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Prevalence of Verotoxin Gene Among the Clinical Isolates of Enteropathogenic Escherichia Coli in Malaysia

KEYWORDS: verotoxin, EPEC, PCR, diarrhea

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ABSTRACT *A total of thirty enteropathogenic Escherichia coli (EPEC) isolated from Kuala Lumpur General Hospital, Malaysia, from children with diarrhea were examined. Polymerase chain reaction used in this study to detect verotoxin (VT) gene proved to be rapid, sensitive, specific and reproducible. Two sets of primers (VT1 and VT2) were used. Both the primers produced a fragment of 348 bp of VT1 gene and 584 bp of VT2 gene, respectively. It was found that 10 E. coli isolates (33%) carried VT1 gene whereas no isolate carried VT2 gene.VT1 was found to be the most common verotoxin among the EPEC strains isolated from clinical sources in Malaysia.*

INTRODUCTION

The term enteropathogenic *Escherichia coli* (EPEC) was proposed by Nester in 1955 to differentiate between the incriminated diarrheal *Escherichia coli* (*E. coli*) and the normal flora. EPEC is a major cause of diarrhea in infants throughout the world. Clinically, EPEC infections are characterized by fever, malaise, vomiting and diarrhea with prominent amount of mucous but no blood [1]. EPEC strains cause a distinctive ultra structural histopathologic lesion in human, a property not shared by nonpathogenic *E. coli* strains and other classes of pathogenic *E. coli*.

A virulence property that has been associated with EPEC is the production of verotoxin (VT). It was reported that some EPEC strains elaborate moderate quantities of cytotoxin very similar or identical to the toxin VT1 and VT2, which might play a role in the pathogenesis of EPEC disease [2]. Many of the cytotoxigenic strains referred to as EPEC serogroups and serotyped by Marques *et al*. [2] and Cleary *et al*. [3] should be more correctly categorized as enterohemorrhagic *E. coli* (EHEC). EPEC upon producing VT1 or VT2 was named as EHEC. There are three types of *E. coli* VTs - VT1, VT2 (human strains) and VT2 variant (porcine strain). Verotoxins, also known as Shiga-like toxin was first identified in *Shigella dysenteriae*, where it is chromosomally encoded, but the genes for its production are transmitted between *E. coli* strains by toxin encoding bacteriophages [4].

PCR is a major advance in molecular diagnostics of pathogenic microorganisms, including *E. coli*. The technique was invented by Kary Mullis in 1985. PCR primers have been developed successfully for several of the categories of diarrheagenic *E. coli*. Advantages of PCR include great sensitivity in *in-situ* detection of target templates [5].

MATERIALS AND METHODS

Bacterial strains

Thirty EPEC isolates were obtained from patients (Kuala Lumpur General Hospital, Malaysia) with diarrhea. *E. coli* O157:H7 EDL933 which carries VT1 and VT2 genes (used as a positive control) and *E. coli* JM101 (used as a negative control) were kindly provided by Dr. Salmah Ismail, University of Malaya. The isolates were cultured onto brain heart infusion agar and brain heart infusion broth.

PCR

The primers used for the PCR were VT1 forward, VT1 reverse, VT2 forward and VT2 reverse. The sequences for these primers were obtained from Cebula et al., 1995 [6]. Expected amplicon size for VT1 is 348 bp, and for VT2 is 584 bp. The primers were synthesized commercially from Biosyntech Company, Malaysia. *Taq* DNA polymerase, dNTP, $MgCl_2$, 10X buffer for PCR were purchased from Promega, USA. Total genomic DNA was extracted by using Colony boiling method [7]. Ten µl of this DNA was used in the PCR. The amplification and cycling parameter were followed as cited by Cebula *et al*. [6]. Amplified products from the PCR reactions were electrophoresed on 1.5% agarose gel and then stained with ethidium bromide. A 1-kb DNA ladder (Advance Biotechnologies Ltd., UK) was run with the gel to estimate the size of the DNA fragment.

Results

PCR

All the 30 *E. coli* isolates were subjected to PCR using two sets of primer (VT1 and VT2). *E. coli* O157:H7 EDL 933 was used as positive control as it harbors both VT1 and VT2 gene. For negative control, sterile deionized water was used instead of template DNA. VT1 and VT2 gene were amplified successfully (348bp and 584bp, respectively). Ten (33%) out of 30 *E. coli* isolates were showed to carry VT1 gene but none of them carried VT 2 gene (Figure 1).

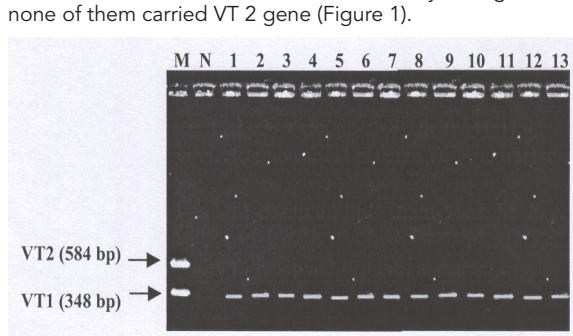

Fig. 1: The PCR results (for the VT1 and VT2 genes) obtained from *E. coli* isolates. Lane M: positive control *E. coli* O157:H7 EDL 933; lane N: negative control; lanes 1-10: *E. coli* (*E. coli* 2, 3, 4, 8, 9, 11, 12, 18, 22, 30 respectively) strains showing positive for VT1 gene and negative for VT2 gene.

DISCUSSION

The objective of this study was to detect the VT gene in EPEC using PCR protocol. Thirty EPEC isolates were examined for the detection of VT gene. The EPEC isolates were isolated from infants with diarrhea. No other clinical symptoms were

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observed from the patients. Previously, PCR assays for detection of VT used a single primer pair, which was capable of amplifying both VT1 and VT2 genes [8]. But most methods now include two primer pairs in the same reaction that yield different sized products for VT1 and VT2 [5]. Therefore, in this study two pairs of primer (VT1 and VT2) were used in polymerase chain reaction (PCR) amplification procedure to detect VT1 and VT2 genes. Primer specific amplification was not detected when the DNA template was isolated from VT-negative *E. coli*, also no false positive amplification was observed from the negative control used, indicating that the method was specific, sensitive and reliable.

Ten EPEC isolates (33%) were found to carry the VT1 gene whereas none of them carried the VT2 gene. These data suggest that VT1 is the most common verotoxin among the EPEC strains isolated from clinical sources in Malaysia. This is in agreement with Nazmul *et al*. [4] where they showed a similar finding in non-O157 *E. coli*. VT2 gene was shown to be dominant among *E. coli* O157 strains isolated from the beef in Malaysia [9]. In another study, VT1 was found to be the most common toxin among the bovine isolates and VT2 the most common in the porcine isolates [10]. Furthermore, Scotland *et al*. [11] also informed that some strains of EPEC belonging to serogroups O26, O55, O111 or O128 produce VT where they found 18 of the 122 EPEC strains to be VT positive after PCR in United Kingdom. In addition, Scotland *et al*. [12] have detected VT genes in 57 of 402 (14%) EPEC isolates using PCR method in the United Kingdom. In this study, the occurrence of only VT1 gene may be due to all the isolates have been originated from clinical isolates and/or due to the geographical distribution of the isolates. It may also be possible that the isolates may be the members of one clone that has become widely distributed.

In summary, the PCR method used here was sensitive, specific and reliable. VT1 was found to be the most common verotoxin among the EPEC strains isolated from clinical sources in Malaysia. How all the VT1-positive EPEC isolates acquired the VT1 gene has yet to be determined. However, studies are in progress to determine the carrier of these VT genes.

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