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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), enterotoxigenic 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).

Cellobiose 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:

tem a: ATAAAATTCTTGAAGACGAAA
tem b: GACAGTTACCAATGCTTAATC
shv a: TTATCTCCCTGTTAGCCACC
shv b: GATTTGCTGATTTCGCTCGG

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 antigene through isolates

Source	NSF	EHEC	O157	H7	O157:H7	%
Beef	29	12	4	2	2	16.7
Stool	44	10	1	6	1	10
Milk	18	7	3	1	1	14.3
Total	91	29	8	9	4	13.8

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 purificated by using genomic DNA purification kit. The results were detected by electrophoresis on 1% agarose gel and exposed to U.V light in which the DNA appeared as compact bands (figure 1).

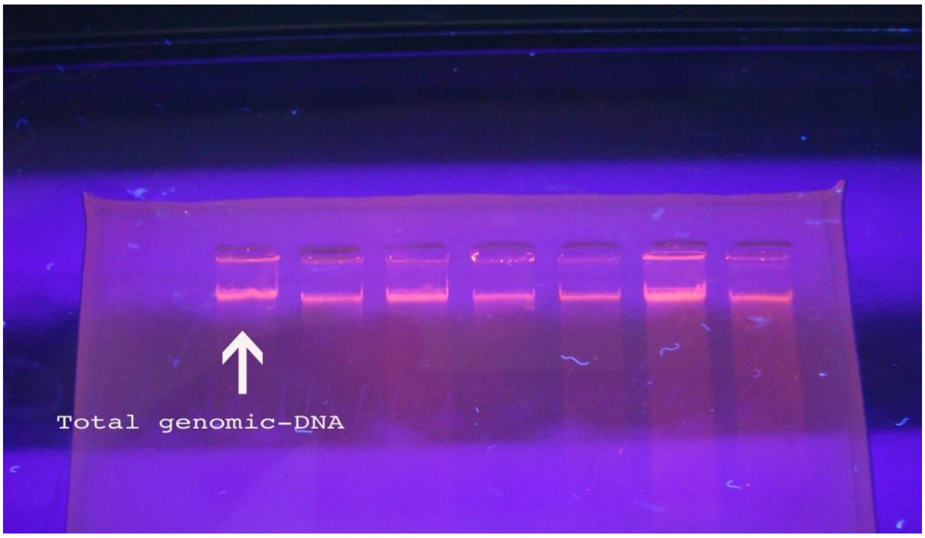

Figure (1): Total genomic DNA extracted from isolates using 1% agarose gel electrophoresis.

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

Source	PCR +ve	*tem*	%	*shv*	%	*tem&shv*	%
Beef	2	2	100	1	50	1	50
Stool	1	1	100	1	100	1	100
Raw milk	1	1	100	0	0	0	0

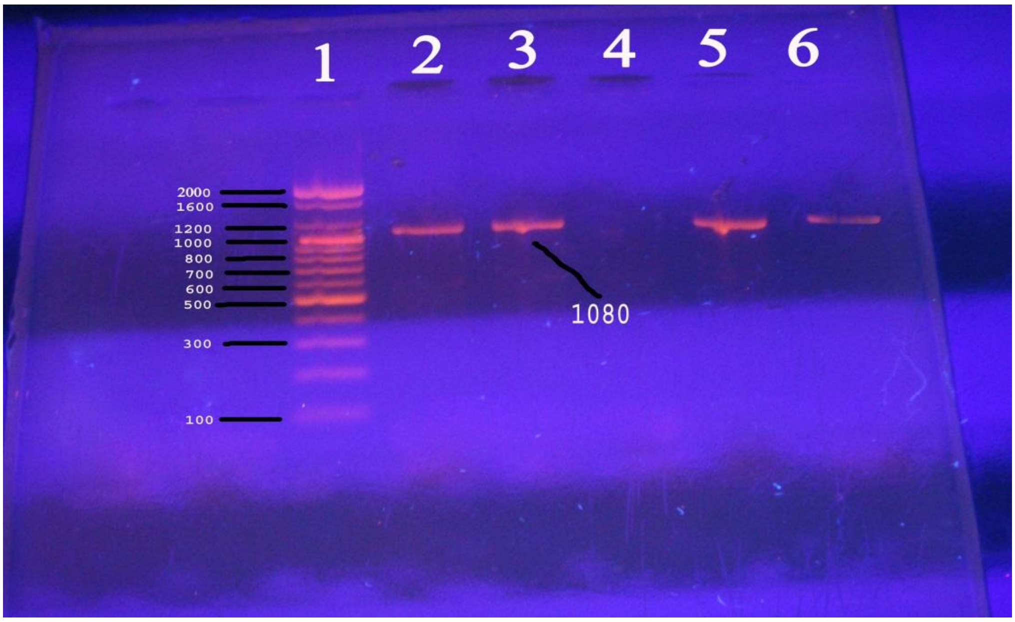

Figure (2): PCR amplification of *E. coli* O157:H7 *tem* gene (1080 bp) .Lane 1,molecular size marker; Lane 2, 3, 5 and 6 *E. coli* O157:H7 *tem* gene isolates; Lane 4 , negative test.

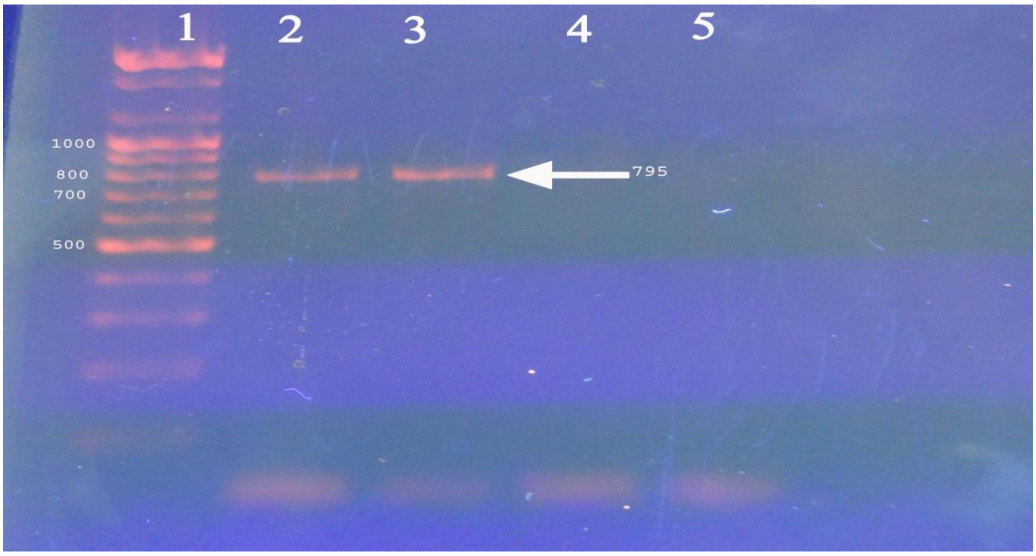

Figure (3): PCR amplification of *E. coli O157:H7 shv* gene (795 bp) Lane 1, molecular size marker; Lanes 2 and 3 *E. coli* O157:H7 *shv* gene; Lane 4 and 5, negative test.

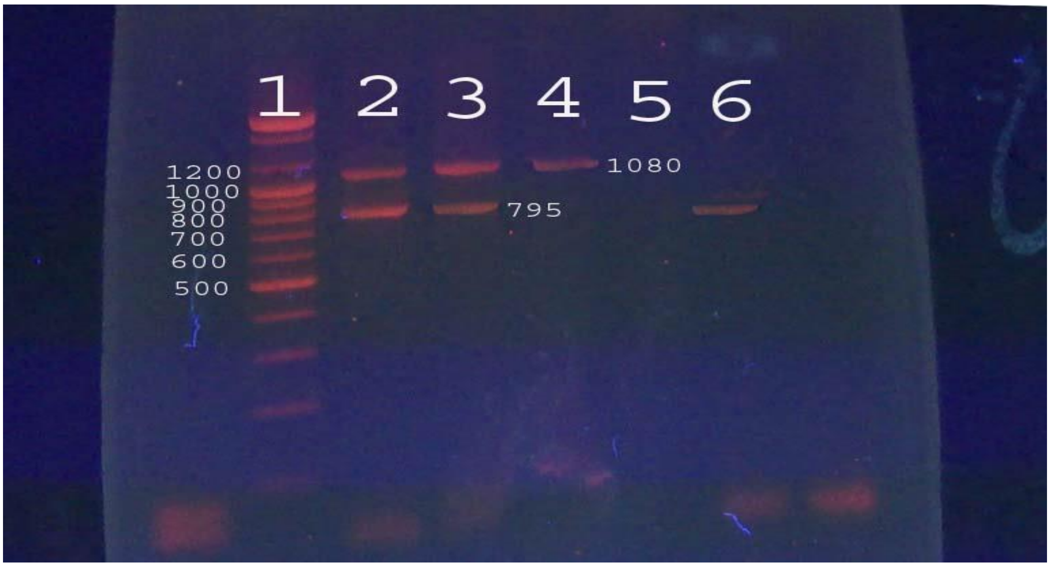

Figure (4): PCR amplification of *E. coli* O157:H7 *tem* and *shv* genes, Lane 1, molecular size marker; Lanes 2-3, *E. coli* O157:H7 *tem* and *shv* genes; Lane 4, *E. coli* O157:H7 *tem* gene; Lane 5, negative test; Lane 6, *E. coli* O157:H7 *shv* gene**.**

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 Dahanasheree 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 Daood, (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 collegues 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 cephaloridine 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 23strains. Taslima, (2012) founded *tem* and *shv* β-lactamases genes presence in 50.5% and 57.1 % respectively.

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