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# Shiga toxin producing Escherichia coli in some chicken products

**Hemmat, M. Ibrahim[1]; Reham. A. Amin[1]; Nahla, A. Abou El Roos[2] ; Rabab, S. Khattab[1]**

[1] *Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University.*

[2] *Animal Health Research Institute, Shiben-El Kom, Egypt.*

A B S T R A C T

A total of 120 random samples of raw chicken products (pane, thigh) and half cooked chicken products (nuggets-pane)and cooked chicken products (luncheon - shawerma) (20 of each) were collected from different butcher's shops and supermarkets in El-Menofia governorate. These samples were examined for isolation and identification of shiga toxin producing *E.coli* . The incidence of *E. coli* were 50%, 40%, 25%, 20% ,10% and 15% of examined samples of chicken thigh, pane (raw), nuggets, pane(half cooked), luncheon and shawerma (cooked) respectively. Moreover, the incidence of serologically identified *E.coli* as Enteropathogenic *E.coli* (*E coli* $O_{114}$ : $H_{21}$, *E coli* $O_{119}$ : $H_4$ , $O_{44}$ : $H_{18}$) was 60%, Enterheamorrhagic *E.coli* (*E coli* $O_{111}$: $H_2$, *E coli* $O_{26}$ : $H_{11}$) was 55%, Enterotoxogenic *E.coli* (*E coli* $O_{125}$: $H_{18}$and *E coli* $O_{127}$: $H_6$) was 30% and Enteroinvasive *E.coli* (*E coli* $O_{124}$) was 15%. The achieved results evident that Enteropathogenic *E. coli* is the most contaminant of our examined samples followed by Enterheamorrhagic *E. coli* then Enterotoxogenic *E.coli* and finally Enteroinvasive *E.coli.* PCR resultes from biochemically positive *E. coli* samples clarified the absence of Stx1 from all isolated Ecoli. strains, while Stx2 is present in O44:H18 , O114:H21 , O119:H4 and O127:H6 isolates and absent from O26:H11 , O111:H2 ,O124 and O125:H18 isolates. The results cleared that PCR is an ideal method for identification of *E. coli*, as it was effective, less labor, more sensitive, reduces effort and time9The public health significance of isolated microorganisms and the possible sources of contamination of chicken meat cuts and products with these organisms as well as suggestive hygienic measures to improve the quality of such items were discussed.

**Key words**: *Shiga toxin, E.coli, Chicken products.*

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## 1. INTRODUCTION

In Egypt, chicken products such as shawarma, nuggets, pane, luncheon are gaining popularity because they represent quick easily prepared chicken meals and solve the problem of the shortage in fresh meat of high price which is not within the reach of large numbers of families with limited income.

Chicken meat provide an animal protein of high biological value for consumers at all ages, where they contain all the essential amino acids required for growth with high proportion of unsaturated fatty acids and low cholesterol value. Moreover, poultry mea is a good source of different types of vitamins as niacin, riboflavin, thiamine and ascorbic acid as well as sodium, calcium, iron, phosphorus, sulpher and iodine *(Amin -Reham, 2007).* Poultry meat is more popular in the consumer market because of advantages such as easy digestibility and acceptance by the majority of people (*Yashoda et*

*al., 2001).*Unfortunately, such products offer ideal medium for microbial growth for they are highly nutritious, have a favorable pH, and are normally lightly salted or not salted at all (Johnston and Tompkin, 1992).

There have been a number of food-borne illnesses resulting from the ingestion of contaminated foods such as chicken meats. Most of the pathogens that play a role in foodborne diseases have a zoonotic origin *(Busani et al., 2006). Escherichia coli (E.coli)* is considered as one of the most common causes of food poisoning outbreaks all over the world *(Mead et al., 1999).*

*Escherichia coli* is a Gram-negative, rod-shaped, flagellated, non-sporulating, and facultative anaerobic bacterium that belongs to Enterobacteriaceae family. Some serogroups of E. coli are able to cause disease and food poisoning. These types of E. coli are generally classified into 6 subgroups including enterotoxigenic E. coli (ETEC), enteropathogenic E. coli (EPEC), enteroinvasive E. coli, entero-hemorrhagic E. coli (EHEC), enteroadherent E. coli, and diffusely adherent E. coli). The EHEC strains are one of the subsets of Shiga toxin (Stx)-producing E. coli (STEC) strains, which are isolated from patients and are responsible for severe clinical symptoms such as hemorrhagic colitis (HC) and the potentially lethal hemolytic uremic syndrome (HUS) ( Karmali et al., 1989). Several studies showed that consumption of contaminated food with STEC strains is the main cause of human infections (Hussein and Sakuma, 2005). It seems that STEC virulence genes have a major role in causing diseases. Shiga toxins, the main virulence factors contributing to pathogenicity, consist of 2 major types, the Stx1, which is identical to Stx of Shigella dysenteriae, and Stx2, which is 56% homologous to Stx1 (Scheutz and Strockbine, 2005).

Shiga toxin-producing Escherichia coli (STEC) are an important cause of haemorrhagic colitis and the diarrhea associated form of the haemolytic uraemic syndrome. Of the numerous serotypes of E. coli that have been shown to produce Shiga toxin (Stx), E. coli 0157:H7 and E. coli 0157:NM (non-motile) are most frequently implicated in human disease.

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).*PCR can be applied on fixed tissues (frozen or formalin fixed) reducing the potential dangers involved in transport and handling of specimens with live virulent pathogens (Reinoso et al., 2004).

The aim of the present study was planned out to examine raw chicken cuts (pane and thigh) semi-cooked products (nuggets, pane) and cooked products (shawarma, luncheon) for isolation and Identification of *E.coli,* and detection of shiga-toxin genes of *E.coli* isolated from examined sample by polymerase chain reaction (PCR).

## 2. MATERIAL AND METHODS

### *2.1. Collection of Samples*

A total 120 random samples of raw chicken products (pane and thigh), half-cooked chicken products (pane, nuggets) and Cooked Products (luncheon and shawrma) (20of each) collected from different supermarkets at Menoufia gover9norate.. The collected samples were transferred directly to the laboratory in an ice-box under complete aseptic conditi999ons without undue delay and then subjected to the following examination.

### *2.2. Preparation of Samples (ICMSF, 1996)*

Twenty-five grams of the examined chicken meat samples were transferred to a sterile polyethylene bag, and 225 ml of 0.1 % sterile buffered peptone water were aseptically added to the content of the bag. Each sample was then homogenized in a blender at 2000 r.p.m for 1-2 minutes to provide a homogenate.The prepared samples were subjected to the following examination:

### *2.3. Isolation and identification of Escherichia coli:*

#### *2.3.1. Pre-enrichment (ICMSF, 1996):*

From the original dilution, one ml was inoculated into MacConkey broth tubes supplemented with inverted Durham's tubes. The inoculated tubes were incubated at 37°C for 24 hours. The development of acid and gas indicate positive coliform.

*2.3.2. Enrichment broth:*

One ml from positive MacConkey broth tube was inoculated into another MacConkey broth tubes and incubated at 44°C for 24 hours. The development of acid and gas indicate positive true fecal type.

*2.3.3. Plating media:*

Loopfuls from positive MacConkey broth tubes were separately streaked onto Eosin Methylene Blue agar medium (E.M.B.), which were then incubated at 37°C for 24 hours. Suspected colonies were metallic green in color. Suspected colonies were purified and inoculated into nutrient agar slope tubes for further identification.

*2.3.4. Morphological examination:*

Gram's staining (Cruickshank *et al*., 1975) Motility test (MacFaddin, 2000)

*2.4 Biochemical identification (ISO, 2007):*

*2.5 Serological Identification:*

The applied technique recommended by Kok *et al*. (1996) was used. by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

*2.6.PCR:*

PCR approaches have been applied to detect different species of several microbial niches, to differentiate closely related species and to recognize single species (Settanni and Corsetti, 2007). The primary advantages of PCR tests are increased sensitivity and less time required to process samples in the laboratory when compared to standard culture methods (Lampel et al., 2000).

## 3. RESULTS

Incidence of *E. coli* isolated from the examined samples of chicken products was illustrated in Table 1. As shown in table 2, the incidence of serologically identified *E. coli* as : Entero-pathogenic *E.coli* (*E coli* O114 : H21, *E coli* O119 : H4 , O44 : H18) was 60%, Enterheamo-rrhagic *E.coli* (*E coli* O111: H2, *E. coli* O26 : H11) was 55%, Enterotoxogenic *E.coli* (*E .coli* O125: H18and *E. coli* O127: H6) was 30% and Enteroinvasive *E.coli* (*E. coli* O124) was 15%. The achieved results evident that the Enteropathogenic *E. coli* is the most contaminants of our examined samples followed by Enterheamorrhagic *E. coli* and Entero-toxogenic E.coli and finally Enteroinvasive *E. coli*.

The results declared in table (3) and Figure (1) the PCR analysis of *E. coli* isolates for the presence of Stx1 and Stx2 genes which are virulence genes in STEC, it clarified the absence of Stx1 from all isolated Ecoli. strains, while Stx2 is present in O44:H18 , O114:H21 , O119:H4 and O127:H6 isolates and absent in O26:H11 , O111:H2 ,O124 and O125:H18 isolates .

Table (1): Incidence of *E. coli* isolated from the examined samples of chicken products (n=20).

| Samples | | | Positive samples | |
|---|---|---|---|---|
| | | | No. | % |
| Chicken | raw | Thigh | 10 | 50% |
| | | Pane | 8 | 40% |
| | half-cooked | Nuggets | 5 | 25% |
| | | Pane | 4 | 20% |
| | cooked | Luncheon | 2 | 10% |
| | | Shawerma | 3 | 15% |
| | | Total (100) | 32 | 32% |

Table (2): Incidence and serotyping of *E.coli* isolated from the examined samples of chicken meat products (n=20).

| *E.coli* strains | Raw product | | | | Half cooked | | | | Cooked product | | | | Strain Characteristics |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pane | | Thigh | | Pane | | Nuggets | | Luncheon | | Shawerma | | |
| | No. | % | No. | % | No. | % | No | % | No. | % | No. | % | |
| $O_{26}$: $H_{11}$ | - | - | - | - | 1 | 5 | 1 | 5 | 1 | 5 | 1 | 5 | EHEC |
| $O_{44}$: $H_{18}$ | 1 | 5 | 1 | 5 | - | - | 1 | 5 | - | - | - | - | EPEC |
| $O_{111}$: $H_2$ | 1 | 5 | 2 | 10 | 1 | 5 | 1 | 5 | 1 | 5 | 1 | 5 | EHEC |
| $O_{114}$: $H_{21}$ | 2 | 10 | 1 | 5 | 1 | 5 | 1 | 5 | - | - | 1 | 5 | EPEC |
| $O_{119}$ : $H_4$ | 1 | 5 | 1 | 5 | - | - | 1 | 5 | - | - | - | - | EPEC |
| $O_{124}$ | 1 | 5 | 2 | 10 | - | - | - | - | - | - | - | - | EIEC |
| $O_{125}$ : $H_{18}$ | 1 | 5 | 2 | 10 | - | - | - | - | - | - | - | - | ETEC |
| $O_{127}$ : $H_6$ | 1 | 5 | 1 | 5 | 1 | 5 | - | - | - | | - | - | ETEC |
| Total | 8 | 40 | 10 | 50 | 4 | 20 | 5 | 25 | 2 | 10 | 3 | 15 | |

EPEC = Enteropathoge1nic *E.coli*
ETEC = Enterotoxigenic *E.coli*
*EHEC= Enterohaemorrhagic *E.coli.*


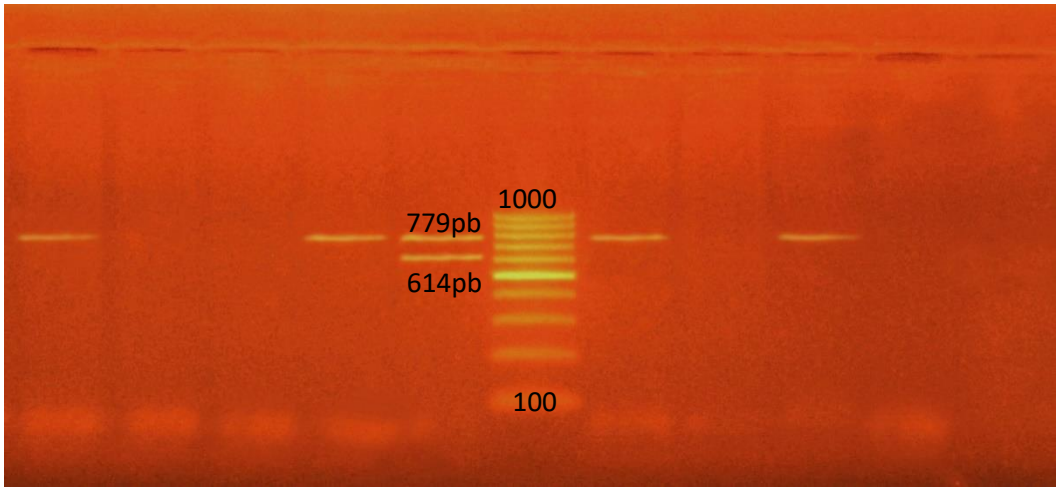


Fig. (1): Agarose gel electrophoresis of multiplex PCR of *stx1*(614 bp), *stx2* (779 bp genes for characterization of Enteropathogenic *E. coli.*
Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive *E. coli* for *stx1*, *stx2*
Lane C-: Control negative.
Lanes 2, 4, 5, 8: sample Positive *E. coli* for *stx2* gene.
Lanes 1, 3, 6, 7: sample Negative *E. coli* for *stx2* genes.
Lanes 1, 2, 3, 4, 5, 6, 7, 8: sample Negative *E. coli* for *stx1* genes.

Table (3): Incidence of virulence genes of Shiga toxin-producing *E.coli* (STEC) in the isolated *E.coli* strains from the examined products by using PCR(n=8)

| Serotype | Stx1 | Stx2 |
|---|---|---|
| O26:H11 | -ve | -ve |
| O44:H18 | -ve | +ve |
| O111:H2 | -ve | -ve |
| O114:H21 | -ve | +ve |
| O119:H4 | -ve | +ve |
| O124 | -ve | -ve |
| O125:H18 | -ve | -ve |
| O127:H6 | -ve | +ve |

***Stx1*:** Shiga- toxin 1 gene
***Stx2*:** Shiga- toxin 2 gene

## 4. DISCUSSION

*E. coli* is considered as a commensal micro-organism in the alimentary tract of most domestic and wild animals as well as human (Miskimin et al., 1976). Presence of *E. coli* in meat indicates a general lack of cleanness during slaughtering, evisceration, dressing, transportation and handling of meat (ICMSF, 1996). As well as, E. coli may be used as an indicator microorganism because it provides an estimate of fecal contamination and poor sanitation during processing (Eisel et al., 1997).
Results achieved in Table (1) indicated that *E. coli* was isolated from 50%, 40%, 25%, 20% ,10% and 15% of examined samples of chicken thigh, pane(raw), nuggets, pane(half cooked), luncheon and shawerma respectively.
Moreover, the data recorded in Table (2) revealed that seven strains of serologically identified *E. coli* isolated from the examined chicken pane(raw) samples recorded as $O_{44}$ : $H_{18}$ EPEC,$O_{111}$: $H_2$ EHEC, and $O_{114}$: $H_{21}$ EPEC, $O_{119}$ : $H_4$ EPEC, $O_{124}$ EIEC $O_{125}$ : $H_{18}$ ETEC, and $O_{127}$ : $H_6$ ETEC , als seven strains of serologically identified *E. coli* isolated from the examined chicken thigh samples and recorded as $O_{44}$ : $H_{18}$ EPEC ,$O_{111}$: $H_2$ EHEC $O_{114}$: $H_{21}$, $O_{119}$ : $H_4$ EPEC, $O_{124}$ EIEC, $O_{125}$ : $H_{18}$EPEC, and $O_{127}$ : $H_6$ .
Concerning, the chicken nuggets samples (half cooked); the serologically identified *E. coli* isolated from the examined samples revealed 5 isolates recorded as one isolates of $O_{26}$: $H_{11}$ EHEC, one isolate of $O_{111}$: $H_2$ EHEC, one isolate of $O_{114}$: $H_{21}$EPEC, and one isolate of $O_{119}$ : $H_4$EPEC and $O_{44}$ : $H_{18}$ EPEC.
There are only 4 strains isolated from pane (half cooked) and recorded as $O_{111}$: $H_2$EHEC and $O_{26}$: $H_{11}$EHEC, $O_{114}$: $H_{21}$EPEC and $O_{127}$ : $H_6$ETEC while only 2 strains are isolated from chicken luncheon and recorded as $O_{111}$: $H_2$EHEC, $O_{26}$: $H_{11}$EHEC,and3 strains are isolated from chicken shawerma and recorded as $O_{111}$: $H_2$EHEC, $O_{26}$: $H_{11}$EHEC,and. $O_{114}$: $H_{21}$EPEC. as recorded in table (3).
Although most strains of *E.coli* are harmless, several are known to produce toxins that can cause diarrhea. The pathogenic groups include: Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Enterohaemorrahgic (EHEC), Entero-invasive (EIEC), Enteroagregative (EAEC), Diffusely Adherent (DAEC) (Nataro And Kaper, 1998).
In table (2) illustrated that the incidence of serologically identified *E. coli* as Entero-pathogenic *E.coli* was 60%, Enterheamo-rrhagic *E.coli* was 55%, Enterotoxogenic *E.coli* was 30% and Enteroinvasive *E.coli* was 15%. The achieved results evident that the Enteropathogenic *E. coli* is the most contaminants of our examined samples followed by Enterheamorrhagic *E. coli* and Enterotoxogenic *E.coli* and finally Entero-invasive *E.coli.*
These results differ from those obtained by *lee et al. (2009)* who isolated entero-toxigenic *E.coli* (34.6%) followed by enterohaemorrhagic *E. coli* (35.9%) and finally enteropathogenic *E.coli* (20.5%).
Enteropathogenic *E.coli* which subsequently was divided into class I that is usually

enteroadherent factor positive (EAF+) and class II that is rarely enteroadherent factor negative (EAF-), and each of them has certain serotypes, while Enterohaemorrhagic *E.coli* which recognized as the primary cause of haemorrhagic diarrhea and Haemolytic Uremic Syndrome (HUS).

The pathogenicity of EHEC appears to be associated with the number of several cytotoxins referred to Shiga- like toxin (SLT) or Vero toxins (VT) (Karmali, 1989).

EPEC was implicated in cases of gastroenteritis, cystitis, colitis, pyelonephritis, peritonitis and puerperal sepsis as well as food poisoning outbreaks *(Doyle, 1990).*

Enterohaemorrhagic *E.coli* has been reported to be probably the most important term of food borne disease *(Cliver, 1990).* An outbreak of *E.coli O111* in south Australia (1995) in which 23 children with HUS were hospitalized *(CDCP, 1995).*

Generally, EPEC strains are the major cause for many cases of infantile diarrhea. In typical cases, symptoms appear within 12 to 36 hours. Clinically, EPEC illness is characterized by fever, malaise, vomition and water stools which occasionally contain mucous.

In this study, E.coli can be found in chicken meat products within greater proportion in raw chicken meat products (pane-thigh)and half cooked products(nuggets- pane) than in cooked chicken meat products (luncheon – shawerma) due to heat treatment or/and freezing, which agree with El-Tahan et al. (2006) who Isolated E.coli only from both nuggets and Luncheon samples collected from Down Town retail markets but sample from Shubra and Nasr city were free. On the other hand our result does not agree with Tolba (1994) who reported that the E.coli could not detected from nuggets. Also Ouf-Jehan (2001) who examined 20 samples of luncheon which collected from different localities from Giza and Cairo governorates, and failed to detect E. coli in the examined luncheon samples.

The polymerase chain reaction (PCR) based diagnostic assays have been developed to target these genes. PCR is considered as a selective and sensitive method that rapidly amplifies specific regions of a gene.

The results recorded in table (3) revealed that $stx_1$ and $stx_2$ genes failed to be detected in the isolated *EHEC* strains ($O_{111}$: $H_2$ *E coli* $O_{26}$ : $H_{11}$).

These results differ from those obtained by (Elsabagh-rasha, 2010) who reported that the chicken fillet revealed two samples (10%) for $stx_1$, while one sample for $stx_2$. While $stx_2$ genes can be detected in the isolated *EPEC* strains($O_{114}$ : $H_{21}$, *E coli* $O_{119}$ : $H_4$ , $O_{44}$ : $H_{18}$), also detected in the isolated ETEC strain(only one $O_{127}$: $H_6$ ).

The ability of shiga toxin production by *E.coli* is usually chromosomal mediated, which may be lost in some strains, especially on subcultivation which may result in loss of some of virulence genes.

These results agreed with (Flanders et al. (1995); China et al. (1996); Lampel et al. (2000); Kong et al. (2002); Brooks et al. (2004); Wang et al. (2007) and Edris-shimaa, 2012) who concluded that PCR technique is more accurate, rapid, highly specific and sensitive than traditional methods for detection of *E. coli.* The traditional methods of *E. coli* identification were able to identify and isolate them, but it was time consuming.

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