Molecular Characterization of *Escherichia Coli* Isolated from Meat and Meat Products in Port-Said Markets

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Abstract
Eight hundred of meat products collected randomly from Port-Said markets for *E. coli* isolation. *E. coli* total prevalence was (43%). *E. coli* presence in minced meat was (19.77%) followed by raw meat as (17.44%), sausage as (17.15%), burger as (16.57%), pastirma as (15.70%), luncheon as (6.10%), salami as (4.07%), and frankfurter as (3.20%). PCR showed that *E. coli* serotypes were positive for (*pho***A**) and (*tsh*) while negative for (*stx*1) and (*Vt*2e). *E. coli* O26, O125, and O157 carried (*eae***A**). *E. coli* O157 was the only serotype that carried (*hly*) and (*stx*2).

Introduction

Meat and meat products are an excellent source of a wide variety of nutrients, high quality proteins, vitamins, and certain minerals. These nutrients are required for growth and multiplication of many microorganisms so they considered as important sources of human infections with a variety of foodborne pathogens as *Enterobacteriaceae* ***Doulgeraki et al. (2012)***. *Escherichia coli* is a Gram-negative rod-shaped bacterium belonged to family *Enterobacteriaceae* that is commonly found as a part of the normal microflora in the intestinal tract of humans and warm-blooded animals ***Meng et al. (2007)***.

E. coli serotypes were categorized according to virulence genes they possess, clinical signs, and mode of transmission into enterotoxigenic (ETEC), enter invasive (EIEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), and enteroaggregative (EAEC) ***Koneman et al. (1997)***. The virulence of *E. coli* is mainly associated with their ability to damage intestinal epithelial cells and two phage-encoded cytotoxins called shiga toxins (*stx*1 and *stx*2) **Wong *et al.*, (2000)**. Other major virulence genes besides shiga toxins are attaching and effacing gene (*eae***A**) and hemolysin gene (*hly*) ***Paton and Paton (1998)***.

The aim of this study was to determine the prevalence of *E. coli* in raw meat and meat-based products, serotyping, and screen virulence genes (*pho***A**, *hly*, *eae***A**, *tsh*, *stx*1, *stx*2, and *Vt*2e) presence in isolated serotypes using PCR.

Material and Methods

Samples collection: 700 meat-based products and 100 fresh raw meat specimens were collected randomly from Port Said governorate during the period between September 2016 to September 2018.

Bacteriological isolation: 25 grams of each product represented the product sample were added aseptically to 225 ml buffered peptone water then were enriched by incubation at 37ºC for 24 hours ***ICMSF (1978)***. Enriched samples were streaked on Eosin Methylene Blue agar (EMB) and MacConkey's agar and incubated at 37°C for 24 hours while on Tryptone Bile Glucuronic Agar (TBX) were incubated first at 37°C for 4 hours then at 44°C for 20 hours ***Koneman et al. (1997)***.

Serological examination: Isolates were submitted to serological typing by slide agglutination test using O somatic antigen ***Edwards and Ewing (1972)***.

Polymerase Chain reaction: For detection of different virulence genes in *E. coli* serotypes, oligonucleotide primers that have specific sequence and amplify a particular product were used Table (1). DNA extraction had been done by following manufacturer's instructions of QIAamp DNA mini kit as shown in Table (2). PCR products were electrophorized using 1.5% agarose gel using Gel casting apparatus (Biometra). The gel was photographed by a gel documentation system and the data analyzed through computer software according to ***Sambrook et al. (1989)***.

Table (1): *Oligonucleotide primers sequences:*

Target gene	Primers sequences 5'-3'	Amplified segment (bp)	Reference
*pho***A**	F: 5-CGTGATCAGCGGTGACTATGAC-3	720 bp	**Hu *et al*., (2011)**
	R: 5-CGATTCTGGAAATGGCAAAAG-3		
hly	F: 5-AACAAGGATAAGCACTGTTCTGGCT-3	1177 bp	**Piva *et al*., (2003)**
	R: 5-ACCATATAAGCGGTCATTCCCGTCA-3		
*eae***A**	F: 5-ATGCTTAGTGCTGGTTTAGG-3	248 bp	**Bisi-Johnson *et al*., (2011)**
	R: 5-GCCTTCATCATTTCGCTTTC-3		
tsh	F: 5-AGTCCAGCGTGATAGTGG-3	620 bp	**Delicato *et al*., (2003)**
	R: 5-GGTGGTGCACTGGAGTGG-3		
*stx*1	F: 5-ACACTGGATGATCTCAGTGG-3	614 bp	**Dipineto *et al*., (2006)**
	R: 5-CTGAATCCCCCTCCATTATG-3		
*stx*2	F: 5-CCATGACAACGGACAGCAGTT-3	779 bp	
	R: 5-CCTGTCAACTGAGCAGCACTTTG-3		
*Vt*2e	F: 5-CCAGAATGTCAGATAACTGGCGAC-3	322bp	**Orlandi *et al*., (2006)**
	R: 5-GCTGAGCACTTTGTAACAATGGCTG-3		

F: Forward primer R: Reverse primer

Table (2): *Cycling conditions of the different primers during cPCR:*

Target gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
*pho***A**	94˚C 5 minutes	94˚C 30 seconds	55˚C 40 seconds	72˚C 45 seconds	35	72˚C 10 minutes
hly	94˚C 5 minutes	94˚C 30 seconds	60˚C 40 seconds	72˚C 1 minute	30	72˚C 10 minutes
*eae***A**	94˚C 5 minutes	94˚C 30 seconds	51˚C 30 seconds	72˚C 30 seconds	35	72˚C 7 minutes
tsh	94˚C 5 minutes	94˚C 30 seconds	54˚C 40 seconds	72˚C 45 seconds	35	72˚C 10 minutes
*stx*1, *stx*2	94˚C 5 minutes	94˚C 30 seconds	58˚C 40 seconds	72˚C 45 seconds	35	72˚C 10 minutes
*Vt*2e	94˚C 5 minutes	94˚C 30 seconds	57˚C 40 seconds	72˚C 40 seconds	40	72˚C 10 minutes

Results and Discussion

Meat products are rich in nutritional composition that also causes the growth of many microorganisms including food pathogenic bacteria ***AL-Mutairi (2011)***. In the present study, *E. coli* prevalence was (43%) that is nearly similar to ***El-Sharkaway et al. (2016)*** who reported *E. coli* as (41%) in meat products while lower than ***Abd El Tawab et al. (2015)*** who isolated *E. coli* from (30.5%) of meat-based samples. *E. coli* isolates were (19.77%) in minced meat, (17.44%) in raw meat, (17.15%) in sausage, (16.57%) in burger, (15.7%) in pastirma, (6.1%) in luncheon, (4.07%) in salami, and (3.2%) in frankfurter. The present results is lower than ***El-Sharkaway et al. (2016)*** as they recorded *E. coli* highest ratio in burger as (29.26%) followed by minced meat as (26.82%), sausage as (24.39%), and pastirma as (19.51%).

Serologically, the prevalence of various *E. coli* serotypes revealed that the most predominant serotype was O125 as (25%) followed by O158 as (20.93%), O111 as (10.47%), O55 as (8.43%), O157 as (5.81%), O26 as (4.07%), O119 as (2.33%), O142 as (2.03%), O114 as (1.74%), both O124 and O136 as (1.45%), O78 as (1.16%), O112 as (0.87%), both O63 and O126 as (0.58%), both O25 and O86 as (0.29%), and un-typed serotypes as (12.5%). ***Abd El Tawab et al. (2015)*** recorded the prevalence of *E. coli* O26 as (15.6%) which is higher than the present study while *E. coli* O111 was (9.4%) which is nearly similar to the current result. *E. coli* O157 was found in (5%) of meat

samples ***Abdul-Raouf et al. (1996)*** which is nearly similar to the present survey. ***Ammar et al. (2016)*** mentioned that (12.8%) of tested meat samples were positive for *E. coli* and O111 was the most prevalent serotype as (40.62%) followed by O26 as (12.5%), O124, O127, and O128 as (9.37%) for each, O78 and O119 (6.25%) for each which is higher than the present study.

Table (3) showed that 12 *E. coli* serotypes (O26, O55, O78, O111, O114, O119, O124, O125, O136, O142, O157, and O158) were subjected to PCR for detection of alkaline phosphates (*pho***A**) gene and hemolysin (*hly*), attaching and effacing (*eae***A**), temperature sensitive hemagglutination (*tsh*), shiga toxin I (*stx*1), shiga toxin II (*stx*2), and verotoxin 2e (*Vt*2e) virulence genes. All isolates were positive for alkaline phosphates (*pho***A**) gene **Figure (1)**. These results agree with ***Chang et al. (1986)*** and ***Kong et al. (1999)*** who reported that *pho***A** gene is a housekeeping gene present in all *E. coli* serotypes.

Hemolysin (*hly*) is significant virulence gene as it can result in extraintestinal injuries ***Bhakdi et al. (1990)***. **Figure (2)** showed that only *E. coli* O157 carried *hly* gene that agrees with ***Chinen et al. (2001)*** while ***Abd El Tawab et al. (2015)*** revealed that *hly* gene was absent in *E. coli* O157.

The detection of attaching and effacing (*eae***A**) gene by PCR viewed in **Figure (3)** showed that three of the examined isolates were positive for the *eae***A** gene. This result agrees with ***Hala et al. (2011)*** who detected the *eae***A** gene in (20%) of *E. coli* isolates but disagree with ***Mohammadi et al. (2013)*** who reported that all of *E. coli* isolates were *eae***A**-negative.

The three *E. coli* serotypes that carried *eae***A** gene were *E. coli* O26, O125, and O157. This goes parallel with ***Dambrosio et al. (2007)*** who recorded that *E. coli* O26 harbored *eae***A** and ***Osek and Gallien (2002)*** who detected presence of *eae***A** in *E. coli* O157. ***Ibrahim et al. (2015)*** reported that *eae***A** was absent in *E. coli* O124 while present in *E. coli* O125 which agrees with the current result.

Osek and Gallien (2002) recorded that *E. coli* O157 that carried *eae***A** also harbored *hly* which is in line with current result. As reported by ***Paton and Paton (1998)*** and ***Karch et al. (1992),*** the combination between these two virulence genes is an important indicator of pathogenicity of *E. coli* for humans than each gene alone. Therefore, isolated *E. coli* O157 can be a potential health risk for man.

In the present study, result of temperature sensitive hemagglutination gene (*tsh*) presented in **Figure (4)** that all *E. coli* serotypes carried *tsh*. This agrees with ***Janben et al. (2001)*** and ***Saidenberg et al. (2013)*** who detected *tsh* gene in (85.3%) and (78.3%) of *E. coli* respectively.

However, *tsh* is mainly isolated from avian pathogenic *E. coli* (APEC), our results confirms its presence in all *E. coli* serotypes. ***Abdulgayeid et al. (2015)*** recorded *tsh* gene in all *E. coli* isolates recovered from buffalo calves' fecal samples. This may be a result of the expression of *tsh* gene in different animal species is underestimated or a poultry-to-buffalo transmission of APEC.

The most important virulence genes that responsible for pathogenicity of *E. coli* are shiga toxins (*stx*1 and *stx*2) ***Vallance and Finlay (2000).*** **Figure (5)** showed that none of *E. coli* serotypes carried *stx*1. Only *E. coli* O157 carried *stx*2 that was absent in all other serotypes.

The present result was parallel with ***Murphy et al. (2005)*** and ***Dambrosio et al. (2007)*** who recorded absence of *stx*1 and *stx*2 in *E. coli* O26 and ***Ibrahim et al., (2015)*** who confirmed that *E. coli* O124 did not carry *stx*1 or *stx*2.

Abd El Tawab et al. (2015) recorded that *stx*1 was absent in *E. coli* O111, O26, and O157 serotypes while *E. coli* O157 carried *stx*2 and produced a particular band at 779 bp which is in line with the current survey. On the other hand, ***Tafida et al. (2014)*** recognized *stx*1 in *E. coli* O157 isolates while *stx*2 was absent and ***Gomez-Aldapa et al. (2013)*** reported that none of *E. coli* O157 isolates had stx1 or stx2.

Figure (6) showed that Verotoxin2e (*Vt*2e) was absent in all *E. coli* serotypes which disagreed with ***Younis et al. (2015)*** who isolated *Vt*2e from (20%) of *E. coli* samples.

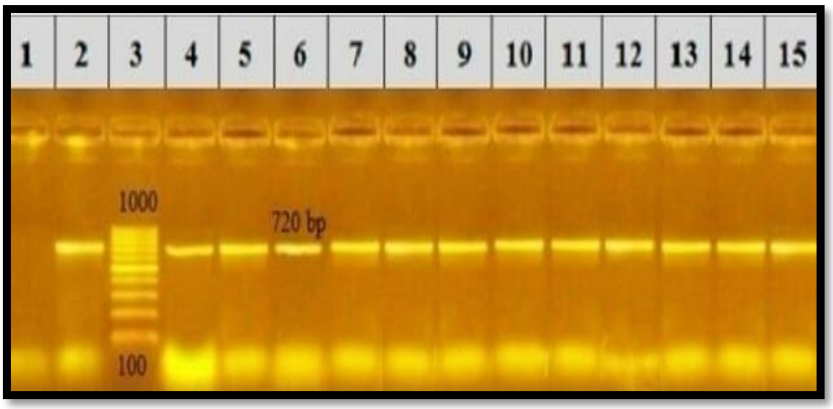

Figure (1): PCR result of (*pho*A) in *E. coli* serotypes.
Lane (1): Negative control, **Lane (2):** Positive control, **Lane (3):** Molecular marker, **Lane (4, 5, 6, 7, 9, 10,11,12,13, 14, and 15):** *E. coli* serotypes *pho*A-positive at 720 bp.

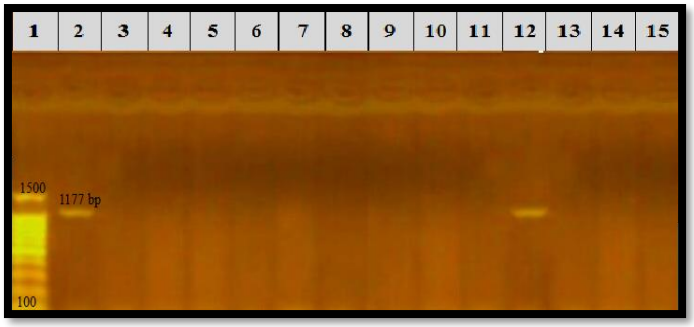

Figure (2): PCR result of (*hly*) virulence gene in *E. coli* serotypes.
Lane (1): Molecular marker, **Lane (2):** Positive control, **Lane (3):** Negative control, **Lane (4):** O78, **Lane (5):** O114, **Lane (6):** O119, **Lane (7):** O124, **Lane (8):** O136, **Lane (9):** O142, **Lane (10):** O55, **Lane (11):** O111, **Lane (12):** O157 with specific band at 1177 bp, **Lane (13):** O26, **Lane (14):** O158, and **Lane (15):** O125.

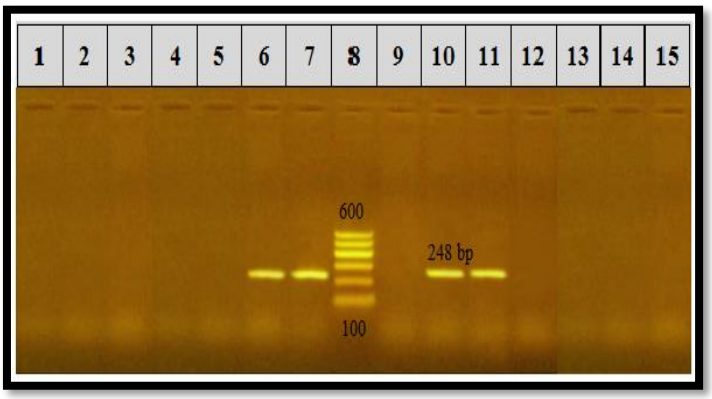

Figure (3): PCR result of (*eae*A) virulence gene in *E. coli* serotypes.
Lane (1): O55, **Lane (2):** O78, **Lane (3):** O111, **Lane (4):** O114, **Lane (5):** O119, **Lane (6):** O26, **Lane (7):** Positive control, **Lane (8):** Molecular marker, **Lane (9):** Negative control, **Lane (10, 11):** O125, O157 with specific band at 248 bp, **Lane (12):** O124, **Lane (13):** O136, **Lane (14):** O142, and **Lane (15):** O158.

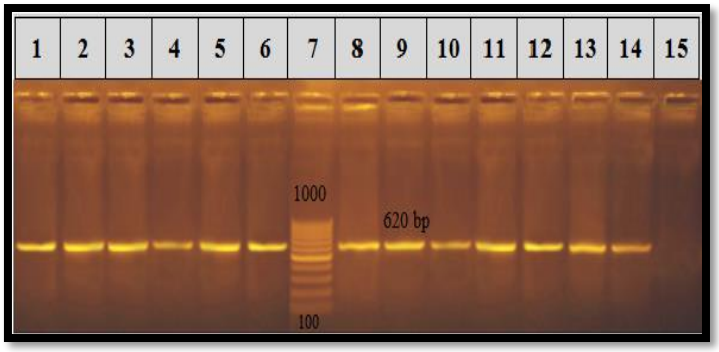

Figure (4): PCR result of (*tsh*) virulence gene in *E. coli* serotypes.
Lane (1, 2, 3, 4, 5, 6, 9, 10,11,12,13, and 14): *E. coli* serotypes *tsh*-positive with 620 bp band, **Lane (7):** Molecular marker, **Lane (8):** Positive control, and **Lane (15):** Negative control.

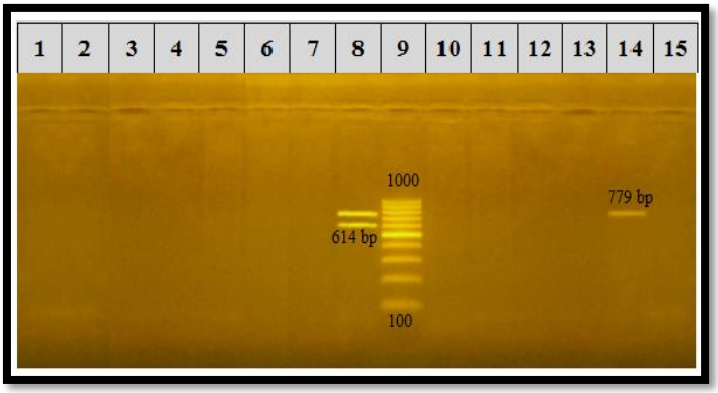

Figure (5): PCR result of (*stx*1) and (*stx*2) virulence gene in *E. coli* serotypes.
Lane (1): O78, **Lane (2):** O114, **Lane (3):** O119, **Lane (4):** O124, **Lane (5):** O136, **Lane (6):** O142, **Lane (7):** Negative control, **Lane (8):** Positive control, **Lane (9):** Molecular marker, **Lane (10):** O55, **Lane (11):** O111, **Lane (12):** O26, **Lane (13):** O158, **Lane (14):** O157 positive for *stx*2 with specific band at 779 bp, and **Lane (15):** O125.

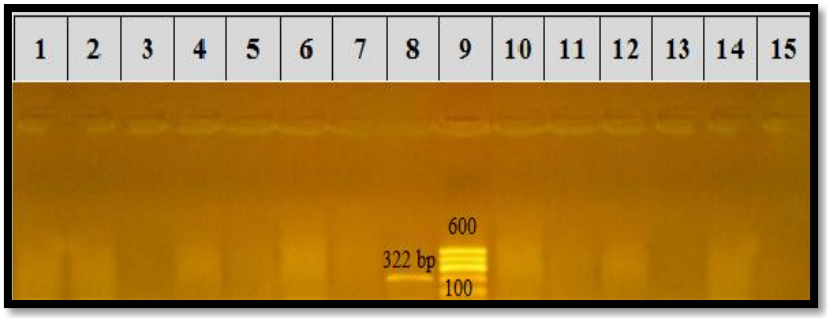

Figure (6): PCR result of (*Vt*2e**) virulence gene in *E. coli* serotypes.**
Lane (1,2,3,4,5,6,10,11,12,13,14, and 15) *E. coli* serotypes *Vt*2e-negative, **Lane (7):** Negative control, **Lane (8):** Positive control, **Lane (9):** Molecular marker.

Table (3) *Distribution of phoA and virulence genes in E. coli serotypes:*

Serotype	*pho***A**	**Virulence genes**					
		hly	*eae***A**	*tsh*	*stx*1	*stx*2	*Vt*2e
O26	+	-	+	+	-	-	-
O55	+	-	-	+	-	-	-
O78	+	-	-	+	-	-	-
O111	+	-	-	+	-	-	-
O114	+	-	-	+	-	-	-
O119	+	-	-	+	-	-	-
O124	+	-	-	+	-	-	-
O125	+	-	+	+	-	-	-
O136	+	-	-	+	-	-	-
O142	+	-	-	+	-	-	-
O157	+	+	+	+	-	+	-
O158	+	-	-	+	-	-	-

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مدى تواجد و التوصيف الجزيئي لاشريشيا كولاي المعزول من اللحوم و منتجاتها من اسواق بورسعيد

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الملخص العربي

أجريت الدراسة على عدد ٨٠٠ منتج من منتجات اللحوم و أثبتت النتائج وجود ميكروب الاشريشيا كولاي بنسبة (٤٣٪) حيث كانت (19.٧٧%) في اللحم المفروم و (١٧.٤٤٪) في اللحم النيئ و (17.15%) في السجق و (١٦.57٪) في البرجر و (١٥.٧٪) في البسطرمة و (6.10%) في اللانشون و (4.07%) في السلامي و (3.2%) في الفكرانكفورتر. الفحص السيرولوجي أوضح تواجد (O25, O26, O55, O63, O78, O86, O111, O112, O114, O119, O124, O125, O126, O136, O142, O157, O158 و عينات لم تصنف سيرولوجيًا). اظهر تفاعل البلمرة المتسلسل أن كل عترات الاشريشيا كولاي المختبرة كانت ايجابية لعوامل الضراوة phoA و tsh. العترات O26 و O125 و O157 كانت تحتوي على عامل الضراوة eaeA. O157 فقط كانت تحتوي على عوامل الضراوة hly وstx2. عوامل الضراوة stx1 و Vt2e لم تتواجد في أي من العترات.