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Characterization of diarrheagenic *E. coli* causing a diarrheal outbreak in the south of Iran, Summer 2015Gholamreza Pouladfar¹, Amir Arasteh-Far¹, Maneli Amin-Shahidi¹, Nasrin Firoozian¹, Bahman Pourabbas¹, Mohsen Moghadami^{1,2}, Fereshteh Fani^{1*}¹Professor Alborzi Clinical Microbiology Research Center, Faculty of Medicine, Shiraz University of Medical Sciences, Shiraz, Fars, Iran²Health Policy Research Center, Shiraz University of Medical Sciences, Shiraz, Islamic Republic of Iran

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ABSTRACT

Objective: To perform a laboratory investigation to identify and characterize the causative pathogens of a diarrheal outbreak in the south of Iran in July 2015.**Methods:** Laboratory investigation was done through standard cultures and molecular methods for causative agent and possible source identification. Antibiotic resistant patterns, extended-spectrum β -lactamase (ESBL) production ability and plasmid profiling were used to characterize the isolated pathogens.**Results:** Out of 16 stool samples received in the lab, 14 were positive for enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and non-O157 enterohaemorrhagic *E. coli* (EHEC). Of 5 EIEC isolates, 3 were similar in terms of plasmid patterns and ESBL production ability and virulence genes (*ipaH+* and *virF+*). The EAEC isolates were positive for at least one of two virulence genes, *agg* and *aap*. Out of 7 EAEC isolates, 2 had the same patterns of plasmid profiles, antibiotic resistance and virulence gene (*aap+*). Of the 7 EAEC isolates, four had ESBL production ability. The two non-O157 EHEC isolates were positive for *stx2* virulent gene and were also susceptible to all tested antibiotics.**Conclusions:** Laboratory investigation showed that the outbreak was caused by mixed diarrheagenic *E. coli* pathogroups, possibly due to waste contamination of drinking water.

1. Introduction

The investigation and control of foodborne disease outbreaks are multi-disciplinary tasks requiring expertise in different fields of clinical medicine, epidemiology and microbiology. Outbreaks of foodborne disease in developing countries are poorly investigated because of lack of an integrated system in which different fields collaborate with each other. Recently, a national foodborne surveillance system was conducted by the Center for Communicable Disease Control, Ministry of Health and Medical Education in Iran[1]. According to the report of this surveillance system, from 2006 to 2011, a total of 2250 outbreaks were reported in Iran[1]. Even though the rate of the outbreaks is high, very limited studies regarding laboratory investigation of causative agent and source detection of those outbreak are available[2,3].

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In July 2015, a diarrheal outbreak occurred among the habitants of Sepidan, a city in the south of Iran. It was detected and controlled by the Health Deputy of Shiraz University of Medical Sciences. The aim of this study was to show that laboratory investigation is a valuable tool in identifying causative agent which in turn could be useful in confirming probable source of the outbreak suggested by the local health authority. We performed a laboratory investigation in order to identify the most common bacterial enteric pathogens including *Shigella* spp., *Salmonella* spp., *Campylobacter* spp. and diarrheagenic *E. coli*. We focused on detecting the five pathogroups of *E. coli*, including Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC) and Enterohaemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC) from stool samples of the patients by molecular method and also showing the relatedness of the isolated organisms in terms of antibiotic susceptibility and plasmid profiling.

2. Material and methods

2.1. Epidemiologic investigation

From 16–22 July 2015, Sepidan local health authorities noticed an increased incidence of watery and bloody diarrheal illness

among the Sepidan inhabitants and the visitors who travelled to this city because of the long weekend vacation (18–19 July). The epidemiological investigation was conducted by the local health authorities and the Iranian Centers for Disease Control and Prevention immediately after the outbreak was reported.

Environmental investigation by local health authorities revealed inadequate chlorination of tap water in the region for a few hours during the period of the outbreak. Furthermore, local authority investigation showed that some temporary washrooms (WC) were installed near the source of drinking water of travellers' camps. Therefore, local health authority concluded that inadequate chlorination of tap water and contamination of water supply could be considered as possible source of contamination.

2.2. Laboratory investigation

2.2.1. Samples

Stool samples from patients were collected and sent to the local laboratory for bacterial pathogen detection. Out of all collected stool samples, 16 fresh stool samples (watery and bloody) were sent to our laboratory (Professor Alborzi Clinical Microbiology Research Center, Shiraz, Iran) as microbiology reference lab in order to be examined for possible bacterial causes. The samples were analyzed to detect 4 pathogenic bacteria including *Shigella* spp., *Salmonella* spp., *Campylobacter* spp. and diarrheagenic *E. coli*.

2.2.2. Bacterial isolation

In order to isolate *Salmonella* and *Shigella* species, stool samples were treated in two different ways: they were inoculated directly on MacConkey agar, XLD (Xylose-Lysine-Desoxycholate) and Hektoen Enteric (HE) agar (Merck, Darmstadt, Germany), and were then incubated at 37 °C for 24 h; they were also initially inoculated on GN enrichment broth before being subcultured on XLD and HE agar. Concerning *Campylobacter* spp., the samples were inoculated on the selective medium for this bacteria – Skirrow's medium. The latter was made using Columbia agar, from Biolife, as the base, to which 7% of lysed blood as well as vancomycin, trimethoprim and polymyxin B (Sigma) were added with final concentrations of 0.01, 0.005 and 2500 IU/L, respectively. The plates were then incubated at 42 °C for 2–3 days under microaerophilic conditions (10% CO₂, 5% O₂ and 83% N₂) created in an Anoxomat (MART®, Microbiology B.V.).

All colonies suspected for *Shigella* and *Salmonella* from HE and XLD agar were identified by biochemical tests, and for the confirmation, API 20E (Biomérieux, France) was used. For diarrheagenic *E. coli* identification, all *E. coli* colonies on MAC were preserved in 15% glycerol at –70 °C to be used at a later stage for group typing by PCR. All the colonies suspected for *Campylobacter* were examined in wet smear as well as microscopically after Gram staining.

2.2.3. PCR to detect diarrheagenic *E. coli* virulence genes

The DNA was extracted using the PEG-200 alkaline buffer method[4]. In order to identify different groups of *E. coli*, their associated specific primers were utilized, including *bfp* and *eaeA* for EPEC; *lt* and *st* for ETEC; *ipaH* and *virF* for EIEC; *stx1*, *stx2*, and *eaeA* for EHEC; and *agg* and *aap* for EAEC (Table 1). PCR was performed in the final volume of 50 µL including 5 µL PCR buffer (Thermo Scientific, Maxima Hot Start *Taq* DNA polymerase, EP0602), 2.5 mmol/L of MgCl₂ (Thermo Scientific, Maxima Hot Start *Taq* DNA polymerase, EP0602), 0.4 ng of mixed dNTP (Thermo Scientific, R0192), 15 picomol of each primer (Bioneer, South Korea), 2.5 IU of *Taq* polymerase (Thermo Scientific, Maxima Hot Start *Taq* DNA polymerase, EP0602), and 2 µL of template. The solutions were then subjected to the following cycling

condition: 94 °C for 5 min, 94 °C for 30 s, 55 °C for 30 s (for *st* gene, the optimal annealing was at 50 °C), 72 °C for 30 s (35 cycles), and a final extension step (72 °C for 8 min) in a thermal cycler (Applied Biosystem, Veriti). Subsequently, 8 µL of the PCR product was subjected to gel electrophoresis (Biorad, Wide mini-sub® Cell GT) employing 1.5% agarose (Invitrogen, 16500), stained by means of GelRed Nucleic Acid Gel Stain (Biotium, 41002), and visualized by gel documentation (UVItect, DBT-08). In each PCR run, genomic DNA from *E. coli* ATCC 35401 (*lt+*, *st+*), *E. coli* ATCC 43887 (*stx1-*, *stx2-*, *eae+*), *E. coli* containing pCVD432 (*aggR+*, *aap+*), and *E. coli* O157:H7 ATCC 43894 (*stx1+*, *stx2+*, *eae+*) were used as positive controls.

Table 1

List of primers used in this study.

<i>E. coli</i> category tested strains	Locus	Primers	Amplicon size (bp)	Ref
EPEC	<i>bfp</i>	F: AATGGTGCTTGCCTTGCTGC	326	[5]
		R: GCCGCTTTATCCAACCTGGTAAG		
	<i>eaeA</i>	F: GACCCGGCACAAGCATAAGC	384	[6]
		R: CCACCTGCAGCAACAAGAGG		
ETEC	<i>lt</i>	F: GGCGACAGATTATACCGTGC	450	[6]
		R: CGGTCTCTATATCCCTGTT		
	<i>st</i>	F: ATTTTCTTTCTGTATTATCTTT	190	[6]
		R: CCGGTACAAGCAGGATTACA		
EIEC	<i>ipaH</i>	F: ATGCGAGAAATTAATATGCTCAG	786	This study
		R: GAATAGCAGAGTTTGATCTGATAAG		
	<i>virF</i>	F: AGCTCAGGCAATGAACTTTGAC	618	[6]
		R: TGGGCTTGATATCCGATAAGTC		
STEC	<i>stx1</i>	F: TTCAGCAAGTGCCTGGCGA	212	This study
		R: CGCTGAATCCCCCTCCATTA		
	<i>stx2</i>	F: GGCGCGTTTTGACCATCTCG	518	This study
		R: GATGATGGCAATTCAGTATAACG		
EAEC	<i>aggR</i>	F: GTATACACAAAAGAAGGAAGC	254	[6]
		R: ACAGAATCGTCAGCATCAGC		
	<i>aap</i>	F: GGCATCTTGGGTATCAGCCTG	313	This study
		R: CCCATTCGGTTAGAGCACTATATT		

E. coli strains that were positive for *aggR* and/or *aap* genes were interpreted as being EAEC and those positive for *stx1* and *stx2*, as STEC. The EIEC were those positive for *virF* and/or *ipaH*[7,8].

2.2.4. Antimicrobial susceptibility testing

Using the Kirby Bauer disc diffusion method, antibiotic susceptibility to commercially available antibiotics (Rosco Neo-Sensitabs Denmark) was determined according to CLSI 2014 guidelines[9]. The antibiotics included cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), ampicillin (10 µg), amikacin (30 µg), co-trimoxazole (25 µg), gentamicin (10 µg), ciprofloxacin (5 µg), meropenem (10 µg) and piperacillin-tazobactam (100–10 µg/mL).

2.2.5. Extended spectrum beta-lactamase (ESBL) production detection

Isolates that were resistant to cefotaxime or ceftazidime were screened for their ability to produce ESBL through the CLSI combination disc method[9], using discs of cefotaxime and ceftazidime along with those with clavulanic acid added. The zone diameters were determined using the HiAntibiotic zone scale (Himedia). A zone diameter of ≥ 5 mm for the latter disc, which was larger than that for either of the agents tested alone, was taken as evidence for a positive ESBL production.

2.2.6. Serotyping of *E. coli* isolates

All *E. coli* isolates were tested for the presence of O157, H7 antigens by agglutination using O157 and H7 commercially available antisera (Microgen Bioproducts, UK).

2.2.7. Plasmid DNA extraction

Plasmid DNA was extracted using QIAprep® Miniprep kit from all *E. coli* isolates. Subsequently extracted plasmid DNA was subjected to gel electrophoresis (Biorad, Wide mini-sub® Cell GT) employing 0.7% agarose (Invitrogen, 16500), stained by means of GelRed Nucleic Acid Gel Stain (Biotium, 41002), and visualized by gel documentation (UVItec, DBT-08). The similarities among the isolates on the basis of their plasmid profiles were analyzed.

3. Results

3.1. Bacterial culture

Sixteen stool samples received in our laboratory were tested for 4 species of bacteria (*E. coli*, *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp.). All stool cultures were negative for *Salmonella* and *Campylobacter* species, whereas two green colonies from HE agar were identified as *Shigella sonnei* (*S. sonnei*) by API20E. In order to re-confirm the isolated *S. sonnei* strains, *S. sonnei* antisera (Microgen Bioproducts, UK) were used. The results of serotyping were not in agreement with the API20E ones. These two isolates would be used at a later stage for *E. coli* group-typing by PCR. A total of twenty-two isolated *E. coli* strains from stool cultures were also subjected to PCR for *E. coli* virulence genes.

3.2. *E. coli* group-typing and serotyping

In total 24 isolates were tested for *E. coli* pathogroups. The PCR detected 14 diarrheagenic *E. coli* strains. They were categorized in three groups including EAEC, EIEC and STEC-non EHEC (Table 2).

Table 2

Classification of diarrheagenic *E. coli* according to amplicon(s) produced by PCR for virulence-associated determinants.

Groups	Virulence associated determinant ^a									
	<i>aggR</i>	<i>aap</i>	<i>ipaH</i>	<i>virF</i>	<i>eae</i>	<i>bfp</i>	<i>lt</i>	<i>st</i>	<i>stx1</i>	<i>stx2</i>
EAEC ^b										
1	-	+	-	-	-	-	-	-	-	-
2	+	+	-	-	-	-	-	-	-	-
3	+	-	-	-	-	-	-	-	-	-
4	+	+	-	-	-	-	-	-	-	-
5	-	+	-	-	-	-	-	-	-	-
6	-	+	-	-	-	-	-	-	-	-
7	-	+	-	-	-	-	-	-	-	+
EIEC ^c										
1	-	-	+	+	-	-	-	-	-	-
2	-	-	+	+	-	-	-	-	-	-
3	-	-	+	-	-	-	-	-	-	-
4	-	-	+	+	-	-	-	-	-	-
5	-	-	-	+	-	-	-	-	-	-
STEC, not EHEC ^d										
1	-	-	-	-	-	-	-	-	-	+
2	-	-	-	-	-	-	-	-	-	+

^a: Virulence genes associated with the *E. coli* groups. *aggR*: Aggregative fimbria; *aap*: Antiaggregation protein (dispersion); *ipaH*: Invasion plasmid antigen; *virF*: Virulence invasion factor; *eae*: Intimin; *bfp*: Bundle-forming pilus; *lt*: Heat-labile enterotoxin; *st*: Heat-stable enterotoxin; *stx*: Shiga toxin; ^b: EAEC, enteroaggregative *E. coli*, *aggR* or *aap* positive; ^c: EIEC, enteroinvasive *E. coli*, *ipaH* or *virF* positive; ^d: STEC, not EHEC, *stx1* or *stx2* positive, *eae* negative; STEC: Shiga-toxin producing *E. coli*; EHEC: Enterohemorrhagic *E. coli*.

Seven out of 14 diarrheagenic *E. coli* strains were positive for *aggR* and/or *aap* genes and were interpreted as being EAEC. Among the 14 diarrheagenic *E. coli*, five were considered as being EIEC since they were positive for either *ipaH* or *virF* genes. The two suspected *S. sonnei* isolates were identified as being EIEC according to PCR results. Two isolates were identified as STEC-non EHEC, and were positive only for *stx2* gene in PCR and negative for O157, H7 antigens. No ETEC or EPEC groups were detected among all tested *E. coli* isolates. None of the 24 *E. coli* isolates was positive for O157 and H7 antigens.

3.3. Antimicrobial susceptibility testing and ESBL production ability

Antimicrobial susceptibility testing was done on all 14 diarrheagenic *E. coli* isolates. Table 3 presents the rate of resistance of isolated pathogens to 10 different antibiotics as well as their ability to produce ESBL. Most of the isolated *E. coli* were resistant to ampicillin (78.6%). All *E. coli* isolates belonged to five patterns: pattern A (resistant to ampicillin, cefotaxime, ceftriaxone and ceftazidime) with three members (EAEC1, EAEC7 and EIEC4); pattern B (resistant to ampicillin, sulfamethoxazole-trimethoprim, cefotaxime, ceftriaxone and ceftazidime) with four members (EAEC3, EIEC1, EIEC2 and EIEC4); pattern C (resistant to ampicillin, sulfamethoxazole-trimethoprim, cefotaxime, ceftriaxone and ceftazidime and piperacillin-tazobactam) with only one member (EAEC2); pattern D (resistant to ampicillin, sulfamethoxazole-trimethoprim, cefotaxime, ceftriaxone and ceftazidime, ciprofloxacin and piperacillin-tazobactam) with only one member (EIEC3); pattern E (resistant to ampicillin) with two members (EAEC5 and EAEC6) and finally pattern F (susceptible to all tested drugs with no evidence of resistance) with three members (EIEC5, STEC1 and STEC2). There was no evidence of resistance to meropenem, amikacin and gentamycin. Resistance to piperacillin-tazobactam and ciprofloxacin was found in 1 and 2 isolates, respectively. The rate of ESBL positive cases in EAEC and EIEC isolates was 57.1% (4/7) and 80% (4/5), respectively.

Table 3

Antibiotic susceptibility pattern of the pathogens isolated from Sepidan outbreak.

Pathogens	Antibiotics										ESBL
	AMP	CTX	CAZ	CRO	MRP	SXT	AMK	GM	CIP	PTZ	
EAEC1	R	R	R	R	S	S	S	S	S	S	Pos
EAEC2	R	R	R	R	S	R	S	S	S	R	Pos
EAEC3	R	R	S	R	S	R	S	S	S	S	Pos
EAEC4	R	S	S	S	S	S	S	S	S	S	Neg
EAEC5	R	S	S	S	S	S	S	S	S	S	Neg
EAEC6	R	S	S	S	S	S	S	S	S	S	Neg
EAEC7	R	R	R	R	S	S	S	S	S	S	Pos
EIEC1	R	R	R	R	S	R	S	S	S	S	Pos
EIEC2	R	R	R	R	S	R	S	S	S	S	Pos
EIEC3	R	R	R	R	S	R	S	S	R	R	Pos
EIEC4	R	R	R	R	S	R	S	S	S	S	Pos
EIEC5	S	S	S	S	S	S	S	S	S	S	Neg
STEC1	S	S	S	S	S	S	S	S	S	S	Neg
STEC2	S	S	S	S	S	S	S	S	S	S	Neg

The pattern of antibiotic susceptibility and ESBL production ability of isolated diarrheagenic *E. coli* are shown in this table. The number after each group refers to the number of isolates in that group. AMP: Ampicillin; CTX: Cefotaxime; CAZ: Ceftazidime; CRO: Ceftriaxone; MRP: Meropenem; SXT: Sulfamethoxazole-trimethoprim; AMK: Amikacin; GM: Gentamycin; CIP: Ciprofloxacin; PTZ: Piperacillin-tazobactam. Pos: Positive; Neg: Negative.

3.4. Plasmid profiling

The extracted plasmid which appeared as bright bands mostly between 2–10 kb on the gel was used in the typing analysis. Figure 1 shows the plasmid patterns of the *E. coli* isolates. Analysis of plasmid DNA of diarrheagenic *E. coli* isolates revealed that all isolates had at least one plasmid and the number of plasmid varied from 1 to 13. In total 7 different profiles were identified among all isolates. Profile 1 with only one band greater than 10 kb includes three EAEC strains (1, 3 and 4) and STEC2. Out of 5 EIEC strains, 4 isolates (1, 2, 4 and 5) had similar plasmid patterns (profile 2) with 13 bands. In this profile, most plasmids ranged from 2 to 10 kb, and one plasmid was smaller than 2 kb and 2 plasmids larger than 10 kb. The EAEC5 and EAEC6 with similar patterns were included in profile 3 with 4 plasmids. Out of the 4 plasmids of profile 3, one was larger than 10 kb and the rest were in the range of 2–4.5 kb. The 4 remaining *E. coli* isolates (EAEC2, EAEC7, STEC1 and EIEC3) had four different plasmid profiles.

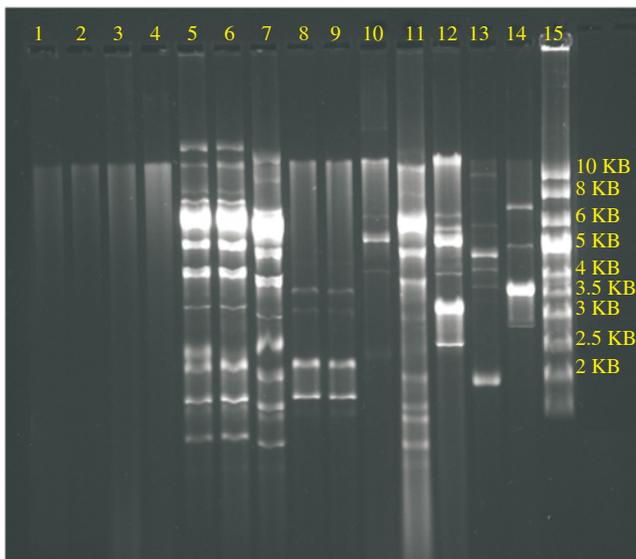


Figure 1. Plasmid profiling of the isolated *E. coli* strains.

Plasmid profiles of the 14 *E. coli* strains isolated from the outbreak, in 0.7% agarose. Lane 1: EAEC (strain 1); Lane 2: EAEC (strain 3); Lane 3: EAEC (strain 4); Lane 4: STEC (strain 2); Lane 5: EIEC (strain 1); Lane 6: EIEC (strain 2); Lane 7: EIEC (strain 5); Lane 8: EAEC (strain 5); Lane 9: EAEC (strain 6); Lane 10: EAEC (strain 7); Lane 11: EIEC (strain 4); Lane 12: EAEC (strain 2); Lane 13: EIEC (strain 3); Lane 14: STEC (strain 1); Lane 15: Supercoiled DNA ladder (2–10 kb).

4. Discussion

Cases were defined as gastroenteritis with diarrhea in any people referred to local health service during 16–22 July, 2015. All patients presented with gastrointestinal complaints such as bloody and watery diarrhea and abdominal pain. The bacterial strains including EIEC, EAEC and STEC-non EHEC were isolated from fecal samples of cases. During environmental investigation local health authorities noticed that inadequate chlorination of tap water had occurred in the region for a few hours. Furthermore, local authority investigation showed that some temporary washrooms (WC) were installed near the source of drinking water of travellers' camps. Environmental investigation excluded food as a possible source of the outbreak, because it was observed that there were patients among those who

ate fast-food, those who ate food in restaurant, and those who ate food brought from home. All of these observations were indicative of a possible waterborne infection even though tap water sample examination at the local laboratory had shown no bacterial growth. Although causative agents of this outbreak from tap water could not be isolated, (we did not receive any suspected water samples) the detection of fecal coliforms from patients' samples was consistent with local health authority report, suggesting contamination of tap water with waste water as a possible source of infection in this outbreak. Mixed enteropathogens in clinical samples of cases with gastroenteritis outbreak have already been reported in Switzerland, Finland and Chile^[10-12]. The source of both outbreaks in Switzerland and Finland was reported to be defective waste water system and sewage contaminated drinking water due to observation of fecal coliforms, respectively.

Many resources are available for the foodborne diseases outbreaks, but too often the outbreaks of foodborne disease remain unrecognized or are not investigated in developing countries because few of the available resources are directed in such countries. Diarrheagenic *E. coli* has been considered as one of the most common causative agents for diarrhea among sporadic cases across the country both in children and adults^[13-16]. To our knowledge this is the first report of diarrheagenic *E. coli* as a causative agent of gastroenteritis outbreak in Iran. However, several outbreaks of gastroenteritis due to diarrheagenic *E. coli* have been previously reported in Japan, Korea, China, Brazil and the Kenya^[17-22].

Antimicrobial resistance patterns are valuable as a guide to empirical therapy, as a typing method, and as an indicator of the dissemination of antimicrobial resistance determinants. By analyzing trends in the resistance patterns of the various *E. coli* isolates, we found that 66.6% (8/12) of the EAEC and EIEC isolates were able to hydrolyze the third generation cephalosporins through ESBL production, while resistance to ciprofloxacin was found only in 7.1% (1/12) of the EAEC and EIEC isolates (Table 3). Interestingly, the similar high rate of ESBL production was observed among diarrheagenic *E. coli* isolated from patients with acute invasive diarrhea referred to emergency departments during the period of 7 months in Shiraz, Iran (unpublished data). Based on antibiotic susceptibility results, STEC-non EHEC isolates were sensitive to all tested antibiotics, whereas the rate of antibiotic resistance in EIEC and EAEC was high (Table 3). The common practice in the country, on an empirical basis, is the third generation of cephalosporins for treatment of acute invasive diarrhea. However, the high rate of ESBL phenotype in the diarrhea-causing *E. coli* in the present study calls for reconsideration of the current empirical therapy protocols for acute invasive diarrhea. As in our study, ciprofloxacin was shown to be more effective *in vitro* against the diarrhea-causing pathogens, and one possibility of changes to therapy protocols would be replacing the currently used cephalosporins with this antibiotic which had been recommended by WHO in 2005, as drug of choice for the treatment of shigellosis for every patient irrespective of their age^[23].

In order to find the possible relatedness of the 14 *E. coli* strains isolated from different patients in the present outbreak, plasmid profiling which has been proven as a useful method of typing for epidemiological studies was used^[24,25]. Based on plasmid profiling, plasmid patterns were similar in 4 EIEC, from which 3 also had the same pattern of antibacterial resistance. Concerning the 7 EAEC strains, 2 strains had the same plasmid and antibiotic resistance

patterns. Of the remaining 5 EAEC strains, 3 isolates showed similar plasmid profiles but not exactly the same pattern of resistance. We found plasmid profiling as a more powerful method of typing compared to antibiotic susceptibility since they distinguished 7 and 5 different patterns, respectively. This has already been shown in identification of *Shigella* epidemic strains in Iran[25].

Information bias due to missing epidemiological data could be considered a limitation of the study. The lack of tap water sample as a possible source of outbreak for laboratory analysis could also influence the results of our investigation.

This study showed that the laboratory investigation along with epidemiological and environmental investigation could be a valuable tool for source identification of the foodborne disease outbreaks. The results of this study could compel decision-makers at national and regional levels in Iran to establish an outbreak control system including laboratory, epidemiological and environmental expertise in order to successfully manage the foodborne disease outbreaks.

Conflict of interest statement

We declare that we have no conflict of interest.

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