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Antimicrobial Resistance, Integron and Class 1 Integron in *E. coli* isolated from Ready-to-Eat Foods

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Abstract
A total of 740 ready-to-eat food samples were collected from roadside shops in Tiruchirappalli, Tamil Nadu, to assess *E. coli* contamination. Among these, 500 (68%) samples tested positive for *E. coli*, while 240 were negative. Biochemical characterization confirmed 162 (32.4%) isolates. Simultaneously, water samples from these shops were analysed, revealing *E. coli* contamination in 295 samples, with bacterial counts ranging from 8.4×10^5 to 9.5×10^8 CFU/ml. Molecular detection confirmed the *uidA* gene in 139 (85.8%) isolates. Antibiotic susceptibility testing of 139 strains revealed 96 (69.1%) as drug-resistant, with 16.7% showing 100% resistance. Multidrug-resistant isolates exhibited 75.9% resistance to streptomycin. Integron integrase was detected in 57 (59.37%) isolates, with 56 (98.2%) carrying the intI1 gene. Contamination was linked to water and human handling ($p\leq0.01$). Implementing hygiene practices among food handlers is crucial to mitigating the risk of *E. coli* contamination.

Keywords: Ready-to-eat foods, antibiotic susceptibility, β-glucuronidase, integron, class 1 integron, public health

Introduction
Foodborne infections are a significant global public health concern, with many cases attributed to contaminated food and water (Mandal, 2017; Gautam *et al.*, 2017) [1] [2]. Among various microbial contaminants, *Escherichia coli* (*E. coli*) is a major water pollutant, primarily introduced through human and animal excreta in water bodies (Gupta *et al.*, 2013) [3]. The ingestion of contaminated meat products and fresh produce is a well-documented source of *E. coli*-related foodborne infections (Naganandhini *et al.*, 2015) [4]. Additionally, *E. coli* serves as a potent reservoir and carrier of resistant plasmids (Ghaly *et al.*, 2017) [5], utilizing insertion sequence mechanisms to mobilize a wide array of antimicrobial resistance genes (Shahreza *et al.*, 2017) [6].

The widespread use of antimicrobials in human infections has exerted selective pressure on *E. coli* strains, driving them to evolve adaptive mechanisms for survival in increasingly toxic environments (Szmolka and Nagy, 2013) [7]. Alarmingly, multidrug resistance-related mortalities are projected to rise to 10 million by 2050, with *E. coli* infections contributing to approximately 30% of these cases (Reid *et al.*, 2017) [8]. The genetic basis of antimicrobial resistance in *E. coli* is largely associated with plasmids and transposons, facilitating the horizontal transfer of resistance genes among diverse bacterial species. Environmental factors further accelerate bacterial adaptation through mutation events and genetic exchange (Stalder *et al.*, 2012) [9].

Integrons play a crucial role in antibiotic resistance, with non-pathogenic *E. coli* capable of acquiring multidrug resistance via horizontal gene transfer (Szmolka and Nagy, 2013) [7]. Class 1 integrons, in particular, harbor the *intI* gene, encoding a site-specific recombinase responsible for integrating and excising gene cassettes at specific recombination sites (*attC* and *attI*), thereby enabling the acquisition of unrelated resistance genes (Kheiri and Akhtari, 2016) [10].

Food and water are recognized as key vehicles for the dissemination of antibiotic-resistant genes among human bacterial pathogens (Kaushik *et al.*, 2019) [11]. *E. coli* can be transmitted to humans through ready-to-eat foods, which are susceptible to contamination during processing and capable of spreading resistance genes to other pathogens (Yu *et al.*, 2016) [12]. In India, roadside foods are widely popular due to their distinctive flavors and

accessibility (Morshdy *et al.*, 2018) [13]. However, studies on the microbial quality and safety of ready-to-eat foods in Tamil Nadu remain limited. This knowledge gap has motivated the present study, which aims to assess the hygienic status of ready-to-eat foods available in Tiruchirappalli, Tamil Nadu.

Materials and Methods

Isolation and identification of *E. coli*

A total of 74 different varieties of ready-to-eat food samples including vegetables (n=37), cooked meat products (n=37) with 10 samples in each variety were collected respectively from different roadside street food vendors, restaurants, hotels and fast-food stalls in Tiruchirappalli, Tamil Nadu between 2021 and 2023. All the samples were collected in new sterile polythene zipper pouch and transported immediately to laboratory in an iced sample box and stored at 4˚C until processing. The collected samples were subjected to bacteriological and biochemical examination within 12 h of collection.

The isolation and identification of *E. coli* was carried out as per United States-Food and Drug Administration, Bacteriological Analytical Manual, Chapter 4A with some modification (Feng *et al.*, 2011) [14]. About 10 g portion of each food sample was finely chopped with sterile disposable knife and smashed uniformly in sterile mortar and pestle. The homogenized mixture was mixed in 90 ml of Buffered Peptone Broth (Himedia) and incubated at 37˚C for 24 h as pre-enrichment. Further, enrichment was done by transferring 1 ml of pre-enriched mixture into 9 ml of Lactose Broth and Tryptone Phosphate Broth (Himedia), respectively and incubated for 18 h at 37˚C. A loopful of enriched culture was streaked on to Xylose Lysine Deoxycholate (XLD) agar and MacConkey (MAC) agar plates and incubated at 37˚C for 18±2 h. Further screening test was performed by re-streaking on Eosin Methylene Blue (EMB) agar plates and incubated for 18±2 h at 37˚C. Colonies with metallic green sheen with dark centered purple were selected and sub cultured in Luria Agar (LA) plates and incubated for 16±2 h at 37˚C.

Simultaneously, water samples were also collected along with ready-to-eat food samples. The water used by shopkeepers for food preparation purposes was collected in a 50 ml sterile polypropylene sample container (Himedia) from respective shops. Utmost care was taken to avoid cross contamination while shifting the water samples to the laboratory in an iced sample box. The collected samples were scrutinized for total bacterial count within 2 h of collection.

The 1 ml of each water sample was serially diluted up to 10^{-6} and 100 µl of each dilution was spread plated on to Nutrient Agar (NA) plates and the plates were incubated at room temperature (25-27˚C) for 24-48 h. Plates with isolated colonies of 30-300 were counted and the results were expressed as Colony Forming Unit (CFU). Microscopic examination of gram staining was performed to confirm gram negative rods. In addition, the suspected colonies from LA plates were directly streaked on to MAC and EMB agar plates and incubated for 18±2 h at 37˚C to determine the presence of *E. coli* (Feng *et al.*, 2011) [14]. Biochemical tests were performed after isolation of *E. coli* from ready-to-eat food and water samples to confirm atypical *E. coli*. Pure cultures from non-selective media were tested for IMViC (Indole, Methyl Red, Voges-Proskauer and Citrate), Triple Sugar Iron (TSI) agar, Lysine Iron Agar (LIA) and urea formation in test tubes and incubated for 18 h at 37˚C. The positive isolates were preserved in NA by stabbing and kept at room temperature.

Detection of β-glucuronidase enzyme activity

The freshly cultured *E. coli* isolates were grown on LA plates and colonies were inoculated in 500µl of 1X Phosphate Buffer Solution. The suspension was boiled for 10 min followed by snap chilling for 5 min and centrifugation at 10,000 rpm for 5 min. The boil lysis DNA extraction was done following the method of Dutta *et al.* (2013) [15]. The supernatant was used as a DNA template. The oligonucleotide primers targeting *uidA* gene (166 bp) were used to confirm the identified *E. coli* isolates in the PCR with the conditions described in Table 1. The reference strain ATCC 25922 used as positive control and EPEC as negative control the amplified PCR products were run on agarose gel electrophoresis with 2% agarose gel at 70V for 40 min. After the run, the gel was stained in ethidium bromide (0.5µg/ml) for 20 min and DNA bands were visualized and imaged under UV trans-illuminator.

Antibiotic resistant pattern of *E. coli*

Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method on Muller Hinton Agar (MHA), as per the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2018) [16]. The 24 h fresh culture of *E. coli* isolated from ready-to-eat food samples was streaked on to LA plates and incubated at 37˚C for 16±2 h. The isolated single colony was mixed in 5ml of Luria Bertani Broth and incubated for 4 to 6 h until the appearance of moderate turbidity. Then the bacterial growth was measured by optical density (OD_{600}) and standardized by adjusting to 1.5×10^8 CFU/ml by diluting the inoculum. With the sterile cotton swab, the bacterial cells were evenly swabbed on to MHA plates. The antibiotic discs (Himedia) with different concentrations used in this study are ampicillin (AMP, 10µg), ceftriaxone (CTR, 30µg), chloramphenicol (C, 30µg), ciprofloxacin (CIP, 5µg), doxycycline hydrochloride (DO, 30µg), gentamicin (GEN, 10µg), norfloxacin (NX, 10µg), ofloxacin (OF, 5µg), streptomycin (S, 300µg), tetracycline (TE, 30µg), co-trimoxazole (COT, 25µg) and cefixime (CFM, 5µg). The antibiotic discs were aseptically placed with sterile forceps in the swabbed MHA plates and incubated for 24 h at 37˚C. After incubation, the diameter of zone size was interpreted with zone size interpretative chart as per CLSI guideline with the quality control of reference strain *E. coli* ATCC 25922 and interpreted as sensitive and resistant (Khan *et al.*, 2019) [17].

Multiple Antibiotic Resistance (MAR) index of *E. coli*

The MAR index was determined for each isolate which exhibited resistance to two or more antibiotics by using the formula MAR=a/b, where ‘a’ represents the number of antibiotics to which the isolate was resistant and ‘b’ represents the number of antibiotics to which the isolate was exposed (Krumperman, 1983) [18].

Detection of Integron and Class 1 integron in multidrug resistant *E. coli*

A single colony of *E. coli* was inoculated with 500µl of 1X Phosphate Buffer Solution (PBS) and boiling template method was performed. The suspension was boiled for 10

min followed by snap chilling for 5 min and centrifuged at 6000 rpm for 10 minutes to isolate plasmid DNA. The supernatant was used as a DNA template. The PCR master mixture, primers and cyclic temperature were carried out as per the methods described by Baloch *et al.* (2017) [19] (Table 2). The integron integrase (*intI)* gene has variable region. The integron positive isolates were further detected with the presence of integron integrase class 1 with the base pairs of 565. The cyclic condition of PCR suggested by Yu *et al.* (2016) [12] was followed (Table 2). The clinical strain of EPEC was used as positive control and the reference strain ATCC 25922 was used as negative control. The amplified mixtures were run on agarose gel electrophoresis with 1% concentration at 90 Volt for 25 min. After the run, gel was stained in ethidium bromide for 20 minutes and the bands were visualized under UV trans-illuminator.

Statistical analysis

The statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS), 20.0 version. The correlation between ready-to-eat food products and water samples are significant at 0.01 level.

Results

Screening of *E. coli* from ready-to-eat samples (food and water samples): A total of 74 different varieties of ready-to-eat food products with 10 samples in each variety were collected respectively from different shops in Tiruchirappalli, Tamil Nadu between 2017 and 2019. Among 740 samples of ready-to-eat food products, 500 samples were positive for *E. coli* and 240 samples were negative for *E. coli*. From among 500 (68%) ready-to-eat food samples, 162 (32.4%) isolates were found to produce pink colonies on MAC agar plates, yellow colonies on XLD agar plates and metallic green sheen with dark purple centered colonies on EMB agar plates. The microscopic examination of these colonies showed pure strains of gram negative, rod shaped bacteria. Further, these suspected colonies were subjected to identification through phenotypic examination with the results of Indole-positive, Methyl Red-positive, Voges-Proskauer-negative, Citrate-negative, H_2S-negative, TSI test-positive and LIA test-positive. The highest count of 8 strains of *E. coli* was isolated from mixed veg salad and boti masala out of 10 samples in each. The second highest contamination was in panipuri. The water samples used for food preparation purposes and collected from respective shops were contaminated with bacteria and the presence of *E. coli* was confirmed. Totally 295 water samples have shown the presence of *E. coli*. The bacterial count in collected water samples were ranging from 8.4×10^5 to 9.5×10^8 CFU/ml

Distribution of *uidA* gene in *E. coli*

From among 162 isolates, *uidA* gene was detected in 139 (85.8%) isolates and 23 (14.2%) isolates were found to be negative for *uidA* gene. It was found that the highest detection of *E. coli uidA* gene were from boti masala [8], mixed veg salad (8) followed by pani puri [7], tuna subway [6], liver fry [6], fish finger [5], brain masala [5] and chicken tandoori [5] out of 10 each non-veg ready-to-eat samples.

Antibiotic susceptibility in *E. coli:* Among the 139 *E. coli* strains isolated from ready-to-eat food samples, antibiotic susceptibility was ranging between 53 and 21%. Higher sensitivity was recorded against ofloxacin (53.9%) and chloramphenicol (53.2%) followed by norfloxacin (50.3%) and co-trimoxazole (50.3%). The susceptibility to other antibiotics such as ampicillin, gentamicin, tetracycline, doxycycline, ceftriaxone, cefixime, ciprofloxacin and streptomycin was ranging below 41%. Similarly, the resistant pattern of *E. coli* isolated from ready-to-eat food samples has indicated higher resistance to streptomycin (58.3%). The resistance against other antibiotics like ceftriaxone, ampicillin, cefixime, ciprofloxacin, gentamicin, tetracycline, doxycycline, co-trimoxazole, norfloxacin, ofloxacin and chloramphenicol were ranging between 58 and 29%.

Distribution of antibiotic resistant *E. coli*

From among 139 *E. coli* strains isolated from ready-to-eat food samples, 96 (69.1%) *E. coli* isolates showed multidrug resistance against streptomycin (80.2%), ceftriaxone (67.7%), ampicillin (66.6%), cefixime (66.6%), ciprofloxacin (62.5%), tetracycline (61.4%), doxycycline (60.4%), gentamicin (60.4%), co-trimoxazole (57.3%), norfloxacin (53.1%) ofloxacin (53.1%) and chloramphenicol (42.7%). The overall antimicrobial susceptibility ranged between 35 and 8%. The highest sensitivity was found against chloramphenicol (35.4%) followed by ofloxacin (33.3%), gentamicin (29.1%), co-trimoxazole (29.1%), norfloxacin (28.1%), tetracycline (22.9%), ceftriaxone (20.8%), ampicillin (18.7%), doxycycline (17.7%), cefixime (15.6%), ciprofloxacin (13.5%) and streptomycin has least susceptibility of 8.3%.

Determination of MAR index

The MAR index was calculated for the multidrug resistant *E. coli* isolates. The MAR index was ranging from 0.16 to 1 with an average of 0.58. Majority of the isolates (n=16) showed an index of 1 followed by 0.9 (n=11), 0.3 (n=11), 0.4 (n=10), 0.2 (9), 0.5 (n=8), 0.7 (n=8), 0.58 (7), 0.6 (n=6), 0.1 (n=6) and 0.8 (n=4). MAR index value higher than 0.2 indicate high risk source of contamination. MAR index revealed 90 isolates (93.8%) with MAR index greater than 0.2 and 6 (6.2%) isolates with less than 0.2. However, 16 (16.7%) *E. coli* isolates from ready-to-eat foods had shown resistant to all the antimicrobials tested with MAR index of 1 (Table 3).

Distribution of integron among multidrug resistant *E. coli:* The presence of integron integrase (*intI)* gene was noticed in 57 (59.4%) *E. coli* isolates with the size of PCR fragments of approximately 750 bp (28 isolates) (29.2%), 1000 bp (17 isolates) (17.7%), 1500 bp (9 isolates) (9.4%), 600 bp (2 isolates) (2.1%) and 2000 bp (1 isolate) (1.1%) and 39 (40.6%) isolates were negative for integron gene out of 96 multidrug resistant *E. coli* strains isolated from the ready-to-eat food sources.

Distribution of class 1 integron among *E. coli* from ready-to-eat foods: Furthermore, the presence of class 1 integron was examined among positive integron integrase gene (*intI*) *E. coli* isolates from ready-to-eat food samples. From among 57 positive isolates, 56 (98.2%) isolates were found to be positive for class 1 integron (*intI*1) gene with 565 base pairs and 1 (1.8%) isolate was negative for *intI*1 gene. The determination of integron integrase class 1 along with antibiotic resistant phenotype was detailed in Table 4.

Table 1. Details of PCR primer and condition used for the detection of *uidA* gene in *E. coli*

Target gene	Primer name	Direction	Sequence 5'-3'	Cyclic condition	Base pairs	Reference
UidA	UAL1939b	Forward	ATGGAATTTCG CCGATTTTGC	94 °C 5 min; 35 cycles 94 °C 10s, 55.2 °C 10s, 72 °C 1 min; 72 °C 10 min	166	Godambe *et al.* (2017) [20]
	UAL2105b	Reverse	ATTGTTTGCC TCCCTGCTGC			

Table 2. PCR primer sequence used in the present study for the detection of integron and class 1 integron

Target gene	Direction	Sequence 5'-3'	Cyclic condition	Base pairs	Reference
intI	Forward Reverse	GGCATCCAAGCAGCAAG AAGCAGACTTGACCTGA	94 °C 5 min; 30 cycles 94 °C 30 sec, 55 °C 45 sec, 72 °C 2 min; 72 °C 10 min	Variable region	Baloch *et al.* (2017) [20]
*intI*1	Forward Reverse	ACGAGCGCAAGGTTTCGGT GAAAGGTCTGGTCATACATG	94 °C 3 min; 35 cycles 94 °C 30 sec, 60 °C 30 sec, 72 °C 1 min 30 sec; 72 °C 5 min	565	Yu *et al.*, (2016) [12]

Table 3. Resistance pattern and MAR index of *E. coli* isolated from ready-to-eat food samples

Number of isolates (n=96)	Resistance against number of antibiotics	MAR index
6	2	0.16
9	3	0.25
11	4	0.33
10	5	0.41
8	6	0.5
7	7	0.58
6	8	0.66
8	9	0.75
4	10	0.83
11	11	0.91
16	12	1
Average MAR 0.58		

Table 4. Presence of integron integrase gene class 1 in *E. coli* isolated from ready-to-eat food samples

Food source	***intI*1 gene positive (565 bp)**	***intI*1 gene negative**	Antimicrobial agent
Steamed corn	+		AMP,DO,TE,COT
Mixed veg salad	+		AMP,CIP,NX,OF,COT,CFM
Mixed veg salad	+		DO,GEN,S,TE
Cutlet	+		CIP,DO,NX,OF,S,TE,COT,CFM
Noodles	+		CTR,C,GEN,S,COT,CFM
Pani puri	+		AMP,CTR,C,CIP,DO,GEN,S,TE,COT
Sprouts masala	+		CTR,GEN,S
Sprouts masala	+		CIP,GEN,S
Fruit salad	+		S,CFM
Chicken sausage	+		AMP,S,COT
Chicken tawa	+		CTR,DO,S,TE
Fried chicken	+		AMP,CTR,C,CIP,DO,GEN,NX,OF,S,TE,CFM
Fried chicken	+		AMP,S,TE,COT
Chicken tandoori	+		AMP,CTR,CIP,S,TE,CFM
Chicken tandoori	+		AMP,DO,S,TE,COT
Chicken tandoori	+		CTR,GEN,S,CFM
Chicken tandoori	+		AMP,CIP,NX,OF
Chicken soup	+		AMP,CTR,C,CIP,DO,GEN,NX,OF,S,TE,COT
Fish fry		-	AMP,CTR,C,CIP,GEN,NX,OF,S,TE,COT,CFM

Chilli fish	+		AMP,CTR,C,CIP,GEN,NX, OF,S,CFM
Sheek kabab	+		CTR,C,DO,GEN, S,TE,COT,CFM
Sheek kabab	+		AMP,CTR,CIP,DO,GEN,N X,OF,S,TE,COT,CFM
Sheek kabab	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Fish finger	+		CTR,C,CIP,DO,GEN,NX,O F,S,TE,COT,CFM
Fish finger	+		AMP,CTR,C,CIP, GEN,NX,OF,S,COT,CFM
Fish finger	+		CTR,C,CIP,DO,GEN,NX,O F,S,TE,COT,CFM
Fish finger	+		AMP,CTR,CIP,DO,NX,OF, TE,COT,CFM
Fish finger	+		CTR, CIP,DO,GEN, S, COT,CFM
Fish kabab	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Prawn kabab	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S, COT,CFM
Prawn fried rice	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE, CFM
Prawn fried rice	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Prawn gravy	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Prawn gravy	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Crab masala	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Veg shawarma	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Bonda chat	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Bhel puri	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Mushroom masala	+		AMP,CTR,C,CIP,DO,GEN, S,TE,COT,CFM
Mushroom masala	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Mushroom masala	+		C,CIP,DO,GEN,NX, S,CFM
Burger	+		AMP,CTR,C,CIP,DO,GEN, NX,OF,S,TE,COT,CFM
Bajji capsicum	+		AMP,CTR,CIP,DO,GEN, OF,S,TE, CFM
Kuli paniyaram	+		CTR, CIP,DO,GEN,NX,OF,S,TE, COT
Jigardhanda	+		AMP,CTR,CIP, NX,OF,S, CFM
Jigardhanda	+		AMP,DO,S,TE,COT
Chicken shawarma	+		C,DO,S,TE,CFM
Chicken shawarma	+		CTR,C,DO,S,TE
Boti masala	+		CTR,CIP,NX,S,CFM
Boti masala	+		AMP,GEN,OF,S,TE,COT
Boti masala	+		AMP,CTR,DO,TE,COT
Boti masala	+		S,CFM
Boti masala	+		AMP,CIP,DO,NX,OF,TE
Tuna subway	+		AMP,CTR,CFM
Chicken keema paratha	+		CIP,DO,TE,COT,CFM
Brain masala	+		AMP,CIP,NX,OF
Brain masala	+		AMP,C,CIP,NX,OF
Total - 57	56 (98.2%)	1 (1.8%)	

AMP - ampicillin; CTR - ceftriaxone; C - chloramphenicol; CIP - ciprofloxacin; DO - doxycycline; GEN - gentamicin; NX - norfloxacin; OF - ofloxacin; S - streptomycin; TE - tetracycline; COT - co-trimoxazole; CFM - cefixime

Discussion

In this present study, a total of 740 food samples were analyzed for the presence of *E. coli* in ready-to-eat food products collected in Tiruchirappalli. The screening of water samples has also confirmed the presence of *E. coli.* The distribution of *E. coli* was predominant in water samples, used for all purposes by street food vendors in Tiruchirappalli. Similar results were reported by Shrimali and Shah (2017) [21]. The current study has shown that 29% of ready-to-eat food samples possessed *E. coli.* The microbial load in 39.8% of water samples might be due to unhygienic handling and leakage of sewer pipe lines mixed up with groundwater or corporation water. The statistical analysis with 0.01 level of significance has also proved a strong correlation between ready-to-eat food products and water as a source of cross contamination.

The fresh ready-to-eat mixed veg salads are not subjected to elimination of old leaves, washing and these might be an important route of *E. coli* transmission. The processed meat used in fish fingers, tuna subway and chicken tandoori which were improperly refrigerated for a prolonged period and shallow fried for few seconds. In Tiruchirappalli, chilled ready-to-eat foods were not maintained at proper temperature. Temperature variation due to flux in electricity creates an enabling environment for the microbes to grow. The organ meats like boti (Goat intestine), liver and brain require more water to cleanse the toxins, but it was cleaned in limited water. That might be the primary reason to have highest *E. coli* contamination. In addition, fast food cooking utensils were not washed and it was deliberately kept oily for convenience of further cooking. Vendors who do not own shops, prepare food at their homes and sell at street, use carts to keep their equipment, dispose garbage and waste water nearby, which attracts many insects, flies, rodents to crawl over spreading foodborne pathogens. Few cooks in the roadside fast-food stalls were observed to smoke beedi and chew tobacco while preparing the food. It was also observed that some hawkers while opening polythene bags for packing food products, open the bags by blowing air by mouth and/or using saliva-wet fingers.

In Tiruchirappalli, vending facilities varied from mobile carts to fixed stalls and restaurants. But, the infrastructure is lacking with limited and restricted access to potable water, toilets, refrigeration and washing waste disposal facilities. Most vending units like fast food stalls, chat stalls and barbecue stalls were located in an unhygienic, open roadside location without basic sanitation, manufacturing quality and safety. Food safety is compromised due to difficulty in controlling the large number of consumers, lack of knowledge about microbial contamination, poor knowledge in basic food safety measures, inadequate public awareness of foodborne illnesses, insufficient food inspection by authorities and periodic lab analysis. There are many laws and policies formulated by Food Safety and Standards Authority of India to regulate street vending and food safety. The main drawback in Tamilnadu is implementation and regulation of such policy and hence, remains unsuccessful due to lack of support from municipal bodies.

In the present study, 139 (85.8%) *E. coli* isolates were confirmed for the presence of *uidA* gene indicates poor quality of the food samples. Previous studies have also corroborated the same with poor sanitation of different varieties of ready-to-eat food samples (Ogodo *et al.*, 2016) [22].

The antibiogram among 139 *E. coli* isolates indicated higher sensitivity towards ofloxacin followed by chloramphenicol, norfloxacin, co-trimoxazole, gentamicin, tetracycline, doxycycline, ceftriaxone, ciprofloxacin, cefixime and streptomycin. Whereas, the 96 multidrug resistant *E. coli* isolates showed increased resistance to streptomycin followed by ceftriaxone, ampicillin, cefixime, ciprofloxacin, tetracycline, gentamicin, doxycycline, co-trimoxazole, norfloxacin, ofloxacin and chloramphenicol. The revelation of resistance against antibacterials in the present study was substantiated by Poirel *et al.* (2018) [23] who have observed that *E. coli* became resistant towards tetracycline as a consequence of selective pressure. The fact that the resistance to these antimicrobial agents might be due to mutation and drug efflux (Spagnolo *et al.*, 2016) [24] could not be ruled out. To control further emergence of antibiotic resistance, the application of hygienic practices in food chain is essential (Lima *et al.*, 2017) [25]. Periodic evaluation of antibiogram of *E. coli* isolates from various samples is essential to determine the incidence of multiple drug resistant strains. Despite these efforts, the spontaneous action of antibiotic resistance continues. Therefore, the new antimicrobial agents must be discovered. Furthermore, unnecessary antibiotic use should be avoided, stricter dosage and course regime should be followed.

In the present study, 16.7% of *E. coli* isolates showed multidrug resistant to all the 12 antibiotics with the MAR index of one, which revealed the presence of horizontal gene transfer between the environment and food source. The collected ready-to-eat food samples showed 59.4% of integron in *E. coli*. The observation of integron from various base pairs in this study were similar to the studies of Baloach *et al.* (2017) [19] and Firoozeh *et al.* (2019) [26]. It is suspected that the cross-contamination would have occurred between the sewage and corporation water lines. The septage decanting station and treatment plant in Tiruchirappalli could be the hotspot for integron dissemination in different bacterial species especially in *E. coli*.

Class 1 integrons are most widespread in clinical and environment (Yu *et al.*, 2003) [27]. In the present study, the observation of 98.2% of class 1 integron from ready-to-eat food samples clearly depicts the unhygienic food preparation, sewage cross contamination in water source and indiscriminate therapeutic use of antibiotics by human as possible sources. The class 1 integron in ready-to-eat products which showed resistance to two or more classes of antimicrobials is more likely to have been routed from natural environment into human microbiota via water and foodborne bacteria. The bacterial stress to various agents resulted in the selection of class 1 integron to acquire resistance gene. The remaining 1.8% of fish fry showed negative for class 1 integron which may have other classes of integron since the presence of integron integrase gene was positive. Considering this, the future use of antibiotics should be carefully managed to avoid further bacterial transformation and adaptation.

Conclusion

The recent information provided here evidently depicts the importance of food hygiene, consumption preferences, good hygienic practices and proper cooking of food products prior to consumption. The presence of class 1 integron in *E. coli* from ready-to-eat foods might be due to dumping of human waste, solid waste in open field and near water bodies and direct discharge of treated sewage water into

Cauvery River from sewage treatment plant. Also, self-medication of antibiotics without physician advice, prolonged use of antibiotics, discontinuing antibiotic drug course during illness in human may enable the commensal *E. coli* to get adapted, shredded in feces and reaches the sewage. Hence, the present study portrays the term integron as human induced lifestyle pollution. Ultimately, the drinking water gets contaminated through multiple ways of human handling. Even though the corporation drinking water is being treated with chlorination, it is recommended to follow simple hygienic measures like proper handwashing to prevent infections, educating proper food handling practices to reduce transmission of *E. coli* in food and water. In addition, creating awareness among common people about proper use of antibiotics and consequent drug resistance in order to reduce the burden of antibiotic residues in the environment is essential to prevent further adaption of *E. coli* towards future antibiotics.

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