Asian Pac J Trop Med
2009;2(3):63-69

Original article

Study of enteropathogens associated with paediatric gastroenteritis

Maysaa El Sayed Zaki

Clinical Pathology, Faculty of Medicine, Mansoura University, Egyt

Abstract

Objective: To determine the etiology of acute diarrhea in children under 5 years of age and to improve knowledge of the etiology of gastrointestinal pathogens using traditional and molecular diagnostic techniques. **Methods:** Various common enteropathogens (viral, bacterial and parasites) associated with diarrhea were investigated by conventional and molecular techniques (PCR) for verotoxin present in *Escherichia coli* in 218 children less than 5 years of age admitted to Mansoura University Children hospital- Egypt. **Results:** The occurrence of enteropathogens identified was as follows: *E. coli* O157:H7 38.8% followed by *Salmonella Spp* 29.4%, *Aeromonas* 20% and *Shigella Spp* 11.8%. Rotavirus was found in of samples 17.1%. Rotavirus was statistically significant in age < 2 years old. The commonest parasites found were *E. histolytica* followed by *Enterobius vermicularis*, *Giardia lambia*, *Hymenolepis nana* and *Ascaries*. Shigella and Salmonella isolates were tested for their susceptibility to common antimicrobial agents and most of the isolates were resistant to ampicillin and trimethoprim/sulfamethoxazole. **Conculsion:** This study demonstrated that rotavirus, *E. coli* O157:H7, Salmonella Spp, and Aeromonas were significant enteropathogens. Rotavirus was significantly associated with infantile gastroenteritis. The results highlight the value of using a combination of traditional and PCR techniques in the diagnosis of enteropathogens related to acute gastroenteritis in children.

Keywords: Acute diarrhea; Children; Parasites; Bacteria

INTRODUCTION

Acute infectious diarrhea is a common disease in young children throughout the world. Estimated incidence rates in developing countries range from 3.5 to 7.0 episodes per child per year during the first 2 years of life and from 2 to 5 episodes per child per year for the first 5 years[1]. Attack rates in children less than 5 years old in developed countries range from 1.0 to 2.5 episodes of diarrhea per child per year, with 0.1 to 0.4 episodes resulting in attendance at a general-practice clinic and 0.001 to 0.003 episodes resulting in hospitalization[2-7].

There are relatively few comprehensive studies of the viral, bacterial, and parasitic etiology of severe acute diarrhea in children admitted to hospital in developed countries[8,9] and only one long-term study (extending over 6 years) describing annual changes in the occurrence of pathogens[10]. In developing countries less reports are available about viral, bacterial, and parasitic etiology of severe acute diarrhea in children.

An important pathogen associated with diarrhea is *Aeromonas hydrophila* (*A. hydrophila*). It is gram-negative bacteria of the family *Vibrionaceae* that are often found in association with hemorrhagic septicemia in cold-blooded animals including fish, reptiles, and amphibians[4]. However, these organisms have also been implicated as primary pathogens in cases of acute diarrhea disease in immunocompetent humans

Correspondence to: Dr. Maysaa El Sayed Zaki, Professor of Clinical Pathology, Faculty of Medicine, Mansoura Univesity, Egypt
Tel: 20502258877
Fax: 502234673
E-mail: may_s65@hotmail.com

of all age groups[11, 12]. Indeed, Poirier et al.[13] reported a case of pneumonia of nosocomial origin caused by *A. hydrophila* that complicated toxic coma, while both *A. hydrophila* and *A. sobria* have been isolated from humans with sepsis, peritonitis, urinary tract infections, and severe muscle degeneration[12].

Hemolytic-uremic syndrome, a life-threatening condition normally associated with infections due to *Escherichia coli* (*E. coli*) O157:H7 has also been associated with *A. hydrophila* infection[14]; and a cytotoxin with homology to Shiga toxin 1 has been identified in both *A. hydrophila* and *A. caviae*[15].

Among the viruses causing gastroenteritis, rotavirus is etiologic agent of greatest medical and epidemiological importance[16]. There are seven antigenically distinct serogroups. Pathogenic strains to human belongs to group A, B and C[17]. Most of isolated rotavirus is detected in newborn with diarrhea[18].

Parasites as Giardia lambia, Entamoeba and Cryptosporidium pavam are common etiological agents in diarrhea. Furthermore *Escherichia coli* with different pathogenic entities, *Salmonella* species, *Shigella*, *Campylobacter jejuni*, and *Yersinia* species are common in children with diarrhea[19].

The present study was carried out to determine the etiology of acute diarrhea in Palestinian children less than 5 years of age and to improve knowledge of the etiology of gastrointestinal pathogens using traditional and molecular diagnostic techniques.

MATERIALS AND METHODS

Patients:

The study included two hundred eighteen children presented to Mansoura Children University hospital from October 2006 to October 2007. Their age ranges were from < 2 years up to 5 years. They were 110 males and 104 females. The main complaints were acute diarrhea. The diagnosis of acute gastroenteritis was defined as diarrhea and/or vomiting of 4 days´duration or less, where no alternative systemic cause was identified.

Fecal specimens were divided into three aliquots and were transported the same day to hospital laboratories of the departments of Bacteriology, Gastroenterology, and Virology, where they were stored at 4℃ until processing. Specimens for bacteriological and virological culture were inoculated into appropriate media on the day of collection. Specimens for examination for viruses, by methods such as enzyme immunoassay (EIA) and negative-stain electron microscopy (EM), were prepared as 10% homogenates in phosphate-buffered saline (PBS), pH 7.0, and were clarified by low-speed centrifugation at 2 000 × g for 10 min. PBS extracts were stored at 4℃ for 1 to 2 weeks until they were tested. Surplus PBS extracts and unextracted feces were stored at −70°C until further tests were required.

Bacteriology

Specimens were examined for pathogenic *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Aeromonas*, and *Plesiomonas* by using xylose-lysine deoxycholate agar (XLD), deoxycholate-citrate agar (DCA), and selenite F enrichment broth, incubated in air at 35°C for 18 to 24 h. DCA was incubated at room temperature for an additional 24 h to enhance recovery of *Y. enterocolitica*. Selenite F broth was subcultured onto XLD incubated in air at 35°C for 18 to 24 h. Isolates were identified by using standard biochemical and serological techniques. Isolates of *Salmonella* and *Escherichia coli* were serotyped. Polymeraase chain reaction was used to identify verototxin genes among isolated *Escherichia coli*. Identification of enteropathogenic *E. coli* (EPEC) was done by slide agglutination with commercial polyvalent sera (bioMérieux).

Antibiotics susceptibility

Antibiotics susceptibility test was performed by disc diffusion method for bacterial enteropathogens.

PCR amplification methods for Detection of Verotoxin producing

E. coli: For VT1 forward primer was (5/ ACCCTGTAACGAAGTT TGAC /3) and the reverse primer was (5/ ATCTCATGCGAC TCTTGAC /3) For amplification, 1.5 mL of boiled bacterial supernatant was used as template and 0.5 mL of AmpliTaq Gold (Perkin Elmer, Roche Molecular Systems, Inc., Branchburg, NJ) was used as the polymerase. The steps from denaturation to elongation were repeated 35 times. For VT2 forward primer was 5' TTAACCACACCCACGGCAGT 3' and the reverse primer was 5' GCTCTGGATGCATCTCTGGT 3'.

The PCR amplification mixture for the detection of VT2 contained 26.2 mL of sterile water, 3.5 mL of 10L PCR buffer solution (Finnzymes, Espoo, Finland), 0.98 mL of 4 dNTP mix (containing 5 mM dATP, 5 mM dCTP, 5 mM dTTP, and 5 mM dGTP), 1.0 mL each of forward and reverse primer (10 mM), and 0.7 mL of DNA polymerase (Dyn50 azyme II; Finnzymes). Predenaturation was carried out for 4.5 min at 95℃, and annealing was carried out for 1 min at 62℃[20,13] Five microlitre of the PCR reaction product run on a 3% high resolution agarose gel and stained with ethidium bromide. The VT1 and/or VT2 product of gave bands at 135 and 346 bp, respectively. Using PCR markers greatly aids in theinterpretation of the results[21, 22].

Table 1 Frequency of isolated enteropathogens.

Isolated organisms	*No.*	%
Bacterial etiology	85/172	49.4
E. coli O157:H7	33/85	38.8
Salmonella Spp	25/85	29.4
Shigella Spp	10/85	11.8
Aeromonas	17/85	20.0
Rotavirus	29/172	17.1
Parasites	58/172	33.7
Total	172	100

Table 2 Clinical manifestation in correlation with the isolated pathogens.

	Rotavirus (*No* = 29)		*E. Coli* (*No* = 33)		*Salmonella* (*No* = 17)		*Shigella* (*No* = 3)		*Aeromonas* (*No* = 17)		*Parasite* (*No* = 58)	
	No	%	*No*	%	*No*	%	*No*	%	*No*	%	*No*	%
Vomiting												
+	20	69.0	18	54.5	16	94.1	2	66.7	11	64.7	25	43.1
-	9	31.0	15	45.5	1	5.9	1	33.3	6	35.3	33	56.9
	χ^2 = 8.96		χ^2 = 0.05		χ^2 = 19.4		FET,		χ^2 = 3.3		χ^2 = 0.1	
	P = 0.002 8		*P* = 0.8		*P* = 0.000 0		*P* = 0.6		*P* = 0.07		*P* = 0.8	
Fever												
+	29	100.0	4	12.1	17	100.0	3	100.0	13	76.5	42	72.4
-	0	0.0	29	87.9	0	0.0	0	0.0	4	23.5	16	27.6
	FET,		FET,		FET,		FET,		FET,		χ^2 = 10.1	
	P = 0.004		*P* = 1		*P* = 0.07		*P* = 1		*P* = 0.7		*P* = 0.001	
Abd. Colic												
+	16	55.2	16	48.5	14	82.4	3	100.0	11	64.7	39	67.2
-	13	44.8	17	51.5	3	17.6	0	0.0	6	35.3	19	32.8
	χ^2 = 2.68		χ^2 = 0.08		χ^2 = 1.9		FET,		FET,		χ^2 = 0.01	
	P = 0.1		*P* = 0.8		*P* = 0.17		*P* = 0.55		*P* = 1		*P* = 0.9	
Dehydration												
+	20	69.0	4	23.5	4	23.5	0	0.0	9	52.9	2	3.4
-	9	31.0	13	76.4	13	76.4	3	100.0	7	41.2	56	96.6
	χ^2 = 9.11		FET,		FET,		FET,		FET,		χ^2 = 0.337	
	P = 0.003		*P* = 0.8		*P* = 0.8		*P* = 0.6		*P* = 0.04		*P* = 0.000	

RESULTS

The study included two hundred eighteen children presented to Mansoura Children University hospital

from October 2006 to October 2007. Their age ranges were from < 2 years up to 5 years. They were 110 males and 104 females. The main complaints were acute diarrhea. Enteropathogens were isolated from one hundred seventy patients. Twenty three patients had mixed infections (13.2%).

Bacterial etiology was the commonest followed by parasites and viral cause (49.4%, 33.7%, and 17.1% respectively), Table 1 and Figure 1. The commonest bacteria was *E. coli* O157:H7 38.8% followed by *Salmonella Spp* 29.4%, *Aeromonas hydrophila* 20% and *Shigella Spp* 11.8%. Rotavirus was found in of samples 17.1%, table1. Rotavirus was statistically significant in age < 2 years old.

The commonest parasites found were Entameoba histolytica (*E. histolytica*) followed by Enterobius vermicularis, Giardia lambia, Hymenolepis nana and Ascaries (Figure 2).

Clinical analysis of symptoms and signs associated with enteropathogens revealed that Rotavirus was significantly associated with vomiting, fever and dehydration. Salmonella was significantly associated with vomiting, Aeromonas was significantly associated with dehydration and parasites infestation was significantly associated with fever(Table2).

Figures 3, 4, 5 described antibiotics susceptibility for isolated *Aromonas*, *E. coli* O157, *Salmonella* and *Shigella* species. *Aeromonas* was found highly susceptible to ciprofloxacin, piperacillin chloramphenicol and ceftriaxone. *E. coli* was highly susceptible to Gentamycin and amekacin. *Salmonella* and *Shigella* were susceptible to ceftriaxone and amoxicillin clavulinic acid.

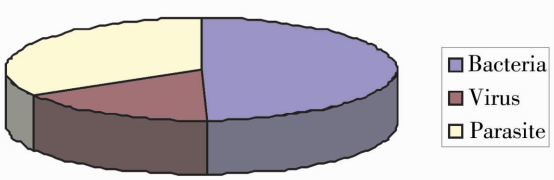

Figure1 Enteropathogens isolated from children

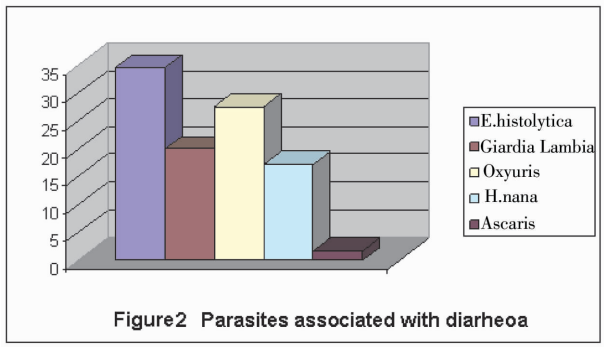

Figure2 Parasites associated with diarheoa

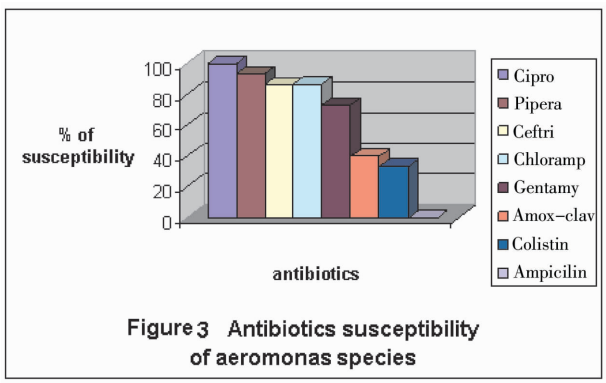

Figure 3 Antibiotics susceptibility of aeromonas species

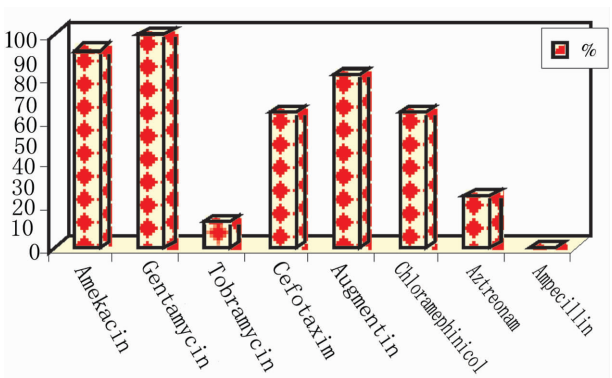

Figure 4 The antibiotic sensitivity pattern of *E. coli* isolated strains to the tested antibiotics by disk diffusion methods.

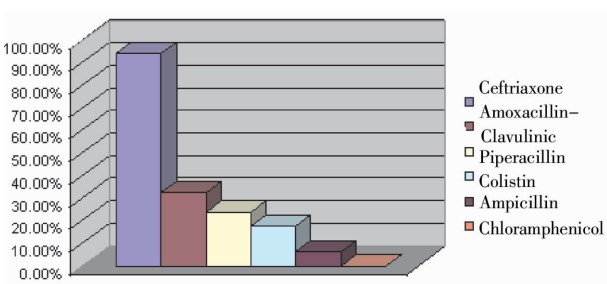

Figure 5 Antibiotics susceptibility of *Salmonella* and *Shigella*.

DISCUSSION

Clarification of the enteropathogens associated with diarrhea diseases in the country is an essential step toward the implementation of effective primary health care activities against the diseases. The results of this study of patients with diarrhea in a developing country may not be completely similar to those that would be obtained in a Western population.

Escherichia coli O157:H7 is an important agent of hemorrhagic colitis and hemolytic uraemic syndrome in children less than five years old and elderly people.

Among the bacterial isolates, Shiga toxin (ST) producing *E. coli* accounted for 38.8%, which was similar to that reported in other countries[17, 23-25]. ETEC, particularly LT-producing *E. coli*, was significantly associated with diarrhea in the patients aged 1 to 5 years in Vientiane in higher percentage 81%[26]. Similar results were obtained in other countries as Morogoro, Tanzania[27], and Spain[28]. Infection with *E. coli O*157 usually manifests as acute, a febrile, painful, bloody diarrhea. Because of the severe complications of ST-producing *E. coli* efficient reservoir and human preventive strategies are important areas of ongoing investigations.

Polymerase chain reaction used to determine verotoxines genes among isolated *E. coli* was rapid method and reliable.

Aeromonas species were found to be common among our patients. Similar results was obtained by Hofer et al., (2006)[29] in São Bento do Una, Pernambuco. Contradictory to other studies with fewer incidences of these enteropathogens in Beijing[30] and India[31]. The difference in incidence can be attributed to the difference in climate and methods of sewage disposal among the countries. Aeromonas infections in man is related to different professional activities such as fishing, surfing, swimming, diving, etc.

The burden of rotavirus disease was greatest in young children aged less than 5 years[32] results show remarkable agreement with the results up to 48% was reported by other studying Tunja[33]. This can be attributed to the difference of patients´ number and environmental factors predisposition for certain infection.

The large percentage of cases (43.5%) where no recognized pathogen was identified raises a number of issues. Firstly, techniques for identifying known pathogens may not have been optimal during the course of this study. Many different organisms may cause diarrhea, and it is not possible in a clinical survey such as this to screen specimens for the entire range of potential enteropathogens In addition, *Clostridium difficile* can cause severe diarrhea in children but was sought only when requested by clinicians. Secondly, even when specifically sought, some pathogens may not be detected because of excretion in insufficient numbers. In particular, intermittent excretion or collection of feces only during the recovery phase may result in failure to isolate the causative pathogen. The inability to identify a pathogen in many infants <6 months of age (60%) may have been influenced by passively acquired maternal antibody that could rapidly reduce microbial shedding to undetectable levels. Thirdly, and of most importance, there may be other (some as yet unknown) pathogens causing severe diarrhea in young children for which diagnostic tests were not available during the course of this study. Our results imply that these elusive pathogens could be of particular importance in diarrhea affecting infants less than 6 months of age. The involvement of small viruses (including caliciviruses and astroviruses) may be clarified when molecular biology techniques are utilized to address this gap in our knowledge.

In the present study Salmonella species and Shigella were isolated from significant number of patients. In Gasa Palestine *Shigella* spp. 9/150 (6%), and *Salmonella* spp. 3/150 (2%) were also associated with diarrhea in children[34]

Parasites causing acute diarrhea were the second common etiology in the present study. Among which *E. histolytic* was the commonest followed by Enterobius vermicularis and Giardia lambia.

Similarly, in study carried out in Nepal of 253 children with persistent diarrhea, 90 (35.5%) had protozoa infections, 63 (24.9%) helminthes infections, 32 (12.6%) had bacterial infections and 16 had mixed infections. Giardia lamblia was the most prevalent (67.7%), followed by Entamaeba histo-

lytica (27.7%)[35].

Though G. lamblia is not a life-threatening parasite, nevertheless, it is still considered as the most common water-borne diarrhea-causing disease. It is important to understand the etiology, frequency, and consequences of acute diarrhea in children. Routine surveillance such as bi-annual follow-up treatments, treating G. duodenalis cysts and other protozoa oocysts detected in ground water sources and continuous health education are the most preventive measures more among poor communities, slightly higher in males than in females with age range of 2-5-year-old children[36].

So, stool microscopy should be routinely performed in children with persistent diarrhea since protozoa infections can be cured with effective treatment and control can be achieved by proper health education.

E. coli, *Aeromonas*, *Shigella* and *Salmonella* isolates were tested for their susceptibility to common antimicrobial agents and most of the isolates were resistant to ampicillin and trimethoprim/sulfamethoxazole with high susceptibility to chloramphnicol, piperacillin and ceftriaxone. Similar pattern of antimicrobial susceptibility was obtained by other studies[34, 37] while The VTEC O157 isolates were 100% resistant to oxytetracycline, chloramphenicol, streptomycin in other study[27]. The discrepancy of antimicrobials susceptibility can be attributed to difference in antibiotics policy in general use among different countries. This sign out that when antimicrobial therapy is appropriate, selection of a specific agent should be made based upon susceptibility patterns of the pathogen or information on local susceptibility patterns.

This study demonstrated that rotavirus, *E. coli* O157:H7, *Salmonella typhi* and *Aeromonas hydrophilia*, were significant enteropathogens besides protozoa like amoeba and Giardia and nematodes as Enterobius vermicularis. The results highlight the value of using a combination of traditional and PCR techniques in the diagnosis of enteropathogens related to gastroenteritis. Antibiotics susceptibility pattern differ according to bacterial species which highlights the importance of antibiotics susceptibility tests for proper treatment.

REFERENCES

1 **Black RE**. Epidemiology of diarrheal disease: implications for control by vaccines. *Vaccine*. 1993; 11:100-106.

2 **Fergusson DM**, Dimond ME, Shannon FT. Morbidity during the pre-school years. *Aust Paediatr J*. 1985; 21:139-145.

3 **Glass RI**, Kilgore PE, Holman RC, Jin S, Smith JC, Woods PA, et al. The epidemiology of rotavirus diarrhea in the United States: surveillance and estimates of disease burden. *J Infect Dis*. 1996;174(Suppl. 1):S5-S11.

4 **Glass RI**, Lew JF, Gangarosa RE, Le Baron C W, Ho MS. Estimates of morbidity and mortality rates for diarrheal diseases in American children. *J Pediatr*. 1991; 118:27-33.

5 **Isaacs D**, Day D, Crook S. Childhood gastroenteritis: a population study. *Br Med J*. 1986; 293:545-546.

6 **Ryan MJ**, Ramsay M, Brown D, Gay NJ, Farrington CP, Wall PG. Hospital admissions attributable to rotavirus infection in England and Wales. *J Infect Dis*. 1996; 174 (Suppl. 1):S12-S18.

7 **Caprioli A**, Pizzella C, Morelli R, Giammanco A, Arista S, Crotti D, et al. the Italian Study Group on Gastrointestinal Infections. Enteropathogens associated with childhood diarrhea in Italy. *Pediatr Infect Dis J*. 1996; 15:876-883.

8 **Chopra AK**, Houston CW. Enterotoxins in Aeromonas-associated gastroenteritis. *Microbes Infect*. 1999; 1: 1129-1137.

9 **Ellis ME**, Watson B, Mandal BK, Dunbar EM, Craske J, Curry A, et al. Micro-organisms in gastroenteritis. *Arch Dis Child*. 1984; 59:848-855.

10 **Durepaire N**, Pradie MP, Ploy MC, Mounier M, Ranger-Rogez S, Martin C, et al. Adenoviruses from stool samples in a university hospital. Comparison with other main enteropathogens (rotavirus, Campylobacter, Salmonella). *Pathol Biol*. 1995;43:601-610.

11 **Aggar WA**, McCormick JD, Gurwith MJ. Clinical and microbiological features of Aeromonas hydrophila-associated diarrhea. *J Clin Microbiol*. 1985; 21:909-913

12 **Chopra AK**, Houston CW. Enterotoxins in Aeromonas-associated gastroenteritis. *Microbes Infect*. 1999; 1: 1129-1137.

13 **Poirier T**, Laurens E, Viaud JY, Poujol B, Lorre G. Nosocomial Aeromonas hydrophila pneumonia complicating toxic coma. *Ann Fr Anesth Reanim*. 1993; 12:72-74

14 **Bogdanovic R**, Cobeljic M, Markovic M, Nikolic V, Ognjanovic M, Sarjanovic L, et al. Haemolytic-uraemic syndrome associated with Aeromonas hydrophila enterocolitis. *Pediatr. Nephrol*. 1991; 5:293-295.

15 **Haque QM**, Sugiyama A, Iwade Y, Midorikawa Y, Yamauchi T. Diarrheal and environmental isolates of Aeromonas spp. produce a toxin similar to Shiga-like toxin 1. *Curr. Microbiol*. 1996; 32:239-245.

16 **Ciarlet M**, Estes MK. Rotavirus and calcivirus infections of gastrointestinal tract. *Curr Opin in Gastr.* 2001;17:10-16.

17 **Jiang B**, Dennehy PH, Spasenberger S, Gentsch JR, Glass RI. First detection of group C rotavirus in fecal specimens of children with diarrhea in the United States. *J Infect Dis.* 1995,172:45-50.

18 **Show RD**. Rotaviruses. In: Richman DD, Whitely RJ, Hayden FG, Eds. Clinical virology. 1st Ed. New York: Churchill Livingstone,1997: 765-780.

19 **Mõnkemûller KE**, Melwilcox C. Gastrointestinal infections in children. *Current Opinion in Gastro.* 2001, 17:35-39.

20 **Eklund M**, Scheutz F, SiitonenA. Clinical isolates of non-O157 Shiga toxin-producing Escherichia coli: serotypes, virulence characteristics, and molecular profiles of strains of the same serotype. *J Clin Microbiol.* 2001;39 :2829-2834.

21 **Beutin L**, Zimmermann S, Gleier K, Rapid detection and isolation of Shiga-like toxin (Verocytotoxin)-producing Escherichia coli by direct 1testing of individual colonies from washed sheep blood agar plates in the VTEC-RPLA assay. *J Clin Microbiol.* 1996;34: 2812-2814.

22 **Beutin L**, Horbach I, Zimmermann S, Gleier K. Comparative evaluation of different diagnostic methods for the detection of verotoxin (Shiga-toxin) producing strains of Escherichia coli (VTEC) in human clinical stool specimens. *J Lab Med.* 1997; 21:537-546..

23 **Bern C**, Glass RI. Impact of diarrheal diseases worldwide. In: Kapikian A Z. , editor. Viral infections of the gastrointestinal tract. 2nd ed. New York, N. Y: Marcel Dekker, 1994: 1-26.

24 **Brandt CD**, Kun HW, Rodriguez WJ, Arrobio JO, Jeffries BC, Stallings EP, et al. Adenoviruses and pediatric gastroenteritis. *J Infect Dis.* 1985;151:437-443.

25 **Creese AL**. Cost effectiveness of potential immunization interventions against diarrhea disease. *Soc Sci Med.* 1986; 23:231-240.

26 **Yamashiro T**, Nakasone N, Higa N, Iwanaga M, Insisiengmay S, Phounane T, et al. Etiological Study of Diarrheal Patients in Vientiane, Lao People's Democratic Republic. *Clin Microbiol.* 1998; 36(8): 2195-2199.

27 **Rajii MA**, Minga UM, Machang'u RS. Prevalence and characterization of verotocytoxin producing Escherichia coli O157 from diarrhoea patients in Morogoro, Tanzania. *Tanzan J Health Res.* 2008; 10(3):151-8.

28 **Villalobos de B LB**, Martínez RE, Blanco AC, Maldonado AJ, Bastardo JW. Molecular detection of shiga toxin-producing (stx1) Escherichia coli and rotavirus in stools of children with diarrhea. *Invest Clin.* 2008;49(3):387-95.

29 **Hofer E**, Reis CM, Theophilo GN, Cavalcanti VO, Lima NV, Henriques Mde F Aeromonas associated with an acute diarrhea outbreak in São Bento do Una, Pernambuco. *Rev Soc Bras Med Trop.* 2006;39(2):217-20 .

30 **Qu F**, Mao YL, Cui EB, Guo TS, Bao CM, Liu LM, et al. The distribution and antimicrobial resistance tendency of pathogens associated with diarrhea in Beijing. *Zhonghua Nei Ke Za Zhi.* 2008;47(4):304-7.

31 **Samal SK**, Khuntia HK, Nanda PK, Satapathy CS, Nayak SR, Sarangi AK, et al. Incidence of bacterial enteropathogens among hospitalized diarrhea patients from Orissa, India. *Jpn J Infect Dis.* 2008;61(5):350-5.

32 **Barnes GL**, Uren E , Stevens KB, Bishop R F. Etiology of Acute Gastroenteritis in Hospitalized Children in Melbourne, Australia, from April 1980 to March 1993. *J Clin Microbiol.* 1998; 36(1): 133-138.

33 **Manrique-Abril FG**, Tigney Diane B, Bello SE, Ospina JM. Diarrhoea-causing agents in children aged less than five in Tunja, Colombia. *Rev Salud Publica (Bogota).* 2006;8(1):88-97.

34 **Abu-Elamreen FH**, Abed AA, Sharif FA. Viral, bacterial and parasitic etiology of pediatric diarrhea in Gaza, Palestine. *Med Princ Pract.* 2008;17(4):296-301.

35 **Mukhopadhyay C**, Wilson G, Pradhan D, Shivananda PG. Intestinal protozoal infestation profile in persistent diarrhea in children below age 5 years in western Nepal. *Southeast Asian J Trop Med Public Health.* 2007;38(1): 13-9.

36 **Dib HH**, Lu SQ, Wen SF. Prevalence of Giardia lamblia with or without diarrhea in South East, South East Asia and the Far East. *Parasitol Res.* 2008;103(2):239-51.

37 **Abu Elamreen FH**, Abed AA, Sharif FA. Detection and identification of bacterial enteropathogens by polymerase chain reaction and conventional techniques in childhood acute gastroenteritis in Gaza, Palestine. *Int J Infect Dis.* 2007;11(6):501-7.