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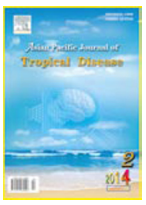

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Molecular study on diarrheagenic *Escherichia coli* pathotypes isolated from under 5 years old children in southeast of Iran

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ABSTRACT

Objective: To determine the phylogenetic groups and prevalence of diarrheagenic *Escherichia coli* (*E. coli*) (DEC) genes from children less than five years of age with diarrhea in southeast of Iran.

Methods: A total of 142 *E. coli* isolates were isolated from diarrheic samples. The isolates were examined for detection of virulence determinants and their phylogenetic background by PCR technique.

Results: The *E. coli* isolates fall into four phylogenetic groups: A (40.14%), B1 (18.31%), B2 (16.90%) and D (24.65%). Eighty isolates were positive for at least one of the examined DEC genes. *E. coli* isolates were classified in enterotoxigenic *E. coli* (52 isolates), enteroaggregative *E. coli* (23), atypical enteropathogenic *E. coli* (9), enteroinvasive *E. coli* (2).

Conclusions: This study demonstrated the importance of enterotoxigenic *E. coli* and enteroaggregative *E. coli* pathotypes in the childhood diarrhea. An epidemiologic surveillance especially for DEC, would be useful in control and prevention of infectious diarrhea in children.

1. Introduction

Gastrointestinal infections due to pathogenic *Escherichia coli* (*E. coli*) are significant causes of morbidity and mortality in children, particularly in developing countries[1]. Clinical categories of *E. coli* comprise commensal, intestinal pathogenic and extra-intestinal pathogenic strains. Diarrheagenic *E. coli* (DEC) pathotypes include enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC) and diffusely adherent *E. coli*[2]. ETEC pathotype defined by the presence of plasmid-encoded enterotoxins, comprise thermostable toxin (*ST*) and the thermolabile toxin (*LT*). ETEC strains are the most common cause of childhood diarrhea among all *E. coli* pathotypes and the major cause of diarrhea in travelers to developing countries[3]. Several virulence factors of EAggEC associated with diarrhea in children. Most of the genes encoding these virulence factors are located in the pAA plasmid, such as probe CVD432 and transcriptional factor encoded by the *aggR* gene. The pAA plasmid also carries the *aap* gene, which secreted low-molecular weight protein that promotes dispersal of EAggEC on the intestinal mucosa and facilitates efficient colonization[4,5]. Outbreaks of EIEC diarrhea are usually food or water-borne. However, through person-to-person transmissions have also been reported[6]. EIEC strains are able to attack intestinal epithelial cells. The invasion plasmid antigen H

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(*ipaH*) gene sequence is used for the diagnosis of EIEC[7,8]. EPEC strains express *eaeA* gene, which produce intimin, and bundle forming pili (*bfpA*) responsible for the attaching and effacing lesions of intestinal microvilli[3,9]. Shiga-toxin-producing *E. coli* or EHEC are principal emerging pathogens that cause food and water-borne diarrheal diseases in humans. All Shiga-toxin-producing *E. coli* strains possess *stx1* and/or *stx2* genes that produce two powerful cytotoxins, called Shiga toxin[10]. The *eaeA* gene of EHEC shares considerable homology with the *eaeA* gene of EPEC. Attaching and effacing *E. coli* strains (*eaeA*+) that harbor the *bfpA* gene are classified as typical EPEC and strains that do not possess *bfpA* gene are classified as atypical EPEC[11,12]. There are important regional differences in the prevalence of different categories of DEC in South and Southeast Asia[13].

Strains of the phylogenetic groups differ in their genotypic and phenotypic characteristics, comprising their antibiotic-resistance profiles, their ability to exploit different sugars sources and their growth rate temperature relationships. Phylogenetically, *E. coli* strains are divided upon amplification of *chuA* and *yjaA* genes and DNA fragment TSPE4.C2. The patterns of amplicons assigned four groups A, B1, B2 and D. DEC strains are derived from groups A, B1 and D, non-pathogenic commensal strains from A and B1, and extra-intestinal pathogenic strains usually belong to groups B2 and D[2,14].

The purpose of this study was to analyze the distribution of phylogenetic groups and occurrence of diarrheagenic genes in *E. coli* isolated from children less than five years of age with diarrhea in southeast of Iran by PCR.

2. Materials and methods

2.1. Sampling and bacteriological identification

One hundred and forty two *E. coli* isolates were obtained from diarrheal samples of children under five years old. Isolates were collected between 2010 and 2012 from children referring to the laboratories of Kerman Province, southeastern Iran. Samples were cultured on Mac Conkey agar and eosin methylene blue (Biolife Laboratories, Milano, Italy). Standard bacteriological methods were used to confirm the *E. coli* isolates. Isolates were stored in Luria-Bertani broth (Invitrogen, Paisley, Scotland) with 30% sterile glycerol at −70 °C for further analysis.

2.2. Reference strains

Five *E. coli* strains were used as positive controls: *E. coli* H10407 for ETEC (*LT*+, *ST*+), *E. coli* 85b for EIEC (*ipaH*+), *E. coli* O42 for EAggEC (probe CVD432+, *aggR*+ and *aap*+), *E. coli* Sakaï for EHEC and atypical EPEC (*stx1*+, *stx2*+ and *eaeA*+) and *E. coli* ECOR62 for (*chuA*+, *yjaA*+ and Tspe4. C2+). *E. coli* strain MG1655 was used as a negative control for virulence genes. All the reference strains were from the bacterial collection of Microbiology Department of Ecole Nationale Vétérinaire Toulouse, France.

2.3. PCR protocol

DNA was extracted from *E. coli* isolates and reference strains by lysis method. All isolates were tested by multiplex PCR assay for the presence of the *LT*, *ST* and *ipaH* genes by Aranda *et al.*[4], for *stx1*, *stx2* and *eaeA* genes by China *et al.* and probe CVD432, *agg*, *aap* genes by Cerna *et al*[15,16]. The phylogenetic groups (A, B1, B2, and D) of each *E. coli* isolate were carried out by triplex PCR method as described previously[17]. The primers used for detecting sequences encoding virulence genes and phylogenetic groups are described in Table 1.

Table 1
Oligonucleotide primers used in this study.

	Gene or probe name	Primer sequence (5′-3′)	Product size (bp)	Reference
ETEC	*LT*	GGC GAC AGA TTA TAC CGT GC	450	[4]
		CGG TCT CTA TAT TCC CTG TT		
	ST	ATT TTT CTT TCT GTA TTG TCT T		
		CAC CCG GTA CAA GCA GGA TT	190	
EAggEC	Probe CVD432	CTG GCG AAA GAC TGT ATC AT	600	[16]
		CAA TGT ATA GAA ATC CGC TGT T		
	aggR	CTA ATT GTA CAA TCG ATG TA	457	
		AGA GTC CAT CTC TTT GAT AAG		
	aap	CTT GGG TAT CAG CCT GAA TG	310	
		AAC CCA TTC GGT TAG AGC AC		
EIEC	*ipaH*	GTT CCT TGA CCG CCT TTC CGA TAC CGT C	600	[4]
		GCC GGT CAG CCA CCC TCT GAG AGT AC		
EPEC & EHEC	*eaeA*	AGG CTT CGT CAC AGT TG	570	[15]
		CCA TCG TCA CCA GAG GA		
	stx1	AGA GCG ATG TTA CGG TTT G	388	
		TTG CCC CCA GAG TGG ATG		
	stx2	TGG GTT TTT CTT CGG TAT C	807	
		GAC ATT CTG GTT GAC TCT CTT		
Phylo-group	*yjaA*	TGA AGT GTC AGG AGA CGC TG	211	[17]
		ATG GAG AAT GCG TTC CTC AAC		
	TspE4.C2	CTG GCG AAA GAC TGT ATC AT	152	
		CGC GCC AAC AAA GTA TTA CG		
	chuA	GAC GAA CCA ACG GTC AGG AT	279	
		TGC CGC CAG TAC CAA AGA CA		

3. Results

3.1. Phylogenetic grouping

The triplex PCR assays for phylotyping of isolates revealed that isolates fall into four phylogenetic groups, whereas 40.14% (57 isolates) belonged to A, 18.31% (26 isolates) to B1, 16.90% (24 isolates) to B2 and 24.65% (35 isolates) to D phylogenetic groups.