Detection of *E. coli* non-O157 carrying verotoxin 1 (VT1) gene from infant diarrhoea patients.

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ABSTRACT

A total of 138 isolates of *E. coli*, obtained from infant diarrhoea patients were provided by General Hospital Kuala Lumpur (GHKL). Confirmation of the *E. coli* strains were done using biochemical tests and api 20E standard test kit. A non-radioactive biotin labeled VT1 probe consisting of the 0.75kb *HincII* fragment from a recombinant plasmid of NTP 705 was used in the hybridization study. 3 clinically isolated strains showed homology with the VT1 probe. With further analysis, these strains were confirmed to be non-O157 *E. coli* strains.

INTRODUCTION

Between 1950 its role as an enteric pathogen was highlighted (Sussman, 1985) and thus, *E. coli* is no longer viewed as a mere opportunist, but special strains that can be considered as a primary pathogen possessing an array of virulence traits that allow the organism to evade host defenses and cause overt disease. The isolation of *E. coli* from feces is now a fairly simple procedure which can be done by most laboratories, using selective media (eg. Eosin Methylene Blue agar, Mac Conkey agar, Bismuth sulphite agar etc.) that reduces the growth of competing microorganisms. Strains of *E. coli* that produce a potent cytoxin active against cultured Vero cells are recognized as important pathogens of man (Konowalchuk et al., 1977). These verotoxigenic (VTEC) strains have been associated with hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) which causes high morbidity and mortality. Neutralization studies indicate that there is more than one verotoxin (VT) (Konowalchuk et al., 1977; Smith et. al., 1983). Specific probes have been developed for use in DNA hybridization tests (Willshaw et. al., 1985). The usefulness of diagnostic DNA probes which consist of virulence genes made hybridization method very useful to detect bacterial species that have similar genes and pathogenic characteristics. Several groups have now cloned the genes specifying VT1 and VT2 (Willshaw et. al., 1985, 1987; Huang et. al., 1986; Kurazono et. al., 1987), and the cloned genes have been used in colony hybridization tests for detecting VT1 and VT2 producing *E. coli*. The technology that uses non-radioactive biotin labeled DNA probes provides a DNA hybridization assay with a high level of specificity and sensitivity. The probe method was significantly more sensitive than the scharbol-MacConkey agar for detecting verotoxigenic *E. coli* (Willshaw et. al., 1985, 1987). Thus, this study was conducted with the objective of determining the pathogenesis on a molecular basis of verotoxin *E. coli* (VTEC) infection in infant diarrhoea in Malaysia.

MATERIAL & METHODS

Bacterial strains

The *E. coli* strains used in this study were obtained from General Hospital Kuala Lumpur (GHKL). *E. coli* ATCC 25922 was used as positive control in all biochemical tests and recombinant clone of NTP 705 was used in the VT1 probe preparation. All *E. coli* strains were subjected to a series of biochemical tests inclusive of citrate utilization, gas production, H2S, indole, nitrate, urease, MRVP, oxidation-fermentation tests, and confirmed with the api 20E standard test kit. All tests were repeated 3 times to ensure the correct results accuracy.

Preparation of cultures
Pure culture strains were stored in 25% sterile glycerol at -70°C. Bacterial cultures were also maintained on nutrient agar slants for 3 to 5 months at 4°C. The cultures were reconstituted by plating onto Brain Heart Infusion (BHI) agar medium and incubated at 37°C for 18-24 hours before use.

**DNA extraction & purification**

Recombinant clone, NTP 705 was grown overnight in Luria Bertani (LB) broth at 37°C with agitation at 180 rpm. Plasmid DNA was extracted using the modified method of Close & Rodriguez, 1982. The plasmid DNA was resuspended in Tris-EDTA (TE) buffer. RNase A was added to the plasmids to a final concentration of 20 mg/ml, and incubated at 37°C for an hour, after which it was purified using phenol-chloroform extraction procedure.

**Preparation of VTI probe**

Plasmid NTP 705 was digested to completion with HincII and electrophoresed at a constant voltage of 12 volts/cm in 1.2% agarose gel in 1X TBE buffer pH 8.0. Fragment of 0.75kb was eluted and purified using Prep-A-Gene System (Bio Rad, USA) and labeled with BioNick Labeling System (Gibco BRL, Life Technologies). The non-radioactive biotinylated probe was stored at -20°C in TE buffer.

**Colonial blot & DNA hybridization**

The presence or absence of a nucleotide sequence homologous to that of VTI gene was examined by the DNA colony hybridization method. The BluGene Nonradioactive Nucleic Acid Detection System was used to detect homologous nucleic acid sequences.

**Analysis of β-glucuronidase activity**

The β-glucuronidase activity was measured in 4-methylumbelliferyl-β-glucuronidase (MUG) agar plate (Dahlen & Linde, 1973). *E. coli* strains were inoculated onto MUG agar and incubated 18-24 hours at 37°C.

**Latex agglutination test**

*E. coli* strains were tested for serotype O157 using Microscreen *E. coli* O157 Test Latex (Microgen, USA). If agglutination occurs in 30 seconds after the mixing of bacterial suspension and the reagents provided, shows the presence of *E. coli* O157.

**Results & Discussion**

The 138 *E. coli* clinical isolates obtained were subjected to a series of basic biochemical tests to confirm the samples were *E. coli* and not any other bacterial strains. *E. coli* ATCC 25922 was chosen as the *E. coli* positive control strain in all biochemical tests.

Colonial blot hybridization was carried out on all 138 *E. coli* strains with recombinant clone NTP 705 was used as VTI positive control. All strains and controls were subjected to the non-radioactive method of colony blot DNA hybridization and detection using biotin labeled probe of NTP 705 to look for isolates carrying the same VTI gene. Only 3 strains hybridized strongly with the VTI probe, showing homology of the nucleic acid sequences with the gene probe. Further analysis of these isolates has been performed in order to confirm whether these strains belong to serotype O157 or other VTEC groups.

Fluorographic procedures with MUG substrate is a common method for *E. coli* identification from human specimen (Kilian & Bulow, 1976; Feng & Hartman, 1982) because 96% of *E. coli* produce β-glucuronidase enzyme (Kilian & Bulow, 1976; Feng & Hartman, 1982) which cleaves the MUG substrate methylumbelliferone and this product will light up under UV light (Doyle & Schoeni, 1984; Ratnam et al., 1988). In this study, the 3 strains were MUG-positive, thus confirmed to be non-O157 because strains of serogroup O157 will give a consistent MUG-negative reaction result (Kilian & Bulow, 1976; Feng & Hartman, 1982). Another specific test was carried out to confirm the status of these 3 strains. As many scientists elsewhere are using latex agglutination test as a presumptive *E. coli* O157:H7 identification, this was the next test done in order to confirm the finding. Moreover, Thompson et al. (Thompson et al., 1990) suggested that latex agglutination test should be used in conjunction with the MUG test for the rapid and accurate detection of *E. coli* serotype O157:H7 strains. Therefore, Microscreen *E. coli* O157 agglutination test was used next as a mean of confirmation of *E. coli* O157. The 3 strains did not show any agglutination with the latex particles and is therefore, confirmed to be non-O157 which produces verotoxin 1 (VT1).
REFERENCES


