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PREVALENCE OF *ESCHERICHIA COLI* O157 IN SOME MEAT PRODUCTS

(With 2 Tables and 2 Figures)

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(Received at 22/12/2008)

تواجد الميكروب القولونى الدمم أو ١٥٧ فى بعض منتجات اللحوم

ايزيس جرجس انطون ، أمانى نبيل ضبع

تم تجميع ٢٥٠ عينة من منتجات اللحوم (٥٠ عينة من كل من اللحم المفروم والبيف برجر والكفتة والسجق الشرقى واللانشون) من أسواق مختلفة فى محافظتى القاهرة والجيزة. وتم فحص العينات بكتريولوجياً لمعرفة مدى تواجد ميكروب القولون المدمم وكانت النتائج على النحو التالى: تم عزل الميكروب من ١ ، ٢ ، ٢ عينة من كل من اللحم المفروم والبيف بيرجر والكفتة على التوالى بنسبة ٢، ٤، ٤% ولم يتم عزل الميكروب من عينات السجق الشرقى واللانشون. ميكروب الأيشريشيا كولاى أو ١٥٧ (O157) أنتج فيروتوكسين الذى ساعد بالتعرف على StxI وذلك بأستخدام تفاعل البلمرة المتسلسل (PCR) لميكروب الأيشريشيا كولاى كان حساساً للمضادات الحيوية التالية سيبروفلوكساسين، أنروفلوكساسين، ستربتوميسين، تراسيكلين وجنتاميسين على الترتيب.

SUMMARY

A total number of 250 meat products (50 each of minced meat, beef burger, kofta, oriental sausage and luncheon) were collected from different markets in Cairo and Giza Governorates. *E. coli* O157 was detected in minced meat, beef burger and kofta, with percentages of 2, 4 and 4%, respectively while the organism failed to be detected in samples of oriental sausage and luncheon. The isolated *E. coli* O157:H7 produce verotoxins which helped in the detection of Stx1 gene by using PCR. The antibiogram activity of the *E. coli* O157:H7 were sensitive to ciprofloxacin, enrofloxacin, streptomycin, tetracycline and gentamicin.

Key words: *Meat products, minced meat, beef burger, sausage, luncheon, E. coli*

INTRODUCTION

E. coli is a normal and healthy part of the intestinal microflora of many worm blooded animals including humans. However, some strains can cause diseases. Verocytogenic *E. coli* including serotype O157: H7 are one of such group causing chronic and potentially fatal illness, related to their ability to produce one or more toxins known as verotoxin or Shiga-like toxin (Uhitil *et al.*, 2001; Dziva *et al.*, 2007; Murphy *et al.*, 2007; Abd Alla *et al.*, 2008).

E. coli O157:H7 was first identified as a foodborne human pathogen in 1982 when it was related to two major outbreaks of hemorrhagic colitis in the USA. It caused symptoms ranging from hemorrhagic colitis to extreme cases of hemolytic uremic syndrome and thrombotic cytopenic purpura (Riley *et al.*, 1983; Griffin and Tauxe, 1991; Padhye and Doyles, 1992).

E. coli O157:H7 is estimated to cause approximately 62500 foodborne illness, 1800 hospitalization and 50 deaths each year in the United State (Mead *et al.*, 1999). From 1982 to 1994 approximately 50% of food borne outbreaks in which the vehicle was identified were associated with the consumption of ground beef (Meng and Doyle, 1998).

Contaminated ground beef is the most common vehicle for the transmission of *E. coli* O157:H7 (WHO, 1997).

It is well established that beef burgers are an important source of *E. coli* O157:H7. The association of this organism with beef burgers led to its nick name as "buger-bug", particularly in the USA (Willshaw *et al.*, 1994; Meng and Doyle, 1998).

There is still only a very limited amount of information on the efficacies of the various protocols in detecting bacterial pathogens especially toxigenic *Escherichia coli* in naturally contaminated food samples. In order to develop toxic gene amplification protocols that have relevance to the meat industry there must be a concerted effort to utilize naturally contaminated samples in the development and evaluation of protocols as well as to initiate multilaboratory about robin evaluations of select protocols. Availability of multilaboratory tested methodologies would provide a means to design pathogen detection strategies at the quality control level rather than an end product confirmatory response to an already documented outbreak (MacDonald *et al.*, 2004).

Food may be derived from a source free from microbial contaminants but becomes contaminated in the course of manufacture transport or sale to food handlers, utensils, air, soil and incomplete hygienic conditions during manufacturing like packaging, storage, slicing and marketing of such products promote the growth and multiplication of various bacteria one of which being *Escherichia coil* (Bryan, 1982).

Polymerase chain reaction is an in-vitro amplification technique for enzymatic synthesis of specific DNA sequences using two oligonueleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA. A repetitive series of cycles involves: template denaturation, primer annealing, and extension of the annealed primers by thermostable DNA polymerase (Erlich *et al.*, 1991). PCR is used now in large scale as a recent techniques for detection of virulence factors in enterotoxigenic *Escherichia coli* serotypes in meat and meat by products (Feng and Monday, 2000; Makino *et al.*, 2000). Enterotoxigenic *E. coli* strains have been known to cause traveler's diarrhea, gastrointestinal infection and diarrhoeal illness. Such strains are known to produce heat-labile enterotoxins (LT) that rare antigenic and similar to cholera enterotoxin and/or a heat-stable enterotoxin (ST) that are of small molecular weight and are nonantigenic (Frank *et al.*, 1977; Niazi and Refai, 1988).

Public health authorities began to reevaluate the role of *Escherichia coli* in food and water illness, the routine laboratory screening for *Escherichia coli* in food is now not restricted only for the isolation, biochemical and serological identification of enteropathogenic Escherichia coil (EPEC) incriminated in food outbreaks, but it is extended to detect enterotoxigenic *Escherichia coli* (ETEC) (Niazi and Refai, 1988).

The present work was planed to illustrate the following:

1- Incidence of *Escherichia coli* in meat products (raw minced meat, oriental sausage, frozen beef burger, Kofta and luncheon).
2- Detection of verotoxin using Vero cell.
3- STX_1 gene detection for identified *E. coli* O157 by PCR using STX_1 specific primers.
4- Antibiotic sensitivity of *E. coli.*

MATERIALS and METHODS

1. Samples:-

A total number of 250 meat products (50 each of minced meat, beef burger, kofta, oriental sausage and luncheon) were collected from different markets in Giza and Cairo Governorates. Samples were packed separately in sterile polyethylene bag and transferred directly to the laboratory with minimum of delay for bacteriological exanimation.

2. Isolation of *E. coli* O157:H7:

According to Feng and Weagant (2002).

2.1. Selective enrichment:-

Twenty five grams from each sample were transferred to 225 ml of Enterohemorrhagic *Escherichia coli* broth (EHEC), blended with a stomcher at a medium speed for one minute and incubated at 37°C for 24 hours.

2.2. Selective plating:

One loopfull of the enrichment was streaked onto telurite cefixime sorbitol MacConkey agar (Oxoid CM7) (TC SMAC) plate and incubated at 37°C ± 0.5°C with shaking For 24 hours.

Typical colonies of *E. coli* O157:H7 (colourless with smoky center 1:2 mm in diameter) were picked up onto Eosen Methylene blue agar (EMB) and incubated at 35°C for 24 hours.

3. Identification of *E. coli* O157:H7 colonies:-

Suspected colonies were picked up, purified and streaked onto slop nutrient agar (Oxoid CM 3) for further identification.

3.2.1. Morphological examination:

Films were prepared and stained with Gram's stain according to the method recommended by Cruickshank *et al.* (1975) for detection of Gram negative, non spore Forming short rods.

3.2.2. Cultural characteristics:

Typical well isolated non sorbitol Fermenting colonies or colorless pale colonies on TC MacConkcy sorbitol agar and then metallic sheen colonies on Eosin Methylene Blue agar (EMB, Oxoid, CM 69) then, were subcultured onto semisolid nutrient agar for biochemical identification.

3.2.3. Biochemical identification (Kerig and Holt, 1984; Quin *et al.*, 2002):

Typical well isolated colonies were picked up and grown into peptone water (Oxoid CM, 9) for 6 hour for the following biochemical

tests (Indole production test, methyl red test, Voges-Proskauer test, Utilization of citrate, hydrolysis of urea, hydrogen sulphide production). Fermentation of sugars like (lactose, sucrose and sorbitol).

3. Serological identification of the isolates:

The method recommended by Edwards and Ewing (1972) by slide agglutination test using polyvalent and monovalent specific antiserum.

4. *E. coli* verotoxin production assay:

Casamino acid-yeast extract fluid media was used to grow *E. coli* isolates for 12 h. at 41°C. Bacteria pellets were removed by centrifugation at 10,000 x g for 10 mm. The supernatant which contained the Shiga-like toxins was filtered with Millipore filter 0.22 µm. Then 100 µl of the filtrate were added to confluent monolayer of Vero cells and incubated at 37°C in 5% CO_2 atmosphere and evaluated for 72 h for cytopathic effect (CPE) (Blanco *et al.*, 1996).

5. PCR detection of *E. coli* isolates:

Nutrient broth cultures were prepared from each *E. coli* isolate, 2 µl aliquots were as templates in 50 µl reaction mixtures contained 200 µM deoxynucleosid triphosphates, 250 mM of each primer and 1 U of Taq polymerase in 10 mM tris HC1 (pH8.3) 50 mM KCI, 2 mM $MgCl_2$, 0.1% gelatin and 0.1% Tween 20. PCR mixtures were subjected to 35 cycle each consisting of 1 µm. denaturation at 95°C, 2 min annealing at 65°C and 1.5 min of elongation 72°C. Final extension at 72 °C for 10 min was also carried out. PCR reaction mixtures were thenj electrophoretic on 2% agarose gels with marker 1-kb DNA ladder (New England Biolabs, Inc.) an stained with ethidium bromide. The designed primer pair was specific for 180 base pair subunit coding region of STX_1 nucleotide 454-633 (Paton and Paton, 1998).

6. Antibiotic sensitivity of *E. coli*

The isolates of *E. coli* were tested for their sensitivity to ciprofloxacin (10 µg), enrofloxacin (10µg), streptomycin (10 µg), tetracycline (30 µg), Penicillin (10 units), ampicillin (10 µg), gentamicin (10 µg), erythromycin (15 µg), rifampin (10 µg), chloramphenicol (30 µg) kanamycin (30 µg), trimethoprim sulphamethazole (25 µg) by the agar disc diffusion method (Bauer *et al.*, 1966).

RESULTS

Bacterial examination:

E. coli O157:H7 was detected from minced meat, beef burger and kofta samples with percentage of 4, 6 and 4%, respectively. The

organism failed to be detected from oriental sausage and luncheon samples.

E. coli isolates which produce of verotoxins (using the result layer assay) for detection of STX_1 gene using PCR assay in 4 serotype of *E. coli* O157 from 5 which positive for verotoxin.

The PCR STX_1 180 pb clear band in the agarose gel of *E. coli* isolates (genotype) were highly correlated to the CPE (cytopathic effect) induced in the vero cells (phenotype) as shown in Figs (1 and 2).

The antibiogram activity of the *E. coli* O157 isolates were sensitive to ciprofloxacin, enrofloxcin, streptomycin, tetracycline and gentamicin but less effective to trimethoprim sulphomethazole, chloramphenicol, kanamycin, rifampin, ampicillin, erythromycin and penicillin.

Table 1: Incidence of *E. coli* O157:H7 in meat products (n* = 50).

Meat products	No. of Samples	No. of suspected colonies		*E. coli* O157:H7	
		No. of samples	%	No.	%
Minced meat	50	6	12	1	2
Beef burger	50	9	18	2	4
Kofta	50	6	12	2	4
Oriental sausage	50	4	8	0	0
Luncheon	50	8	16	0	0

* Number of examined samples.

Table 2: Antibiotic sensitivity test for isolated *E. coli* O157:H7 (5 isolates).

Chemotherapeutic agent	Concentrate	Sensitivity	Percentage
Ciprofloxacin	10 µg	5	100
Euroflacin	10 µg	5	100
Streptomycin	30 µg	4	80
Tetracycline	10 µg	3	60
Gentamicin	10 µg	3	60
Trimethoprim-sulphate	25 µg	2	40
Chloramphenicol	30 µg	2	40
Kanamycin	30 µg	2	40
Rifampin	5 µg	1	20
Ampicillin	30 µg	1	20
Erythromycin	10 µg	1	20
Penicillin	10 units	1	20

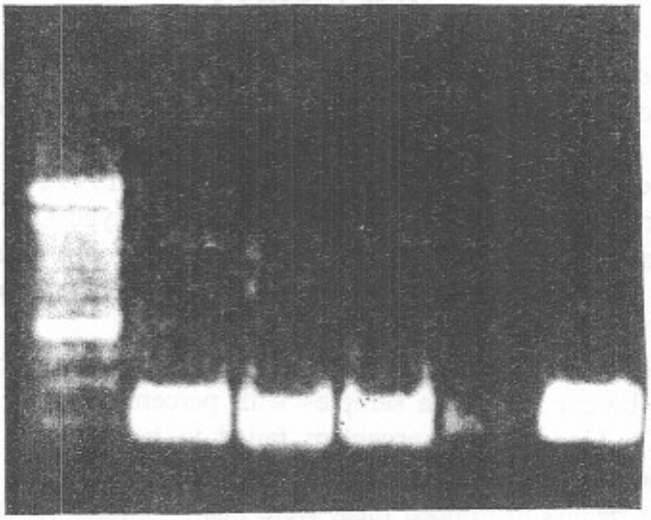

Fig. 1: Cytopathic effect due to production of verotoxin from *E.* coli O157:H7 on vero-cells.

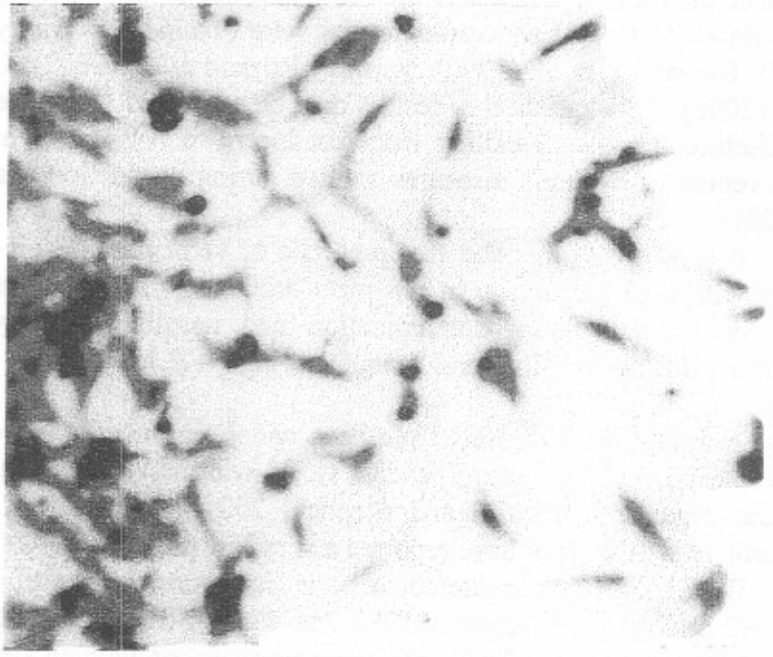

Fig. 2: PCR reaction uixtuses were electrophoresed on 2% agarose gels stand with ethodium bromide positive (prob a, 2, 3 and 5) show clear 180 pb bands L and M is 1-Kb DNA ladder Nuclear marker (England biobabs, Inc.).

DISCUSSION

E. coli 157:H7 has emerged as a major cause of both sporadic cases and out breaks of bloody diarrhea throughout the world (Meng *et al.*, 2001). Most outbreaks of *E. coli* O157:H7 infections are food borne of which beef are the principal vehicle (Tuttle *et al.*, 1999).

Verotoxin-producing *E. coli* especially serotype O157 has been incriminated as a causative agent in single cases and outbreaks of several potentially fatal disease in humans (Borie *et al.*, 1997; Chapman *et al.*, 2000).

E. coli O157:H7 was detected in 1, 3 and 2 isolates from minced meat beef burger and kofta samples with percentage of 4, 6 and 4% respectively. However the organism failed to be detected in oriental sausage and luncheon samples (Table, 1).

The results in minced meat were nearly similar to that reported by Abdel Hakiem *et al.* (1998), Carney *et al.* (2006), while higher incidences were achieved by Abdel-Raouf *et al.*, (1996), Saleh (2001); Sayed *et al.* (2001); Chinen *et al.* (2001); Kassem and Sabry (2003); Magwira *et al.* (2005) and lower results were obtained by Ansay *et al.* (1999); Heuvelink *et al.* (1999); Vernozy-Rozand *et al.* (2002); Chaheo *et al.* (2005). This variation in results may be attributed to difference in manufacture practice, handling from producers to consumers and the effectiveness of hygienic measures applied during production (Sayed *et al.*, 2001).

It is worth to mention that presence of verocytotoxin-producing *E. coli* with high percentage in raw meat products provides evidence to direct or indirect faecal contamination and unsatisfactory hygienic conditions during handling and manufacturing (IFR Information Sheet, 2003).

Although minced meat have been widely implicated as vehicles of *E. coli* O157:H7 infection, studies world-wide have either failed to find the organism, (Fantilli and Stephan, 2001; Uhitil *et al.*, 2001; Dontorou *et al.*, 2003) or have reported a very low prevalence.

Beef burger is considered to be an important source of *E. coli* O157:H7 and in USA between 1982 and 1991, Fifteen percentage of outbreaks of *E. coli* O157:H7 infections were linked with this product (Willshaw *et al.*, 1994; Meng and Doyle, 1998).

Beef burgers were examined in several countries for the presence of *E. coli* O157:H7. Nearly similar results were reported by Magwira *et*

al. (2005); Kassem and Sabry (2003), while high results were achieved by Matter and Vasquez (1998) and Saleh (2001), and low results were reported by Ansay *et al.* (1999). Moreover, Qualio *et al.* (1997); Silveira *et al.* (1999); Dontorou *et al.* (2003) failed to isolate *E. coli* O157:H7.

Minced meat and Burger were the most frequent food items incriminated in transmitting *E. coli* O157:H7 to humans (Anon, 1994).

Kofta made from minced beef meat with some seasonings and spices which may add a substantial number of microorganisms. It was examined in Egypt with high incidence by Saleh (2001). The higher incidence of *E. coli* O157:H7 in Kofta than in minced meat may be due to addition of spices which may be contaminated with bacteria of fecal origin, use of poor quality ingredients in this product, poor sanitation during preservation, handling and improper cooling and refrigeration and cross contamination from unclean equipment and infected workers.

Development of oriental sausage was primary driven as an economic utilization of low quality meat as meat and trimmings were comminuted, salted spiced, nitrates and made into sausage.

E. coli O157:H7 failed to be detected in samples of oriental sausage which may be attributed to the manufacturing technique or presence of nitrate in this product. These findings were in agreement with Saleh (2001); Kasseenbory *et al.* (2004).

In the present study, *E. coli* O157 was not detected in luncheon samples, (Table 1). This may be due to the exposure to high temperature during processing and the highly acidic nature of this meat product. In an experimental study carried by Weagand *et al.* (1994), it was observed that *E. coli* O157 died rapidly in acid foods at room temperature, while survived for weeks at refrigeration temperature.

The presence of *E. coli* O157 in meat could be attributed to the contamination from feces of infected animals as indicated by Suthienkul *et al.* (1990) who recorded that shiga like toxin producing *Escherichia coli* were found in 11% to 84% of fecal matter of cattle before slaughter, from 8% to 28% of fresh beef specimen at slaughter houses using a DNA probe (Chapman *et al.*, 1992).

This wide variation in carriage rate of cows to *E. coli* O157 may be explained in part by the variable efficiencies of the isolation protocols, the season and geographical area may also have an effect on prevalence figures (Chapman *et al.*, 2000; Synge, 2000).

Screening *E. coli* O157 for production of verotoxins using the vero cell assay and detection of Stxt gene using the PCR. All isolates of

E. coli O157 were positive in the vero cell toxicity assay were positive in the Stxt-PCR detection except one isolates. This expressure paterns were recorded by Paton and Paton (1998); Belanger *et al.* (2002). This phenomenon was explained due to gene switch off or due to the expression of the toxins in undetectable amounts. Other authors reported that toxins profile of *E. coli* isolates can be changed and altered over time, as the genes responsible for its expression were unstable and coded by phage that can be integrated or lost due to many different factors (Gyles, 1994; Lee *et al.*, 1996).

The antibiogram activity or the *E. coli* isolates regardless of their serovar higher sensitivity to ciprofloxacin, enrofloxacin, streptomycin, tetracycline and gentamicin similar antibiogram profiles were also manifested by Blanco *et al.* (1996) and Cid *et al.* (1996).

In conclusion, *E. coli* O157 was detected in minced meet, beef burger and kofta which plays an important role in infection to man. Therefore stricted hygienic measures and biosifty roles should be imposed at many levels ranging from farms, slaughter houses to home to minimize risk of spread of *E. coli* O157 to man.

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