Molecular characteristics of *E. coli* contaminating from meat products

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ABSTRACT

A total number of 200 meat products samples (50 each of frozen weight of 25 gram of minced meat, frozen beef burger, frozen kofta and traditional Egyptian sausage) were collected from different markets in Cairo and Giza governorates and examined by multiplex polymerase chain reaction method for detecting virulence genes of Escherichia *coli* strains previously isolated from frozen minced meat, frozen beef burger, frozen kofta and traditional Egyptian sausage. The present study detected the virulence genes *stx*1, *stx*2, *eae*A and *hly*A genes among 10 *Escherichia coli* strains. Results revealed that presence of one or more virulence genes in *E. coli* strains isolated from the examined meat products.

KEY WORDS: *E. Coli*, meat product, PCR, virulence genes.

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1. INTRODUCTION

Culture methods are labor-intensive and time-consuming while PCR assay based on the sequence of the *E. coli* gene ensure highly sensitive and specific results *(China, et al 1996)*. Molecular methods detecting virulence genes have been developed to overcome this problem. Most of these methods detect the virulence genes of *Escherichia coli* (Cebula *et al*., 1995; Feng and Monday, 2000; Fortin *et al*., 2001; Gannon *et al.,* 1997; Guion *et al*., 2008; Oberst *et al.,* 1998; Sharma, 2002; Sharma and Dean-Nystrom, 2003; Yoshitomi *et al*., 2006). The production of Shiga toxin (Stx) is a characteristic trait of Shiga toxin-producing Escherichia coli (STEC), of which there are several hundred known serotypes, many of which have not been implicated in illness. A subset of STEC, referred to as enterohemorrhagic E. coli (EHEC), is comprised of pathogenic strains and includes serotype O157:H7, a recognized pathogen worldwide, as well as others, such as O26:H11, O111: H8, and O103:H2, that also cause human infections (Karsh *et al.,* 2005).

The aim of this article is to detect the virulence genes in previously isolated E. coli strains from frozen minced meat, frozen beef burger, frozen kofta and traditional Egyptian sausage.

2. MATERIALS AND METHODS

2.1. Materials used for PCR:

Reagents used for agrarose gel electrophoresis: Agarose powder, Biotechnology grade (Bioshop[R], Canda inc. lot No: OE16323). It prepared in concentration 2% inTAE buffer. Tris acetate EDTA (TAE) electrophoresis buffer (50×liquid concentration) (Bioshop[R], Canda inc. lot No: 9E11854). The solution diluted to 1× by adding 1 ml stock solution t049 ml double dist. Water to be used in the preparation of the gel or as a running buffer. Ethedium bromide solution (stock solution) biotechnology grade (Bioshop ® Canda Inc, Lot No: 0A14667): The stock solution was diluted by 25µl /200ml double distilled water and stored covered at 4ºC. It was used for staining of PCR products that electrophoreses on agarose gel to be visualized by UV light. Gel loading buffer (6×stock solution) (Fermentas, lot No: ooo56239). The components were dissolved in sterile double distilled water and stored covered with aluminum foil at room temperature. DNA ladder (molecular marker): 100 bp (Fermentas, lot No: 00052518). 5X Taq master (Fermentas): Containing polymerase enzyme, Magnesium chloride (Mg Cl2), Deoxy nucleotide triphosphate (dNTP) and PCR grade water.

2.2. Primer sequences of E. coli used for PCR identification system:

Application of PCR for identification of shiga toxins (*stx*1 & *stx*2), intimin (*eae*A) and

haemolysin (*hyl*A) genes of *E. coli* was performed by using primers (Pharmacia Biotech) as shown in the following table 1.

2.3. *DNA Extraction using QIA amp kit (Shah* et al., *2009):*

After overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100ºC for 20 minutes. Accurately, 50-200 µl of the culture were placed in Eppendorf tube and the following steps were carried out: Equal volume from the lysate (50-200 µl) was added, addition of 20-50µl of proteinase K, then incubation at 56 ºC for 20-30 min. After ncubation, 200 µl of 100% ethanol was added to the lysate. The solution was added to the column and centrifuged at 8000 rpm for 1 min. then the filtrate was discarded. The sediment was washed using AW1 buffer (200 µl), the column was centrifuged at 8000 rpm / 1 min, and the filtrate was discarded. Washing was applied by using the AW2 buffer (200µl), the column was centrifuged at 8000 rpm / 1 min. and the filtrate was discarded. The column was placed in a new clean tube then, 25-50 µl from the Elution buffer was added, centrifuged at 8000 rpm/1min. Then the column was discarded. The filtrate was put in clean tube containing the pure genomic DNA Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

2.4. *Amplification reaction of E. coli (Fagan et al., 1999):*

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR assays were carried out in 1 ml of nucleic acid template prepared by using reference EHEC isolates (approximately 30 ng of DNA), 10 mM Tris-HCl (pH 8.4), 10 mM KCl, 3 mM MgCl2; 2 mM concentrations of each primer, 0.2 mM concentrations of each 29-deoxynucleoside 59-triphosphate, and 4 U ofAmpliTaq DNA polymerase (Perkin-Elmer). Amplification conditions consisted of an initial 95°C denaturation step for 3 min followed by 35 cycles of 95°C for 20 secs, 58°C for 40 s, and 72°C for 90 sec. The final cycle was followed by 72°C incubation for 5 min. The reference strains were *E. coli* O157:H7 Sakai (positive for stx1, stx2, eaeA and hylA) and *E. coli* K12DH5α (a nonpathogenic negative control strain) that does not possess any virulence gene (kindly from Prof. Mohamed Hassan, Fac. Vet. Med., Benha Univ.). Amplified DNA fragments were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

Table (1). Primer sequences of *E. coli* virulence genes:

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stx1 (F)	5′ ACACTGGATGATCTCAGTGG ′3		Dhanashree and Mallya (2008)
Stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3	614	
Stx2 (F)	5′ CCATGACAACGGACAGCAGTT ′3		Dhanashree and Mallya (2008)
Stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3	779	
eaeA (F)	5′ GTGGCGAATACTGGCGAGACT ′3		Mazaheri et al. (2014)
eaeA (R)	5′ CCCCATTCTTTTTCACCGTCG ′3	890	
hylA (F)	5′ ACGATGTGGTTTATTCTGGA ′3		Fratamico et al. (1995)
hylA (R)	5′ CTTCACGTGACCATACATAT ′3	165	

Table (2) Component of PCR Master Mix.

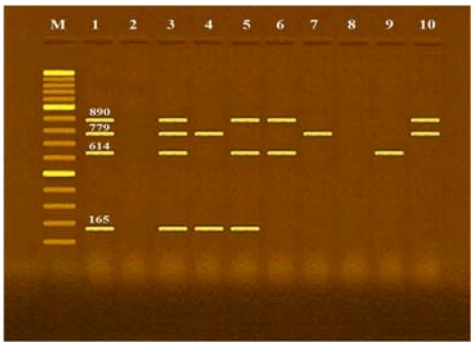
Component	Volume / Reaction
Emerald Amp GT PCR mastermix (2x premix)	12.5 *µl*
PCR grade water	4.5 *µl*
Forward primer (20 pmol)	1 *µl*
Reverse primer (20 pmol)	1 *µl*
Template DNA	6 *µl*
Total	25 *µl*

Table (3) Cycling conditions of the different primers during PCR.

Target gene	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension
stx1	95˚C 3 min.	95˚C 20 sec.	58˚C 20 sec.	72˚C 1.5 min.	72˚C 5 min.
stx2	95˚C 3 min..	95˚C 20 sec.	58˚C 20 sec.	72˚C 1.5 min.	
eaeA	95˚C 3 min.	95˚C 20 sec.	58˚C 20 sec.	72˚C 1.5 min.	
hylA	95˚C 3 min.	95˚C 20 sec.	58˚C 20 sec.	72˚C 1.5 min.	

3. RESULTS

Results from photo (1) the electrophotoretic pattern of *E. coli* by using specific pair primers specific to *(stx1)* with amplification of 614-bp, *stx2* (779bp), *eaeA* (890bp) and *hylA* (165bp) genes and table (4) Occurrence of virulence genes of diarrheagenic *E. coli* isolated from the examined meat products, show that O26 Positive strains for stx1, stx2, eaeA and hly genes, O55 Positive strain for stx2 and hly genes, O111 Positive strain for stx1, eaeA and hly genes, O114): Positive strains for stx1 & eaeA genes, O119 Positive strain for stx2 gene, O125 Positive strain for stx1 gene, O125 Positive strain for stx2 & eaeA genes and O124 Negative strain for stx1, stx2, eaeA and hly genes.



Photograph (1): Agarose gel electrophoresis of multiplex PCR of stx1 (614 bp), stx2 (779 bp), eaeA (890 bp) and hly (165 bp) genes for characterization of *Enteropathogenic E. coli.* Lane M: 100 bp ladder as molecular size DNA marker. Lane 1: Control positive *E. coli* for stx1, stx2, eaeA and hly genes. Lane 2: Control negative. Lane 3 (*E. coli* O26): Positive strains for stx1, stx2, eaeA and hly genes. Lane 4 (*E. coli* O55): Positive strain for stx2 and hly genes. Lane 5 (*E. coli* O111): Positive strain for stx1, eaeA and hly genes. Lanes 6 (*E. coli* O114): Positive strains for stx1 & eaeA genes. Lane 7 (*E. coli* O119): Positive strain for stx2 gene. Lane 8 (*E. coli* O124): Negative strain for stx1, stx2, eaeA and hly genes. Lane 9 (*E. coli* O125): Positive strain for stx1 gene. Lane 10 (*E. coli* O125): Positive strain for stx2 & eaeA genes.

Table (4): Occurrence of virulence genes of diarrheagenic *E. coli* isolated from the examined meat products.

E. coli Serovars	*Stx*1	*Stx*2	*eae*A	hylA
O26 : H11	+	+	+	+
O55 : H7	-	+	-	+
O111 : H2	+	-	+	+
O114 : H21	+	-	+	-
O119 : H4	-	+	-	-
O124	-	-	-	-
O125 : H21	+	-	-	-
O128 : H2	-	+	+	-

Stx1: Shiga- toxin 1 gene, Stx2: Shiga- toxin 2 gene, Eae: intimin gene and hylA: haemolysin gene.

4. DISCUSSION

Photo (1) showed the electrophotoretic pattern of *E. coli* by using specific pair primers specific to *(stx1)* with amplification of 614-bp, *stx2* (779bp), *eaeA* (890bp) and *hylA* (165bp) genes.

Results obtained from Table (4) revealed that occurrence of one or more virulence genes in *E. coli* strains isolated from the examined meat products. *E. coli O26:H11* have the 4 virulence genes, while O124 have none of the virulence genes. The other strains express one (*E. coli O119:H4* and *O125:H21*) or more virulence genes (*O55:H7, O111:H2, O114:H21* and *O128:H2)*. *E. coli* strains possess that genes were more toxigenic and hazardous to consumer health more than other types which does not contain that gene. The combination of serotyping and *stx* genotyping was found useful for identification and for assignment of food-borne *E. coli* to groups with potential lower and higher levels of virulence for humans *(Beutin, et al 2007)*. Similar results were obtained by *Helmy (2003)*, *Beutin, et al (2007)* and *Pradel, et al (2008)*.

The major characteristic of *E. coli* linked to virulence is the production of one or more virulence genes (*Stx*1*, Stx*2*, eae*A and *hyl*A) Toxin and adherence factors may be necessary to cause disease *(Tizpori, 1987* and *Paton and Paton, 1998)*. Culture methods are labor-intensive and time-consuming while PCR assay based on the sequence of the *E. coli* gene ensure highly sensitive and specific results *(China, et al 1996)*.

The DNA sequences of the genes in the O-antigen gene clusters can be utilized to design PCR based assays for the detection or identification of specific *E. coli* serogroups. PCR assays were developed to detect or identify *E. coli* on the basis of the virulence genes. The use of the PCR assays provides the ability to detect, identify, and type this serogroup, eliminating the use of the more labor-intensive serotyping procedure. *(Pradel, et al.,2008)*.

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