Shiga toxin producing Escherichia coli in some chicken products

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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 O114: H21, E coli O119: H4, O44: H18) was 60%, Enterheamorrhagic E. coli (E coli O111: H2, E coli O26: H11) was 55%, Enterotoxogenic E. coli (E coli O125: H16 and E coli O127: H6) was 30% and Enteroinvasive E. coli (E coli O124) 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 results 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 time. The 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.

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 meat is a good source of different types of vitamins as niacin, riboflavin, thiamine and ascorbic acid as well as sodium, calcium, iron, phosphorus, sulphur 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
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Unfortunately, such products offer an 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 the 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, enterohemorrhagic 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 Stockbine, 2005).

Shiga toxin-producing Escherichia coli (STEC) are an important cause of haemorrhagic colitis and the diarrhea associated form of the haemolytic uremic 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 (paw 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 samples by polymerase chain reaction (PCR).

2. MATERIAL AND METHODS

2.1. Collection of Samples

A total 120 random samples of raw chicken products (paw and thigh), half-cooked chicken products (paw, nuggets) and Cooked Products (luncheon and shawarma) (20 of each) collected from different supermarkets at Menoufia governore.. The collected samples were transferred directly to the laboratory in an ice-box under complete aseptic conditions 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: Enteropathogenic E. coli (E. coli O114 : H21, E. coli O119 : H4 , O44 : H18) was 60%, Enterheamorrhagic E. coli (E. coli O111: H2, E. coli O26 : H11) was 55%, Enterotoxogenic E. coli (E. coli O125: H18 and 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 Enterotoxogenic 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).

<table>
<thead>
<tr>
<th>Samples</th>
<th>Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>raw Thigh</td>
<td>10</td>
</tr>
<tr>
<td>raw Pane</td>
<td>8</td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
</tr>
<tr>
<td>half-cooked Nuggets</td>
<td>5</td>
</tr>
<tr>
<td>half-cooked Pane</td>
<td>4</td>
</tr>
<tr>
<td>cooked Luncheon</td>
<td>2</td>
</tr>
<tr>
<td>cooked Shawerma</td>
<td>3</td>
</tr>
<tr>
<td>Total (100)</td>
<td>32</td>
</tr>
</tbody>
</table>
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Table (2): Incidence and serotyping of *E. coli* isolated from the examined samples of chicken meat products (n=20).

<table>
<thead>
<tr>
<th><em>E. coli</em> strains</th>
<th>Raw product</th>
<th>Half cooked</th>
<th>Cooked product</th>
<th>Strain Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pane</td>
<td>Thigh</td>
<td>Pane</td>
<td>Nuggets</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>O_{26}: H_{11}</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>O_{44}: H_{18}</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>O_{111}: H_{2}</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>O_{119}: H_{4}</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>O_{124}: H_{21}</td>
<td>2</td>
<td>10</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>O_{125}: H_{18}</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>O_{127}: H_{6}</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>40</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

EPEC = Enteropathogenic *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)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Stx1</th>
<th>Stx2</th>
</tr>
</thead>
<tbody>
<tr>
<td>O26:H11</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>O44:H18</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>O111:H2</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>O114:H21</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>O119:H4</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>O124</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>O125:H18</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>O127:H6</td>
<td>-ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

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

4. DISCUSSION

*E. coli* is considered as a commensal microorganism 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 cleanliness 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 shawarma 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 O44: H18 EPEC, O111: H2 EHEC, and O114: H21 EPEC, O119: H4 EPEC, O124 EIEC, O125: H18 ETEC, and O127: H6 ETEC, as seven strains of serologically identified *E. coli* isolated from the examined chicken thigh samples and recorded as O44: H18 EPEC, O111: H2 EHEC, O114: H21, O119: H4 EPEC, O124 EIEC, O125: H18 EPEC, and O127: H6. 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 O26: H11 EHEC, one isolate of O111: H2 EHEC, one isolate of O114: H21 EPEC, and one isolate of O119: H4 EPEC and O44: H18 EPEC.

There are only 4 strains isolated from pane (half cooked) and recorded as O111: H2 EHEC and O26: H11 EHEC, O114: H21 EPEC and O127: H6 ETEC while only 2 strains are isolated from chicken luncheon and recorded as O111: H2 EHEC, O127: H11 EHEC, and 3 strains are isolated from chicken shawarma and recorded as O111: H2 EHEC, O26: H11 EHEC, and O127: H1 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), Enteroaggregative (EPEC), Enterohaemorrhagic (EHEC), Enteroinvasive (EIEC), Enteroaggregative (EAEC), Diffusely Adherent (DAEC) (Nataro and Kaper, 1998).

In table (2) illustrated that the incidence of serologically identified *E. coli* as Enteropathogenic *E. coli* was 60%, Enterheamorrhagic *E. coli* was 55%, Enterotoxigenic *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 Enterotoxigenic *E. coli* and finally Enteroinvasive *E. coli*.

These results differ from those obtained by Lee et al. (2009) who isolated enterotoxigenic *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, pylonephritis, 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, vomiting 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 – shawarma) 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 stx1 and stx2 genes failed to be detected in the isolated EHEC strains (O111: H2 E coli O26 : H11).

These results differ from those obtained by (Elsabagh-rasha, 2010) who reported that the chicken fillet revealed two samples (10%) for stx1, while one sample for stx2. While stx2 genes can be detected in the isolated EPEC strains(O114 : H31, E.coli O119 : H4, O44 : H18), also detected in the isolated ETEC strain (only one O127: H6).

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.

5. REFERENCES


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