RESEARCH ARTICLE

Using Polymerase Chain Reaction (PCR) Technique For Detection E. coli O157:H7 Bacteria From Raw Cow’s Milk and Local SOFT Chesses.

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Abstract

E. coli O157:H7 has emerged as an important gastrointestinal pathogen of man can cause severe illness and death. The present study was undertaken to detect the E. coli O157:H7 in 125 samples (75 raw milk, 25 pasteurized milk and 25 soft Arab chesses). Samples were collected from different location (source) in Baghdad city (Abu Ghraib, Al Kadhimiya, Al-A’amiya and Fudaliyah). During the period from October 2015 to January 2016, all samples were screened to detect the presence of non-sorbitol fermenting colonies (NSF) on sorbitol MacConkey agar supplemented with Cefixime, Pasteurium tolerate (CT-SMAC). A total of 125 samples, 28 (37.3%) from raw milk, 0 (0%) from pasteurized milk and 14 (56%) from soft Arab cheese (NSF), with chromogenic media 10 (13.3%) from raw milk, and 4 (16%) from soft Arab chesses which appear purple on the media. E. coli isolates were serotyped as E. coli O157:H7 by latex agglutination test, 7 (9.3%) isolates of raw milk, and 2 (8%) isolates of soft Arab chesses. All the latex agglutination positive isolates were positive to result of the vitek2 test. The specificity of the primers was evaluated by specific primers (uidR and flicH7 genes) 7 (9.3%) 2 (8%) isolates of raw milk, and soft Arab chesses samples respectively. In this study was evaluated based on Polymerase chain reaction (PCR) technique, that amplified the uidR and flicH7 genes.

Introduction:

Escherichia coli (E. coli), a bacterium that is a common inhabitant of warm blooded animals gut, including man. Most strains of E. coli are harmless; however, some strains such as E. coli O157:H7 can cause severe food borne disease and are referred to as enterohemorrhagic E. coli (WHO, 1996).

E. coli O157:H7 is one of hundreds of gram negative E. coli and is the most well known enterohemorrhagic strain (EHEC) (Peacock et al. 2001).

Enterohaemorrhagic Escherichia coli (EHEC) is the main cause of the recent outbreaks of diarrhea, hemolytic uremic syndrome (HUS) and hemorrhagic colitis (Kwon and Cho, 2015).

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*E. coli* O157:H7 serotype are worldwide zoonotic and major foodborne pathogens responsible for the majority of severe cases of human enterohemorrhagic *Escherichia coli* (EHEC) diseases (Dulo, 2014).

Milk and its products are considered a kind of proteins, fats, salts rich nutrient with sweet taste, easy digested, cheap markets, and consumers for raw milk and their products have existed in many parts of the world. Milk is a highly nutritious medium for growth and transmission of several types of microorganism especially *E. coli* O157:H7. Such contaminants may render the milk and its products unsafe to use and expose the consumers to risk of infection (USDA, 2009; Robert, 2008).

Furthermore, *E. coli* O157:H7 has been recognized as a major food safety concern due to its low infectious dose. It is highly virulent, an inoculation of fewer than 10–100 CFU of *E. coli* O157:H7 is sufficient to cause infection (Coffey et al. 2011). *E. coli* O157:H7 pathogens were usually linked with a wide variety of foods such as milk, meats, meat products, dairy products and fresh products, contaminated from the field with wildlife droppings (Bell 2002; Fedio et al. 2011; Mora et al. 2007; Pennington 2010).

Raw milk was first recognized as a vehicle of transmission of *E. coli* O157:H7 in 1986 (Martin et al., 1986; Oberst et al., 1998) when children from Wisconsin, USA, developed HC and HUS after drinking raw milk from dairy farms. Since then, there have been several reports of outbreaks associated with this pathogen following consumption of raw milk (Borczyk et al., 1987; Doyle, 1991; Duncan et al., 1987; Karna et al., 2007; Mai et al., 1987).

The verocytotoxigenic *E. coli* (VTEC) enter the food chain via fecal contamination of milk, contamination of meat with intestinal contents during slaughter or contamination of fruit and vegetables by contact with contaminated manure. It is also transmitted through contact withinfected people, animals or animal waste. Moreover, contaminated water which used for irrigating or washing vegetables can also be source of infection for humans or animal (Paton and Paton, 1998).

Therefore, the present study was planned and aimed to detect and characterize *E. coli* O157:H7 from diarrheic and apparently healthy calves, raw milk and from children suffer from diarrhea, which can achieve through isolation and identification of *E. coli* O157:H7. Serological detection of *E. coli* O157 by using Latex agglutination and confirmation of the *E. coli* O157:H7 using polymerase chain reaction (PCR).

**Materials and Methods:**
A total of one hundred fifty random raw milk and soft Arab chesses samples were collected from deferent locations (source) in Baghdad city (Abu Ghraib, Al Kadhimiya, Al-A’amiriya and Fudaliyah). During the period from October 2015 to January 2016. The samples were brought to the laboratory on crushed ice.

**Culture conditions:**
Immediately in the laboratory, 25 ml of each sample was aseptically transferred to 225 ml of modified tryticase soy broth (mTSB, Himedia, India) containing 20 mg/L novobiocin, followed by incubation at 18–24 h at 41.5 °C according to ISO 16654 (2001). The enriched culture were plated onto sorbitol MacConkey agar supplemented with cefixime (0.05 mg/ L) and potassium tellurite (2.5 mg/ L) (CT-SMAC, Oxoid, UK) and incubated at 41 °C for 24 h. Non-sorbitol fermenting (NSF) colonies from each CT-SMAC plate were selected and streaked onto plates containing HiCrome EC 0157:H7 Selective agar Base and were incubated at 37 °C for 24 h. Subsequently, one isolate from the subculture was further tested for agglutination with an *E. coli* O157:H7 latex test kit (Oxoid, UK) to detect the somatic antigen O157.

**Molecular Detection of (uidRand flicH7) gene by using Multiplex PCR technique**
was done by using commercially available DNA extraction and purification kit (Geneaid, Korea). The purified DNA was detected by electrophoresis in 1% agarose gel with addition of ethedium bromide. Bromophenol blue stain added to the DNA sample and visualizes the DNA by U.V. light.

**uidR:** F: 5′-TGTTACGGTCTGTAGAAAGGCC-3′
R: 5′-AAAAACTGCGCTGCAACAGCAT-3′
152bp (Bej et al., 1991)

**FlicH7:** F: 5′-GCGCTGTGATGTTCTATCGAG-3′
R: 5-CAACGTTGACTTTATCGCCAT -3
625bp (Gannon et al.,1997)

This was done by using customize primers shown above. The PCR reaction mixture contains 12.5 μl of green master mix, 5 μl of purified bacterial DNA, 1 μl of each forward and reverse primers and 5.5 μl deionized water for total volume reaction (25 μl).

PCR tubes were transferred to the thermalcycler (after centrifuged for 10 seconds) to start theamplification reaction according to specific program for each gene. The results of the PCRwere performed in post amplification process.10 μl from amplified sample was directly loaded in a 2% agarose gel containing 0.5 μl /25ml ethidium bromide with the addition of loading buffer and DNA size. Marker as standard in electrophoresis and the gel was run at 75 V. at 1 hr, then the products were visualized by UV transilluminator (Sambrook et al., 1989).

Two concentrations of agarose gel were prepared (1% and 1.5%) as we needed. The concentration of 1% agarose was used in electrophoresis after DNA purification process, while 1.5% agarose was used after PCR detection.

The results of the PCR were performed in post amplification process.10 μl from amplified sample was directly loaded in a 1.5% agarose gel containing 0.5 μl /ml ethidium bromide with the addition of loading buffer and DNA size. Marker as standard in electrophoresis and the gel was run at 75 V. at 1 hr, then the products were visualized by UV transilluminator.

**Results and Discussion:**

According to the results of culturing on CT- SMAC, in the current study percentage of NSF E. coli was 37.3% of raw milk samples and 56% of soft Arab cheeses shown in table (1). in the current study percentage of NSF isolates in raw milk samples was 37.3% which similar to the study contact by Roopnarine et al (2007) who showed that the percentage was 37.3%, but less than the percentage by Abbas et al (2012) which was 57.34% from 150 raw milk samples, whereas higher than Abd Al-Azeem et al (2013)

who showed isolation percentage 6% . The explanation of difference in isolation percentage between this study and other studies may be related to the difference in the serotypes of NSF E. coli and to facilities techniques used in detection and diagnosis of this bacteria. In the current study NSF E. coli was isolated on selective enrichment (TSB-V) supplemented with vancomycin (De-Boer and Heuvelink, 2000). This medium permits the growth of E. coli and inhibit a broad range of contaminants including Proteus spp. Also it is necessary for the increase of bacteria to the level in which easily detected as low (10 cells) infection dose (Twardon et al., 2006). March and Ratnam, (1989) and Silveria etal. (1999) stated that the latex test is simple, highly efficient and reliable test in detecting E. coli O157:H7 with 100% sensitivity and specificity. It was evident from the results shown in Table (1)

In the present study, the overall prevalence of E. coli O157:H7 latex agglutination test was 9.3% from raw milk samples This is convenient with the results showed by Al-Hasnawi (2010) Which was 11%. And less than that by Abbas et al (2012 ; Murinda et al., 2002 and Daoed et al., 2007). In All theseprevious studies, the prevalence percentage were less than the results in the current study, this specific the presence of E.coli O157:H7 of tested samples included in the present study with higher prevalence due to unhygienic measurement that lead to higher contamination rates, suggested that spread of E. coli O157:H7 in the raw milk which were serve as a main source of infection and the risk of acquiring is high (Jamshidi et al., 2008).The positive percentage of E.coli O157:H7 in the present studyto latex agglutination test from soft Arab cheeses (12%) was less than that byAbbas et al(2013) which was 15(33.34%) . The results showed that geographical or locational difference of thefarmer, contaminatedenvironmental conditions and unhygienic measures had an effect on the isolation percentage of E.coli O157:H7 (Spano et al., 2003).
Table 4.1: Frequency of NSF and latex agglutination confirmed E. coli in raw milk and soft Arab cheeses.

<table>
<thead>
<tr>
<th>Isolates source</th>
<th>Total samples</th>
<th>Non sorbitol fermenting</th>
<th>O157 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
<td>75</td>
<td>28 (37.3%)</td>
<td>7 (9.3%)</td>
</tr>
<tr>
<td>Soft Arab Chesses</td>
<td>25</td>
<td>14 (56%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>42</td>
<td>9</td>
</tr>
</tbody>
</table>

In the current study, E. coli 0157:H7 was isolated from raw milk with percentage 9.3% (7/75) and that was higher than results obtained by Abbas et al. (2012) the percentage of PCR positive for VT1 of E. coli O157:H7 isolates from raw milk samples (4.67%) in Basrah city, Brenjchi 2011 (0.77%) in Mashhad, Abdel-Fattah et al. 2008 (2.5%) in Egypt, and Abd Al-Azeem et al. 2013 (1%).

In this study, E. coli O157:H7 was isolated from soft Arab cheeses with percentage (8%) which is similar to the study conducted by Paneto et al. (2007) who showed that percentage was (6%) in Turkey, whereas higher than the percentage by Abbas et al. (2013) which was The percentage of PCR positive for VT1 of E. coli O157:H7 isolates of soft cheese was (3.34%) in Bashar city, but less than the percentage by Vernezy-Roza et al. (2005) who showed that percentage was (13%) in France.

The high percentage recorded in the present study can be attributed to the use of the primers explored for e1111 gene specific for all types of E. coli and the fliC H7 gene specific for the h7 flagellar antigen of E. coli O157:H7. The multiplex PCR assay developed was found to be highly specific as it produced PCR products of 152 bp E. coli specific and 625 bp E. coli O157:H7 specific (Kumaret al., 2013). The results showed that geographical or locational difference of the farmer, contaminated environmental conditions, and unhygienic measures had an impact on the isolation percentage of E. coli O157:H7 (Spano et al., 2003).

The rate of positive PCR isolates for E. coli O157:H7 in Table 1:

<table>
<thead>
<tr>
<th>Isolates source</th>
<th>Total</th>
<th>fliC H7 gene</th>
<th>uidR gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw milk</td>
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<td>7 (9.3)</td>
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</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

Photo 1: Gel electrophoresis image of amplicons by multiplex PCR from selected E. coli O157:H7 strains. Showing the optimization of annealing temperature for E. coli O157:H7 structural and virulence genes detection using 1.5% agarose gel.
agarose gel at 8volt/cm for 2 hour. Lane 1,12: DNA ladder (100 bp),lane 2,11: negative control, lanes 3-10: amplicons obtained using 62 °C.

In the current study E.coli O157:H7 was isolated from raw milk with percentage 9.3% (7/75) and that was higher than results obtained by Abbas et al (2012) the percentage of PCR positive for vt1 of E.coli O157:H7 isolates from raw milk samples (4.67%) in Basrah city, Brenjchi 2011(0.77%) in Mashhad, Abdel-Fattah et al 2008 (2.5%) in Egypt, and Abd Al-Azeem et al 2013 (1%).

In the present study E.coli O157:H7 was isolation from soft Arab cheeses with percentage (8%) which similar to the study contact by Paneto et al (2007) who showed that percentage was (6%) in Turkey, whereas higher than the percentage by Abbas et al (2013) which was The percentage of PCR positive for vt1 of E.coli O157:H7 isolates of soft cheese was (3.34%) in Bashar city, but less than the percentage by Verneyzo-Rozandet al (2005) who showed that percentage was (13%) in France.

The high percentage recorded in the present study can be attributed to the primer explored in the assay were targeted against the uidA gene specific for all types of E.coli and the fliC7 gene specific for the h7 flagellar antigen of E.coli O157:H7. The multiplex PCR assay developed was found to be highly specific as it produced PCR products of 152 bp E.coli specific and 625 bp E.coli O157:H7 specific (Kumaret et al., 2013). The results showed that geographical or locational difference of the farmer, contaminated environmental conditions and unhygienic measures had an impact on the isolation percentage of E.coliO157:H7 (Spano et al., 2003).

Reference:-


