Molecular detection of β-lactamase (tem and shv) genes in *Escherichia coli* O157:H7 isolated from different sources in Basra, Iraq

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**ABSTRACT**

A total of 91 non-sorbitol fermenting *Escherichia coli* previously isolated from stool of hospitalized children suffering from diarrhea, beef samples and raw milk samples were used in this study. Among these, 29 strains were found enterohemorrhagic *E. coli* (EHEC) and only four strains were identified as *Escherichia coli* O157:H7 depending on latex agglutination test. *Escherichia coli* O157:H7 were further examined by amplifying both *tem* and *shv* genes. The results revealed that all isolates were positive to *tem* gen, 50% of them carrying *shv* gene and 50% of isolates carrying both *tem* and *shv* genes.

**Keywords:** *E. coli*, *tem*, *shv*, EHEC, stool, milk, beef.

1. Introduction

*Escherichia coli* O157:H7 is a major cause of food and water-borne illnesses characterized by bloody diarrhea, hemorrhagic colitis (HC) and life-threatening hemolytic uremic syndrome (HUS) in developed nations across the globe (Paton and Paton, 1998). *E. coli* strains that can cause diarrheal illness are assigned to six specific virulence groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes, and distinct O:H serogroups. These categories include: enteropathogenic *E. coli* strains (EPEC), enteroaggregagenic *E. coli* strains (ETEC), enteroinvasive *E. coli* strains (EIEC), diffuse-adhering *E. coli* strains (DAEC), enteroaggregative *E. coli* strains (EAggEC), and enterohemorrhagic *E. coli* table (2-1) strains (EHEC), (Doyle and Beuchat, 2007). Enterohemorrhagic are represented by a single serotype of *E. coli* called *E. coli* O157:H7, which causes diarrheal set of symptoms discrete from that of enteroinvasive *E. coli* (EIEC) and Shigella in that there is copious bloody discharge without fever (Michael et al., 1997). This strain appear to play a role as food borne pathogens and have to be considered in view of food safety aspects (Stephan et al., 2004). EHEC strains are important enteropathogen among young children (Alikhani et al., 2007), and more prevalent in many diarrheal cases in children (Jensen et al., 2007). Extended spectrum β-lactamase has been classified into different types. Initially, the 2 most frequent types of ESBL were TEM types and SHV types. ESBL-producing Escherichia coli now represents an emerging cause of infection in many areas of the world (Moland et al., 2003). Beta-lactamases of TEM- and SHV-type are found in the functional groups 2b, 2be and 2br, some TEM can also be found
in group 2ber (Bush and Jacoby 2010). The parental enzyme TEM-1 was discovered in the mid-1960’s from a Greek patient, a few years later the SHV-1 was described (Ambler, 1991). The first ESBL encountered was SHV-2 in the mid 1980’s (Kliebe, 1985). More than 180 TEM and 120 SHV variants are known today (Jacoby and Bush, 2012).

2. Materials and Methods

2.1. Bacterial strains: A total of 91 non-sorbitol fermenting Escherichia coli previously isolated from stool of hospitalized children suffering from diarrhea, beef samples and raw milk samples were used in this study (Khudaier et al. 2012).

2.2. Confirmation of E. coli O157:H7 (NSFEC). Celllobiose Fermentation test and potassium cyanide (KCN) test were done to confirm the non-sorbitol fermenting ability of E. coli strains used through the study. (MacFaddin, 2000; Collee et al., 1996).

2.3. Latex agglutination Test for E. coli O157 :H7 This test was used for serotyping of E. coli O157:H7 by using commercial kit (Wellcolex E.coli O157:H7, Remel) to detect the somatic antigen O157 and flagellar antigen H7.

2.4. Detection of tem and shv Genes by PCR Assay This procedure was done by using commercially available DNA extraction and purification kit (Promega, USA). The β-lactemase (tem and shv) genes were studied according to protocol of (Ashraf et al., 2005). This was done by using specific primers (below). Oligonucleotide Primers Sequences Used for PCR Amplification of tem, shv genes of 1080 bp were:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>tem</td>
<td>ATAAAATTCTTGGAAGACGAAA</td>
</tr>
<tr>
<td>tem</td>
<td>GACAGTTACCAATGCTTAATC</td>
</tr>
<tr>
<td>shv</td>
<td>TTATCTCCCTGTAGCCACC</td>
</tr>
<tr>
<td>shv</td>
<td>GATTTGCTGATTTCCGCTCGG</td>
</tr>
</tbody>
</table>

PCR product was analyzed by gel electrophoresis in 1% agarose containing ethidium bromide 0.5μg/ml (Sambrook et al., 2001).

3. Results

3.1. Bacterial isolation:

E. coli isolates (91) were screened to detect non-sorbitol fermenting E. coli (NSFEC). Twenty nine NSFEC were detected in beef samples, 44 in stool samples and 18 in raw milk samples (table 1). Twenty nine strains were found enterohemorrhagic E. coli (EHEC), 12 from beef, 10 from stool and 7 from milk. Latex agglutination test was used to detect serotype O157:H7 in non- sorbitol fermenting isolates. We found that two isolates from beef, 1 isolate from stool and 1 isolate from raw milk samples, were found to be E. coli O157:H7.
Table (1): Number of EHEC isolates from NSF *E. coli* and distribution of O157 and H7 antigen through isolates

<table>
<thead>
<tr>
<th>Source</th>
<th>NSF</th>
<th>EHEC</th>
<th>O157</th>
<th>H7</th>
<th>O157:H7</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>29</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>16.7</td>
</tr>
<tr>
<td>Stool</td>
<td>44</td>
<td>10</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Milk</td>
<td>18</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>14.3</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>29</td>
<td>8</td>
<td>9</td>
<td>4</td>
<td>13.8</td>
</tr>
</tbody>
</table>

NSF: non-sorbitol fermenting *E. coli*;  EHEC: enterohemorrhagic *E. coli*

### 3.2. Detection of *tem* and *shv* Genes by PCR Assay

The *E. coli* O157:H7 isolates were further examined by PCR technique using two pairs of primers to amplify both *tem* and *shv* genes. The results revealed that all (100%) of the isolates were positive for *tem* gene, 2 (50%) isolates yielded amplification products with *shv*-PCR specific primers and 2 (50%) isolates were identified as carrying both *tem* and *shv* genes. The DNA of all isolates was extracted and purified by using genomic DNA purification kit. The results were detected by electrophoresis on 1% agarose gel and exposed to UV light in which the DNA appeared as compact bands (figure 1).

![Figure (1): Total genomic DNA extracted from isolates using 1% agarose gel electrophoresis.](image)

### 3.3. Distribution of PCR Positive for *tem* and *shv* Genes in *E. coli* O157:H7.

The results of PCR amplification performed on the DNA extracted from all the studied isolates were confirmed by electrophoresis analysis. By this analysis the strands of DNA which are resulted from the successful binding between specific oligonucleotide primers for (*tem* and *shv*) genes and isolates extracted DNA. These successful binding appeared as single band for each gene under the
U.V light using ethidium bromide as a specific DNA stain. The electrophoresis was also used to estimate DNA weight depending on DNA marker (100 bp DNA ladder) PCR products corresponding to tem (1080 bp) were found in all four E. coli O157:H7 (figure 2), while PCR products corresponding to shv genes (795 bp) were appeared in only 2 E. coli O157:H7 isolates (Figure 3). Two (100%) isolates of E. coli O157:H7 in beef samples were harbored tem gene while only one (50%) isolate of E. coli O157:H7 was harbored both genes (tem and shv). On the other hand, the E. coli O157:H7 which isolated from stool samples harbored tem and shv gene while E. coli O157:H7 which isolated from raw milk samples harbored tem gene only (Table 2; figure 4).

Table (2): Distribution tem and shv genes in E. coli O157:H7 isolates

<table>
<thead>
<tr>
<th>Source</th>
<th>PCR +ve</th>
<th>tem</th>
<th>%</th>
<th>shv</th>
<th>%</th>
<th>tem&amp;shv</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>2</td>
<td>2</td>
<td>100</td>
<td>1</td>
<td>50</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Stool</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>1</td>
<td>100</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Raw milk</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure (2): PCR amplification of E. coli O157:H7 tem gene (1080 bp). Lane 1, molecular size marker; Lane 2, 3, 5 and 6 E. coli O157:H7 tem gene isolates; Lane 4, negative test.
4. Discussion

The classical screening medium for *E. coli* O157:H7 is sorbitol MacConkey agar. This method exploits the fact that *E. coli* O157:H7, unlike 90% of *E. coli* isolates does not ferment sorbitol rapidly (Doyle, 1991). Other studies reported that sorbitol MacConkey agar medium is a useful, rapid, and reliable screening aid for the detection *E. coli* O157:H7, but it is not generally useful of EHEC strains of serotypes other than *E. coli* O157:H7 (March and Ratnam, 1989). The occurrence of NSFEC in children stool, beef and raw milk samples which detected by conventional microbiological methods were 38.9%, 67.4% and 48.6%
respectively. The present occurrence of NSFEC in children stool sample was higher than the result of obtained by Dunah et al., (2010) who reported 15.6%. It is also lower than the finding of A’iaz (2008) and Dhanashree and Shrikar (2008) who detected 57.5% and 73.9%, respectively. The level of contamination of beef by NSFEC of the present study (67.4%) was found to be higher than the 21.88% which recorded by Ghareeb (2005) on cattle meat in Baghdad and much higher than the 7.75% and 7% prevalence reported by Dahanashree and shrikar, (2008); Jamshidi et al., (2008), respectively. However, there finding was lower than 92.46% prevalence reported by Karger et al., (2011). The frequency of NSF E. coli isolated in the present study (48.6%) in raw milk samples was higher than the prevalence reported by Ropnarine et al., (2007) who recorded that NSFEC isolates from milk was 37.5%, also much higher than Daoood, (2007) who showed that isolation rate was 10%. The present results differed from the results of El-safey and Abdul-Raouf, (2003), Mansouri-Najand and Khalili, (2007) who mentioned much lower rates of NSFEC isolates in raw milk 5%; 4.46%, respectively. It represents public health hazards due to fact that food poisoning outbreaks would be difficult to treat and this pool of multidrug resistant EHEC in food supply represents reservoir for transferable resistance genes. The significantly high frequency of resistant EHEC for these antimicrobials was probably an indication of their infrequent usage in livestock for prophylactic or nutritional purposes. Extended spectrum β-lactamase has been reported from all parts of the world. However, prevalence varies widely even in closely related regions. The true incidence is difficult to determine because of the difficulty in detecting ESBL production and due to inconsistencies in testing and reporting (Yusha et al., 2010). Prevalence of ESBL in many parts of the world was (10-40%) among E. coli (Rupp and Paul, 2003). The prevalence of ESBLs in Europe is higher than in the USA but lower than in Asia and South America (Girlich et al., 2004). In 2007 in Asia pacific region was found to harbour plasmid borne ESBLs 62% in E. coli (Bell et al., 2007). ESBL production rates were 96%, 70% and 43% in E. coli in Iran, India and Iraq respectively in 2009, 2010 and 2011 (Ali, 2009; Sharma et al., 2010; Al-Charrakh et al., 2011). There were a limited number of studies on prevalence of ESBL showing a high rate in Bangladesh, where E. coli 43.2% in 2004 (Rahman et al., 2004) and in Bangladesh it was E. coli 47.83% in 2010 (Haque and Salam, 2010). In India in 2010 tem and shv in E. coli were 30% and 38% respectively (Sharma et al., 2010). Very recently in 2011 in India Manoharan and his colleagues found tem in E. coli (39.2%) (Manoharan et al., 2011). The β-lactamases detection in the present study was not helpful to provide any information about the type of β-lactamase enzymes. However, up to our knowledge, there was no published data regarding the frequency of tem and shv genes among clinical isolates of E. coli in Iraq. An attempt was made to evaluate the frequency of tem and shv β-lactamase genes in 4 β-lactamase producing E. coli O157:H7 isolates obtained from beef, stool and milk. Based on the type of β-lactamase genes, E. coli O157:H7 isolates in the present study were divided into three categories; first category, 4/4 (100%) isolates yielded amplification products with tem-PCR specific primers. Secondly, 2/4 (50%) isolates gave PCR products with shv-specific primers. Third category, 2/4 (50%) isolates produce both tem and shv enzymes. The tem genes are by far the most widespread with unknown origin (Widemann et al., 1989). tem-1, which is responsible for most of the ampicillin resistance in; 94% of E. coli strains isolated in Spain, 89% of E. coli strains isolated in Hong Kong, and in 78% of E. coli strains isolated in London.
Livermore, et al., 1986). However, Bradford, (2001) reported that up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1. This enzyme has the ability to hydrolyze penicillins and early cephalosporins such as cephalothin and cephalaridine TEM -2 β-lactamase is widespread in *E. coli*, although they are much rare than TEM -1. The classical TEM -1 and TEM -2 enzymes have minimal activity against newer cephalosporins (Sirot, 1995). In the past 20 years, however, there have been an increasing emergency of ESBLs, which attack many newer cephems and monobactams as well as third generation cephalosporins and anti-Gram-negative bacterial penicillins (Bradford, 2001). Most of these enzymes are mutant of TEM-1 and TEM -2 such as TEM -3, TEM -4, TEM -10, TEM -27, TEM-92(Bradford, 2001). Although strains that produce ESBL are characteristically resistant to new cephalosporins and/or aztreonam, many strains producing these enzymes appear susceptible or intermediate to some or all of these agents in vitro, while expressing clinically significant resistance in infected patients (Paterson and Bonomo, 2005). It was recently reported by Aysha, et al., (2011) what agree with present results the presence of *tem* and *shv* genes in all the 23 strains. Taslima, (2012) founded *tem* and *shv* β-lactamas genes presence in 50.5% and 57.1% respectively.

**References**


Bell, JM.; Chitsaz, M.; Turnidge, JD.; Barton, M.; Walters, LJ. and Jones, RN. (2007). Prevalence and significance of a negative extended-spectrum β-lactamase (ESBL) confirmation test result after a positive ESBL screening test result for isolates of *Escherichia coli* and *Klebsiella pneumoniae*; results from the SENTRY Asia-Pacific Surveillance Program, J.Clin. Microbio. 45(5):1478-1482.


Dunah, CS. ; De, N. and Adamu, MT. (2010). A study on the prevalence of Escherichia coli O157:H7 among patients attending some public hospitals in Adamawa State, Nigeria. Report and opinion. 2(3)


Taslima, Y. (2012). Prevalence of ESBL among Escherichia coli and Klebsiella spp. in a tertiary care hospital and molecular detection of important ESBL producing genes by multiplex PCR: MS.c thesis. Department of Microbiology & Immunology Mymensingh Medical College Mymensingh
