Biochem. Cell. Arch. Vol. 18, No. 1, pp. 31-35, 2018 | www.connectjournals.com/bca | *ISSN 0972-5075*

MULTIPLEX PCR RAPID AND SENSITIVE SCREENING METHOD FOR DETECTION OF LOCAL STRAINS OF *ESCHERICHIA COLI* O157 : H7 IN HILLA CITY

Zainab H. Alsaadi[1], Adnan H. Tarish[2] and Eiman A. Saeed[1]

[1]Department of Microbiology, College of Medicine, Babylon University, Babylon, Iraq.
[2]Pediatric Branch, College of Medicine, Babylon University, Babylon, Iraq.

(Accepted 12 February 2018)

ABSTRACT : This study was performedto investigate molecular characterization of Shiga toxin-producing *E. coli* associated with acute diarrhea (bloody , watery or both) that collected from Children under 5 years old in Babylon province.For the detection of the pathogenic *E. coli* O157:H7 virulence genes (*stx1, stx2, eaeA, hlyA*) in a multiplex PCR protocol using fourspecific primer pairs. The target genes produced species-specific amplicons at (614bp), (779bp), (890bp) and (165bp), respectively. A total of 500 stool samples were collected, cultured on theSorbitol MacConkey agar and *E. coli* 0157: H7 Chromogenic Agar Base with cefixime tellurite supplement. Out of the 500 samples, 223 (44.6%) were non-sorbitol fermenting (NSF). *E. coli* isolates were serotyped as *E. coli* O157:H7 11(2.2%), byserological detection of O157 somatic antigen by using slide agglutination of heat-treated organisms and Vitek2 system. All latex agglutination positive isolates were positive to mPCR test. The results obtained show that the established PCR protocol is suitable for a rapid and specific analysis of the pathogenic *E. coli* O157:H7 in clinical samples for the assessment of microbiological risks.

Key words : *Escherichia coli* O157:H7, Shiga-like toxins, Multiplex PCR.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) strains represent one of the most important groups of food-borne pathogens that can cause several human diseases such as hemorrhagic colitis (HC) and hemolytic – uremic syndrome (HUS) worldwide (Januszkiewicz and Rastawicki, 2016). Hemorrhagic colitis occasionally progresses to hemolytic uremic syndrome (HUS), an important cause of acute renal failure in children and morbidity and mortality in adults (Adams *et al*, 2016). Enterohemorrhagic *E. coli* O157:H7 (EHEC O157:H7) has been known to cause these syndromes since the 1980s, but clinical cases and outbreaks caused by members of other EHEC serogroups are increasingly recognized (Centre for Food Security and Public Health, 2016). The ability of STEC strains to cause disease is associated with the presence of wide range of virulence factors including those encoding Shiga toxin (Mayer *et al*, 2012). The ability of *E. coli* O157:H7 to cause severediseases in humans is related to their capacity to secreteshiga toxins (Stx1 and Stx2) and variants of these toxins, which associated with hemolytic uremic syndrome (HUS). Stx is essential in the pathogenesis of HUS, which has been mostly related to Stx2-producing isolates (Guirro *et al*, 2014). Another virulence associated factors of most STEC isolates associated with severe disease are intimin and haemolysin (Karger and Homayoon, 2015).

MATERIALS AND METHODS

Sampling

A total of 500 stool samples were collected from children under 5 years old with diarrhea from both sexes, who attended to Babylon Maternity and Pediatrics Hospital in Hilla city, 208 females and 292 males.

Stool samples were collected from children, who hospitalized with acute diarrhea (bloody, watery or both) and persistent diarrhea symptoms during the period from July 2016 to February 2017. A detailed information of the patients were obtained, including age, sex, source of drinking water, type of feeding and antibiotics usage.

Isolation of *E. coli* O157:H7

The fecal swabs were collected by using sterile disposable wooden swabs, they were inoculated into MacConkey agar and incubated aerobically at 37ºC for 24hrs. Rose pink coloured colonies with typical appearance of *E. coli* were re-streaked on Eosin Methylene Blue agar, then incubated for 24hrs. at 37°C. The green metallic sheen colonies were considered as *E. coli*.