

Molecular Screening of β -glucuronidase and Class 1 Integron of *Escherichia coli* from Ready-to-Eat Foods in Tiruchirappalli, Tamil Nadu

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Abstract

Ready-to-eat food products procured from different roadside shops in Tiruchirappalli, Tamil Nadu were screened for *Escherichia coli*. A total of 500 samples from 250 vegetable and 250 meat products were collected from different hotels, restaurants and street food vendors in Tiruchirappalli, Tamilnadu. Out of 500 ready-to-eat food samples, 162 (32.4%) *E. coli* strains were isolated. The ready-to-eat meat products had higher bacterial count than the vegetable food samples collected due to unhygienic handling, improper storage, inadequate temperature to maintain processed meat and improper cooking. Biochemically identified *E. coli* colonies were screened for housekeeping gene *uidA* and 139 (85.8%) *E. coli* isolates were confirmed to possess β -glucuronidase activity. In addition, antibiotic susceptibility assay was performed using 12 antibiotics. From 139 *E. coli* strains, 96 (69.1%) isolates showed multidrug resistance. Among them, 16.7% showed 100% resistance to all the antibiotics tested. Whereas, multidrug resistant *E. coli* isolates showed increased resistance (75.9%) to streptomycin followed by 70-50% level of resistance to ceftriaxone, ampicillin, cefixime, ciprofloxacin, tetracycline, gentamicin, doxycycline, co-trimoxazole, norfloxacin, ofloxacin and chloramphenicol. Furthermore, drug resistant *E. coli* isolates 56 (58.3%) were detected with the presence of *int1*. The source of contamination was found to be water and human handling. Drinking water supply from corporation might have been contaminated with fecal waste source is being discharged into Cauvery river which might disseminate horizontal gene transfer.

Keywords: Ready-to-eat, *E. coli*, *uidA* gene, Multidrug resistance, Class 1 integron, Public health

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INTRODUCTION

The World Health Organization (2014), on antimicrobial resistance surveillance, reported that *E. coli* is one among the nine bacteria responsible for common infections in the community¹. Bacterial evolution enables their adaptation to most of the ecosystems². It is suspected that mortalities due to multidrug resistance will increase to 10 million by 2050 and in that, *E. coli* infection's contribution will be 30%³. *E. coli* is a significant member of intestinal non-pathogenic bacteria, which may possess antibiotic drug resistance⁴. *E. coli* is the highly potential reservoir and carrier of resistant plasmids⁵. Antibiotic resistant genes encoded with integrons are regarded as genetic pollutants⁶.

Integrons are mobile genetic elements with plasmids and transposons catalyzed by integrase recombination which promote dissemination of antimicrobial resistant gene among Enterobacteriaceae. *E. coli* involve insertion sequence mechanisms which are unique to mobilize a wide range of antimicrobial resistant genes⁷. Integrons encoding antibiotic resistance present in *E. coli* has a tendency to stockpile multidrug resistance via horizontal gene transfer⁸. The presence of mobile integrons, multidrug resistant plasmids and class 1 integron play a major role in spreading of resistant gene in fresh produce and animal foods. *E. coli* can be transferred to human through ready-to-eat foods that are easily contaminated during and post-processing, storage and easily spread resistance genes to other pathogens⁹. *E. coli* is an active reservoir of integrons which transfer antibiotic resistant genes of different classes of antibiotics including aminoglycoside, fluoroquinolones, cephalosporins, tetracycline, phenicols, and sulfonamides through water and food chain¹⁰. The multidrug resistant non-pathogenic *E. coli* mobilizes the resistant gene through food, water and soil.

Mutation contributes bacterial adaptation and horizontal gene transfer that occurs much in the environment¹¹. The food and water bodies are regarded as a vehicle for dissemination of antibiotic resistant genes among human bacterial pathogens due to non-selective use of antimicrobials¹². A better understanding of dissemination of antibiotic resistant gene into the human microbiome through food is essential to prevent multidrug resistant

infections¹³. The spontaneous process of resistance in commensal *E. coli* makes as fitness genes and adapted the commensal *E. coli* to disseminate antibiotic resistance genes¹⁴. Antibiotic resistance among pathogenic and non-pathogenic bacteria is a global threat. The present study was undertaken to study the antibiotic resistance and to detect the class 1 integron among non-pathogenic *E. coli* from different ready-to-eat foods obtained in Tiruchirappalli, Tamil Nadu.

MATERIALS AND METHODS

The isolation and identification of *E. coli* was carried out as per The United States – Food and Drug Administration, bacteriological analytical manual with some modification¹⁵.

Collection of sample

A variety of 500 different ready-to-eat food samples such as vegetable (n=250), meat products (n=250) were purchased from different roadside street food vendors, restaurants, hotels and fast food stalls in Tiruchirappalli, Tamilnadu. All the samples were collected in a new polythene zipper pouch and brought 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.

Isolation and identification of *E. coli*

About 10 g of each sample was smashed uniformly in sterile mortar and pestle and mixed in 90 ml of buffered peptone broth (Himedia, Mumbai) 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 16±2 h at 37°C¹⁵. A loop 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 h¹⁶. 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 sub cultured in luria agar (LA) plates and incubated for 16±2 h at 37°C. Biochemical tests were performed after isolation to confirm atypical *E. coli*. Pure cultures from non-selective media were tested for indole, methyl red, voges-proskauer, citrate, triple sugar iron (TSI)

agar, lysine iron agar (LIA) and urea formation in test tubes and incubated for 18 h at 37°C^{15,16}.

Molecular screening 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 (PBS). The suspension was boiled for 10 min followed by 5 min snap chilling and centrifuged at 10,000 rpm for 5 min¹⁶. The supernatant was used for molecular confirmation. The oligonucleotide primers targeting *uidA* gene (166 bp) were used to confirm the identified *E. coli* isolates (Table 1). The molecular assay was carried out in a 20µl reaction mixture containing 10µl of 2X PCR master mix (Himedia) with 1µl of each forward and reverse primers (Eurofins, India), 3µl of DNA and 5µl of water¹⁶. The amplified products were run on 2% agarose gel at 70V for 40 min and stained in 0.5µg/ml of ethidium bromide for 20 min¹⁹ and visualized under UV trans-illuminator.

Antibiotic susceptibility of *E. coli*

Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method on Muller Hinton Agar, as per the guidelines of Clinical and Laboratory Standards Institute¹⁷. 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 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 (OD600) and standardized by adjusting to 1.5×10⁸ CFU/ml by diluting the inoculum. With the sterile cotton

swab, the bacterial cells were taken from the bacterial suspension and 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. The diameter of zone was interpreted with zone size interpretative chart with the quality control of reference strain *E. coli* ATCC 25922¹⁸.

Detection of integron integrase class 1 (*int1*) in *E. coli*

The antibiotic resistant *E. coli* strains were investigated for class 1 integron, a genetic element which disseminate antibiotic resistance via horizontal gene transfer. Boiling template method was performed¹⁶. The suspension was centrifuged at 6000 rpm for 10 minutes to isolate plasmid DNA (Table 2). The presence of integron integrase class 1 was detected with the primers targeting 565 base pairs. The amplified mixtures were run on agarose gel electrophoresis with 1% concentration⁹ at 90 Volt for 25 min. After the run, 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 food and class 1 integron are significant at 0.01 level.

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	Ref.
<i>uidA</i>	UAL1939b	Forward	ATGGAATTCGCCGATTTTGC	94°C 5 min; 35 cycles 94°C 10s, 55.2°C 10s, 72 °C 1 min; 72 °C 10 min	166	19
	UAL2105b	Reverse	ATTGTTTGCTCCCTGCTGC			

Table 2. The primer sequence used for the detection of class 1 integron

Target gene	Direction	Sequence 5'-3'	Cyclic condition	Base pairs	Ref.
<i>int1</i>	Forward	ACGAGCGCAAGGTTTCGGT	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	9
	Reverse	GAAAGGTCTGGTCATACATG			

RESULTS

A total of 500 food products were procured respectively from different shops in Tiruchirappalli, Tamil Nadu. Among 500 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. 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, H₂S-negative, TSI test-positive and LIA test-positive.

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 out of 250 vegetable ready-to-eat food samples, 80 (32%) *E. coli* strains were identified and 64 (80%) of them were confirmed positive for *uidA* gene. Among them, 16 (20%) isolates were negative. On the other hand, 82 (32.8%) *E. coli* isolates were identified among 250 ready-to-eat meat products and 75 (91.46%) of them were positive for *uidA* gene and 7 (8.53%) *E. coli* isolates were found to be absence of *uidA* gene. The positive *uidA* gene in ready-to-eat meat products were higher than ready-to-eat vegetable food samples (Table 3).

Totally, 139 (vegetable = 64 and meat products = 75) *E. coli* isolates were analyzed for drug resistance. *E. coli* from both vegetable and meat products showed highest resistance against streptomycin (62.5% and 54.7%) respectively. Similarly, lower resistance was recorded against chloramphenicol in both vegetable (21.9%) and meat products (36%) respectively. In general, meat products showed higher resistance pattern than vegetable food samples except streptomycin. In meat products, other antibiotics such as ceftriaxone (54.7%), ampicillin (50.7%), cefixime (50.7%), tetracycline (46.7%), ciprofloxacin (45.3%), co-trimoxazole (45.3%), doxycycline (45.3%), norfloxacin (44%), gentamicin (42.7%), ofloxacin (41.3%) have shown higher resistance. In vegetable food samples, ampicillin (42.2%) followed by gentamicin (42.2%), ceftriaxone (40.6%), ciprofloxacin (40.6%), cefixime (40.6%), doxycycline (37.5%), tetracycline (37.5%), co-trimoxazole (34.4%), ofloxacin (31.2%) and norfloxacin (28.1%) showed higher resistance

From among 139 *E. coli* strains, 96 (69.1%) (vegetable = 42 and meat products = 54) isolates were found to possess multidrug resistance. The 96 *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%),

Table 3. A detailed positive report of *E. coli* isolated from ready-to-eat food products

Food products	No. of samples	No. of <i>E. coli</i> positive isolates	No. of <i>uidA</i> positive <i>E. coli</i>	Total no. of multidrug resistant isolates	Total no. of <i>Int1</i> positive samples
Vegetables	250	80	64	42	20
Meat products	250	82	75	54	36
Total	500	162 (32.4%)	139 (85.8%)	96 (69.1%)	56 (58.3%)

Table 4. Statistical analysis between ready-to-eat foods and class 1 integron

Ready-to-eat food samples	Mean	Standard Deviation	N
<i>E. coli</i> isolated from vegetable and meat products	2.1622	2.19233	500
Presence of <i>int1</i> among vegetable and meat products	.5405	.76720	500
Correlation	Class 1 integron (<i>int1</i>)		
<i>E. coli</i> isolated from ready-to-eat food samples	.657**		

** Correlation is significant at the 0.01 level.

doxycycline (60.4%), gentamicin (60.4%), co-trimoxazole (57.3%), norfloxacin (53.1%) ofloxacin (53.1%) and chloramphenicol (42.7%). 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%. Furthermore, *E. coli* isolated from 16 (16.7%) ready-to-eat food samples (vegetable=7 and meat products=9) have shown 100% resistance against all the antibiotics used in this study.

The presence of integron integrase class 1 (*intI1*) was examined among 96 multidrug resistant *E. coli* isolates from ready-to-eat food samples. From among 96 multidrug resistant isolates, 56 (58.3%) isolates were found to be positive for class 1 integron gene with 565 base pairs and 40 (41.7%) isolate were negative for *intI1* gene. Also, this study proved a strong correlation between food and class 1 integron at 0.01 level of significance (Table 4).

DISCUSSION

In this study, 32.4% of *E. coli* were isolated from 500 samples and 139 (85.8%) confirmed with the presence of β -glucuronidase enzyme activity which confirms the occurrence of *E. coli* which indicates poor quality of the food samples. There is a high probability of cross-contamination from water source, deprived hygienic practices in preparation area, inappropriate temperature, shallow cooking, improper cleaning of meat, unwashed vegetables, uncleaned utensils, knives and low quality of raw materials. The isolation of *E. coli* collected from roadside vendors of Tiruchirappalli was substantiated by the observation of Edward *et al.* (2012) who have reported 100% *E. coli* contamination in 15 samples of already prepared pre-packaged fruits sold in port^{20,21}. Though it is observed that the deep oil fried snacks and kebab dishes were seemed to be hot, charred and well-cooked, in reality, only the superficial layers were roasted and inner part of the meat or marinated vegetable remains uncooked. Similar detection of *uidA* gene were reported from *E. coli* isolated from various water food samples²².

The overall analysis among 139 *E. coli*

isolates, 96 multidrug resistant *E. coli* isolates from meat products (n=54) showed increased resistance to streptomycin followed by ceftriaxone, ampicillin, cefixime, ciprofloxacin, tetracycline, gentamicin, doxycycline, co-trimoxazole, norfloxacin, ofloxacin and chloramphenicol. Whereas the multidrug resistant *E. coli* isolates from vegetable samples (n=42) showed maximum resistance against streptomycin and gentamicin followed by ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, doxycycline, norfloxacin, ofloxacin, tetracycline, co-trimoxazole and cefixime. The revelation of resistance against antibacterials in the present study was substantiated by Wistrand-Yuen *et al.* (2018) who have observed that *E. coli* became resistant towards tetracycline, cephalosporin and penicillin as a consequence of selective pressure²³. The fact that the resistance to these antimicrobial agents might be due to mutation and drug efflux²⁴ could not be ruled out. The present study was substantiated by the observation of Lambrecht *et al.* (2019) that humans can be exposed to antibiotic resistant *E. coli* by contact with a contaminated natural environment and by inadequately cooked food through cross-contamination²⁵. This record of multidrug resistant *E. coli* in 54 (56.2%) meat products in the present study coincided with the report of Jans *et al.* (2018) that antimicrobial resistance was prevalent in meat and seafood at retail level of > 50%²⁶.

E. coli from ready-to-eat food samples has shown resistance towards more than one antimicrobial agents and generated a concern for public health. In addition, 16.7% of multidrug resistant *E. coli* isolates have showed 100% resistance to all the twelve antibiotics. The ready-to-eat meat products showed higher resistance to *E. coli*. Overall, highest antibiotic resistance among 139 *E. coli* isolates was noticed against Streptomycin. The fact that the water source used in food processing and unhygienic handling is an indication of the possible route of transmission of resistance since the water body receives antibiotic residues due to indiscriminate use by humans²⁷.

Class 1 integrons are considered as most widespread of multidrug resistance in clinical, environment and are evidenced to have activity only in human. A part of class 1 integron is found in chromosomes of environmental bacteria²⁸. In

the present study, the observation of 58.3% 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 is more likely to have been routed from natural environment into human microbiota via water and foodborne microorganisms. The bacterial stress to various antimicrobials, non-antimicrobial agents, heavy metals used in the agriculture field in the form of fertilizer resulted in the selection of class 1 integron to acquire resistance gene. The remaining 41.7% showed negative for class 1 integron which may have other classes of integron or absence of integron integrase gene.

Possible source of contamination

The Cauvery water existing for the population through Tiruchirappalli corporation supply to individual homes and bore well connections with hand pump²⁹. Disposal of wastewater from sewage treatment plant into Cauvery river, pollute the water. Also, the sewer pipe lines are connected nearby the corporation water lines. There is a high chances of cross-contamination through pipe lines. Hence, the domestic usage of fecal contaminated water in food processing and vessel washing purposes might be the reason to acquire multidrug resistance and horizontal gene transfer.

CONCLUSION

This study has clearly shown the prevailing microbial contamination of *E. coli* in street foods in Tiruchirappalli due to unhygienic practices and locations. Adequate awareness to the consumers and proper hygienic routines to the street food industry must be provided through camps. Apart from handling and cross-contamination, current conventional cooking methods are not adequate to kill heat-resistant strains of *E. coli*. Gradual increase of heat to certain target temperature will be lethal and kills *E. coli* in food. Miserably, options for treating drug resistance is diminishing due to overuse of antibiotics. The new antimicrobial agents have been discovered. The mobile integrons are widely distributed and abundant in human ecosystem. As described above, the class 1 mobile integrons is an efficient

tool for bacterial adaptation. This allows the extra-intestinal pathogenic and non-pathogenic *E. coli* to overcome human activity to control bacterial growth. The non-pathogenic *E. coli* from food and water sources are the active reservoirs of multidrug resistance determinants transferable to human. Considering this, the future use of antibiotics should be carefully managed to avoid further bacterial transformation and adaptation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Professor KS designed the work. AJ performed the experiments, generated data and wrote the manuscript. Professor KS read and approved the manuscript.

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ETHICS STATEMENT

Not applicable.

DATA AVAILABILITY-

All the data generated during the study are included in this manuscript.

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