Multiplex polymerase chain reaction (PCR) assay for simultaneous detection of shiga-like toxin (stx1 and stx2), intimin (eae) and invasive plasmid antigen H (ipaH) genes in diarrheagenic Escherichia coli

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Despite the fact that diarrheagenic Escherichia coli (DEC) has been identified as a major etiologic agent of diarrhea in children worldwide, few studies have been performed in Iran to evaluate the etiology of these organisms. To evaluate the etiology of shiga toxin-producing E. coli (STEC), enteropathogenic E. coli (EPEC) and enteroinvasive E. coli (EIEC) in children with diarrhea in Iran a total 300 stool specimens from children with diarrhea were tested for the detection of E. coli. Out of 300 samples, 39 were identified as E. coli by biochemical tests and were subjected for serogrouping. The most prevalent serogroups among these isolates were serogroup IV, followed by III,I and II respectively. A single multiplex polymerase chain reaction (MPCR) was designed for the detection of target genes of stx1/stx2, eae and ipaH in DEC. The dominating strain was EPEC (55.6%), followed by STEC (25%) and EIEC (19.4%).

Key words: Multiplex polymerase chain reaction (PCR), diarrheagenic Escherichia coli, shiga-like toxin.

INTRODUCTION

Diarrheagenic Escherichia coli (DEC) is an important agent of childhood diarrhea which represents a major public health problem in developing countries and is now being recognized as emerging entero-pathogens in the developed countries (Nataro and Kaper, 1998; Soltan Dallal., 2001; Mitchell et al., 2005; Akinjogunla et al., 2009). DEC was usually transmitted through food or water contaminated with human or animal faeces. Person-to-person transmission might also take place, but is probably less common (Wood et al.1983; Harris et al.1985; Nataro et al.1998). Poor sanitation, personal hygiene and environmental conditions are some of the factors that facilitate the transmission of the disease. Thus, DEC is more prevalent in the developing countries (Galane et al., 2001; Campos et al., 2004; Kalantar et al., 2011).

Based on their virulence factors, diarrheagenic E. coli have been classified into six groups such as, enteropathogenic E. coli (EPEC), enterohemorrhagic E. coli (EHEC), enteroinvasive E. coli (EIEC), enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAEC)
Table 1. Isolated DEC types in two hospitals.

<table>
<thead>
<tr>
<th>DEC types</th>
<th>EPEC</th>
<th>EHEC</th>
<th>EIEC</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>14</td>
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<tr>
<td>Total</td>
<td>20</td>
<td>9</td>
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<td>3</td>
</tr>
</tbody>
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A, Ali Asghar pediatrics hospital; B, pediatrics department of Imam Khomeini hospital.

Figure 1. Gel electrophoresis of virulence genes of detected DEC types. Lines 1, 5 and 11, EPEC (eae gene); Lines 2, 10, 12 and 13, EHEC (Stx1/2 gene); Lines 3, 6 and 14, EIEC (ipaH gene); Lines 4 and 7, EHEC (eae and stx1/2 genes); Line 8, positive control; Line 15, negative control.

and diffusely adherent E. coli (DAEC).

Commonly isolated diarrheagenic E. coli in children are EPEC strains, which contained pathogenicity island (locus of enterocyte effacement or LEE containing eae gene). The eae gene is responsible for encoding proteins involved in the formation of attaching and effacing (A/E) lesions on host intestinal cells. EHEC or shiga toxin-producing E. coli (STEC) is the cause of hemolytic uremic syndrome, which may contain the locus of enterocyte effacement and by definition either or both of the shiga toxins (stx1 and stx2). Some of EHEC strains harbors the chromosomal gene of eae which is responsible for the encoding of the outer membrane protein intimin, same as EPEC strains. The ipaH gene in EIEC strains is similar to shigella species, which causes shigella-like dysenteric enteritis in human (Katia et al., 2007; Maricel et al., 2005; Sunabe and Honma, 1998; Stacy-Phipps et al., 1995; Rappelli et al., 2001). Multiplex polymerase chain reaction (PCR) systems have been used to reduce the number of tests needed for diagnosis of diarrheagenic E. coli (Osek, 2001; Pass et al., 2000; Paton and Paton, 2002; Rappelli et al., 2001; Ratchrachenchai et al., 1997; Rich et al., 2001). The potential of diarrheagenic E. coli to cause diarrhea in children in other developing countries have been reported previously (Mitchell et al., 2005; Maricel et al., 2005). Therefore, the aim of this study is to use multiplex PCR to simultaneously detect diarrhea-agenic E. coli such as EPEC, EHEC and EIEC in fecal samples of children less than 5 years with diarrhea.

MATERIALS AND METHODS

A total of 300 stool samples were collected from children with diarrhea in Ali Asghar pediatrics hospital and pediatrics department of Imam Khomeini hospital in Tehran, from April to Jul 2008. The samples were cultured on hekteon enteric agar (MERCK) and incubated at 37°C for 24 h.

The following schema outlines the cultivation, biochemical
RESULTS

300 stool samples were tested in which 39 were identified as E. coli by biochemical tests (Table 2). Out of 39 E. coli isolated which were subjected for serogrouping were classified as EPEC and most of them were isolated from among children under one year age. The serogroup IV was the dominant serogroup, followed by III, I and II respectively as it is shown in table 3. Out of these 39 identified, 36 were confirmed as DEC by PCR method. Out of 36 DEC, 20 (55.6%) possessed eae, 7 (19.4%) ipaH and 9 (25%) both stx1/2 and eae genes, which are designating as EPEC, EIEC and EHEC respectively. The three strains which were identified as E. coli by biochemical tests do not have eae, stx1/2 or ipaH genes. Out of 39 isolates which were identified as E. coli, 20 have eae, 9 stx1/2 and 7 ipaH genes, which are classified as EPEC, EHEC and EIEC, respectively (Figure 1).

DISCUSSION

It is widely accepted that the characteristics of several specific virulence genes are sufficient for the identification of six categories of DEC strains (Nataro and Kaper, 1998; Katia et al., 2007).

Historically, EPEC was defined as a category of E. coli belonging to certain serogroups that had been associated with outbreaks of infantile gastroenteritis. Several studies (Smith et al., 1990; Knutton et al., 1991; Scotland et al., 1991; Robins-Browne et al., 1993; Law et al., 1994; Morelli et al., 1994) have recently demonstrated that this group of organisms is actually quite heterogeneous in the possession of putative virulence properties. EPEC strains associated with outbreaks (Moyenuddin et al., 1989; Robins-Browne et al., 1993) and it is of significant value in the detection of EPEC in developing countries. The serogroup IV was the dominant serogroup. Some of these serotypes could be originally avirulent and so they have contributed to the larger number of avirulent strains.
in these serogroups. (Soltan Dallal et al., 2006; Galane and Le Roux 2001; Savulescu et al., 2007).

In this study, we used multiplex PCR to detect the presence of target genes of stx1/stx2, eae and ipaH in EPEC, EHEC and EIEC. In this study, eae gene was dominating in isolates while EPEC was the dominating strain to cause diarrhea in children. Results of a similar study in Iran (Soltan Dallal., 2001; Alikhani et al., 2006; Kalantar et al., 2011), also showed that, EPEPC is dominating DEC type causing diarrhea in children which is correlated with our results. The high percentage of stx1/2 genes (231%) was obtained in this study and the percentage of stx1/2 genes in girls was more than that of boys. In total, 3 out of 39 tested E. coli did not have eae, stx1/2 and ipaH genes, perhaps these strains belong to ETEC, EAEC or DAEC that were not included in this study. Also, 3 out of 39 isolates possessed simultaneously both eae and stx1/2 genes, this might be due to virulence factors of EHEC and other DEC strains which are mobile within bacterial populations (Agin et al., 1996; Estelle et al., 2006; Boerlin, 1999).

This study also showed that, multiplex amplification of nucleic acid can be used as a replacement for conventional method in detection of DEC strains in Iranian children and for epidemiological study of these pathogens, particularly the emerging strains such as EHEC and EIEC in Iran.

Acknowledgment

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Table 2. isolated DEC types in two hospitals. A, Ali Asghar pediatrics hospital; B, pediatrics department of Imam Khomeini hospital.

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Table 3. serological results of EPEC serogroups

<table>
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<th>percentage</th>
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<tr>
<td>100</td>
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REFERENCES


