Malaysian Journal of Science 24: 86 (2005)

Detection and molecular characterization of verotoxin in enteropathogenic *Escherichia coli* and non-O157 diarrheagenic *Escherichia coli*

Nazmul M.H.M

Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

A total of 50 *E. coli* isolates were examined. Thirty three isolates were EPEC, isolated from Miri hospital (2001), Malaysia, from children with diarrhea. Remaining 17 isolates were non-O157 diarrheagenic *E. coli*, isolated from GHKL (1998), Malaysia, from patients with diarrhea. All these isolates were examined for the detection of verotoxin (VT) and were further characterized.

Plasmids were detected in 34 isolates with the POR of 68%. These 34 isolates appear to harbor 1 or more plasmids with the maximum of 4 plasmids. The overall sizes of the plasmid DNA range from the lowest 1.8 kb to the highest 96 kb. For polymerase chain reaction, two sets of primers (VT1 and VT2) were used. Both the primers produce a fragment of 348 bp of VT1 gene and 584 bp of VT2 gene respectively. Twenty *E. coli* isolates (40%) carried VT1 gene whereas none of them carried VT2 gene. Among them,16 isolates are EPEC and remaining 4 isolates are non-O157 *E. coli*.

The amplified PCR product of VT1 gene from EDL933 *E. coli* was used as DNA probe and was labeled by Horse Radish Peroxidase for southern hybridization study. After hybridization, VT1 probe showed homology with the chromosome of 18 isolates. In all these 18 isolates VT1 gene is most probably located on chromosome. Among them 16 were EPEC isolates and 2 were non-O157 *E. coli* isolates. In another two isolates (*E. coli* 129 and *E. coli* 141), the VT1 probe showed homology with the 7.2 kb plasmids, indicating the VT1 gene to be located on the 7.2 kb plasmid. Both the isolates are non-O157 *E. coli*.

Bacteriophage induction from all the VT1 positive *E. coli* isolates showed that all the VT1 positive isolates did not harbor any lysogenic bacteriophage. PCR was carried out using the 7.2 kb plasmids of non-O157 *E. coli* 129 and *E. coli* 141 as template DNA to reconfirm the hybridization results. A fragment size of 348 bp of VT1 gene was successfully amplified which confirms that in both the isolates the VT1 gene is located on 7.2 kb plasmid. None of the other *E. coli* isolates which also carries 7.2 kb plasmid showed VT1 gene amplification after PCR. However, nucleotide sequencing made from the VT1 gene isolated from 7.2 kb plasmid of *E. coli* 129 showed 97% homology with the known VT1 gene bank which proves that to be the similar gene carried by *E. coli* O157 and other ancestors.

In conclusion, we believe to report for the first time that VT1 gene to be located on a 7.2 kb plasmid of non-O157 *E. coli,* and there is no lysogenic phage present in them, which provides a new basis for strategies to prevent EPEC and non-O157 *E. coli* infection.