Phenotypic, genomic confirmation and antibiotic studies of *Escherichia coli* from ready-to-eat foods vended in Tiruchirappalli, Tamil Nadu

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Abstract
The study was intended to isolate, identify and confirm molecularly the presence of Escherichia coli in most commonly vending ready-to-eat food products from street food shops of Tiruchirappalli. A total of 74 ready-to-eat food samples of vegetable, seafood, chicken and mutton products were purchased. Fifty out of 74 samples had Escherichia coli and 162 positive strain were identified by biochemical examinations.

Antibiotic susceptibility test was performed and isolates were found to be susceptible (40.3%), intermediate (15.4%) and resistant (44.3%) against twelve antibiotics. A high percentage of 51.2% isolates was susceptible to ofloxacin whereas 59.2% isolates showed resistance to streptomycin. An alarming multidrug resistance was observed with 115 strains. Among these, 21 isolates including vegetable (11), seafoods (8) and chicken (2) samples exhibited 100% resistance to all the twelve antibiotics. The prevalence of Escherichia coli in ready-to-eat vegetable products was found to be higher than ready-to-eat seafood, chicken and mutton products. The results depicted lack of food safety awareness and fecal cross contamination. Using uncleaned poor quality raw materials causes high level of microbial contamination. The presence of antibiotic resistant Escherichia coli in ready-to-eat foods would possess a serious threat to public health.

Keywords: *Escherichia coli*, *uidA* gene, ready-to-eat foods, public health, antibiotic resistance.

Introduction

Escherichia coli (E. coli) is a versatile facultative anaerobe that can grow in several extra intestinal environments[18]. Globally, a wide variety of street foods have been linked with *E. coli* infection[8,14]. The roadside foods are unique for their flavors and accessibility[16] in India. Meat products are rich sources of animal protein[23] and raw salads are considered as a healthy diet[17]. Ready-to-eat food serves as a vehicle for many diseases in developing countries where hygienic standards are not strictly followed[10].

The consumption of meat products and fresh produce is linked with *E. coli infection*, as they harbor enormous quantity of microbial contamination[4]. *E. coli* produces β-glucuronidase enzyme which is frequently used as enzymatic marker that encodes *uidA* gene to discriminate *E. coli* from other coliforms[2,5,20]. The antimicrobial susceptibility and antibiotic resistance pattern of *E. coli* constantly vary from time to time in different geographical areas[11]. The ready-to-eat food products are usually ingested directly without further processing which possesses a risk of oral exposure to antibiotic resistant microbes[7].

The indiscriminate use of antimicrobials[19] to treat infections has generated the emergence of antibiotic resistance under enormous antimicrobial pressure on *E. coli* strains[21] to play a major role in the emergence of antibiotic resistance[15]. The increasing incidence of multidrug resistant *E. coli* is an important public health concern[13]. Henceforth, this study is indented to screen antibiotic resistant *E. coli* isolated from ready-to-eat food samples in Tiruchirappalli.

Material and Methods

Phenotypic identification of *E. coli*: Seventy-four ready-to-eat food items including vegetables (37), seafoods (11), chicken (21) and mutton (5) products were collected from different roadside food stalls in Tiruchirappalli, Tamil Nadu. The samples were transported in an iced box to laboratory and stored at 4˚C until processing. The samples were analyzed within 2 h of collection. A 25g portion of each food sample was finely smashed in mortar and pestle. The mixture was transferred in 250ml of buffered peptone broth (BPB) for pre-enrichment and incubated at 37˚C for 24 h.

Further one ml of pre-enriched mixture was enriched with 9 ml of lactose broth and incubated for 24 h at 37˚C. A loop full of culture from lactose broths was streaked onto MacConkey (MAC) agar plates and incubated at 37˚C for overnight. The suspected colonies were reconfirmed by streaking on to Eosin Methylene Blue (EMB) agar plates and incubated for 24 h at 37˚C. All the typical colonies were subjected to various biochemical tests of IMViC, Triple Sugar Iron (TSI) agar, Lysine Iron Agar (LIA) and urea formation and incubated for overnight at 37˚C.

Genomic confirmation: The biochemically identified strains through methods were confirmed by molecular techniques. Genomic DNA of *E. coli* was extracted by boil lysis method[6]. The suspension of single colony of *E. coli* in 500µl of 1X Phosphate Buffer Solution (PBS) was boiled for 10 min followed by snap chilling for 5 min and centrifugated at 10,000 rpm for 5 min. The supernatant was used as a DNA template immediately after extraction. PCR assay was carried out with 1µl of 10 pico moles of each forward

UAL1939b (ATGGAATTTCGCCGATTTTGC) and reverse UAL2105b (ATTGTTTGCCTCCCTGCTGC) primers (Eurofins, India) targeting 166 bp of *uidA* gene.

The DNA was denatured at 94 °C for 5 min and amplified for 35 cycles at 94 °C for 10 s, 55.2 °C 10s and 72 °C 1 min. A final extension incubation of 10 min at 72 °C was included[9]. The amplified PCR products were run on 2% agarose gel electrophoresis at 70V for 40 min. After the run, the gel was stained in ethidium bromide and DNA bands were visualized and imaged under UV trans-illuminator.

Antibiotic susceptibility test: Antibiotic susceptibility test was performed by Kirby-Bauer disc diffusion method as per the guidelines of Clinical and Laboratory Standards Institute[24]. The twelve antibiotics used in this study were, ampicillin (AMP, 2 mcg), ceftriaxone (CTR, 30 mcg), chloramphenicol (C, 30 mcg), ciprofloxacin (CIP, 5 mcg), doxycycline hydrochloride (DO, 30 mcg), gentamicin (GEN, 10 mcg), norfloxacin (NX, 10 mcg), ofloxacin (OF, 5 mcg), streptomycin (S, 300 mcg), tetracycline (TE, 30 mcg), co-trimoxazole (COT, 25 mcg) and cefixime (CFM, 5 mcg).

A single colony of *E. coli* was transferred in 5ml of 1X PBS and incubated for 4 to 6 h. The bacterial growth was measured by optical density (OD_{600}) and adjusted to 1.5×10^8 CFU/ml. Sterile cotton swabs with bacterial culture were swabbed onto Muller-Hinton agar (MHA) plates. Aseptically, antibiotic discs were placed on swabbed agar plates and incubated for 24 h at 37˚C.

Results and Discussion

E. coli in ready-to-eat foods is one of the most important causes of gastrointestinal disturbances all over the world[16]. Seventy-four ready-to-eat food products are randomly collected. Fifty samples had *E. coli* out of 74 ready-to-eat foods. The enriched samples with pink colonies on MAC agar plate and the same colonies which produced metallic green sheen with dark centered purple colonies on EMB agar plates were selected. The phenotypic identification with biochemical tests confirmed 162 positive *E. coli* in ready-to-eat foods.

Further, *E. coli* specific *uidA* gene was confirmed with all the identified colonies in 74 food samples. The highest number of 80 strains were isolated from vegetables followed by seafoods (32), chicken (31) and mutton (19) samples (Table 1). The higher prevalence of *E. coli* in vegetable ready-to eat food products was observed to be the vehicle of *E. coli* transmission[22].

Partially-cooked vegetables are used in fast foods and raw vegetables are used as toppings in chat items. The vegetables used in cooking are not subjected to pasteurization, elimination of old leaves, washing, rinsing and proper storage as these are important points of transmission of *E. coli*. The pre-cut raw vegetables were stored in open buckets when vehicles passed by these chopped vegetables are highly exposed to dust. The improper handling practices enable *E. coli* to multiply and cause gastrointestinal discomfort.

In this study, the ready-to eat vegetable samples carried more *E. coli*. Most of the street food shops do not own a refrigerator. Hence, the seafoods are not stored at chilled temperature. Instead they marinate the seafoods in masala and leave it till the customer orders. The cross contamination in seafoods with seafoods is higher when cold storage is not administered. The meat products are marinated with masala manually in open air with naked hands and cooked at high temperature for short time. This makes the meat remaining uncooked inside.

E. coli contamination in meat and meat products could pose serious threat to public health[12]. Lack of food safety awareness, lack of hygienic practices in preparation area, improper handling, fecal cross contamination through water when processing the food materials by street food vendors are the primary causes of foodborne illnesses.

Genomically confirmed 162 *E. coli* isolates were subjected to antibiotic susceptibility test against 12 different antibiotics. Overall percentage of susceptible (40.3%), intermediate (15.4%) and resistant (44.3%) was observed. A high level of antibiotic resistance among *E. coli* was observed against streptomycin (59.2%) followed by ceftriaxone (50%), ampicillin (48.7%), cefixime (48.1%), ciprofloxacin (45.6%), gentamycin (44.4%), doxycycline (42.5%) tetracycline (43.2%), co-trimoxazole (41.9%), chloramphenicol (32.7%), norfloxacin (36.4%) and ofloxacin (37.6%) (Table 2).

Table 1
Prevalence of *E. coli* strains in various types of ready-to-eat food samples

Types of samples	Total no. of samples collected	Total no. of *E. coli* positive samples	Total no. of *E. coli* negative samples	Total no. of *E. coli* isolated	Total no. of *uidA* positive *E. coli*
Vegetable	37	25	12	80	80
Seafood	11	9	2	32	32
Chicken	21	13	8	31	31
Mutton	5	3	2	19	19
Total	74	50	24	162	162

Table 2
Antibiogram profile of *E. coli* against different antibiotics

Antibiotic name	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	67 (41.3)	16 (9.8)	79 (48.7)
Ceftriaxone	57 (35.1)	24 (14.8)	81 (50)
Chloramphenicol	79 (48.7)	30 (18.5)	53 (32.7)
Ciprofloxacin	54 (33.3)	34 (20.9)	74 (45.6)
Doxycycline	63 (38.8)	30 (18.5)	69 (42.5)
Gentamycin	73 (45)	17 (10.4)	72 (44.4)
Norfloxacin	78 (48.1)	25 (15.4)	59 (36.4)
Ofloxacin	83 (51.2)	18 (11.1)	61 (37.6)
Streptomycin	33 (20.3)	33 (20.3)	96 (59.2)
Tetracycline	65 (40.1)	27 (16.6)	70 (43.2)
Co-trimoxazole	78 (48.1)	16 (9.8)	68 (41.9)
Cefixime	55 (33.9)	29 (17.9)	78 (48.1)

Researchers have observed that food animals fed with antibiotics at lower concentration to promote growth may be the causative factor of developing resistance in bacteria[1,3]. The antibiotic resistance noticed in *E. coli* from meat products could be linked with the above observation, though firm evidences are needed to confirm the same. On the other hand, majority of the isolates were susceptible to ofloxacin (51.2%), chloramphenicol (48.7%), norfloxacin (48.1%), co-trimoxazole (48.1%), gentamicin (45%), ampicillin (41.3%), tetracycline (40.1%), doxycycline (38.8%), ceftriaxone (35.1%), cefixime (33.9%), ciprofloxacin (33.3%) and streptomycin (20.3%). Noticeably, 115 (71%) *E. coli* isolates have multidrug resistance. This result shows an increased antibiotic resistance among *E. coli* due to indiscriminate use of antibiotic. Among these isolates, 21 (12.9%) *E. coli* strains were 100% resistant to all the 12 antibiotics. Similar results were observed and it was suggested that access to clean water, health education to sellers and proper disposal of waste materials are more important to improve the quality of food[12].

Conclusion

The results of this study revealed that ready-to-eat street foods which are cheap and economically convenient to buy, are not healthy due to *E. coli* contamination. Multidrug resistance in *E. coli* emphasized the need for creating awareness among public regarding self-medication of antibiotics and proper actions to be addressed to improve food safety.

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