

Determination of Adhesion Virulence Factors of Enteropathogenic *Escherichia coli* (*eaeA*⁻, *bfpA*⁻) Isolates from Asymptomatic Individuals Compared to those with Diarrhea

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Abstract

Objective: The aim of this study was to investigate the prevalence of *toxB*, *paa*, *lpf* and *iha* adhesion genes in enteropathogenic *Escherichia coli* (EPEC) isolates lacking in two important adhesion factors, the *eaeA* and *bfpA* genes.

Methods: We examined a total of 70 serologically confirmed EPEC (*eaeA*⁻, *bfpA*⁻) isolates. DNA from the isolates was extracted by the phenol-chloroform method. *toxB*, *paa*, *lpf* and *iha* adhesion genes in the EPEC isolates were detected by polymerase chain reaction. Data were analyzed by SPSS software and statistical analysis using the chi square test. P-values less than 0.05 were considered significant.

Results: PCR was positive for the *toxB* gene in 2 (2.85%), *paa* in 3 (4.28%), *lpf* in 32 (45.71%) and *iha* in 15 (21.42%) of the 70 strains. Statistically, none of the *toxB*, *paa*, and *lpf* genes were associated with diarrhea. However, the *iha* gene showed a weak significant relation to diarrhea (P=0.11).

Conclusion: The main mechanism of pathogenicity for EPEC is attachment and effacement. Therefore, EPEC (*eaeA*⁻, *bfpA*⁻) should have another adhesin factor, which should be investigated. EPEC strains (*eaeA*⁻, *bfpA*⁻) that possess the *lpf* gene are common. Further investigations of the virulence properties of these strains are necessary in order to elucidate the role of these virulence factors in diarrhea among Iranian children.

Keywords: Enteropathogenic *Escherichia coli*, Adhesion Virulence Factors, Diarrhea, Serology

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[7] (EHEC pO157 plasmid-encoded protein) ToxB
pO157
(IrgA homologue adhesion) Iha
.[8] IrgA

aeA EPEC 70
36 34 *bfpA*

1381-1390

)
[9] 24
.[10]

Eosin Methylene) (MacConkey Agar)
(Blue
IMViC
ODC (Lysine Decarboxylase) LDC
(Ornithine Decarboxylase)
API 20 E
EPEC

[13] *stx* .[12 11] (Porcine A/E-associated adhesin) Paa [6]

EPEC
) *ae* .
(Gene Encoding Intimin
Locus)
(of Enterocyte Effacement Pathogenicity Island
Bundle Forming)] *bfp*
EPEC Adherence) EAF [(Pilus: BFP
EPEC (Factor
(Atypical) (Typical)
ae EPEC .
EPEC *bfpA*
.[2 1] *ae*
EPEC
(Attaching and Effacing) -
BFP .[3]
.[5 4]
EPEC
bfp ae

PCR [15] *bfpA* [14] *eaeA*

620 DNA EPEC
 (1:24:25) - -
 8000×g 10

10
 8000×g -80
 -20 TE- RNase DNA
 [16]

PCR

DNA

25 PCR DNA
 BHI
 (Merck) (Heart Infusion Broth Brain)
 Shaker) 24
 (Incubator
 Genet) 10x 2/5 (Depleted Water: DDW 12000×g 5
 Genet) 25 MgCl₂ 1/5 (Bio
 Bioneer) 10 dNTPs 0/5 (Bio
 (Primers) 0/5 (EDTA] 600
 () 10 Tris- 0/5 (Ethylenediaminetetraacetic Acid)
 5 *Taq* DNA polymerase 0/2 25 SDS 26 [1 NaCl 1 HCl
 DNA 1 (Genet Bio) K 3 (Sodium Dododecyl Sulfate)
 60

PCR 1

		()					
6	EH41	798	1: 50:	94 48	AGCAGGCATAACGCAAG	GAACTGTAGATGGGTAC	<i>lpf</i>
			1: 30:	72 94			
7	EDL933	605	45: 30:	60 72	CTCGAGAGTGCCTTTCCTGG	GGATCCATGAGGAACATAA	<i>paa</i>
			1: 1/5:	94 72			
8	EDL933	602	1: 1/5:	94 72	TTCTTACCTGATCTGATGCAGC	ATACCTACCTGCTCTGGATTGA	<i>toxB</i>
			30: 1:	94 56			
8	EDL933	1305	1: 1/5:	56 72	GTATGGCTCTGATGCGATG	CAGTTCAGTTTCGCATTCACC	<i>iha</i>

