Genotypic Detection of Enterohaemorrhagic E. coli (EHEC) among Diarrheagenic Patients in Egypt

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Abstract: Enterohaemorrhagic E. coli is recognized as important food borne pathogen, responsible for sporadic cases of serious outbreaks worldwide. The morbidity and mortality associated with several recent outbreaks due to EHEC have highlighted the threat this organism poses to public health. This study was conducted to identify and characterize the virulence traits. A total of 110 samples from human were collected from ‘Abou El-Reesh’ Children’s Hospital, Cairo, Egypt. E. coli isolates 91% (n = 100) were identified by conventional microbiology culture and were phenotypically characterized using biochemical and motility tests. Multiplex PCR reactions were applied for the detection of virulence genes (stx1 and stx2) while uniplex PCR was applied for the detection of eaeA virulence gene. Among all isolated E. coli only one was sorbitol-nonfermenting EHEC, which also harbored stx1, stx2 and eaeA virulence genes.

Key words: Enterohaemorrhagic E. coli • Shiga Toxins • PCR • Egypt

INTRODUCTION

Enterohaemorrhagic Escherichia coli is one of the five groups of E. coli which are recognized as aetiological agents of diarrhoea, [1]. It has been implicated in food borne, waterborne and airborne outbreaks all over the world [2]. For instance, the EHEC outbreak started in Germany in May 2011 with 3,368 cases including 36 deaths (European Center for Disease Prevention and Control; http://www.ecdc.europa.eu/en/Pages/home.aspx).

Shiga toxins are the major virulence factors of EHEC which have cytotoxicity effects for human and animal eukaryotic cells and cause dangerous details like HUS [3]. Two major classes of Shiga toxins have been found in EHEC, Stx1 and Stx2 [4]. Both types of toxins have similar structures and mode of action. Shiga toxins possess an AB5 structure (the holotoxin is approximately 70 kDa), composed of a pentamer of subunit B (7.7 kDa monomers) linked to a single 32 kDa A subunit [5]. In addition, the B-pentamer plays a crucial role in the binding to the target cell, while the A subunit is responsible for the enzymatic activity of the toxin, inhibition of protein synthesis [6].

The present study was aimed at investigating the prevalence of shiga-toxin producing E. coli strains from clinical cases in one of the Governarates’ ‘Cairo’ in Egypt. The virulence characteristics of isolates were also studied.

MATERIALS AND METHODS

Screening, Isolation and Identification of EHEC: Stool samples were collected from 110 patients with acute diarrhoea at early stages enteric illness before antibiotics therapy on admission to ‘Abou El-Reesh’ Children’s Hospital, Egypt. A loopful of the stool was inoculated on air dried MacConkey (Oxoid, UK) agar plates and incubated at 37°C for 24 h. E. coli-like colonies that were pink to rose red colonies were selected for further examining the morphology and biochemical properties testing of growing colonies. Gram staining was evaluated...
Table 1: Sequences and predicted lengths of PCR products of multiplex PCR assays for the simultaneous identification of Enterohemorrhagie E. coli according to Rappelli et al.[9]

<table>
<thead>
<tr>
<th>Primer</th>
<th>Oligonucleotide sequence(5’ to 3’) Target gene</th>
<th>PCR product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stx1</td>
<td>F:GAAGAGTCCGTGGGATTACG \nR: AGCGATGCAGCTATTAATAA</td>
<td>Stx1 450</td>
</tr>
<tr>
<td>Stx2</td>
<td>F*: GGGTACTGTGGCTGCTGTACTGG \nR: GCTCTGAGATGCATCTCGGT</td>
<td>Stx2 130</td>
</tr>
<tr>
<td>eaeA</td>
<td>F: TGATAAGCTGAGCTCAATCC \nR: CTGAACCAGATCGTAACGGC</td>
<td>eaeA 229</td>
</tr>
</tbody>
</table>

(*) This primer was modified from GGGTACTGTGGCTGCTGTACTGG according to Rappelli et al. [9] to be GGGTACTGTGCCTGCTGTACTGG according to the blasting with stx2 gene using the NCBI database and Bioinformatics tool following the procedure described by Merchant and Packer [7] and E. coli-like colonies were subjected to different biochemical tests, including sugar fermentation tests, indole production test, Methyl-Red and Voges-Proskauer (IMIVC) tests and Sorbitol MacConkey (SMAC) (Oxoid,UK) agar following the standard methods described by Cowan [8].

In this study, however, in addition to routine culture for bacterial biochemical identification, were used for bacterial identification, CHROM Agar we used (Oxoid, USA), a new assay with simple usage, for better confirmation the identification of Escherichia coli. Moreover, Biochemical profile was performed by API 20E (bioMérieux, France) according to the manufacturer instructions.

Genotypic Characterization of Isolated EHEC: All isolated E. coli strains were subjected to molecular screening of commonly reported virulence genes. This characterization was performed through multiplex and Uniplex PCR reactions for stxl, stx2 genes and eaeA gene respectively(Table 1). Initially, 3 pure colonies of each E. coli strain were picked from MacConkey agar plates and suspended in 200 µl v/v PCR grade (mili Q) water. The bacterial suspension was then boiled for 5 min in thermostatic water bath (D3165-Kottermann, Germany) and reconstituted directly into ice bath until use. Finally, the suspension was centrifuged at 1400 g for 5 min and the supernatant was collected and used in the PCR reactions. All premiers and PCR reactions were designed and conducted according to Rappelli et al. [9] with minor modifications. All necessary positive controls (using standard E. coli strains) and negative controls (without any DNA general, out of the 100 E. coli isolates used in the addition) were conducted within each PCR set up.

RESULTS

Screening, Isolation and Identification of E. coli Strains: Among the screened collected 110 clinical specimens, 100 (91%) were verified as E.coli bacterial isolates (Fig. A) by showing pink to rose red colonies surrounded by a narrow zone of precipitated bile on MacConkey agar. While on EMB agar, they were showing metallic green in color. Other morphological, Gram-negative E. coli isolates differ in physical appearance in that some isolates were non motile rod with no flagella while other isolates were motile rod with flagella. In Addition, purple colonies on CHROM Agar (Fig.1B) and API 20E biotype was clearly established these organisms as E. coli.

Moreover, one of them (1%) harbored EHEC (’sorbitol-negative E. coli ) out of 100 E.coli bacterial isolates in samples stool samples (Fig. 3B) by using Sorbitol MacConkey (SMAC). It was showed colourless to pale, flat and smooth, circular or serrated colonies at the edge after inoculated (SMAC) (Oxoid,UK) agar plates. However, the biochemical reactions of EHEC colonies indicate that they were similar to E. coli by using API 20E but they were differed in their reactions for ornithine decarboxylase and the fermentation of dulcitol, rhamnose, sucrose and melibiose in API-20E, thus colonies has 5144572 biochemical profile code(Fig.1A).

Genotypic Characterization of Isolated EHEC: Multiplex PCR analysis of 100 E.coli stool samples from diarrhoeal patients were detected by a single reaction of one combination of Stx1 and Stx2 primers. It gave distinct and adequate amplification of their respective targets: stx1, stx2 which showed PCR products 130 bp and 510 bp respectively (Fig.2).Moreover, Uniplex PCR analysis of primers for amplification of eaeA which showed PCR products 229 bp (Fig.2). While no products were obtained from the EHEC negative control.
Fig. 1: Photographic picture showing the biochemical reactions profiles of EHEC clinical isolate using API 20 kit system (A) and the growth of the isolated E. coli strains on CHROM Agar (B).

Fig. 2: The PCR products, which have been amplified by the sets of multiplex PCR reactions using DNA from isolated EHEC strain. Lane M is 100-bp ladder, lane 1 eaeA gene, lane 2 stx1 gene and stx2 gene and Lane 3 (negative control).

Fig. 3: The recorded prevalence (91%) of the isolated E. coli strains compared to the E. coli free samples collected from 110 clinical samples (A) and the recorded prevalence (1%) of the isolated EHEC strain out of 100 of the clinical E. coli samples (B).

The distributions of EHEC infection with various virulence traits were as follows: (100%) One patients harboured E. coli containing both stx1 and stx2 genes and were also 100% positive for Intimin gene.

DISCUSSION

The isolated bacterial isolates showed typical culture and biochemical characteristics to members belonging to genus E. coli [8]. Indeed, EHEC is associated with a broad spectrum of illnesses in humans including non-bloody and bloody diarrhoea, haemorrhagic colitis (HC) and the often deadly haemorrhagic uremic syndrome (HUS) [10]. However, some studies have suggested that there is an interesting phenomenon in developing countries in which EHEC is much less frequently isolated than other diarrheagenic E. coli as reported by Akbar, [11] in Kalar town in Iraq and other countries, as by Alikhani et al. [12], by Güney et al. [13] in Turkey, by Ghenghesh et al. [14] in Libya and by Raji et al. [15] in East Africa, This was simply confirmed in the present study where EHEC had low prevalence (1%).

Although, EHEC and enteropathogenic E.coli EPEC share eaeA (the intimin structural gene), but the major virulence factors defining the characteristics of EHEC are Stx1 and Stx2. In the present study only 1 (1%) isolates was identified to be EHEC had eae gene and both stx1 and stx2 genes.

The present study, most frequently observed of both stx1 and stx2 genes were detected together in 1 (100%) isolate out of 100 E. coli. This result was in contrast to the studying of Shiga toxin-producing Escherichia coli strains by Wieler et al. [16], stx1 genes alone in 107 strains (61%) included was the higher prevalence.
Our result was similar to the study of prevalence of enterohemorrhagic *Escherichia coli* O157 in cattle feces during slaughter by Elder *et al.* [17], 58.4% of total EHEC isolates included for both *stx1* and *stx2* genes together was higher prevalence. This difference in results subject that excretion of Shiga toxin-producing *Escherichia coli* is affected by several factors like food, age, stress and seasonal changes.

**CONCLUSION**

Although around 100 *E.coli* isolates have been collected, only one was geneotypically characterized as EHEC. This isolate harbored both genes of Stx proving potential risks, therefore, genotypic characterization is crucial to show any possible sequestered risks.

**REFERENCES**