

PREVALENCE OF *E. COLI* WITH SPECIAL CONCERN TO SHIGA-TOXIGENIC *E. COLI* O157 AND O111 IN STREET-VENDED SANDWICHES

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Abstract

Escherichia coli is one of the predominant enteric species in the human gut. However, there are small groups of *E. coli*, sometimes referred to as pathogenic *E. coli* that can cause severe diarrheal diseases in humans. Shiga toxigenic *Escherichia coli* (STEC), is one of the pathogenic *E. coli*, and certain strains appear to be of greater virulence for humans, especially those belonging to serogroups O111 and O157. Meat based sandwiches are well-recognized sources of *E. coli*. Ninety meat based sandwiches from street vendors (30 of each of liver, kofta and hawawshy) were collected in Assiut city. Twenty one (23.3%) of the examined sandwiches were contaminated with *E. coli*. Duplex PCR was used targeting for portions of the *rfb* (O-antigen-encoding) regions of *E. coli* serotypes O157 and O111, generating PCR products of 259 and 406 bp, respectively, that is specific for STEC, the share of STEC presence were 12 out of 90 (13.3%) of the examined samples. Overall, the obtained results detection of STEC in the street vended sandwiches was of a great importance as an indication of the unhygienic practices that followed, so it is advised to conduct further heating before consumption and need of health education programs as well as hygienic practice for the food handlers and vendors.

Keywords: *E. coli*, STEC, *rfb* regions, street vended sandwiches

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1. INTRODUCTION

The consumption of foods contaminated with food borne pathogenic microorganisms and microbial toxins are responsible for deaths, illnesses, hospitalization, and economic losses. Due to their widespread nature, food borne diseases (FBD), especially gastrointestinal infections, have negative effects on human health. The Food and Agricultural Organization defines street foods as ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers, especially in streets and other similar public places.¹ The hygienic aspects of vending operations are a major source of concern for food control officers; Stands are often crude structures, and running water and toilets may not be readily available. The washing of hands, utensils, and dishes is often done in buckets or bowls. Refrigeration is unavailable and disinfection is rarely carried out. Flies, insects and rodents may be attracted to sites where there is no sewage disposal.² The majority of street food vendors are uninformed of good hygiene practices (GHP).

Escherichia coli is an important component of the intestinal microflora of humans and warm-blooded mammals. While *E. coli* typically harmlessly colonizes the intestinal tract, several *E. coli* clones have evolved the ability to cause a variety of diseases within the intestinal tract and elsewhere in the host. Those strains that cause enteric infections are generally called diarrheagenic or pathogenic *E. coli* strains. *E. coli* is a member of the faecal coliform group and is a more specific indicator of faecal pollution than other faecal coliform. These types of *E. coli* are generally classified into 6 subgroups including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC).³ The EHEC strains are one of the subsets of Shiga toxin (Stx)-producing *E. coli* (STEC) strains. Shiga toxin-producing *Escherichia coli* (STEC, Vero cytotoxin-producing *Escherichia coli*, VTEC) strains are capable of producing Shiga toxins (which include verotoxin) are the most

important cause of acute renal failure in childhood due to haemolytic uremic syndrome (HUS). The strains that produce HUS and/or haemorrhagic diarrhoea and/or and thrombotic thrombocytopenic purpura (TP) are termed enterohemorrhagic *E. coli* (EHEC) strains. Shiga toxin-producing *Escherichia coli* (STEC) strains have emerged as important human enteric pathogens. Most human infections caused by STEC result from the consumption of contaminated foods, especially those of bovine origin, such as undercooked ground beef and unpasteurized cows' milk, and by person-to-person contacts. Within the STEC family, certain strains appear to be of greater virulence for humans, for example, those belonging to serogroups O111 and O157. The principal strain is *E. coli* O157:H7 and the other important non-O157 STEC among EHEC are as serogroups O111 and O26. Domestic ruminants, mainly cattle, have been implicated as the principal reservoir of the STEC pathogens. STEC strains that are pathogenic to humans have been shown to belong to a broad range of O serogroups. Nevertheless, it seems that especially enterohaemorrhagic *Escherichia coli*-related food-borne illness O157 and O111 are responsible for the majority of severe cases.⁴ Ruminants are the primary reservoirs of STEC and human infection is through consumption or contact with contaminated foods such as vegetables, fruits.⁵

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.⁶

Few researches on detection of STEC in ready to eat sandwiches especially street vended sandwiches, so this study is carried out for detection of *E. coli* contamination in street vended sandwiches which is a good indication of fecal contamination. Also for specific detection of STEC belonging to serogroups O157 and O111 as important food-borne pathogens which can cause severe disease using Duplex PCR assay.

2. MATERIALS AND METHODS

Collection and Preparation of the sample:

Ninety Ready to eat meat based sandwiches samples of liver, kofta and hawawshy (30 of each) were collected from street vendors in Assiut city. Samples were transported to the laboratory and microbiological analysis was carried out immediately.

Isolation and identification of *E. coli*

Ten grams of each sample were mixed in 90 ml brain heart infusion broth and incubated at 35° C for 20–24 hours then streaked on MacConkey agar for 24 hrs at 35° C. The lactose positive colonies were picked and streaked on EMB agar and incubated at 35°C for 24 hours. The green metallic sheen colonies were considered to be *E. coli*. Identification of *E. coli* was done by biochemical test as TSI, IMViC, catalase and sugar fermentation.⁷

Identification of STEC serogroups O157 and O111:

The identified *E. coli* isolates by conventional methods were examined for detection of STEC serogroups O157 and O111 by duplex PCR assay with the use of specific primer sets.

DNA template preparation

DNA was extracted from the test strains using boiling method. One millilitre of a broth culture was centrifuged at 15,000 rpm for 3 min. The cell pellet was suspended in 200- μ L sterile distilled water and vortexed vigorously. The cell suspension was boiled for 10 min; cooled at -20°C for 10 min then centrifuged at 15000 rpm for 3min. The supernatant was collected and used as the DNA template solution for the duplex PCR.

Oligonucleotide primers

Oligonucleotides primers used for PCR were synthesized by Roche, Germany, based on published data.⁸ Table 1 summarizes the primer pairs sequences. The primer pair, O157F and O157R, was targeted *rfbE* gene specific to *E. coli* O157 producing an amplicon of 259 base pairs. The primer pair, O111F and O111R, was targeted *rfb* gene specific to *E. coli* O111 producing an amplicon of 406 base pair.

Table 1: primer pairs used for detection of *E.coli* O157 and O111 the Duplex PCR

Primer	Sequence	specificity	Amplicone size
O157F O157R	CGGACATCCATGTGATATGG TTGCCTATGTACAGCTAATCC	nt 393–651 of <i>rfbE</i> O157:H7	259
O111F O111R	TAGAGAAATTATCAAGTTAGTTCC ATAGTTATGAACATCTTGTTTAGC	nt 24–429 of ORF 3.4 of <i>E.coli</i> O111 <i>rfb</i> region	406

Table 2: Prevalence of *E.coli* in the examined street vended sandwiches meat samples

Samples	No. of the examined samples	No. of positive samples	
		No.	%
Liver	30	9	30.0%
Kofta	30	8	26.6%
Hawawshy	30	4	13.3%
Total	90	21	23.3%

Table 3: Prevalence of STEC serogroups O157 and O111 in the examined street vended sandwiches samples

Samples	Incidence of <i>E.coli</i> O157		Incidence of <i>E.coli</i> O111		Total	
	No	%	No	%	No.	%
Liver	3	10%	3	10%	6	20%
Kofta	Nd	0	6	20%	6	20%
Hawawshy	Nd	0	Nd	0	Nd	0
Total	3	3.3%	9	10%	12	13.3%

N d: not detected

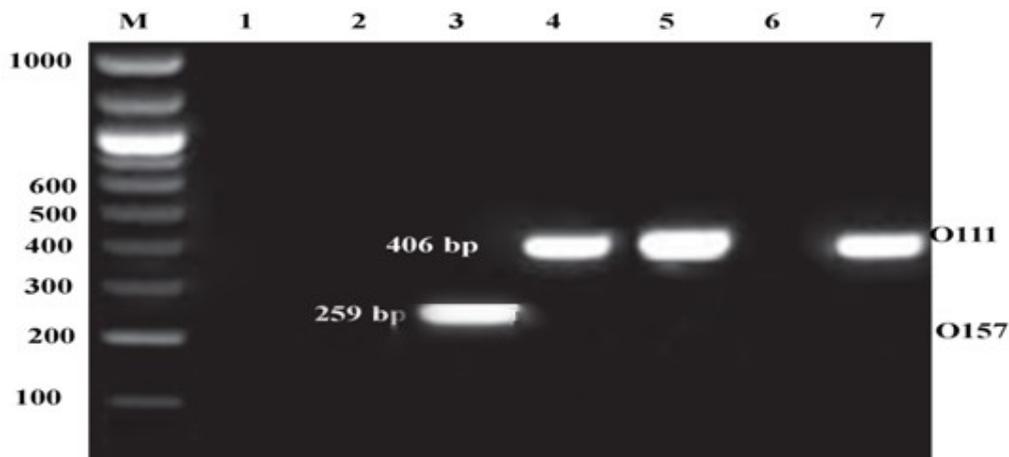


Fig. (1). Duplex-PCR amplification patterns of isolate of *E.coli* analyzed by 1% agarose gel electrophoresis. Lanes M: molecular weight marker (100 bp); 1 and 6 negative *E.coli* O111 and O157; lane 3, Positive *E. coli* O157; lane 4, 5 and 7 positive *E.coli* O111 strains.

Duplex PCR technique

The duplex PCR amplification was performed using a Techne cyclogene 48-Well Thermal Cycler. The duplex PCR reaction mixture was included: 25uL of master mix, 200 nM of each primer (forward and reverse), 4ul of template, and water to make final volume of 50 uL. A

negative control was containing sterile distilled water instead of the DNA template.

Amplification conditions was carried out as follows: initial denaturation at 95°C for 10 min; 30 cycles of 94°C for 40 s, 60°C for 40 s and 72°C for 1 min; and a final elongation step at 72°C for 7 min. Amplicons were visualized

after running at 100V for 1 h on a 1% agarose gel containing ethidium bromide. A 100 bp DNA ladder (Genscript, NJ, USA) was used as a size marker.

3. RESULTS AND DISCUSSION

The presence of *E. coli* and other *Enterobacteriaceae* such as *Enterococcus faecalis* in food is indicative of faecal contamination⁹ and suggests poor hygiene during preparation, handling and storage, lack of reheating and improper vending temperatures.

The prevalence of *E. coli* in the examined street vended meat based sandwiches samples as shown in table (2) were 21 out of 90 examined samples (23.3%). The examined liver sandwiches showed the highest contamination incidence (30%; 9/30) followed by kofta sandwiches (26.6%; 8/30), while the examined hawawshy sandwiches showed the lowest incidence (13.3%; 4/30). *E. coli* may contaminate sandwiches during processing through contamination and/or faecal material as a result of poor sanitary practices, improper handling and improper hygiene conditions.² Relatively lower rate of *E. coli* (2.96%) found by Büyükyörük et al.¹⁰ and they referred the main reason for this low rate might be the high temperature application used by the vendors to make sandwiches just before collecting samples.

On the contrary, much higher incidence was recorded by Mugampoza et al.¹¹ who detected *E. coli* at 100% of the examined street vended food samples, and Bostan et al.¹² and Fang et al.¹³ detected *E. coli* in RTE foods at rates of 71.9% (n=96) and 88% (n=50), respectively.

Shiga toxin-producing *Escherichia coli* (STEC) are of significant public health threats. Although STEC O157 are recognized food borne pathogens, non-O157 STEC such as O111 are also important causes of human disease. Centre for Infectious Diseases and Prevention identified that non-O157 groups have been responsible for over 70% of EHEC associated illness.⁶

Table 3 showed the prevalence of STEC serogroups O157 and O111 in the examined street vended sandwiches samples by using Duplex PCR. Twelve out of 90 (13.3%) samples were contaminated with STEC. The incidence of *E. coli* O157 was 10 % (3/30) in the examined liver sandwiches, and the incidence of *E. coli* O111 in the examined liver and kofta sandwiches were 10% (3/30) and 20% (6/30), respectively. *E. coli* O157 not detected in the examined kofta and hawawshy sandwiches samples and *E. coli* O111 not detected in hawawshy sandwiches samples.

Molecular methods based on PCR amplification are reliable alternatives to conventional serogrouping technique for the detection of the target pathogens in meat samples. As shown in Figure1. Duplex-PCR amplification pattern was used for detection of STEC serogroups O157 and O111 in the examined samples targeting for portions of the *rfb* (O-antigen-encoding) regions of *E. coli* serotypes O157 and O111, generating PCR products of 259 and 406 bp, respectively. Rasheed et al.¹⁴ couldn't isolate STEC pathogen from the examined street vended samples and they stated that efficient cooking and other good hygienic prophylactic measures are needed to decrease the incidence of STEC in food items. Also a quantitative microbial risk assessment carried out on *E. coli* O157 in beef burgers produced in the Republic of Ireland concluded that burgers cooked were rare constituted a significant risk to the consumer,¹⁵ while Pavithra and Ghosh¹⁶ found 36 Out of 215 samples from fast foods, meat shops and fish stalls samples (16.7%) were identified with *E. coli*; twelve out of 100 samples from fast foods. Out of 36 *E. coli* positive samples, nine (25%) possessed the gene encoding shiga toxin (*stx 1*) gene including two samples from fast food at two different time schedule.

4. CONCLUSIONS

In conclusion the results obtained from this research proved that RTE street vended meat based sandwiches are important source of

STEC and represents a potential threat on the public health. So it is advised to conduct further heating before consumption and need of health education programs as well as hygienic practice for the food handlers and vendors.

5. REFERENCES

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