

Molecular Characterization of pathogenic *E. coli* isolated from meat and their products

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Abstract

This study was planned to throw the light on the prevalence of *Escherichia coli* (*E. coli*) in meat and meat products and determine its virulence gene that may be considered a significant food safety threat. Therefore two hundred samples of meat and meat products; minced meat, kofta, sausage, beefburger, pastirma, luncheon and hot dog (25 of each) were randomly collected from supermarkets, butcher shops and street vendors in Ismailia city. Bacteriological examination revealed that the prevalence of *E. coli* was 17% of the total collected samples. Serological identification of *E. coli* isolated from meat, minced meat, beefburger and pastirma revealed that strains of *E. coli* were belong to serotypes O:157 K:-, O:91 K:-, O:103 K:-, O:125 K:70 respectively, while O:26 K:60 serotype was isolated from kofta and sausage. Strains from luncheon and hot dog were Untyped, in addition to isolated *E. coli* strains from all samples were untyped by using Anti-Coli I, II and III. Molecular characterization of typed strains of *E. coli* using Real-Time PCR (RT-PCR) showed that serotype O:26 K:60 was isolated from kofta and harbored both *vt1* and *vt2*, while O:26 K:60, O:103 K:- and O:91 K:- serotypes isolated from sausage, beefburger and minced meat respectively, were harbored *vt2* gene only.

Key words: *E. coli*, Virulence genes, meat and meat products.

Introduction

Meat and meat products have an important role in human nutrition as they are desirable foodstuffs. They are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients (Biesalski, 2005). On the other hand, they are considered an ideal culture medium for growth of many organisms because of their high

moisture, high percentage of nitrogenous compounds of various degree of complexity, plentiful supply of minerals, accessory growth factors and some fermentable carbohydrates (glycogen) of a favorable pH for most of enteric microorganisms (Mohammed, 2011). Changes in consumer eating habits have increased the demand for a wide

variety of raw, frozen, pre-cooked and further processed meat items. As a result, the meat industry has continued to seek ways to increase the acceptability, lengthen the shelf life overall meat quality and safety of meat products (*Selvan et al, 2007*).

Meat products may be contaminated with microorganisms from meat handlers, which carry of pathogenic microorganism during the processes of manufacturing, packing and marketing. Improper cooking, refrigeration or storage may lead to meat borne illness. Food-borne pathogens are the leading causes of illness and death in developing countries costing billions of dollars in medical care, medical and social costs (*Fratmico et al, 2005*).

Microbial quality of meat and their products plays an important role in increasing public health issue all over the world (*Ahmed and Ismail, 2010*). However the use of proper hygienic practices in handling food of animal origin and proper heating of such foods before consumption are important control measures for the prevention of *E. coli* infections (*Michael, 1991*).

E. coli is an important member of the normal intestinal microflora of humans and other mammals (*Kaper et al, 2004*). It constitutes a significant risk to human health and remains an important cause of infant mortality in developing countries (*Chen and Frankel, 2005*). *E. coli* is commonly non virulent but some strains have

adopted pathogenic or toxigenic virulence factors that make them virulent for humans and animals. *E. coli* is the most predominant species in all food poisoning cases associated with some meat products (*Gi et al, 2009*). Various studies indicate that enteropathogenic *E. coli* cause diarrheal disease either by invasion of the intestinal mucosa after attachment to host epithelial cells through pili or by elaboration of enterotoxins (*Sack, 1975*).

Real-time PCR (RT-PCR) is a more efficient way of testing large numbers of samples and it is a single-step, closed-tube method that eliminates contamination. RT-PCR is very sensitive and can detect small amounts of samples (*Klein, 2009*) additionally; this method is highly sensitive making it more robust and reproducible than conventional PCR.

Due to the high incidence of food-borne infections, there is an urgent need for control and/or prophylaxis for food poisoning outbreaks associated with meat products. It depends greatly on investigating the causative agents in food (meat products), eliminating them to ensure food safety and to protect public health from microbial contamination of food. In this study, a RT- PCR assay was used for detection of 2 virulence genes (*vt1and vt2*) in *E. coli* strains obtained from examined samples.

Materials and Methods

1. Samples collection:

Two hundred samples of meat and meat products; minced meat, kofta, sausage, beefburger, pastirma, luncheon and hot dog (25 of each) were randomly collected from different retailers in Ismailia city. Each sample was aseptically transported in ice-box to laboratory within 24 hour for bacteriological examinations.

2. Preparation of the samples:

The technique recommended by *APHA (2001)* was used for samples preparation. 25 gram of sample was aseptically added to 225 ml peptone saline and then homogenized in a stomacher for 2 min then incubated at 40°C for 24hr.

3. Isolation and biochemical identification of *E. coli*: according to *Konemann et al., (1993)*.

4. Serotyping of isolated *E. coli*:

Table (1): *Oligonucleotide primers used in the PCR technique*

Virulence gene	Primer
<i>vt1</i>	* Forward Primer: <i>vt1</i> -F28 Sequence: 5`-ACG TTA CAG CGT GTT GCR GGG ATC-3` * Reverse Primer: <i>vt1</i> -R28 Sequence: 5`-TTG CCA CAG ACT GCG TCA GTR AGG-3`
<i>vt2</i>	* Forward Primer: <i>vt2</i> -F28 Sequence: 5`-TGT GGC TGG GTT CGT TAA TAC GGC-3` * Reverse Primer: <i>vt2</i> -R28 Sequence: 5`-TCC GTT GTC ATG GAA ACC GTT GTC-3`

Results

1. Results of isolation and biochemical identification of *E. coli*

Typical *E. coli* metallic sheen green colonies on Eosin Methylene Blue were subjected to several biochemical tests. It was positive for Indole, Methylene red, catalase,

The technique recommended by (*Edwards and Ewing, 1972*) was used for serotyping the isolates.

5. Molecular characterization and detection of *E. coli* virulence gene:

a- Extraction of bacterial DNA:

DNA extraction was performed using the GF-1 Tissue DNA extraction kit (**vivantis**), according to the manufacturer's instructions listed in user Guide (Version 3.1).

b- Real-Time PCR:

SYBR Green Real-time PCR was done according to (*Dafni-Maria et al. 2012*)

Primers targeting the species specific virulence gene [verotoxin1, verotoxin2 (*vt1-vt2*)] were synthesized by (**Biolegio**), the primers used are listed in Table (1).

Nitrate reduction and negative for Oxidase, Voges-Proskauer, Simmon's citrate, Urease, H₂S and gelatin liquefaction. Ferment lactose and glucose with acid and gas. The highest rate was from samples collected from meat (44%) followed by minced meat (28%), kofta (20%), sausage (16%),

beefburger (12%) and pastirma (8%). The lowest rate was from samples collected from luncheon and hot dog (4%). The total percentage was 17% from the total samples as shown in Table (2).

2. Results of serological identification of isolated *E. coli*

Anti-Coli I, II, III polyvalent antisera were used for serotyping of *E. coli* and determination of their antigenic structure. The results revealed the identification of 6 strains isolated from meat and meat products as shown in Table (3), where one strain of *E. coli* isolated from meat was belong to serotype O:157 K:-, one strain isolated from minced meat was belong to serotype O:91 K:-, two strains isolated from kofta and sausage were identified as O:26 K:60, one from 3 *E. coli* strains isolated from beefburger was serotyped as O:103 K:-. One from 2 *E. coli* strains isolated from pastirma was serotyped as O:125 K:70, while one *E. coli* strain isolated from each luncheon and hot dog samples were untyped. On the

other hand, the results demonstrated the presence of untyped strains of *E. coli* among different samples of meat and meat products according to the used Anti-Coli groups.

3. Results of molecular characterization and detection of *E. coli* virulence genes

Serologically identified *E. coli* (six serotypes) were submitted for molecular detection and determination of verotoxin1, 2 (*vt1* – *vt2*) virulence gene by RT-PCR. Table (3) showed the positive serotypes for *vt1* – *vt2* gene and confirmed to be virulent *E. coli* isolates.

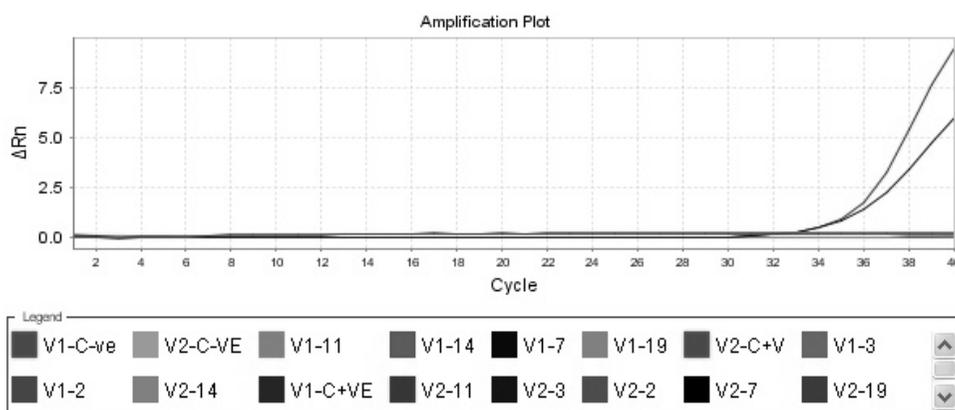
The result showed that one serotype (O:26 K:60 from kofta) was harbored *vt1* gene Figure (1), while four serotypes (O:26 K:60 from both kofta and sausage, O:91 K:- , O:103 K:- from minced meat and beefburger respectively) were harbored *vt2* gene Figure (2). Taking into account that the additional peak appeared in the two Figures represent the control positive.

Table (2): Prevalence of *E. coli* in meat and meat products

Type of samples	Samples No.	<i>E. coli</i> isolates	
		No.	%
Meat	25	11	44
Minced meat	25	7	28
Kofta	25	5	20
Sausage	25	4	16
Beefburger	25	3	12
Pastirma	25	2	8
Luncheon	25	1	4
Hot dog	25	1	4
Total	200	34	17

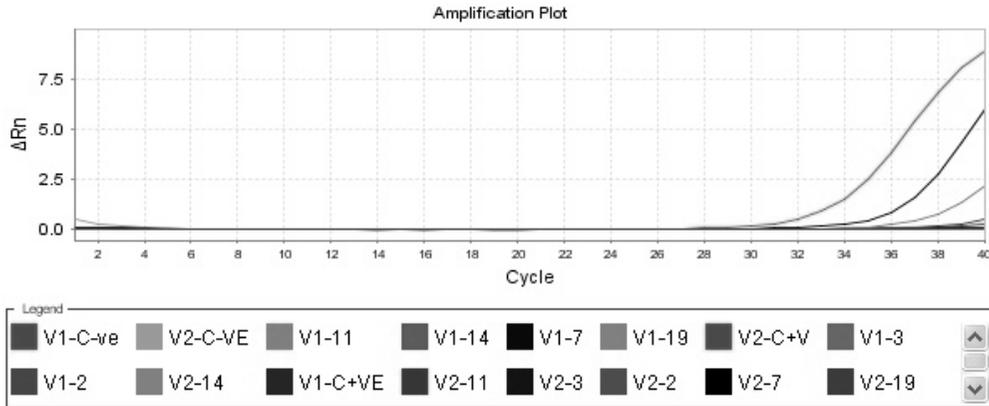
Table (3): Serotyping of the *E. coli* isolated from meat and meat products

Type of samples	No. of positive <i>E. coli</i>	<i>E. coli</i>		Virulence gene	
		Serotypes	No.	vt1	vt2
Meat	11	Untyped	10	-	-
		O:157 K:-	1	-	-
Minced meat	7	Untyped	6	-	-
		O:91 K:-	1	-	+ve
Kofta	5	Untyped	4	-	-
		O:26 K:60	1	+ve	+ve
Sausage	4	Untyped	3	-	-
		O:26 K:60	1	-	+ve
Beefburger	3	Untyped	2	-	-
		O:103 K:-	1	-	+ve
Pastirma	2	Untyped	1	-	-
		O:125 K:70	1	-	-
Luncheon	1	Untyped	1	-	-
Hot dog	1	Untyped	1	-	-



2: meat, 3: pastirma, 7: kofta, 11: beefburger, 14: minced meat, 19: sausage

Fig (1): The amplification plots for all samples targeting vt1.



2: meat, 3: pastirma, 7: kofta, 11: beefburger, 14: minced meat, 19: sausage
Fig (2): The amplification plots for all samples targeting *vt2*.

Discussion

Meat and meat products are recognized as a major source of food-borne pathogens that cause food poisoning in humans. The source of infection is not determined in the majority of food born disease outbreaks. Currently the most important pathogen associated with meat and meat products is *E. coli* (Gi et al, 2009).

The present work was made in order to evaluate the role of *E. coli* among meat and meat products in Egypt, also to determine the virulence genes characteristics of *E. coli* using RT- PCR.

In the present study, a total of 200 samples of meat and meat products; minced meat, kofta, sausage, beef burger, pastirma, luncheon and hot dog, were examined for presence of *E. coli* strains. The percentage of *E. coli* was 17 % (34 isolates) as shown in Table (2). These results were agree with those obtained by (Saleh et al, 2010) where the percentage of *E. coli* recovered

from pastirma was (8%) and from luncheon was (4%), on the other hand disagree with those obtained by (Hanan, 1991) who detected *E. coli* in pastirma, luncheon, minced meat and sausage in a percentage of (27%, 52%, 33% and 45%) respectively.

Out of 34 *E. coli* strains, only 6 were serotyped by using commercially available antisera as shown in Table (3). Distribution of 6 *E. coli* serotypes recovered from meat, minced meat, pastirma and beefburger were belonged to (O:157 K:-, O:91 K:-, O:125 K:70 and O:103 K :-) respectively, on the other hand O26:K60 serotype were isolated from both kofta and sausage. The rest of isolated strains were untypable. Similar findings have been reported by (Robert et al, 2006) who isolated *E. coli* O91 from ground beef, (Seran et al, 2012) isolated O:157 K:- serotype from meat, (Mohammed, 2011) isolated O:26 K:60 from kofta and (George et al, 2012) who

previously recorded O:26 K:60 from Sausage, While *E. coli* O:125 K:70 recovered from pastirma was not reported in available literature.

The difference in the rate of isolation of *E. coli* and its serotypes may attribute to difference in localities, methods of sampling and total number of samples. Also the variation in the results obtained by different investigators may be due to difference in manufacturing practices, handling and difference in time of exposure. High contamination level of *E. coli* in examined raw meat may indicates unsanitary conditions. They are indicators of fecal pollution at slaughterhouse which begin from skinning and direct contact with knives and workers hands. Also, during evisceration contamination may come from intestinal contents as well as from water during rinsing and washing of carcasses. Undercooked meat products have caused many food poisoning incidents associated with *E. coli* which is present in the feaces, intestines and hide of healthy cattle from where it can potentially contaminate meat during the slaughtering process (Duffy et al, 2003). *Enterobacteriaceae* were very useful as indicators of bad hygiene or bad treatment of food products and their presence in large number indicates a big possibility of the multiplication of *E.coli* and other pathogens (Nissen et al, 2011).

While *E. coli* serotyping is an important technique for making the proper diagnosis and epidemiological investigations during food-borne outbreaks, it cannot be relied on alone for categorizing a strain of *E. coli*, so the identification of specific virulence genes must also be performed (Barlow et al, 1999). PCR is a powerful molecular biology technique for the detection of virulence genes. It is not only highly sensitive and specific, but it also provides rapid and reliable results. It can help to distinguish *E. coli* isolates from meat and meat products through detection of virulence genes (Kimata et al, 2005). Verotoxins (*vt1* and *vt2*) are thought to play a prominent role in the pathogenesis of *E. coli* infections (O'Brien et al, 1992) that is a frequent cause of severe human diseases including bloody diarrhea and hemolytic uremic syndrome (HUS) (Manning et al, 2007). In this study, using of SYBR Green RT- PCR for identification of tested *E. coli* strains was highly specific with the primers chosen for the detection of *vt1* and *vt2* virulence genes. Results for the molecular characterization of six serotyped strains of *E. coli* showed that *E. coli* O:26 K:60 serotype isolated from kofta was harbored both *vt1* and *vt2*, while O:26 K:60, O:103 K:- and O:91 K:- serotypes isolated from (sausage, beef burger and minced meat) respectively, were harbored *vt2* gene only Table (3), Figures (1)

and (2). These results agreed with (**Bhong *et al*, 2008**) who detected *vt1* and *vt2* in *E. coli* serotype by using SYBR Green RT-PCR.

The variation in the presence of virulence encoding genes among different serotypes isolated from different sources revealed that the mechanisms of pathogenesis depends mainly on the presence of different virulence factors not to the different serotypes. (**Aranda *et al*, 2004**), The qualitative Real-Time PCR assay was rapid, sensitive and used as an alternative method to conventional methods for studying *vt1* and *vt2* virulence genes expression in *E. coli* (**Fitzmaurice *et al*, 2004**).

Conclusion

- The raw meat and meat products samples were found to be contaminated with *E. coli*.
- In addition to the conventional methods used for isolation and identification of *E. coli*, PCR is required as rapid, accurate and specific tool for detection of pathogenic *E. coli* and their virulence genes.
- Application of good hygiene practices (GHP) during meat processing is essential to control the hazards of *E. coli* food poisoning.

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المخلص العربي

" التوصيف الجزيئي للايشيريشيا القولونية الممرضة المعزولة من اللحوم و منتجاتها "

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استهدفت هذه الدراسة إلقاء الضوء على مدى تواجد ميكروب الايشيريشيا كولاي في اللحوم و بعض منتجاتها وتحديد جين الضراوه بها والذي قد يمثل تهديدا لسلامة الاغذية لذلك تم فحص عدد ٢٠٠ عينة من اللحم و منتجاتها [لحم مفروم ، كفته ، سجق ، بيف برجر ، بسطرمة ، لانشون ، هوت دوج] (٢٥ عينة من كل منها) تم تجميعهم عشوائيا من السوبرماركت و محلات الجزاره و الباعه الجائلين في محافظة الاسماعيليه و أظهرت نتائج الفحص البكتريولوجي والتعريف البيوكيميائي أن عدد ٣٤ عينة (١٧%) كانت موجبه لميكروب الايشيريشيا كولاي، كما أوضحت نتائج التصنيف السيرولوجي لميكروب الايشيريشيا كولاي المعزول من عينات اللحم و اللحم المفروم و البيف برجر و البسترمة أن عزرات الايشيريشيا كولاي كانت تنتمي الى مجموعات O:157 K:- ، O:91 K:- ، O:103 K:- ، O:125 K:70 على التوالي، بينما كانت السلالات المعزولة من الكفته و السجق

تنتمى الى مجموعة O:26 K:60 . بالاضافه الى سلالات لم يتم تصنيفها سيولوجيا باستخدام المجموعات السيولوجيه Anti-Coli I, II, III . و باستخدام اختبار تفاعل انزيم البلمرة المتسلسل ذو الوقت الحقيقى لتحديد جينات الضرواه $vf1$, $vf2$ ، أسفرت النتائج ان ميكروب الايشيريشيا كولاي (O:26 K:60) المعزوله من الكفته أظهر تحديد جينات الضرواه $vf1$, $vf2$ مقارنة بالمعزوله من السجق و الايشيريشيا كولاي (-:O:103 K:- ، -:O:91 K:-) المعزوله من البيف برجر و اللحم المفروم كانت تحتوي على جين الضراوه $vf2$ فقط.