Conventional and rapid detection of *Escherichia coli* in some beef products

R.R. Shawish¹, A.M. Elbagoury¹ and A.M. Edris²

1 Department of Food Hygiene & Control, Faculty of Veterinary Medicine University of Sadat City.  
2 Department of Food Control, Faculty of Veterinary Medicine, Moshtoher Benha University.

Abstract

A total of 250 random meat product samples (50 each of minced meat, beef burger, beef sausage, beef kofta and beef luncheon) were collected from different super markets at Menofia, Quliobia and Cairo governorates for detection of *Escherichia coli* using conventional method and Polymerase chain reaction. Conventional method indicated that *E. coli* could be isolated from of minced meat, beef burger, beef sausage, beef kofta and beef luncheon in percentage of 46%, 28%, 32%, 36% and 16% respectively. While by using Polymerase chain reaction(PCR) detected that *E. coli* in minced meat, beef burger, beef sausage, beef kofta and beef luncheon in a percentage of 38%, 22%, 30%, 32% and 12% respectively. In conclusion meat products constitute an important reservoir of *Escherichia coli* infection to man and PCR reaction is the most rapid, sensitive and efficient approach for detection of *E. coli*.

1. INTRODUCTION

Meat products may be derived as raw materials from a source less in microbial contamination but becomes contaminated in the course of manufacture, transport or sale from food handlers, utensils, air, soil and incomplete hygienic condition during manufacturing steps like packaging, storage, slicing and marketing of such products that promoting the growth and multiplication of various bacteria one of which being *E. coli*.

In Egypt, meat products such as minced meat, kofta, sausage, beef burger and luncheon are gaining popularity as they represent quick easily prepared meat 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.

Contamination of such products with some food borne microorganisms during further processing make us in need to rapid and accurate methods for detection of such organisms as using Polymerase chain reactions (PCR) techniques. PCR technique have been recommended as accurate and rapid method to detect food borne pathogen and or their toxins in food and also characterization of the isolated microorganisms as *E. coli* (Reinoso et al., 2004).

*E. coli* is a major component of the normal intestinal flora of human and other mammals and the commensal *E. coli* strains from the normal intestinal flora are usually harmless to the host and only cause disease in immunocompromised hosts or when the gastrointestinal barriers are breached. However, some specific *E. coli* strains represent primary pathogens with an enhanced potential to cause disease after acquiring specific virulence attributes. These virulence attributes are normally encoded on genetic elements that can be exchanged between different strains or on those elements once having been mobile but later becoming fixed
into the genome. Specific combinations of virulence factors form different pathotypes based on the various human diseases caused by *E. coli* can cause (Li et al., 2005).

Pathogenic *E. coli* have been broadly classified into two major categories: the diarrheagenic *E. coli* and the extraintestinal pathogenic *E. coli*. Among the diarrheagenic *E. coli*, there are currently six categories: enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC)/Shiga toxin-producing *E. coli* (STEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusively adherent *E. coli* (DAEC). *E. coli* strains causing extraintestinal infections have been collectively called extraintestinal pathogenic *E. coli* (ExPEC), which includes two major pathotypes: uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) (Xiaodong, 2010). They are phylogenetically and epidemiologically distinct from commensal and diarrheagenic strains. Compared to human commensal strains, which usually derive from phylogenetic groups A and B1, most of ExPEC strains belong to the B2 and D groups and harbor various virulence factors which allow them to induce diseases in both healthy and compromised hosts.

Uropathogenic *E. coli* (UPEC), which belong to the ExPEC, are the principal cause of community-acquired urinary tract infections “UTI” (70–95%) and a large portion of nosocomial UTIs (50%) in the U.S while Neonatal meningitis *E. coli* (NMEC) which belong to ExPEC are the main cause of meningitis in newly born infants (Xiaodong, 2010).

*E. coli* are the most important food borne pathogen causing food poisoning and many other disease conditions among food consumers. However, there is therefore still a need for rapid and accurate methods for detection of *E. coli* in food, therefore the present study was planned out to secure the isolation and identification of *E. coli* by conventional method and direct PCR technique.

### 2. MATERIAL AND METHODS

#### 2.1. Collection of the samples

A grand total of 250 random meat product samples (50 each of minced beef, raw kofta, beef burger, fresh sausage and beef luncheon) were collected from different super markets at Menofia, Cairo and El-Kalyobia governrate. The collected samples were transferred under hygienic conditions in an ice box to the laboratory without undue delay to be examined bacteriologically for isolation and identification of *E. coli* using both conventional method and PCR technique.

#### 2.2. Preparation of the samples (APHA, 1992)

The samples were divided into two parts, one part used for isolation and identification of *E. coli* through traditional method and other part used for isolation and identification of *E. coli* through recent PCR technique. Twenty five grams of each examined meat product samples were transferred to 225 ml of sterile buffered peptone water 0.1% then homogenized by stomacher (Seward stomacher 80 Biomaster, serial No. 46464, England) for 2 minutes to provide a homogenate of 1/10 dil.

#### 2.3. Isolation and identification of *E. coli* by conventional method was recommended by (APHA, 1992).

#### 2.4. PCR detection *E. coli* in beef meat products. (Daly et al., 2002).

##### 2.4.1. Preparation of samples:

25 g of each sample was added to 225 of brain heart infusion broth and stomached in stomacher (Seward stomacher 80 Biomaster, serial No. 46464, England) for 2 minutes then incubated overnight.

##### 2.4.2. Genomic DNA extraction:

One ml of an overnight incubated broth was centrifuged at 13000 rpm for 2 minutes at 4°C and the sediment was suspended in equal volume of Tris-EDTA buffer. Furthermore, 100µl of lysozyme solution (10 mg/l), 100 µl of proteinase K enzyme (0.3 mg/l) and 1% dodecyl sulphate were added.

The DNA lysate was extracted once with chloroform/isoamyl alcohol (24:1, ratio by volume), then extracted with phenol/
chloroform/isoamyl alcohol (25:24:1, ratio by volume). The aqueous phase was mixed with isopropanol alcohol and incubated at -20°C for 30 minutes. The precipitated DNA was spooled out, rinsed in 70% ethanol and dissolved in 0.5 ml of Tris EDTA buffer.

2.4.3. DNA amplification for PCR reaction:
The PCR reaction mix (50 µl) for each sample was consistent of:
10 µl extracted DNA
2.5 µl primers mix.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Predicted size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>5’- CTGGAAGAGGCTAGCCTGGACGAG -3'</td>
<td>366 bp</td>
<td>Yokoigawa et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>5’- AAAATCGGCACCGGTTGAGCGGTC -3'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 µl deoxynucleoside triphosphate (dNTP-mix)
5 µl 10x buffer
1 µl Taq-DNA polymerase enzyme (5000 U/ml)
30.5 µl ultra-pure deionized water.

The reaction mixture was overlaid with mineral oil and was incubated in the thermal cycler as follows:
- The first initial cycle: 95°C for 6 minutes (denaturation), 35°C for 2 minutes (annealing) and 72°C for 1.5 minutes (extension).
- The consequent 35 cycles: 95°C for 20 seconds (denaturation), 35°C for one minute (annealing) and 72°C for 1 minute (extension).
- The final extension step at 72°C for 5 minutes then kept at 4°C (hold temperature).

2.4.4. Separation of PCR amplicons by Gel Electrophoresis:
After the amplification was completed the amplified products were analyzed on agarose gel (consisted of 2% agarose and 5 µL of ethidium bromide in 1 x Tris –Acetate EDTA (TAE) buffer. The samples were electrophoresed at 100 volts for one hour, shown under ultra violet transilluminator and photographed. Visible bands of appropriate size of 366 bp were considered positive.

3. RESULTS

Table (1): Comparison between incidence of E. coli in the examined meat product samples by conventional and PCR method.

<table>
<thead>
<tr>
<th>Meat products</th>
<th>No. of samples</th>
<th>Conventional method</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Minced meat</td>
<td>50</td>
<td>23</td>
<td>46</td>
</tr>
<tr>
<td>Beef burger</td>
<td>50</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Beef sausage</td>
<td>50</td>
<td>16</td>
<td>32</td>
</tr>
<tr>
<td>Beef kofta</td>
<td>50</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td>Beef luncheon</td>
<td>50</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

Table (2): Acceptability of the examined meat products samples according to the (EOSQ, 2005).
EOSQ = EGYPTIAN ORGANIZATION FOR STANDARDIZATION AND QUALITY CONTROL, 2005.

<table>
<thead>
<tr>
<th>Meat products</th>
<th>EOSQ</th>
<th>permissible limit</th>
<th>Acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Minced meat</td>
<td>2005/1694</td>
<td>free</td>
<td>27</td>
</tr>
<tr>
<td>Beef burger</td>
<td>2005/1688</td>
<td>free</td>
<td>36</td>
</tr>
<tr>
<td>Beef sausage</td>
<td>2005/1972</td>
<td>free</td>
<td>34</td>
</tr>
<tr>
<td>Beef kofta</td>
<td>2005/1694</td>
<td>free</td>
<td>32</td>
</tr>
</tbody>
</table>
Lane (M): MW marker = 100 bp DNA ladder (Promega).
Lane 1: positive control
Lane 2, 3, 4, 5, 6 show several bands at level of 366bp.
Lane 7: negative control

4. DISCUSSION

Every treatment done to the meat from the point of slaughtering until it is ready for consumption will add to the bacterial load of this meat. Thus, meat products are considered as a major vehicle of most reported food borne outbreak, and may be contaminated with several types of organisms through long chain of preparation, handling of raw meat, equipment, processing, distribution storage and retailing. This makes us in need to apply rapid and accurate methods for detection of such microorganisms by using PCR technique.

Presence of *E. coli* in raw food of animal origin can be expected because of the close association of this food with the animal environment and contamination of the carcass from fecal material, hide during slaughtering and dressing procedures. These organisms are destroyed by heat processing of foods. Thus, the presence of *E. coli* in a heat processed food means either process failure or more commonly, post processing contamination from equipment, employees or from contact with contaminated raw foods (*National Academy of Sciences, 1985*).

The results recorded in table (1) showed comparison between using conventional method and the PCR technique for *E. coli* detection in examined meat products. It is obvious that 46%, 28%, 32% , 36% and 16% of examined minced meat, beef burger, beef sausage, beef kofta and beef luncheon samples, respectively were contaminated with *E. coli* using conventional method or normal isolation method. These results were agreed with that reported by Gobran (1985), Morshdy (1985), Nashed-Heba et al. (1993), Abou- Hussien-Reham (2004) and Hassan (2012). But lower
results were detected by Zaki - Eman (1990), Ahmed (1992), El-Feky (1994), Fathi and Thabet (2001), Ouf – Jehan (2001) and Eleiwa - Nesreen (2003). Presence of E. coli in food is considered as indicator of faults during preparation, handling, storage or service. It is also, considered as indicator of fecal contamination, besides, it may induce severe diarrhea in infants and young children, as well as food poisoning and gastroenteritis among the adults. E. coli was also isolated by Caserio and Pantano (1980), Hefnawy (1980), Gouda (1991) and Mousa et al. (1993), and Hassan (2007). E. coli also can be detected by direct PCR technique in examined minced meat, beef burger, beef sausage, beef kofta and beef luncheon by the following ratio 38%, 22%, 30%, 32% and 12% respectively. These results were agreed with that revealed by Stampi-Serena (2004) and Hassan (2012) who could detect E. coli in 30.2% of tested meat product samples specially in minced meat and burger using PCR technique, but lower incidence could be detected by Abongo and Momba (2009) who isolated E. coli from 2.8% of examined meat product samples. Also lower incidence was detected by Lee et al. (2009) who stated that the incidence of E. coli was 4.1%.

From the previous results it is concluded that isolation can be detect more by using PCR as PCR technique requires specific count per gram to detect the microorganism and isolation can be detected even small count. (Li et al., 2005) and (Lee et al., 2009). In the other hand PCR is considered as rapid technique for detection of E. coli as it can detect it within 10 hours, including a 6 hours enrichment step. (Gordillo et al., 2011). Isolation of E. coli in food testing laboratories is very important, and this rapid E. coli detection help the strategy which will contribute to quality control in food industries. (Takahashi et al., 2009).

Photo (1) agarose gel showing 4 positive strains of E. coli by PCR. We can detect bands of PCR products which indicate presence of E. coli in the examined meat product samples.

Regarding table (2): the results declared that 27, 36, 34, 32 and 41 samples of minced meat luncheon, beef burger, beef, sausage, beef kofta and beef luncheon were free from E. coli that means the acceptability were 54%, 72%, 68%, 64% and 82%, respectively.

Regarding to these results it was concluded that minced meat and beef kofta were of low acceptability this might be mainly attributed to the manner of handling each product, the number of processing operations that the product subjected to them, amount of post processing contamination and storage condition and shelf life of each product.

5. REFERENCES


الملخص العربي

الكشف التقليدي والسرعة من ميكروب الآشريشيا كولاي
في بعض منتجات اللحوم البقري

رياض ربيع شاويش، عبد الرحمن محمود الباجوري، أبو بكر مصطفى إدريس
قسم الرقابة الصحية على الأغذية - كلية الطب البيطري - جامعة مدينة السادات
قسم الرقابة الصحية على الأغذية - كلية الطب البيطري - جامعة بنها

أجريت هذه الدراسة على 250 عينة من منتجات اللحوم (اللحم المفرود والنقاق والبقر والكفتة البقرية واللائحة البقرية) بواقع 50 عينة من كل منتج والمجموعة من أسواق محافظة القاهرة والقاهرة والقليوبية وذلك للكشف عن وجود ميكروب الآشريشيا كولاي في منتجات اللحوم بالطرق التقليدية وهي العزل وبالطرق الحديثة مثل تقنية تفاعلات البلمرة المتسلاة PCR.

تم الكشف عن وجود ميكروب الآشريشيا كولاي باستخدام طرق العزل التقليدية فوجد في كل من اللحم المفرود بنسبة 46% وفي البرجر البقري بنسبة 28% وفي النقاق بنسبة 33% وفي الكفتة البقرية بنسبة 36% وفي اللائحة بنسبة 16% و باستخدام تفاعل البلمرة المتسلسل للكشف عن ميكروب الآشريشيا كولاي يوجد في كل من اللحم المفرود بنسبة 38% وفي البرجر البقري بنسبة 22% وفي النقاق بنسبة 30% وفي الكفتة البقرية بنسبة 32% وفي اللائحة بنسبة 12%.

وأثبتت الدراسة أن تفاعل البلمرة المتسلسل دقيق وسريع في الكشف عن ميكروب الآشريشيا كولاي كما أنها توفر الوقت والعمالة داخل العمل.