC prevalence rate was reported higher in fecal samples of younger sheep (22.7%) at slaughter than older animals (0–1.9%) at pasture [71]. Similarly, in New Zealand, a higher prevalence rate of STEC was reported in slaughtered lamb (3.8%) than ewes (0.9%) at pasture [72]. Another study was reported on a group of older Scottish beef cattle potentially related with a lower risk of E. coli O157 shedding [73].

Even though several factors such as study design, sample collection and isolation methods used have a profound impact on the prevalence rate of E. coli O157. Despite this, the intrinsic factors (age, sex etc.) and extrinsic factors (season, diet, and climate etc.) have also a significant impact on the prevalence rate of E. coli O157 [74]. Keeping in view these differences thus limits the application of our study which could be one of the noticeable reasons behind the low prevalence rate 2/400 (0.5%) of E. coli O157 reported in our findings.

**Conclusion**

The present study revealed a low prevalence rate of 2/400 (0.5%) reported in sheep 1/75 (1.33%) and buffalo 1/80 (1.25%) RAJ samples at District Rawalpindi and Islamabad, Pakistan. However, it cannot be underestimated as healthy adult sheep and buffalo was possibly essential carriers of STEC O157 in these regions. But, as compared to Rawalpindi, the Islamabad region was at high risk because STEC O157 with 4 clinical relevant virulent genes (stxl, stx2, eae and hlyA) were detected in positive RAJ sample of buffalo...
which may possibly act as a serious public health consequence in future.

**Recommendations**

Data about the study of disease transmission of STEC O157 in these specific areas Rawalpindi and Islamabad as well in other parts of Pakistan are rare. Subsequently, more data is required for the study of disease transmission of STEC O157, distribution of virulence genes and their subtypes in *E. coli* separates from small and large ruminants and transmission of STEC O157 from these living organisms is to devise proper control systems. These control procedures would ultimately help in diminishing the increasing number of human STEC cases in Pakistan and further keep away from possible losses to Pakistan’s economy.

**Acknowledgements** We would like to thank Matiuallah (Department of Microbiology, Hazara University Mansehra, Pakistan) for his generous suggestion and help in revision of the final draft manuscript.

**References**

1. Chileshe J, Ateba CN (2013) Molecular identification of *Escherichia coli* O145: H28 from beef in the North West Province, South Africa. Life Sci J 10(4):1171–1176
2. Karmali MA, Gannon V, Sargeant JM (2010) Verocytotoxin-producing *Escherichia coli* (VTEC). Vet Microbiol 140(3–4):360–370
3. Sekhar MS, Sharif NM, Rao TS (2017) Serotypes of sorbitol-fermenting *Escherichia coli* (SP-STEIC) isolated from freshwater fish. Int J Fish Aquatic Sci 5:503–505
4. Oporto B, Ocejo M, Alkorta M, Marimón JM, Montes M, Hurtado A (2019) Zoonotic approach to Shiga toxin-producing *Escherichia coli*: integrated analysis of virulence and antimicrobial resistance in ruminants and humans. Epidemiol Infect. https://doi.org/10.1017/S0950268819000566
5. Hunt JM (2010) Shiga toxin–producing *Escherichia coli* (STEC). Clin Lab Med 30(1):21–45
6. Fernandez TF (2008) *E. coli* O157: H7. Vet World 1(3):83
7. Pintara A, Jennison A, Rathnayake IU, Mellor G, Huygens F (2020) Core and accessory genome comparison of Australian and international strains of *O157 Shiga toxin-producing Escherichia coli*. Front Microbiol 11:2162
8. Mian AH, Fatima T, Qayyum S, Ali K, Shah R, Ali NM (2020) A study of bacterial profile and antibiotic susceptibility pattern found in drinking water at district Mansehra, Pakistan. Appl Nanosci 10:5435–5439. https://doi.org/10.1007/s13204-020-01411-0
9. Xia X, Meng J, McDermott PF, Ayers S, Blickenstaff K, Tran TT, Zhao S (2010) Presence and characterization of Shiga toxin-producing *Escherichia coli* and other potentially diarrheagenic *E. coli* strains in retail meats. Appl Environ Microbiol 76(6):1709–1717
10. Elson R, Grace K, Vivancos R, Jenkins C, Adak GK, O’Brien SJ, Lake IR (2018) A spatial and temporal analysis of risk factors associated with sporadic Shiga toxin-producing *Escherichia coli* O157 infection in England between 2009 and 2015. Epidemiol Infect 146(15):1928–1939
11. Persad AK, Lejeune JT (2015) Animal reservoirs of Shiga toxin-producing *Escherichia coli*. Enterohemorrhagic *Escherichia coli* and other shiga toxin-producing *E. coli*. ASM Press, Washington, DC
12. Rigobelo EC, Santo E, Marin JM (2008) Beef carcass contamination by Shiga toxin-producing *Escherichia coli* strains in an abattoir in Brazil: characterization and resistance to antimicrobial drugs. Foodborne Pathog Dis 5:811–817
13. Joris A, Vanrompay D, Verstraete K, De Reu K, De Zutter L (2012) Enterohemorrhagic *Escherichia coli* with particular attention to the German outbreak strain O104:H4. VDT 81(3):3–10
14. Kiranmayi C, Krishnaiah N, Mallika EN (2010) *Escherichia coli* O157: H7—an emerging pathogen in foods of animal origin. Vet World 3(8):382
15. Mughimi-Gras L, Van Pelt W, Van der Voort M, Heck M, Friesema I, Franz E (2018) Attribution of human infections with Shiga toxin-producing *Escherichia coli* (STEC) to livestock sources and identification of source-specific risk factors, The Netherlands (2010–2014). Zoonoses Public Health 65(1):e8–e22
16. Rahimi E, Montaz H, Anari MMH, Alimoradi M, Momen M, Riahi M (2012) Isolation and genomic characterization of *Escherichia coli* O157: NM and *Escherichia coli* O157: H7 in minced meat and some traditional dairy products in Iran. Afr J Biotech 11(9):2328–2332
17. Kim JS, Lee MS, Kim JH (2020) Recent updates on outbreaks of Shiga toxin-producing *Escherichia coli* and its potential reservoirs. Front Cell Infect Microbiol 10:273
18. Anonymous (2012) Summary of notifiable diseases—the United States, 2010. MMWR Morb Mortal Wkly Rep 59:1–111
19. Ecce E (2013) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA J 11:3129
20. On S, Lim E, Lopez L, Cresse P, Pirie R (2011) Annual report concerning foodborne disease in New Zealand. Environmental Science and Research Limited (ESR), Christchurch, New Zealand, p 130
21. Carbonari CC, Fittipaldi N, Teatro S, Athey TB, Pianciola L, Masana M, Melano RG, Rivas M, Chinen I (2016) Whole-genome sequencing applied to the molecular epidemiology of shiga toxin-producing *Escherichia coli* O157:H7 in Argentina. Genome Announc. 1:10. https://doi.org/10.1128/genomeA.01341-16
22. Qayyum S, Basharat S, Mian AH, Qayum S, Ali M, Chughtai P, Shahzad M (2020) Isolation, identification and antibacterial study of pigmented bacteria. Appl Nanosci 10:4495–4503. https://doi.org/10.1007/s13204-020-01363-5
23. Qayyum S, Nasir A, Mian AH, Rehan S, Qayum S, Siddiqui MF, Kaloom S (2020) Extraction of peroxidase enzyme from different vegetables for biodetoxification of vat dyes. Appl Nanosci 10:5191–5199. https://doi.org/10.1007/s13204-020-01348-4
24. Group OW (2012) Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet network, 2010. Commun Dis Intell Q Rep 36:E213
25. Ali NH, Farooqui A, Khan A, Khan AY, Kazmi SU (2010) Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. J Infect Dev Ctries 4:382–388
26. Fatima T, Mian AH, Khan Z, Khan AM, Anwar F, Tariq A, Sardar M (2020) Citrus sinensis a potential solution against superbugs. Appl Nanosci 10:5077–5083. https://doi.org/10.1007/s13204-020-01408-9
27. Irshad H, Binyamin I, Ahsan A, Riaz A, Shahzad MA, Qayyum M, Yousaf A (2020) Occurrence and molecular characterization of *Shiga Toxin-producing Escherichia coli* isolates recovered from cattle and goat meat obtained from retail meat shops in Rawalpindi and Islamabad, Pakistan. Pak Vet J 40(3):10. https://doi.org/10.29261/pakvetj/2020.045
28. Mohsin M, Haque A, Ali A, Sarwar Y, Bashir S, Tariq A, Afzal A, Ifikhar T, Saeed MA (2010) Effects of ampicillin, gentamicin,
and cefotaxime on the release of Shiga Toxins from Shiga Toxin-producing Escherichia coli isolated during a diarrhea episode in Faisalabad, Pakistan. Foodborne Pathog Dis 7:785–90

29. Razzaq A, Shamsh S, Nawaz A, Nawaz A, Ali A, Malik K (2016) The occurrence of Shiga toxin producing E. coli from raw milk. Pure Appl Biol 5(2):270–276

30. Shahzad K, Muhammad K, Sheikh A, Yaqub T, Rabbani M, Hussain T, Anjum A, Anees M (2013) Isolation and molecular characterization of Shiga toxin producing E. coli O157. J Anim Plant Sci 23:1618–1621

31. Jeshvsee SS, Chai LC, Pui CF, Son R (2012) Optimization of multiplex PCR conditions for rapid detection of Escherichia coli O157: H7 virulence genes. Int Food Res J 192(1)

32. Radu S, Ling OW, Rusul G, Karim MIA, Nishibuchi M (2001) Detection of Escherichia coli O157: H7 by multiplex PCR and their characterization by plasmid profiling, antimicrobial resistance, RAPD and PFGE analyses. J Microbiol Methods 46:131–139

33. Irshad H, Cookson A, Hotter G, Besser T, On S, French N (2012) Epidemiology of Shiga toxin-producing Escherichia coli O157 in very young calves in the North Island of New Zealand. N Z Vet J 60:21–26

34. Fortin NY, Mulchandani A, Chen W (2001) Use of real-time polymerase chain reaction and molecular beacons for the detection of Escherichia coli O157: H7. Anal Biochem 289:281–288

35. Perelle S, Dilasser F, Grout J, Fach P (2004) Detection by 5′-nuclease PCR of Shiga-toxin producing Escherichia coli O26, O55, O91, O103, O111, O113, O145 and O157: H7, associated with the world’s most frequent clinical cases. Mol Cell Probes 18:185–192

36. Stromberg ZR, Redweik GA, Mellata M (2018) Detection, prevalence, and pathogenicity of non-O157 Shiga toxin-producing Escherichia coli from cattle hides and carcasses. Foodborne Pathog Dis 15(3):119–131

37. Sharma VK, Dean-Nystrom EA (2003) Detection of enterohemorrhagic Escherichia coli O157: H7 by using a multiplex real-time PCR assay for genes encoding intimin and Shiga toxins. Vet Microbiol 93:247–260

38. Mori L, Perales R, Rodriguez J, Cervera M, Davies R, Cesare AD, Herman L, Hilbert F, Skandamis P, Suffredi E, Jenkins C, Niskanen T, Scheutz F, Felicio MTDS, Messens W, Bolton D (2020) Pathogenicity assessment of Shiga toxin-producing Escherichia coli (STEC) and the public health risk posed by contamination of food with STEC. EFSA J 18(1):e05967. https://doi.org/10.2903/j.efsa.2020.5967

39. Momtaz H, Farzan E, Rahimi E, Safarpoor Dehkordi F, Sououd N (2012) Molecular characterization of Shiga toxin-producing Escherichia coli isolated from ruminant and donkey raw milk samples and traditional dairy products in Iran. Sci World J 2012(2):20. https://doi.org/10.1100/2012/231342

40. de Boer E, Heuvelink AE (2000) Methods for the detection and isolation of Shiga toxin-producing Escherichia coli. J Microbiol Methods 42(2):102–108

41. Wang F, Yang Q, Kase JA, Meng J, Clotilde LM, Lin A, Ge B (2013) Current trends in detecting non-O157 Shiga toxin-producing Escherichia coli in food. Foodborne Pathog Dis 10(8):665–677

42. EFSA Biobaz Panel, Koutsounanis K, Allende A, Alvarez-Ordóñez A, Bover-Cid S, Chemaly M, Davies R, Cesare AD, Herman L, Hilbert F, Skandamis P, Suffredi E, Jenkins C, Niskanen T, Scheutz F, Felicio MTDS, Messens W, Bolton D (2020) Pathogenicity assessment of Shiga toxin-producing Escherichia coli (STEC) and the public health risk posed by contamination of food with STEC. EFSA J 18(1):e05967. https://doi.org/10.2903/j.efsa.2020.5967

43. McPherson AS, Dhungel OP, Ward MP (2015) Comparison of recto-anal mucosal swab and faecal culture for the detection of Escherichia coli O157 and identification of super-shedding in a mob of Merino sheep. Epidemiol Infect 143(13):2733–2742

44. Rice DH, Sheng HQ, Wynia SA, Hovde CJ (2003) Recto anal mucosal swab culture is more sensitive than fecal culture and distinguishes Escherichia coli O157: H7-colonized cattle and those transiently shedding the same organism. J Clin Microbiol 41(11):4924

45. Williams KJ, Ward MP, Dhungel OP (2015) Longitudinal study of Escherichia coli O157 shedding and super shedding in dairy heifers. J Food Prot 78(4):636–642

46. De Boer E, Heuvelink AE (2000) Methods for the detection and isolation of Shiga toxin-producing Escherichia coli. J Appl Microbiol 88(S1):1335-1435

47. Vimon A, Vernozy-Rozand C, Delignette-Muller ML (2006) Isolation of E. coli O157: H7 and non-O157 STEC in different matrices: review of the most commonly used enrichment protocols. Lett Appl Microbiol 42(2):102–108

48. Conrad CC, Stanford K, McAllister TA, Thomas J, Reuter T (2016) Competition during enrichment of pathogenic Escherichia coli may result in culture bias. Facets 1(1):114–126

49. Pires SM, Morabito S, Niskanen T, Scheutz F, Felicio MTDS, Messens W, Bolton D (2020) Pathogenicity assessment of Shiga toxin-producing Escherichia coli (STEC) and the public health risk posed by contamination of food with STEC. EFSA J 18(1):e05967. https://doi.org/10.2903/j.efsa.2020.5967

50. Bonardi S, Alpigiani I, Toffoli R, Vismarra A, Zecca V, Greppi C, Brindani F (2015) Shiga toxin-producing Escherichia coli O157, O26 and O111 in cattle faeces and hides in Italy. Vet Record 177(13):389–395

51. Williams KJ, Ward MP, Dhungel OP (2015) Longitudinal study of Escherichia coli O157 shedding and super shedding in dairy heifers. J Food Prot 78(4):636–642

52. De Boer E, Heuvelink AE (2000) Methods for the detection and isolation of Shiga toxin-producing Escherichia coli. J Appl Microbiol 88(S1):1335-1435

53. Momtaz H, Farzan E, Rahimi E, Safarpoor Dehkordi F, Sououd N (2012) Molecular characterization of Shiga toxin-producing Escherichia coli isolated from ruminant and donkey raw milk samples and traditional dairy products in Iran. Sci World J 2012(2):20. https://doi.org/10.1100/2012/231342

54. Brusa V, Piñeyro PE, Galli L, Linares LH, Ortega EE, Padera ML, Leotta GA (2016) Isolation of Shiga toxin-producing Escherichia coli from ground beef using multiple combinations of enrichment broths and selective agars. Foodborne Pathog Dis 13(3):163–170

55. Gill A, Huszczynski G, Gauthier M, Blais B (2014) Evaluation of eight agar media for the isolation of shiga toxin—producing Escherichia coli. J Microbiol Methods 96:6–11

56. Lupindu AM (2018) Epidemiology of Shiga toxin-producing Escherichia coli O157: H7 in Africa in review. South Afr J Infecct Dis 33(1):24–30

57. Shridhar PB, Noll LW, Shi X, An B, Cernicchiaro N, Renter DG, Bai J (2016) Multiplex quantitative PCR assays for the detection and quantification of the five major non-O157 Escherichia coli serogroups in cattle feces. J Food Prot 79(1):66–74

58. Shridhar PB, Noll LW, Shi X, An B, Cernicchiaro N, Renter DG, Bai J (2016) Multiplex quantitative PCR assays for the detection and quantification of the five major non-O157 Escherichia coli serogroups in cattle feces. J Food Prot 79(1):66–74

59. Verhaegen B, De Reu K, De Zutter L, Verstraete K, Heyndrickx Messens W, Bolton D (2020) Pathogenicity assessment of Shiga toxin-producing Escherichia coli (STEC) and the public health risk posed by contamination of food with STEC. EFSA J 18(1):e05967. https://doi.org/10.2903/j.efsa.2020.5967

60. Shridhar PB, Noll LW, Shi X, An B, Cernicchiaro N, Renter DG, Bai J (2016) Multiplex quantitative PCR assays for the detection and quantification of the five major non-O157 Escherichia coli serogroups in cattle feces. J Food Prot 79(1):66–74

61. Shridhar PB, Noll LW, Shi X, An B, Cernicchiaro N, Renter DG, Bai J (2016) Multiplex quantitative PCR assays for the detection and quantification of the five major non-O157 Escherichia coli serogroups in cattle feces. J Food Prot 79(1):66–74
1. Sethulekshmi C, Latha C, Anu CJ (2018) Occurrence and quantification of Shiga toxin-producing *Escherichia coli* from food matrices. Vet World 11(2):104

2. Macori G, McCarthy SC, Burgess CM, Fanning S, Duffy G (2020) Investigation of the causes of shigatoxigenic *Escherichia coli* PCR positive and culture negative samples. Microorganisms 8(4):587

3. Perera A, Clarke CM, Dykes GA, Fegan N (2015) Characterization of Shiga toxigenic *Escherichia coli* O157 and non-O157 isolates from Ruminant Feaces in Malaysia. Biomed Res Int 2015:382403

4. Murphy BP, McCabe E, Murphy M, Buckley JF, Crowley D, Fanning S, Duffy G (2016) Longitudinal study of two Irish dairy herds: low numbers of Shiga toxin-producing *Escherichia coli* O157 and O26 Super-Shedders Identified. Front Microbiol 7:1850

5. Thomas KM, McCann MS, Collery MM, Logan A, Whyte P, McDowell DA, Duffy G (2012) Tracking verocytotoxigenic *Escherichia coli* O157, O26, O111, O103 and O145 in Irish cattle. Int J Food Microbiol 153(3):288–296

6. Bibbal D, Loukiadis E, Kérourédan M, Ferré F, Dilasser F, de Garam CP, Brugère H (2015) Prevalence of carriage of Shiga toxin-producing *Escherichia coli* serotypes O157: H7, O26: H11, O103: H2, O111: H8, and O145: H28 among slaughtered adult cattle in France. Appl Environ Microbiol 81(4):1397

7. McCabe E, Burgess CM, Lawal D, Whyte P, Duffy G (2019) An investigation of shedding and super-shedding of Shiga toxigenic *Escherichia coli* O157 and *E. coli* O26 in cattle presented for slaughter in the Republic of Ireland. Zoonoses Public Health 66(1):83–91

8. Fox J, Corrigan M, Drouillard J, Shi X, Oberst R, Nagaraja T (2007) Effects of concentrate level of diet and pen configuration on the prevalence of *Escherichia coli* O157 in finishing goats. Small Rumin Res 72:45–50

9. Mersha G, Asrat D, Zewde B, Kyule M (2010) The occurrence of *Escherichia coli* O157: H7 in feces, skin and carcasses from sheep and goats in Ethiopia. Lett Appl Microbiol 50:71–76

10. Menrath A, Wieler LH, Heidemanns K, Semmler T, Fruth A, Kemper N (2010) Shiga toxin producing *Escherichia coli*: identification of non-O157: H7-super-shedding cows and related risk factors. Gut Pathog 2(1):1–9

11. Mir RA, Weppelmann TA, Elzo M, Ahn S, Driver JD, Jeong KC (2016) Colonization of beef cattle by Shiga toxin-producing *Escherichia coli* during the first year of life: a cohort study. PLoS ONE 11(2):e0148518

12. Kilonzo C, Atwill ER, Mandrell R, Garrick M, Villanueva V, Hoar BR (2011) Prevalence and molecular characterization of *Escherichia coli* O157: H7 by multiple-locus variable-number tandem repeat analysis and pulsed-field gel electrophoresis in three sheep farming operations in California. J Food Prot 74(9):1413–1421

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.