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Molecular characterization of Shigatoxigenic *Escherichia coli* (STEC) in sheep and goat in Krishna district of Andhra Pradesh

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) strains represent an emerging threat in foodborne bacterial pathogens. The present study was designed to determine prevalence of STEC, antibiogram profile, ESBLs and genetic diversity. Among the 468 samples analyzed, 172 (36.75%) were confirmed to be *E. coli* by species specific PCR. Out of the 172 confirmed *E. coli* isolates, 42 (24.41%) isolates carried either *stx*1, *stx*2 or both indicating them to be STEC. Along with shiga toxins, virulence genes *eae*A and *hly*A genes were also present in some of the isolates. The antibiogram profile of STEC isolates revealed highest resistance against ampicillin (90.47%) followed by tetracycline (80.95%), amoxicillin-clavulanic acid (76.19%), co-trimoxazole (71.42%), ceftriaxone (69.04%), ciprofloxacin (54.76%), chloramphenicol (21.42%) and amikacin (14.28%). Extended spectrum β-Lactamase (ESBL) genes were detected in 10 isolates, with bla_{TEM} in seven isolates and $bla_{CTX-M-9}$ in three isolates. Both ERIC and REP-PCR genotyping methods differentiated STEC into different strains and are considered to be highly suitable genotyping methods and can be effectively utilized for epidemiological investigation of isolates.

Keywords: Antibiogram profile, *E. coli*, ESBL, STEC, Genotyping, Virulence genes,

Shiga-toxin-producing *Escherichia coli* (STEC) are zoonotic foodborne pathogens capable of causing serious human illness (Majowicz *et al.* 2014). In 2018, more than 8,000 STEC infections were reported in Europe, with over 37% of cases requiring hospitalization and further medical treatment (EFSA and ECDC 2019).

Small ruminants have been recently recognized as an important reservoir of STEC which can secrete the bacteria through the faeces (Wani *et al.* 2004). Due to absence of clinical manifestation, these reservoir animals enter the food chain and spread STEC either with their faeces or intestinal contents during slaughter (Asakura *et al.* 2014). Leading to complications such as haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Peacock *et al.* 2001). The virulence of STEC is primarily associated with the presence of shiga toxin-encoding genes (*stx*1 and/or *stx*2) as well as other virulence factors, such as the intimin-encoding *eae*A gene and the enterohemolysin *hly*A gene (Scheutz *et al.* 2012). The *stx*2 is more strongly associated with severe diseases and HUS syndrome than *stx*1 (Kawano *et al.* 2008). STEC have become significant food-borne pathogens, leading to gastroenteritis that can range from mild diarrhoea to severe haemorrhagic colitis. The pathogen STEC O157: H7 was recognized for the first time during an outbreak in 1982 in the United States (Fernandez *et al.* 2009). Recent epidemiological studies have identified several non-O157 serogroups, such as O26, O45, O91, O103, O104, O111, O113, O121 and O145 associated with severe human diseases in the United States, Europe and Latin American countries (Brooks *et al.* 2005). Hence, the present study was conducted to detect the occurrence of STEC from sheep and goat as well as from farmers/workers and the farm environment and to assess their antimicrobial resistance and to assess the genetic diversity of Shigatoxigenic *E. coli* isolates from different sources using DNA fingerprinting techniques.

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MATERIALS AND METHODS

Sample collection and enrichment of media: A total of 468 samples were collected from 18 different farms in Krishna district of Andhra Pradesh including feed (n=2) and farm water sample (n=3) as well as 20 faecal samples with (n=5) from each adult sheep and goat, lambs and kids and hand swabs (n=1) of farm workers from each farm. About 10 g of faecal samples mixed with 10 ml of water were inoculated in 90 ml of PBS at pH 7.4 whereas 10 g of feed samples were homogenized in 90 ml of PBS. All hand swabs collected from sheep and goat farmers were transferred to 10ml Tryptone Soya Broth (TSB). One ml each of PBS suspensions were inoculated in 9 mL of TSB and incubated at 37°C aerobically for 18-24 h. Enriched samples were streaked onto MacConkey agar and incubated at 37°C for 18-24 h. From each plate, pink colonies were picked up and streaked on Eosin Methylene

Blue (EMB) agar and incubated at 37°C for 24 h. The isolates with characteristic metallic sheen were identified as presumptive E. coli colonies and were subjected to biochemical and molecular tests for further confirmation. The enriched broth was used for further DNA extraction and antibiogram studies.

Molecular detection of E. coli by PCR targeting E. coli 16SrRNA gene: Extraction of DNA from the *E. coli* was carried out by conventional boiling and rapid cooling method (Naidu *et al.* 2021). The concentration of DNA was determined with spectrophotometer and adjusted to 50 ng/µL. All the presumptive *E. coli* were further confirmed by species specific targeting E16SrRNA gene (Sun *et al*. 2011) (Supplementary Fig.1.)

Detection of Putative Virulent genes of E. coli: Detection of stx1 and stx2 was done using m-PCR assay or both indicating as STEC. It also characterized virulence gene profile (eaeA, hlyA along with stx1 and stx2) in confirmed E. coli isolates (Paton and Paton, 1998) (Supplementary Fig.2.)

Identification and Confirmation of E. coli O157: The confirmed STEC isolates were further streaked on Sorbitol MacConkey Agar supplemented with cefixime and tellurite. The pale yellow sorbitol non fermenting colonies were presumed to be *E. coli* O157 isolates (Ojo *et al.* 2010) and the presumptive *E. coli* O157 isolates were confirmed through amplification ofe *rfb*O157 gene using PCR (Desmarchelier *et al.* 1998) (Supplementary Fig. 3.)

Antimicrobial Susceptibility Testing: Antibiogram of STEC isolates was carried by Kirby Bauer disc diffusion method on Muler Hinton Agar (Bauer *et al*. 1966). Susceptibility patterns of STEC were studied as per the zone diameter interpretative breakpoints for *E. coli* as given in Clinical and Laboratory Standards Institute guidelines (CLSI 2021). The nine antibiotics that were tested included ampicillin (AMP, 10 µg), amoxicillin-clavulanic acid (AMC, 30 µg), ceftriaxone (CTR, 30 µg), ciprofloxacin (CIP, 5 µg), tetracycline (TE,30 µg), chloramphenicol (C, 30 µg), amikacin (AK, 30 µg), imipenem (IPM, 10 µg), and co-trimoxazole (COT, 25 µg) procured from M/s. HiMedia Laboratories (Mumbai).

Detection of Extended Spectrum β-Lactamase Production: For phenotypic confirmation of ESBL production, phenotypic screening test (PST) was done by testing their susceptibility against four antimicrobial agents: cefotaxime, ceftazidime, ceftriaxone and aztreonam. Resistance to at least one of the four antibiotics used was considered as positive PST followed by CDM (Combination Disc Method) carried out using ceftazidime (30 µg), ceftazidime plus clavulanic acid (30/10 µg), cefotaxime (30 µg), cefotaxime plus clavulanic acid (30/10 µg); ceftriaxone (30 µg), ceftriaxone plus sulbactam (30/10 µg). ESBL production was confirmed when zone diameter around the combination discs was ≥5 mm when compared to discs containing respective cephalosporin alone (CLSI 2021).

Molecular Detection of ESBL genes: Two m-PCR assays- m-PCR I for the amplification of bla_{TEM}, bla_{SHV},

Table 1. Occurrence of *E. coli* in different samples of sheep and goat farms

Farm	Faecal samples	Feed samples	Water samples	Farm workers
				Hand swab
A	12/20 (60%)	0/2 (0%)	1/3 (33.3%)	0/1 (0%)
B	8/20 (40%)	0/2 (0%)	1/3 (33.3%)	0/1 (0%)
C	8/20 (40%)	0/2 (0%)	0/3 (0%)	0/1 (0%)
D	7/20 (35%)	0/2 (0%)	0/3 (0%)	1/1 (100%)
E	8/20 (40%)	1/2 (50%)	1/3 (33.3%)	0/1 (0%)
F	8/20 (40%)	0/2 (0%)	1/3 (33.3%)	0/1 (0%)
G	8/20 (40%)	1/2 (50%)	1/3 (33.3%)	0/1 (0%)
H	8/20 (40%)	0/2 (0%)	0/3 (0%)	1/1 (100%)
I	8/20 (40%)	0/2 (0%)	1/3 (33.3%)	0/1 (0%)
J	11/20 (55%)	0/2 (0%)	1/3 (33.3%)	0/1 (0%)
K	8/20 (40%)	0/2 (0%)	1/3 (33.3%)	0/1 (0%)
L	9/20 (45%)	0/2 (0%)	0/3 (0%)	1/1 (100%)
M	7/20 (35%)	0/2 (0%)	0/3 (0%)	0/1 (0%)
N	9/20 (45%)	0/2 (0%)	0/3 (0%)	0/1 (0%)
O	11/20 (55%)	0/2 (0%)	0/3 (0%)	0/1 (0%)
P	9/20 (45%)	1/2 (50%)	0/3 (0%)	0/1 (0%)
Q	9/20 (45%)	0/2 (0%)	0/3 (0%)	0/1 (0%)
R	9/20 (45%)	0/2 (0%)	1/3 (33.3%)	0/1 (0%)
TOTAL	157/360 (43.61%)	3/36 (8.33%)	9/54 (16.66%)	3/18 (16.66%)

Table 2. Distribution of virulence factors and genes in *E. coli* isolates from 18 sheep and goat farms

Source	No of isolates analyzed	Virulence genes								
		*Stx*1 (%)	*stx*2 (%)	*stx*1+*eae*A (%)	*stx*1+*stx*2+*hly*A (%)	*stx*1+*eae*A+*hly*A (%)	*stx*2+*eae*A+*hly*A (%)	*stx*1+*hly*A (%)	*stx*1+*stx*2 (%)	*stx*2+*eae*A (%)	*stx*2+*hly*A (%)
Adult sheep	33	0	1	0	3	0	0	0	0	0	0
Faecal samples			(3.03)		(9.09)						
Adult goat	38	1	1	0	4	0	0	2	0	0	0
Faecal samples		(2.63)	(2.63)		(10.52)			(5.26)			
Lamb	41	0	0	1	6	0	0	2	2	1	0
Faecal samples				(2.439)	(14.63)			(4.87)	(4.87)	(2.43)	
Kid	45	0	0	0	8	1	3	3	0	0	1
Faecal samples					(17.77)	(2.22)	(6.66)	(6.66)			(2.22)
Feed sample	3	0	0	0	0	0	0	0	0	0	0
Water sample	9	0	0	0	2	0	0	0	0	0	0
					(22.22)						
Farm workers Hand swab	3	0	0	0	0	0	0	0	0	0	0
Grand total	172	1	2	1	23	1	3	7	2	1	1
		(0.58)	(1.16)	(0.58)	(13.37%)	(0.58)	(1.74)	(4.06)	(1.16)	(0.58)	(0.58)

bla_{OXA} and m-PCR II for bla_{CTX-M} genes were carried out (Dallenne et al. 2010) (Supplementary Fig.4.)

Genetic Diversity of STEC isolates: Genetic diversity among STEC isolates was analysed by ERIC-PCR and REP-PCR (Mohapatra and Mazumder 2008).

RESULTS AND DISCUSSION

In our current study, *E. coli* were isolated in 210 out of 468 samples collected. The overall prevalence of *E. coli* was 172 (36.75%, 172/468) by PCR. The findings were in accordance with the prior study conducted by Elsayed *et al.* (2018) where they reported an overall prevalence of 41.7% in sheep and goat. The high occurence in some farms might be due to poor sanitation and hygiene and lack of regular disinfection practices which exacerbate bacterial growth and also due to presence of carriers in the farm (Ray and Singh 2022). Highest occurrence of *E. coli* was found in the faecal samples 157 (43.61%, 157/360) followed by water samples 9 (16.66%, 9/54), hand swabs of farm workers 3 (16.66%, 3/18) and feed samples 3 (8.33%, 3/36) (Table 1).

The highest prevalence of *E. coli* in faecal samples is probaly due to *E. coli* being natural inhabitants in gastrointestinal tract of mammals (Zaheri *et al*. 2020). The occurrence of *E. coli* in hand swabs was attributed to poor hygiene practices like improper hand cleaning, which elevated the risk of food contamination (Campos *et al*. 2009).

Among the 172 *E. coli* isolates, 42 isolates (24.41%) from 18 sheep and goat farms were positive for *stx*1/*stx*2 or both indicating STEC. Along with *stx*1 and *stx*2, *eae*A and *hly*A genes were also detected in some of the isolates. The virulence gene profile of STEC isolates revealed predominance of *stx*1 and *hly*A (83.33% each, 35/42) followed by *stx*2 (78.57%, 33/42) and *eae*A (14.28%, 6/42). Three isolates harboured only single gene (*stx*1 in one isolate, *stx*2 in two isolates). Remaining 39 isolates carried more than one virulence gene with *stx*1 and *hly*A in 16.66% (7/42) isolates, *stx*1 and *stx*2 in 4.76% (2/42) isolates, *stx*2 and *eae*A in 2.38% (1/42), *stx*2 and *hly*A in 2.38% (1/42), *stx*2, *eae*A and *hly*A in 7.14% (3/42) STEC isolates, combination of *stx*1, *stx*2 and *hly*A in (54.76%, 23/42) STEC isolates, *stx*1, e*ae*A and *hly*A in (2.38%, 1/42) isolate (Table 2).

Among the different samples collected from various sources, STEC were recorded in the faecal samples and water samples. No STEC were detected in feed samples and hand swabs of the farm workers (Table 3). Contamination of water, either directly or indirectly, with faeces significantly increases the likelihood of STEC presence in the water (Beutin *et al.* 1993).

Age wise, highest prevalence of STEC was found among kids (17.77%, 16/90) followed by lambs (14.44%, 13/90) adult goat (7.77%, 7/90) and adult sheep (4.44%, 4/90) (Table 4). High prevalence of STEC in kids and lambs may be attributed to the weaning stress, low immune response, gut microbial changes and dietary factors (Ndegwa *et al.*

Table 3. Occurrence of STEC among different samples of sheep and goat farms

Farm	Faecal samples (%)	Feed sample (%)	Water sample (%)	Hand swabs of farm workers (%)	Total (%)
A	7/20 (35)	0/2 (0)	0/3 (0)	0/1 (0)	7/26 (26.92)
B	1/20 (5)	0/2 (0)	0/3 (0)	0/1 (0)	1/26 (3.84)
C	1/20 (5)	0/2 (0)	0/3 (0)	0/1 (0)	1/26 (3.84)
D	1/20 (5)	0/2 (0)	0/3 (0)	0/1 (0)	1/26 (3.84)
E	7/20 (35)	0/2 (0)	0/3 (0)	0/1 (0)	7/26 (26.92)
F	2/20 (10)	0/2 (0)	0/3 (0)	0/1 (0)	2/26 (7.69)
G	2/20 (10)	0/2 (0)	0/3 (0)	0/1 (0)	2/26 (7.69)
H	1/20 (5)	0/2 (0)	0/3 (0)	0/1 (0)	1/26 (3.84)
I	1/20 (5)	0/2 (0)	0/3 (0)	0/1 (0)	1/26 (3.84)
J	4/20 (20)	0/2 (0)	1/3 (33.33)	0/1 (0)	5/26 (19.23)
K	1/20 (5)	0/2 (0)	0/3 (0)	0/1 (0)	1/26 (3.84)
L	1/20 (5)	0/2 (0)	0/3 (0)	0/1 (0)	1/26 (3.84)
M	1/20 (5)	0/2 (0)	0/3 (0)	0/1 (0)	1/26 (3.84)
N	1/20 (5)	0/2 (0)	0/3 (0)	0/1 (0)	1/26 (3.84)
O	2/20 (10)	0/2 (0)	0/3 (0)	0/1 (0)	2/26 (7.69)
P	2/20 (10)	0/2 (0)	0/3 (0)	0/1 (0)	2/26 (7.69)
Q	2/20 (10)	0/2 (0)	0/3 (0)	0/1 (0)	2/26 (7.69)
R	3/20 (15)	0/2 (0)	1/3 (33.33)	0/1 (0)	4/26 (15.38)
TOTAL	40/360 (11.11)	0/36 (0)	2/54 (3.70)	0/18 (0)	42/468 (8.97)

2020).

The confirmed STEC isolates were cultured on CT-SMAC agar in order to differentiate the *E. coli* O157 and non-O157 serotypes. Among the 42 STEC isolates, one was identified as highly pathogenic *E. coli* O157 using PCR. It was isolated from faecal sample of lamb of farm E carried and the *stx*1, *stx*2 and *hly*A virulence genes. Which further enhanced its pathogenicity.

Among the 42 STEC isolates, highest resistance was observed against ampicillin (90.47%) followed by tetracycline (80.95%), amoxicillin-clavulanic acid (76.19%), co-trimoxazole (71.42%), ceftriaxone (69.04%), ciprofloxacin (54.76%), chloramphenicol (21.42%) and amikacin (14.28%). STEC isolates showed highest sensitivity against imipenem (95.23%), followed by amikacin (80.95%), chloramphenicol (73.80%), ciprofloxacin (42.85%), ceftriaxone (30.95%), co-trimoxazole (23.80%), amoxicillin-clavulanic acid (16.66%) and ampicillin and tetracycline (7.14% each) (Table 5). The high resistance to tetracycline is primarily due to its widespread use in food animal production, both as a growth promoter and antibiotic therapy. The high resistance level to amoxicillin can be attributed to the widespread clinical use against various aerobic gram-negative enteric bacilli. The high resistance to sulfamethoxazole might be associated with its extensive use in enteric bacterial and parasitic infections (Elsayed *et al.* 2018).

All the 42 isolates obtained from various sources in the present study were resistant to at least three or more classes of antibiotics, considering as MDR (Ramatla *et al.* 2024). antibiotics tested and were indicated as suspected ESBL producers. Out of 17 suspected ESBL producers, 12 (28.57%) were confirmed as ESBL producers using CDM.

Table 4. Occurrence of STEC in sheep and goat of different ages (faeces)

Farm	Adult sheep (%)	Adult goat (%)	Lamb (%)	Kid (%)	Total (%)
A	2/5 (40)	1/5 (20)	2/5 (40)	2/5 (40)	7/20 (35)
B	0/5 (0)	0/5 (0)	0/5 (0)	1/5 (20)	1/20 (5)
C	0/5 (0)	0/5 (0)	1/5 (20)	0/5 (0)	1/20 (5)
D	0/5 (0)	1/5 (20)	0/5 (0)	0/5 (0)	1/20 (5)
E	0/5 (0)	2/5 (40)	2/5 (40)	3/5 (60)	7/20 (35)
F	0/5 (0)	1/5 (20)	1/5 (20)	0/5 (0)	2/20 (10)
G	0/5 (0)	0/5 (0)	1/5 (20)	1/5 (20)	2/20 (10)
H	0/5 (0)	0/5 (0)	0/5 (0)	1/5 (20)	1/20 (5)
I	0/5 (0)	0/5 (0)	0/5 (0)	1/5 (20)	1/20 (5)
J	0/5 (0)	1/5 (20)	1/5 (20)	2/5 (40)	4/20 (20)
K	1/5 (20)	0/5 (0)	0/5 (0)	0/5 (0)	1/20 (5)
L	0/5 (0)	0/5 (0)	0/5 (0)	1/5 (20)	1/20 (5)
M	0/5 (0)	0/5 (0)	1/5 (20)	0/5 (0)	1/20 (5)
N	1/5 (20)	0/5 (0)	0/5 (0)	0/5 (0)	1/20 (5)
O	0/5 (0)	0/5 (0)	1/5 (20)	1/5 (20)	2/20 (10)
P	0/5 (0)	0/5 (0)	1/5 (20)	1/5 (20)	2/20 (10)
Q	0/5 (0)	0/5 (0)	1/5 (20)	1/5 (20)	2/20 (10)
R	0/5 (0)	1/5 (20)	1/5 (20)	1/5 (20)	3/20 (15)
Total	4/90 (4.44)	7/90 (7.77)	13/90 (14.44)	16/90 (17.77)	40/360 (11.11)

Table 5. Antimicrobial susceptibility patterns of STEC isolates

S.No	Antibiotic disc (Concentration)	Interpretative criteria (mm)					
		Sensitive		Intermediate		Resistant	
		No (42)	%	No (42)	%	No (42)	%
1	Penicillin Ampicillin (AMP 10µg)	3	7.14	1	2.38	38	90.47
2	Beta-lactamase inhibitor Amoxicillin-Clavulanic acid (AMC 30µg) (20/10)µg	7	16.66	3	7.14	32	76.19
3	Cephalosporins Ceftriaxone (CTR 30µg)	13	30.95	0	0	29	69.04
4	Fluoroquinolones Ciprofloxacin (CIP 5µg)	18	42.85	1	2.38	23	54.76
5	Tetracyclines Tetracycline (TE 30 µg)	3	7.14	5	11.90	34	80.95
6	Phenolics Chloramphenicol (C 30 µg)	31	73.80	2	4.76	9	21.42
7	Aminoglycosides Amikacin (AK 30µg)	34	80.95	2	4.76	6	14.28
8	Carbapenems Imipenem (IPM 10 µg)	40	95.23	2	4.76	0	0
9	Sulfonamides Co-Trimoxazole (Trimethoprim/ Sulfamethoxazole)25µg (COT 1.25/23.75 µg)	10	23.80	2	4.76	30	71.42

The STEC isolates had an Multiple antibiotic resistance (MAR) index exceeding 0.2. If MAR index greater than 0.2 it implies that the strains originated from an antibiotic contaminated environment. Which may be attributed to the prolonged and non-judicious use of antibiotics in both animals and humans within the study area (Elsayed *et al.* 2018) (Table 6).

Of the 42 STEC isolates, 17 (40.47%) were found be resistant to at least one or more of the cephalosporin

Of the 12 CDM positive isolates, bla_{TEM} gene was detected in 7 (16.66%) isolates and bla_{CTXM}-Group 9 gene in 3 (7.14%) isolates. Isolates carrying ESBL genes were also found to possess two or three combinations of virulence genes in all, contributing to severe health complications in humans and leading to the failure of beta-lactam therapy. The co-existence of virulence genes and antimicrobial resistance genes in STEC isolates has gained a significant threat to public health (Zhao *et al.* 2022). The O157 isolate

Table 6. MAR index among STEC isolates

MAR index label	Sample ID	No. of isolates	Total no of antibiotics the isolate was resistant to	Total no. of antibiotics tested	MAR index value/ each isolate	Total MAR index
		(A)	(B)	(C)	(B/C)	(A*B/C)
MAR 1	EFK3, LFK2, RFG2	3	7	9	0.77	2.31
MAR 2	AFL1, AFL3, DFG2, BFK1 AFS2, JFW1 JFK3, RFK4	8	6	9	0.66	5.28
MAR 3	AFG2, EFG3, EFL2, EFK4, FFL1, GFL2, JFG1, PFL1, IFK3, EFK2, FFG1, RFL3	12	5	9	0.55	6.6
MAR 4	AFK1, HFK1, JFL2, KFS2, MFL2, NFS2, OFL2, PFK1, QFL4, RFW2, OFK1, EFG2, EFL1, GFK2, JFK2	15	4	9	0.44	6.6
MAR 5	AFS3, CFL2, QFK2, AFK2	4	3	9	0.33	1.32
TOTAL		42				22.11
					Average MAR – 22.11/42 - 0.52	

exhibited multidrug resistance to five classes of antibiotics and harbored virulence genes that represent a significant public health concern, while no ESBL genes were detected.

ERIC-PCR typing of 43 STEC (along with standard) revealed 1-8 fragments per isolate ranging from~ 180 bp to ~1500 bp whereas REP-PCR typing revealed 3-10 fragments per isolate, ranging from ~120 bp to ~3200 bp. Out of the 43 STEC (along with standard) isolates analyzed, only 41 ERIC patterns were obtained as 2 isolates (AFK1 and DFG2) did not yield any bands by ERIC -PCR fingerprinting. Out of the 43 STEC (along with standard) isolates analyzed, only 40 REP patterns were obtained as 3 isolates (GFK2, IFK3 and RFG2) did not yield any bands by REP -PCR fingerprinting.

Cluster analysis of ERIC-PCR profiles differentiated STEC isolates into eight clusters (Fig .1). Cluster I & II consists of two STEC isolates each which were closely related with 90% similarity cut off. Both of these isolates which were obtained from the same farm explains the close relation. Cluster III consists of three STEC isolates (CFL2, EFG3, S) where CFL2 clustered separately from EFG3 and S with 80% similarity cut off. Cluster IV consists of seven STEC isolates which is sub- clustered into two sub-clusters where sub-cluster 1 comprised of three STEC isolates in which IFK3 was clustered separately from other two isolates. Sub-cluster 2 comprised of three STEC isolates in which OFL2 clustered separately from other two isolates with 80% similarity cut-off. The isolate (HFK1) was away from both the sub-clusters. Cluster V comprised of six STEC isolates which were further grouped into three sub-clusters each comprising of two STEC isolates each with 90% similarity cut off. Cluster VI comprised of three STEC isolates in which AFS3 clustered separately from other two isolates. Cluster VII comprised of five STEC isolates. Cluster VIII comprised of five STEC isolates. Eight STEC isolates were found to be unclustered with isolates indicating wider genetic diversity.

Cluster analysis of REP-PCR profiles differentiated STEC isolates from different sources into nine clusters (Fig. 2). Cluster I comprised of three STEC isolates where PFK1 clustered separately from other two isolates. Cluster II comprised of four STEC isolates which were sub-clustered into two sub-clusters, where sub-cluster 1 comprised of two STEC isolates with 90% similarity among the isolates and sub-cluster 2 had two STEC isolates. Cluster III comprised of two STEC isolates with 90% similarity. Cluster IV comprised of five STEC isolates. Cluster V comprised of three STEC isolates where OFK1 clustered separately from other two isolates. Cluster V1 comprised of four STEC isolates (LFK2, AFS3, AFK1, AFG2). Of these four isolates, isolate LFK2 was distantly related than the other three isolates and these three isolates were obtained from the same farm. Cluster VII comprised of five STEC isolates which were further sub-clustered into two sub-clusters where sub-cluster 1 had two STEC isolates with 90% similarity and sub-cluster 2 comprised of three STEC isolates. Cluster VIII comprised of three STEC isolates. Cluster IX comprised of six STEC isolates which were separated into two sub-clusters where sub-cluster 1 comprised of three STEC isolates and sub-cluster 2 comprised of three STEC isolates. Four isolates along with standard were found be unclustered with other isolates indicating wider genetic diversity.

Discriminatory power for ERIC-PCR was 0.9988 and REP-PCR was 0.9966. Both ERIC and REP-PCR

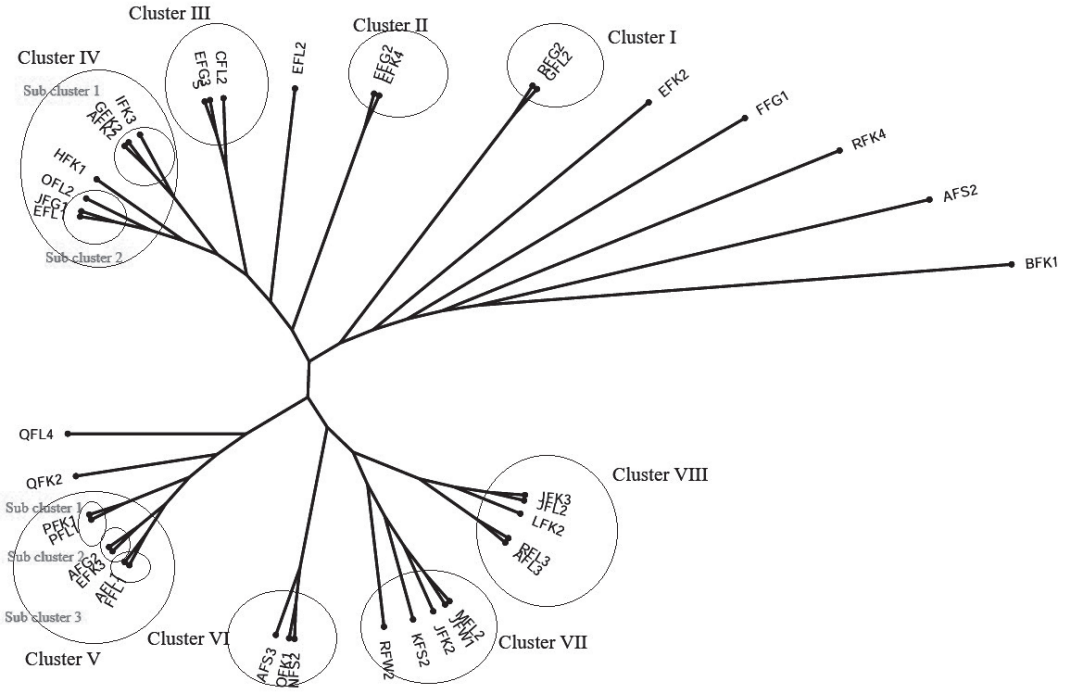

Fig. 1 Cluster analysis of ERIC-PCR fingerprints of STEC isolates from different sources

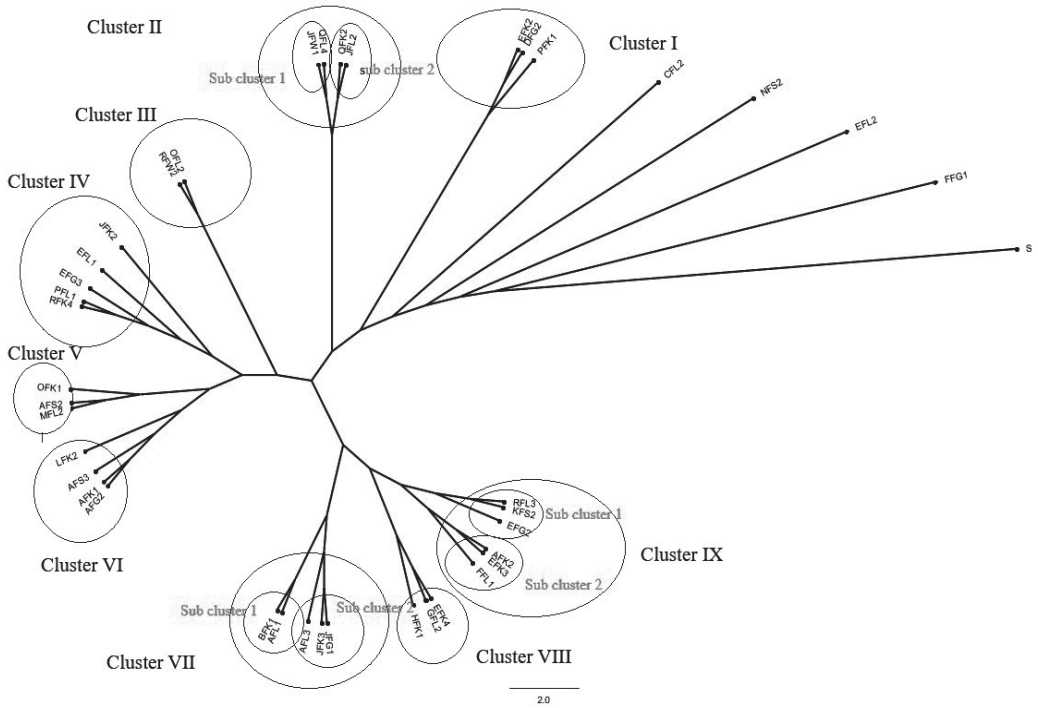

Fig. 2 Cluster analysis of REP-PCR fingerprints of STEC isolates from different Sources

genotyping methods are considered to be highly suitable genotyping methods since discriminatory power > 0.9 is considered as highly significant (Hunter and Gaston 1998). Therefore, ERIC and REP-PCR fingerprinting methods can be effectively utilized for epidemiological investigation of isolates.

This study demonstrated the occurrence of STEC in sheep and goat farms, with the highest prevalence detected in faecal samples, confirming their role as major reservoirs. The isolates harbored important virulence genes (*stx1, stx2, hlyA, eaeA*), with an O157 serotype identified, underscoring their zoonotic potential. High levels of multidrug resistance, ESBL production, and elevated MAR indices highlighted the impact of indiscriminate antimicrobial use and pose a significant public health threat. The genetic diversity revealed through ERIC-PCR and REP-PCR further indicated circulation of heterogeneous STEC strains in the study area. These findings emphasized the importance of strengthened farm hygiene, rational antibiotic use, and a One Health approach to reduce the risk of STEC transmission to humans**.**

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