Iranian J Publ Health, Vol. 38, No.3, 2009, pp.70-77

Original Article

Serotypes of Enteropathogenic *Escherichia coli* Isolated from Children Under 5 Years of Age

**MM Aslani[1], MY Alikhani [2]*

[1]Dept. of Microbiology, Pasture Institute of Iran, Tehran, Iran,
[2]Dept. of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences,Iran

(Received 2 Mar 2009; accepted 11 Aug 2009)

Abstract

Background: The purpose of this study was to find out the frequency of different serotypes of enteropathogenic *Escherichia coli* (EPEC) among healthy/diarrheal cases.

Methods: A total of 191 strains, 111 from diarrheal and 80 from asymptomatic persons were examined. Determination of the EPEC serogroups was performed by agglutination tests using polyvalent and monovalent O antiserum. PCR-RFLP analysis of the flagellin-encoding (*fli*C) gene and agglutination tests using polyvalent and monovalent sera against H antigens (H1 to H 56) according to the instructions of the manufacturer was performed.

Results: Seventeen (8.9%) strains were non-motile and untypable with conventional serotyping method that showed as H^-. Forty-three *fli*C restriction patterns were found for motile and non-motile serotypes. Each motile serotype was characterized by one or two *fliC* specific restriction patterns. O142:H48 (6.8%), O86:H48 (6.3%), O111:H21 (4.7%) and O127:H21 (4.2%) were the most prevalent serotypes, and O55:H12/45, O86:H48, O127:H21, O142:H48, O126:H48 and O126:H19 serotypes were the most frequently agents in diarrheal cases, compared to asymptomatic children ($P< 0.05$). There were common EPEC serotypes in diarrheal and asymptomatic children, however some serotypes either found only in diarrheal cases or isolated from asymptomatic persons.

Conclusion: Some serotypes were isolated more frequently from diarrheal cases than asymptomatic persons. The conventional serological method using antisera is the basis for the H typing system in *E-coli*, but it is impossible to serotype non-motile bacteria. PCR-RFLP analysis of *fli*C gene is a practical method in identifying the H variant in motile and non-motile EPEC serotypes and is useful for epidemiological studies.

Keywords: *Epidemiology*, *EPEC Serotypes*, fliC *gene*, *PCR-RFLP*, *Diarrhea*

Introduction

Diarrhea is one of the most common causes of morbidity and mortality among infants and children in developing world (1). Enteropathogenic *Escherichia coli* (EPEC) strains are diarrheagenic *E-coli*, which usually are classified by a combination of oligosaccharides (O), flagella (H), and capsular (K) antigens. They are associated with outbreaks of infantile diarrhea among children in developing countries (2-4). In contrast to the limited importance of EPEC in industrialized countries, EPEC is a major cause of diarrhea in developing countries (5). Studies in Brazil (6), Mexico (7), and South Africa (8) have shown that 30-40% of infant diarrhea can be accredited to EPEC. These strains are also an important cause of disease in nosocomial outbreaks, outpatient clinics, patients referred to hospitals, and in urban and rural areas (5).

Classification of EPEC by O and H antigens or serotyping is an important epidemiological tool. Although serotyping offers a very specific and reliable method for classification of EPEC serotypes, identification of the total serotypes is a time consuming process, needs expert technicians, requires large panel of specific H antisera, is subjective and cross-reactive and is limited to a few reference laboratories all around the world, and it fails to identify non-motile strains (9). Non-motile strains are relatively common among EPEC serogroupes (6, 10) and are not type able with the traditional antisera; these strains should

70 ***Corresponding author:** Tel: +98 21 66405535, E-mail:mmaslani@yahoo.com

be marked as "H ¯" However, by using the restriction analysis of *fli*C gene (encoding flagellin) both motile and non-motile strains of *E. coli* and EPEC are type able (9, 11, 12). This method has been used to characterize flagellin genes from other bacterial species (13-15).

The *E. coli* species consist of various serotypes, ranging from highly pathogenic to nonpathogenic strains. It has also been noted in a number of studies (16), that certain serotypes are far more likely to be associated with some of the virulence factors of diarrheagenic *E. coli*, than other serotypes.

The purpose of this study was to find out the frequency of different serotypes among healthy/ diarrheal cases in two provinces at the northern and south-west of Iran.

Materials and Methods

Strains of E. coli

Fecal specimens were obtained from randomly selected children aged less than 5 yr old, with diarrhea and without clinical symptoms who were currently not taking any antibiotics therapy in Golestan Province (Northern Iran) and Ilam Province (south-west Iran) in 2003. Diarrhea was defined as three or more liquid or semi liquid stools defecation per day. The healthy children had not any gastrointestinal symptoms for at least one week prior to the collection of the specimens. Stool specimens were collected on swabs, and transported to the laboratory in Cary Blair medium.

Characterization of E. coli *strains*

The stool specimens were inoculated onto MacConkey agar plates and incubated at 37 ˚C for 24 h. Three to five colonies of each sample identified by biochemical assay (17) as *E. coli* were selected for subculture and serogrouping. Determination of the EPEC serogroups and the flagella antigens was performed by agglutination tests using polyvalent and monovalent sera against O antigens (O26, O55, O86, O111 ,O114, O119, O124, O125, O126, O127,O128, O142) and flagella H antigens (H1 to H 56) according to the instructions of the manufacturer (Bio-Rad Co and Statens Serum institute, Copenhagen, Denmark, respectively). A strain giving clumping with 4% saline was defined as rough.

Amplification of the fliC *gene*

The entire coding sequence of the *fliC* gene was amplified by PCR with the primers: fFSA (5′- CAA gTC ATT AAT ACA AAC AgC C-3′) and rFSA (5′- gAC ATA TTG gAC ACT TCg gT-3′) designed for detection of flagella antigen encoded gene in *E. coli* strains (9). A 50µl reaction mixture contained 1 µl of template DNA (approximately 50 ng), 25 Pmol of each primer, 200 µM deoxyribonucleotide triphosphate mixture (Fermentas), 8µl of 10X PCR buffer (Fermentas), 1.5 mM MgCl2, 2.5U of Taq polymerase (Fermentas). The standard cycling condition after the initial denaturation step of 5 min at 94 ˚C, were 1 min at 94 ˚C, 1 min at 57 ˚C, and 2 min at 72 ˚C for 35 cycles and final extension step of 5 min at 72 ˚C. For some strains, the annealing temperature was reduced to 54 to 55 ˚C to increase the amount of product that was amplified. PCR products (10 µl) were separated by electrophoresis in 0.8% agarose gel (Bioprobe) in Tris-borate buffer(0.089 M Tris-base,0.089 M boric acid, 2.5 mM EDTA-Na2, pH 8), with the 1 kbp DNA ladder(Fermentas) as a molecular size marker. There was second band in some strains. To prevent the amplification of the second band, we used stringent annealing temperature at 57 ˚C.

Restriction patterns

The *fli*C PCR product was digested with *Hha*I restriction endonuclease (Roche) according to the manufacturer's instructions and incubated overnight at 37 ˚C. The restriction fragments were separated on a 2% agarose gel (1% standard agarose, Bioprobe and 1% Metaphor agarose, FMC Bioproducts) for 5 h at 5V/cm. A 100 bp (Fermentas) and 50 bp(Roche) DNA ladder were used as a molecular size marker. The restriction fragments were stained with ethidium bromide. An image file was generated and saved as TIFF format. Analysis and interpretation of patterns were

done using the Taxotron package. This package contains the data of all O:H serotypes (Taxolab, Institut Pasteur, Paris, France).

Statistical methods

The χ^2 test was used to verify differences between groups (statistical significance, $P< 0.05$)

Results

The EPEC strains were isolated from 44.9% of the children with diarrhea versus only 7.2% of healthy individuals ($P< 0.05$). The 191 EPEC strains belonged to 11 serogroups and 35 serotypes (H-types). The O: H serotypes found in this study are listed in Table 1. Seventeen (8.9%) strains were non-motile and untypable with conventional serotyping method that marked as H^- (Table2). The O142:H48 (6.8%), O86:H48 (6.3%), O111:H21 (4.7%) and O127:H21 (4.2%) were the most prevalent serotypes, followed by serotypes O111: H^- and O55:H12/45 (each identified in 3.1%) and O111:H9 (2.6%) and O119: H^-, O86:H30, O111:H2 (each identified in 2.1%) (Table 1). However, serotypes O55:H12/45, O86:H48, O127: H21, O142:H48, O126:H48 and O126:H19 were the most frequently identified agents in diarrheal cases, compared to asymptomatic children ($P< 0.05$).

RFLP patterns for the fliC *gene*

The flagellin gene was amplified in 191 EPEC strains. Most EPEC strains produced a single band ranging from 0.9 to 1.8 kb. Forty-three restriction patterns were obtained for 191 motile and non-motile strains under study. The RFLP patterns (F type) for each serotype (Fig. 1) in the different O serogroups is shown in Table 2. A distinct pattern was associated with each of 25 H types (Table 1). However, one pattern (F12/45) was shared by two H types (H12 and H45). For some of the flagellar types, more than one RFLP pattern was observed (Table 1). RFLP patterns of 17 non-motile EPEC strains were identical to some of those observed for the motile strains (Table 2). The distribution of EPEC serotypes in diarrheal and asymptomatic children is shown in Table 3. The number of each O: H serotypes found in each group are given in parenthesis. Of all, three were common serotype in both groups, however some serotype found only in diarrheal cases or isolated from healthy/asymptomatic persons. A characterization rate of 82 different serotypes from191 isolates is shown in Table 3.

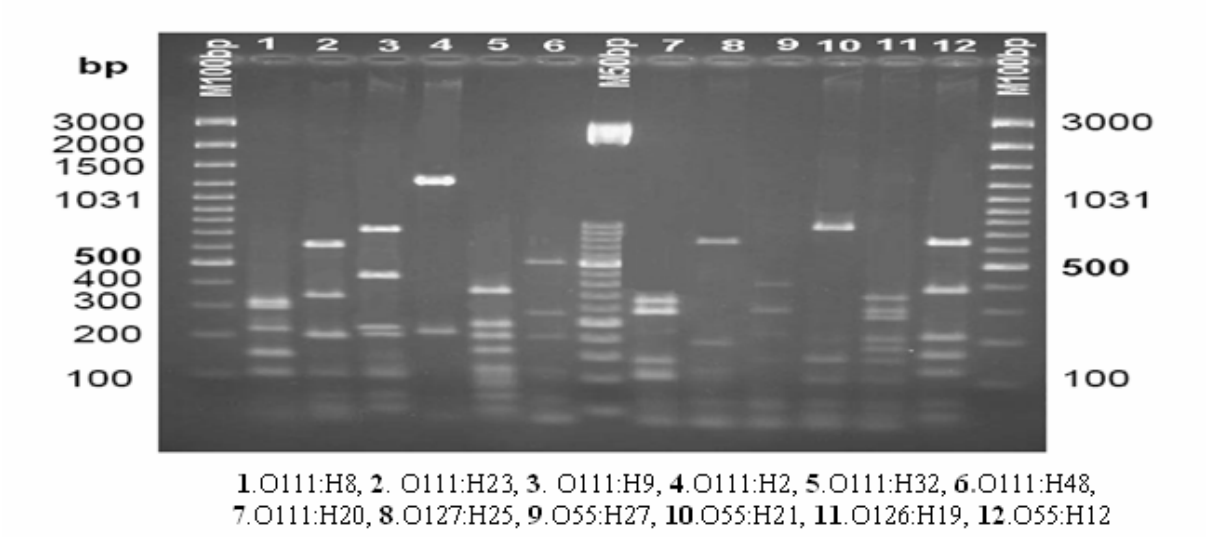

Fig. 1: HhaI restriction profiles of amplified fliC genes

Table 1: *flic* gene restriction analysis of motile EPEC serotypes

Serogroups (No)	H-type (No)	F-type (No)
O26(6)	H25(1), H29(1), H28(1), H12/45(1), H33(1), H10(1)	F25a(1), F29 (1), F28(1), F12/45(1), F33(1), F10(1)
O55(13)	H27(2), H21(1), H34(2), H12/45(6), H48(2)	F27(2), F21c(1), F34(2), F12/45(6), F48(2)
O86(22)	H4(1), H18(1), H30(4), 48(12), H19(1), H17(2), H9(1)	F4a(1), F18(1), F30(4), F48(12) F19c(1), F17(2), F9a(1)
O111(48)	H8(2), H20(2), H34(2), H12/45(2), H10(2), H21(9), H18(1),H28(2), H24(1), H9(5), H48(1), H14(2), H3(2), H6(2), H43(1), H19(1), H23(1), H17(1), H37(2), H2(4), H4(2), H26(1)	F8b(2), F20(2), F34(2), F12/45(2), F10(2), F21a(1), F21b(1), F21c(7) F18(1), F28(2), F24(1), F9a(5), F48(1), F14(2), F3(2), F6(2), F43(1), F19a(1), F23(1), F17(1), F37(2), F2a(3), F2c(1), F4a(2), F26(1)
O114(3)	H10(2), H2(1)	F10(2), F2a(1)
O119(4)	H34(1), H23(1), H4(1), H27(1)	F34(1), F23(1), F4a(1), F27(1)
O125(2)	H15(2)	F15(2)
O126(9)	H2(1), H28(2), H: unknown (1), H19(3), H20(1), H38(1)	F2a(1), F28(2), F: unknown (1), F19a(3), F20(1), F38(1)
O127(32)	H25(1), H47(3), H6(2), H10(2), H4(2), H9(2), H19(1), H11(1), H4 (1), H42(1), H17(1), H20(1), H37(1), H21(8), H28(2), H32(2), H2(1)	F25b(1), F47a(3), F6(2), F10(2) F4a(2), F9a(2), F19a(1), F11(1), F4a(1), F42(1), F17(1), F20(1), F37(1), F21a(3), F21b(5), F28(2), F32(2), F2a(1)
O128(6)	H2(1), H9(1), H10(2), H12/45(2)	F2a(1), F9a(1), F10(2), F12/45(2)
O142(29)	H37(1), H42(2), H6(3), H48(16), H7(3), H11(2), H5(1), H17(1)	F37(1), F42(2), F6(3), F48(16), F7(3), F11(2), F5(1), F17(1)

Table 2: *fliC* gene restriction analysis of non-motile EPEC serotypes

Serogroups (No)	H-type (No)	F-type (No)
O55(1)	H$^-$ (1)	F2a(1)
O86(1)	H$^-$ (1)	F21a(1)
O111(6)	H$^-$ (6)	F6(1), F12/45(1), F9a(1), F2a(1), F32(1), F48(1),
O119(4)	H$^-$ (4)	F26(1), F9a(2), F44(1)
O125(1)	H$^-$ (1)	F12/45(1)
O126(1)	H$^-$ (1)	F2a(1)
O127(1)	H$^-$ (1)	F21a(1)
O142(2)	H$^-$ (2)	F33(1), F48(1)

Table 3: Distribution of EPEC serotypes in diarrheal and asymptomatic children

Serogroups (No)	Diarrheal (No)	Asymptomatic (No)
O26(6)	H25(1), H29(1), H28(1), H10(1)	H12/45(1), H33(1)
O55(14)	H21(1), H34(1), H12/45(6), H48(2)	H27(2), H34(1), H2. NM(1)
O86(23)	H18(1), H30(1), 48(11), H19(1), H17(2), H9(1)	H4(1), H30(3), H48(1), H21. NM(1)
O111(54)	H8(1), H20(1), H34(1), H12/45(1), H10(2), H21(3), H18(1),H24(1), H9(2), H48(1), H3(1), H6(2), H37(1), H2(3), H4(1)	H8(1), H20(1),H34(1),H12/45(1), H12/45.NM(1), H21(6), H28(2), H9(3), H9.NM(1), H48.NM(1), H14(2), H3(1),H6(1), H6.NM(1), H43(1), H19(1), H23(1), H17(1), H37(1),H2(2), H2.NM(1), H4(1),
O114(3)	H2(1)	H10(2)
O119(8)	H23(1), H4(1), H27(1), H44.NM(1)	H34(1), H26.NM(1), H9.NM(2)
O125(3)	H15(2)	H12/45.NM(1)
O126(10)	H2(1), H28(2), H: unknown (1), H19(2), H20(1)	H19(1), H38(1), H2.NM(1)
O127(33)	H25(1), H47(1), H6(1), H10(1), H9(1), H19(1), H4 (1), H37(1), H21(8), H21.NM(1), H28(1), H32(1), H2(1)	H47(2), H6(1),H10(1), H4(2), H9(1), H11(1), H42(1), H17(1), H20(1), H28(1), H32(1)
O128(6)	H9(1), H10(1), H12/45(1)	H10(1), H12/45(1), H2(1)
O142(31)	H42(1), H6(1), H48(13), H48.NM(1), H7(1), H11(1), H5(1), H17(1), H33.NM(1)	H37(1), H42(1), H6(2), H48(3), H7(2), H11(1)

Discussion

Diarrhea is a significant problem all around the world and is responsible for considerable morbidity and mortality, especially in the developing countries (18). Diarrhea also is a problem in the some developed countries, but the course of the disease is generally mild, and the mortality has decreased. However, the expenditure of medical care and the absence of patients from work or school cause financial losses. Moreover, in recent years the diarrhea outbreaks have increased in the industrialized countries. Recently it was reported in England that infectious intestinal disease reaches to 20% each year (19).

The EPEC strains were isolated from 44.9% of the children with diarrhea versus only 7.2% of healthy individuals ($P< 0.05$). The results showed that EPEC continues to be associated with infantile diarrhea in our area.

Systematic O serotyping of *E.coli* began in the early 1930s (5), and many studies showed that the O serotype of *E.coli* are generally associated with pathogenesis (20-21). O serotyping became important tools to classify *E.coli* in clinical settings. It has been shown repeatedly that antigenic typing of *E. coli* is extremely useful in epidemiological studies (9, 22-24). A characterization rate of 82 different serotypes from191 isolates

(Table 3) suggest that the mean number of each serotype isolated is around two. The serotypes found can thus be divided into two groups, those isolated singly and those found twice or more frequently in this study. This study shows the variety of EPEC serotypes in children with and without diarrhea. However, there are certain serotypes, which are much more likely to be encountered in diarrhea than others. Serotypes O55:H12/45, O86:H48, O127:H21, O142:H48, O126:H48 and O126:H19 were significantly associated with diarrhea in children ($P< 0.05$). Serotypes O55:H6 and O111:H2 (12), O111:H2 (25) and O111: H^- (2) were reported as most frequent isolates in different geographical areas. These data show that some EPEC serotypes have been clearly associated with infantile diarrhea since the time these bacteria were first described.

A strong relationship between serotypes and pathotypes of *E. coli* has been widely reported (16). Many virulence factors are plasmid or phage mediated and there are apparently a strong correlation between strains of certain serotypes carrying certain virulence factors (23). It was found a strong relation between O157:H7 serotype and shiga toxin and it were found also that common ovine Shiga toxigenic *E. coli* (STEC) serotypes and human isolates of the same serotype have the same toxin type (26). This association suggests that there is a strong connection not just between serotype and pathotype but also between serotype and the subtypes of some virulence factors. In spite of this data, we need more studies to elucidate the association of EPEC serotypes and diarrhea. These studies should be done in different areas all around the world and all EPEC isolates should be fully serotyped according to O and H antigens.

Rapid and reliable detection and typing methods are required for successful microbiological surveillance and investigation of infectious diseases. Traditionally, pathogenic bacteria from stools have been analyzed using conventional culturing methods and O and H serotyping, which are time-consuming and laborious, needs a lot of antisera and only a few reference centers in the world are able to provide a full serotyping service for *E. coli*, and flagella typing is not applicable for non-motile strains. Furthermore, some isolates are generally not very motile and their flagellation needs to be enhanced by several passages on semisolid agar. In comparison to traditional serotyping method, the DNA based typing methods is high throughput, specific, and sensitive and also avoids most cross-reactions (27). They enable the analysis to be done within 24 h, or even within one working day, and provide more specific detection of the desired organism (5, 27).

Our study showed that the *fliC* gene could be amplified in all EPEC strains and *Hha*I restriction analysis of the *fliC* gene could be used for a flagella identification system. Because *fliC* gene restriction patterns can be obtained in any laboratory equipped with a thermocycler and electrophoresis apparatus, identification of RFLP patterns is likely to be more readily available than serotyping. Nevertheless as with traditional serotyping, this technique has its limit in distinguishing H12 and H45. The RFLP analysis has a number of advantages over serotyping: It is less time-consuming, it eliminates the troublesome use of series H antisera that usually is expensive, and it allows finding the serotype of non-motile strains that are rather frequent in some EPECO serogroups.

Our study showed both the large variety of *E. coli* serotypes present in the diarrheal cases and healthy persons but also that some serotypes appear more frequently in diarrheal cases. Since it seems there is association between certain serotype and diarrheal diseases, it is important to make compile the data regarding serotypes of diarrheagenic *E. coli* present in infectious cases and healthy human intestine especially in infant and children.

Acknowledgments

The authors are thankful to Miss F Shoraj, and Mr M Aslani for technical help Dr H Shojaei for critical discussion. The authors declare that there is no conflict of interests.

References

1. Snyder JD, Merson MH **(**1982). The magnitude of the global problem of acute diarrhoeal disease: a review of active surveillance data. *Bull WHO*, 60: 605-13.
2. Giammanco A, Maggio M, Giammanco G, Morelli R, Minelli F, Caprioli A (1996). Characteristics of *Escherichia coli* Strains Belonging to Enteropathogenic *E. coli* Serogroups Isolated in Italy from Children with Diarrhea. *J Clin Microbiol,* 34: 689-94.
3. Donnenberg MS, Kaper JB (1992). Enteropathogenic *Escherichia coli*. *Infect Immun,* 60: 3953-61.
4. Law D (1994). Adhesion and its role in the virulence of enteropathogenic *Escherichia coli*. *Clin Microbiol Rev,* 7: 152-73.
5. Nataro JP, Kaper JB (1998). Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev,* 11(1): 142-201.
6. Gomes TA, Vieira MA, Wachsmuth IK, Blake PA, Trabulsi LR (1989). Serotype-specific prevalence of *Escherichia coli* strains with EPEC adherence factor genes in infants with and without diarrhea in Sao Paulo, Brazil. *J Infect Dis,* 160(1): 131-35.
7. Cravioto A, Molina J, Manjarrez A, Eslava C (1996). Enteropathogenic *Escherichia coli*: The Mexican experience. *Rev Microbiol,* 27(Supple): 21-24.
8. Robins-Browne RM, Levine MM, Rowe B, Gabriel EM (1982). Failure to detect conventional enterotoxins in classical enteropathogenic (serotyped) *Escherichia coli* strains of proven pathogenicity. *Infect Immun,* 38: 798-801.
9. Machado J, Grimont F, Grimont PA (2000). Identification of *Escherichia coli* flagellar types by restriction of the amplified *fliC* gene. *Res Microbiol,* 151(7): 535-46.
10. Campos LC, Whittam TS, Gomes TAT, Andrade JRC, Trabulsi LR (1994). *Escherichia coli* serogroup O111 includes several clones of diarrheogenic strains with diferrent virulence properties. *Infect Immun,* 62: 3282-88.
11. Fields PI, Blom K, Hughes HJ, Helsel LO, Feng P, Swaminathan B (1997). 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,* 35(5): 1066-70.
12. Botelho BA, Bando SY, Trabulsi LR, Moreira-Filho CA (2003). Identification of EPEC and non-EPEC serotypes in the EPEC O serogroups by PCR-RFLP analysis of the *fliC* gene. *J Microbiol Methods,* 54(1): 87-93.
13. Alm RA, Guerry P, Trust TJ (1993). Distribution and polymorphism of the flagellin genes from isolates of *Campylobacter coli* and *Campylobacter jejuni*. *J Bacteriol,* 175: 3051-57.
14. Kilger G, Grimont PAD (1993). Differentiation of *Salmonella* phase 1 flagellar antigen types by restriction of the amplified *fliC* gene. *J Clin Microbiol,* 31: 1108-10.
15. Winstanley C, Coulson MA, Wepner B, Morgan JAW, Hart CA **(**1996). Flagellin gene and protein variation amongst clinical isolates of *Pseudomonas aeruginosa*. *Microbiology,* 142: 2145-51.
16. Lior H (1994). Classification of *Escherichia coli* In: C.L. Gyles, Editor, *Escherichia coli in domestic animals and humans*, CAB International, Wallingford, UK.
17. Ewing WH (1986). *Edward and Ewing's Identification of Enterobacteriaceae*. 4th ed. NY: Elsevier Science.
18. Guerrant RL, Hughes JM, Lima NL, Crane J (1990). Diarrhea in developed and developing countries: magnitude, special settings, and etiologies. *Rev Infect Dis,* 12(Suppl 1): S41–50.

19. Wheeler JG, Sethi D, Cowden JM, Wall PG, Roderigues LC, Tompkins DS, Hudson MJ, Roderick PJ (1999). Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The infectious intestinal diseases study Executive. *BMJ, 17*; 318 (7190): 1046–50.
20. Yayue L, Dan L, Boyang C, Weiging H, Yanqun L, Fenxia L, Xi G, David AB, Lu F, Lei W (2006). Development of a serotype-specific DNA Microarray for Identification of some Shigella and Pathogenic Escherichia coli strains. *J Clin Microbiol,* 44(12): 4376-83.
21. Wang LH, Curd W, Reeves PR (1998). Sequencing of *Escherichia coli* O111 O-antigen cluster and dentification of O-111-specific genes *J Clin Microbiol,* 36(9): 3182-87.
22. Orskov F, Orskov I (1992). *Escherichia coli* serotyping and disease in man and animals. *Can J Microbiol,* 38(7): 699-704.
23. Vu-Khac H, Holodfa E, Pilipcines E, Blanco M, Blanco JE, Dahbi G, Mora A, Lopez C, Gonzalez EA, Blanco J (2007). Seotypes, virulence genes, intimin types and PFGE profiles of *Escherichia coli* isolated from piglets with diarrhoea in Slovakia. *Vet J*, 174(1): 176-87.
24. Blanco M, Lazo L, Blanco JE, Dahbi G, Mora A, Lopez C, Gonzalez EA, Blanco J (2006). Seotypes, virulence genes, intimin types and PFGE profiles of *Escherichia coli* isolated from Cuban pigs with diarrhea. *Int Microbiol*, 9(1): 53-60.
25. Elias WP, Barros SF, Moreira CG, Trabulsi LR, Gomes TA (2002). Enteroaaggregative *Escherichia coli* strains among classical enteropathogenic *Escherichia coli* O serogroups. *J Clin Microbiol,* 40(9): 3540-41.
26. Ramachandaran V, Hornitzky MA, Bettelheim KA, Walker MJ, Djordjevic SP (2001). The common ovine Shiga toxin 2- containing *Escherichia coli* serotypes and human isolates of the same serotype have the possess a Stx2d toxin type. *J Clin Microbiol,* 39(6):1932-37.
27. Ballmer K, Korczak BM, Kuhnert P, Slickers P, Ehricht R, Hachler H (2007). Fast DNA serotyping of Escherichia coli by use of an oligonucleotide microarray. *J Clin Microbiol,* 45(2): 370-79.