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Prevalence of β-lactamase Producing Shiga Toxigenic *E. coli* (STEC) in Retail Meats and Chicken Cloacal Swabs

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ABSTRACT

Foodborne illness caused by multidrug resistant STEC is one of the most important global public health problems in the world. So, the present study was undertaken to know the prevalence of β-lactamase (ESBL and *AmpC*) producing STEC in retail meats and chicken cloacal swabs by PCR. A total of 183 samples (135 foods of animal origin and 48 chicken cloacal swabs) collected from retail meat shops and poultry farms in and around Krishna district were subjected to cultural isolation and confirmation of β-lactamase producing STEC by different PCR assays. The overall prevalence of *E. coli* was found to be 37.15% (68/183) by species-specific PCR. The STEC specific virulence genes *stx1, hlyA* and *stx2* were detected in 10.29%, 2.941% and 1.470%, of *E. coli* isolates, respectively and no *eae A* gene was identified. ESBL and/or *AmpC*-producing *E. coli* were found in 80.88% (55/68) of isolates with *bla*TEM being the predominant gene (87.27%) followed by *bla*CTX-M-2 (9.09%), *bla*OXA (7.27%), *bla* DHA (3.63%) and CIT (1.81%). β-lactamase activity was detected in 66.66% of STEC isolates. These findings revealed that retail meats are the potential source of ESBL and/or *AmpC*-producing *E. coli*.

HIGHLIGHTS

- Prevalence of β-lactamase producing STEC in retail meats
- Retail meats were found to be potential source for β-lactamase producing STEC

Keywords: *E. coli*, PCR, STEC, ESBL and *AmpC* β-lactamases

E. coli is a Gram-negative, rod-shaped, flagellated non-sporulating and facultative anaerobic bacteria that belongs to Enterobacteriaceae family. Although, *E. coli* is a commensal that can be found in intestines of variety of animals including man, some pathogenic strains can cause debilitating fatal diseases in humans and animals (Jafari *et al.*, 2012). These pathogenic *E. coli* are classified into Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enterohaemorrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC) and Enteroaggregative *E. coli* (EAgEC). Among these, EHEC which are also known as shiga toxin producing *E. coli* (STEC), are considered as the most dangerous group that cause a variety of diseases ranging from mild diarrhoea to severe bloody

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diarrhoea called haemorrhagic colitis (HC) and even leads to life-threatening sequelae such as haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic Purpura (TTP) (Brett *et al.,* 2003; Perera *et al.,* 2015). As STEC is endemic in cattle and other domestic animals, undercooked meat and unpasteurized milk act as the main source of infection. STEC has also emerged as one of the most virulent groups associated with cases of food borne illness in humans. Retail meats derived from food animals could potentially serve as transmission vehicles for STEC and other diarrheagenic *E. coli* strains (Xia *et al.,* 2010).

STEC strains produce cytotoxins known as shiga like toxins (*stx*) that are classified into two major classes shiga like toxin1(*stx*1) and shiga like toxin2 (*stx*2) between which the amino acid profile is 56% similar. *Stx*1 is 98% analogous to the shiga toxin of *Shigella dysenteriae* (Jackson *et al.,* 1987). In addition to *stx* 1 and *stx* 2, most disease causing STEC strains also produce a protein called intimin (*eaeA*) that is involved in the enterocyte attaching and effacing and enables the intimate adherence of STEC to the intestinal epithelium of the host (Yu and Kaper, 1992). Further a specific plasmid encoded haemolysin called EHEC haemolysin (hlyA) is involved in extraction of iron from the blood released into the intestine (Beutin *et al.,* 1995).

In food producing animals, broad spectrum antibiotics are not only used to treat bacterial infections but also used for disease prevention (metaphylaxis and prophylaxis) and growth promotion. Nowadays, the antibiotics usage at subtherapeutic doses is increasing due to increased demand for livestock products globally, especially in South and Southeast Asia, due to rising incomes (Brower *et al.,* 2017). This massive and indiscriminate use of broad-spectrum cephalosporins among animals could be an important reason for the occurrence of ESBL-producing bacteria among food producing animals and in meat products (Sabia *et al.*, 2017). These resistant bacteria will be excreted through faeces and enter into environment that can contaminate the foods of animal origin during animal slaughter and carcass processing. The human gut could be colonized with these resistant bacteria particularly ESBL and *AmpC* β-lactamase producing strains through consumption of contaminated meat and cause fatal infections and treatment failure which results in serious consequences in patients. In case of β-lactamase producing STEC infections, antibiotic treatment results in higher occurrence of the infection due to liberation of toxins stimulated by the non-lethal concentration of antibiotics (Puii *et al.,* 2019). A number of studies in various countries have proposed meat as a source of β-lactamase producing bacteria (Agerso *et al.,* 2012). Recent studies in the Netherlands have reported that the predominant ESBL genes and plasmids in *E. coli* obtained from broiler meat are present even in human clinical isolates (Egervärn *et al.*, 2014). In the past few decades, foodborne illness caused by *E. coli* mainly ESBL and/or *AmpC* β-lactamase producing *E. coli* was one of the most important global public health problems in the world since they were isolated from livestock, animal feed and water in increasing prevalence and pathogenicity (Gilliss *et al.*, 2013). So, the aim of present study was to know the prevalence of β-lactamases producing STEC in meats of food animals collected from retail shops and also in chicken cloacal swabs.

MATERIALS AND METHODS

Standard control and primers

ATCC (American Type Culture Collection) culture of *E. coli* (ATCC 25922) was used as standard positive control and *Proteus mirabilis* (ATCC 12453) was used as negative control. Oligonucleotide primers were custom synthesized from M/s. Bioserve Biotechnologies Pvt. Ltd. (Hyderabad).

Sample collection

A total of 183 samples comprising 135 food samples of animal origin (17 chicken, 35 mutton, 15 beef, 20 pork and 48 fish) collected from retail meat shops and 48 poultry cloacal swabs collected from poultry farms in and around Krishna district, Andhra Pradesh. The study was carried out during the period of February 2019 to January 2020, in the Department of Veterinary Public Health and Epidemiology, N.T.R College of Veterinary Science, Gannavaram, India.

Ten grams of meat samples were inoculated into 90ml of Tryptose soya broth (TSB) and homogenized, while cloacal swabs were directly inoculated into 10 ml TSB broth test tubes and incubated at 37ºC for 24h. After incubation, the samples were streaked on MacConkey and EMB

agar plates and incubated at 37ºC for 24h. Identification of isolates was done based on colony morphology (MacConkey – pink color colonies and EMB-greenish metallic sheen colonies) and the colonies were enriched in TSB for further confirmation by species-specific PCR.

DNA extraction from enriched broth samples

DNA was extracted by boiling and snap chilling method (Suresh *et al.*, 2018). About 1.5 mL of enriched broth cultures were taken into microcentrifuge tubes and centrifuged at 8000 rpm for 10 min. Supernatant was discarded, 50 µL of nuclease-free water was added and placed in boiling water bath at 100°C for 10 min, then immediately snap chilled for 10 min and centrifuged at 10,000 rpm for 5 min. The supernatant was used for PCR assays as a DNA template.

Confirmation of *E. Coli* by species-specific-uniplex PCR

Presumptive colonies of *E. coli* were confirmed molecularly by species-specific genes (Table 1). PCR assay was optimized in 25 µL reaction mixture containing 2 µL of DNA template, 12.5 µL of 2x master mix (EmeraldAmp GT PCR Master Mix, Takara), 1.5 µL each of forward and reverse primers (10 pmol/µL) and the rest of the volume is made by adding nuclease-free water. The cycling conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles of 95°C for 1 min, 50 °C for 50 sec and 72 °C for 1 min and a final extension step at 72 °C for 10 min. PCR products were subjected to gel electrophoresis using 1% agarose with ethidium bromide as fluorescent dye (Sambrook & Russell, 2001) and visualized using Gel Documentation unit (BIORAD).

Table 1: Primer sequences for *E. Coli* species specific PCR (Sun *et al.,* 2011)

Primer	Sequence (5'-3')	Amplicon size (bp)
E16S: F	ATCAACCGAGATTCCCCCAGT	231
E16S: R	TCACTATCGGTCAGTCAGGAG	

Detection of virulence genes in *E. Coli* isolates

All the confirmed *E. coli* isolates were screened for the presence of virulence genes (Table 2). PCR assay was optimized in 25 µL reaction mixture containing 2.5 µL of DNA template, 12.5 µL of 2x master mix (EmeraldAmp GT PCR Master Mix, Takara), 0.3 µL each of forward and reverse primers (10 pmol/µL) and the rest of the volume is made by adding nuclease-free water. The cyclical conditions followed were according to the Paton and Paton (1998).

Table 2: Primers used for detection of virulence genes in *E. Coli* (Paton and Paton, 1998)

Primer	Target gene	Primer sequence	Amplicon size (bp)
stx 1: F	stx 1	ATAAATCGCCATTCGTTGACTAC	180
stx 1: R		AGAACGCCCACTGAGATCATC	
stx 2: F	stx 2	GGCACTGTCTGAAACTGCTCC	255
stx 2: R		TCGCCAGTTATCTTGACATTCTG	
eaeA: F	eaeA	GACCCGGCACAAGCATAAGC	384
eaeA: R		CCACCTGCAGCAACAAGAGG	
hlyA: F	hlyA	GCATCATCAAGCGTACGTTCC	534
hlyA: R		AATGAGCCAAGCTTGTTAAGCT	

Antimicrobial resistance profile of *E. Coli* isolates

All the confirmed *E. coli* isolates were subjected to antibiotic sensitivity test against 14 different antibiotics like cefepime, ceftriaxone, amikacin, tetracycline, ceftazidime, gentamicin, piperacillin/tazobactam, ceftazidime+clavulanic acid, ofloxacin, chloramphenicol, amoxicillin+clavulanic acid, ampicillin, cefotetan, imipenem on Muller Hinton agar by using Kirby Bauer disc diffusion method (Bauer *et al.*, 1966). Direct colony of each isolate was suspended in PBS (pH 7.4) and the turbidity was adjusted to 0.5 McFarland units (equivalent to an approximate cell density of 1.5×10^8 CFU/ml having absorbance of 0.132 at wavelength of 600nm). The diameter of inhibition zones was measured and susceptibility patterns of *E. coli* species were interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2018).

Detection of ESBL production in *E. Coli* isolates by phenotypic methods

Screening of *E. coli* isolates for resistance against third-generation cephalosporins like cefotaxime (30 µg),

ceftazidime (30 µg), ceftriaxone (30 µg) and monobactams like aztreonam (30 µg) was done by disc diffusion method (Bauer *et al.*, 1966) on Mueller-Hinton agar using commercial discs (HiMedia, Mumbai). Resistance to at least one of the four antibiotics used was considered as a positive screening test (PST) for ESBL and AmpC β-lactamase production (Drieux *et al.*, 2008; CLSI, 2018). PST positive isolates were further subjected to combination disc method (CDM) for phenotypic confirmation (PCT) of ESBL production, in which cephalosporin discs along with combination disc containing cephalosporin plus clavulanate/sulbactam were used i.e. ceftazidime (CAZ, 30 µg), ceftazidime + clavulanic acid (CAC, 30/10 µg), cefotaxime (CTX, 30 µg), cefotaxime + clavulanic acid (CEC, 30/10 µg) and ceftriaxone (CTR, 30 µg), ceftriaxone + sulbactam (CIS, 30/10 µg). ESBL production was confirmed by an increased inhibition zone diametre of ≥ 5 mm in case of combination discs (Drieux *et al.*, 2008; CLSI, 2018).

Identification of ESBL genes by m-PCR

All PCT positive *E. coli* isolates DNA were subjected to two m-PCR assays to detect different ESBL genes (*bla*TEM, *bla*SHV, *bla*OXA, *bla*CTX-M-1, *bla*CTX-M-2 and *bla*CTX-M-9 groups). PCR assays were optimized in 25 µL reaction mixture containing 2 µL of DNA, 12.5 µL of 2X master mix (EmeraldAmp GT PCR Master Mix, Takara), 0.5 µL of forward and reverse primers (10 pmol/µL) and the rest of the volume is made by adding nuclease-free water. The primers and PCR conditions were followed according to Dallenne *et al*. (2010).

Detection of *AmpC* β-lactamase genes in *E. Coli* by m-PCR

m-PCR assay for the detection of *bla*AmpC genes was performed to all the PST positive isolates since PST positive but PCT negative organisms may produce *AmpC* or both ESBL and *AmpC* β-lactamases. PCR was optimized in 25 µL reaction mixture containing 2 µL of DNA, 12.5 µL of 2X master mix (EmeraldAmp GT PCR Master Mix, Takara), 0.2 µL of forward and reverse primers (10 pmol/µL) and the rest of the volume is made by adding nuclease-free water, under standardized cycling conditions described by Manoharan *et al.* (2012).

RESULTS AND DISCUSSION

In the present study, *E. coli* were detected in 45.18% (61/135) of raw meat samples and 14.58% of chicken cloacal swabs (7/48) by PCR. The overall prevalence of *E. coli* was found to be 37.15% (68/183) which is almost similar to the findings of Prabhu Kishore (2017) who reported 34.03% of *E. coli* in the present study area. Among various raw meat samples, highest prevalence was recorded in fish (62.50%) followed by beef (60.00%), chicken (35.29%), pork (30.00%) and mutton (28.57%) (Table 3 & Fig. 1). Highest prevalence of *E. coli* was recorded in fish samples as water bodies can be easily prone to contamination with municipal sewage.

Table 3: Prevalence of *E. Coli* in samples

No. of samples	*E. Coli* positives	Prevalence of *E. Coli* (%)
Chicken (17)	6	35.29
Pork (20)	6	30.00
Beef (15)	9	60.00
Mutton (35)	10	28.57
Fish (48)	30	62.50
Total (135))	61	45.18
Cloacal swabs (48)	7	14.58
Grand Total (183)	68	37.15

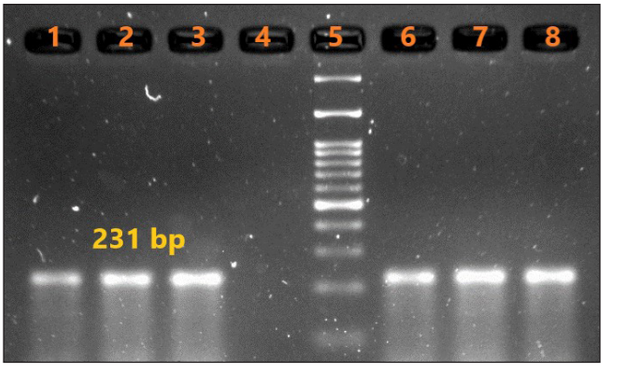

Fig. 1: Gel photograph of species-specific PCR for *E. Coli.* Lane 1: Positive control of *E. coli* (ATCC 25922) (231 bp); Lane 4: Negative control; Lane 5: DNA ladder (100 bp); Lane 2, 3, 6, 7, 8: E. coli (231 bp) isolated from different samples

Among the 61 *E. coli* isolates recovered from the raw meat samples, 14.75% (9/61) of isolates carried at least one of the STEC gene with *stx*1 being the predominant

gene detected (77.77%, 7/9) followed by *hlyA* (22.22%, 2/9), *stx*2 (11.11%, 1/9) and no *eaeA* gene was detected. Shiga toxins were detected in 50.00% (3/6) of chicken isolates, 30.00% (3/10) of mutton isolates, 22.22% (2/9) of beef isolates and 3.33% (1/30) of fish isolates (Table 4 and Fig. 2). In our study, *stx*1 gene was recovered from six isolates (3-chicken, 1- beef, 1-fish and 1-mutton), *stx*2 from one beef isolate, *hlyA* from one mutton isolate and both *stx*1 and *hlyA* were found in one mutton isolate. In the present study, overall prevalence of STEC in different raw meats (14.75%) was comparable with the findings of Bai *et al.* (2015) who reporteded 19.39% prevalence of STEC in different raw meats in China. No isolate recovered from the chicken cloacal swabs was positive for STEC. However, 50.00% (3/6) of *E. coli* isolates recovered from the chicken meat samples were positive for shiga toxins. This might be due to the use of contaminated water during slaughter or from the unhygienic human handlers, knives and meat cutting boards.

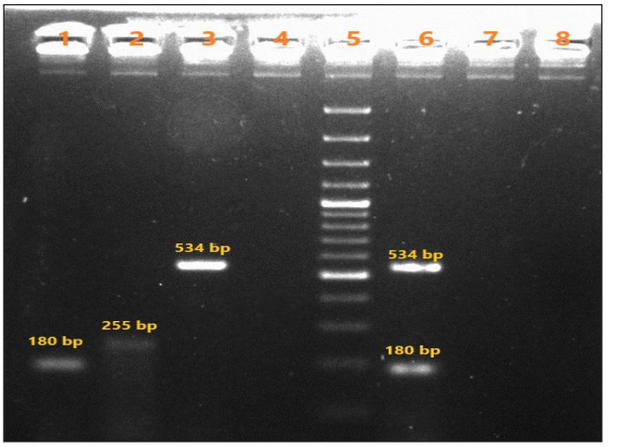

Fig. 2: Gel photograph of PCR assay targeting shiga toxin genes. Lane 1: *E. coli* isolate with *stx1* (180 bp) gene; Lane 2: *E. coli* isolate with *stx2* (255 bp) gene; Lane 3: *E. coli* isolate with *hlyA* (534bp) gene; Lane 4: Negative control; Lane 5: DNA ladder (100 bp); Lane 6: *E. coli* isolate with *stx1* (180bp) and *hlyA* (534bp)

Table 4: Detection of shiga toxins in *E. coli* isolated from different samples

Shiga toxins	Chicken isolates (6)	Pork isolates (6)	Beef isolates (9)	Mutton isolates (10)	Fish isolates (30)	Cloacal swab isolates (7)
Stx1	3	—	1	1	1	—
Stx2	—	—	1	—	—	—
hlyA	—	—	—	1	—	—
Stx1+ hlyA	—	—	—	1	—	—
Prevalence (%)	50%	—	22.22%	30.00%	3.33%	—

Table 5: Antibiotic sensitivity test of *E. coli* isolates by phenotypic method

Sl. No.	Antibiotic discs (conc)	Antibiotic sensitivity of *E. coli* isolates		
		Sensitive	Intermediate	Resistant
1	Amikacin (AK-30 µg)	52 (76.47%)	13 (19.11%)	3 (4.41%)
2	Amoxyclav (AMC-20/10 µg)	15 (22.05%)	19 (27.94%)	34 (50.00%)
3	Ampicillin (AMP-10 µg)	34 (50.00%)	13 (19.11%)	21 (30.88%)
4	Cefepime (CPM-30 µg)	40 (58.82%)	25 (36.76%)	3 (4.41%)
5	Cefotetan (CTN-30 µg)	55 (80.88%)	11 (16.17%)	2 (2.94%)
6	Ceftazidime (CAZ-30 µg)	48 (70.58%)	13 (19.11%)	7 (10.29%)
7	Ceftazidime/Clavulanic acid (CAC-30/10 µg)	14 (20.58%)	16 (23.52%)	38 (55.88%)
8	Ceftriaxone (CTR-30 µg)	50 (73.52%)	12 (17.64%)	6 (8.82%)
9	Chloramphenicol (C-30 µg)	35 (51.47%)	17 (25.00%)	16 (23.52%)
10	Gentamicin (GEN-10 µg)	54 (79.41%)	1(1.47%)	13 (19.11%)
11	Imipenem (IPM-10 µg)	48 (70.58%)	20 (29.41%)	0
12	Ofloxacin (OF-5 µg)	45 (66.17%)	19 (27.94%)	4 (5.88%)
13	Piperacillin/Tazobactam (PIT-100/10 µg)	53 (77.94%)	13 (19.11%)	2 (2.94%)
14	Tetracycline (TE-30 µg)	6 (8.82%)	3 (4.41%)	59 (86.76%)

All the *E. coli* isolates were subjected to antibiotic sensitivity test against fourteen antibiotics. Among these, highest levels of resistance was observed against tetracyclines identically detected in 59 (86.76%) isolates followed by ceftazidime/clavulanic acid in 38 (55.88%), amoxyclav in 34 (50.00%), ampicillin in 21 (30.88%), chloramphenicol in 16 (23.52%), gentamicin in 13 (19.11%), ceftazidime in 7 (10.29%), ceftriaxone in 6 (8.82%), ofloxacin in 4 (5.88%), amikacin and cefepime in 3 each (4.41%), cefotetan and piperacillin/ tazobactam in 2 (2.94%) each. None of the isolates were resistant to imipenem which is in accordance with Rasheed *et al.* (2014). Papich (2002) reported that resistance to carbapenems was extremely rare in veterinary medicine.

All the 68 *E. coli* isolates were subjected to PST, PCT and m-PCR for the detection of β-lactamases (ESBL's and *AmpC* β-lactamases) out of which 80.88% (55/68) of isolates were positive for β-lactamases production by both phenotypic and molecular methods. Recent surveys have shown that 70–90% of Enterobacteriaceae in India are ESBL producers and colonization of this type of bacteria in humans is widespread (Brower *et al.*, 2017). Heavy use of antibiotics could be one of the risk factors for the occurrence of ESBL-producing organisms (Paterson and Bonomo, 2005). Of the 55 β-lactamases producing *E. coli* isolates, 52 isolates were found positive for only ESBL's (TEM, OXA and CTX-M-2), 2 isolates for *AmpC* β-lactamases (DHA and CIT) and 1 isolate for both ESBL's and *AmpC* β-lactamases (TEM and DHA). Prevalence rate of β-lactamase *E. coli* among different samples was 100% in fish (30/30) and chicken cloacal swabs (7/7), 83.33% (5/6) in chicken, 80% (8/10) in mutton, 50% (3/6) in pork and 22.22% (2/9) in beef with *bla*TEM being the predominant gene detected (87.27%, 48/55) followed by *bla*CTX-M-2 (9.09%, 5/55), *bla*OXA (7.27%, 4/55), *bla*AmpC gene DHA (3.63%, 2/55) and CIT (1.81%, 1/55) among all the tested *E. coli* isolates (Table 6 and Fig. 3). A clear prevalence of only TEM-type β-lactamases were found in *E. coli* isolated from chicken (5 isolates), mutton (6 isolates), beef (2 isolates), pork (3 isolates), cloacal swabs (4 isolates) and fish (23 isolates). *bla*TEM was the predominant gene detected (87.27%) whereas Nadimpalli *et al.* (2019) and Nguyen *et al.* (2016) reported CTX-M to be the predominant gene among the foods of animal origin isolates. This variation might be due to differences in usage of different antibiotics in various geographical regions. TEM was also found in combination with OXA in two cloacal swab isolates, with CTX-M-2 in two fish isolates and with DHA in one fish isolate. Single CTX-M-2 gene was found in two fish isolates and in one cloacal swab isolate. OXA was found in two mutton isolates whereas *AmpC* β-lactamases were found in two fish isolates, with DHA in one and CIT in the other isolate. In the present study 66.66% (6/9) of STEC isolates harboured β-lactamase genes. Higher incidence of 59.38% β-lactamase producing STEC was reported from North East India (Puii *et al*., 2019).

Table 6: Prevalence of β-lactamase genes in *E. coli* isolated from different samples

Samples	*E. coli* Positive samples	ESBLs			AmpC β-lactamases	
		TEM	OXA	CTXM-2	DHA	CIT
Chicken (17)	6	5	—	—	—	—
Pork (20)	6	3	—	—	—	—
Beef (15)	9	2	—	—	—	—
Mutton (35)	10	6	2	—	—	—
Fish (48)	30	26	—	4	2	1
Cloacal swabs (48)	7	6	2	1	—	—
Total (183)	68	48	4	5	2	1

In the present study, prevalence of β-lactamases in fish and chicken cloacal swab isolates was 100% since non-therapeutic usage of antibiotics was particularly common in poultry production and aquaculture compared to other food animal production (Sivaraman, 2018). In aquaculture, application of antibiotics occurs en masse by inclusion of antibiotics in fish food. This wide application of antibiotics in fish food contributes to leaching of antibiotics into the water and pond sediments from unconsumed food and through faeces. Thus, it exerts selection pressures on the sediment and water micro flora and leads to development of resistance. In addition, fish farming is usually integrated with sewage or industrial waste water or with land agriculture. These unhygienic measures, overcrowding and other manipulations act as stressors to the fish and promote an increased use of antibiotics as prophylaxis. All the isolates (100%) from chicken cloacal swabs were carrying ESBLs which is in agreement with the studies reporting that healthy chickens frequently carry ESBL/*AmpC*-producing *E. coli* in their rectum (Dierikx

et al., 2013; Reich *et al.,* 2013). As healthy chickens are harbouring ESBLs, prevalence of ESBLs in chicken samples would be expected due to significant faecal contamination during slaughter (Nguyen *et al.*, 2016). Less prevalence of β-lactamases in beef isolates was reported since the average annual consumption of antimicrobials to produce one kilogram of beef meat was low (45mg/1kg meat) when compared to chicken (148mg/1kg meat) and pork (172mg/1kg meat) (Van Boeckel *et al*., 2015).

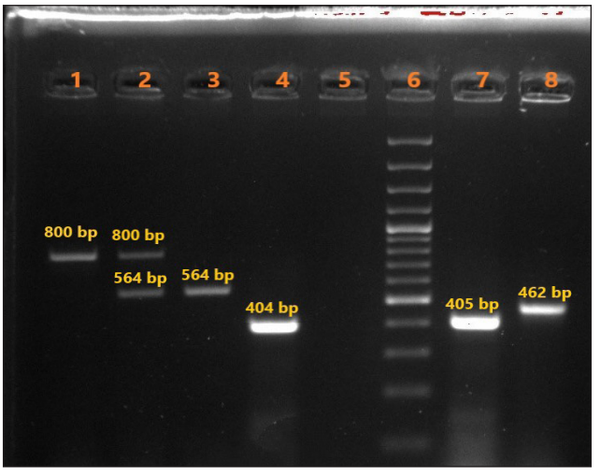

Fig. 3: Gel photograph showing ESBL and *AmpC* β-lactamase genes in *E. Coli* isolates. Lane 1: *E. coli* isolate with bla_{TEM} (800 bp) gene; Lane 2: *E. coli* isolate with bla_{TEM} (800 bp) and bla_{OXA} (564 bp) gene; Lane 3: *E. coli* isolate with bla_{OXA} (564 bp) gene; Lane 4: *E. coli* isolate with $bla_{CTX\text{-}M\text{-}2}$ (404 bp) gene; Lane 5: Negative control; Lane 6: DNA ladder (100 bp); Lane 7: *E. coli* isolate with bla_{AmpC} DHA (405 bp) gene; Lane 8: *E. coli* isolate with bla_{AmpC} CIT (462 bp) gene

CONCLUSION

This study revealed the high prevalence β-lactamases especially in STEC isolates (66.66%) isolated from foods of animal origin which can be due to indiscriminate use of antibiotics in food animals. In developing countries like India, where antibiotics can be easily available due to poor monitoring, it is difficult to control the indiscriminate use of antibiotics in food producing animals. So, creating awareness among the livestock rearers about the use of antibiotic alternatives in growth promotion and disease treatment can decrease the use of antibiotics and ultimately results in decreased prevalence of virulent antimicrobial resistant strains.

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