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ORIGINAL ARTICLE

Molecular and phenotypic characterization of atypical enteropathogenic *Escherichia coli* serotypes isolated from children with and without diarrhea

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Background: We characterized 36 atypical enteropathogenic *Escherichia coli* (EPEC) serotypes isolated from children with and without diarrhea in Iran. Because the identification of atypical EPEC based on biochemical features is rather difficult and time consuming, we used a combination of three approaches, including a polymerase chain reaction-based method, culture adherence assay, and the restriction analysis of *fliC* gene (*fliC*-restriction fragment length polymorphism), to identify *E coli* serotypes.

Methods: To distinguish typical and atypical EPEC strains, the presence of EPEC attaching effacing A gene (*eaeA*) gene and EPEC-attaching factor (EAF) plasmid were analyzed. All *E coli* strains were identified based on the detection of the *eaeA*⁺, bundle-forming pili A gene (*bfpA*⁻), EAF⁻ or *eaeA*⁻, *bfpA*⁺, EAF⁻ profiles and the absence of *stx* (encoded for shiga toxin) gene as atypical EPEC.

Results: All strains studied belonged to 5 atypical EPEC serogroups and 15 serotypes based on the virulence profiles. Of 36 atypical EPEC serotypes, 22 (61.2%) and 14 (38.8%) strains isolated from diarrheal and healthy cases, respectively. O142:H48 (19.5%) and O111:H21 (11.1%) serotypes were the most prevalent isolates, followed by serotypes O111: H⁻ and O86:H48 (5.6% each).

Conclusions: The characteristics of the atypical EPEC serotypes from children with diarrhea were significantly different from those without diarrhea. The compilation of data on atypical

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EPEC strains presented here indicates the importance of a combined approach of conventional and molecular tests to study the virulence and epidemiology of EPEC serotypes in human subjects.

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Introduction

Enteropathogenic *Escherichia coli* serotypes (O:H types) have been associated with infantile diarrhea and are responsible for morbidity and mortality in developing countries.^{1,2} Diarrhea because of EPEC is the result of signals triggered by the pathogen-host membrane interaction stimulating reorganization of the cytoskeleton of the affected cell, involved in accumulation of polymerized actin, loss in microvillus structure, and effacement of the intestinal villi.^{2–4} The attaching and effacing (A/E) lesion encoded by genes in a pathogenicity island of the EPEC chromosome is called the locus of enterocyte effacement. This region encodes an outer membrane protein (intimin) and proteins of a Type III secretion system.^{1,4,5} Bundle-forming pili (BFP) encoded by the EPEC-attaching factor (EAF) plasmid promote the localized adherence of bacteria to epithelial cells but are not essential for the development of A/E lesions. Furthermore, they facilitate the occurrence of the A/E lesion.^{1,6,7} *E coli* strains, which are able to induce the A/E lesion and are *stx* gene (encoded for shiga-like toxin) negative, are considered to be EPEC strains. The EPEC strains that harbor the EAF plasmid are designated as typical EPEC, and those without the plasmid are called atypical EPEC.¹ Epidemiological studies have indicated that atypical EPEC serotypes are associated with human and animal hosts and are considered to be human emerging pathogens.^{2,8–10} Typical and atypical EPEC strains belong to two different sets of serotypes and show different adherence patterns.² The typical strains present only the localized adherence (LA) pattern, whereas atypical strains may have the localized-adherence like (LAL) pattern,¹¹ the diffuse adherence (DA), or the aggregative adherence (AA) pattern. The LAL pattern is a characteristic of the strains of most serotypes and is mediated mainly by intimin.¹² EPEC strains can be identified by the detection of virulence genes, but identification of serotypes is also important for epidemiological purposes. Certain serotypes of *E coli* are more associated with some of virulence factors than the others.¹³ Recent studies have showed that the restriction analysis of *fliC* gene (encoding flagelin) can be used to type motile and nonmotile *E coli* serotypes.^{14–16} Although in our previous study¹⁷ the association of atypical EPEC serogroups with diarrhea was statistically significant, more characterization of the strains are needed to elucidate the role of atypical EPEC serotypes in diarrheal diseases among Iranian children. The objective of the present study was to characterize atypical EPEC serotypes by phenotypic and molecular methods. It was anticipated that such a study may also provide a baseline for the presence of the virulence factors characterizing *E coli* strains, which are still important causes of morbidity and mortality among infants and children in the developing world.

Methods

Bacterial strains

A total of 36 EPEC strains isolated from children (less than 10 years old) with and without diarrhea were included in this study.

Examination of virulence genes by polymerase chain reaction

All strains were examined by polymerase chain reaction (PCR) with the specific primers for the presence of the virulence genes; *eaeA*,¹⁸ *bfpA*,¹⁹ *stx*,²⁰ and EAF²¹ plasmid. Prototype EPEC strain 2348/69 (serotype O127: H6), which expressed intimin, BFP, and EAF and *E coli* EDL933 (*stx*⁺) strain was used as positive control in PCR assays, and *E coli* HB101 was included in the experiment as a negative control.

EPEC serotyping

Serotyping was performed by standard procedure²² using antisera to EPEC flagellar H antigens—H1—H56. Flagellar H antigens were identified by agglutination test using H-specific antisera according to the manufacturer's instruction (Statens Serum Institut, Copenhagen, Denmark).

fliC PCR-restriction fragment length polymorphism analysis

The protocol developed by Machado et al.¹⁴ was used with some modifications. The entire coding sequence of the *fliC* gene was amplified by PCR with specific primers.¹⁴ The amplified *fliC* gene was digested with *HhaI* restriction endonuclease (Roch) and incubated overnight at 37°C. The restriction fragments were separated by electrophoresis in 2% agarose gel (1% standard agarose, SIGMA and 1% Metaphor agarose, Cambrex, Rockland, ME, USA) in Tris-borate buffer (0.089 M Tris-base, 0.089 M boric acid, 2.5 mM EDTA-Na₂, pH 8), for 5 hours at 5 V/cm. The 100 bp (Fermentas) and 50 bp (Roch) DNA ladders were used as molecular size markers. Digitization and interpretation of patterns was carried out using the Taxotron package (Taxolab, Institute Pasteur, Paris, France).

Adherence assays

The test to detect adherence to HeLa cells (National cell Bank of Iran, Institute Pasteur of Iran) was performed as described by Scaletsky et al.²³ *E coli* strains showing no adherence after a period of 3 hours of incubation were

submitted to a 6-hour adherence test to D-mannose (1% wt/vol). EPEC strain 2348/69 (serotype O127: H6), *E coli* strains E17-2 (serotype O3: H2), and C1845 (serotype O75: NM) showing LA, AA, and DA were used respectively as positive control in adherence assays and *E coli* K12 was used as a negative control.

Results

Bacterial strains

All *E coli* strains were identified based on the detection of the *eaeA*⁺, *bfpA*⁻, *EAF*⁻ or *eaeA*⁻, *bfpA*⁺, *EAF*⁻ profiles and the absence of *stx* gene as atypical EPEC. The 36 *E coli* strains studied belonged to five EPEC serogroups (O86, O111, O114, O127, and O142) based on these virulence profiles. EPEC serogroups were isolated as the sole pathogen from 61.2% (22 of 36) of children with diarrhea compared with 38.8% (14 of 36) in those without diarrhea (Table 1).

fliC RFLP patterns

To investigate the *fliC* restriction fragment length polymorphism (RFLP) patterns (F-types) of 36 atypical EPEC clinical isolates, 32 motile and 4 nonmotile strains (Table 1) were subjected to PCR with specific primers¹⁴ and subsequently were digested with *HhaI*. All strains demonstrated a single PCR product of 0.9–2.6 kb. After digestion with *HhaI*, 17 different F-type were observed among the 36 motile and nonmotile strains (Table 1). When a particular H type showed two restriction patterns, the letter “a” or “b” was used to designate these patterns. The *fliC* genes H6, H10, H11, H17, H19, H20, H24, H28, H33, H36, H47, and H48 showed a single and specific restriction pattern, whereas the *fliC* genes H2, H4, H8, H9, and H21 showed two patterns (a and b). The pattern “b” was predominant among the different H types. We found that four (11.1%) nonmotile strains presented RFLP patterns identical to some of those observed for the motile strains.

Table 1 Characterization of atypical EPEC strains isolated from healthy infants and diarrheal cases

Genetic profile	Adherence pattern	Clinical status	F-type	Serotype (O:H)	Serogroups (n) ^a
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F17	H17	O86 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F48	H48	O86 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	A	F8b	H8	O111 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F24	H24	O111 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	A	F9a	H9	O111 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F9a	H ⁻	O111 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	A	F21b	H21	O111 (2)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F21b	H21	O111 (2)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F48	H48	O111 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	A	F6	H ⁻	O111 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	A	F19	H19	O111 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	A	F17	H17	O111 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	A	F10	H10	O114 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F10	H10	O127 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F28	H28	O127 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F21b	H ⁻	O127 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	A	F4a	H4	O127 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F21b	H21	O127 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	A	F47	H47	O127 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F2a	H2	O127 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F33	H ⁻	O142 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	A	F6	H6	O142 (1)
<i>eaeA</i> ⁺ / <i>bfpA</i> ⁻	LAL	D	F48	H48	O142 (3)
<i>eaeA</i> ⁻ / <i>bfpA</i> ⁺	NA	D	F48	H48	O86 (1)
<i>eaeA</i> ⁻ / <i>bfpA</i> ⁺	NA	A	F20	H20	O111 (1)
<i>eaeA</i> ⁻ / <i>bfpA</i> ⁺	UDP	D	F6	H6	O127 (1)
<i>eaeA</i> ⁻ / <i>bfpA</i> ⁺	UDP	D	F48	H48	O142 (3)
<i>eaeA</i> ⁻ / <i>bfpA</i> ⁺	NA	A	F48	H48	O142 (1)
<i>eaeA</i> ⁻ / <i>bfpA</i> ⁺	UDP	D	F36	H36	O142 (1)
<i>eaeA</i> ⁻ / <i>bfpA</i> ⁺	NA	A	F11	H11	O142 (1)

^a All strains are *EAF* and *Stx* negative.

A = asymptomatic person; *bfpA* = bundle-forming pili A gene; D = diarrhea; *eaeA* = EPEC attaching effacing A gene; *EAF* = EPEC-attaching factor; EPEC = enteropathogenic *Escherichia coli*; LAL = localized adherence-like; NA = nonadherence; *Stx* = shiga toxin; UDP = undefined pattern.

Analysis of results between H serotypes and *fliC* RFLP pattern

We did not find any difference between H serotypes and the *fliC* RFLP patterns. The 36 atypical EPEC strains belonged to five serogroups and 15 serotypes (Table 1). Four nonmotile isolates with different O-types (O111, O127, and O142) were also studied. A cryptic or nonexpressed *fliC* gene was amplified in all cases and F-patterns were easily identified (Table 1). Serotypes O142:H48 (19.5%) and O111:H21 (11.1%) were the most prevalent, followed by serotypes O111:H⁻ and O86:H48 (5.6% each). The serotypes O111:H21, O111:H⁻ and O142:H48 were isolated from both healthy and diarrheal cases; and serotype O86:H48 was only identified in cases with enteritis (Table 1).

Virulence factors in *E coli* strains with different *fliC* RFLP patterns

All strains showed a positive reaction with one or more primers for *eaeA*, *bfpA*, and *EAF* genes. On the basis of these positive reactions, two different combinations of virulence factor genes were identified: *eaeA*⁺, *bfpA*⁻, *EAF*⁻, *stx*⁻, and *eaeA*⁻, *bfpA*⁺, *EAF*⁻, *stx*⁻ (Table 1). These profiles were recognized as the definitive combination for atypical EPEC. The distribution of these virulence genes according to the F types identified is shown in Table 1. Of the 17 *fliC* RFLP patterns, 12 (70.6%) showed the characteristic atypical EPEC combination of *eaeA*⁺, *bfpA*⁻, *EAF*⁻, *stx*⁻, 3 (17.6%) *eaeA*⁻, *bfpA*⁺, *EAF*⁻, *stx*⁻, and 2 (11.8%) two profile combinations.

Adherence pattern

The adherence phenotypes (Fig. 1) and combinations of virulence genes are summarized in Table 1 for each F type. Out of 36 atypical EPEC isolates, 32 (88.9%) adhered to HeLa cells and 4 (11.1%) did not (Fig. 1C). Strains with *eaeA*⁺, *bfpA*⁻, *EAF*⁻, *stx*⁻ were usually of LAL pattern (Fig. 1A), characterized by the presence of less-compact clusters of bacteria observed only after 6 hours of incubation, but the strains with the *eaeA*⁻, *bfpA*⁺, *EAF*⁻, and *stx*⁻ profile displayed a characteristic adherence patterns that were different from any pattern described in the literature and were therefore assigned the undefined pattern (Fig. 1B).

Discussion

Regarding the ability of atypical EPEC to cause diarrhea, it is recommended that more studies are necessary to elucidate their virulence mechanisms because doubt still exists as to the pathogenic role of some of these serotypes. Recently, it has been described that atypical EPEC strains hybridize with the *eae* probe but not with the *EAF* probe.²⁴

In our study, 36 *E coli* strains are classified as atypical EPEC based on *eaeA*⁺, *bfpA*⁻, *EAF*⁻, and *stx*⁻ or *eaeA*⁻, *bfpA*⁺, *EAF*⁻, and *stx*⁻ profiles. The *eaeA*⁺, *bfpA*⁻, and *EAF*⁻ patterns are the most frequently (75%) observed array of virulence factors in this study. This combination was characteristic of strains, which showed usually LAL adherence

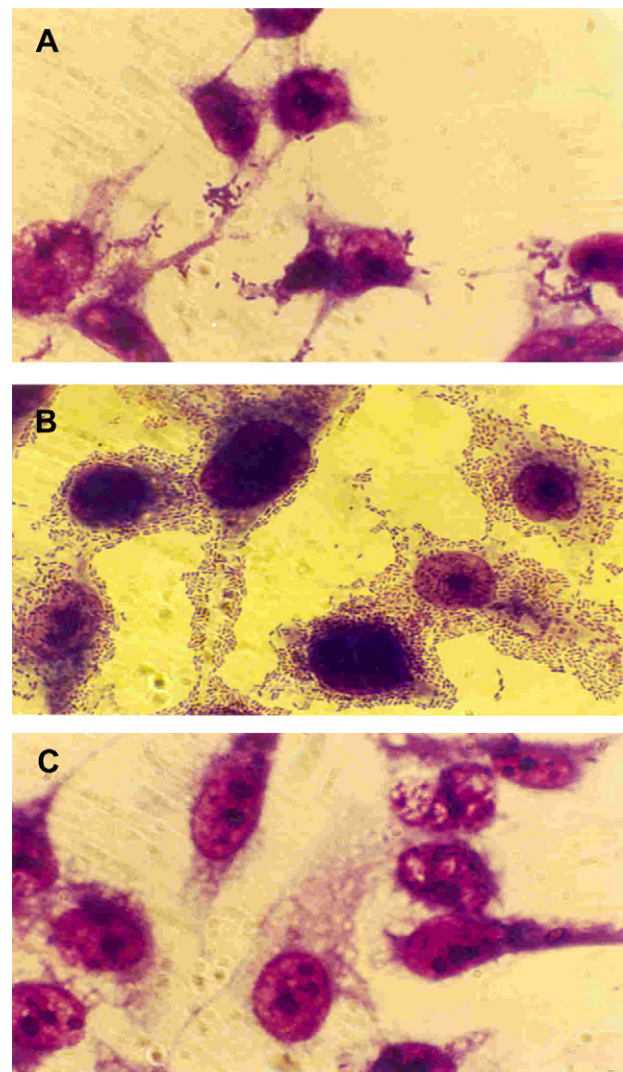


Figure 1. HeLa cell adherence assay with atypical EPEC strains. (A) Localized adherence-like pattern; (B) Undefined adherence pattern; (C) Nonadherence pattern. EPEC = enteropathogenic *Escherichia coli*.

phenotype (which appears after 6 hours of incubation). Regarding the LAL strains, it has been generally accepted that the adhesion of EPEC (not atypical strains) to cultured epithelial cells is mediated initially by BFP and later by intimin.¹² As all 27 LAL strains lacked BFP, it is likely that the delayed appearance and the weaker density of this pattern are because of the lack of BFP production by these strains. It is noteworthy that these *eaeA*⁺ strains are isolated with relatively high frequency from patients with sporadic diarrhea.¹¹ Of the 36 atypical strains, 9 (25%) showed *eaeA*⁻, *bfpA*⁺, and *EAF*⁻ profile with nonadherence or undefined pattern. The absence of intimin may explain why *bfpA*⁺ strains did not show LAL pattern.

Atypical EPEC strains belonged to different serotypes (Table 1). A number of H antigens, including H2, H8, H11, H21, H30, H32, H36, and H46 have previously been identified by conventional H serotyping in *E coli* strains that cause diarrhea in humans.^{25,26} Using *fliC*-RFLP, we have demonstrated that 36 atypical EPEC strains isolated from patients

and healthy cases belonged to 17 different H serotypes possessing *fliC* genes. To avoid difficulties associated with the conventional H serotyping, the *fliC* RFLP method was used primarily for the detection and identification of the H7 antigen in nonmotile shiga toxin producing *E coli* O157 strains.²³ We demonstrated that the *fliC* RFLP is a reliable, rapid, and easy-to-perform method for determination of the H types of *E coli* clinical isolates, including nonmotile strains belonging to the different H clone untypable by serotyping. The results showed that the *fliC* RFLP method is superior to H serotyping because it correctly identified the H types of all strains investigated. The *fliC* RFLP method, which allows determination of the H types of isolates in 48 hours, can be particularly used in epidemiological investigations when prompt information about the H type is needed.

A marker of EPEC pathogenicity is the presence of the EAF plasmid, which codes for the fimbria denoted BFP, responsible for the LA pattern characteristic of typical EPEC.¹ None of the strains isolated here was positive in the PCR assay for EAF, although 9 of 36 were positive for *bfpA*. Serotypes isolated from children's diarrhea, such as the atypical O119:H2 and the typical O142:H6, have been reported as EAF-negative, *bfpA*-positive EPEC strains in Brazil,^{2,27} despite the importance of the EAF plasmid for the regulation of chromosomal genes involved in the form of the A/E lesion.²⁸ Serotypes O86:H48, O86:H17, O127:H10, O127:H21, O127:H6, O127:H2, O111:H48, O111:H24, and O142: H⁻ was only identified in cases with enteritis. Thus, this study confirms the view that certain serotypes of atypical EPEC may be more virulent for human than other serotypes.

In conclusion, our results highlights the wide variation in atypical EPEC serotypes in these strains and demonstrate advantage of this valuable strain collection, and further analysis of atypical EPEC strains in relation to their virulence and epidemiology is needed to assess their significance as human pathogens.

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References

- Ochoa TJ, Barletta F, Contreras C, Mercado E. New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. *Trans R Soc Trop Med Hyg* 2008;**102**:852–6.
- Trabulsi LR, Keller R, Gomes TAT. Typical and atypical enteropathogenic *Escherichia coli*. *Emerg Infect Dis* 2002;**8**: 508–13.
- Frankel G, Phillips AD, Trabulsi LR, Knutton S, Dougan G, Matthews S. Intimin and the host cell—is it bound to end in Tir (s)? *Trends Microbiol* 2001;**9**:214–8.
- Vallance BA, Finlay BB. Exploitation of host cells by enteropathogenic *Escherichia coli*. *Proc Natl Acad Sci USA* 2000;**97**: 8799–806.
- Kozub-Witkowski E, Krause G, Frankel G, Kramer D, Appel B, Beutin L. Serotypes and virutypes of enteropathogenic and enterohaemorrhagic *Escherichia coli* strains from stool samples of children with diarrhoea in Germany. *J Appl Microbiol* 2008; **104**:403–10.
- Donnenberg MS, Kaper JB. Enteropathogenic *Escherichia coli*. *Infect Immun* 1992;**60**:3953–61.
- Girón JA, Ho ASY, Schoolnik GK. An inducible bundle-forming pilus of enteropathogenic *Escherichia coli*. *Science* 1991;**254**: 710–3.
- Joel MA, Tabarelli GF, Aranda KRS, Fabbriotti SH, Fagundes-Neto U, Mendes CMF, Scaletsky I C.A. Typical Enteropathogenic and Atypical Enteropathogenic Types of *Escherichia coli* Are the Most Prevalent Diarrhea-Associated Pathotypes among Brazilian Children. *J Clin Microbiol* 2007;**45**: 3396–9.
- Vieira MAM, Andrade JRC, Trabulsi LR, Rosa ACP, Dias AMG, Ramos SRTS, et al. Phenotypic and genotypic characteristics of *Escherichia coli* strains of non-enteropathogenic *E. coli* (EPEC) serogroups that carry *eae* and lack the EPEC adherence factor and shiga toxin DNA probe sequences. *J Infect Dis* 2001;**183**: 762–72.
- Viljanen MKT, Peltola T, Junnila SYT, Olkkonen L, Jarvinen H, Kuistila M, et al. Outbreak of diarrhea due to *Escherichia coli* O111:B4 in school children and adults: association of Vi antigen-like reactivity. *Lancet* 1990;**336**:831–4.
- Rodrigues J, Scaletsky ICA, Campos LC, Gomes TAT, Whittan ST, Trabulsi LR. Structure and virulence factors in strains of *Escherichia coli* of the classic serogroup O55. *Infect Immun* 1996;**64**:2680–6.
- Pelayo JS, Scaletsky IC, Pedroso MZ, Sperandio V, Giron JA, Frankel G. Virulence properties of atypical EPEC strains. *J Med Microbiol* 1999;**48**:41–9.
- Bettelheim KA, Beutin L, Gleier K, Pearce JL, Luke RKJ, Zimmermann S. Serotypes of *Escherichia coli* isolated from healthy infants in Berlin, Germany and Melbourne, Australia. *Comp Immun Microbiol Infect Dis* 2003;**26**:55–63.
- Machado J, Grimont F, Grimont PA. Identification of *Escherichia coli* flagellar types by restriction of the amplified *fliC* gene. *Res Microbiol* 2000;**151**:535–46.
- Botelho BA, Bando SY, Trabulsi LR, Moreira-Filho CA. Identification of EPEC and non-EPEC serotypes in the EPEC O serogroups by PCR-RFLP analysis of the *fliC* gene. *J Microbiol Methods* 2003;**54**:87–93.
- Fields PI, Blom K, Hughes HJ, Helsel LO, Feng P, Waminathan B. Molecular characterization of the gene encoding H antigen in *Escherichia coli* and development of a PCR-restriction fragment length polymorphism test for identification of *E. coli* O157:H7 and O157:NM. *J Clin Microbiol* 1997;**35**:1066–70.
- 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**: 1159–63.
- 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**:144–8.
- Gunzburg ST, Tornieporth NG, Riley LW. Identification of EPEC by PCR-based detection of the bundle-forming pilus gene. *J Clin Microbiol* 1995;**33**:1375–7.
- 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**:543–8.
- Franke J, Franke S, Schmidt H, Schwarzkopf A, Wieler LH, Baljer G, et al. Nucleotide sequence analysis of enteropathogenic *Escherichia coli* (EPEC) adherence factor probe and development of PCR for rapid detection of EPEC harboring virulence plasmids. *J Clin Microbiol* 1994;**32**:2460–3.
- Ørskov F, Ørskov I. Serotyping of *Escherichia coli*. *Methods Microbiol* 1984;**1**:43–112.

23. Scaletsky ICA, Silva MLM, Trabulsi LR. Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells. *Infect Immun* 1984;45:534–6.
24. Kaper JB, Mac Daniel TK, Jarvis KG. Molecular genetics of enteropathogenic *Escherichia coli*. *Rev Microbiol* 1996;27 (Suppl. 1):82–8.
25. Afset JE, Anderssen E, Bruant G, Harel J, Wieler L, Bergh K. Phylogenetic backgrounds and virulence profiles of atypical enteropathogenic *Escherichia coli* strains from a case-control study using multilocus sequence typing and DNA microarray analysis. *J Clin Microbiol* 2008;46:2280–90.
26. Giammanco A, Maggio M, Giammanco G, Morelli R, Minelli F, Scheutz F, et al. Characteristics of *Escherichia coli* strains belonging to enteropathogenic *E. coli* serogroups isolated in Italy from children with diarrhea. *J Clin Microbiol* 1996;34: 689–94.
27. Trabulsi LR, Campos LC, Whittam TS, Gomes TAT, Rodrigues J, Gonçalves AG. Traditional and non-traditional enteropathogenic *Escherichia coli* serogroups. *Rev Microbiol* 1996;27:1–6.
28. Kaper JB, Gomez-Duarte OG. The *per* regulator of enteropathogenic *Escherichia coli*. *Mol Microbiol* 1997;23:179–81.