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# MULTIDRUG-RESISTANT *stx1* HARBORING *ESCHERICHIA COLI* FROM MEAT SHOP AND FAST FOOD

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## ABSTRACT

*Escherichia coli* have public health impact on humans by causing gastroenteritis including hemorrhagic colitis and hemolytic uremic syndrome. A total of 215 samples collected from fast foods, swabs from meat shops and fish markets were examined. Thirty-six samples (16.74%) were identified as *E. coli* with various serogroups like O8, O19, O22, O41, O60, O101, O116 and O147, respectively. Only nine (25%) *E. coli* possessed the gene encoding shiga toxin (*stx* 1). Isolated *E. coli* were multidrug-resistant with complete resistance to fluroquinolones (ciprofloxacin, norfloxacin, ofloxacin, and lomefloxacin respectively). Isolation of *stx* 1 bearing *E. coli* with multidrug resistance from fast food and meat sources is a clear indication of environmental contamination and is a matter of public health concern.

## PRACTICAL APPLICATION

Detection of diarrheogenic serogroups of *stx 1* harboring *Escherichia coli* with multidrug resistance in fast foods and meat shops is applicable in creating awareness among public about the unhygienic practices followed in the fast food centers and retail meat shops and also the improper disposal of fecal and antibiotic wastes, which is the cause for such contaminations. This study also insists the need of health education and hygienic practice for the food handlers.

## INTRODUCTION

Millions of people suffer from preventable foodborne diseases every year. Foodborne diseases are the growing public health problem and imply a great impact worldwide. *Escherichia coli* is an autochthonous member of the gastrointestinal tract of humans and warm-blooded animals with and without genetic determinants for pathogenicity. Pathogenic *E. coli* are responsible for three major types of clinical infections like enteric and diarrheal diseases, urinary tract infections, sepsis and meningitis, respectively (Maheux *et al.* 2009). There are several pathovars (enteropathogenic *E. coli* – EPEC, enterotoxigenic *E. coli* –ETEC, enteroinvasive *E. coli* – EIEC, enterohemorrhagic *E. coli* – EHEC, diffusely adherent *E. coli* – DAEC, enteroaggregative *E. coli* – EAggEC) with certain sets of serogroups and virulence factors causing enteric and diarrheal disease. EHEC is a group of *E. coli* pathovars which has recently gained its significance in foodborne gastroenteritis. EHEC exhibits its pathogenicity by the production of two immunologically noncross-reactive shiga toxin (stx), *stx*1 and *stx*2 (Gavin and Thomson 2004; Bonyadian *et al.* 2010) with or without *eae* gene responsible for attaching and effacement in enterocytes (Mainil and Daube 2005).

EHEC is the most common source of primary or secondary contamination in raw foods of animal origin (Mainil and Daube 2005). Presence of *E. coli* in food and/or water is an indication of fecal contamination and stands as a case of public health concern. The Food Safety and Standards Authority of India has been instituted in 2006 to regulate and monitor the food safety in India to curb food associated health problem. But it has yet to take firm steps to regulate the existing unhygienic practices in food industry (FICCI 2010). Therefore, several diarrheal outbreaks are reported

occasionally (Ramamurthy 2008). However, very few studies have been carried out to investigate microbiological standards of meat based fast food in India (Khan *et al.* 2002; Hazarika *et al.* 2007). During the turn of the millennium and with the influence of globalization, fast food (mostly meat based) is available in majority of Indian metros and suburbs. The quality of such fast food has not been examined adequately.

Beyond identification of *E. coli* in food, serotyping of *E. coli* strains in food and environmental samples is important to understand the epidemiology. To date, EHEC prevalence studies focused only on *E. coli* O157 : H7, because of its initial predominance in human infection. There is little attention to the risk posed by non-O157 serogroups. Centre for Infectious Diseases and Prevention identified that non-O157 groups have been responsible for over 70% of EHEC associated illness (Monaghan *et al.* 2012). From 1982 to 2002, a total of 350 outbreaks were reported from 49 states of U.S.A., accounting for 8,598 cases of *E. coli* O157 infection (Rangel *et al.* 2005). A report on the occurrence of EHEC serotypes in ground beef samples shows the prevalence of EHEC in Egypt and Brazil (Uhitil *et al.* 2001). Outbreaks in Asia (Mitsuhiro 2008), Tanzania (Raji *et al.* 2008) and Germany (Jores *et al.* 2005) including the recent outbreak of a novel strain of *E. coli* O104 : H4 in Northern Germany causing neurological syndrome in adults and outbreak of O157 clone causing hemolytic uremic syndrome in China (Xiong *et al.* 2012) pose the considerable importance of the emerging pathogen in food and food-associated sources.

With reference to the Indian scenario, prevalence of food/waterborne *E. coli* infection were reported in Kolkata (Khan *et al.* 2002; Ramamurthy 2008), Mangalore (Dhanashree and Mallya 2008), Cochin (Hatha *et al.* 2004), Himachal Pradesh (Gupta and Gupta 2009), which indicated the environmental contamination by diarrheagenic *E. coli*. A study on surface water and potable water in Lucknow city encounters the presence of EHEC (Ram *et al.* 2011) and EPEC in Kolkata (Ghosh *et al.* 1991). Vellore, which has tropical wet-and-dry climate, is a developing city in Tamil Nadu, India located 150 km away from the sea shore, has slum areas practicing open defecation and improper sewage channels in few places. Villages near Vellore city attained epidemiological importance because of some diarrheal outbreaks (Kang *et al.* 2001). Reports on pathogenic *E. coli* associated incidence in Vellore city to date described the clinical importance of uropathogenic *E. coli* (Naveen and Mathai 2005) and EAggEC in villages around Vellore (Pai *et al.* 1997). Occurrence of pathogenic *E. coli* in fast foods of retail outlets of Vellore city markets, food handlers and meat stalls has not been studied. The present study has thus been carried out with an objective of examining the presence of pathogenic *E. coli* in fast food including meat based food, water, and raw meat from Vellore city, India.

## MATERIALS AND METHODS

### Collection of Samples

A total of 215 samples were collected for a period of 18 months (May 2008–November 2009) from retail butcher shops and counters in markets and from the fast food centers located throughout Vellore city. Samples were collected randomly from handlers' palms (20), slaughter platforms (20), knives (20), water (20) and fast foods like hot dogs (12), burgers (10), pizzas (5), chicken rolls (30), vegetable rolls (7), salads (5), sandwiches (15) spinach (10), sausages(6) and fish stall samples (15). Swabs were taken from full palm area, full surface area of platform (approximately 900 $cm^2$), full surface area of knife (approximately 120 $cm^2$); 10 mL of washed water and 1.0 g each of hot dog, burger, pizza, chicken roll, vegetable roll, salad, sandwich, spinach, sausage and fish samples were collected in sterile vials and were brought to the laboratory within 1 h using an ice box.

### Microbiological Examination of Samples

Swabs were streaked onto MacConkey agar (HiMedia, Mumbai, India) and incubated at 37C for 24 h. Solid samples were suspended in sterile 0.2 mL of phosphate buffered saline (PBS, 0.2 M, pH 7.2) and 0.1 mL of the suspension was pour plated onto MacConkey agar and was incubated at 37C for 24 h. Water samples were filtered through a 0.45-μ membrane, and filtrates were suspended in sterile 0.2 mL of PBS and 0.1 mL of the suspension was pour plated onto MacConkey agar and was incubated at 37C for 24 h. The lactose-fermenting (LF) colonies (2–5) were selected and inoculated to peptone water (pH 7.2) and were subjected to biochemical tests, (viz., Indole, MR-VP, citrate utilization, triple sugar iron agar, catalase, oxidase). All the LF colonies showing prototype reaction for *E. coli* like, Indole, MR and catalase positive; VP, citrate, oxidase and gram stain negative were selected and subcultured onto eosine methylene blue agar (EMB; Himedia). Motility of the isolate was examined by inoculating into Mannitol Motility Agar and also by Hanging drop method. All the isolates were numbered following the code PV and stored in nutrient agar stabs, slants at 4C and in 20% glycerol-tryptic soy broth at −30C for subsequent works.

### Serotyping of *E. coli*

All the confirmed *E. coli* isolates from 215 different food samples were serogrouped at National *Salmonella* and

**TABLE 1.** PRIMERS USED FOR THE PATHOGENIC CHARACTERIZATION AND VALIDATION OF *ESCHERICHIA COLI*

| Virulence factor coding genes | Primers oligonucleotide sequences | Amplicon size | References |
|---|---|---|---|
| *stx 1* | 5′CAACACTGGATGATCTCAG 3′ 5′CCCCCTCAACTGCTAATA 3′ | 350 bp | Pal *et al*. 1999 |
| *stx 2* | 5′ATCAGTCGTCACTCACTGGT 3′ 5′CTGCTGTCACAGTGACAAA3′ | 110 bp | Pal *et al*. 1999 |
| *eae* | 5′AAACAGGTGAAACTGTTGCC 3′ 5′CTCTGCAGATTAACCCTCTGC3′ | 554 bp | Yu *et al*. 1992 |
| *shrkag* | 5′AACTGGTTACCTGCCGTGAG-3′ 5′TGGTGATGGTGGTGGTAATG-3′ | 110 bp | Rathina Kumar and Ghosh Asit 2012 |

*Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh, India using polyvalent and monovalent *E. coli* antisera. In brief, *E. coli* isolates were harvested and suspended in 0.2 mL PBS (0.1 M phosphate buffer saline, pH 7.2) from subcultured Luria agar. A 10 µL of *E. coli* suspension and 10 µL of polyvalent antisera were mixed and rocked on a grease-free clinical glass slide for 30 s and agglutination was noticed. Following the agglutination with polyvalent antisera, respective monovalent antisera (O) were used accordingly and serogrouped.

## Antimicrobial Susceptibility Test

The *E. coli* isolates were tested for susceptibility to a panel of 10 antimicrobials including major fluroquinolones using reference *E. coli* (ATCC 25922) strain. The assay was done by the disc diffusion method on Muller Hinton Agar (MHA; Hi-Media), following Kirby–Bauer protocol (Bauer *et al*. 1966), using cultures of *E. coli* of exponential phase and was equivalent to McFarland's standard (0.5). The following antibiotic discs (concentration in µg) from Hi-Media were used: Ampicillin (Amp, 10), Chloramphenicol (C, 30), Ciprofloxacin (Cf, 5), Lomefloxacin (Lm, 10), Nalidixic acid (Na, 30), Norfloxacin (Nf, 10), Oflaxacin (Of, 5), Nitrofurantoin (Nit, 300), Streptomycin (S, 300) and Tetracycline (T, 30), respectively. MHA plates were seeded with inoculum and antibiotic discs were placed aseptically. The plates were incubated at 37C overnight and diameter of the zones of inhibition was measured. The isolates were classified as susceptible or resistant on the basis of the standard charts received with the discs from manufacturer.

## Validation of *E. coli* and Detection of Virulence Gene by Polymerase Chain Reaction (PCR)

The isolates were subjected to PCR for the validation of *E. coli* with the designed primer *shrkag* in a simplex PCR, detection of virulence genes namely *stx* 1, *stx* 2 in a multiplex PCR and *eae* in a simplex PCR following the methods described elsewhere (Yu and Kaper 1992; Pal *et al*. 1999; Rathina Kumar and Ghosh Asit 2012). The primers used for the analysis are tabulated with conditions (Tables 1 and 2). Amplified PCR products were separated using 0.8% agarose gel (HiMedia), stained with ethidium bromide (EtBr) and documented using Gel Doc system (Gel Doc System, Vilber Lourmat, megabioprint 1000/26 Mx).

## Salt Aggregation Test (SAT)

The SAT was performed using different molar concentrations of ammonium sulphate (Ljungh and Wadström 1982). *E. coli* isolates were harvested and suspended in 0.2 mL of 0.1 M PBS (pH 7.2) from subcultured Luria agar. A 10 µL of *E. coli* suspension was mixed with equal volume of ammonium sulfate solution of different concentration (0.01 M, 0.05 M, 0.10 M, 0.15 M, and 0.20 M) on a clean glass slide rocked and observed for agglutination after 20 s at room temperature.

## Hemagglutination Assay (HA)

Blood samples from human (groups O+, O-, A+, B+) and bovine was collected in equal volume of Alsever solution (NaCl, 0.42%; Na-citrate, $2H_2O$, 0.8%; citric acid; $H_2O$, 0.055% and D-glucose 2.05%) and centrifuged at 1,000 rpm for 5 min and the pellet was washed with 10 mL of PBS and was resuspended in 5 mL of PBS and stored in the refrigerator (Ghosh *et al*. 1993). The bacterial suspension of 20 µL volume was added to equal volume of the prepared erythrocyte suspension on a clean concave slide and rocked well. The sensitivity of mannose and other sugars like glucose, fructose, lactose, sucrose and starch on hemagglutination was tested by adding 20 µL of 0.5% sugar to 20 µL of erythrocyte suspension before adding the bacterial suspension.

**TABLE 2.** POLYMERASE CHAIN REACTION CONDITIONS USED IN THIS STUDY

| PCR step | *shrkag* | *stx 1* and *stx 2* | *eae* |
|---|---|---|---|
| Preheating | 94C for 30 s | 94C for 5 min | Not required |
| Denaturation | 94C for 1 min | 94C for 1 min | 94C for 1 min |
| Annealing | 60C for 1.5 min | 50C for 1 min | 52C for 1.5 min |
| Extension | 72C for 1.5 min | 72C for 1 min | 72C for 1.5 min |
| Final extension | 72C for 7 min | 72C for 7 min | 72C for 7 min |
| Number of cycles | 30 cycles | 35 cycles | 30 cycle |

### Statistical Analysis

The samples collected and the number of isolates identified as *E. coli* was tested for analysis of variance (ANOVA) single factor with a *P*-value of 0.5 and *F*-test with the *P*-value of 0.05. The values less than the assumed *P*-value are considered to be significant.

## RESULTS

Out of 215 samples from fast foods, meat shops and fish stalls, 36 samples (16.7%) were identified with *E. coli*. Sixteen *E. coli* was isolated (32%) from 50 beef stall samples, two from 50 chicken stall samples (4%), 12 from 100 fast food samples (12%) and six from 15 fish market samples (40%), respectively. Among fast food samples, three *E. coli* from 12 hot dogs, two from 10 burgers, two from six sausages, one from 15 sandwich fillings, four from 30 chicken roll stuffing were isolated, respectively.

Isolates examined with biochemical tests viz. Indole, MR–VP, citrate utilization, triple sugar iron agar, catalase, oxidase, sugars (glucose, fructose, sorbitol, salicin, rhamnose, lactose, galactose, sucrose, arabinose), Gram staining, and motility showed reactions, like indole, MR and catalase positive; VP, citrate, oxidase and gram-stain negative with motility and were identified as *E. coli* and preserved. On EMB agar, isolates showed characteristic metallic sheen. However, four of the isolates namely PV 2, PV9, PV21 and PV25 were found to be nonmotile. All the isolates were validated by PCR analysis using designed primer, *shrkag*, amplicon size 110 bp (Fig. 1).

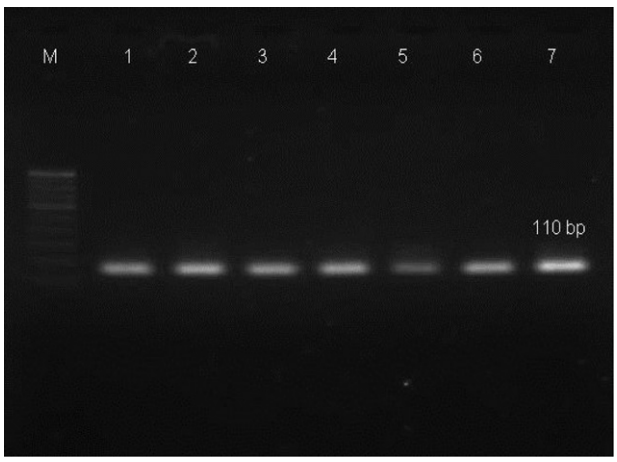


**FIG. 1.** POLYMERASE CHAIN REACTION ANALYSIS OF ISOLATED *ESCHERICHIA COLI* USING DESIGNED PRIMER, *shrkag*
This validates the isolates as *E. coli* with amplicon size 110 bp. M represents molecular weight marker of 1 kb. Lane 1–7 represents strains PV 1, PV 2, PV 3, PV 4, PV 5, PV 6 and PV 7, respectively.

The statistical analysis of 36 *E. coli* isolated from 215 samples subjected to ANOVA single factor analysis with the *P*-value less than 0.5 and *F*-test with *P*-value less than 0.05 revealed that the distribution of *E. coli* in the samples collected was significant. Value obtained from ANOVA single factor (0.12349) and *F*-test (0.009), respectively.

Isolated strains were O serogrouped. The serotyping of 36 *E. coli* revealed that 21 strains were serotypable (58.3%) and were distributed to eight different serogroups (O8, O19, O22, O41, O60, O101, O116, and O147 respectively) (Table 3). The *E. coli* with O19, O41, O60 and O147 were encountered rarely, whereas O116, O22, O101 were moderate. The most dominating serogroup among samples was O8 with seven isolates (19.4%). Six strains of *E. coli* were rough (16.6%) and nine (25%) were untypable (UT). Among contaminated samples, two samples of hotdogs (collected at different time intervals) were with O8; three samples of raw beef with O8, O60 and O116; one sample of sausage and chicken roll each with O22, respectively (Table 3). The result showed notable diversity of strains isolated from variety of samples including fast food (Table 3).

All *E. coli* isolates were multiple antimicrobial resistant. Strains showed resistance from 5.5% against streptomycin to 100% against four major fluroquinolones: ciprofloxacin, lomefloxacin, norfloxacin and ofloxacin, respectively. Isolated *E. coli* showed resistance to nalidixic acid (91.6%), nitrofurantoin (77.7%), tetracycline (72.2%), chloramphenicol (47.2%), and ampicillin (19.46%) respectively. Sixteen (44.4%) were resistant to seven antibiotics used. Isolated *E. coli* showed 15 different cluster of drug resistance with maximum of eight strains (22.2%) showing the pattern, Cf, Lm, Nx, Of, Na, Nit, T; followed by Cf, Lm, Nx, Of, Na, Nit, T, C (13.9%); Cf, Lm, Nx, Of, Na, Nit, C (11.1%) and Cf, Lm, Nx, Of, Na, Nit, T, A, C (8.33%), respectively. The isolated strains appeared to be multiclonal with respect to MDR property although some strains shared same antibiotic-resistance patterns and were isolated from different samples at different time period (Table 3).

The PCR analysis of 36 *E. coli* showed that nine (25%) strains carried the Shiga toxin producing gene, *stx* 1 (Fig. 2; Table 3). None of the isolates possessed for intimin gene, *eae* and Shiga toxin gene-2, *stx* 2. Out of these nine isolates, three belong to O116, two were UT and remaining four belongs to O8, O19, O22 and O60 respectively. The cell surface hydrophobicity as revealed by the SAT was found positive among 17 strains (47.2%) only at the optimum concentration of 0.05 M. Only 6 (PV 2, PV 21, PV 25, PV 32, PV 33, PV 35) were positive for hemagglutination with different groups of human erythrocytes, except bovine erythrocyte, suggesting the adherence property to enterocytes. Also there was no effect on hemagglutination in presence of different sugars like, mannose, glucose, fructose,

**TABLE 3.** DISTRIBUTION OF ISOLATED *stx1* PRODUCING, MDR *E. COLI* FROM MEAT AND MEAT-BASED FAST FOOD

| Sample (source) | Isolate | Resistance pattern | Serogroup | Gene* |
|---|---|---|---|---|
| Raw beef (B) | PV 7 | Cf, Lm, Nx, Of,Na, C, T | Rough | |
| | PV 10 | Cf, Lm, Nx, Of, Na, Nit, T | O8 | |
| | PV 12 | Cf, Lm, Nx, Of, Na, Nit, C | O116 | *stx* 1 |
| | PV 14 | Cf, Lm, Nx, Of, Nit | O60 | *stx* 1 |
| | PV 21 | Cf, Lm, Nx, Of, Na, Nit, T | Untypable | |
| Handler (B) | PV 11 | Cf, Lm, Nx, Of, Na, C | O8 | |
| | PV 17 | Cf, Lm, Nx, Of, Na, Nit, C, T | O116 | |
| | PV 26 | Cf, Lm, Nx, Of, Na, C | O22 | *stx* 1 |
| Knife (B) | PV8 | Cf, Lm, Nx, Of, Na, Nit, T | O8 | |
| | PV9 | Cf, Lm, Nx, Of, Na, Nit, T | Untypable | |
| | PV28 | Cf, Lm, Nx, Of, Na, Nit, C | O19 | *stx* 1 |
| Water (B) | PV 15 | Cf, Lm, Nx, Of, Na, Nit, C T | Rough | |
| | PV 23 | Cf, Lm, Nx, Of, Na, Nit, C, T,Amp | O8 | |
| | PV 24 | Cf, Lm, Nx, Of, Na, Nit, C | O8 | |
| Slaughter platform (B) | PV29 | Cf, Lm, Nx, Of, Na, Nit, C | O116 | *stx* 1 |
| | PV30 | Cf, Lm, Nx, Of, Na, Nit, T | O22 | |
| Raw chicken (C) | PV13 | Cf, Lm, Nx, Of | Untypable | *stx* 1 |
| | PV27 | Cf, Lm, Nx, Of, Na, C | O116 | *stx* 1 |
| Raw fish (F) | PV35 | Cf, Lm, Nx, Of, Na, T, Amp | Rough | |
| Water (F) | PV31 | Cf, Lm, Nx, Of, Na, Nit, C, T, Amp | O41 | |
| Handler (F) | PV32 | Cf, Lm, Nx, Of, Na, Nit, C, T, Amp, S | Untypable | |
| Knife (F) | PV36 | Cf, Lm, Nx, Of, Na, Nit, T, Amp | O147 | |
| | PV33 | Cf, Lm, Nx, Of, Na, Nit, C, T, Amp | O101 | |
| | PV34 | Cf, Lm, Nx, Of, Na, Nit, T, Amp | O101 | |
| Chicken roll (FF) | PV1 | Cf, Lm, Nx, Of, Na, Nit, T | Untypable | *stx* 1 |
| | PV2 | Cf, Lm, Nx, Of, Na, T | Untypable | |
| | PV5 | Cf, Lm, Nx, Of, Na, Nit, C, T | Rough | |
| | PV22 | Cf, Lm, Nx, Of, Na, Nit, T | O22 | |
| Hot dog (FF) | PV3 | Cf, Lm, Nx, Of, Na, Nit, T | Rough | |
| | PV4 | Cf, Lm, Nx, Of, Na, Nit, C, T | O8 | *stx* 1 |
| | PV16 | Cf, Lm, Nx, Of, Na, Nit, C, T | O8 | |
| Veg burger (FF) | PV6 | Cf, Lm, Nx, Of, Na, T | Untypable | |
| | PV18 | Cf, Lm, Nx, Of, Na, Nit, T | Rough | |
| Veg sandwich (FF) | PV19 | Cf, Lm, Nx, Of, Na, Nit, T | Untypable | |
| Sausage (FF) | PV20 | Cf, Lm, Nx, Of, Nit, T | O22 | |
| | PV25 | Cf, Lm, Nx, Of, Nit, Na | Untypable | |

* Genes *stx* 2 and *eae* negative.

B, beef stall; C, chicken stall; F, fish stall; FF, fast food; Amp, ampicillin; C, chloramphenicol; Cf, ciprofloxacin; Lm, lomefloxacin; Na, nalidixic acid; Nf, norfloxacin; Of, oflaxacin; Nit, nitrofurantoin; S, streptomycin; and T, tetracycline.

lactose, sucrose and starch respectively. The *stx* 1 possessing *E. coli* were found distributed among different food samples (Table 3).

## DISCUSSION

The study presents the basic information about the bacteriological quality of raw meat; utensils used at the source of meat preparation and of the processed meat based fast food in Vellore city. As a matter of microbiological convenience and simplicity, fecal contamination was monitored by the demonstration of *E. coli* in samples. Isolation of *E. coli* from fast food is a great threat to public health. With a few limitations, study was carried out with moderate sample size (ANOVA single factor: 0.12349 and *F*-test: 0.009) of various food samples for demonstration of contaminant *E. coli* with drug resistance and virulence properties. It is the first attempt to report the hygienic character of fast food in southern India. India is the second most populous country with diversity in socioeconomy and with the burden of diseases like diarrhea. Open defecation is still a practice in the neighborhood of the sampling area and the market does not maintain the hygienic standard. Hence contamination is an easy access to the raw materials for preparation of meat, to the knives, cutting block surfaces, water used etc. As a result, 16.7% of the samples were found contaminated with *E. coli*. Of the samples, 32% of beef stall samples and 40% of the fish stall samples were contaminated with *E. coli*. The

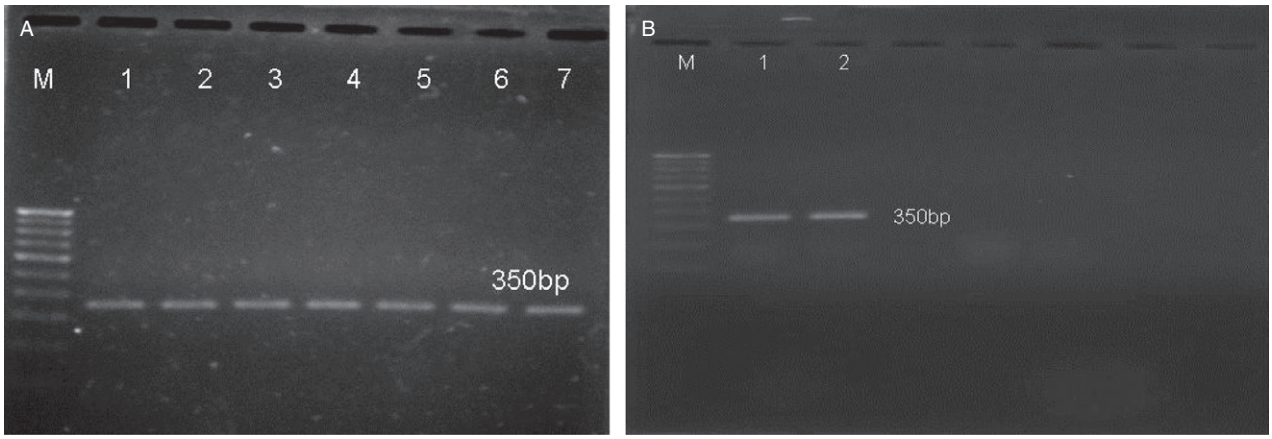


**FIG. 2.** PATHOGENIC CHARACTERIZATION OF ISOLATED *ESCHERICHIA COLI* USING THE PRIMER ENCODING GENE *stx* 1 (AMPLICON 350 BP) (A) Lane 1–7 represents strains PV 1, PV 4, PV 12, PV 13, PV 14, PV 26 and PV 27 respectively. (B) Lane 1 and 2 represents strains PV 28 and PV 29 respectively. M in (A) and (B) represents molecular weight marker of 1 kb.

contamination was as high as 12% among fast food. Thus, the study hints that the hygienic standard of such products are to be quarantined for the benefit of society. The Federation of Indian Chambers of Commerce and Industry survey on challenges in food processing sector (http://www.ficci.com/SEDocument/20073/Food-Processing-Bottlenecks-study.pdf; FICCI 2010) reported the possible bottlenecks in food processing units in India, which were (1) inadequate infrastructural facility; (2) absence of comprehensive national level policy; and (3) lack of trained manpower, etc. Most of the small restaurants cannot maintain the standards of food safety and hygiene. The poor hygienic condition of food may lead to the outbreak in a community. Khan *et al.* (2002), Dhanashree and Mallya (2008) reported pathogenic *E. coli* from beef in India. Similarly, Dambrosio *et al.* (2007) isolated pathogenic strains of *E. coli* from minced beef in Italy; Ryu *et al.* (2012) observed the prevalence of MDR *E. coli* in commercial and cooked foods in Korea; and Castro-Rosas *et al.* (2012) reported from ready-to-eat-salads in Mexico. Hence the problem of *E. coli* contamination is a common feature and is worldwide and an obvious cause of gastroenteritis (Scharlach *et al.* 2012).

Isolated *E. coli* represents different O-serogroups. However, distribution of O-serogroups observed in this study did not symbolize the EHEC causing infection to humans but to cattle. As reported elsewhere, serotypes O8:K5, O8:K25, O101:K28, O101: K30 were isolated from diarrheic calves (Braaten and Myers 1977) and O22, O60 with several other serovars from neonatal diarrheic calves (Tripathi and Soni 1984). Serogroups like O8, O22 and O101 were reported in diarrheic buffalo and cow calves (Minakshi *et al.* 1992); O101 (Hussain *et al.* 2003); and O8, O22, O60, O101, O116 from diarrheic calves (Wani *et al.* 2004). Thus, the serogroups like O8. O22, O60, O101 and O116 isolated in our study are assumed to be of cattle origin while, O19, O41, and O147 are possibly of other origin (Orden *et al.* 1999) including humans (Czirok *et al.* 1976).

The resistance pattern of MDR strains presents a great challenge to the treatment of infections. Non-O157 strains from humans and animals have developed antibiotic resistance and many are resistant to multiple antimicrobials used (Khan *et al.* 2002). In this study, all the isolates were found to be resistant to multiple drugs. Presence of MDR strains in meat-based food samples has been reported in several studies (Dambrosio *et al.* 2007; Ryu *et al.* 2012). Isolation of MDR *E. coli* from fast food and meat sources gives a red alert of fecal contamination. This property enhances the virulence of strains too. Again, use of fluroquinolones to combat *stx*-related gastroenteritis might influence the over production of toxin, as shown in a study (Zhang *et al.* 2000). Emergence of resistant strains is the result of creating selective pressure on bacteria which leads to the acquisition of new genetic material by antimicrobial susceptible bacteria from resistant strains by conjugation, transformation and transduction.

The cell surface hydrophobicity revealed by SAT among 17 isolates is an important observation which reveals the interaction between host and the pathogens contributed by fimbrial appendages and nonfimbrial surface proteins. Hemagglutination assay indicates the interaction of carbohydrate binding proteins present on the fimbriae and the proteins present on the surface of the erythrocytes (Ghosh *et al.* 1993). In this study, six isolates showed sugar resistant hemagglutination and thus possessed the adherence ability to enterocytes and possibly pathogenicity.

The prevalence of non-O157 *E. coli* with *stx* has been reported in many places (Hazarika *et al.* 2007; Ram *et al.* 2011; Matulkova *et al.* 2012; Monaghan *et al.* 2012). Besides

multidrug resistance, isolated non-O157 *E. coli* strains harbored *stx* 1 in the meat-based food and other samples. The importance of detecting such pathotype in food stuff was recently highlighted by the outbreak in Northern Germany (Scharlach *et al*. 2012). Out of 36 *E. coli* positive samples, nine (25%) possessed *stx* 1 gene including two samples from fast food at two different time schedule. *Stx* harboring strains isolated in our study did not have the gene for intimin. Similar observation with *stx* producing *E. coli* without intimin gene was reported elsewhere (Bonyadian *et al*. 2010), suggesting the various display of genetic architecture among the circulating *E. coli* population in different geographic milieu.

In conclusion, *E. coli* of different serogroups in fast food is the clue of fecal contamination of human or/and nonhuman origin. Possession of *stx* gene with MDR property among isolated *E. coli* strains indicates the prevalence of pathogenic *E. coli* in the study area. This also specifies the quality of preparation and prepared food for consumers in Vellore city. This study provides the first-hand evidence that fast food centers and retail meat shops are potentially vulnerable to fecal contamination. There is a need to educate people of all economic strata regarding personal hygiene, food safety, proper cooking practices and delivery. Further study on controlling such contamination may help in solving the problem of food hygiene and environmental health.

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