Diarrheagenic *Escherichia coli* in acute gastroenteritis in infants in North-West Italy

Gabriella Amisano[1], Stefania Fornasero[1], Giuseppe Migliaretti[1], Stefano Caramello[1], Valentina Tarasco[2], Francesco Savino[2]

[1]Department of Public Health and Microbiology, University of Turin, Italy;
[2]Department of Pediatrics, Regina Margherita Children Hospital, University of Turin, Italy

SUMMARY

Enteropathogenic *Escherichia coli* may cause diarrhoea in infancy, but it is not routinely detected and regarded as a major causative agent. The aim of the present study was to estimate the incidence of enteropathogenic *E. coli* infection and to investigate its epidemiology and pathogenesis from faecal specimens in infants hospitalized for acute gastroenteritis. Between March 2008 and June 2009, faecal samples were collected and examined to recognize diarrhoeal aetiology, especially for *E. coli*, by cultural identification and multiplex-PCR.

E. coli were isolated in 75 of 160 collected samples (46,88%); 10 samples of which (6,3%) had been positively recognised for pathogenic genes. Data showed that the presence of diarrheagenic *E. coli* infection was 6.3%, but it becomes 5% considering *E. coli* as a unique agent responsible for diarrhoea. The datum is not statistically meaningful because of the small sample (p>0,05).

Bacterial pathogens were also isolated in 60 samples (37,5% of the total collected samples): 15 *Salmonella spp.*, 8 *Klebsiella pneumoniae*, 9 *Klebsiella oxytoca,* 11 *Citrobacter freundii*, 5 *Pseudomonas aeruginosa*, 2 *Serratia spp.*, 7 *Enterobacter cloacae,* 1 *Shigella spp.*, 2 *Campylobacter spp*.

Rotavirus was the predominant pathogenic single etiologic agent identified. It was found in 35 samples (21.88% of the overall collected samples), while Adenovirus, serotypes 40 or 41, was isolated in 2 samples (1.3%). Rotavirus infection was found predominantly in winter with respect to autumn.

Data provide an interesting epidemiologic survey of enteropathogenic *E. coli*, which is not usually detected, although it may have potential clinical implications.

Abbreviations: CDEC, detaching *E. coli*; DAEC, diffusely adherent *E. coli*; EAggEC, enteroaggregative *E. coli*; EHEC, enterohaemorrhagic *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*.

KEY WORDS: Acute gastroenteritis, Infants, *Escherichia coli*, Molecular identification, *Polymerase chain reaction*

Received April 19, 2010 *Accepted* August 10, 2010

INTRODUCTION

Diarrheal disease is still a health problem, especially in developing countries, where it is considered one of the foremost causes of death in children, accounting for approximately 2 million deaths each year worldwide (Kosek *et al.,* 2003; Bryce *et al.*, 2005). Further, acute gastroenteritis could affect subjects of any age and status and also represents an important cause of hospitalization and morbidity in developed countries (Caprioli *et al.,* 1996).

The features of acute diarrhea vary from place to place depending on local meteorology, geography, and socioeconomic variables.

Knowledge of the major etiologic agents of this disease is important for epidemiological surveillance and correct treatment (Yamashiro *et al.*, 1998).

The different diarrhoeal syndromes can be caused by bacterial, viral and parasitic infections of ei-

Corresponding author
Gabriella Amisano, PhD
Dipartimento di Sanità Pubblica e Microbiologia
Università degli Studi di Torino
Via Santena, 5bis - 10126 Torino, Italy
E-mail: gabriella.amisano@unito.it

ther single or multiple aetiology (Ochoa *et al*., 2004; Robins-Browne *et al.*, 2004a; O'Ryan *et al.*, 2005).
Diarrheagenic *E. coli* pathotypes represent a leading bacterial cause of pediatric diarrhea in developing regions, with some responsible for traveler's diarrhea, and are also an emerging cause of diarrhea in industrialized countries (Nataro & Kaper, 1998; Choen *et al*., 2005). *E. coli* is usually found in the commensal intestinal bacterial flora, but it could become a pathogen through acquisition of genetic determinants, which may enhance adhesiveness or toxicity. These two factors make *E. coli* particularly aggressive in infants (Nowrouzian *et al*., 2003).
E. coli strains associated with diarrhea have been classified into six groups, based on clinical, epidemiological and molecular criteria: enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAggEC) and diffusely adherent *E. coli* (DAEC) (Nataro and Kaper, 1998; Guion *et al*., 2008).
Our interest was to investigate the prevalence of the enteropathogenic *E. coli* in clinically relevant (i.e., hospitalization-requiring) infantile gastroenteritis, using highly sensitive molecular techniques as diagnostic tools. A further aim was to determine the distribution of co-infections between *E. coli*, bacterial and viral agents, and the correlation between gastrointestinal infection and infant age.

METHODS

Subjects and sample collection

Between March 2008 and June 2009, a total of 160 stool samples from infants admitted to the Department of Pediatric and Adolescence Science (Regina Margherita Children Hospital, Turin, Italy) with a diagnosis of acute gastroenteritis were submitted to the laboratory.
Infants mean age was 272±148 days, they were 92 males and 68 females, born at term and appropriate for gestational age. Patients presented diarrhea, defined by stool volume higher than 10 ml/kg/die or liquid stools, with or without vomiting. Infants were excluded in case of vomiting but no diarrhoea, or if they suffered from concomitant illness, chronic or malformative diseases.
At enrolment, each infant underwent a clinical examination and parents were interviewed to obtain data on gestational age, kind of delivery (spontaneous or caesarean), birth weight, and type of feeding (exclusively or partial breast-feeding or formula-feeding).
Informed consent was obtained from each child's parent.
Stool samples were requested from enlisted patients within the first 48 hours of hospitalization. Children had not received any antibiotic therapy in the week preceding the sampling.
About 5-10 g faeces were collected for each subject directly from the nappy. Each sample was stored at -80°C, immediately after collection, in a numbered screw-capped plastic container in aerobic conditions, until they were processed.

Detection and isolation of *E. coli* strains

Faecal samples were collected from all infants. Coliform strains were isolated by homogenizing fresh samples (10% wt/vol) with sterile saline (0.9% NaCl). The homogenates were filtered through a 100 µm metal sieve and serially diluted in the same solution. One hundred microlitres of each dilution were seeded on the selective McConkey-sorbitol agar plates (VWR™ MERCK, Germany), utilizing quadrant streaking methods to produce isolated colonies, and incubated overnight at 37°C in 5% CO_2 atmosphere.
From each plate three different colonies, identified as *E. coli* on the basis of their morphology were inoculated in 5 ml TSB and incubated overnight at 37°C.
An aliquot of overnight bacterial culture was subjected to biochemical identification by API 20E system (Biomérieux, France).
The isolated strains were also screened for possible haemolytic activity on Blood Agar plates containing 5% sheep erythrocytes washed three times with PBS (pH 7.2). The plates were incubated at 37°C in aerobic conditions and were examined for haemolytic activity.
DNA was extracted with the NucleoSpin Tissue method genomic DNA (Macherey-Nagel, USA) and was stored at -20°C until further use.
For each isolate one µl of DNA obtained as described above, corresponding to about $5x10^3$ cells, was processed by each multiplex PCR assay for the presence of genes *lt* and *st* (Moseley *et al.*, 1982; Sears & Kaper, 1996) (ETEC), of genes *eaeA*

TABLE 1 - *Primer sequences and predicted lengths of PCR products.*

Target gene	*Oligonucleotide sequence (5' To 3')*	*Amplicon Size (Bp)*	*Reference*
elt	F,TCTCTATGTGCATACGGAGC R,CCATACTGATTGCCGCAAT	322	Orjan & Strockbine (1993)
sta	F,TCTTTCCCCTCTTTTAGTCAGTC R,CCAGCACAGGCAGGATTAC	170	Rappelli et al. (2001)
eaeA	F,TGATAAGCTGCAGTCGAATCC R,CTGAACAGATCGTAACGGC	229	Calundungo et al. (1994)
bfpA	F,CACCGTTACCGCAGGTGTGA R,GTTGCCGCTTCAGCAGGAGT	450	Calundungo et al. (1994)
stx1	F,GAAGAGTCCGTGGGATTACG R,AGCGATGCAGCTATTAATAA	130	Pollard et al. (1990)
stx2	F,GGGTACTGTGCCTGTTACTGG R,GCTCTGGATGCATCTCTGGT	510	Calundungo et al. (1994)
uidA	F,CCAAAAGCCAGACAGAGT R,GCACAGCACATCAAAGAG	623	McDaniels et al. (1996)

(Jerse *et al.*, 1990) and *bfpA* (Donnenberg *et al.*, 1992) (EPEC), and of genes stx_1 and stx_2 (O'Brien & Holmes, 1987) (EHEC). A primer pair specific for the *uidA* gene coding for β-glucuronidase (McDaniels *et al.*, 1996) was also used for the confirmative species identification of *E. coli*. Details of the nucleotide sequence, the specific reference, and the predicted length of resulting amplicon for each primer pair are listed in Table 1.

PCR was performed in a 25 µl reaction mixture containing 12,5 µl PCR Master Mix (2X) (Fermentas, Canada), 1 µM (each) primer, 1 µl template DNA and water nuclease-free to 25 µl.

The thermocycle program used for all amplifications consisted of the following time and temperature profile: 5 min at 94°C; 30 cycles of 1 min at 94°C, 1 min of annealing (ranging from 55°C to 58°C), 1 min at 72°C; 7 min at 72°C after the final cycle before cooling at 4°C.

The amplification products were electrophoresed through a 2% agarose gel and visualized with UV transilluminator after ethidium bromide staining. A 100 bp DNA ladder (Invitrogen, USA) was used as a molecular size marker in gel. Positive controls for PCR were *E. coli* ATCC 35401 (ETEC st^+/lt^+), *E. coli* ATCC 43887 (EPEC $bfpA^+/eaeA^+$) and *E. coli* ATCC 35150 (EHEC $stx1^+/stx2^+/eaeA^+$); *E. coli* ATCC 25922 was used as negative control. The eventual cytotoxic activity and identification of any verocytotoxin strains were determined by Vero cell culture assay (Karmali *et al.*, 1985).

Isolation of other enteric pathogens

All faecal samples were examined for Rotavirus and Adenovirus using enzyme immunoassay tests. Specimens were tested for Rotavirus antigen by using a commercial ELISA kit (Rotaclone, Meridian Diagnostics) (Coffin *et al.*, 2006). Adenovirus was detected using commercial ELISA kit (Adenoclone, Meridian Diagnostics) (Rodriguez-Baez *et al.*, 2002) with subsequent determination of serotypes 40 and 41 by Adenoclone 40, 41 (Meridian Diagnostics).

All samples from hospitalized patients were examined for other enteric pathogens in addition to potential pathogenic *E. coli*, Rotavirus and Adenovirus, including *Campylobacter* spp, *Salmonella* spp., *Shigella* spp., using standard microbiology procedures (World Health Organization, 1987).

Statistical Analysis

In order to describe the characteristics of the studied sample, the main information was shown using classical descriptive indicators. Odds Ratio (OR) indicator and relative 95% confidence intervals (95% CI) were estimated [in the text showed in square brackets] to describe possible

different risks of pathogenic *E. coli* infections considering age (13-21 months *versus* 0-12 months).

RESULTS

E. coli were isolated in 75 of 160 collected samples (46,88%). Ten samples of the *E. coli* group (13.33%) had been positively recognised for genes related to pathogenesis: six were EPEC (two typical EPEC with the *eae* and *bfpA* genes and four atypical EPEC with only eae genes) (60% of pathogens), and four ETEC (one with the *lt* and *st* genes, one with only *st* gene and two with *lt* genes) (40% of pathogens).
All isolates were negative in Vero cell cytotoxicity test.
Others bacterial potentially pathogens were isolated in 60 samples (37,5% of the overall collected samples): 15 *Salmonella spp.*, 8 *Klebsiella pneumoniae*, 9 *Klebsiella oxytoca,* 11 *Citrobacter freundii*, 5 *Pseudomonas aeruginosa*, 2 Serratia *spp.*, 7 *Enterobacter cloacae,* 1 *Shigella spp.*, and 2 *Campylobacter spp*.
Rotavirus was found in 35 samples (21.88% of the overall collected samples) and Adenovirus, serotypes 40 or 41, in 2 samples (1.3%).
In 8 of the 10 subjects with enteropathogenic *E. coli* infection, *E. coli* was the only potentially pathogenic microorganism observed, while in 2 of them there was a coinfection between *E. coli* and one or more infective agents such as viruses (Rotavirus). Therefore, data showed that the presence of diarrheagenic *E. coli* infection was 6.3%, but it becomes 5% considering *E. coli* as a unique agent responsible for diarrhea. The datum is not statistically meaningful, probably because of the small sample ($p>0.05$).

Table 2 shows the presence of pathogenic *E. coli* strains isolated on the basis of different age of patients. 3/106 infants aged 0-12 (2.8%) and 7/54 infants aged 13-21 months (12,9%) were positive for diarrheagenic *E. coli* [13-21 months *versus* 0-12 months OR=5.11, 95% CI from 1.12 to 26.28]. This is a significant difference even if the 95% CI shows a high variability of the estimated risk.
The seasonal prevalence of pathogenic *E. coli* had a strong reduction in autumn and winter (from October to January) and few seasonal peaks in March-May.
The data on Rotavirus and Adenovirus gastroenteritis showed both viruses presented a remarkable reduction in the summer months (from June to September).

DISCUSSION

The present study was performed to identify the incidence of *E. coli* as a potential aetiologic agent of diarrheal disease in a Children's Hospital in North-West Italy. Previous reports have evaluated the prevalence of various non-Shiga toxin-producing *E. coli* as a cause of childhood diarrhea in the EU and in the US (Nataro & Kaper, 1998; Caeiro *et al.,* 1999; Knutton *et al.*, 2001; Choen *et al.*, 2005).
Our study showed the importance of pathogenic *E. coli* gastroenteritis in clinical practice and in the surveillance of incidence and complications. This is possible by means of institutes such as the Istituto Superiore di Sanità in Italy, Enter-Net in the EU (Fischer, 1999) and Pulse-Net in the US. However, diarrheagenic *E. coli* strains are not routinely sought as stool pathogens in clinical laboratories.

TABLE 2 - *Correlation between the presence of pathogenic E. coli strains and age of patients.*

	Aetiological agent			
AGE (months)	*Pathogenic E. coli*	*Not pathogenic E. coli*	*Not E. coli*	*Negative*
13-21	7	28	12	7
0-12	3	37	48	18
	OR*=5.11; 95% CI from 1.12 to 26.28			

*OR was calculated comparing the pathogenic E. coli category with over all other categories.

We observed two categories of diarrheagenic *E. coli* associated with clinical illness: EPEC (typical and atypical) and ETEC. EPEC infection is primarily an illness concerning children less than 2 years of age (Nguyen *et al.*, 2006). Atypical EPEC are now predominant in industrial countries, while typical EPEC are more frequently isolated in developing areas. Atypical EPEC constituted the *eaeA*-positive isolates. This finding is in accordance with reports from the UK (Knutton *et al.*, 2001) and Brazil (Dulguer *et al.*, 2003).

E. coli was the only potential pathogen isolated in 5% of the analysed samples; although this result is not statistically significant due to the small number of positive samples, 5% of children were discharged without identification of the aetiological agent responsible for gastroenteritis. This lack of evaluation is associated with the risk bound to the possible spread of the infection.

Rotavirus was the predominant single pathogenic etiologic agent identified (21.88%) (Rodriguez-Baez *et al.*, 2002; Coffin *et al.*, 2006; Forster *et al.*, 2009), while routine bacterial enteropathogens (*Salmonella spp., Shigella spp., Campylobacter spp.)* were identified in 18 (11.25%) of studied subjects.

Rotavirus and diarrheagenic *E. coli* co-infection has been shown in some subjects and it could be considered a risk factor for more highly symptomatic illness (Davidson *et al.*, 2002). This result is not supported by statistical significance, probably because of the small sample size, but it yields interesting clinical information, so its importance should be argued.

In our study, enteropathogenic *E. coli* were more often isolated in 13-21 months-old infants than in 0-12 month-old babies. Our data do not explain the reason for such difference, but we can hypothesize that it could be related to the different type of feeding among the two groups or more probably by attendance at nursery and infant school with the consequent infection risk.

As concerns differences in seasonal prevalence of specific pathogens, epidemic curves need to be considered before proposing probable microorganisms causing acute gastroenteritis in children (O'Ryan *et al.*, 2005). In accord with previous reports conducted in temperate climates, our data describe a strong reduction of acute diarrhea due to enteropathogenic *E. coli* in cold months (Robin-Browne, 1984; Robins-Browne *et al.*, 2004b). The seasonal variation was similar in Rotavirus and Adenovirus and in accord with references (Dennehy, 2000; Staat *et al.*, 2002); Rotavirus still represents the leading winter gastroenteric agent (Dennehy, 2000; Carraturo *et al.*, 2008; Payne *et al.*, 2008).

The present research suggests that diarrheagenic *E. coli* may be an important and unrecognized cause of diarrhea in infancy, not only in developing countries but also in developed areas, such as Italy. Further study of the pathogenic mechanisms of these *E. coli* may have significant implications for the approach to diagnostic and treatment protocols for acute gastroenteritis in children.

A role of primary importance to reduce the diffusion of these pathogenic agents is played by improvements in sanitary measures (i.e. hand-washing after every nappy change and a clean water supply) (Sobel *et al.*, 2004) and food hygiene (Beutin, 2006).

The results of this study allow us to point out the importance of the introduction of potential pathogenic *E. coli* search also in routine diagnostic searches.

The finding of diverse *E. coli* types and particularly the detection of EPEC and ETEC strains stress the need for enhanced surveillance of gastroenteritis agents in infants with more active characterization of the *E. coli* isolated strains.

REFERENCES

Beutin L. (2006). Emerging enterohaemorrhagic *Escherichia coli*, causes and effects of the rise of a human pathogen. *J. Vet. Med. B Infect. Dis. Vet. Public. Health.* **53**, 299-305.

Bryce J., Boschi-Pinto, C., Shibuya, K., Black, RE, WHO Child Health Epidemiology Reference Group. (2005). WHO estimates of the causes of death in children. *Lancet.* **365**, 1147-1152.

Caeiro J.P., Mathewson J.J., Smith M.A., et al. (1999). Etiology of outpatient pediatric nondysenteric diarrhea: a multicenter study in the United States. *Pediatr. Infect. Dis. J.* **18**, 94-97.

Calundungo R., Maddau G., Rappelli P., Cappuccinelli P., Leite F., Francisco M. and Colombo M. (1994). *Escherichia coli* and enteritis in Angola. *Lancet.* **344**, 538-539.

Caprioli A., Pezzella C., Morelli R., et al. (1996). Enteropathogens associated with childhood diarrea in Italy. The Italian Study Group on

Gastointestinal Infections. *Pediatr. Infect. Dis. J.* **15**, 876-883.

CARRATURO A., CATALANI V., TERGA L. (2008). Microbiological and epidemiological aspects of Rotavirus and enteric Adenovirus infections in hospitalized children in Italy. *New Microbiol.* **31**, 329-336.

CHOEN M.B., NATARO J.P., BERNSTEI D.I., ET AL. (2005). Prevalence of diarrheagenic *E. coli* in acute childhood enteritis: a prospective controlled study. *J. Pediatr.* **146**, 54-61.

COFFIN S., ELSER J., MARCHANT C., SAWYER M., POLLARA B., FAYORSEY R., NELSON L., LAWLEY D., GOVEIA, M., STEK J., HILLE D., DINUBILE M. (2006). Impact of acute *Rotavirus* gastroenteritis on pediatric outpatient practices in the United States. *J. Pediatr. Infect. Dis.* **25**, 584-589.

DAVIDSON G., BARNES G., BASS D., ET AL. (2002). Infectious diarrhea in children: working group report of the first world congress of pediatric gastroenterology, hepatology, and nutrition. *J. Pediatr. Gastroenterol. Nutr.* **35** (Suppl 2), S143-150.

DENNEHY P.H. (2000). Trasmission of *Rotavirus* and other enteric pathogens in the home. *Pediatr. Infect. Dis. J.* **19** (Suppl. 10), S103-105.

DONNENBERG M.S., GIRON J.A., NATARO J.P., KAPER J.B. (1992). A plasmid-encoded type IV finbrial gene of enteropathogenic *Escherichia coli* associated with localized adherence. *Molecular Microbiology* **6**, 3427-3437.

DULGUER M.V., FABBRICOTTI S.H., BANDO S.Y., MOREIRA-FILHO C.A., FAGUNDES-NETO U., SCALETSKY I.C. (2003). Atypical enteropathogenic *Escherichia coli* strains: phenotypic and genetic profiling reveals a strong association between enteroaggregative *E. coli* heat-stable enterotoxin and diarrhea. *J. Infect. Dis.* **188** (11), 1685-1694.

FISCHER I.S. (1999). The Enter-net international surveillance network- how it works. *Eurosurveill.* **4**, 52-55.

FORSTER J., GUARINO A., PAREZ N., MORAGA F., ROMÁN E., MORY O., TOZZI A.E., DE AGUILETA A.L., WAHN U., GRAHAM C., BERNER R., NINAN T., BARBEROUSSE C., MEYER N., SORIANO-GABARRÓ M. AND THE ROTAVIRUS STUDY GROUP. (2009). Hospital-based surveillance to estimate the burden of rotavirus gastroenteritis among European children younger than 5 years of age. *Pediatrics.* **123**, e393-400.

GUION C.E., OCHOA T.J., WALKER C.M., BARLETTA F., CLEARY T.G. (2008). Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. *J. Clin. Microbiol.* 4**6**, 1752-1757.

JERSE A.E., YU J., TALL B.D., KAPER J.B. (1990). A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc. Natl. Acad. Sci USA*. **87**, 7839-7843.

KARMALI M.A., PETRIC M., LIM C., FLEMING P.C., ARBUS G.S., LIOR H. (1985). The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J. Infect. Dis.* **151**, 775-782.

KNUTTON S., SHAW R., PHILLIPS A.D., ET AL. (2001). Phenotypic and genetic analysis of diarrhea-associated *Escherichia coli* isolated from children in the United Kingdom. *J. Pediatr. Gastroenterol. Nutr.* **33**, 32-40.

KOSEK M., BERN C., GUERRANT R.L. (2003). The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bull. W.H.O.* **81**, 197-204.

MCDANIELS A.E., RICE E.W., REYES A.L., JOHNSON C.H., HAUGLAND R.A., AND STELMA G.N. JR. (1996). Confirmational identification of *Escherichia coli,* a comparison of genotypic and phenotypic assays for glutamate decarboxylase and beta-D-glucuronidase. *Appl. Environ. Microbiol.* **62**, 3350-3354.

MOSELY S.L., ECHERVERRIAM P., SERIWATANA J., ET AL. (1982). Identification of enterotoxigenic *Escherichia coli* by colony hybridization using enterotoxin gene probes. *J. Infect. Dis.* **145**, 863-869.

NATARO J.P., KAPER J.B. (1998). Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* **11**, 142-201.

NGUYEN R.N., TAYLOR L.S., TAUSCHEK M., ROBINS-BROWNE R.M. (2006). Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhea in children. *Emerg. Infect. Dis.* **12**, 597-603.

NOWROUZIAN F., HESSELMAR B., SAALMAN R., STRANNEGARD I.L., ABERG N., WOLD A.E., ADLERBERTH, I. (2003). *Escherichia coli* in infants' intestinal microflora: colonization rate, strain turnover, and virulence gene carriage. *Pediatr. Res.* **54**, 8-14.

O'BRIEN A.D., HOLMES R.K. (1987). Shiga and Shiga-like toxins. *Microbiol. Rev.* **51**, 206-220.

OCHOA T.J., SALAZAR-LINDO E., CLEARY T.G. (2004). Evaluation of children with persistent infectious diarrhea. *Semin. Pediatr. Infect. Dis.* **16**, 259-263.

ORJAN O., STROCKBINE N. (1993). PCR detection of heat-stable, heat-labile, and Shiga toxin genes in *Escherichia coli*. In: Persing, D. H., Smith, T. F., Tenover, F. C., and White, T. J. Eds molecular microbiology. Principles and applications. Washington, D.C., American Society for Microbiology, 271-276.

O'RYAN M., PRADO V., PICKERING L.K. (2005). A millennium update on pediatric diarrheal illness in the developing world. *Semin. Pediatr. Infect. Dis.* **16**, 125-136.

PAYNE D.C., STAAT M.A., EDWARDS K.M., SZILAGYI P.G., GENTSCH J.R., STOCKMAN L.J., CURNS A.T., GRIFFIN M., WEINBERG G.A., HALL C.B., FAIRBROTHER G., ALEXANDER J., PARASHAR U.D. (2008). Active, population-based surveillance for severe rotavirus gas-

troenteritis in children in the United States. *Pediatrics.* **122**, 1235-1243.

POLLARD D.R., JOHNSON W.M., LIOR H., TYLER S.D., AND ROZEE K.R. (1990). Rapid and specific detection of verotoxin genes in *Escherichia coli* by the polymerase chain reaction. *J. Clin. Microbiol.* **28**, 540-545.

RAPPELLI P., MADDAU G., MANNU F., COLOMBO M.M., FIORI P.L., CAPPUCCINELLI P. (2001). Development of a set of multiplex PCR assays for the simultaneous identification of enterotoxigenic, enteropathogenic, enterohemorrhagic and enteroinvasive *Escherichia coli*. *New Microbiol.* **24**, 77-83.

ROBINS-BROWNE R.M. (1984). Seasonal and racial incidence of infantile gastroenteritis in South Africa. *Am. J. Epidemiol.* **119**, 350-355.

ROBINS-BROWNE R.M., BORDUN A.M., TAUSCHEK M., BENNET-WOOD V.R., RUSSEL J., OPPENDISANO F., LISTER N.A., BETTELHEIM K.A., FAIRLEY C.K., SINCLAIR M.I., HELLARD M.E. (2004a). *Escherichia coli* and community-acquired gastroenteritis, Melbourne, Australia. *Emerg. Infect. Dis.* **10**, 1797-1805.

ROBINS-BROWNE R.M., BORDUN A-M, TAUSCHEK M., BENNETT-WOOD V., RUSSELL J., OPPEDISANO F., ET AL. (2004b). Atypical enteropathogenic *Escherichia coli*: a leading cause of community-acquired gastroenteritis in Melbourne, Australia. *Emerg. Infect. Dis.* **10**, 1797-1805.

RODRIGUEZ-BAEZ N., O'BRIEN R., QIU S.Q., ET AL. (2002). *Astrovirus, Adenovirus*, and *Rotavirus* in hospitalized children: prevalence and association with gastroenteritis. *J. Pediatr. Gastroenterol. Nutr.* **35**, 64-68.

SEARS C.L., KAPER J.B. (1996). Enteric bacterial toxins: mechanisms of action and linkage to intestinal secretion. *Microbiol. Rev.* **60**, 167-215.

SOBEL J., GOMES T.A., RAMOS R.T., ET AL. (2004). Pathogen-specific risk factors and protective factors for acute diarrheal illness in children aged 12-59 months in Sao Paulo, Brazil. *Clin. Infect. Dis.* 38, 1545-1551.

STAAT A., AZIMI P.H., BERKE T., ET AL. (2002). Clinical presentations of *Rotavirus* infection among hospitalized chldren. *Pediatr. Infect. Dis. J.* **21**, 221-227.

WORLD HEALTH ORGANIZATION (1987). Programme for control of diarrhoeal diseases. In *Manual for Laboratory Investigation of Acute Enteric Infections* (CDD/93.3, rev.1), pp. 9-20. Geneva: World Health Organization.

YAMASHIRO T., NAKASONE N., HIGA N., IWANAGA M., INSISIENGMAY S., PHOUNANE T., ET AL. (1998). Etiological study of diarrhoeal patients in Vientiane, Lao People's Democratic Republic. *J. Clin. Microbiol.* **36**, 2195-2199.