Mustansiriyah Journal of Pure and Applied Sciences, Print ISSN: 2957-9910. Online ISSN: 2957-9929, Vol. 3, No. 3, 2025, Pages 135-140
DOI: 10.47831/mjpas.v3i3.252

MJPAS

MUSTANSIRIYAH JOURNAL OF PURE AND APPLIED SCIENCES

Journal homepage*:*
https://mjpas.uomustansiriyah.edu.iq/index.php/mjpas

RESEARCH ARTICLE - BIOLOGY

Prevalence of Enteroinvasive *E. coli* and Enterotoxiogenic *E. coli* Among Children with Severe Diarrhea in Al-najaf Al-ashraf

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Article Info.

Article history:

Received
23 June 2024

Accepted
5 Augest 2024

Publishing
30 June 2025

Abstract

Diarrhea is the second most important disease that cause death in children under 5 years old. This research aimed to investigate the prevalence of Enteroinvasive *E. coli* (EIEC) and Enterotoxogenic *E. coli* (ETEC) associated with diarrhea among children less than 5 years. One hundred stool samples have been collected from children under 5 years old whom suffering from acute diarrhea. All samples are cultured on MacConkey agar and Eosin Methylene Blue (EMB) for detection of Diarrheagenic *E. coli* (DEE). DNA extraction was carried out and PCR technique. was used for amplification of *stp* gene for detection of ETEC and *ipaH* gene for detection of EIEC. The results showed wide distribution of DEC (45%) among male and female where a high percentage of infection occurred among male (60%) in Compression with female (40%). The results of Agarose gel electrophoresis observed that 33% of isolates were possess *ipaH* which revealed that these isolates were belong to EIEC while 22% of isolates were possess *stp* gene which revealed that these isolates were belong to EHEC. In conclusion, diarrhea may associate with EIEC and EHEC in addition to EPEC.

The official journal published by the College of Education at Mustansiriya University

Keywords: Enterotoxogenic *E. coli* (ETEC); Enteroinvasive *E. coli* (EIEC); Children with Acute Diarrhea; polymerase chain reaction PCR.

1. Introduction

Diarrhea is the second most important disease that cause death among children under 5 years old where the reports of WHO confirmed that this disease affect 1.7 million person yearly and annually cause death to 525,000 children under 5 years old [1].

Diarrheagenic E. coli represented one of the most causes of diarrhea which involved many Gram-negative serotypes that belong to the enteric bacteria that cause diarrhea which include: Enteropathogenic E. coli (EPEC), Enterohemorrhagic E. coli (EHEC), Enteroinvasive E. coli (EIEC), Enteroaggregative E. coli (EAEC), and Shiga like toxin E. coli (STEC). DEC are classified depending on somatic (O), flagellar (H) and capsular (K) antigens [2].

EIEC is similar to Shigella dysentery that cause dysentery due to its ability to adhesion, penetration, and invasion of the lining of large intestine Causing its death which resulted acute diarrhea [3]. It is non-motile, non-lactose fermenter causing colon and acute diarrhea infection. Its ability to invasion depends on presence of plasmid with molecular weight 140 Mega Dalton coding for outer membrane protein (OMP), where E. coli 0124 represented a wide distribution serotype [4]. Most ETEC that produce heat stable entero- toxin (8T) occupy 8th among enteric pathogens that cause diarrhea leading to the death. Also, repeated diarrhea led to long-term consequence such as male nutrition, stop growth and chronic enteritis [5]. ETEC when attach to the epithelium release either LT (heat libeled toxin) or ST that effect on enteric cell by disruption of homogenous electrolyte balance leading to fluid loss and secretory diarrhea [6].

EHEC cause Hemolytic- Uremic syndrome due its ability to produce cytotoxins so it's called STEC which have the ability to produce or inhibit the protein inside target cells as the symptoms of disease are progress [7]. EHEC and EPEC acts in the same pattern in their ability to target mucous membrane of intestine in a mechanism called Attaching and Effacing. ipaH gene (code for invasion plasmid antigen H which located in multiple copies on chromosome or plasmid) play an important role in invasion

of epithelial layer lining the intestine. Such plasmid with molecular weigh 60 KD is widely distributed among EIEC and are code and possess ipaH gene a specific gene that used in diagnosis of EIEC from different sources of infection [8].

Due to the wide distribution of diarrhea resulted from bacterial infection, this research aimed to investigate the prevalence of EIEC and EHEC among children under 5 years old with a cute diarrhea.

Nomenclature & Symbols

EHEC	Enterohemorrhagic *E. coli*	LT	Labile toxin
EIEC	Enteroinvasive *E. coli*	OMP	Outer Membrane Protein
EMB	Eosin methylene blue	PCR	Polymerase chain reaction
IpaH	Invasion plasmid antigen-H	Stp	Heat Stable toxin
		SL	Stable toxin

2. Methods

2.1. Specimens Collection

One hundred stool samples have been collected from children under 5 years old with acute diarrhea whom admitted to the AL Zahra Teaching Hospital and Middle Euphrates Hospital during the period from 1-October-2020 to 15- November – 2020.

2.2. Bacterial Isolation

All samples were cultural directly on MacConkey agar and Eosin Methylene Blue (EMB) for primary isolation and Identification of DEC. Further identification was carried out using biochemical tests as described previously [9], 10].

2.3. DNA Extraction

Alkaline lysis method that described previously [11] has been followed up for extraction of genomic DNA from 30 randomly selected bacterial isolates. The purity and concentration of extracted DNA have been measured using DNA - RNA spectrophotometer (Bio droop, England).

2.4. PCR techniques

Amplification of *stp* and *ipaH* genes for genotypic detection of EHEC and EIEC respectively was carried out using a set of primers as illustrated in Table 1. The content of PCR mixture is prepared with:

1. A final volume 20µl by mixing 5µl of master mix (INTRON, Korea).
2. 1.5µl of each F and R primers (Final concentration of each one 10 Pmol µ1-1) as illustrated in Table 2.
3. 8µl of nuclease free water, and 4µl of DNA template.

Table 1. Components of the reaction mixture

Mix Components	Volume\20 Ml	Manufacturer(origin)
(dGTP,dATP,dCTP,dTTP,dNTP)	Each 250 Mm	
Taq DNA Polymerase	1 U Paragraph	INRON(Korea)
Reaction Buffer with $Mgcl_2$ (1.5Mm)	1 x	
Tracking dye and Stabilizer	—	

Then The mixture was applen centrifuge for short spin, and then transferred to the thermo cycler. The amplification process includes 3 stages:

1. Primary denaturation at 95°C5min^{-1} followed by 30 cycles of denaturation at 95°C30sec^{-1}.
2. Annealing at 51°C1min^{-1} for *stp* and 50°C1min^{-1} for *ipaH*.
3. Elongation at 72°C45sec^{-1}.
4. With a final extension at 72°C5min^{-1}.

Table 2. The sequences of synthesized oligonucleotide (INTRON, Korea)

Genes	Sequences 5'⟶ 3'	Molecular weight of amplicon (bp)	References
ipaH	F-GCTGGAAAAACTCAGTGCCT-R-CCAGTCCGTAAATTCATTCT-	474	[12]
Stp	F-TCTTTCCCCTCTTTTAGTCAG-R-ACAGGCAGGATTACAACAAAG	166	[13]

2.5. Electrophoresis technique

All amplicons were electrophoresed using a stained 1% Agarose gel, documentation system was used to visualize the gel (Biometra, USA).

3. Results and Discussion

The results of isolation and identification of DEC depending on microscopic and biochemical tests showed that 45 (45%) isolates were belong to DEC. A high percentage of infection with DEC occurred in male (60%) in comparison with female (40%) as shown in Fig. 1.

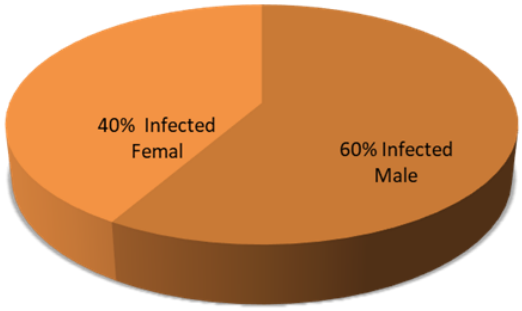

Fig. 1. The percentage of DEC among children with diarrhea of both sexes.

Thirty isolates have been selected for further genotypic detection of EIEC and EHEC. The results of Agarose gel electrophoresis of stp amplicon showed that 10 (3.3%) isolates were possess the gene by appearance of amplicon with molecular weight 166bp as shown in Fig. 2, while the results of electrophoresis of ipaH amplicon showed that only 15 (50%) isolates gave positive results for amplification by appearance of amplicon with molecular weight 474bp as displayed in Fig. 3.

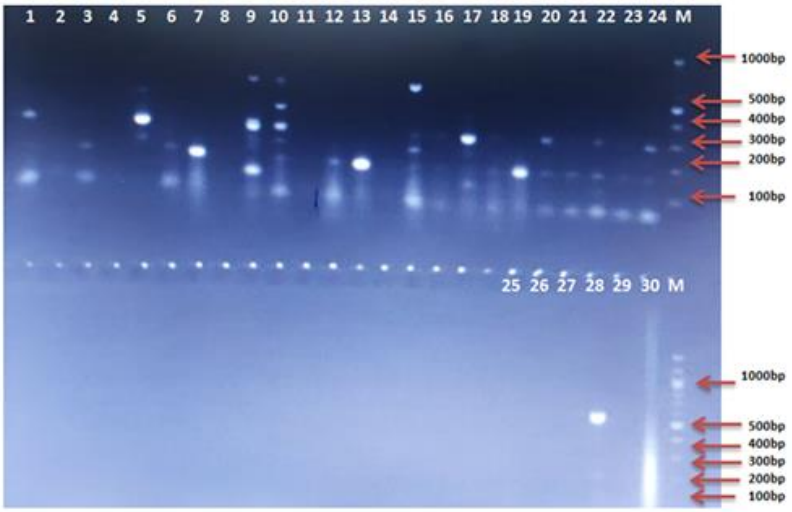

Fig. 2. Agarose gel electrophoresis of stp amplicon (166bp). Line M: DNA ladder (100bp). Line 9, 10, 12, 13, 15-24, 28: positive results for amplification. Line 1-8, 11, 14, 25, 26, 27, 29, and 30: negative results for amplification.

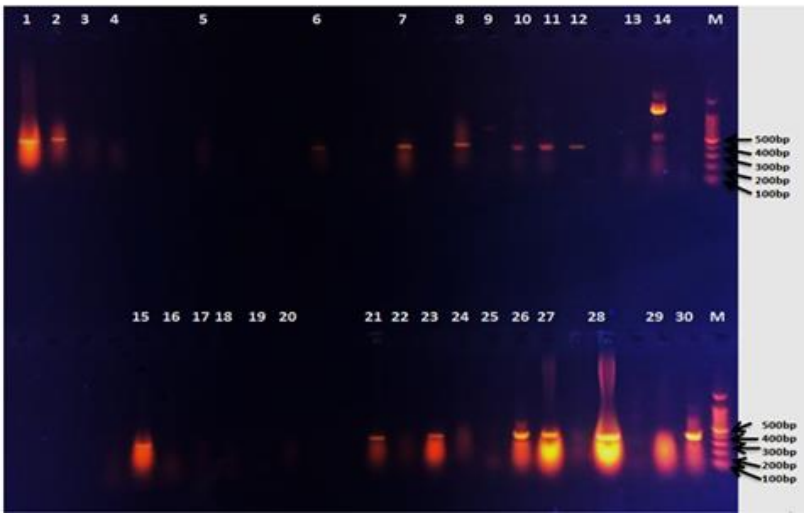

Fig 3. Agarose gel electrophoresis of ipah amplicon (474bp). Line M: DNA ladder (100bp). Line 1, 2, 6-12, 14, 15, 21, 23, 26, 27, 28, and 30: positive results for amplification. Line 3, 4, 5, 6, 13, 16, 17, 18, 19, 20, 22, 24, 25, and 29: negative results for amplification.

Diarrhea is the second disease that cause death among children worldwide where different group and serotypes of DEC were associated with the spreading of disease [14]. *stp* represented one of the most important virulence factors coding for heat stable toxin that inhibit sodium absorption and stimulate secretion of chloride which led to fluid diarrhea [15]. Many studies improved that strains which possess *stp* gene were belong to ETEC [16] The present study improved a wide distribution of isolates that possess *stp* gene which lead to moderate and acute diarrhea among children [17].

On the other hand, the present research showed highly prevalence of *ipaH* which is one gene of EIEC and it is located on plasmid or chromosome [18]. Many studies improved wide distribution of *ipaH* among EIEC [2]. Also, some studies reported that isolates which possess *ipaH* were belong to either *Shigella* or EIEC while isolates lack that gene were belong to other groups of DEC [19]. Most EIEC were associated with moderate and acute diarrhea among children under 5 years old [20].

4. Conclusion

Wide distribution of acute diarrhea associated with gastroenteritis among Iraqi children with high prevalence of ETEC and EIEC which responsible for moderate and acute diarrhea among children under 5 years old.

Acknowledgement

This is a text of acknowledgement. Do not forget the people who have assisted you with your work. Do not exaggerate with thanks. If your work has been paid by a Grant, mention the Grant name and number here.

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