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Prevalence of verotoxin gene among the clinical isolates of non-O157 diarrheagenic *Escherichia coli in Malaysia.*

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ABSTRACT

A total of 48 non-O157 diarrheagenic *Escherichia coli* isolated from General Hospital Kuala Lumpur, Malaysia, from children with diarrhea were examined. This study proved the polymerase chain reaction used to detect verotoxin (VT) gene was sensitive, specific, rapid 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 12 *E. coli* isolates (25%) carried VT1 gene whereas no isolate carried VT2 gene.VT1 was found to be the most common verotoxin among the non-O157 diarrheagenic *Escherichia coli* strains isolated from clinical sources in Malaysia.

Keywords: *verotoxin,* non-O157 *E. coli,* PCR, diarrhea

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INTRODUCTION

Non-O157 verotoxigenic *Escherichia coli* (VTEC) is a typical *Escherichia coli.* The most common non-O157 VTEC isolated from sporadic cases of hemolytic uremic syndrome (HUS) was reported to be O26:H11 [1]. The particular *E. coli* serotypes now known to be VTEC were present in humans and animals many years ago, but have acquired the ability to produce VT and probably other virulence factors. Non-O157 *E. coli* is commonly present in food and food production animals. It is an emerging pathogen. There is an increase in problems associated with these emerging pathogens [2]. In recent years increased attention has been focused on infections caused by isolates of *E. coli* serotypes other than O157. Infections with non-O157 *E. coli* are increasingly recognized in many countries [3]. Many non-O157:H7 isolates that are associated with outbreaks do not possess either *eae* or the pO157 plasmid [4] indicating that there must be additional, as yet unknown, virulence factors that distinguish pathogenic from non-pathogenic non-O157 *E. coli* strains. The virulence profiles of most non-O157 *E. coli* are unknown [3]. Therefore, easy detection, isolation, and characterization of non-O157 *E. coli* isolates are necessary for improving our knowledge of these organisms [5, 6].

A virulence property that has been found to be associated with non-O157 *E. coli* is the production of verotoxin (VT). 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 [7, 8].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 [9]. PCR method was used by many researchers to detect the VT gene. In our previous study we managed to detect VT gene from enteropathogenic *E. coli* strains which made us interested to detect the VT gene from non-O157 diarrheagenic *E. coli* using the similar protocol.

MATERIALS AND METHODS

Bacterial strains

Forty eight confirmed non-O157 diarrheagenic *E. coli* isolates were obtained from children (General Hospital Kuala Lumpur, 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 [10]. 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*. [11]. 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 48 *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). Twelve (25%) out of 48*E. coli* isolates were showed to carry VT1 gene but none of them carried VT 2 gene (Figure 1).

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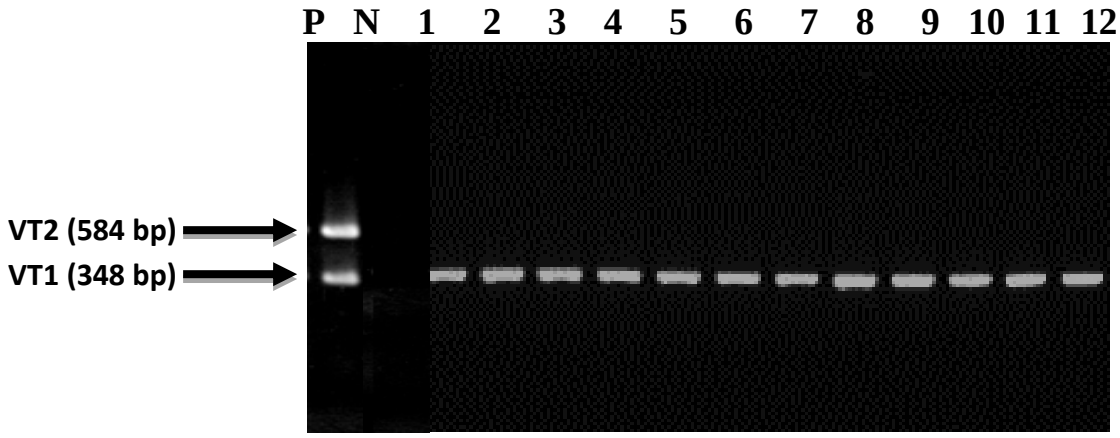

Figure 1: The PCR results (for the VT1 and VT2 genes) obtained from *E. coli* isolates. Lane P: positive control *E. coli* O157:H7 EDL 933; lane N: negative control; lanes 1-12: *E. coli* (*E. coli*2, 3, 6, 7, 9, 11, 15, 20, 32, 38, 45 and 47respectively) strains showing positive for VT1 gene and negative for VT2 gene.

DISCUSSION

The objective of this study was to detect the VT gene in non-O157 *E. coli* using PCR protocol. Forty eight non-O157 *E. coli* isolates were examined for the detection of VT gene. The non-O157 *E. coli* isolates were isolated from patients with diarrhea. No other clinical symptoms were 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 [12]. But most methods now include two primer pairs in the same reaction that yield different sized products for VT1 and VT2 [13]. 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.

Twelve non-O157 *E. coli* isolates (25%) 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 non-O157 *E. coli* strains isolated from clinical sources in Malaysia. This is in agreement with Nazmul *et al.* [9] 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 [14].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 [15]. Furthermore, Scotland *et al*. [16] also informed that some strains of *E. coli* belonging to serogroups O26, O55, O111 or O128 produce VT where they found 18 of the 122 *E. coli* strains to be VT positive after PCR in United Kingdom. In addition, Scotland *et al*. [17] have detected VT genes in 57 of 402 (14%) *E. coli* 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 non-O157 *E. coli* strains isolated from clinical sources in Malaysia. How all the VT1-positive non-O157 *E. coli* 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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