Molecular detection and antimicrobial resistance of diarrheagenic *Escherichia coli* strains isolated from diarrheal cases

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ABSTRACT

الأهداف: لقد تم توثيق انتشار مقاومة المضادات الميكروبية في البكتريا كمشكلة خطيرة على مستوى العالم. تعتبر سلالة عصيات اسكريتشيا المسهلة السبب الرئيسي للإسهال في الدول النامية.

الطريقة: أجريت دراسة على شريحة عرضية في عام 2005م بمعهد باستيور بإيران لتحديد سلالة عصيات اسكريتشيا المعزولة من الأطفال المصابين بالإسهال وأيضاً من اجل تحديد نموذج قابلية المعزولات للعوامل المضادة للميكروبات. تم اكتشاف جينات عامل السمية لعصيات أي كولي المسهلة بواسطة سلسلة تفاعل الخمائر الناقلة.

النتائج: كان من بين عصيات إي كولي المسهلة والبالغ عددها 193 والمكتشفة بواسطة تفاعل سلسلة الخمائر الناقلة معزولات 86(44.5%) (STEC) و 74(38.4%) (EPEC) و 19(9.8%) (enteroaggregative *E. coli* isolates) و 14 (7.3%) (enterotoxigenic *E. coli* isolates). تم تحديد القابلية لأثني عشر من العوامل السريرية المهمة المضادة للميكروبات ل193 سلالة من عصيات كولي المسهلة. تمت ملاحظة حوادث عالية من المقاومة لعقار تيتراسيلين (63%) وأمبيسيلين (62%) وستريبتومايسين (56%) و أوموكسيسيلين/حمض كلافولانيك (44.5%) وتريميثوبيريم/سلوفاميثازول (39.5%) و سيفالوتين (37%).

خاتمة: تعتبر سلالة (STEC) و سلالة (EPEC) مع المقاومة العالية لعقار تريتاسيسيلين وأمبيسيلين ولكن قابلية عالية للكيونولونيس من بين أهم أكثر العوامل المسببة للإسهال في إيران. تقترح هذه الدراسة أن مقاومة المضادات الميكروبية منتشرة بين سلالات عصيات إي كولي لدى المرضى الإيرانيين. ينبغي تحديث الإرشادات للاستعمال المناسب للمضادات الحيوية في الدول النامية.

Objectives: To identify and classify Iranian isolates of diarrheagenic *Escherichia coli* (*E. coli*) on the basis of presence of virulence genes and to determine antibiotic susceptibility of isolated strains.

Methods: The current cross-sectional study was conducted in 2005 at the Pasteur Institute, Tehran, Iran. One hundred and ninety-three diarrheagenic *E. coli* isolated from diarrheal patients in different regions of Iran were included in current study. Virulence factor genes for diarrheagenic *E. coli* were detected by polymerase chain reaction.

Results: Of the 193 diarrheagenic *E. coli* detected by PCR, 86 (44.5%) were Shiga toxin-producing *E. coli* (STEC), 74 (38.4%) enteropathogenic *E. coli* (EPEC), 19 (9.8%) enteroaggregative *E. coli*, and 14 (7.3%) enterotoxigenic *E. coli* isolates. Susceptibility to 12 clinically important antimicrobial agents was determined for 193 strains of diarrheagenic *E. coli*. A high incidence of resistance to tetracycline (63%), ampicillin (62%), streptomycin (56%), amoxicillin/clavulanic acid (44.5%), trimethoprim/sulfamethoxazole (39.5%), and cephalothin (37%) was observed.

Conclusion: The STEC and EPEC strains with high resistance to tetracycline and ampicillin, but highly susceptible to quinolones are among the most important causative agent of diarrhea in Iran. This study suggests that antimicrobial resistance is widespread among *E. coli* strains colonizing Iranian patients. Guidelines for appropriate use of antibiotics in developing countries require updating.

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Resistance to antibiotics is very common in bacterial isolates all around the world.[1-4] Regular surveillance of antibiotic resistance provides data for antibiotic therapy and resistance control.[5,6] Information on antimicrobial resistance patterns is important in choosing the appropriate antibiotic therapy, as even if microbiology laboratory services are accessible, antibiotic susceptibilities patterns will generally be known after 72 hours, and acute enteritis is an important cause of mortality and morbidity among children in developing countries. Diarrhea caused by multidrug-resistant bacteria is an important public health dilemma among children and is set as a research priority of the control of diarrheal disease program for developing countries by the World Health Organization. The strains of the different diarrheagenic *Escherichia coli (E. coli)*, namely, Enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC), are among the most important causes of acute enteritis.[7] In Iran, based on WHO guidelines, children with diarrhea are treated by oral rehydration salt (ORS). However, in most cases, an antibiotic (currently ampicillin or co-trimoxazole) is also administered. Therefore, treatment of patients with antibiotics requires knowledge of the antimicrobial resistance patterns of the most prevalent diarrheagenic bacteria. Antimicrobial therapy should be restricted to severe diarrheal cases as well as traveler's diarrhea.[8] However, because of overuse and misuse of antibiotics in the treatment of diarrhea, antibiotic resistance is progressively increasing among enteric pathogens in our country. The main purpose of this study was to identify and classify Iranian isolates of diarrheagenic *E. coli* on the basis of presence of virulence genes and to determine antibiotic susceptibility of isolated strains.

Methods

Bacterial strains. One hundred and ninety-three diarrheagenic *E. coli* isolated from diarrheal patients in different regions of Iran were included in the current study. Ethical approval was obtain for this study. These strains included rural and urban isolates of Central, Western, and Northern parts of Iran including strains from Tehran, the capital city of Iran. The isolates were identified by a combination of conventional and molecular bacteriological methods at Pasteur Institute, Tehran, Iran in 2005.[9]

PCR detection. The organisms were classified as EAEC, EPEC, ETEC, and STEC based on the specific virulence genes detected by polymerase chain reaction (PCR). For DNA extraction, a loopful of bacterial growth was taken directly from the confluent area of the culture, suspended in 0.5 ml of sterile, distilled water and boiled for 20 minutes. These samples were subjected to 5 different PCR reactions targeting Shiga toxinogenic (stx) gene,[10] *Escherichia coli* attaching and effacing (eae) gene,[11] heat-labile (LT) enterotoxin (eltB) encoding gene,[12] heat-stable (ST) enterotoxin (estA) encoding gene,[12] and pCVD 432 plasmid.[13] The primers and amplification procedures are shown in **Table 1**. The *E. coli* strains that carried eae gene and were negative for stx gene were considered as EPEC. The strains that were positive by PCR for pCVD432 were interpreted as EAEC and those positive for stx gene and positive or negative for eae gene as STEC. The *E. coli* strains positive for LT-producing gene, ST-producing gene, or both, were considered to be ETEC. Positive controls were *E. coli* ATCC 35401 (LT+, ST+), *E. coli* ATCC 43894 (stx+, eae+), and *E. coli* RH6420 (*E. coli* 17-2, EAEC). The negative control was sterile distilled water.

Table 1 - Amplimer and primers of PCR for diarrheagenic *Escherichia coli*.

Pathogen	Target	Primer pair	Amplimer (No. of bp)	Reference
STEC	stx	F, GAACGAAATAATTTATATGT R, TTTGATTGTTACAGTCAT	900	Lin et al[10]
EPEC	eae	F, CATTATGGAACGGCAGAGGT R, ATCTTCTGCGTACTGCGTTCA	790	Beaudry et al[11]
ETEC-LT	eltB	F, TCTCTATGTGCATACGGAGC R, CCATACTGATTGCCGCAAT	322	Blomen et al[12]
ETEC-ST	estA	F, GCTAAACAAGTAGAGGTCTTCAAAA R, CCCGGTACAGAGCAGGATTACAACA	147	Blomen et al[12]
EAEC	PCVD432	F, CTGGCGAAAGACTGTATCAT R, CAATGTATAGAAATCCGCTGTT	630	Schmidt et al[13]

E. coli - Escherichia coli, STEC - shiga toxin-producing *E. coli*, EPEC - enteropathogenic *E. coli*, ETEC - enterotoxigenic *E. coli*, EAEC - enteroaggregative *E. coli*, stx - shiga toxin gene, eae - EPEC attaching and effacing gene, eltB - heat labile enterotoxin gene, estA - heat stable enterotoxin gene, pCVD432 - CVD432 plasmid, bp - base pairs

Antimicrobial susceptibility testing. Antimicrobial susceptibility pattern was determined by Kirby-Bauer disk diffusion method according to recommendation proposed by the clinical and laboratory standards institute (CLSI).[14] The antibiotics tested were ampicillin (AM-10), cefoxitin (FOX-30), chloramphenicol (C-30), ceftriaxone (CRO-30), cephalothin (CF-30), gentamicin (GM-10), nalidixic acid (NA-30), ciprofloxacin (CIP-5), tetracycline (Te-30), sulfamethoxazole/trimethoprim (SXT), amoxicillin/clavulanic acid (AmC-30) and streptomycin (S-10) (BD BBLTM Sensi- DiscTM). The *E. coli* ATCC 25922, ATCC 35218, and *Pseudomonas aeruginosa* ATCC 27853 were tested along with the isolates for quality control purposes. The result was documented according to performance range of inhibition zone of the control strains.[14]

Data interpretation. Data were interpreted by susceptible, intermediate, or resistant percent of strains to an antimicrobial agent according to CLSI interpretative criteria.[14] It was considered that in vitro resistance to cephalothin, a commonly tested cephalosporin, may not predict resistance to other cephalosporins including cefazolin, cefuroxime, cefpodoxime, cefprozil, and loracarbef.[14]

Results. ***Distribution of strains.*** Of the 193 diarrheagenic *E. coli* detected by PCR, 86 (44.5%) were STEC, 74 (38.4%) EPEC, 19 (9.8%) EAEC, and 14 (7.3%) ETEC isolates. The 2 types of ETEC according to the type of enterotoxin synthesized was as follow: 7 strains (50%) of the ST toxin, 6 strains (42.9%) of the LT toxin, and one strain (7.1%) had both ST and LT genes.

Antimicrobial resistance. The results for the antibiotic susceptibility testing of the different group of diarrheagenic *E. coli* strains are shown in **Tables 2 & 3.** The results showed that the highest rates of resistance (resistance plus intermediate) were to chloramphenicol, tetracycline, amoxicillin/clavulanic acid, streptomycin, and ampicillin **Table 2**. The EPEC and STEC strains showed the highest rates of resistance to ampicillin and tetracycline. However, the rates of resistant strains among EAEC and ETEC strains were higher than that of EPEC and STEC strains. These antibiotics were tetracycline, amoxicillin/clavulanic acid and ampicillin **Table 2**. Of the 193 diarrheagenic *E. coli* isolates, 63% were resistant to Tetracycline, 62% were resistant to Ampicillin, 56% were resistant to Streptomycin, 44.5% were resistant to Amoxicillin/Clavulanic acid, 39.5% were resistant to SXT, 37% were resistant to Cephalothin, 31% were resistant to Chloramphenicol, 2.5% were resistant to Ciprofloxacin, and 2% were resistance to Nalidixic acid. Of the few *E. coli* strains resistant to Ciprofloxacin, one strain was EAEC and 5 strains were ETEC.

Table 2 - Antimicrobial susceptibility of diarrheagenic *E. coli* isolates from diarrheal cases.

Antibiotic	Diarrhoeagenic *E. coli*			
	R		S	
	n	%	n	%
Ampicillin (Am-10)	120	(62)	73	(38)
Cefoxitin (Fox-30)	8	(4)	185	(96)
Chloramphenicol (C-30)	60	(31)	133	(69)
Ceftriaxone (CRO-30)	13	(7)	180	(93)
Cephalothin (Cf-30)	72	(37)	121	(63)
Gentamicin (GM-10)	12	(6)	181	(94)
Nalidixic Acid (NA-30)	7	(4)	186	(96)
Ciprofloxacin (CIP-5)	6	(3)	187	(97)
Tetracycline (Te-30)	121	(63)	72	(37)
Sulfamethoxazole and Trimethoprim (SXT)	76	(39)	117	(61)
Amoxicillin/Clavulanic acid (Am/C-30)	86	(45)	107	(55)
Streptomycin (S-10)	108	(56)	85	(44)

R - resistance, S - sensitive, *E. coli - Escherichia coli*

Discussion. Diarrheal diseases due to the different diarrheagenic *E. coli* are a health problem in many parts of the world.[7] It has been estimated that infectious diseases cause 9.2 million deaths in the developing countries, and diarrheal diseases are the fourth most common cause of mortality.[15] Most mild diarrhea cases are effectively treated by ORS therapy and only patients with severe or persistent diarrhea should receive antibiotics. Antimicrobial resistance of diarrheagenic *E. coli* is usually frequent towards beta-lactam antibiotics. Surveillance information about antimicrobial resistance should be up-to-date and used in clinical management and treatment guidelines.[16]

Of the 193 *E. coli* isolates studied, approximately 55% displayed resistance to one or more antibiotics, including ampicillin, tetracycline, streptomycin, sulfamethoxazole-trimethoprim, and cephalothin. These data are in accordance with previous studies indicating that use of these drugs played a key factor in the emergence of antimicrobial-resistant *E. coli*.[17,18]

In this study, amplification of the eaeA gene was used to detect EPEC strains, and 74 (38.4%) strains were found to have this gene. Approximately 60% of EPEC strains were resistant to ampicillin and tetracycline.

Table 3 - Antimicrobial susceptibility of different diarrheagenic *E. coli* isolates from diarrheal cases.

Antibiotic	EPEC n = 74				STEC n = 86				EAEC n = 19				ETEC n = 14			
	R		S		R		S		R		S		R		S	
Ampicillin (Am-10)	44	(59)	30	(41)	49	(57)	37	(43)	16	(84)	3	(26)	11	(78)	3	(22)
Cefoxitin (Fox-30)	2	(3)	72	(97)	2	(3)	84	(97)	2	(11)	17	(89)	2	(14)	12	(86)
Chloramphenicol (C-30)	19	(26)	55	(74)	23	(27)	63	(73)	17	(89)	2	(11)	1	(7)	13	(93)
Ceftriaxone (CRO-30)	4	(6)	70	(94)	3	(4)	83	(96)	1	(5)	18	(95)	5	(36)	9	(64)
Cephalotin(CF-30)	37	(50)	37	(50)	30	(35)	56	(65)	1	(5)	18	(95)	4	(29)	10	(71)
Gentamicin (GM-10)	3	(4)	71	(96)	3	(4)	83	(96)	2	(11)	17	(89)	4	(29)	10	(71)
Nalidixin Acid (NA-30)	0	(0)	74	(100)	1	(1)	85	(99)	2	(11)	17	(89)	4	(29)	10	(71)
Ciprofloxacin (CIP-5)	0	(0)	74	(100)	0	(0)	86	(100)	1	(5)	18	(95)	5	(36)	9	(64)
Tetracycline (Te-30)	41	(55)	33	(45)	51	(59)	35	(41)	17	(89)	2	(11)	12	(86)	2	(14)
Sulfamethoxazole and Trimethoprim (SXT)	26	(35)	48	(65)	30	(34)	56	(96)	13	(68)	6	(32)	7	(50)	7	(50)
Amoxicillin/Clavulanic acid (Am/C-30)	20	(27)	54	(73)	37	(43)	49	(57)	17	(89)	2	(11)	12	(86)	2	(14)
Streptomycin (S-10)	39	(53)	35	(47)	44	(51)	42	(49)	15	(79)	4	(21)	10	(71)	4	29

E. coli - Escherichia coli, EPEC - enteropathogenic *E. coli*, STEC - shiga toxin-producing *E. coli*, EAEC - enteroaggregative *E. coli*, ETEC - enterotoxigenic *E. coli*, R - resistant, S - sensitive, data expressed as n (%)

Since these drugs are recommended for management of different infections in children, *E. coli* isolates should be monitored for further dissemination of ampicillin and tetracycline resistance. However, EPEC isolates from this study were susceptible to cephalosporins and ciprofloxacin that are important antimicrobials for treating infection caused by multi resistant EPEC strains.

The STEC strains are among the most important agents of diarrhea in Iran.[19,20] Human infections with STEC strains are commonly transmitted from cattle or dairy products especially undercooked meat or unpasteurized milk and usually are connected with the rural environment.[21] Resistance to 2 or more classes of antimicrobials was found in 45% of STEC strains. In the last decades since antimicrobial agents have been used for the aim of disease prevention or growth promotion in animals, livestock on farm is frequently exposed to antimicrobial substances, so the resistance phenotype can give a selective advantage to bacteria. As a result, humans are more likely to be exposed to these organisms via food and direct and indirect transmission from animals.[22]

There is a controversy on the association of EAEC with acute diarrhea,[23] however, many reports suggest that they are associated with persistent diarrhea.[24,25] The highest frequencies of antimicrobial-resistant phenotype were observed for EAEC. Nearly more than 80% of strains were resistant to ampicillin, chloramphenicol, tetracycline, and amoxicillin/clavulanic acid. As long-term antimicrobial therapy is usually recommended for EAEC persistent diarrhea, the indirect selection for multiresistant strains will increase the antimicrobial-resistant pathogen, and also facilitate the spread of mobile resistance elements to these bacteria.

Antimicrobial resistance in ETEC as a cause of diarrhea in children[7] was more frequent than that of other groups of diarrheagenic *E. coli*. The ETEC were significantly more resistant to ciprofloxacin and nalidixic–acid than EPEC and STEC. This group of *E. coli* also showed higher resistance to ampicillin, tetracycline, amoxicillin/clavulanic acid and sulfamethoxazole-trimethoprim.

Our results showed that application of antibiotics as chemoprophylactic agents or growth promotion in agriculture is important in the selection of antimicrobial-resistant phenotypes. In the current study, the presence of 75% resistant *E. coli* to ampicillin that showed a dual resistance to streptomycin and tetracycline could be indicative of spread of mobile genetic elements or plasmids. The results also suggest that antimicrobial resistance is wide spread among diarrheagenic *E. coli* strains.

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References

1. Calva JJ, Sifuentes-Osornio J, Ceron C. Antimicrobial resistance in fecal flora: longitudinal community-based surveillance of children from urban Mexico. ***Antimicrob Agents Chemother*** 1996; 40: 1699-702.
2. Bouzari S, Jafari A, Zarepoor M. Distribution of genes encoding toxins and antibiotic resistance patterns in diarrhoeagenic *Escherichia coli* isolates in Tehran. ***East Mediterr Health J*** 2007; 13: 287-293
3. Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. ***Clin Infect Dis*** 1998; 26: 341-345.
4. Nys S, Okeke IN, Kariuki S, Dinant GJ, Driessen C, Stobberingh EE. Antibiotic resistance of faecal *Escherichia coli* from healthy volunteers from eight developing countries. ***J Antimicrob Chemother*** 2004; 54: 952-955.
5. O'Brien T. The global epidemic nature of antimicrobial resistance and the need to monitor and manage it locally. ***Clin Infect Dis*** 1997; 24 Suppl 1: 2-8.
6. Okeke IN, Fayinka ST, Lamikanra A. Antibiotic resistance in *Escherichia coli* from Nigerian Students, 198-1998. ***Emerg Infect Dis*** 2000; 6: 393-396.
7. Nataro JP, Kaper JB. Diarrhoeagenic *Escherichia coli*. ***Clin Microbiol Rev*** 1998; 11: 142–201.
8. Nguyen TV, Le PV, Le CH, Weintraub A. Antibiotic resistance in diarrhoeagenic *Escherichia coli* and Shigella strains isolated from children in Hanoi, Vietnam. ***Antimicrob Agents Chemother*** 2005; 49: 816-819.
9. Forbes BE, Sahm DF , Weissfeld AS. Bailey & Scott's Diagnostic Microbiology. 12th ed. Mosby, Elsevier, USA: The C.V. Mosby Company; 2007. p. 363-378.
10. Lin Z, Kurazono H, Yamasaki S, Takeda Y. Detection of various variant verotoxin genes in *Escherichia coli* by polymerase chain reaction. ***Microbiol Immunol*** 1993; 37: 543-548.
11. Beaudry M, Zhu C, Fairbrother JM, Harel J. Genotypic and phenotypic characterization of *Escherichia coli* isolates from dogs manifesting attaching and effacing lesions. ***J Clin Microbiol*** 1996; 34: 144-148.
12. Blomen I, Lofdahl S, Stenstrom TA, Norberg R. Identification of enterotoxigenic *Escherichia coli* isolates: a comparison of PCR DNA hybridization, ELISAs and bioassays. ***J Microbiol Methods*** 1993; 17: 181-91.
13. 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: 701-705.
14. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests Approved standard. 7th ed. Wayne (PA): NCCLS; 2003.
15. Murray CJL, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. ***Lancet*** 1997; 349: 1269-1276.
16. Williams RJ, Ryan MJ. Surveillance of antimicrobial resistance-an international perspective. ***BMJ*** 1998; 317: 651.
17. Stephan R, Schumacher S. Resistance patterns of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from animals, food and asymptomatics human carriers in Switzerland. ***Lett Appl Microbiol*** 2001; 32: 114-117.
18. Threlfall EJ, Ward LR, Forst JA, Willshaw GA. The emergence and spread of antibiotic resistance in food-borne bacteria. ***Int J Food Microbiol*** 2000; 62: 1-5.
19. Aslani MM, Bouzari S. An epidemiological study on Verotoxin-producing *Escherichia coli* (VTEC) infection among population of northern region of Iran (Mazandaran and Golestan provinces). ***Eur J Epidemiol*** 2003; 18: 345-349.
20. Aslani MM, Badami N, Mahmmoodi M, Bouzari S. Verotoxin-producing *Escherichia coli* (VTEC) infection in randomly selected population of Ilam Province (Iran). ***Scand J Infect Dis*** 1998; 30: 473-476.
21. Beutin L, Bulte M, Weber A, Zimmermann S, Gleier K. Ivestigation of human infection with verocytotoxin-producing strains of *Escherichia coli* (VTEC) belonging to serogroup O118 with evidence for zoonotic transmission. ***Epidemiol Infect*** 2000; 125: 47-54.
22. Mainil J. Shiga/verocytotoxins and Shiga/verotoxigenic *Escherichia coli* in animals. ***Vet Res*** 1999; 30: 235-257.
23. Albert MJ, Faruque SM, Faruque AS, Neogi PK, Ansaruzzaman M, Bhuyan NA, et al. Controlled study of *Escherichia coli* diarrheal infections in Bangladeshi children. ***J Clin Microbiol*** 1995; 33: 973-977.
24. Kahali S, Sarkar B, Rajendran K, Khanam J, Yamasaki S, Nandy RK, et al. Virulence characteristics and molecular epidemiology of enteroaggregative *Escherichia coli* isolates from hospitalized diarrheal patients in Kolkata, India. ***J Clin Microbiol*** 2004; 42: 4111-4120.
25. Fang GD, Lima AM, Martins CV, Nataro JP, Guerrant RL. Etiology and epidemiology of persistent diarrhea in northeastern Brazil: a hospital-based, prospective, case-control study. ***J Pediatr Gastroenterol Nutr*** 1995; 21: 137-144.

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Farouk AE, Batcha MF, Greiner R, Salleh HM, Salleh MR, Sirajudin AR. The use of a molecular technique for the detection of porcine ingredients in the Malaysian food market. ***Saudi Med J*** 2006; 27: 1397-1400.

Rafay AM, Nsanze HN. Multi-drug resistance of *Escherichia coli* from the urinary tract. ***Saudi Med J*** 2003; 24: 261-264.