Detection of *Escherichia coli* O157:H7 Strain by conventional and molecular methods from diarrheal children in Baghdad.

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Abstract: *Escherichia coli O157:H7* is a pathotype of *Enterohemorrhagic Escherichia coli* (EHEC) which is considered as a public health problem bacteria induced food-borne diarrheas, bloody diarrhea and hemolytic uremic syndrome that occurring at any age group. Three hundred stool samples obtained from children with bloody diarrhea, their age ranged from one month to five years whom visited or admitted as "out-patients" to Al-Eskan pediatrics hospital, Al-Kadhumia pediatric hospital or from private clinic in Baghdad**.** EHEC was found in 37 (12.33%) patients. The highest rate 18 (48.64%) were in infants aged (3-12) months and 12 out of 37(32.43%) in age group (13-24) months. A 25 isolates were positive on Sorbitol MacConkey Agar with Cefixime and Tellurite (SMAC-CT). Commercial latex agglutination test revealed that *E.coli*:O157:H^7 was found in 14(56%) out of 25 culture positive cases while the other 11 (44%) isolates were *E.coli* O157: H^- The results of PCR amplification of (vt1 gene) showed that 37 (12.33%) out of 300 stool samples were produce (130 pb) amplified band**.** All culture positive sample (n=25) were also positive by PCR.
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1. Introductions

Enterohemorrhagic Escherichia coli (EHEC) as a subgroup of Shiga-toxin (Stx)-producing *E. coli* (STEC) are characterized by certain serotypes that are frequently occurring in outbreaks and are associated with severe clinical illnesses such as hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) (Sabine et al.,2013; Levine, 19872). These microorganisms are classified into pathotypes, and one of these pathotype is Shiga toxin (STEC) or *Escherichia coli O157:H7*, which is considered public health problem bacteria which induced food-borne diarrheas, bloody diarrhea and hemolytic uremic syndrome that occurring at any age group (Kaper *et al*., 2004). The name of *Verotoxigenic Escherichia coli* (VTEC) refers to their ability to produce toxins with a cytopathic effect on Vero cells. These bacteria produce two types of toxin: shiga toxin 1 (Stx1), which is most closely related to that produced by *S. dysenteriae*, and shiga toxin 2 (Stx2), which has several variants (Andreoli, 1999). EHEC are organisms with demonstrated human pathogenicity while most VTEC belong to *E coli* serogroup O157. Verotoxin production has been identified in more than 200 serogroup (Banatvala *et al*., 2001).

Hemolytic uremic syndrome is the most common cause of acute renal failure in children and the incidence of this syndrome is increasing worldwide(Kaper *et al*., 2004). *E. coli O157:H7* is believed to cause more than 80 percent of the STEC infections that lead to hemolytic uremic syndrome (Andreoli, 2002). Some assays for the detection of diarrheagenic *E. coli* are available, such as biochemical reactions, serotyping, phenotypic assays based on virulence characteristics, and molecular detection methods (Nataro and Kaper, 1998). Among these, PCR, one of the molecular biology-based detection methods, is a commonly used method that gives rapid, reliable results and that also has a high sensitivity and a high specificity (Bellin *et al*., 2001; Presterl *et al*., 1999). In the present study we investigate the prevalence of *E. coli* pathotype among children with diarrhea in Baghdad by conventional and molecular methods

2. Material and Methods

Over five months from January 2015 to May 2015, Three hundred stool samples obtained from children with bloody diarrhea whom visited or admitted as "out-patients" to Al-Eskain pediatrics hospital, Al-Kadhumia pediatric hospital or from private clinic in Baghdad were enrolled in this study. Stool samples were divided into two portions. One portion was for the direct stool examinations and the second portions was inoculated in tetrathionate broth for 24 hrs at 37 ºC and then inoculated in MacConkey agar for isolation and identification of lactose fermenter *E.Coli*. Isolated *E.coli* is subcultured in Sorbitol MacConkey Agar (SMAC-CT). Tis medium is modified MacConkey Agar using sorbitol instead of lactose with cefixime and tellurite. Cefixime inhibits *Proteus* spp. and tellurite inhibits non-O157 *E. coli*

and other organisms, thus improving the selectivity of SMAC-CT for *E. coli* O157:H7. Differentiation of enteric microorganisms is achieved by the combination of sorbitol and the neutral red indicator. Colorless or pink to red colonies are produced depending upon the ability of the isolate to ferment the carbohydrate sorbitol. *E. coli* O157:H7 considered as sorbitol negative. This medium prepared as follows: peptone (20gm), Bile salts (1.5gm), Sodium chloride (5gm), Neutral red (0.03 gm), Crystal violet (0.001 gm), D-Sorbitol (10gm), Cefixime (0.05 mg),Potassium Tellurite (2.5 mg) and Agar (15 gm). These ingredients were suspended in one litter of distilled water; PH was adjusted to (7.1) sterilized at 121 ºC for 15 minutes, left to cool before pouring into plates (Zadik *et al*.,1993).

For confirmatory identification of *E. coli O157:H7* Latex agglutinations test for *E. coli O157:H7* (OXOID-England) were also used in current study. The stranded isolates *E. coli* ATCC25922 used as a negative control.

In order to detect Enterohemorrhagic *Escherichia coli* (EHEC) shiga toxin 1 (vt1) gene, a polymerase chain reaction technique was used.DNA was extracted from each sample using AccuPrep stool DNA extraction kit (Bioneer Korea). The extraction protocol as recommended by the manufacturer data sheet. Purity and concentrations of all DNA samples were determined using nano-drop instrument (ActgeneNAS-99 Taiwan). The general ratio (A 260/280) equal to (1.8) perform better for PCR reaction. Ready to used Master Mix (AccuPower PCR premix from Bioneer, korea) were used in this study, 1 µl DNA template (sample and control) and specific primer 1 µl for forward and 1 µl for reverse (provided from AccuOligo Bioneer, Korea) were added to PCR Master mix tube. Free nuclease distilled water was used to complete the total volume to 20 µl then mixed briefly by vortex and spin. The sequences of the primers selected for use in the amplification completely matched the sequences of the corresponding genes of EHEC in the GenBank database (Trung *et al*., 2005). The primer sequences of (vt1) target gene (shiga toxin 1 vt1) were forword 5'-GAAGAGTCCGTGGGATTACG-3' and 5' -AGCGATGCAGCTATTAATAA-3 for reverse with Amplified size (130 bp).

The thermocycling conditions with a cleaver scientific thermal cyclers (TC 32/ 80- UK) were as follows: 96°C for 4 min, 94°C for 20 s, 55°C for 20 s, and 72°C for 10 s for 30 cycles, with a final 7-min extension at 72°C. PCR products were resolved in 1.5% (wt/vol) agarose gel (Merck- Germany) at 120 mV for 1hour. A molecular marker (100 bp DNA ladder; Bioneer) was run concurrently. The DNA bands were visualized and photographed under UV light after the gel was stained with ethidium bromide.

3. Results:

This study involved 300 Iraqi patients with bloody diarrhea, their age ranged from one month to five years. EHEC was found in 37 (12.33%) patients, the highest rate 18 (48.64%) were in infants aged (3-12) months and 12 out of 37(32.43%) in age group (13-24) months. The lowest rates (18.9%) were in children over two years. Culture result showed that 25 isolates were positive on Sorbitol MacConkey Agar with Cefixime and Tellurite (SMAC-CT). All culture positive sample (n=25) were also positive by PCR. Commercial latex agglutination test revealed that *E.coli*:O157:H^7 was found in 14(56%) out of 25 culture positive cases while the other 11 (44%) isolates were *E.coli* O157: H^-. The results of PCR amplification of (vt1 gene) showed that 37 (12.33%) out of 300 stool samples were produce, (130 pb) amplified band (Figure 1).

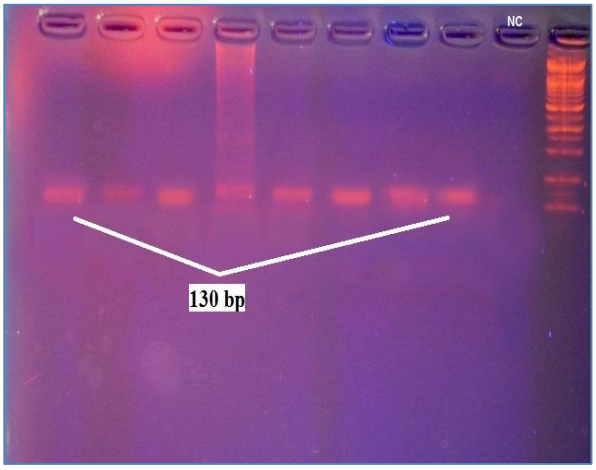

Figure 1: Gel electrophoresis of shiga toxin 1 vt1 gene product by PCR of *E. coli O157:H7*; lane 1:100bp ladder; lane 2: NCnegative;lane 3-10: PCR positive products (130 bp) for vt1 gene; 1.5% agarose at 120 mV for 1hour.

4. Discussion:

Escherichia coli O157:H7 (STEC) is one of hundreds of *E. coli* strains. Although the most strains are harmless and live in the intestine of healthy human and animals, STEC strain produces a powerful toxin and can cause severe illness. (Vijay *et al*., 2003).

PCR is a powerful molecular biology technique for the detection of target DNA in various clinical specimens and for the detection of many kinds of pathogens. It is not only highly sensitive and specific, but it also provides rapid and reliable results for stool samples, it can help to distinguish diarrheagenic *E. coli* from those of the normal flora.

The present study showed that the risk developing of bloody diarrhea caused by *E. coli* O157:H7 was in first two years of life and the lowest rate was in children over two years of life. Patients selection was restricted to those who had bloody diarrhea because *E.coli O157* is mostly associated with this clinical feature. Results in the present study are in agreement with some previous studies by Khanjar *et al*., (2014) and it was in disagreement with findings of Trung *et al*., (2005) were they reported that *E. coli* O157:H7 was not found in any stool samples of children under two years old.

The new medium considered as selective and differential medium for the detection of *Escherichia coli* serotype O157:H7. (TC-SMAC) gave substantial suppression of non-0157 strains and also inhibited non sorbitol fermenting (NSF) bacteria such as proteus species (March and Ratnam, 1986; Baqir *et al*.,2008). Results in current study showed that all isolated on Sorbitol MacConkey Agar with Cefixime and Tellurite (SMAC-CT) gave positive results with PCR, therefor the sensitivity of the (SMAC-CT) media was estimated to be (100%) compare with PCR. These results corresponded to results mentioned by Khanjar *et al*., (2014) and Masoumeh *et al*., (2012).

Regarding latex agglutination test, results in the present study showed that *E.coli*:O157:H7 was more prevalent (56%) than *E.coli* O157: H^- (44%).This finding agreed with Pai *et al*., (1988) and disagreed with Zaid, (2000), who found that *E.coli* O157: H^- more prevalent than *E.coli*:O157:H7 and this discrepancy probably due to that *E.coli* O157: H^- was in fact is *E.coli*:O157:H7 strains but lost their flagella (Karmali *et al*., 2014).

The present finding of PCR demonstrated that among 300 children who suffering from bloody diarrhea, 37 (12.33%) were EHEC O157:H7 and this percentage was higher than those reported by Khanjar *et al.*, (2014). High incidence in the recent study may be due to the use of significant parameters and methods to detect these pathogens. This result was in accordance with Faten (2013). This result indicates that this pathogen was considered as a one of the most important causes of gastrointestinal infection in children.

In conclusion the results in the current study provided evidence that *E.coli* o157:H7 is an important cause of bloody diarrhea in pediatric population and must attract more attention about control strategies. PCR assay is very important in the identification of this causative pathogen because of high sensitivity and must be used as diagnostic test in hospital laboratories.

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