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MANTHANA PHENGMAK : PCR DETECTION OF SHIGA TOXIN AND ENTEROTOXIN GENES IN *Escherichia coli* ASSOCIATED WITH DIARRHOEA. THESIS ADVISOR : AMORNRUT LEELAPORN, Ph.D., PODJANEE KOMOLPIS, M.D., BOONCHUAY EAMPOKALAP, M.Sc. 124 p. ISBN 974-661-838-5

Enterovirulent *Escherichia coli* (*E. coli*) is among the common bacterial pathogens capable of causing diarrhoeal disease. In this study, the optimized conditions for PCR technique were developed to detect two groups of enterovirulent *E. coli*: Shiga toxin-producing *E. coli* (STEC) and enterotoxigenic *E. coli* (ETEC). Both STEC and ETEC were determined in a total of 211 diarrhoeal patients who attended at Bamrasnaradura Infectious Diseases Hospital (BIDH), Nonthaburi, Thailand. Patients included 58 non-bloody diarrhoeal children (group A), 61 non-bloody diarrhoeal adults (group B), 37 bloody-diarrhoeal children (group C), and 55 bloody-diarrhoeal adults (group D). One hundred and three normal control subjects (52 children in group E and 51 adults in group H) were also included. Furthermore, other bacterial enteric pathogens were isolated and identified by culture method routinely used in laboratory. The use of three pairs of primers with optimized conditions for detection of STEC, heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST)-producing *E. coli* (ETEC) simultaneously in a single reaction and multiplex PCR using 3 primers for detection and differentiation of *stx* genes including, *stx1* and *stx2/stx2v* was established. Results obtained by PCR and culture method revealed that 181 isolates of bacterial pathogens were found in 112 (53%) from a total of 211 diarrhoeal patients. STEC and ETEC were detected in 2 and 14 cases, respectively. STEC isolates were detected in patients in group A and group D. Patient in the latter group carried 2 strains of STEC one of which harboured *stx1* gene while the other contained both *stx1* and *stx2* genes. ETEC was detected in children (11 cases) more than in adult patients (3 cases) with statistically significant difference ($p < 0.05$). In control group, enteric bacteria were identified from group E (2 cases) only; one case containing Stx1 & Stx2v-producing *E. coli* and *Aeromonas hydrophila* and the other one carrying LT-ST ETEC. However, direct detection of toxin genes from faecal specimens yielded unsatisfactory results (23.5% from faecal culture positive for STEC and ETEC). In addition, the use of sorbitol MacConkey agar followed by identification of *E. coli* O157:H7 seems to be an inappropriate method for detecting STEC in routine diagnostic laboratory in Thailand. Furthermore, other enteric pathogens detected in this study included *Vibrio* spp. (30%), *Shigella* spp. (20%), *Plesiomonas shigelloides* (18%), *Aeromonas* spp. (13%), *Salmonella* spp. (6%) and *Campylobacter* spp. (1%). These data indicate the major contribution of enterovirulent *E. coli* to diarrhoeal illness and that PCR is a suitable technique for detection of these organisms.