J Food Sci Technol (April 2021) 58(4):1247–1261
https://doi.org/10.1007/s13197-020-04634-9

ORIGINAL ARTICLE

Extended-spectrum beta-lactamase (ESBL) producing and multidrug-resistant *Escherichia coli* in street foods: a public health concern

M. Sivakumar[1] · G. Abass[1] · R. Vivekanandhan[1] · Anukampa[1] · D. K. Singh[1] · Kiran Bhilegaonkar[1] · Suman Kumar[1] · M. R. Grace[1] · Zunjar Dubal[1]

Revised: 23 June 2020 / Accepted: 3 July 2020 / Published online: 10 July 2020

Abstract Antimicrobial resistance (AMR) pattern and virulence genes of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from foods of animal origin were evaluated. Based on combination disc method and ESBL E test, 42 of the 213 *E. coli* isolates were confirmed as ESBL producers where a high presence was observed in raw foods (60.62%), environmental samples (46.73%) and ready to eat foods (42.99%) of which 31(26.49%), 3(6.97%) and 7(15.21%) samples harbored ESBL *E. coli*, respectively. Higher contamination rates were observed in samples collected from meat vendors (54.36%), milk vendors (48.88%) and egg vendors (45.20%) of which 16.1%, 11.11% and 2.05%, respectively were ESBL *E. coli*. Among the 42 ESBL isolates, 85.71% (36/42) were multidrug-resistant. On polymerase chain reaction (PCR) analysis, expression of beta-lactamase genes viz., *blaCTXM* was noted in 69.04% (29/42) ESBL isolates, *blaTEM* in 66.66% (28/42) and *blaOXA-1* in 19.04% (8/42) isolates, while *blaSHV* was not detected in any of the isolates. Other AMR genes viz., *blaAmpC*, *sul1*, *sul2*, *tet(A)*, *tet(B)*, *catI*, *dhfrI*, *aac(3)-IIa(aacC2)*, *aph(3′)-Ia(aphA1)*, *qnrB*, *qnrS* were detected by PCR in 39, 28, 29, 3, 9, 5, 17, 11, 6, 6 and 33 isolates, respectively. None of the isolates harbored chloramphenicol (*floR*) and plasmid-mediated quinolone resistance (PMQR) (*qnrA*) genes. However, 21 isolates were positive for class I integron (*int1*), 5 for EPEC (*eae*) and 9 for ETEC (*lt*) while none were carrying *bfp* or *stII* genes. All ESBL producing isolates formed a single group when subjected to enterobacterial repetitive intergenic consensus (ERIC PCR) genotyping. The presence of multidrug-resistant ESBL *E. coli* in street foods of animal origin raises the issues of food safety and public health.

Keywords AMR genes · ESBL *E. coli* · PCR · Street food · Virulence

Introduction

Street food vending is one of the most popular and fast-growing sectors in many developing countries including India. These foods are not only appreciated for their unique flavors and convenience but they also have a vital role in maintaining the nutritional status of the low-income urban populations and are source of livelihood for the poor entrepreneurs (WHO 1996). Street-vended foods pose a serious public health threat due to lack of knowledge about the microbial status of raw materials, foods and their handlers; inappropriate protection from flies, pest and rodents; lack of basic food safety measures; inadequate public awareness about health hazards present in the foods and lack of sufficient resources for inspection and laboratory analysis of food samples (WHO 1996). In spite of the many benefits of street-vended foods, they may also act as a potential vehicle for foodborne pathogens such as *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens, Salmonella* spp., and *Bacillus cereus* during preparation, post-cooking and other handling stages (Paudyal et al. 2017; Noor 2019). Microbiological quality of several street foods such as raw fish, chilly, avocado, cooked potato, panipuri, chaats, eggrolls, samosa, kachori, puchkka, alu chop, vegetable momo, pork

✉ Zunjar Dubal
drzunjar@yahoo.co.in

[1] Division of Veterinary Public Health, ICAR-Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly, Uttar Pradesh 243 122, India

momo, alu-cheura, vegetable chowmein, jhal-muri, mutton momo, sya-faley and chicken sold at the street of various countries have been assessed where > 31% samples were contaminated with *E. coli, Salmonella, P. aeruginosa, S. aureus, Klbesiella* spp.and *Campylobacter* spp. with the viable bacterial load of $> 1.7 \times 10^5$ CFU/g (Sharma and Mazumdar 2014; Kharel et al. 2016; Eromo et al. 2016; Siddabathuni 2019; Birgen et al. 2020). Amongst these, *E. coli* is a common foodborne pathogen and appears frequently in many of the street-vended foods all over the world including India (Siddabathuni 2019). Antimicrobial resistance is now an emerging global health problem and many patients succumb to death due to drug-resistant organisms and the unavailability of appropriate antibiotics. Development of antimicrobial resistance due to indiscriminate use of antimicrobials in food production chain, especially in poultry rearing is becoming a major concern with food products of animal or poultry origin are considered as one of the source of antibiotic resistance dissemination. Nevertheless, foods of plant origin, particularly salads and ready-to-eat street foods/meals also play a significant role in dissemination of antibiotic resistance and are becoming a major concern (Campos et al. 2015). Multidrug resistant and ESBL producing *E. coli* have been isolated from raw meat, vegetable salad, egg surface, unpasteurized milk, raw fish and water by various workers, indicating serious public health issues (Rasheed et al. 2014; Odonkor and Addo 2019; Silva et al. 2019). Extended-spectrum beta-lactamase (ESBL) producing *E. coli* have been isolated from foods of animal origin and vegetable salad, as well (Bhoomika et al. 2016; Siddabathuni 2019). ESBL producing *E. coli* inactivates beta-lactam antibiotics containing oxyimino group, especially 3rd and 4th generation cephalosporins and monobactam (except cephamycins or carbapenems) (Guenther et al. 2011). They are also resistant to other class of antibiotics like fluoroquinolones, aminoglycosides, trimethoprim, sulfonamide, chloramphenicol, and tetracycline (Abayneh et al. 2018) which leaves only a few antibiotics for therapy. Therefore the health professionals do not have other options than to use the last resort antibiotics like carbapenems which recently leads to the development of resistance against carbapenems too (Dagher et al. 2018). ESBL producing *E. coli* was prevalent in several food animal species at farm level and in products thereof (Apostolakos et al. 2019). In India, many researchers have reported the occurrence of ESBL producing *E. coli* in hospital settings (Oberoi et al. 2013; Rath et al. 2014; Singh et al. 2016). However, limited reports are available from food-producing animals and their products (Ghatak et al. 2013; Karet al. 2015; Bhoomika et al. 2016; Pruthvishree et al. 2018; Senthil Murugan et al. 2019). In fact, very few studies demonstrated the AMR and virulence genes in *E. coli* isolates recovered from foods of animal origin particularly from ready to eat food sold at Indian streets. Therefore, the present work was undertaken to determine the AMR and virulence profile of *E. coli* isolate circulating in foods of animal origin sold at the street and its associated environment.

Materials and methods

Collection and processing of samples

A cross-sectional study was carried out from September 2015 to May 2017, in which a total of 430 samples comprising foods of animal origin and the associated environment from different roadside food vendors located in Delhi and Bareilly cities were collected by adopting all aseptic precautions. In this study, samples were collected randomly only once from each vendor at different localities of Delhi namely, New Delhi, Old Delhi, Hazarat Nizamuddin and Anand Vihar; and from Delapeer, Air Force station, Sahdana, Rajendra Nagar and Izatnagar localities in Bareilly, depending on the availability of food articles with the vendors. For convenience and as per the degree of processing, the samples were categorized as environmental [consisting of hand swab (HS), table swab (TS), cloth swab (CS) and plate swab (PS)]; raw foods (consisting of raw chicken, raw egg, raw milk, raw paneer and raw fish); ready to eat foods (consisting of lassi, rasmalai, burfi, pedha, curd, rasgulla, salad, chutney and masala); and cooked foods (consisting of chicken gravy, omelette, cooked fish, boiled egg and boiled milk).

To collect swab samples, each swab was kept in a screw-capped tube or test tube containing 10 mL sterile maintenance medium (0.9% NSS + 0.1% peptone) (Vaidya et al. 2007). Moistened swabs were rubbed for 30 s on l0 × 10 cm area of table, plate, and cloth individually, while an entire surface area of hand (palm) was covered for hand swab samples. The individual swab then transferred to respective tubes containing maintenance medium. Approximately 50–100 g of each food sample was collected separately in a sterile polythene bag. All the samples were then transported in a cold chain to Food Borne Infection Laboratory, Division of Veterinary Public Health, IVRI and processed as soon as possible or kept at 4 °C till further processing.

Isolation and phenotypic identification of ESBL producing *E. coli* isolates

All the samples were processed for isolation and identification of *E. coli* on MacConkey and Eosin Methylene Blue agar followed by Gram staining and biochemical characterization (Indole, MR-VP and Citrate Utilization test) by

standard procedures. For initial screening of ESBL production, all the confirmed *E. coli* isolates were streaked on to the MacConkey agar plate supplemented with 2 mg/L cefotaxime (Costa et al. 2009). Isolates showing growth on these plates were then screened for susceptibility to 3rd and 4th generation cephalosporins and monobactam by disc diffusion assay using commercially available discs (BD BBL™, USA) of cefpodoxime (CPD 10 µg), ceftazidime (CAZ 30 µg), cefotaxime (CTX 30 µg), cefepime (FEP 30 µg) and aztreonam (ATZ 30 µg). Control strains such as *E. coli* (ATCC 25922) and *K. pneumoniae* (ATCC 700603) were used during ESBL detection. The isolates exhibiting reduced susceptibility to any of these antibiotics were further subjected to combination disc method and ESBL E-test (CLSI 2015; Kar et al. 2015).

Phenotypic detection of antimicrobial resistance

All the phenotypically confirmed ESBL producing *E. coli* isolates were screened for different antibiotics by disc diffusion assay. Commercially available antibiotic discs (BD BBL™,USA) viz., ampicillin (10 µg), amoxicillin–clavulanic acid (AMC 20/10 µg), carbenicillin (CB 100 µg), cefoxitin (FOX 30 µg), ceftriaxone (CRO 30 µg), chloramphenicol (C 30 µg), ciprofloxacin (CIP 5 µg), doxycycline (D 30 µg), gentamicin (GM 10 µg), kanamycin (K 30 µg), nalidixic acid (NA 30 µg), sulfadiazine (SD 250 µg), sulfamethoxazole and trimethoprim (SXT 23.75/1.25 µg) and tetracycline (TE 30 µg) were tested against these isolates and results were expressed as sensitive, intermediately resistant or resistant (CLSI 2015).

Detection of antimicrobial resistance and putative virulence genes by PCR

All the phenotypically confirmed isolates were subjected to PCR for amplification of antimicrobial resistance and virulence genes. The DNA was extracted from each *E. coli* isolate by standard heat lysis protocol and analyzed for the presence of major beta-lactamase genes such as bla_{TEM}, $bla_{\text{CTX-M}}$, bla_{SHV}, $bla_{\text{OXA-1}}$ and bla_{ampC}; plasmid-mediated quinolone resistance (PMQR) determinants such as *qnr*A, *qnr*B, and *qnr*S; class I integron [*int*1]; sulphonamide resistance [*sul*1 and *sul*2]; tetracycline resistance [*tet*(A) and *tet*(B)]; chloramphenicol resistance (*catI* and -*floR*); trimethoprim resistance (*dhfrI*) and gentamicin resistance [*aac(3)-IIa*(*aacC2*) and *aph(3)-Ia(aphA1)*] genes. Multiplex PCR was performed for determination of virulence genes of Shiga toxin producing *E. coli*/enterohemorrhagic *E. coli* (STEC/EHEC; *stx 1, stx 2*), enteropathogenic *E. coli* (EPEC: *eaeA*, *bfp*) and enterotoxigenic *E. coli* (ETEC: *lt, stII*) while uniplex PCR for hemolysin (*hlyA*) and enteroaggregative *E. coli* (EAEC: *aggR*).

The uniplex and multiplex PCR assays were performed in a reaction mixture comprising 2.5 µL of 10X *Taq* buffer with 25 mM $MgCl_2$, 2.5 µL of 2 mM each dNTP, 10 pmol each primer, 1 U of dream *Taq* polymerase (Thermo Scientific, USA), 2 µL DNA template and nuclease-free water to make volume up to 25 µL. The details of primer used, cycling conditions and expected product size are listed in Table 1. The PCR cycling conditions for all the reactions were set at 94 °C for 5 m, followed by 30 cycles of 94 °C for 30 s, annealing temp. for 30 s and 72 °C for 30 s and a final step at 72 °C for 7 m. The annealing temperature for respective primer pair is presented in Table 1. Agar gel electrophoresis was carried out in 1.5% agarose gel containing 2 µl ethidium bromide (10 mg/mL) in tris-borate buffer and was visualized with a UV transilluminator and documented (UVP, UK). The PCR product of bla_{TEM} and $bla_{\text{CTX-M}}$ genes were sequenced from Eurofins India Ltd. for further confirmation of the results.

Statistical analysis

The data were statistically analyzed using the Statistical Package for Social Sciences (SPSS v20) software package. Data frequencies and cross tabulations were used to summarize descriptive statistics. The chi-square test was performed on the data at a level of significance of 5%. The null hypothesis was that the type of sample among the particular source of sample did not influence the *E. coli* occurrence. The Pearson chi-square value was adopted for denoting significance. $P \leq 0.05$ was assigned as significant.

Phylogenetic analysis of the ESBL producing *E. coli* isolates by ERIC PCR

All the ESBL producing *E. coli* isolates were subjected to enterobacterial repetitive intergenic consensus (ERIC)-PCR as described by Versalovic et al. (1991). Details of primers, cyclical conditions and amplification size are given in Table 1. The PCR reaction mixture was the same as described earlier except 30 pmol of each primer. The amplicons were analyzed on 2% agarose gel and images were analyzed using the Doc-It®LS Image Acquisition Software (UVP, UK). By comparing differences in the banding pattern, phylogenetic relationship among the isolates was established. The similarity was calculated by the dice coefficient using the unweighted pair group method with arithmetic mean (UPGMA) method.

Table 1 Primer sequence used for corresponding gene in PCR assay

SI. No	Gene	Primer	Annealing temperature (°C)	Product size (bp)	References
1	bla_{TEM}	F-ATGAGTATTCAACATTTCCG R-CTGACAGTTACCAATGCTTA	55	867	Bhattacharjee et al. (2007)
2	bla_{CTXM}	F-CAATGTGCAGCACCAAGTAA R-CGCGATATCGTTGGTGGTG	60–65[a]	540	Dutta et al. (2013)
3	bla_{SHV}	F-TCGCCTGTGTATTATCTCCC R-CGCAGATAAATCACCACAATG	52	768	Maynard et al. (2004)
4	bla_{OXA-1}	F-GCAGCGCCAGTGCATCAAC R-CCGCATCAAATGCCATAAGTG	60	198	Maynard et al. (2004)
5	bla_{AmpC}	F-CCCCGCTTATAGAGCAACAA R-TCAATGGTCGACTTCACACC	57	631	Shahid et al. (2012)
6	*sul*1	F-CGGCGTGGGCTACCTGAACG R-GCCGATCGCGTGAAGTTCCG	65	433	Kerrn et al. (2002)
7	*sulI*2	F-CGGCATCGTCAACATAACC R-GTGTGCGGATGAAGTCAG	60	722	Maynard et al. (2004)
8	*qnrA*	F-ATT TCTCACGCCAGGATTTG R-GATCGGCAAAGGTTAGGTCA	56	516	Ciesielczuk et al. (2013)
9	*qnrB*	F-GATCGTGAAAGCCAGAAAGG R-ATGAGCAACGATGCCTGGTA	56	476	Ciesielczuk et al. (2013)
10	*qnrS*	F-GCAAGTTCATTGAACAGGGT R-TCTAAACCGTCGAGTTCGGCG	56	428	Ciesielczuk et al. (2013)
11	*tet*(A)	F-GTGAAACCCAACATACCCC R-GAAGGCAAGCAGGATGTAG	60	888	Maynard et al. (2004)
12	*tet*(B)	F-CCTTATCATGCCAGTCTTGC R-ACTGCCGTTTTTTCGCC	60	774	Maynard et al. (2004)
13	*catI*	F-AGTTGCTCAATGTACCTATAACC R-TTGTAATTCATTAAGCATTCTGCC	60	547	Maynard et al. (2004)
14	*floR*	F-CGCCGTCATTCCTCACCTTC R-GATCACGGGCCACGCTGTGTC	60	215	Maynard et al. (2004)
15	*dhfrI*	F-AAGAATGGAGTTATCGGGAATG R-GGGTAAAAACTGGCCTAAAATTG	60	391	Maynard et al. (2004)
16	*aac(3)-IIa* (*aacC2*)	F-CGGAAGGCAATAACGGAG R-TCGAACAGGTAGCACTGAG	60	740	Maynard et al. (2004)
17	*aph(3)-Ia(aphA1)*	F-ATGGGCTCGCGATAATGTC R-CTCACCGAGGCAGTTCCAT	60	600	Maynard et al. (2004)
18	*Int*1	F-GGTCAAGGATCTGGATTTCG R-ACATGCGTGTAAATCATCGTC	60	481	Levesque et al. (1995)
19	ERIC	F-ATGTAAGCTCCTGGGGATTCAC R-AAGTAAGTGACTGGGGTGAGCG	50	104–1750	Versalovic et al. (1991)
20	*stx*1	F-CAGTTAATGTGGTGGCGAAGG R-CACCAGACAATGTAACCGCTG	62	348	Cebula et al. (1995)
21	*stx*2	F-ATCCTATTCCCGGGAGTTTACG R-GCGTCATCGTATACACAGGAGC	62	584	Cebula et al. (1995)
22	*eae*	F-TCAATGCAGTTCCGTTATCAGTT R-GTAAAGTCCGTTACCCCAACCTG	62	482	Vidal et al. (2004)
23	*bfp*	F-GGAAGTCAAATTCATGGGGGTAT R-GGAATCAGACGCAGACTGGTAGT	62	254	Vidal et al. (2004)
24	*lt*	F-GCACACGGAGCTCCTCAGTC	62	218	Vidal et al. (2004)

Table 1 continued

SI. No	Gene	Primer	Annealing temperature (°C)	Product size (bp)	References
		R-TCCTTCATCCTTTCAATGGCTTT			
25	*stII*	F-AAAGGAGAGCTTCGTCACATTTT	62	129	Vidal et al. (2004)
		R-AATGTCCGTCTTGCGTTAGGAC			
26	*hlyA*	F-AGCTGCAAGTGCGGGTCTG	62	569	Wang et al. (2002)
		R-TACGGGTTATGCCTGCAAGTTCAC			
27	*aggR*	F-GTATACACAAAAGAAGGAAGC	52	254	Ratchtrachenchai et al. (1997)
		R-ACAGAATCGTCAGCATCAGC			

[a]95°C × 5 m/95 °C × 30 s—65 °C × 30 s—72 °C × 30 s (5 cycles) 95 °C × 30 s—62 °C × 30 s—72 °C × 30 s (10 cycles)/95 °C × 30 s—60 °C × 30 s—72 °C × 30 s (15 cycles)/95 °C × 30 s—58 °C × 30 s—72 °C × 30 s (15 cycles)/72 °C × 7 m

Results and discussion

Presence of ESBL producing *E. coli* in street-vended foods and associated environment

Several researchers indicated the presence of ESBL producers in foods of animal origin such as chicken meat and raw milk (Ghatak et al. 2013; Bhoomika et al. 2016; Senthil Murugan et al. 2019), which calls for a focus on their occurrence and dissemination in food-producing animals. Unfortunately, this knowledge is inadequate from India. Therefore, for determining the AMR and virulence genes in *E. coli* isolates, a total of 430 street-vended foods of animal origin and associated environmental samples were processed for isolation of *E. coli* where 213 (49.53%) samples were found positive/contaminated based on cultural, morphological and biochemical characterization (Table 2). Of these 213 isolates, 42 (19.71%) were ESBL producers based on the results of a combination disc method and ESBL E test. Noticeably, more than 40% food samples from different parts of India were found to be contaminated with *E. coli* wherein > 10% isolates were ESBL producers (Bhoomika et al. 2016). However, < 10% ESBL producing isolates of *E. coli* has also been reported earlier (Abayneh et al. 2018). A higher prevalence of 73.58% ESBL producers were observed in human clinical samples in North East India (Bora et al. 2014).

In the present study, high presence of *E. coli* was observed in raw food samples (60.62%), followed by environmental samples (46.73%) and ready to eat foods (42.99%), of which, 31(26.49%), 3(6.97%) and 7(15.21%) samples harbored ESBL producers, respectively (Table 2). Fortunately, only one cooked food sample harbored ESBL producing *E. coli*. Presence of ESBL producing *E. coli* in ready-to-eat foods and cooked foods is of public health significance. It has been proven from the earlier studies that the foods are a transmission vector for ESBL producing bacteria, probably from reservoirs, food animals and food handlers and once infected it can cause an outbreak (Lavilla et al. 2008; O'Connor et al. 2017). Among different food vendors, higher contamination of *E. coli* was observed in samples collected from meat vendors (54.36%), followed by milk vendors (48.88%) and egg vendors (45.20%) with ESBL producing *E. coli* showing a similar trend with 16.1%, 11.11%, and 2.05%, respectively. On comparison of samples collected from two cities, it was noted that higher occurrence of ESBL producing *E. coli* in samples from Delhi (17.54%) than Bareilly (6.96%). Usually, raw foods and environment play an important role in the contamination of a final food product and the same was observed in the present study also. Bhoomika et al. (2016) also noted the higher occurrence of *E. coli* in raw milk samples (81.11%), followed by chicken meat (66.32%) and chevon meat (46.34%), while Sharma and Bist (2010) reported 75% prevalence in chevon, pork and poultry meat from Mathura city of India.

Phenotypic and PCR based detection of antimicrobial resistance and putative virulence genes

Antibiotic resistance in *E. coli* is of particular concern because of its diverse origin, common inhabitance of animal and human intestine, a frequent cause of urinary tract infections, diarrhea and other infections, and ease with which the organism acquires and transfers antibiotic resistance determinants not only within strains of *E. coli* but also with other species of bacteria (Osterblad et al. 2000). While analyzing the drug resistance profile of 42 ESBL isolates of the present study, it was of concern to

Table 2 Details of ESBL producing *E. coli* isolated from different samples collected from Delhi and Bareilly

Description of samples	Type of samples	Delhi					Bareilly				
		No. of samples analyzed	No. of *E. coli* isolated	*P* value	No. of ESBL *E. coli* isolated	*P* value	No. of samples analyzed	No. of *E. coli* isolated	*P* value	No. of ESBL *E. coli* isolated	*P* value
Environmental	HS	5	1	< 0.001	0	–	17	9	0.174	1	0.174
	TS	1	0		0		16	7		1	
	CS	0	0		0		16	11		0	
	PS	3	1		0		18	5		1	
	Water	0	0		0		16	9		0	
	Sub-total	9	2 (22.22%)		0		83	41 (49.39%)		3 (3.61%)	
Raw foods	Raw chicken	14	13	< 0.001	10	< 0.001	25	19	0.049	4	0.021
	Raw egg	17	10		0		16	8		0	
	Raw milk	10	5		0		39	24		10	
	Paneer	21	17		5		26	9		0	
	Raw fish	0	0		0		10	6		1	
	Raw kabab	0	0		0		15	6		1	
	Sub-total	62	45 (72.58%)		15 (24.19%)		131	72 (54.96%)		16 (12.21%)	
Ready to eat foods	Lassi	2	1	< 0.001	0	< 0.001	2	0	0.228	0	0.928
	Rasmalai	0	0		0		5	0		0	
	Burfi	0	0		0		5	2		0	
	Pedha	2	1		0		3	0		0	
	Curd	4	2		0		3	0		0	
	Rasgulla	2	2		0		2	0		0	
	Salad	14	9		3		28	13		1	
	Chutney	7	4		1		20	9		2	
	Masala	0	0		0		8	3		0	
	Sub-total	31	19 (61.29%)		4 (22.58%)		76	27 (35.52%)		3 (3.94%)	
Cooked foods	Chicken gravy	1	0	< 0.001	0	< 0.001	1	0	0.031	0	–
	Omelette	3	0		0		7	0		0	
	Cooked fish	1	0		0		0	0		0	
	Boiled egg	4	1		1		6	0		0	
	Boiled milk	3	2		0		4	0		0	
	Cooked kabab	0	0		0		8	4		0	
	Sub-total	12	3 (25%)		1 (8.33%)		26	4 (15.38%)		0	
Total		114	69 (60.52%)		20 (17.54%)		316	144 (45.56%)		22 (6.96%)	

HS hand swab, *TS* table swab, *CS* cloth swab, *PS* plate swab

*Significant at 5% level of significance

note that, 36 (85.71%) isolates were resistant to ≥ 3 class of antimicrobials indicating multi-drug resistant (MDR) *E. coli*. Further, these isolates were absolutely resistant to ceftriaxone, cefepime, cefotaxime, ceftazidime, aztreonam, ampicillin and carbenicillin. The details of the phenotypic and genotypic antimicrobial resistance and virulence profile of ESBL producing *E. coli* isolates are described in Table 3. It is alarming to note that, the *E. coli* isolates of the previous study showed complete sensitivity against sparfloxacin and ciprofloxacin (100%) followed by nitrofurantoin (87.5%, 92.00%), chloramphenicol (87.5%, 88.00%) and tetracycline (100%, 80.00%) (Dubal et al. 2009). However, the present study isolates showed resistance against other antibiotics as well, like sulfadiazine (97.61%), cefpodoxime (95.25%), nalidixic acid (80.95%), tetracycline (78.57%), ciprofloxacin (57.14%), sulphamethoxazole and trimethoprim (76.19%), doxycycline (69.04%), kanamycin (40.47%), chloramphenicol (21.42%), amoxicillin–clavulanic acid (19.04%) and gentamicin (14.28%). However, all the isolates were sensitive to cefoxitin (Table 3). Similar findings were also noted earlier (Kar et al. 2015; Abayneh et al. 2018).

Screening for the presence of ESBL genes revealed the predominance of a bla_{CTXM} gene in 69.04% (29/42) ESBL isolates, followed by bla_{TEM} gene in 66.66% (28/42) and $bla_{\text{OXA-1}}$ gene in 19.04% (8/42) isolates, while bla_{SHV} was not detected in any of the isolate (Table 3). Besides these genes, other AMR genes such as bla_{ampC} was detected in 39 (92.85%) ESBL isolates followed by sulfonamide-resistant *sul*1 and *sul*2 genes in 28 (66.66%) and 29 (69.04%) isolates, respectively. In a study conducted by Tekiner and Ozpinar (2016), a total of 250 food samples of animal origin were screened for ESBL-producing *Enterobacteriaceae* in which 55 were phenotypically positive while in a genotypic analysis, 53 isolates showed the presence of bla_{TEM}, 36 for bla_{CTXM}, and 19 for bla_{SHV} genes. The sequence obtained for bla_{TEM} genes (KX465109, KX465110, and KX465114) and $bla_{\text{CTX-M}}$ genes (KX465112, KX465113, and KX465114) were submitted to GenBank. Overdevest et al. (2011) also reported a lower percentage by genotypic tests i.e. 30.2% as against 42.74% by phenotypic tests. They also stated that, the prevalence of ESBL genes differed among the 4 meat groups: 71 (79.8%) in chicken, 4 (4.7%) in beef, 1 (1.8%) in pork, 2 (9.1%) in mixed or ground meat and 1 (11.1%) in other types of meat where $bla_{\text{CTX-M-1}}$ (58.1%) was the most common genotype in chicken meat followed by $bla_{\text{TEM-52}}$ (14%) and $bla_{\text{SHV-12}}$ (14%) and $bla_{\text{CTX-M-1}}$ (62.5% of ESBL) in other types of meat. On the other hand, Kar et al. (2015) also revealed bla_{SHV}, bla_{CTXM} and bla_{TEM} genes in 17, 13 and 9 out of 18 ESBL *E. coli* isolates besides 9 isolates carried sulfonamide resistance gene (*sul1*) and 2 carried the bla_{AmpC} gene.

In addition to the presence of beta-lactamase genes, the isolates of the study also carried other AMR genes, namely trimethoprim resistance gene (*dhfrI*) in 17 (40.47%) isolates, aminoglycoside resistance genes *aac(3)-IIa (aacC2)* and *aph(3)-Ia(aphA1)* in 11 (26.19%) and 6 (14.28%) isolates; tetracycline resistance genes *tet*(A) and *tet*(B) in 3 (7.14%) and 9 (21.42%) isolates; chloramphenicol resistance gene *catI* in 5 (11.90%); plasmid-mediated quinolones resistance (PMQR) genes (*qnrB* and *qnrS*) in 6 (14.28%) and 33 (78.57%) isolates, respectively (Table 3; Fig. 1). However, none of the isolates harbored genes responsible for chloramphenicol (*floR*) and plasmid-mediated quinolone resistance (PMQR) (*qnrA*) with the exception of *Int*1 gene in 21 (50%) isolates (Table 3). Contrast to our findings, Ahmed et al. (2010) reported *dfr1, dfr17*, *dfr12* and *dfr9* in 40.3%, 28%, 17.3% and 0.3% isolates with an overall of 93% (260/279). They also detected tetracycline resistant genes in 86.8% (172/198) [*tet*(A) (18%) and *tet*(A + B) (11%)] and chloramphenicol resistant *catI* gene in 73.5% (75/102) isolates. Mobile genetic elements such as transposons, plasmids, and class 1 integrons play a vital role in dissemination of multiple antibiotic resistances as well as virulence markers (Singh et al. 2005). ESBLs genes are usually located on large plasmids, and these also carry other AMR genes (Rawat and Nair 2010).

In the present study, only five isolates were found to be carrying *eae* gene (11.90%) (atypical EPEC); nine carrying *lt* gene (21.42%) (atypical ETEC), while none carrying *bfp* or *stII* genes. It is interesting to note that, the isolates carrying antimicrobial genes possess fewer virulence characteristics, which may probably be due to acquiring of AMR genes in place of virulence genes in these isolates; an observation that needs to be further investigated and elaborated in future studies. Similar findings have been reported by other researchers in the past. Franz et al. (2015) reported 17.1% pathogenic variants from 170 ESBL-producing *E. coli* isolated from wastewater and surface water where only 8.3% constituted potential gastrointestinal pathogens (4.1% EAEC, 1.8% EPEC, 1.2% EIEC, 1.2% ETEC and no STEC). In another study, 7.2% (8/111) and 0.7% (1/141) of ETEC and EAEC isolates showed resistance to ceftriaxone and cefotaxime, respectively (Kim et al. 2014) and *astA*, *tsh* and *iucD* genes were present in 4, 3 and 3 isolates, respectively among 18 ESBL producing *E. coli* isolates and none carried characteristic virulence genes of STEC (*stx1, stx2*), EPEC (*eaeA*) or *hlyA* (Kar et al. 2015). To know the virulence profile in non ESBL *E. coli* isolates, we also screened remaining 171 isolates for detection of virulence gene by PCR wherein 1.75% (3/171), 2.92% (5/171), 1.75% (3/171) and 0.58% (1/171) isolates content *stx1, eae, lt*, *aggR* genes, respectively.

Table 3 Antimicrobial resistance and virulence profile of *E. coli* isolated from street vended foods of animal origin and associated environment

Isolate code	Type of sample	Type of vendor	Combination disc method	ESBL E-test	Antimicrobial resistance gene profile by PCR	Virulence gene profile by PCR	Class 1 integron	Antimicrobial resistance profile by disc diffusion assay					
								ATM	CPD	CTX	CAZ	AMC	CRO
DE2	RC	MEV	+	+	bla_{TEM},bla_{CTX-M},bla_{AmpC},*qnrS*,*sul*1,*sul*2	–	*int*1	R	R	R	R	I	R
DE3	RC	MEV	+	+	bla_{CTX-M},bla_{OXA-1},bla_{AmpC},*qnrS*,*tet*(B), *dhfrI*,*sul*1,*sul*2	–	*int*1	R	R	R	R	I	R
DE4	RC	MEV	+	+	bla_{TEM},bla_{CTX-M},bla_{AmpC},*qnrS*,*sul*1,*sul*2	*lt*	–	R	R	R	R	I	R
DE5	RC	MEV	+	+	bla_{TEM},bla_{CTX-M},bla_{AmpC},*qnrS*,*sul*1,*sul*2, *aph(3)-Ia(aphA1)*	*lt*	*int*1	R	R	R	R	I	R
DE6	RC	MEV	+	+	bla_{TEM},bla_{CTX-M},bla_{AmpC},*qnrS*,*sul*1,*sul*2, *dhfrI*	*lt*	*int*1	R	R	R	R	I	R
DE7	RC	MEV	+	+	bla_{TEM},bla_{CTX-M}, bla_{OXA-1},bla_{AmpC},*qnrS*, *tet*(B),*catI*, *sul*1,*sul*2,*aph(3)-Ia(aphA1)*	–	*int*1	R	R	R	R	R	R
DE8	RC	MEV	+	+	bla_{TEM},bla_{OXA-1},bla_{AmpC}, *qnrS*,*tet*(B),*catI*, *sul*2	–	*int*1	R	R	R	R	I	R
DE10	RC	MEV	+	+	bla_{TEM},bla_{CTX-M},bla_{OXA-1},bla_{AmpC},*qnrS*, *tet*(B),*dhfrI*,*sul*1,*sul*2,*aac(3)-IIa* (*aacC2*)	*lt*	*int*1	R	R	R	R	I	R
DE11	RC	MEV	+	+	bla_{TEM},bla_{AmpC},*qnrS*,*catI*,*sul*1,*sul*2	*lt*	*int*1	R	R	R	R	I	R
DE13	RC	MEV	+	+	bla_{CTX-M},bla_{AmpC},*catI*,*sul*1,*sul*2	*lt*	–	R	R	R	R	I	R
DE34	C	MEV	+	+	bla_{CTX-M},bla_{AmpC},*qnrS*, *sul*1	–	–	R	R	R	R	I	R
DE35	BE	EV	+	+	bla_{TEM},bla_{AmpC},*qnrB*,*qnrS*,*sul*1,*aac(3)-IIa*(*aacC2*)	–	*int*1	R	R	R	R	I	R
DE36	S	EV	+	+	bla_{CTX-M},bla_{AmpC},*qnrB*,*qnrS*,*aac(3)-IIa* (*aacC2*),*aph(3)-Ia(aphA1)*	–	*int*1	R	R	R	R	I	R
DE37	S	MEV	+	+	bla_{TEM},bla_{CTX-M},bla_{AmpC},*qnrS*	–	–	R	I	R	R	R	R
DE38	S	MEV	+	+	bla_{TEM},bla_{AmpC},*qnrB*,*qnrS*,*tet*(B),*aac(3)-IIa* (*aac C2*),*sul*2		*int*1	R	R	R	R	I	R
DE52	P	MLV	+	+	bla_{TEM},bla_{AmpC},*qnrS*,*catI*,*dhfrI*,*aph(3)-Ia(aph A1)*,*sul*2	–	*int*1	R	R	R	R	I	R
DE53	P	MLV	+	+	bla_{CTX-M}, bla_{AmpC},*qnrS*, *sul*1	–	–	R	R	R	R	R	R
DE54	P	MLV	+	+	bla_{TEM},bla_{CTX-M},bla_{AmpC},*qnrS*,*tet*(B), *aac(3)-IIa*(*aacC2*),*sul*1	–	–	R	R	R	R	R	R
DE55	P	MLV	+	+	bla_{AmpC},*qnrS*,*dhfrI*,*sul*1,*sul*2	–	*int*1	R	R	R	R	R	R
DE58	P	MLV	+	+	bla_{CTX-M},bla_{AmpC},*qnrS*,*sul*1,*sul*2	–	*int*1	R	R	R	R	I	R

Table 3 continued

Isolate code	Type of sample	Type of vendor	Combination disc method	ESBL E-test	Antimicrobial resistance gene profile by PCR	Virulence gene profile by PCR	Class 1 integron	Antimicrobial resistance profile by disc diffusion assay					
								ATM	CPD	CTX	CAZ	AMC	CRO
DE76	C	MEV	+	+	*bla*$_{TEM}$,*bla*$_{AmpC}$, *qnrS, sul*1, *sul*2	–	–	R	R	R	R	I	R
DE78	C	EV	+	+	*bla*$_{TEM}$,*bla*$_{CTX-M}$, *bla*$_{OXA-1}$,*bla*$_{AmpC}$,*tet*(A), *dhfrI,sul*1, *sul*2,*aac(3)-IIa* (*aacC2*)	–	*int*1	R	R	R	R	I	R
DE80	TS	MEV	+	+	*bla*$_{TEM}$,*bla*$_{CTX-M}$,*bla*$_{AmpC}$,*qnrB,qnrS,dhfrI, sul*2	*eae*	–	R	R	R	R	I	R
DE83	RF	MEV	+	+	*bla*$_{TEM}$,*bla*$_{CTX-M}$,*bla*$_{AmpC}$,*qnrS,dhfrI, sul*1,*sul*2	–	*int*1	R	R	R	R	I	R
DE85	RC	MEV	+	+	*bla*$_{CTX-M}$, *bla*$_{OXA-1}$,*bla*$_{AmpC}$,*qnrB,dhfrI, aph(3)-Ia (aphA1),sul*1,*sul*2	–	–	R	I	R	R	R	R
DE87	RC	MEV	+	+	*bla*$_{CTX-M}$,*bla*$_{AmpC}$,*qnrS,dhfrI,tet*(B),*sul*2	*eae*	*int*1	R	R	R	R	R	R
DE88	RM	MLV	+	+	*bla*$_{AmpC}$	–	–	R	R	R	R	I	R
DE90	RM	MLV	+	+	*bla*$_{CTX-M}$,*bla*$_{AmpC}$,*qnrS,tet*(B),*dhfrI,sul*1	–	*int*1	R	R	R	R	I	R
DE99	RM	MLV	+	+	*bla*$_{TEM}$,*bla*$_{CTX-M}$,*bla*$_{AmpC}$,*sul*1	*eae, lt*	–	R	R	R	R	I	R
DE100	RM	MLV	+	+	*bla*$_{CTX-M}$,*bla*$_{AmpC}$,*qnrS,dhfrI,aac(3)-IIa* (*aacC2*),*sul*2	–	–	R	R	R	R	I	R
DE101	RM	MLV	+	+	*bla*$_{TEM}$,*bla*$_{CTX-M}$,*bla*$_{OXA-1}$,*bla*$_{AmpC}$,*qnrS, dhfrI, sul*2	*eae, lt*	–	R	R	R	R	I	R
DE102	RM	MLV	+	+	*bla*$_{TEM}$,*bla*$_{CTX-M}$,*bla*$_{AmpC}$,*qnrS*,*tet*(A),*dhfrI, aph(3)-Ia(aphA1),sul*1,*sul*2	*eae, lt*	–	R	R	R	R	R	R
DE104	RM	MLV	+	+	*bla*$_{TEM}$,*bla*$_{CTX-M}$,*bla*$_{AmpC}$,*qnrS,dhfrI,aac(3)-IIa* (*aacC2*),*sul*2	–	*int*1	R	R	R	R	I	R
DE105	RM	MLV	+	+	*bla*$_{TEM}$,*bla*$_{CTX-M}$, *bla*$_{OXA-1}$,*bla*$_{AmpC}$,*qnrS, tet*(A),*dhfrI,aac(3)-IIa* (*aacC2*), *sul*2	–	*int*1	R	R	R	R	I	R
DE107	RM	MLV	+	+	*bla*$_{TEM}$,*bla*$_{CTX-M}$,*bla*$_{AmpC}$,*qnrB,qnrS,dhfrI, tet*(B),*sul*1,*sul*2	–	*int*1	R	R	R	R	I	R
DE108	RM	MLV	+	+	*bla*$_{TEM}$, *bla*$_{CTX-M}$,*bla*$_{AmpC}$,*qnrS,aac(3)-IIa* (*aacC2*),*sul*1,*sul*2	–	–	R	R	R	R	I	R
DE114	PS	MEV	+	+	*bla*$_{AmpC}$,*qnrS,aac(3)-IIa* (*aacC2*),*sul*1,*sul*2	–	–	R	R	R	R	I	R
DE115	RC	MEV	+	+	*bla*$_{TEM}$,*bla*$_{AmpC}$,*sul*1	–	–	R	R	R	R	I	R
DE116	RK	MEV	+	+	*bla*$_{AmpC}$,*qnrS,sul*1,*sul*2	–	–	R	R	R	R	S	R
DE119	S	MEV	+	+	*bla*$_{TEM}$	–	–	R	R	R	R	I	R
DE120	HS	MEV	+	+	*bla*$_{TEM}$,*sul1*	–	–	R	R	R	R	I	R
DE121	RC	MEV	+	+	*bla*$_{TEM}$, *bla*$_{CTX-M}$	–	–	R	R	R	R	I	R
Total						*lt*-9	21	42	40	42	42	8	42

Table 3 continued

Isolate code	Type of sample	Type of vendor	Combination disc method	ESBL E-test	Antimicrobial resistance gene profile by PCR	Virulence gene profile by PCR	Class 1 integron	Antimicrobial resistance profile by disc diffusion assay					
								ATM	CPD	CTX	CAZ	AMC	CRO
						eae-5							

Isolate code	Antimicrobial resistance profile by disc diffusion assay												
	FEP	FOX	AM	CB	TE	D	G	K	C	SD	SXT	CIP	NA
DE2	R	S	R	R	R	R	S	I	S	R	R	I	R
DE3	R	S	R	R	R	R	S	I	S	R	R	I	R
DE4	R	S	R	R	R	R	S	I	S	R	R	R	R
DE5	R	S	R	R	R	R	S	I	R	R	R	R	R
DE6	R	S	R	R	R	R	S	I	S	R	R	I	R
DE7	R	S	R	R	R	R	S	R	R	R	R	R	R
DE8	R	S	R	R	R	R	S	I	S	R	R	S	R
DE10	R	S	R	R	R	R	S	R	S	R	R	R	R
DE11	R	S	R	R	R	R	S	I	R	R	R	R	R
DE13	R	S	R	R	R	R	S	I	S	R	I	R	R
DE34	R	S	R	R	I	I	I	R	R	R	R	R	I
DE35	R	S	R	R	R	R	R	R	R	R	R	R	R
DE36	R	S	R	R	R	R	R	R	I	R	R	R	R
DE37	R	S	R	R	I	I	I	I	I	R	I	R	I
DE38	R	S	R	R	R	R	R	R	R	R	R	R	R
DE52	R	S	R	R	R	R	S	R	S	R	R	R	R
DE53	R	S	R	R	R	R	R	R	R	R	R	R	R
DE54	R	S	R	R	R	I	R	R	S	R	R	R	I
DE55	R	S	R	R	R	R	I	R	I	R	R	R	R
DE58	R	S	R	R	R	R	I	R	S	R	I	R	R
DE76	R	S	R	R	I	I	I	I	R	I	R	R	R
DE78	R	S	R	R	S	I	S	I	S	R	I	I	I
DE80	R	S	R	R	R	R	I	I	I	R	R	I	R
DE83	R	S	R	R	R	R	I	R	I	R	R	R	R
DE85	R	S	R	R	I	I	S	I	S	R	I	I	R
DE87	R	S	R	R	R	R	I	R	R	R	R	R	R
DE88	R	S	R	R	R	I	I	R	I	R	R	I	I
DE90	R	S	R	R	R	R	I	R	I	R	R	R	R

Table 3 continued

Isolate code	Antimicrobial resistance profile by disc diffusion assay												
	FEP	FOX	AM	CB	TE	D	G	K	C	SD	SXT	CIP	NA
DE99	R	S	R	R	R	I	S	I	S	R	R	I	I
DE100	R	S	R	R	I	S	S	I	S	R	S	I	S
DE101	R	S	R	R	R	R	S	I	S	R	R	R	R
DE102	R	S	R	R	R	R	S	I	S	R	R	I	R
DE104	R	S	R	R	R	R	S	I	S	R	R	R	R
DE105	R	S	R	R	R	I	S	S	S	R	R	I	R
DE107	R	S	R	R	R	R	S	I	S	R	R	R	R
DE108	R	S	R	R	I	R	S	I	S	R	R	I	R
DE114	R	S	R	R	I	I	S	I	I	R	I	I	I
DE115	R	S	R	R	R	R	S	S	S	R	R	I	R
DE116	R	S	R	R	S	S	S	I	S	R	I	S	S
DE119	R	S	R	R	R	R	R	R	I	R	I	I	R
DE120	R	S	R	R	R	R	S	R	S	R	S	R	R
DE121	R	S	R	R	R	I	S	I	S	R	R	I	I
Total	42	0	42	42	33	29	6	17	9	41	32	24	34

RC raw chicken; *C* chutney; *BE* boiled egg; *S* salad; *P* paneer; *TS* table swab; *RF* raw fish; *RM* raw milk; *PS* plate swab; *RK* raw kabab; *HS* hand swab; *MEV* meat vendor; *EV* egg vendor; *MLV* milk vendor; *R* resistant; *I* intermediate resistant; *S* sensitive; *ATM* aztreonam; *CPD* cefpodoxime; *CTX* cefotaxime; *CAZ* ceftazidime; *AMC* amoxycillin-clavulanic acid; *CRO* ceftriaxone; *FEP* cefepim; *FOX* cefoxitin; *AM* ampicillin; *CB* carbenicillin; *TE* tetracycline; *D* doxycycline; *G* gentamicin; *K* kanamycin; *C* chloramphenicol; *SD* sulfadiazine; *SXT* sulphamethoxazole + trimethoprim; *CIP* ciprofloxacin; *NA* nalidixic acid; *MDR* multi drug resistance

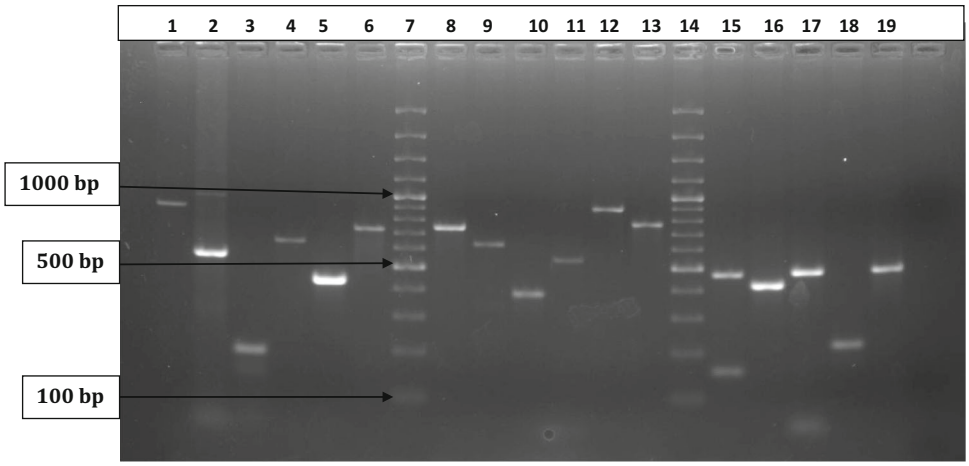

Fig. 1 Agarose gel electrophoresis of PCR products with representative *E. coli* isolates carrying the various tested resistant genes. Lane 1 (DE2): *bla*TEM (867 bp), lane 2 (DE4): *bla*CTXM (540 bp), lane 3 (DE3): *bla*OXA-1 (198 bp), lane 4 (DE3): *bla*ampC (631 bp), lane 5 (DE5): *sul*1 (433 bp), lane 6 (DE6): *sul*2 (722 bp), lane 7, 14: 100 bp plus ladder, lane 8 (DE35): *aac(3)-IIa(aacC2)* (740 bp), lane 9 (DE36): *aph(3′)-Ia(aphA1)* (600 bp), lane 10 (DE10): *dhfrI* (391 bp), lane 11 (DE13): *catI* (547 bp), lane 12 (DE105): *tet*(A) (888 bp), lane 13 (DE87): *tet*(B) (774 bp), lane 15 (DE107): *qnr*B (476 bp), lane 16 (DE8): *qnr*S (428 bp), lane 17 (DE101): *eae* (482 bp), lane 18 (DE102): *lt* (218 bp), lane 19 (DE104): *int*1(481 bp)

Analysis of ERIC PCR

All the 42 ESBL producing strains were characterized by ERIC PCR to determine the genetic diversity and phylogenetic relationship among the isolates. All the isolates were found typable by ERIC PCR and produced amplicons of 104–1750 bp with a total of nine different distinct types/clades. A comparison of the clustering patterns generated nine distinct types/clades with discriminatory power (D value) of 0.82. All the isolates under study formed two main clusters (A and B) with the heterogeneity of 71.2%. The two main clusters further divided into subclusters and formed 6 clades (B, A3, A4, A5, A6, and A7) (Fig. 2). Clade (B) was formed by a single isolate of raw chicken (DE8). Eight isolates of raw chicken (DE2, DE3, DE4, DE5, DE6, DE11, DE87, and DE115) were grouped in clade A4. Two isolates, one isolate from chutney and one from plate swab were grouped in clade A5. In clade B, one isolate of raw chicken (DE8) and in clade A3, one isolate of hand swab (DE120) was grouped. It is interesting to note that, maximum number of isolates, including six from raw milk (DE88, DE90, DE101, DE102, DE104 and DE107); 5 from paneer (DE52, DE53, DE54, DE55 and DE58); three from raw chicken (DE7, DE10, DE13); two each from salad (DE36 and DE119) and chutney (DE34 and DE78); one each from raw kabab (DE116), raw fish (DE83), boiled egg (DE35), hand swab (DE121) and table swab (DE80) were grouped in clade A6. Further, all five isolates from paneer samples were grouped closely in clade A6. In clade A7, four isolates from raw milk (DE99; DE100; DE105 and DE108) and two isolates from salad (DE37 and DE38) were grouped together. These clustering patterns could imply a common source of origin of these isolates.

The similarities between the ERIC profiles among isolates from diverse sources as identified in clades B, A5, A6, and A7 indicates that ERIC PCR fingerprints were effective in differentiating the isolates from various sources. Further, 100% typeability of ERIC PCR reaffirms the fact that this technique is very reliable in genotyping of isolates and hence is a useful tool in food microbiology. ERIC-PCR has been reported as an effective tool in typing *E. coli* isolates from various sources including animals (Wan et al. 2011) and water (Casarez et al. 2007). Oltramari et al. (2014) genotyped 95 *E. coli* isolates from milk and reported high genetic diversity among the isolates. They further reported common clustering of isolates from four milk samples processed in four different dairies, suggesting a single clone contaminant per milk sample and hence a common source of contamination. The relatedness between isolates from different sources reveals that there is a need to follow proper hygiene measures along with good manufacturing practices so as to prevent contamination of the raw materials used in the preparation of street-vended foods. This would reduce the transmission of these pathogens to humans thus reducing the risk of food-borne illnesses.

Conclusion

It is concluded from the present findings that the street-vended foods and their environment may be the potential source of ESBL producing and multidrug resistance *E. coli*

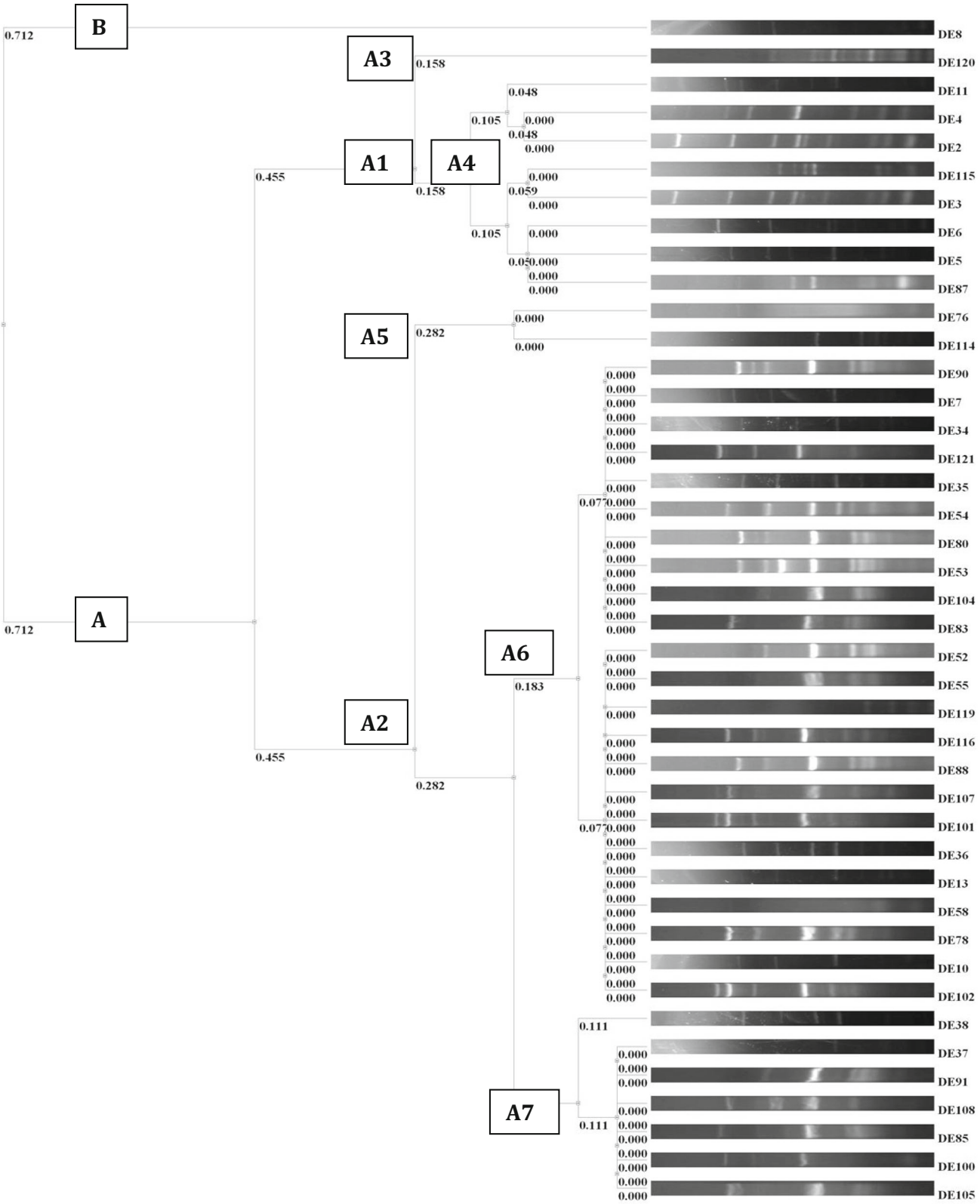

Fig. 2 Phylogenetic analysis of ESBL *E. coli* isolates isolated from street-vended foods of animal origin and associated environment. The unweighted pair group with arithmetic mean method (UPGMA) was used to summarize the similarity of ERIC PCR profile of the strain in a dendrogram

harboring multiple AMR and virulence genes. Raw food (60.62%), environmental samples (46.73%) and ready to eat foods (42.99%) were found to be major source of *E. coli*, of which, 31(26.49%), 3(6.97%) and 7(15.21%) samples harbored ESBL producers, raising food safety and public health concern. However, due to effective cooking practices followed in India, only one cooked sample harboring ESBL producing *E. coli*. Owing to the ease in acquiring and transferring antibiotic resistance determinants and difficulties in treating the patients infected with MDR ESBL producing *E. coli,* a continuous surveillance of street foods and associated environment for foodborne pathogens and their antimicrobial and virulence profiling is necessary.

Acknowledgements The authors are thankful to the Director and Joint Director (Research), Indian Veterinary Research Institute, Izatnagar, India for providing necessary facilities for the study. The work supported by grants from Indian Council of Agricultural Research, New Delhi, India is duly acknowledged.

Compliance with ethical standards

Conflict of interest We do not have any commercial or associative interest that represents a conflict of interest in connection with the research work submitted.

References

Abayneh M, Tesfaw G, Abdissa A (2018) Isolation of extended-spectrum β-lactamase- (ESBL-) producing *Escherichia coli* and *Klebsiella pneumoniae* from patients with community-onset urinary tract infections in Jimma University Specialized Hospital, Southwest Ethiopia. Can J Infect Dis Med Microbiol 2018: 4846159

Ahmed MO, Clegg PD, Williams NJ, Baptiste KE, Bennett M (2010) Antimicrobial resistance in equine fecal *Escherichia coli* isolates from North West England. Ann Clin Microbiol Antimicrob 9:12

Apostolakos I, Mughini-Gras L, Fasolato L, Piccirillo A (2019) Assessing the occurrence and transfer dynamics of ESBL/pAmpC-producing *Escherichia coli* across the broiler production pyramid. PLoS One 14(5):e0217174

Bhattacharjee A, Sen MR, Anupurba S, Prakash P, Nath G (2007) Detection of OXA-2 group extended-spectrum- b-lactamase-producing clinical isolates of *Escherichia coli* from India. J Antimicrob Chemother 60:703–704

Bhoomika SS, Patyal A, Gade NE (2016) Occurrence and characteristics of extended-spectrum β-lactamases producing *Escherichia coli* in foods of animal origin and human clinical samples in Chhattisgarh, India. Vet World 9:996–1000

Birgen BJ, Njue LG, Kaindi DM, Ogutu FO, Owade JO (2020) Determinants of microbial contamination of street-vended chicken products sold in Nairobi county, Kenya. Int J Food Sci. https://doi.org/10.1155/2020/2746492

Bora A, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Ahmed G (2014) Prevalence of bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* from Northeast India. Indian J Pathol Microbiol 57(2):249–254

Campos J, Gil J, Mourao J, Peixe L, Antunes P (2015) Ready-to-eat street-vended food as a potential vehicle of bacterial pathogens and antimicrobial resistance: an exploratory study in Porto region, Portugal. Int J Food Microbiol 206:1–6

Casarez EA, Pillai SD, Di Giovanni GD (2007) Genotype diversity of *Escherichia coli* isolates in natural waters determined by PFGE and ERIC-PCR. Water Res 41:3643–3648

Cebula TA, Payne WL, Feng P (1995) Simultaneous identification of strains of *Escherichia coli* serotype O157:H7 and their Shiga-like toxin type by mismatch amplification mutation assay-multiplex PCR. J Clin Microbiol 33:248–250

Ciesielczuk H, Hornsey M, Choi V, Woodford N, Wareham D (2013) Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. J Med Microbiol 62:1823–1827

CLSI (2015) Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standard Institute, CLSI document, Wayne, pp M100–S124

Costa D, Vinue L, Poeta P, Coelho AC, Matos M, Saenz Y, Torres C (2009) Prevalence of extended spectrum beta-lactamase producing *Escherichia coli* isolates in faecal samples of broilers. Vet Microbiol 138:339–344

Dagher C, Salloum T, Alousi S, Arabaghian H, Araj GF, Tokajian S (2018) Molecular characterization of carbapenem resistant *Escherichia coli* recovered from a tertiary hospital in Lebanon. PLoS ONE 13(9):e0203323

Dubal ZB, Avasthe RK, Haque N, Toppo S, Murugkar HV, Barbuddhe SB (2009) Efficacy of medicinal plant extracts and antimicrobials on different serotypes of *Escherichia coli*. Indian J Ani Sci 79(1):13–16

Dutta TK, Warjri I, Roychoudhury P, Lalzampuia H, Samanta I, Joardar SN, Chandra R (2013) Extended-spectrum β-lactamase producing *Escherichia coli* isolates possessing the shiga toxin gene (stx1) belonging to the O64 serogroup associated with human disease in India. J Clin Microbiol 51(6):2008–2009

Eromo T, Tassew H, Daka D, Kibru G (2016) Bacteriological quality of street foods and antimicrobial resistance of isolates in Hawassa, Ethiopia. Ethiop J Health Sci 26(6):533–542

Franz E, Veenman C, van Hoek AH, de Roda HA, Blaak H (2015) Pathogenic *Escherichia coli* producing extended spectrum β-lactamases isolated from surface water and wastewater. Sci Rep 5:14372

Ghatak S, Singha A, Sen A, Guha C, Ahuja A, Bhattacharjee U, Jana PS (2013) Detection of New Delhi metallo-beta-lactamase and extended-spectrum beta-lactamase genes in *Escherichia coli* isolated from mastitic milk samples. Transbound Emerg Dis 60:385–389

Guenther S, Ewers C, Wieler L (2011) Extended-spectrum beta-lactamases producing *E. coli* in wildlife, yet another form of environmental pollution? Front Microbiol 2:1–13

Kar D, Bandyopadhyay S, Bhattacharyya D, Samanta I, Mahanti A, Nanda PK, Singh RK (2015) Molecular and phylogenetic characterization of multidrug-resistant extended spectrum beta-lactamase producing *Escherichia coli* isolated from poultry and cattle in Odisha, India. Infect Genet Evolut 29:82–90

Kerrn M, Klemmensen T, Frimodt-Moller N, Espersen F (2002) Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. J Antimicrob Chemother 50:513–516

Kharel N, Palni U, Tamang JP (2016) Microbiological assessment of ethnic street foods of the Himalayas. J Ethn Foods 3(3):235–241

Kim JS, Kim J, Kim SJ, Jeon SE, Oh KH, Cho SH, Chung GT (2014) Characterization of CTX-M-type extended-spectrum beta-lactamase producing diarrheagenic *Escherichia coli* isolates in the Republic of Korea during 2008–2011. J Microbiol Biotechnol 24:421–426

Lavilla S, GonzaÌez-López JJ, Miro E, Domínguez A, Llagostera M, Bartolome RM, Mirelis B, Navarro F, Prats G (2008) Dissemination of extended-spectrum b-lactamase-producing bacteria: the food-borne outbreak lesson. J Antimicrob Chemother 61:1244–1251

Levesque C, Piche L, Larose C, Roy PH (1995) PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob Agents Chemother 39:185–191

Maynard C, Bekal S, Sanschagrin F, Levesque RC, Brousseau R, Masson L, Larivière S, Harel J (2004) Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal *Escherichia coli* isolates of animal and human origin. J Clin Microbiol 42:5444–5452

Noor R (2019) Insight to foodborne diseases: proposed models for infections and intoxications. Biomed Biotechnol Res J 3:135–139

Oberoi L, Singh N, Sharma P, Aggarwal A (2013) ESBL, MBL and AmpC beta-lactamases producing superbugs havoc in the intensive care units of Punjab India. J Clin Diag Res 7(1):70–73

O'Connor C, Philip RK, Kelleher J et al (2017) The first occurrence of a CTX-M ESBL-producing *Escherichia coli* outbreak mediated by mother to neonate transmission in an Irish neonatal intensive care unit. BMC Infect Dis 17(1):16

Odonkor ST, Addo KK (2019) Prevalence of multidrug-resistant *Escherichia coli* isolated from drinking water sources. Int J Microbiol. https://doi.org/10.1155/2018/7204013

Oltramari K, Cardoso RF, Patussi EV, Santos ACB, Mikcha JMG (2014) Genetic heterogeneity of *Escherichia coli* isolated from pasteurized milk in State of Paraná, Brazil. Braz J Pharma Sci 50(2):337–343

Osterblad M, Hakanen A, Manninen R, Leistevuo T, Peltonen R, Meurman O, Kotilainen P (2000) A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. Antimicrob Agents Chemo 44:1479–1484

Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Kluytmans J (2011) Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. Emerg Infect Dis 17:1216–1222

Paudyal N, Anihouvi V, Hounhouigan J, Matsheka MI, Sekwati-Monang B, Amoa-Awua W, Asagbra A (2017) Prevalence of foodborne pathogens in food from selected African countries-A meta-analysis. Int J Food Microbiol 249:35–43

Pruthvishree BS, Vinodh Kumar OR, Sinha DK, Malik YPS, Dubal ZB, Desingu PA, Singh BR (2017) Spatial molecular epidemiology of carbapenem-resistant and New Delhi metallo beta-lactamase (blaNDM)-producing *Escherichia coli* in the piglets of organized farms in India. J Appl Microbiol 122(6):1537–1546

Rasheed MU, Thajuddin N, Ahamed P, Teklemariam Z, Jamil K (2014) Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. Rev Inst Med Trop Sao Paulo 56(4):341–346

Ratchtrachenchai OA, Subpasu S, Ito K (1997) Investigation on enteroaggregative Escherichia coli infection by multiplex PCR. Bull Dep Med Sci 39:211–220

Rath S, Dubey D, Sahu MC, Padhy RN (2014) Surveillance of ESBL producing multidrug-resistant *Escherichia coli* in a teaching hospital in India. Asian Pac J Trop Dis 4(2):140–149

Rawat D, Nair D (2010) Extended-spectrum β-lactamases in Gram negative bacteria. J Glob Infect Dis 2(3):263–274

Senthil Murugan MS, Sinha DK, Vinodh Kumar OR, Yadav AK, Pruthvishree BS, Vadhana P, Singh BR (2019) Epidemiology of carbapenem-resistant *Escherichia coli* and first report of blaVIM carbapenemases gene in calves from India. Epidemiol Infect 147:e159

Shahid M, Sobia F, Singh A, Khan HM (2012) Concurrent occurrence of blaampC families and blaCTX-M genogroups and association with mobile genetic elements ISEcp1, IS26, ISCR1, and sul1-type class 1 integrons in *Escherichia coli* and Klebsiella pneumoniae isolates originating from India. J Clin Microbiol 50:1779–1782

Sharma I, Mazumdar JA (2014) Assessment of bacteriological quality of ready to eat food vended in streets of Silchar city, Assam, India. Indian J Med Microbiol 32:169–171

Sharma I, Bist B (2010) Antibiotic resistance in *Escherichia coli* isolated from raw goat, pig and poultry meat in Mathura city of Northern India. Assam Uni J Sci Technol Bio Environ Sci 6(1):89–92

Siddabathuni A (2019) Bacteriological analysis of ready-to-serve foods from a South Indian City: a potential source for drug resistant pathogens. J Microbiol Infect Dis 9(2):83–89

Silva V, Nunes J, Gomes A, Capita R, Alonso-Calleja C, Pereira JE, Torres C, Igrejas G, Poeta P (2019) Detection of antibiotic resistance in *Escherichia coli* strains: can fish commonly used in raw preparations such as sushi and sashimi constitute a public health problem? J Food Prot 82(7):1130–1134

Singh N, Pattnaik D, Neogi DK, Jena J, Mallick B (2016) Prevalence of ESBL in *Escherichia coli* isolates among ICU patients in a tertiary care hospital. J Clin Diag Res 10(9):DC19–DC22

Singh R, Schroeder CM, Meng J, White DG, Mc Dermott PF, Wagner DD, Zhao S (2005) Identification of antimicrobial resistance and class 1 integrons in Shiga toxin-producing *Escherichia coli* recovered from humans and food animals. J Antimicrob Chem 56(1):216–219

Tekiner IH, Ozpinar H (2016) Occurrence and characteristics of extended spectrum beta-lactamases-producing *Enterobacteriaceae* from foods of animal origin. Braz J Microbio l47(2):444–451

Vaidya VM, Paturkar AM, Waskar VS, Zende RJ, Dubal ZB (2007) Analysis of sources of contamination in organized poultry slaughterhouse. Indian J Comp Microbiol Immunol Infect Dis 28:48–52

Versalovic J, Koeuth T, Lupski R (1991) Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acid Res 19(24):6823–6831

Vidal R, Vidal M, Lagos R, Levine M, Prado V (2004) Multiplex PCR for diagnosis of enteric infections associated with diarrheagenic *Escherichia coli*. J Clin Microbiol 42:1787–1789

Wan L, Wang ZB, Yan Q, Wang X, Lei Y, Zuo L, Guo WZ (2011) Genetic diversity of *Escherichia coli* isolated from commercial swine farms revealed by enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) and repetitive extragenic palindrome PCR (REP-PCR). Afr J Biotechnol 10:10543–10550

Wang G, Clark CG, Rodgers FG (2002) Detection in *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O157: H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. J Clin Microbiol 40:3613–3619

WHO (1996) Essential safety requirements for street-vended foods. Revised Edition. Food Safety Unit, Division of Food and Nutrition, World Health Organization, pp 2–3