International Journal of Enteric Pathogens

Int J Enteric Pathog. 2016 August;4(3):e36326. Original Article

http://enterpathog.com

doi 10.15171/ijep.2016.01

Prevalence of *Escherichia coli* Pathotypes Among Children With Diarrhea in Babol, Northern Iran

Fatemah Moshtagian[1], Majid Alipour[2*], Yousef Yahyapour[3]

[1]Department of Microbiology, Pharmaceutical Science Branch, Islamic Azad University, Tehran, Iran
[2]Department of Microbiology, Babol Branch, Islamic Azad University, Babol, Iran
[3]Infectious Diseases & Tropical Medicine Research Center and Department of Microbiology, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran

***Corresponding Author:**
Majid Alipour,
Department of Microbiology, Babol Branch, Islamic Azad University, Babol, Iran
Email: alipourrna@yahoo.com

Published Online March 26, 2016

Keywords:
Diarrheagenic *Escherichia coli*, Prevalence, PCR

Abstract

Background: Diarrheagenic *Escherichia coli* (DEC) are major causes of diarrhea in the world particularly among infants and young children.

Objectives: The aim of this study was to determine the prevalence of DEC strains in stool samples from children under 5 years old.

Patients and Methods: Stool specimens were collected from 200 children under 5 years visiting hospital due to gastroenteritis. *E. coli* pathotypes were detected by using conventional culture techniques and polymerase chain reaction (PCR).

Results: Sixty-eight (34%) out of 200 specimens were positive for DEC. Different pathotypes would show the following profiles: 43 (21.5%) for enteropathogenic *E. coli* (EPEC); 18 (9%) for enterotoxigenic *E. coli* (ETEC) including 10 (55.5%) *st* positive, 6 (33.3%) *lt* positive and 2 (11.1%) *st* and *lt* both positive; 6 (3%) for enteroaggregative *E. coli* (EAEC) and 1 (0.5%) for enteroinvasive *E. coli* (EIEC). Enterohemorrhagic *E. coli* (EHEC) was not isolated from any of the *E. coli* strains tested.

Conclusions: This study shows that DEC is a common cause of childhood diarrhea in Babol. EPEC and ETEC were the most frequent pathotypes in the population under study.

Received January 20, 2016; **Revised** February 7, 2016; **Accepted** February 29, 2016

Background

Escherichia coli is one of the predominant facultative anaerobes in the human gastrointestinal tract. Many strains of *E. coli* are harmless and even provide many health benefits to the host. However, there are small groups of *E. coli* that have evolved and developed pathogenic strategies that can cause a broad spectrum of disease, including severe diarrheal disease.[1] Diarrheagenic *Escherichia coli* (DEC) are conveniently classified into 6 major pathotypes according to their virulence genes and including enterotoxigenic *E. coli* (ETEC), Shiga toxin producing *E. coli* (STEC, also referred to as verotoxigenic *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC).[2] EIEC is also the only *E. coli* pathotype to invade and multiply within host epithelial cells, and can cause invasive inflammatory colitis and dysentery, but most symptomatic infections are characterized by watery diarrhea indistinguishable from that produced by other diarrheagenic *E. coli* pathotypes.[3] EHEC cause hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS).[4] ETEC is an important cause of diarrheal disease in infants (6-18 months), young children, and the elderly in the developing world. It is also known to be the major cause of "traveler's diarrhea" acquired by tourists visiting developing nations.[5] The main distinguishing feature of EPEC is the ability to induce a characteristic histopathology called the attaching and effacing (A/E) lesion.[6] EAEC is increasingly recognized as an emerging enteric pathogen and cause of persistent diarrhea (greater than 2 weeks duration) in children and adults in both developing and developed countries.[7] The pathogenicity of DAEC strains is inadequately understood but it has been associated with diarrhea in young children under 12 months which is typically mild without blood in the feces.[8] In many studies, a different prevalence of DEC strains has been reported. In studies conducted in Iran, Bangladesh and Jordan a high prevalence of DEC strains was detected.[9-11] The identification of diarrheagenic *E. coli* using biochemical and serological tests is unreliable. Therefore, several polymerase chain reaction (PCR) tests have been developed to amplify the target regions present in virulence genes and identify strains of DEC.[12]

Objectives

The objective of the present study was to determine the prevalence of DEC among children with diarrhea in

Babol, Northern Iran. Since DAEC is not well defined as a distinct pathotype, its detection was not included in this study.

Patients and Methods

Isolation and Identification of *Escherichia coli*

Stool samples, from June to October 2014 were collected from hospitalized children under 5 years old in the Babol. A total of 200 stool samples from diarrheal patients were processed by directly inoculating the fecal matter onto MacConkey Agar and culturing overnight at 37°C. The suspected pink colonies were then cultured on eosin methylene blue (EMB) agar plates to see the metallic sheen color of the *E. coli*. Stool samples were a metallic green sheen colony from each plate with a typical *E. coli* morphology was selected and examined by biochemical tests, including indole, methyl red, Voges–Proskauer, citrate and urease tests. The isolates that were positive to indole and methyl red tests but negative to Voges–Proskauer, citrate and urease tests were identified as *E. coli*.

Reference Strains

The following DEC reference strains were used as positive controls: ETEC ATCC 35401 (*elt*, *est*), EHEC ATCC 43889 (*hlyA*), EPEC ATCC 43887 (*eae*), EIEC ATCC 43893 (*ial*) and EAEC ATCC 29552 (*pCVD432*).

DNA Templates for PCR Reactions

All isolated *E. coli* strains were grown on Luria-Bertani (LB) broth at 37°C for 24 hours. Genomic DNA was extracted according to the method described by Gómez-Duarte et al.[13] Bacteria were first harvested from 1.5 mL of an overnight LB broth culture, suspended in 200 µL of sterile water, and boiled at 100°C for 10 minutes. Following centrifugation of the lysate, the supernatant containing a crude DNA extract was used as a DNA template on a multiplex PCR for identification of *E. coli* pathotypes.

Polymerase Chain Reaction Detection of Diarrheagenic *Escherichia coli*

Two multiplex PCR assays were performed for the detection of five pathotypes of DEC. The oligonucleotide primers used in this study are listed in Table 1.

Multiplex PCR 1 contained primer mix 1 for the detection of *elt* and *est* for the enterotoxin of ETEC, *hlyA* for the plasmid encoded enterohemolysin of EHEC and, *pCVD432* for the nucleotide sequence of the EcoR1- Pst DNA fragment of EAEC. Multiplex PCR reaction 2 contained primer mix 2 specific for *eaeA* for the structural gene of intimin of EPEC and *ial* for the invasion associated locus of the invasion plasmid found in EIEC.[14-18]

Multiplex Polymerase Chain Reaction

PCRs were performed with a 20 µL reaction mixture containing 2 µL of the solution containing DNA, 2 µL of 10x PCR buffer, 0.4 µL of 10mM mixture of deoxynucleoside triphosphates, 0.6 µL of 50mM $MgCl_2$, 0.2 µL of 5 U of Taq DNA polymerase, 1 µL of each primer (20 pmol), and 10 µL of distilled water. The reactions were performed as follows: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 1 minute, annealing at 55°C (for multiplex PCR 1) and 57°C (for multiplex PCR 2) 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes. Positive and negative controls were conducted for all DEC strains which were positive in the multiplex PCR. The PCR products were electrophoresed on a 2% agarose gel and visualized with UV transilluminator after EtBr staining.

Results

From the 200 stool samples, DEC strains were identified in 80 of the samples, and DEC was detected in 68 (34%) of the diarrheic children under 5 years old within the period of the study. Of the 68 DEC strains detected by PCR, 43 (21.5%) were EPEC, 18 (9%) ETEC, 6 (3%) EAEC and 1 (0.5%) EIEC isolates. Among 18 ETEC isolates, *st* gene was detected in 10 (55.5%), *lt* gene in 6 (33.3%) and both *lt* and *st* genes were present in 2 (11.1%) isolates. The frequency of each DEC pathotype is shown in Table 2.

EHEC was not isolated from any of the *E. coli* strains tested. The most frequently isolated was EPEC. The amplified products for the *elt* gene and the *est* gene (ETEC) were of 322 bp and 170 bp, respectively. The PCR product

Table 1. PCR Primers Used in This Study

Target Organism	Target Gene	Primer Sequence (5′-3′)	Size of Product (bp)	Reference
EIEC	*ipaH*	F-CTCGGCACGTTTTAATAGTCTGG R-GTGGAGAGCTGAAGTTTCTCTGC	933	Vidal et al,[17] 2005
	ial	F-CTGGTAGGTATGCTGAGG R-CCAGGCCAACAATTATTTCC	320	Svenungsson et al,[18] 2000
EAEC	*pCVD432*	F-CTG GCG AAA GAC TGT ATC AT R-CAA TGT ATA GAA ATC CGC TGT T	630	Schmidt et al,[16] 1995
ETEC	*elt*	F-TCTCTATGTGCATACGGAGC R-CCATACTGATTGCCGCAAT	322	Rappelli et al,[14] 2001
	est	F-TCTTTCCCCTCTTTTAGTCAGTC R-CCAGCACAGGCAGGATTAC	170	
EHEC	*hlyA*	F-GCATCATCAAGCGTACGTTCC R-AATGAGCCAAGCTGGTTAAGCT	534	Aslantas et al,[15] 2006
EPEC	*eae*	F-TGATAAGCTGCAGTCGAATCC R-CTGAACCAGATCGTAACGGC	229	Rappelli et al,[14] 2001

Table 2. Diarrheagenic *Escherichia coli* Among Children With Diarrhea

DEC	No. (%)
EPEC	43 (21.5)
ETEC	18 (9)
EAEC	6 (3)
EIEC	1 (0.5)
DEC negative	132 (66)
Total	200 (100)

Abbreviations: DEC, diarrheagenic *E.coli*; EPEC, enteropathogenic *E.coli; ETEC,* enterotoxigenic *E.coli; EAEC,* enteroaggregative *E. coli*; EIEC, enteroinvasive *E.coli.*

for *eae* gene was of 229 bp. The amplified products for the *ipaH* gene and the *ial* gene (EIEC) were 933 bp and 320 bp, respectively. The PCR product for *pCVD432* gene (EAEC) was 630 bp (Figures 1 and 2).

Discussion

Among the bacterial pathogens, *E. coli* plays an important role in causing diarrhea in children under 5 year old. In the present study, 68 (34%) isolates were identified as DEC strains by multiplex PCR. Hegde et al[19] reported a prevalence of 26% DEC strains from 200 stool samples from children with diarrhea in India. In a study conducted by Gómez-Duarte et al[13] in Colombia, a lower prevalence of 14.4% has been reported. The prevalence of DEC varies in the world from region to region and even between countries. Albert et al[9] reported that in children with diarrhea, EPEC had the highest prevalence. Alikhani et al[20] identified that EPEC was the most prevalent pathotype among DEC. In another study in Iran, Alikhani et al[11] reported that the most frequently identified DEC was EPEC (47.5%). Usein et al[21] also showed that 9% of the children with diarrhea carried EPEC isolates. In this study, EPEC was the predominant *E. coli* pathotype (21.5%). The reason for different rates of identification of the EPEC may be due to health status in various areas. In our study, the second most common pathotype of DEC was the ETEC 18 (9%). The prevalence of ETEC was lower in our study than in some previous studies. The rate of isolation of the ETEC reported by Wolk et al[22] and Viboud et al[23] was 20.7% and 18.3%, respectively. In studies conducted by Hegde et al[19] and Nguyen et al,[24] the rate of isolation of the ETEC was 3.5% and 2.2%, respectively. In this study, the detection rate for EAEC was 3.0%. Our finding is approximately similar to those reported by Aranda et al,[25] Ifeanyi et al,[26] and Aslani et al,[27] in which they found that the rate of EAEC was 2%, 2% and 10.7%, respectively. The very low percentage (0.5%) prevalence of EIEC obtained in this study is in close agreement with the study reported by Hegde et al,[19] who reported a prevalence of EIEC in children with diarrhoea as 1.5% in India. In the present study, we did not isolate any EHEC strains. The results of current investigation are consistent with the studies reported by Moyo et al[28] and Nguyen et al.[24] In conclusion, EPEC was the most commonly identified DEC strain in the region studied. Therefore, further studies are needed to investigate virulence properties and antimicrobial resistance of EPEC strains.

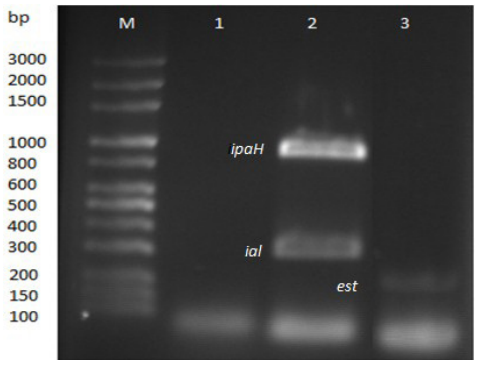

Figure 1. Agarose Gel Electrophoresis of Clinical Isolates of DEC From Pure Cultures. Lane M, DNA ladder; Lane 1, negative control; Lane 2, EIEC (933 bp-*ipaH*), EIEC (320 bp- *ial*); Lane 3, ETEC (170 bp-*est*).

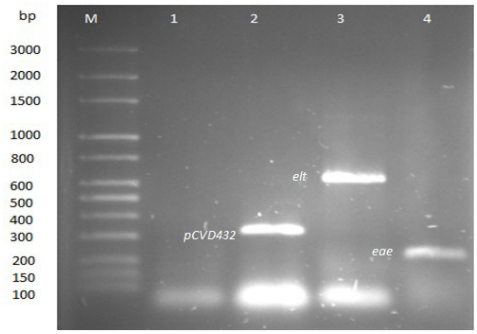

Figure 2. Agarose Gel Electrophoresis of Clinical Isolates of DEC From Pure Cultures.
Lane M, DNA ladder; Lane 1, negative control; Lane 2, ETEC (322 bp- *elt*); Lane 3, EAEC (630 bp- *pCVD432*); Lane 4, EPEC (229 bp- *eae*).

Acknowledgments

The authors thank Seyed Ali Sajadi from Amirkola children's hospital for the excellent technical assistance.

Authors' Contribution

FM performed practical experiments and collected samples. MA collected data, set up the tests, wrote the manuscript, and performed interpretation of the results. YY contributed to the performing of the experiments and writing of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Disclosures

None.

Funding/Support

No funding of any kind has been received for this study.

References

1. Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev. 1998;11(1):142-201.
2. Belanger L, Garenaux A, Harel J, Boulianne M, Nadeau E, Dozois CM. Escherichia coli from animal reservoirs as a potential source of human extraintestinal pathogenic E. coli. FEMS Immunol Med Microbiol. 2011;62(1):1 -10. doi:10.1111/j.1574-695X.2011.00797.x.
3. Bolton DJ. Verocytotoxigenic (Shiga Toxin-Producing)

Escherichia coli: virulence factors and pathogenicity in the farm to fork paradigm. Foodborne Pathog Dis. 2011;8(3):357-365. doi:10.1089/fpd.2010.0699.
4. Vallance BA, Chan C, Robertson ML, Finlay BB. Enteropathogenic and enterohemorrhagic Escherichia coli infections: emerging themes in pathogenesis and prevention. Can J Gastroenterol. 2002;16(11):771-778.
5. Qadri F, Svennerholm AM, Faruque AS, Sack RB. Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clin Microbiol Rev. 2005;18(3):465-483. doi:10.1128/CMR.18.3.465-483.2005.
6. Trabulsi LR, Keller R, Tardelli Gomes TA. Typical and atypical enteropathogenic Escherichia coli. Emerg Infect Dis. 2002;8(5):508-513. doi:10.3201/eid0805.010385.
7. Huang DB, Nataro JP, DuPont HL, et al. Enteroaggregative Escherichia coli is a cause of acute diarrheal illness: a meta-analysis. Clin Infect Dis. 2006;43(5):556-563. doi:10.1086/505869.
8. Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. Nat Rev Microbiol. 2004;2(2):123-140. doi:10.1038/nrmicro818.
9. Albert MJ, Faruque SM, Faruque AS, et al. Controlled study of Escherichia coli diarrheal infections in Bangladeshi children. J Clin Microbiol. 1995;33(4):973-977.
10. Shehabi AA, Bulos NK, Hajjaj KG. Characterization of diarrhoeagenic Escherichia coli isolates in Jordanian children. Scand J Infect Dis. 2003;35(6-7):368-371.
11. Alikhani MY, Hashemi SH, Aslani MM, Farajnia S. Prevalence and antibiotic resistance patterns of diarrheagenic Escherichia coli isolated from adolescents and adults in Hamedan, West Iran. Iran J Microbiol. 2013;5(1):42-47.
12. Toma C, Lu Y, Higa N, et al. Multiplex PCR assay for identification of human diarrheagenic E. coli. J Clin Microbiol. 2003; 41(6): 2669-71.
13. Gómez-Duarte OG, Arzuza O, Urbina D, et al. Detection of Escherichia coli enteropathogens by multiplex polymerase chain reaction from children's diarrheal stools in two Caribbean-Colombian cities. Foodborne Pathog Dis. 2010;7(2):199-206. doi:10.1089/fpd.2009.0355.
14. Rappelli P, Maddau G, Mannu F, Colombo MM, Fiori PL, Cappuccinelli P. Development of a set of multiplex PCR assays for the simultaneous identification of enterotoxigenic, enteropathogenic, enterohemorrhagic and enteroinvasive Escherichia coli. New Microbiol. 2001;24(1):77-83. doi:10.1128/JCM.43.10.5362-5365.2005.
15. Aslantaş O, Erdoğan S, Cantekin Z, Gülaçti, Evrendilek GA. Isolation and characterization of verocytotoxin producing Escherichia coli O157 from Turkish cattle. Int J Food Microbiol. 2006:106(3):338-342. doi:10.1086/313770.
16. Schmidt H, Knop C, Franke S, Aleksic S, Heesemann J, Karch H. Development of PCR for screening of enteroaggregative Escherichia coli. J Clin Microbiol. 1995;33(3):701-705.
17. Vidal M, Kruger E, Durán C, et al. Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic Escherichia coli associated with enteric infections. J Clin Microbiol. 2005;43(10):5362-5365.
18. Svenungsson B, Lagergren A, Ekwall E, et al. Enteropathogens in adult patients with diarrhea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases. Clin Infect Dis. 2000;30(5):770-778. doi:10.1016/j.ijfoodmicro.2005.08.005.
19. Hegde A, Ballal M, Shenoy S. Detection of diarrheagenic Escherichia coli by multiplex PCR. Indian J Med Microbiol. 2012;30(3):279-284. doi:10.4103/0255-0857.99485.
20. Alikhani MY, Mirsalehian A, Aslani MM. Detection of typical and atypical enteropathogenic Escherichia coli (EPEC) in Iranian children with and without diarrhea. J Med Microbiol. 2006;55(Pt 9):1159-1163. doi:10.1099/jmm.0.46539-0.
21. Usein CR, Tatu-Chitoiu D, Ciontea S, Condei M. Damian M. Escherichia coli pathotypes associated with diarrhea in Romanian children younger than 5 years of age. Jpn J Infect Dis. 2009;62(4):289-293.
22. Wolk M, Ohad E, Shafran R, et al. Epidemiological aspects of enterotoxigenic Escherichia coli diarrhoea in infants in the Jerusalem area. Public Health Rev. 1995;23(1):25-33.
23. Viboud GI, Jouve MJ, Binsztein N, Vergara M, Rivas M, Quiroga M, et al. Prospective cohort study of enterotoxigenic Escherichia coli infections in Argentinean children. J Clin Microbiol. 1999;37(9):2829-2833.
24. Nguyen TV, Le Van P, Le Huy C, Gia, K N, Weintraub, A. Detection and characterization of diarrheagenic Escherichia coli from young children in Hanoi, Vietnam. J Clin Microbiol. 2005;43(2):755-760. doi:10.1128/JCM.43.2.755-760.2005.
25. Aranda KR, Fagundes-Neto U, Scaletsky IC. Evaluation of multiplex PCR for diagnosis of infection with diarrheagenic Escherichia coli and Shigella spp. J Clin Microbiol. 2004;42(12):5849-5853. doi:10.1128/JCM.42.12.5849-5853.2004.
26. Ifeanyi CI, Ikeneche NF, Bassey BE , Al-Gallas N, Ben Aissa R, Boudabous A. Diarrheagenic Escherichia coli pathotypes isolated from children with diarrhea in the Federal Capital Territory Abuja, Nigeria. J Infect Dev Ctries. 2015;9(2):165-174. doi:10.3855/jidc.5528.
27. Aslani MM, Alikhani MY, Zavari A, Yousefi R, Zamani AR. Characterization of enteroaggregative Escherichia coli (EAEC) clinical isolates and their antibiotic resistance pattern. Int J Infect Dis. 2011;15(2):136-139. doi: 10.1016/j.ijid.2010.10.002.
28. Moyo SJ, Maselle SY, Matee MI Langeland N, Mylvaganam H. Identification of diarrheagenic Escherichia coli isolated from infants and children in Dar es Salaam, Tanzania. BMC Infect Dis. 2007;7:92. doi:10.1186/1471-2334-7-92.