Prevalence of E. coli and detection of virulent genes by multiplex PCR in meat products

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A B S T R A C T

A total of 100 meat products samples of minced meat, kofta, beef burger and sausage (25 samples of each), weight of each sample 10 g were collected from different shops and supermarkets in Cairo governorate, to be investigated for the presence of E. coli and detection of virulent genes by modern technique (PCR). The obtained results indicated that the incidence of E. coli isolated from the examined samples were (4) 16%, (7) 28%, (6) 24% and (11) 44% of minced meat, kofta, beef burger and sausage, respectively. Actually E. coli strains isolated from 18 positive E. coli were 9 strains O26, O155:H7, O103, O111:H4, O114:H21, O119:H6, O125:H21 and O128:H2. These isolated strains were investigated by using Multiplex PCR to detect presence of virulent genes (stx1, stx2 and eaeA) in each isolated strain. The results obtained reported that E. coli O26 & O111 posses (3) genes stx1, stx2 and eaeA genes, E. coli O103, O119 & O128 carry (2) genes stx1 and stx2, E. coli O155 carry (2) genes stx1 and eaeA genes, E. coli O125 carry (2) genes stx2 and eaeA genes, E. coli O114 posses (1) gene stx1gene. While virulence genes were not detected in E. coli O124.

Keywords: E.coli, Meat Products, PCR.

1. INTRODUCTION

During the last decade, the increase of human population in relative to the great development in human life caused a great demand of easily prepared meals contained high level of animal protein. However, meat products are generally excellent sources of protein containing a good balance of the essential amino acids and having a high biological value (Biesalski, 2005). Food borne diseases remain a major problem and one of public health concern. Epidemiological data show an increasing incidence of infectious diarrhea (Osservasalute, 2008). It is reported that large number of human illness outbreaks have been traced worldwide during the past 23 years due to consumption of under-cooked ground beef and other beef products contaminated with Shiga toxin-producing E. coli (STEC). Because most STEC outbreaks in the epidemiological studies have focused on the prevalence of this serotype in beef cattle worldwide, however, additional STEC serotypes (e.g., members of the O26, O91, O103, O111, O118, O145 and O166 serogroups) have been isolated from beef and caused human illnesses ranging from bloody diarrhea and hemolytic colitis to the life-threatening hemolytic uremic syndrome (HUS) (Little et al., 2008). Application of multiplex PCR for detection of non-O157:H7 STEC virulence genes as (stx1, stx2, eae, hly, etpD, katP6) not only improve the detection
efficiency but also increase the accuracy and mentioned that traditional detection approaches for non-O157 STEC are both time and labour consuming in diseases surveillance (Wang et al., 2013).

Therefore, the present study was planned out to throw out light on: Conventional recovery methods, to detect prevalence of E. coli in examined meat products. Bacteriological and serological identification of the isolates. Molecular characterization of E. coli strains using Polymerase chain reaction (PCR) for detection of virulent genes of isolated E. coli strains.

2. MATERIAL AND METHODS

2.1. Collection of samples

One hundred random samples of meat products represented by minced meat, kofta, beef burger and sausage (25 of each), sample weight 10gm were collected from different supermarkets and from retail stores in Cairo governorate. The collected samples were aseptically collected in sterile polyethylene bags. All samples were examined bacteriological for detection of E. coli.

2.2. Isolation and identification E. coli

The technique recommended by (APHA, 1992) by using Eosin Methylene Blue (EMB) agar media. Suspected colonies for E. coli were morphologically and biochemically identified.

2.3. Serotyping of E. coli

E. coli isolates were serologically identified according to (Kok et al., 1996) by using rapid diagnostic E. coli antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the enteropathogenic types.

2.4. In-Vitro anti-microbial sensitivity test

The isolated E. coli strains were subjected to antimicrobial susceptibility test by the single diffusion method according to (Mary and Usha, 2013).

2.5. Detection of Virulence genes of isolated E. Coli strains by multiplex PCR

Application of PCR for identification of shiga toxins (stx1 & stx2) and intimin (eaeA) genes of E. coli was performed essentially by using Primers (Pharmacia Biotech) as shown in the table (1).

Table (1): Primers sequences, target genes and amplicon size of the used genes

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Oligonucleotide sequence(F-R)</th>
<th>Product (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1 (F)</td>
<td>5’ACACTGCGATGACTGACGTTG’3’</td>
<td>614</td>
<td>(Phanadore and Malys, 2008)</td>
</tr>
<tr>
<td>stx1 (R)</td>
<td>5’CTGAAATCCCCTCCATTGT’3’</td>
<td>719</td>
<td>(Phanadore and Malys, 2008)</td>
</tr>
<tr>
<td>stx2 (F)</td>
<td>5’CCATAGAACCAGACGACGTTT’3’</td>
<td>690</td>
<td>(Delevery et al., 2013)</td>
</tr>
<tr>
<td>stx2 (R)</td>
<td>5’CCGCTGAAATCGAAGAGT’3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. RESULTS

It is evident from the results recorded in table (2) that that the incidence of E. coli in minced meat, kofta, beef burger and sausage were 4(16%), 7(28%), 6(24%) and 11(44%), respectively. Table (3) show the percentage of the accepted examined meat products according to ESS (2005) of E. coli, acceptable samples were 84%, 72%, 76%, 56% in minced meat, kofta, beef burger and sausage, respectively.

Table (2): Incidence of isolated E. coli from examined meat product samples

<table>
<thead>
<tr>
<th>products</th>
<th>Number of +Ve isolates</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minced meat (n=25)</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Kofta (n=25)</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>Beef burger (n=25)</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>Sausage (n=25)</td>
<td>11</td>
<td>44</td>
</tr>
</tbody>
</table>

Results achieved in table (4) show the serological identification of E. coli isolated from examined meat product samples, were belonged to the following serotypes E. coli O26, O55, O103, O111, O114, O119, O124, O125, O128.
Prevalence of E. coli and detection of virulent genes by multiplex PCR

Table (3): Acceptability of the examined samples of meat products based on their contamination with E. coli according to ESS (2005)

<table>
<thead>
<tr>
<th>Products</th>
<th>Number of isolates</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minced meat (n=25)</td>
<td>21</td>
<td>84</td>
</tr>
<tr>
<td>Kofta (n=25)</td>
<td>18</td>
<td>72</td>
</tr>
<tr>
<td>Beef burger (n=25)</td>
<td>19</td>
<td>76</td>
</tr>
<tr>
<td>Sausage (n=25)</td>
<td>14</td>
<td>56</td>
</tr>
</tbody>
</table>

Table (4): Incidence of pathogenic E. coli serotypes in examined meat products

<table>
<thead>
<tr>
<th>E. coli strains</th>
<th>M</th>
<th>K</th>
<th>B</th>
<th>S</th>
<th>Strain character</th>
</tr>
</thead>
<tbody>
<tr>
<td>O26</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>EHEC</td>
</tr>
<tr>
<td>O55:H7</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>EPEC</td>
</tr>
<tr>
<td>O103</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>EHEC</td>
</tr>
<tr>
<td>O111:H4</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>EHEC</td>
</tr>
<tr>
<td>O112:H21</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>EPEC</td>
</tr>
<tr>
<td>O113:H8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>EPEC</td>
</tr>
<tr>
<td>O124</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>EIEC</td>
</tr>
<tr>
<td>O125:H21</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>ETEC</td>
</tr>
<tr>
<td>O128:H2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>ETEC</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>11</td>
<td></td>
</tr>
</tbody>
</table>

M=Minced meat, K=Kofta, B=Beef burger, S=Sausage

Photo (1) revealed that 9 E. coli strains investigated by multiplex PCR to detect presence of virulence genes stx1, stx2 and intimin (eaeA). From recorded results found that E. coli O26 & O111 posses (3) genes stx1, stx2 and eaeA genes, E. coli O103, O119 & O128 carry (2) genes stx1 and stx2, E. coli O55 carry (2) genes stx1 and eaeA genes, E. coli O123 carry (2) genes stx2 and eaeA genes, E. coli O114 posses (1) gene stx1 gene, while E. coli O124 carry no genes.

4. DISCUSSION

The presence of E. coli in contaminated food products is commonly attributed to fecal contamination when they are improperly handled and/or when inactivation treatments fail. The adaptation of E. coli at low pH and a_w levels can vary at different temperatures depending on the serotype (Valero et al., 2010).

Shiga toxin (stx) producing E. coli (STEC) contamination in food and water is one of the most recognized concerns and a major financial burden in human hygiene control worldwide. Rapid and highly reliable methods of detecting and identifying STEC causing gastroenteric illnesses are crucial to prevent food borne outbreaks. A number of tests have been developed and commercialized to detect STEC using molecular microbiology techniques. Most of these are designed to identify virulence factors such as Shiga toxin and intimin as well as E.coli O and H antigen serotype specific genes. In order to screen pathogenic STEC without relying on O:H serotyping, we developed a rapid detection and genotyping assay for STEC virulence genes using a PCR for detection of major virulence genes, Shiga toxin 1 and 2 (stx1 and stx2), intimin (eae) (Goji et al., 2015).

The incidence of E. coli in (Table1) revealed that minced beef was 16% which is nearly similar to results obtained by (Barlow et al., 2006) and (Filliol et al., 2008) which were 16% and 17%, respectively. On the other
hand higher figure obtained by El-Gohary, 1993& Hugo et al., 2012 & Zakarya and Fouad 2013 which were 75%, 38.1%, and 25%, respectively.

In kofta the incidence of *E. coli* was 28% the results is nearly similar to that reported by Torky (2004), 30%, higher results were obtained by Abdalla and Hassan (2000), 40% while lower result obtained by El-Sherif (2009) 10%. The incidence of *E. coli* in beef burger was 24% nearly similar results obtained by Aouf (2001) 30%, while lower results recorded by Ahmed (1992) and El- Sherif (2009) were 6.6% and 10%, higher results reported by (Fathi et al.,1994) and El-Mossalami (2003) were 77.78% and 35%, respectively while in sausage the incidence of *E. coli* was 44% these result nearly similar to results obtained by El-Mossalami (2003) 40%. On the other hand higher results obtained by El-Gohary (1993) with percentage 78%. Lower figure obtained by Ahmed (1992) and (Zakarya and Fouad, 2013)16.6%, and 15%, respectively.

Presence of *E. coli* in meat products were unaccepted and hazard on consumer health also disagreed with ESS (Egyptian standard specification) of such meat products and indicates inadequate sanitary conditions during stages of manufacturing, dirty equipment and improper handling. (Table 2) show percentage of the accepted examined meat products according to ESS (2005) of *E. coli*.

The serotypes of *E. coli* isolated in this study as shown in (table3) were 9 *E. coli* strains belonged to following serotypes: *O*26, *O*55, *O*103, *O*111, *O*114, *O*119, *O*124, *O*125, *O*128. PCR based methods, as multiplex PCR is very useful as it allows the simultaneous detection of several pathogens by introducing different primers to amplify DNA regions coding for specific genes of each bacterial strain targeted (Tourn et al., 2005).

So these 9 *E. coli* strains were investigated by using multiplex PCR to detect presence of virulence genes *stx1*, *stx2* and *intimin* (*eaeA*). From recorded results found that *E. coli* *O*26 & *O*111 posses (3) genes *stx1*, *stx2* and *eaeA* genes, *E. coli* *O*103, *O*119 & *O*128 carry (2) genes *stx1*and *stx2*, *E. coli* *O*55 carry (2) genes *stx1*and *eaeA* genes, *E. coli* *O*125 carry (2) genes *stx2* and *eaeA* genes, *E. coli* *O*114 posses (1) gene *stx1*gene, while *E. coli* *O*124 carry no genes. The strains which were positive for *eaeA* gene which encodes intimin, an important binding protein of pathogenic STEC as *E. coli* *O*26, *O*111, *O*55 and *O*125 more virulent than other strains not carry this gene and considered more toxigenic and hazardous to consumer health.

Applying Modern technique as PCR based detection of Shiga toxin-producing *E. coli* (STEC) in a routine microbiology laboratory over 16 years, molecular characterization of strains.

Shiga toxin-producing *E. coli* (STEC) is a heterogeneous group of bacteria causing disease ranging from asymptomatic carriage and mild infection to hemolytic uremic syndrome (HUS). Characterize STEC detected by use of PCR for detection of *stx1*, *stx2* and *eae* genes from 996 through 2011. STEC isolates were characterized with respect to serogroup or serotype, (Haugum et al., 2014).

5. REFERENCES


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