Using Culture and PCR Technique for Detection of Escherichia coli O157:H7 in Duhok Cheese

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ABSTRACT: This study was aimed to investigate the presence of Escherichia coli O157:H7 in Duhok cheese by culture and Polymerase Chain Reaction (PCR) technique. The cheese samples (n:120) were purchased from different supermarkets and shops from Zakho, Akre, Amedy and Duhok cities during September 2011 to February 2012.

X-gal positive, MUG negative, indole positive colonies were found as 10% (12/120) of samples. These colonies were confirmed by biochemical tests (API E 20 test, latex agglutination tests) and results showed that 7 (5.83%) of E. coli isolates from cheese were positive for O157:H7. These isolates were also confirmed by PCR assay using specific primers for rfbO157 and flicH7. The numbers of positive isolates for rfbO157 and flicH7 were 3.33 and 0.83%, respectively. The PCR results confirmed as 0.83% of samples were E. coli O157:H7.

Key words: Duhok cheese, Escherichia coli O157:H7, Cultural technique, PCR technique

Kültür ve PZR Yöntemi ile Duhok Peynirinde Escherichia coli O157:H7’nin Araştırılması


X-gal pozitif, MUG negatif, indol pozitif olan %10 (12/120) oranında şüpheli koloni tespit edilmiştir. Şüpheli kolonilere biyokimyasal testler uygulanmış (API E 20 ve latex ağlütinasyon testi) ve %7 (5,83) oranı olan E. coli O157:H7 tespit edilmiştir. Bu izolatlar rfbO157 ve flicH7 primerleri kullanılarak PCR yöntemi ile doğrulama işlemi uygulanmıştır. %3.33 oranında O157 ve 0.83 oranında H7 antijeni pozitif olarak tespit edilmiştir. PCR analizlerinin sonunda örneklerin %0,83’unun E. coli O157:H7 olduğu gözlenmiştir.

Anahtar Kelimeler: Duhok peyniri, Escherichia coli O157:H7, Kültür teknigi, PZR teknigi

INTRODUCTION

Enterohaemorrhagic Escherichia coli (EHEC) serotype O157:H7 is a foodborne pathogen. The serotype O157:H7 is a predominant serotype among enterohaemorrhagic E. coli, and associated with foodborne outbreaks. This bacteria causes diarrhea, bloody diarrhea, life-threatening post diarrheal disorder (hemorrhagic colitis and hemolytic uremic syndrome (HUS)) (Karmali, 1989; Andreoli et al., 2002).

Raw milk is considered as a good medium for growth of many different pathogenic bacteria included E. coli O157:H7. Since healthy domestic animals, especially cattle, sheep and goats, can harbor STEC and O157:H7 in their feces (Blanco et al., 2003; Beutin et al., 2004). Transmission occurs through consumption of undercooked meat, unpasteurized dairy products and vegetables, or water contaminated by feces of carriers. Also, person-to-person transmission has also been documented (Beutin et al., 2004).

Cheese is an important milk product produced in almost every part of the world. It is widely consumed by the majority of people. Different studies showed that 1-5% of foodborne infections were related to consumption of milk and dairy products, that 53% of cases of foodborne infections caused by contaminated cheese and that Enteropathogenic E. coli (EPEC) is the causative agent of 18.33% of these cases (Schrade and Yager, 2001). Contaminated unpasteurized dairy products such as raw milk and raw-milk cheese have been incriminated in recent foodborne STEC outbreaks (Honish et al., 2005; CDC, 2007). The ability of survival of the pathogen in raw goat milk lactic (soft) cheeses, aged cheddar cheese made from unpasteurized milk (Schlessert et al., 2006), feta cheese and yogurt (Morgan et al., 1993; Govaris et al., 2002) were documented.

This study was aimed to investigate the incidence of E. coli O157:H7 in local Duhok cheese collected from Northern Iraq /Duhok Province under Duhok, Amedy, Zakho, Akre cities. Local cheese is a conventional soft cheese which is manufactured from unsterilized raw milk in farmer houses and frequently consumed in Northern Iraq.

MATERIAL and METHODS

The samples of local cheese (n:120) were obtained from different supermarkets and shops in Northern Iraq/Duhok Province under the following districts; Duhok city center (n:40), Amedy (n:20), Zakho (n:28)

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and Akre (n:32). The samples were collected from September 2011 to February 2012. The samples were brought to the laboratory immediately in ice pack.

**Microbiological Analyses**

A total of 25 g sample was enriched in 225 mL Butterfield's phosphate buffer at 37°C ± 1°C for 18-24 h. The enriched culture was plated on TC-SMAC (BD, BBL, 222226) and R&F *E. coli* O157:H7 agar (Merck, 1.04036.0500) and incubated at 37°C ± 1°C for 18-24 h. On TC-SMAC typical O157:H7 colonies are colorless or neutral/gray with a smoky center and 1-2 mm in diameter. Sorbitol-fermenting bacteria such as most *E. coli* appear as pink to red colonies. On *E. coli* O157:H7 agar, *E. coli* O157:H7 colonies are black to blue-black colonies. *E. coli* O157:H7 (ATCC 43895) was used as a control. Suspected typical colonies were tested for O157 antigen by latex agglutination (RIM *E. coli* O157:H7 Latex Test (Remel, Lenexa, KS, 800-255-6730). Also the suspected colonies were tested for purity with TSAYE. *Coli Complete (CC) disc* (BioControl, Bellevue, WA) was placed a in the heaviest streak area on the TSAYE plate and incubated at 37°C ± 1°C for 18-24 h. Blue color on and around the disc (indicative for coliforms) and blue fluorescence around the disc was checked under long wave UV (365 nm) light (indicative of *E. coli*). X-gal (+), MUG (-), indole (+) colonies were assumed as *E. coli* O157:H7 (Feng et al., 2011). Then the colonies were re-purified on Blood agar for API 20E test for confirmation (Stampi et al., 2004) and polymerase chain reaction (PCR) assay using the O-antigen-encoding region of *rfb*O157 and *flisc*H7 generic primers as described before (Gannon et al., 1997; Paton and Paton, 1998).

**RESULTS and DISCUSSION**

Out of 120 traditional cheese samples 12 (10%) were determined as *E. coli*. The results showed that 7 (5.83%) of *E. coli* isolates from cheese were positive for O157:H7 (Table 2).

Among *E. coli* isolates, 3.33% were positive for *rfb*O157 and 0.83% were positive for *flisc*H7 (Table 3).

Rahimi et al. (2011) reported that 5 (4.2%) cheese samples were positive for *E. coli* O157 by PCR test. Abdul-Raouf et al. (1996) reported that 6% of raw cow milk samples examined in Egypt were contaminated with *E. coli* O157:H7. Cheese containing unpasteurized milk was implicated as the vehicle of transmission in outbreaks of infectious intestinal disease (Altekruse et al., 1998). Incidence of *Escherichia coli* O157 in raw milk and white pickled cheese manufactured from raw milk in Turkey was studied. According to the results, *E. coli* O157 was determined in 1% of the total raw milk samples and in 4% of the cheese samples (Öksüz et al., 2004). Our findings were not differed greatly from other regions.

**DNA Extraction**

DNA of *E. coli* that isolated from cultural method was extracted from few colonies grown on Mac Conkey Agar (Merck) plates by boiling method. One loopful of *E. coli* from agar plates was suspended in 100 µl of sterile deionized water in an eppendorf tube and a bacterial suspension was made by using vortex. The bacterial suspension was boiled at 100°C for 5 minutes and centrifuged at 10,000 x g for 2 min. The supernatant was used as DNA template for PCR (Tobias and Vutukuru, 2012).

**PCR Preparation**

All the PCR reactions were performed in 20 µl final volume containing 0.5 µl of templet DNA, 10 µl of ReddyMix (containing KAPA2G FastHotStart DNA Polymerase, buffer, 0.2 µM of each dNTP, 1.5 mM of MgCl₂, at the final concentration of 1.5 mM) (Tetchum, Sweden). Additional MgCl₂ to the final concentration of 2 mM, and each of the primers (MWG, Germany) to the final concentration of 10 µM were added for the final PCR reaction. The thermo cycling conditions for all the PCRs were as follows: 95°C for 2 min, 95°C for 15 s, 52°C for 8 s, and 10 s at 72°C for 30 cycles, with a final 2 min extension at 72°C, and all the PCRs were performed in the MJ Research PTC-200 ThermalCycler. Amplified samples were evaluated by 1.5% agarose gel electrophoresis in Tris-borate-EDTA buffer and EtBr staining and illuminated by UV-transilluminator and documented by a gel documentation apparatus and 1000bp DNA ladder was used as a marker for mPCR assay. The expected size of products for *rfb*O157 and *flisc*H7 genes amplification were 259 and 625 bp, respectively. Primers were listed in Table 1.

### Table 1. Primers used for detection of *E. coli* O157:H7

<table>
<thead>
<tr>
<th>Primers</th>
<th>Primer sequence (5′–3′)</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157 Forward</td>
<td>CCGACATCCATGTTGTATGG</td>
<td>259</td>
<td>(Paton and Paton, 1998)</td>
</tr>
<tr>
<td>Reverse</td>
<td>TGGCTATGTAACGTTATCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>flisc</em>H7 Forward</td>
<td>GCGCTGTGAGTTCTATCGAGC</td>
<td>625</td>
<td>(Gannon et al., 1997)</td>
</tr>
<tr>
<td>Reverse</td>
<td>CAACGTTGACTTTTATCGCCATCC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Culture and API test results

<table>
<thead>
<tr>
<th>Region</th>
<th>Samples</th>
<th>Positive samples by culture method</th>
<th>Positive samples by API test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duhok center</td>
<td>40</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Amedy</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zakho</td>
<td>28</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Akre</td>
<td>32</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>12</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 3. PCR results for *E. coli* O157:H7

<table>
<thead>
<tr>
<th>Samples</th>
<th>Positive samples from Biochemical tests</th>
<th>Positive samples from API test</th>
<th>rfbO157</th>
<th>flic H7</th>
<th>O157: H7</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>12 (10%)</td>
<td>7 (5.8%)</td>
<td>4 (3.33%)</td>
<td>1</td>
<td>1 (0.8%)</td>
</tr>
</tbody>
</table>

The occurrence of *E. coli* in cheese samples may be due to lack of proper sanitation and absence of pasteurization of milk used for cheese production. To prevent the contamination of traditional cheese, strict hygienic procedures must be followed.

We can conclude that the routine microbiological examination should be done in producing factories, groceries and other food plants. Hygienic procedures should be applied for personnel whom involved in handling and preparing of food. Food safety information, training of staff, consumers should be organized and controlled by the governments and always work in partnership for eliminating food related EHEC illness.

REFERENCES


