J. Agric. Sci. Mansoura Univ., 34 (5): 4551 - 4563, 2009

DETECTION OF E.COLI O157:H7 IN MEAT AND ITS PRODUCTS BY CONVENTIONAL AND REAL TIME PCR TECHNIQUES

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ABSTRACT

A total of 68 samples including (raw minced meat, Luncheon, Pasterma, and sandwiches of cooked beef, cooked liver, cooked sausage, kofta and burger) were randomly collected from butchers, take away meals' markets and street vendors in different regions of Great Cairo (Cairo, Giza and Shobra El-Khema). The evaluation of microbial load of the collected samples revealed that, no samples contained Total Bacterial Count (TBC) more than the permissible limits, 26.5%, 10.3%, 10.3%, 5.9%, 35.4% and 86.8% of the examined samples contained Total Fungal Count (TFC), Total Coliform Count (TCC), E.coli (Faecal coliform count), Staphylococcus count, Salmonella and Bacillus cereus counts (BC) more than the permissible limits, respectively.

The E.coli (Faecal coliform) positive samples (7) together with another 7 samples contained the highest cfu/g of TCC were selected to be tested with Standard (St) as well as Real Time (RT) PCR techniques to detect the presence of E.coli serotype O157:H7.

The result of St Polymerase Chain Reaction (PCR) assay indicated positive results (specific bands) in 78.6% of the examined samples. On the other hand, by using specific RT PCR Kit for the detection and differentiation between E.coli O157:H7 and other E.coli serotypes, the obtained results revealed that, all St PCR positive samples showed a separate plateau differed from that obtained from the E.coli O157:H7 positive control sample, indicating that the present serotype (E.coli O55:H7 as clarified by the kit user manual) has nearly the same primer sequence as E.coli O157:H7 which led to false positive result using St PCR technique. This result indicated that, RT PCR is considered as an important, specific and accurate method for the detection and identification of food poisoning organisms.

INTRODUCTION

The term "street foods" describes a wide range of ready-to-eat foods which are mainly prepared in public places, notably streets. Like fast foods, the final preparation of street foods occurs when the customer orders the meal which then can be consumed where it is purchased or taken away. Street foods and fast foods are low in cost compared to restaurant meals and offer an attractive alternative to home-cooked food (FAO, 1986). Microbial quality of these foods must undergo strict monitoring schemes as it is considered -in most cases- as the main cause of food poisoning outbreaks.

EOS (2005) stated that the permissible limits of microbial loads for different food categories are summarized as follows:

- Total Aerobic Count must not exceed 10^6 cfu/g in minced meat, kofta and sausage, while in both luncheon and pasterma the number should not

exceed 10^4 cfu/g. On the other hand, it should not be more than 10^3 cfu/g in burger.

- Total Spore forming anaerobes; must not exceed 10^2 cfu/g in all food categories under study except for both luncheon and pasterma which must be free.
- Total Yeast and Mould counts must not be more than 10^2 cfu/g in all food categories under study.
- Total coliform count must not be more than 10^2 cfu/g except for pasterma which must be free.
- Total count of *staphylococcus aureus* bacteria must not be more than 10^2 cfu/g except for both Luncheon and Pasterma which must be free.
- All products must be free from *E. coli*, *Salmonella, Bacillus cereus* and other disease causing microorganisms.

Escherichia coli is a gram-negative bacterium that generally inhabits the intestinal tract of humans and animals. However, some of isolates of this organism are pathogenic, and these
enterovirulent *E. coli* isolates are important food-borne pathogens associated with severe gastrointestinal and circulatory system diseases, such as hemorrhagic colitis (HC), hemorrhagic-
uremic syndrome (HUS), and thrombotic thrombocytopenic purpura, in humans (Jones, (1999), Karmali, (1989). *E. coli* O157:H7 is a major strain which causes these kinds of food-borne outbreaks all over the world. In 1975, *E. coli* O157:H7 was first isolated from clinical samples, but it was not reported in association with outbreaks until 1982 (Karch *et al.,* (1999). In 1996 there were some large outbreaks in Japan which originated in Sakai City, Osaka (Michino *et al.,* 1999). These outbreaks affected more than 17,000 people. A total of 106 children developed HUS, and 13 of them children died (Karch *et al.,* 1999).

Similar outbreaks have been reported in Australia, Canada, the United States, various European countries, and Africa (Effler, *et al.,* (2001), Fegan and Desmarchelier. (2002), Galanis *et al.,(* 2003), Michino *et al.,* (1999), Rocchi, and Capozzi. (1999), Sharma and Carlson. (2000). The pathogenicity of *E. coli* O157:H7 is associated with a number of virulent factors, including Shiga-like toxins 1 and 2 (encoded by the *stx*1 and *stx*2 genes, respectively) and intimin (encoded by the *eaeA* gene). Shiga-like toxins are believed to play a major role in the pathogenesis of HC and HUS through a cytopathic effect on the vascular endothelial cells of the kidneys and intestines (Sharma *et al.,* 1999). Strains isolated from patients with HC usually produce both Shiga-like toxins 1 and 2, and strains that produce only *stx*1 are uncommon (Guan and Levin. 2002).

Cattle are generally considered the major reservoir for this organism, although it has also been isolated from sheep (Kudva *et al.,* 1996), goats (Bielaszewska *et al.,* 1997), dogs, deer, horses, and seagulls (Karch *et al.,* 1999). An important aspect of this organism is the fact that the ingestion of 10 to 100 of these organisms may be sufficient to cause an infection.

Among the most important sources of human infection are direct contact with cattle and other ruminants, contaminated bathing water,

contaminated beef products, unpasteurized milk, vegetables, fruits, and drinking water contaminated with waste materials (Eva and Marianne. 2003). The detection and the correct identification of this strain are important parts of food hygiene.

Traditional methods for the identification of *E. coli* O157:H7, such as biochemical and serotype tests, carried out from 5 to 7 days.

In recent years, some molecular methods were developed to detect and identify this food-borne pathogen, such as PCR and enzyme-linked immunosorbent assay. PCR is a rapid and easy to use method and can provide a preliminary characterization (Davis *et al.,* (2003), Fortin *et al.,* (2001). The use of the PCR method to detect pathogens, however, has some shortcomings, such as some false-positive or false-negative results for more complex samples and a low sensitivity with more primer sets. At the same time, the ethidium bromide used to stain the electrophoresis gel after PCR is a harmful chemical and its application is time-consuming.

The TaqMan detection system is a new qualitative and quantitative system that uses a fluorogenic hybridization probe to detect the target genes; and it has previously been demonstrated to be a rapid, high-throughput, semiautomatic PCR scheme for the identification of *E. coli* (Oberst *et al.,* (1998), Sharma *et al.,* (1999), *Salmonella* (Sharma and Carlson. (2000), and *Listeria* spp. (Bassler *et al.,* (1995).

The objective of this study is to screen the microbiological quality of food derived from meat and its products, along with detection of the incidence of E.coli O157:H7 in it. In addition to assessing the utility of the TaqMan PCR system for the detection and identification of the *stx*2 gene (which is responsible for the biosynthesis of Shiga-like toxin 2) of *E. coli* O157:H7 in different meat and meat products' samples.

MATERIALS AND METHODS

Materials:

1- Samples:

Meat and meat products' samples were collected from Egyptian local market and street vendors. The distribution details are illustrated in Tables (1).

2- *Escherichia coli* O157:H7 strain:

Escherichia coli O157:H7 was isolated and was chemically, serologically and molecular biologically identified and kindly supplied by Food Safety and Biotechnology Laboratory, RCFF, ARC, Giza Egypt.

3- Primers:

Forward, reverse primers and prope sequences of the used *Stx2* gene are illustrated in Table (2).

Table (1): Distribution of the sources of collected samples:

Sample type / Source		Giza	Cairo	Qualioubeya	Total
Raw		2	1	1	4
Total		2	1	1	4
Cooked	Liver	7	4	5	16
	Sausage	8	4	6	18
	Kofta	7	3	0	11
	Burger	1	0	0	1
Total		23	11	11	45
Processed	Luncheon	5	3	2	10
	Pasterma	4	2	3	9
Total		9	5	5	19
All					68

Table (2): Sequences of Primers' and probe sequences of used in this study.

Item	Sequence
Stx2 *Forward primer*	ACC ACA TCG GTG TCT GTT ATT AAC C
Stx2 *Reverse primer*	CGG TAG AAA GTA TTT GTT GCC GTA TT
Stx2 *Prope sequence*	TTT GCT GTG GAT ATA CGA GGG CTT GAT GTC TAT

Methods:

Culture media used in the microbiological assay:

Tryptic glucose agar medium was used for enumeration of total microbial counts, Malt extract agar medium was used for yeast and mold count, McConkey broth purple was used for the enumeration of coliforms and violet red agar was prepared for the determination of fecal *Escherichia coli.* Some pathogenic bacteria were confirmed on selective media such as; *Bacillus cereus* agar used for enumeration of *Bacillus cereus*, Baird parker agar used for enumeration of *Staphylococcus aureus*, Brillient green agar used for counting of *Salmonella* spp. and Tetrathionate broth and Bismuth sulphite agar for isolation and identification of Salmonella spp. (Difco, 1984).

DNA extraction from the examined samples:

All tested samples were enriched in Buffered Peptone Water (BPW) for 24 hrs at 37ºC before being subjected to DNA extraction. DNA extraction was performed using Prepman Ultra sample preparation reagent, (Applied Biosystem, USA), according to the user manual attached to the Kit as follows:

One ml from the enriched BPW was transferred to 2 ml microcentrifuge tube and spinned for 3 min at 16000 rpm. The resulted supernatant was discarded and the obtained pellet was eluted using 200 ul of Prepman Ultra sample preparation reagent. The tube then was incubated in a boiling water bath for 10 min with frequent mixing. After reaching room temperature, the tube was centrifuged at 16000 rpm for 3 min, then 50 ul from the supernatant was transferred to a new labeled microcentrifuge tube (Ligozzi and Fontana, 2003).

TaqMan RT-PCR assay for detection of *E. coli* O157:

PCR was performed in a reaction mixture with a total volume of 25 ul containing 1 ul of extracted DNA, 0.5 mM of *stx*2 *E. coli* O157 primers (Table 2), 0.2 mM of fluorogenic probe, and TaqMan Universal Master Mix (Applied

Biosystems). The Master Mix contained AmpErase uracil-*N*-glycosylase (UNG), deoxynucleoside triphosphate with dUTPs, 6-carboxyrhodamine as an internal passive fluorogenic reference, and an optimized buffer component. Amplification and detection were carried out in optical-grade 96-well plates in an ABI Prism 7000 sequence detection system (Applied Biosystems) with an initial step of 50°C for 2 min, which is the required optimal AmpErase UNG enzyme activity, and then at 95°C for 10 min, to activate the AmpliTaq Gold DNA polymerase and to deactivate the AmpErase UNG enzyme, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The reaction conditions for amplification and the parameters for fluorescence data collection were programmed into a Dell laptoplinked directly to the ABI Prism 7000 sequence detection system by using the SDS 1.6 application software, according to the manufacturer's instructions. After real-time data acquisition, the threshold, which was defined as being 10-fold higher than the baseline, was determined; and the cycle threshold (*CT*) value was manually set so that it intersected the amplification curves in the linear
region of the semi log plot (Ching *et al.,* 2005).

Standard PCR assay for detection of *E. coli* O157:

The same primer sequence was used also (forward and reverse only) in applying a conventional PCR assay. Reaction volumes were 50 ul and reaction mixtures consisted of (final concentration) 10mM Tris HCl pH 9, 50 mM KCl, 0.1% Triton X-100, 200 uM of each dNTP (Applied Biosystem, USA), 2 mM MgCl, 1.5 U of Taq polymerase (Applied Biosystem, USA) and 0.7 uM of the stx2 primers (forward and reverse). The thermal profile was done as mentioned before using Biometra thermal cycler, USA. Following amplification, 10 ul of 50% sucrose solution were added to the PCR mixtures resulting in a total volume of 60 ul from which 25 ul were pipette into wells in 1.8% melting agarose (Fisher Scientific, USA). The PCR reaction samples were separated by horizontal gel electrophoresis (Biorad, USA) and digital images were obtained using gel documentation system, USA (Guan and Levin, 2002).

RESULTS AND DISCUSSION

Microbial Content of the examined samples:

Data in Table (3) illustrate the microbial content of raw and processed beef samples collected from Giza Governorate. It is clear that, all collected raw meat were unfit for consumption as one of them contained TFC, E.coli, BC and Salmonella and the other contained E.coli and Salmonella more than the permissible limits. Also, none of the examined processed meat samples were fit for consumption as all exceeded the permissible limits in one or more of the analyzed parameter(s). It is clear also from this table that, Pasterma samples contained TFC more than the permissible limits which may be attributed to using certain types of spices which contained high numbers of TFC and also may be due to long storage period during processing. It was noticed that, BC was found in all processed meat samples

which indicated bad hygienic measures during processing, as this type of gram positive bacteria is present in contaminated hands and utensils.

Table (3): Microbiological criteria of raw and processed beef samples collected from Giza governorate:

M.O / S. No.	TBC	TFC	TCC	FC (E.coli)	B.C	Staph.	Salmonella
Raw							
1	$10x10^2$	$13X10^3$	22X10	D	19X10	$27X10^2$	D
2	$35x10^2$	ND	$100X10^2$	D	ND	$27X10^2$	D
Processed							
1 (L)	$30x10^2$	25X10	ND	ND	$20X10^2$	$40X10^2$	ND
2 (L)	$60x10^2$	$12X10^2$	ND	ND	5X10	$14X10^2$	ND
3 (L)	$10x10^2$	$12X10^2$	5X10	ND	10X10	20X10	D
4 (L)	$20x10^2$	25X10	ND	ND	10X10	$13X10^2$	ND
5 (L)	60x10	8X10	13X10	ND	15X10	35X10	ND
6 (P)	$40x10^3$	$46X10^3$	ND	ND	$15X10^2$	$32X10^2$	ND
7 (P)	$25x10^3$	$80X10^3$	20X10	ND	$30X10^2$	$60X10^2$	D
8 (P)	50x10	$40X10^3$	ND	ND	6X10	ND	ND
9 (P)	$14x10^4$	$80X10^3$	20X10	D	$70X10^2$	40X10	D

M.O= Microorganism S. No.= Sample number TBC= Total Bacterial Count TFC= Total Fungal Count TCC= Total Coliform Count FC= Faecal coliform B.C= Bacillus cereus Staph.= Staphylococcus count D= Detected ND= Not Detected L = Luncheon P= Pasterma

Table (4) shows the microbial content of cooked meat sample in Giza Governorate which revealed that, only one sample (Kofta) out of 23 samples was fit for human consumption and all the other samples contained one or more source(s) of contamination. The inclusion of BC and Staphylococcus count which are incriminated as food poisoning bacteria as well as indicator bacteria in numbers exceeded the permissible limits indicated the bad hygienic measures followed during cooking. Salmonella also was detected in 6 out of 23 samples which indicated cross contamination with contaminated stuffs during cooking.

Table (5) indicates that, the raw meat sample collected from Cairo Governorate was fit for consumption as all measured parameters were within the permissible limits. Concerning processed meat samples, 2 out of 5 samples were fit for human consumption and the other 3 samples contained one or more contaminant(s). High TFC was also noticed in Pasterma samples as in Giza Governorate and bad hygienic measures were cleared by the presence of BC in 3 samples. Although all cooked meat samples in Cairo Governorate were unfit for consumption, they showed better microbial content compared to samples collected from Giza Governorate as about 9 out of 11 (81.8%) of the examined samples contained only one contaminant which was BC indicating non hygienic measures.

Data in Table (6) discusse the microbial content of beef samples collected from Qualioubeya Governorate which showed that, the collected raw meat sample was unfit for consumption as it contained BC bacteria which must not be present in this type of meat. One of the 5 processed meat samples was fit for consumption and the other 4 samples contained one or

more contaminant(s). The inclusion of Total and Faecal Coliform Bacteria in processed meat samples indicated bad hygienic measures during processing and also indicated that the processing procedures could not overcome these types of bacteria in case that they were present in the raw ingredients. All cooked meat samples collected from Qualioubeya Governorate were unfit for consumption as they contained BC (100%), E.coli (9.09%), Salmonella (18.18%) and TFC (18.18%) more than the permissible limits.

Table (4): Microbiological criteria of cooked beef samples collected from Giza governorate:

M.O / S. No.	TBC	TFC	TCC	FC (E.coli)	B.C	Staph.	Salmonella
1 (L)	30x104	6X10	ND	ND	8X10	22X10	ND
2 (L)	$26x10^3$	40x10	ND	ND	9X102	10X103	ND
3 (L)	$12x10^2$	5X10	ND	ND	10X10	10X102	ND
4 (L)	20x10	9X10	ND	ND	15X10	22X10	ND
5 (L)	60x104	100X103	ND	ND	35X103	100X103	ND
6 (L)	$56x10^2$	15X102	ND	ND	10X103	30X102	ND
7 (L)	40x103	20X104	ND	ND	40X103	80X102	ND
8 (S)	$20x10^3$	27X10	ND	ND	6X10	9X10	ND
9 (S)	40x10	20X10	ND	ND	2X10	3X10	ND
10 (S)	32x102	3X10	ND	ND	4X10	3X10	ND
11 (S)	$14x10^2$	20X102	ND	ND	16X10	4X10	ND
12 (S)	20x103	40x103	ND	ND	40X103	14X102	ND
13 (S)	20x103	60x103	ND	ND	20X103	11X103	ND
14 (S)	$50x10^3$	15x104	ND	ND	30X103	20X10	ND
15 (S)	$28x10^3$	ND	3x10	ND	ND	20X102	D
16 (K)	$23x10^2$	$30x10^2$	40x10	D	10X10	12X10	D
17 (K)	$40x10^2$	$80x10^2$	5x10	ND	25X102	25X102	D
18 (K)	27x103	ND	20x102	ND	ND	59X103	D
19 (K)	15x10	8x10	30x10	ND	70X10	5X10	D
20 (K)	$15x10^3$	$30x10^2$	ND	ND	ND	25X102	ND
21 (K)	30x102	95x102	ND	ND	50X10	2X10	ND
22 (K)	24x103	50x103	ND	ND	10X103	15X102	ND
23 (B)	3x10	60x103	18x10	ND	12X10	29X10	D

M.O= Microorganism S. No.= Sample number TBC= Total Bacterial Count TFC= Total Fungal Count TCC= Total Coliform Count FC= Faecal coliform B.C= Bacillus cereus Staph.= Staphylococcus count D= Detected ND= Not Detected L= Liver S= Sausage K= Kofta B= Burger

Generally, data obtained from this study indicated that, 75% of raw, 84.2% of processed and 97.8% of cooked beef samples contained one or more reason(s) to be considered as unfit for human consumption as stated by EOS (2005). Also, 51.5% of the examined samples were unfit due to only one contaminant, 22.1%, 10.3%, 4.4%, 4.4% and 0% were unfit due to the presence of 2, 3, 4, 5 and 6 contaminants respectively. In raw beef samples, 50%, 75%, 50% and 25% of tested samples contained E.coli, BC, Salmonella and TFC more than the permissible limits, while all samples contained TBC, TCC and Staphylococcus count within the permissible limits. All processed meat samples contained TPC within the permissible limits, while 36.8%, 10.5%, 5.3%, 37.7%, 26.3 and 42.1% of the tested samples contained TCC, E.coli, Staphylococcus count, BC, Salmonella and TFC in numbers more than the permissible limits, respectively.

Table (5): Microbiological criteria of Beef samples collected from Cairo governorate:

M.O / S. No.	TBC	TFC	TCC	FC (E.coli)	B.C	Staph.	Salmonella
Raw							
1	13X10	38X10	ND	ND	ND	9X10	ND
Processed							
1 (L)	33X102	25X10	ND	ND	20X102	40X102	ND
2 (L)	40X103	13X10	ND	ND	ND	2X10	ND
3 (L)	25X10	30X10	ND	ND	ND	20X10	ND
4 (P)	100X102	18X103	11X10	ND	26X10	ND	ND
5 (P)	50X103	40X103	ND	ND	19X102	30X102	ND
Cooked							
1 (L*)	15X102	11X10	8X10	D	35X10	11X10	D
2 (L*)	80X102	25X10	2X10	ND	16X102	40X10	D
3 (L*)	30X10	5X10	ND	ND	4X10	17X10	ND
4 (L*)	16X102	ND	ND	ND	100X10	30X10	ND
5 (S)	90X10	6X10	ND	ND	10X10	14X10	ND
6 (S)	11X103	43X10	ND	ND	24X10	30X10	ND
7 (S)	7X10	ND	ND	ND	4X10	14X10	ND
8 (S)	67X10	2X10	ND	ND	65X10	13X10	ND
9 (K)	35X10	30X10	ND	ND	18X102	ND	ND
10 (K)	100X102	21X10	ND	ND	24X10	22X10	ND
11 (K)	40X10	ND	ND	ND	80X10	13X10	ND

M.O= Microorganism S. No.= Sample number TBC= Total Bacterial Count
TFC= Total Fungal Count TCC= Total Coliform Count FC= Faecal coliform
B.C= Bacillus cereus Staph.= Staphylococcus count D= Detected ND= Not Detected
L= Luncheon P= Pasterma L*= Liver S= Sausage K= Kofta

Table (6): Microbiological criteria of Beef samples collected from Qualioubeya governorate:

M.O / S. No.	TBC	TFC	TCC	FC (E.coli)	B.C	Staph.	Salmonella
Raw							
1	80X10	27X10	ND	ND	14X10	17X102	ND
Processed							
1 (L)	23X102	25X10	ND	ND	20X10	30X10	ND
2 (L)	20X10	60X10	ND	ND	4X10	65X10	ND
3 (P)	20X102	50X10	ND	ND	ND	ND	ND
4 (P)	30X103	20X103	16X10	ND	18X102	12X103	D
5 (P)	80X103	25X103	11X10	D	70X102	5X10	D
Cooked							
1 (L*)	80X10	15X10	ND	ND	30X102	3X10	ND
2 (L*)	12X103	25X102	ND	ND	20X102	40X10	ND
3 (L*)	45X10	40X102	ND	ND	100X10	25X10	ND
4 (L*)	25X10	54X10	2X10	ND	4X10	6X10	D
5 (L*)	25X103	25X104	ND	ND	18X102	15X10	ND
6 (S)	23X102	30X10	ND	ND	3X10	20X10	ND
7 (S)	15X103	75X102	ND	ND	100X102	6X10	ND
8 (S)	9X10	3X10	ND	ND	35X10	12X0	ND
9 (S)	25X10	37X10	ND	ND	100X10	6X10	ND
10 (S)	30X103	30X103	8X10	D	25X102	27X10	D
11 (S)	25X102	40X102	ND	ND	4X10	21X10	ND

M.O= Microorganism S. No.= Sample number TBC= Total Bacterial Count
TFC= Total Fungal Count TCC= Total Coliform Count FC= Faecal coliform
B.C= Bacillus cereus Staph.= Staphylococcus count D= Detected ND= Not Detected
L= Luncheon P= Pasterma L*= Liver S= Sausage

Obtained data also showed that, all cooked meat samples contained TPC and TCC within the permissible limits while 6.7%, 6.7%, 93.3%, 22.2%

and 20% of the examined samples contained E.coli, Staphylococcus, BC, Salmonella and TFC more than the permissible limits.

As an overview of the resulted data, all tested samples contained TPC within permissible limits while 26.5%, 10.3%, 10.3%, 5.9% 35.4% and 86.6% of the tested samples contained TFC, TCC, E.coli, Staphylococcus count and BC in numbers more than the permissible limits respectively.

E.coli O157:H7 detection by PCR:

Traditional methods based on biochemical characteristics are labor-intensive, and the total time required for determination of the identities of the pathogens is typically about 72 h. Rapid detection techniques directed at similar immunological and genetic targets are therefore of great interest. Immunological methods based on the detection of Shiga-like toxins have been developed. However, these methods cannot differentiate *E. coli* O157:H7 from other less virulent enterohemorrhagic and enteropathogenic *E. coli* strains. Other methods, based on the detection of O157 somatic and H7 flagellar antigens, are equally inadequate because of their lack of specificity.

PCR offers the ability to determine the absolute and relative amounts of pathogens in complex matrices, and assays that were recently developed for the identification of *E. coli* O157:H7 are based on the detection of genes encoding Shiga-like toxins (Belanger *et al., (*2002), intimin (Oberst *et al., (*1998), and O antigen (Fortin *et al., (*2001). However, those single PCR methods sometimes lack specificity, for example, the targets for the H7 flagellar antigen genes may cross-react with *E. coli* O55:H7 and so will fail to identify *E. coli* O157:NM (where NM is nonmotile).

According to previous reports, strain of *E. coli* O157 isolated usually produce both Shiga-like toxins 1 and 2. Isolates that produce only *stx*1 are uncommon (Guan and Levin, (2002). Therefore, in this study we used primers and probes to detect and identify *E. coli* O157 isolates producing Shiga-like toxin 2 by targeting the particular gene *stx*2 *E. coli* O157.

Data in Tables (7&8) illustrated the sources, distribution and PCR analysis results of the tested samples for the presence of E.coli O157:H7 by PCR. It is clear that, 14 out of 68 tested samples were selected for this reason (2 raw, 5 cooked and 7 processed meat samples). Seven samples were positive for E.coli (4 from Giza, 1 from Cairo and 2 from Qualioubeya) and other 7 contained TCC in higher numbers (5 from Giza, 1 from Cairo and 1 from Qualioubeya).

Images (1&2) showed the result of traditional PCR analysis of the selected 14 samples which revealed that, although only 7 samples were positive for E.coli during screening of the microbial flora, 11 out of 14 samples were positive for E.coli O157:H7. This difference (4 samples) in the number of positive samples may be due to the inclusion of E.coli in number less than the detection limit of the used method (more than 10 cfu/g) leading to failure of its detection by direct dilution and plating but after enrichment (before DNA extraction) the number increased and hence could be detected by PCR technique. Confirming this result with RT-PCR indicated that, the amplification blots of all positive samples showed a separate plateau (Image

3) differed than the plateau of the control positive *E. coli* O157:H7 DNA (applied Biosystem, USA) which indicated the inclusion of *E. coli* O55:H7 not O157:H7 as mentioned in the instruction manual of the test kit. These findings were supported by the findings of Cui *et al.,(* 2003), Ching *et al.,* (2005) who stated the same conclusion by using RT-PCR in E. coli O157:H7 detection and identification.

Image (1 & 2): Electrophoresed Agarose gel result of the tested samples:

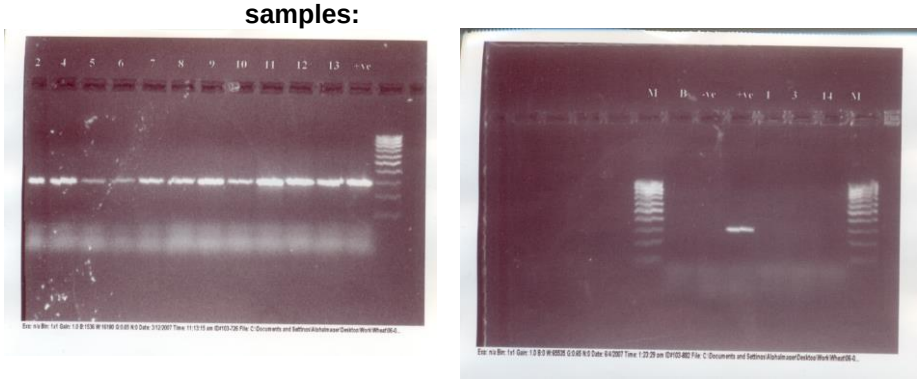

Image 1 shows the specific bands for the 11 positive samples while image 2 shows the specific band for the control positive DNA only while no specific bands are shown for 3 tested samples, one control negative DNA and a blank reaction

Image 3: Amplification blots of the examined 11 E. coli O157:H7 positive samples by using RT PCR

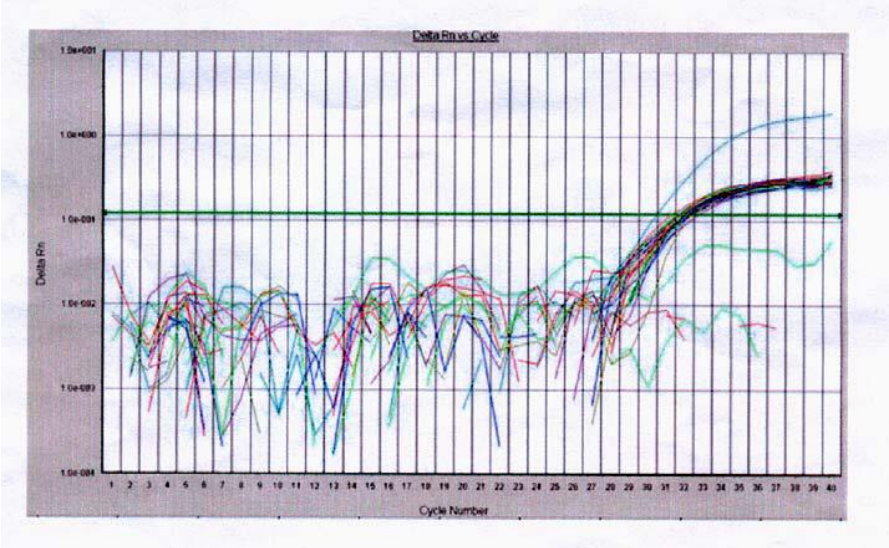

Table (7): Sources of samples tested for E.coli O157:H7 by PCR:

Place / Type	Giza		Cairo		Qualioubeya		Total
	F	C	F	C	F	C	
Raw	2	0	0	0	0	0	2
Cooked	1	2	1	0	1	-	5
Processed	1	3	0	1	1	1	7
Total	**4**	**5**	**1**	**1**	**2**	**1**	**14**

F= Faecal coliform positive samples C= Total Coliform positive samples

Table (8): Distribution and result of analysis of the selected samples for PCR analysis:

Code	Type	Source	Primary result	Result of St PCR	Result of RT-PCR
1	C (Kofta)	Giza	TCC +ve	No band	NT
2	C (Kofta)	Giza	TCC +ve	Specific band	O155:H7
3	Raw (Ground beef)	Giza	E. coli +ve	No band	NT
4	Raw (Ground beef)	Giza	E. coli +ve	Specific band	O155:H7
5	P (Pasterma)	Qualioubeya	E. coli +ve	Specific band	O155:H7
6	P (Luncheon)	Giza	TCC +ve	Specific band	O155:H7
7	P (Luncheon)	Giza	TCC +ve	Specific band	O155:H7
8	C (Kofta)	Giza	E. coli +ve	Specific band	O155:H7
9	P (Pasterma)	Giza	TCC +ve	Specific band	O155:H7
10	P (Pasterma)	Cairo	TCC +ve	Specific band	O155:H7
11	P (Pasterma)	Qualioubeya	TCC +ve	Specific band	O155:H7
12	P (Pasterma)	Giza	E. coli +ve	Specific band	O155:H7
13	C (Sausage)	Qualioubeyya	E. coli +ve	Specific band	O155:H7
14	C (Liver)	Cairo	E. coli +ve	No band	NT

TCC +ve = Total Coliform Count Positive sample NT = Not Tested
E. coli +ve = E. coli positive sample C = Cooked, P = Processed

In summary, the used TaqMan real-time PCR method used for the detection of *E. coli* O157 showed good specificity and sensitivity and also saved substantial time because of the preparation of samples minimum time of pre-culture. The shortening of the processing time and the increase in the specificity for pathogen detection are critical for the safety and sanitation of our food supply.

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الكشف عن بكتيريا E. coli O157:H7 في اللحوم و منتجاتها بإستخدام تفاعل البلمرة المتسلسل التقليدي و اللحظي

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****معمل سلامة الغذاء و البيوتكنولوجي – المركز الإقليمي للأغذية و الاعلاف - مركز البحوث الزراعية**

تم تجميع عدد ٦٨ عينة من اللحم المفروم و اللانشون و البسطرمة و سندويتشات اللحم المطبوخ (الكبدة المطبوخة و السجق و الكفتة و الهامبرجر)عشوائياً من الجزارين و محلات الأغذية السريعة و الباعة الجائلين في منطقة القاهرة الكبرى (القاهرة و الجيزة و شبرا الخيمة) للكشف عن المحتوى الميكروبي لها و الذي بين أن المحتوى من العدد الكلي للبكتيريا في جميع العينات المختبرة لم تتعد الحدود المسموح بها بينما تخطت ٢٦,٥% و ١٠,٣% و ١٠,٣% و ٥,٩% و ٣٥,٤% و ٨٦,٨% من العينات المختبرة الحدود المسموح بها للعدد الكلي للفطريات و العدد الكلي لبكتيريا القولون و العدد الكلي لبكتيريا الاشريشيا كولاي و العدد الكلي لبكتيريا الاستافيلوكوكاس و العدد الكلي للسالمونيللا و العدد الكلي لبكتيريا الباسيلس سيريس على الترتيب.

كذلك تم إختبار العينات الموجبة العدد الكلي لبكتيريا القولون و العدد الكلي لبكتيريا الاشريشيا كولاي (١٤ عينة) بإستخدام تفاعل البلمرة المتسلسل للكشف عن تواجد بكتيريا الإشريشيا كولاي O157:H7. وقد أعطت النتائج المتحصل عليها بإستخدام تفاعل البلمرة المتسلسل التقليدي نتيجة إيجابية لعدد ١١ عينة من العينات المختبرة ، بينما أثبتت النتائج المتحصل عليها بإستخدام تفاعل البلمرة المتسلسل اللحظي أن هذه النتيجة الموجبة هي لبكتيريا الاشريشيا كولاي O55:H7 التي تتشابة مع بكتيريا الاشريشيا كولاي O157:H7 في التركيب الجيني لمنطقة الأسواط التي تستخدم في حركة البكتيريا.

وعلى ذلك فقد أثبت هذه الدراسة أن إستخدام تفاعل البلمرة المتسلسل اللحظي يعتبر من أهم و أدق طرق التحليل الحديثة للكشف عن و السيطرة على البكتيريا المسببة للتسمم الغذائي.