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Molecular Characterization of *Escherichia Coli* Isolated from Poultry Meat and its Products

Zakaria H. Elbayoumi[1], Reyad R. Shawish[2], Mohamed Hamada[3], Hanady R. Esmail[4]
[1,2,3]Department of Food Hygiene & Control, Faculty of Veterinary Medicine, University of Sadat City, Egypt
[4]Veterinarian, Directorate of Veterinary Medicine, Shebin El-Kom, El-Menofia Governorate, Egypt

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***Correspondence to:**
zico76us@yahoo.com

ABSTRACT

This study was conducted on chicken breast, chicken thigh, Shish Taouk, chicken shawarma, chicken nuggets and chicken luncheon (35 of each) collected from different supermarkets at Menoufia Governorates for isolation and identification of *E. coli* and using PCR for detection of virulence gene. The obtained results indicated that the incidence of *E. coli* was 14.3%, 20%,14.3%, 17.1%, 14.3% and 20% of examined samples of chicken breast, chicken thigh, Shish Taouk, chicken shawarma, chicken nuggets and chicken luncheon, respectively. Moreover, the incidence of serologically identified *E. coli*O_{78},O_{26}: H_{11}, O_2: $H_{6,}$ O_{91}:H_{21},O_{44}:H_{18}, O128:H2,O_1:H_7, O_{103}:H_2, O_{127}:H_4,O_{114}:H_4 *and* O_{158}.PCR results showed shiga toxin 2 gene(*stx2*) detected in (O_1), (O_2), (O_{114})& (O_{128}), while shiga toxin 1 gene(*stx1*) detected in (O_{44}), (O_{127}) & (O_{158}), also (O_{78}) & (O_{91}) Positive *E. coli*strains for *stx1* and *stx2* genes, and(O_{26}) & (O_{103}) Positive *E. coli*strains for *stx1*, *stx2*and *eaeA* genes.

1. INTRODUCTION

The production of poultry meat products has increased throughout the world due to its specific sensory attributes and the consumer's belief that white meat is healthier than red meat. Poultry products are highly perishable foods. Therefore, the industry is focused on methods to increase the overall safety and quality of poultry products (Colak *et al.*, 2011) poultry meat and its products are very popular food throughout the world and no wonder since They are delicious, nutritious and considered as a good and cheap source of protein characterized by good flavor and easily digested on the other hand, they rank first or second in foods associated with food borne disease as *E. coli* in most of the countries all over the world where USA ranked third of the reported food-borne disease outbreaks. Many researchers have reported that poultry meat and its products were contaminated by several pathogenic bacteria (Basaran Khraman and Ak 2012 and Urumova *et. al.*, 2014).

E. coli is a commensal inhabitant of the gastrointestinal tract of mammals and birds, is also the causative agent of several diseases in animals and human worldwide. Pathogenic *E. coli* strains have been divided into intestinal pathogenic *E. coli* and extra intestinal pathogenic *E. coli* (ExPEC) depending on the location of the infection they are causing. EPEC strains are responsible for a variety of infections, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia (Russo and Johnson, 2000 and Kaper*et al.*, 2004). At least six different categories of pathogenic *E.coli* causing enteric infections have been identified and further characterized *(Alfredo et al., 2010)*.These pathotypes are: Verocytotoxigenic *E. coli* (VTEC), Enterotoxigenic *E. coli* (ETEC), Enter-invasive*E. coli* (EIEC), Enteropathogenic *E. coli (*EPEC), Enteroaggregative *E. coli* (EAggEC), Diffusely adherent *E. coli* (DAEC) Also, certain strains of *E.coli* known as verocytotoxin-producing *E. coli* (VTEC) produce a potent poison, or toxin, which causes illnesses ranging from mild diarrhea through to very

severe inflammation of the gut. Occasionally this can cause complications such as kidney failure, and anemia. The most important toxin-producing strain associated with human illness is known as *E. coli* O_{157} (Nagwa et al., 2012**).** Most *E. coli* strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, and neonatal meningitis. It can also be characterized by severe abdominal cramps, diarrhea that typically turns bloody within 24 hours, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and Gram-negative pneumonia (Todar, 2007). STEC represent a hazardous public health problem worldwide causing various human gastrointestinal tract diseases, including watery or bloody diarrhea and might develop a life-threatening disease, such as hemorrhagic colitis (HC), Thrombotic Thrombocytopenic Purpura (TTP) and Hemolytic Uremic Syndrome (HUS). The latter is characterized by thrombocytopenia, microangiopathic hemolytic anemia and acute renal failure (Pennington, 2010).

Polymerase Chain Reaction (PCR) based methods have been identified as a powerful diagnostic tool for the detection of pathogenic microorganisms (Malorny et al., 2003). Compared to other methods of detection, these methods are rapid, highly specific and sensitive in the identification of target organisms (Wang et al., 2007).

The aim of the present study was to determine the occurrence, serovars and virulence gene profile of *E. coli* isolated poultry meat (chicken thigh and chicken breast) and some of its product (chicken shawarma, Shish Taouk, chicken nuggets and chicken luncheon).

2. MATERIAL AND METHODS

Collection of Samples: A total 210 random samples of freshraw chicken cuts (chickenbreast and chickenthigh), some half-cooked chicken products (chickenshawarma, chickennuggets and Shish Taouk**)** and Cooked Products(chickenluncheon)(35 of each) collected from different supermarkets at Menoufia governorate.

2.1. Preparation of Samples according to *(APHA, 1992)*

2.2 Isolation and identification of *Escherichia coli* according to *(ICMSF, 1996*)

2.3 Polymerase Chain Reaction (PCR) of *E. coli*

1. Materials used for PCR:

1.1. Reagents used for agarose gel electrophoresis:

1.1.1. Agarose powder, Biotechnology grade (Bioshop^R, Candainc.lot No: OE16323).

1.1.2. Tris acetate EDTA (TAE) electrophoresis buffer (50×liquid concentration) (Bioshop^R,Candainc. lot No: 9E11854).

1.1.3. Ethedium bromide solution (stock solution) biotechnology grade (Bioshop ® CandaInc, Lot No: 0A14667):

1.2. Gel loading buffer (6×stock solution) (Fermentas, lot No: ooo56239).

1.3. DNA ladder (molecular marker):

100 bp (Fermentas, lot No: 00052518).

1.4. 5X Taq master (Fermentas):

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

2.3.B. 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 of Ampli Taq 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* O_{157}:H_7 Sakai (positive for *stx1*, *stx2* and *eaeA)* and *E. coli* K12DH5α (a nonpathogenic negative control strain) that does not possess any virulence gene. 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 size.

Table (1) Primer sequences of *E. coli* used for PCR identification system

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

Table (2): Cycling conditions of the different primers during PCR.

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

Table (3) Incidence of identified E. coli serotypes isolated from the examined samples of chicken meat products (n=35).

Samples / **Isolated Bacteria**	**Raw Products**				**Half cooked**						**Cooked Products**		Types
	Breast		**Thigh**		**Nuggets**		**Shish Taouk**		**Shawrema**		**Luncheon**		
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	
O_2: H_6	-	-	2	5.7	1	2.9	-			-	2	5.7	
O_{114}: H_4	-	-	-	-	-	-	-			-	1	2.9	
O_{44}: H_{18}	-	-	-	-	1	2.9	-			-	-	-	
O_1: H_7	-	-	-	-	-	-	2	5.7		-	-	-	
O_{78}	3	8.6	2	5.7	2	5.7	-	-	3	8.6	-	-	
O_{158}	-	-	-	-	-	-	-			-	1	2.9	EPEC
O_{26}: H_{11}	2	5.7	-	-	-	-	-	-	2	5.7	1	2.9	
O_{91}: H_{21}	-	-	2	5.7	-	-	-	-	-	-	1	2.9	
O_{103}: H_2	-	-	-	-	-	--	2	5.7	-	-	-	-	EHEC
O_{128}: H_2	-	-	-	-	2	5.7	-	-	-	-	-	-	ETEC
O_{127}: H_4	-	-	-	--	-	-	2	5.7	-	-	-	-	
Total	**5**	**14.3%**	**7**	**20%**	**5**	**14.3%**	**6**	**17.1%**	**5**	**14.3%**	**7**	**20%**	

3. RESULTS

3.1 Prevalence of *E. coli* isolated from poultry meat and some of its products:

A total of 210 chicken cuts and some of chicken by product were examined bacteriologically, incidence of *E. coli* was 14.3%, 20%, 14.3%, 17.1%, 14.3% and 20% of examined sample of chicken breast, chicken thigh, Shish Taouk, chicken shawarma, chicken nuggets and chicken luncheon, respectively.

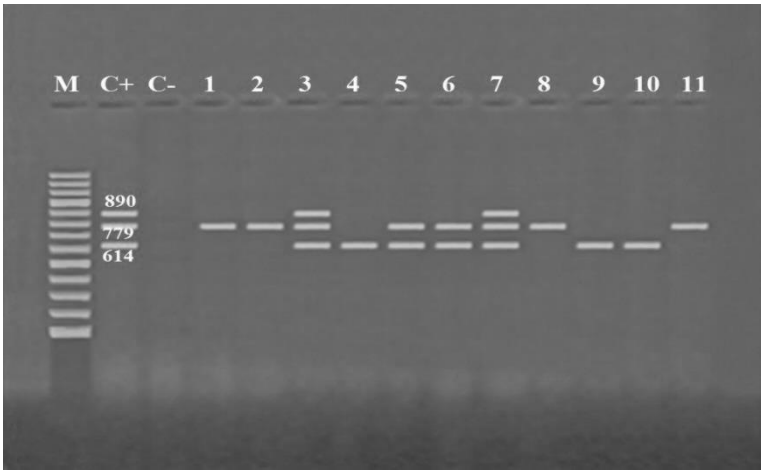
3.2 Results of PCR amplification of the *stx1, stx2* and *eae A* genes of Enteropathogenic *E. coli* serogroups*:*

The genomic DNA of *Enteropathogenic E. coli* serogroups were tested using 3 sets of primers for detection of 3 virulence genes that play a role in virulence of *Enteropathogenic E. coli*. the genes were shiga toxin 1 gene(*stx1*), shiga toxin 2 gene(*stx2*) and intimin gene (*eaeA*). It was applied on random isolated *Enteropathogenic E. coli* serogroups (O_1,O_{127} and O_{103} from Shish Taouk; O_2 and $O_{91}$from thigh;

O_{114} and O_{158} from luncheon;O44 and O_{128} from nuggets; $O_{78}$from breast and O_{26} from Shawarma). PCR results showed shiga toxin 2 gene(*stx2*) detected in (O_1), (O_2), (O_{114})& (O_{128}), while shiga toxin 1 gene(*stx1*) detected in (O_{44}), (O_{127}) & (O_{158})**,** also (O_{78}) & (O_{91}). Positive *E. coli*strains for *stx1* and *stx2* genes, and**(**O_{26}) & (O_{103}) Positive *E. coli*strains for *stx1*, *stx2*and *eae A* genes The genomic DNA of *Enteropathogenic E. coli* serogroups were tested using specific primer for the *stx1*gene. the *stx1*gene was amplified in (O_{44}), (O_{127}), (O_{158})**,** (O_{78}), (O_{91}), **(**O_{26}) and (O_{103}) which giving product at (614 bp**)** as showing in Photograph (1).

While genomic DNA of *Enteropathogenic E. coli* serogroups were tested using specific primer for the *stx2* gene. the *stx2*gene was amplified in (O_1), (O_2), (O_{114}), (O_{128}), (O_{78}), (O_{91}), (O_{26}) and (O_{103}) which giving product at (779 bp) as showing in Photograph (1).

And genomic DNA of *Enteropathogenic E. coli* serogroups were tested using specific primer for the *eae A* gene. The *eae A* gene was amplified in(O_{26}) & (O_{103}) which giving product at(890 bp) as showing in Photograph (1).



Photograph (1): Agarose gel electrophoresis of multiplex PCR of *stx1* (614 bp), *stx2* (779 bp) and *eaeA* (890 bp) genes characterization of *Enteropathogenic E. coli.* Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *E. coli* for*stx1, stx2*and *eaeA* genes. Lane C-: Control negative. Lanes 1 (O1), 2 (O2), 8 (O114)& 11 (O128): Positive *E. coli*strains for *stx2* gene. Lanes 4 (O44), 9 (O127) & 10 (O158): Positive *E. coli*strains for *stx1* gene. Lanes 5 (O78) & 6 (O91): Positive *E. coli*strains for *stx1* and *stx2* genes. Lanes 3 (O26) & 7 (O103): Positive *E. coli*strains for *stx1, stx2*and *eaeA* genes.

4. DISCUSSION

The incidence of *E. coli* in poultry meat and some of its products revealed that incidence of isolation of *E. coli* in the examined samples of (chicken breast, chicken thigh, Shish Taouk. chicken Shawarma, chicken Nuggets and chicken Luncheon),respectively. Was (14.3 % (5), 20% (7), 14.3% (5), 17.1% (6), 14.3% (5) and 20% (7), respectively. incidence of isolation of *E. coli* in the examined samples of thigh and breast 20% (7) and 14.3 % (5),respectively. These results nearly agreed with other authors Vaidya *et al.,* (2005) isolated *E.coli* from examined breast with percentage rate 14.57 % on the other hand , lower incidence from thigh and breast reported by Mohamed (2004) isolated *E. coli* from the examined samples of chicken breast with percentage of 7.5% and in chicken thigh with percentage of 2.5% , Marionette *et al.,* (2009) isolated *E. coli* from the examined samples of chicken breast and thigh with percentage of 6.67% , 10%,respectively. Edris - Shimaa *et al.*, (2011) isolated *E. coli* from the examined samples of chicken breast with percentage of 12% and in chicken thigh with percentage of 16%, Hassanin *et al.,* (2014) isolated *E. coli* from examined breast with percentage rate 10%,Khaled *et al.,* (2015) isolated *E. coli* from examined breast with percentage rate 10%. and Riyad (2011)isolated *E. coli* from the examined samples of chicken breast and thigh with percentage of 8.7% in both samples.

On the other side higher incidence from thigh and breast was documented by several authors AL-Dughaym and Altabari (2010) isolated *E. coli* from the examined samples of chicken thigh with percentage of 60% , Ruban and fairoze (2011) isolated *E. coli* from examined thigh and breast in the range

of 42 to 88 % , Cook *et al.,* (2012) isolated *E. coli* from the skin-off chicken breasts, 33 (33%) and from the skin-on chicken breasts, 77 (41%), James Andrews (2013) isolated *E. coli* from examined breast with percentage of 65% , Robert Roos (2013) isolated *E. coli* from examined breast with percentage of 65.2% , *E. coli* was isolated from the examined thigh with percentages of 33.33% , Edris *et al.,* (2015) isolated *E.coli* from examined thigh and breast with percentages of 88% , 70%, respectively. Khalafalla *et al.,* (2015) isolated *E. coli* from both examined thigh and breast with percentages of 100% and Khaled *et al.,* (2015) isolated *E. coli* from examined thigh with percentages of30%.

While Mohamed (2004) and Mohamed Hamada *et al.,* (2008) failed to isolate *E. coli*O_{157}:H_7 from examined thigh and breast.

Incidence of isolation of *E. coli* in the examined samples of chicken luncheon, nuggets and shawarma, were 20%, 14.3% and 17.1%,respectively. Some authors nearly agreed with our results in luncheon as Rady *et al*., (2011) who isolated *E. coli* with percentage of 20%.

Lower incidence of E. *coli* from chicken luncheon reported by Fawzy (2004), Naglaa *et al.,* (2009)and Samaha *et al.,* (2012)isolated *E. coli* with percentage of 8%,10% and 8%, respectively. Also, higher incidence of *E. coli* in chicken luncheon reported by El Sabagh-Rasha (2010), Hashim (2003)and Sharaf-Eman and Sabra-Sherifa (2012) isolated *E. coli* with percentage of 35%, 19 (22.35) % and 25%, respectively.

Khalifa and Abd-El Shaheed (2005), Riyad (2011) and Mostafa-Hemmat *et al.,* (2014) failed to isolate *E. coli* from luncheon also Ransom and Hill (2001), Hashim (2003) and Mohamed Hamada *et al.,* (2008) failed to isolate *E. coli* O_{157}:H_7 from luncheon.

Higher incidence of *E. coli* in chicken shawarma, chicken nuggets and Shish Taouk reported by Mahmoud (2006), Eglezos *et al.,* (2008) and Naglaa *et al.,* (2009) *E. coli* percentage was 35.6%, 47% and 20%, respectively. Also, higher incidence of *E. coli* in chicken shawarma reported by Hassanin *et al.,* (2014), Mohamed -Walaa (2014), Sharaf- Eman and Sabra -Sherifa (2012) and Nimri –Laila *et al.,* (2014) 33.3%, 33.3%, 20% and 29.0%, respectively.

On the other side lower incidence of *E. coli* in chicken shawarma documented by Saad *et al., (*2015) isolated *E. coli* with percentageof10%.*Hamid and Majid (2008),* Abd El-Rahman et al., (2010) reported low incidence of *E. coli* in Shish Taouk *and nuggets* with percentage of *5.5 %,* 10.6%, respectively.

Fatin, S. *et al.,* (2015), Rawaish - Rabab (2014), Odu and Akano (2012) and Abdalhamid *et al.,* (2013) also reported low incidence of *E. coli* in shawerma 6.67%, 6.67%, 13.6% and 8%, respectively. Samaha *et al.,* (2012) reported low incidence in nuggets 12%.

On the other side higher incidence from *E. coli* in chicken nuggets and Shish Taouk reported by AL-Dughaym and Altabari (2010), Hassanin *et al.,* (2014) isolated E. *coli* with percentage of 60% and 26.7%, respectively.

Also, higher incidence reported by Mohamed -Walaa (2014), Saad *et al., (*2015) isolated E. *coli* with percentage of 26.7%, 25% of examined shish and nuggets, respectively.

Tareq *et al.,* (2013) failed to isolate *E. coli* O_{157}:H_7 from chicken shawerma, Mostafa-Hemmat *et al.,* (2014), Fulden Karadal *et al.,* (2013) failed to isolate *E. coli* and *E. coli* O_{157}:H_7 from chicken nuggets, respectively.

The variation of the results between different authors may be due to the differences in manufacture practices, handling from producers to consumers, storage and the effectiveness of hygienic measures applied during production. The presence of *E. coli* in food of animal origin is considered as indicator of faults during preparation, handling, storage or services (Tebbut, 1999).

The incidence of identified E. *coli* serotypes in poultry meat and some of its product. The results reported in table (3) revealed that the isolated serotypes of *E. coli* in chicken luncheon were O_2: H_6, O_{144}:H_4, O_{158}, O_{26}:H_{11} and O_{91}:H_{21} with incidence of 2(5.71%), 1(2.9%), 1(2.9%), 1(2.9%) and 1(2.9%), respectively. this result agrees with El Sbagh-Rasha (2010) who isolated O_{26}:K_{60}, O_{55}:K_{59}, O_{111}:K_{58} *and* O_{124}:K_{72} on the other hand this result not agree with Fawzy (2004) who cannot detect any serotypes that present in luncheon and isolated O_{55}: K_{59} and O_{124}: K_{72}.

The results reported in table (5) revealed that the isolated serotypes of *E. coli* in thigh were O_2: H_6, O_{78} and O_{91}:H_{21} with incidence of 2(5.71%),2(5.71%)and 2(5.71%), respectively and serotypes in breast were O_{78} and O_{26}:H_{11} with incidence of 3(8.6) and 2(5.71%), respectively. this result agrees with Edris *et al.,* (2015)who isolated O_{78} from thigh and O_{26}, O_{78} from breast, Marionette *et al.,* (2009) O_{78}:K_{80} from breast and thigh, Mostafa-

Hemmat *et al.,* (2014) isolated O_{78}: k_{80} from thigh and Hassanin *et al.,* (2014) isolated O_{26}: k_{60} from breast.

on the other hand, this result not agree with Khalafalla *et al.,* (2015) who cannot detect any serotypes that present in thigh and breast and isolated O_{157} and O_{18},Edris-Shimaa*et al*., (2011)cannot detect any serotypes that present in thigh and breast but isolated O_{55}: K_{59} and O_{119}: K_{69} $(B_{14})$and Riyad (2011)cannot detect any serotypes that present in thigh and breast but isolated O_{124}:K_{72}, O_{119}:K_{69}, O_{128}:K_{67}, *respectively.*

The results reported in table (3) revealed that the isolated serotypes of *E. coli* in chicken shawarma were O_{26}:H_{11} *and* O_{78} with incidence of 2(5.71%)*and* 3(8.6%), respectively. this result agrees with Saad *et al., (*2015) isolated O_{26}*(5%).* on the other hand, this result not agree with Mahmoud (2006), Nimri–Laila *et al.,* (2014) who cannot detect any serotypes that present in chicken shawarma but isolated *E. coli*O_{157}: H_7 with incidence of 11.1 %, Rawaish-Rabab (2014)and Fatin, *et al.,* (2015) also not agree but isolated serotype O_{119}:H_4.

The results reported in table (3) revealed that the isolated serotypes of *E. coli* in chicken nuggets were O_2: H_6, O_{44}:H_{18}, O_{78} and O_{128}:H_2 with incidence of 1(2.9%),1(2.9%), 2(5.71%)and 2(5.71%), respectively. this result not agree with Saad *et al., (*2015) who isolated O_{119}:H_6(5%), O_{86}(5%), O_{125}:H_{21}(5%), O_{124}(5%) and O_{26}(5%). Also, this result not agree with Abd El-Rahman et *al.,* (2010) who isolated O_{103}: k_3(12%) and *E. coli* O_{119}: K_{69} (8%).

The results obtained showed that the examined thigh samples are more contaminated than other samples and this may have attributed to exposure of thigh samples to fecal contamination by worker's hands during evisceration. The presence of *E.coli* in high numbers indicates the presence of organisms originating from fecal pollution. This is due to improper slaughtering techniques, contaminated surfaces and/or handling of the meat by infected food handlers *(Neletal., 2004).*Also, the presence of these pathogens can be due to contamination taking place during the meat processing at slaughter house or due to the poor handling of the retailers of meat(Kagambèga *et al.*, 2011).The presence of *E.coli* in the examined chicken products considered as indicator for improper handling or unhygienic conditions (Hashim, 2003).

The present study was directed to recognize some virulence genes that may play a role in virulence of *Enteropathogenic E.coli* by using one of the recent development molecular biological techniques (PCR). the genes were shiga toxin 1 gene(*stx1*), shiga toxin 2 gene(*stx2*) and intimin gene(*eaeA*). it was applied on random isolated *Enteropathogenic E. coli* serogroups (O_1,O_{127} and O_{103} from Shish Taouk; O_2 and $O_{91}$from chicken thigh; O_{114} and O_{158} from chicken luncheon;O_{44} and O_{128} from chicken nuggets;$O_{78}$from breast and O_{26} from chicken shawarma(Photograph1). PCR results showed shiga toxin 2 gene(*stx2*) detected in (O_1), (O_2), (O_{114})& (O_{128}), while shiga toxin 1 gene(*stx1*) detected in (O_{44}), (O_{127}) & (O_{158}), also (O_{78}) & (O_{91}) Positive *E. coli*strains for *stx1* and *stx2* genes, and(O_{26}) & (O_{103}) Positive *E. coli*strains for *stx1, stx2*and *eaeA* genes(Photograph1).

Shiga toxins are central to the pathogenesis of bloody diarrhea and hemolytic uremic syndrome through cytopathic effect on vascular endothelial cells of kidney, intestine, central nervous system and other organs (Brogden *et al.,* (2000), Ethelberg (2004). Although Stx1 and Stx2 have similar structures and modes of action their toxicities appear to be distinct. Stx2 was 1000 times more cytotoxic than Stx1 towards human renal microvascular endothelial cells, the putative target of Shiga toxins in the development of HUS (Louise and Obrig 1995).

Although fimbriae and OMPs have been identified which may be associated with STEC adhesion, most studies have concentrated on intimin as a potential adhesin. There is a precedent for this as intimin was shown to be involved in both initial bacterial attachment (Hicks *et al.,1998*) and in intimate attachment (Frankel *et al.,199*8) of EPEC. Intimate attachment is necessary for full expression of the effects of EPEC on the host cell cytoskeleton. Mutants at the eaeA locus remain capable, however, of subtler cytoskeletal alterations and of inducing host-cell tyrosine kinase activity (Rosenshine *et al.*,1992;) We speculate that this signal transduction event results in elevated intracellular calcium concentrations and fluid secretion (Baldwin *et al.,* 1991) Loss of microvilli caused by EPEC attaching and effacing lesions is an alternative mechanism for EPEC diarrhea. While malabsorption may be a factor in protracted EPEC infection, the acute onset of diarrhea in volunteers (in as little as 2.9 h) indicates that secretory mechanisms are also operative. Interestingly, the eaeA mutant was not completely avirulent; diarrhea developed in 4 of 11 volunteers. Since diarrhea does not develop in volunteers who ingest nonpathogenic *E. coli*). This suggests that

intimine attachment is not essential for the development of diarrhea.

These results agree with Zende *et al.*, (2013)O_2 harbored the gene for SLT2 and 2 (18.18%) for heat -stable enterotoxin a (STa). None of the strain contains SLT1 and LT genes. These results disagree with Wageh *et al.,* (2015) O_{127}, O_{114} *and* O_{78} harbored ast A gene, which is a major determinant for *ETEC.*

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