Etiology of childhood diarrhea in Zliten, Libya.

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ABSTRACT

Objectives: To determine the etiological agents of diarrhea in children from a small semi-urban city in Libya and the association of age, gender, seasonal variation, breast-feeding, source of water for drinking, or antibiotic use with the isolation of enteropathogens and whether such agents are community or hospital acquired.

Methods: Using standard microbiological techniques we examined stool samples from 169 children (70 females) aged a few days to 12 years with acute diarrhea for viral, bacterial and parasitological agents. We used the disc diffusion method to determine the susceptibility of bacterial pathogens to antimicrobial agents. We carried out the study between April 2000 to March 2001.

Results: We detected a single agent in 44.4%, rotavirus in 26.6%, *Salmonella* in 13.6%, and *Cryptosporidium* in 13% of patients and other enteric pathogens, *Shigella* in 3.6%, *Aeromonas* in 5.5%, *Entamoeba histolytica/dispar* in 11.8, and *Giardia lamblia* in 1.2%. Serotyping of isolated *Salmonella* resulted in 21 being *Salmonella enteric serotype heidelberg* and 3 *Salmonella enteritidis.* We detected both serotypes in one child. More than 75% of the isolated *Salmonella* were resistant to 6 different commonly used antimicrobial agents.

Conclusion: We found rotavirus, non-typhoid *Salmonella* and *Cryptosporidium* to be the most important enteric agents associated with childhood diarrhea in Zliten. The isolated bacterial pathogens showed high resistant rates, particularly among the *Salmonella*, to the commonly used antimicrobial agents. The ease of which one can obtain these drugs in Zliten may play a role in such resistance.

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Diarrhea remains one of the principle causes of morbidity and mortality in children. In the 1990s diarrhea accounted for a median of 21% of all deaths of children under 5 years of age in these developing areas and countries, being responsible for 2.5 million deaths per year.[1] The causative agents of acute diarrhea, in order of decreasing frequency are bacteria, viruses, and protozoa.[2] Although, treatment of patients with symptoms of infectious diarrhea with antibiotics remains controversial, we should initiate antimicrobial therapy for those who are severely ill and for patients with risk factors for extraintestinal spread of infection after obtaining appropriate blood and fecal cultures.[3] The presently accepted treatment of all acute infectious diarrheal diseases is rehydration, antibiotic treatment (when indicated), and nutritional therapy.[2]

Published studies regarding the problem of childhood diarrhea in Libya are very few and carried out mainly in the 2 major cities of the country (Tripoli and Benghazi). Furthermore, such studies are either dealt with a single agent (namely rotavirus) or viral and bacterial, but not parasitical agents.[4,5] Therefore, the present study was undertaken to determine the etiological agents of diarrhea (including viral, bacterial and parasitical agents) in children from a small semi-urban city in Libya with special emphasis on the role of antimicrobial resistant enteric bacteria. We determined

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the association of age, gender, seasonal variation, breast feeding, source of water for drinking, or antibiotic use with the isolation of enteropathogens, and whether such agents are community or hospital acquired.

Methods. Zliten is a coastal city on the Mediterranean Sea located 160 kilometers east of Tripoli with a population of approximately 100. The main activities of the population are agriculture, trade, and small-scale businesses. We included in the study, 169 children (70 females) aged a few days to 12 years (73.4% [124/169] being ≤1 year and 14.2% [24/169] being >1-≤2 years) with acute diarrhea (≥3 liquid stool/day) admitted to Al-Shafa Private Clinic and the Central Hospital in Zliten from April 2000 to March 2001.

We obtained clinical history from parents, and through physical examination by a consultant pediatrician (MD). We recorded clinical symptoms including fever, vomiting, abdominal pain, dysentery, convulsions, and dehydration in a standard proforma. We also recorded type of feeding practices (breast, artificial or both [mixed]), history of taking antibiotics, source of drinking water (treated or untreated), and recent travel abroad. Furthermore, we examined freshly collected liquid stools from patients microscopically for the presence of blood, mucous and white blood cells (WBCs). To isolate *Salmonella*, *Shigella* and *Aeromonas*, stool specimens were cultured directly on the following media: *Salmonella-Shigella* agar (SSA), MacConkey agar and blood agar supplemented with 15 mg/L ampicillin (ABA). Specimens were also inoculated into alkaline peptone water (APW, pH 8.6) and selenite F broth (SFb). All media were incubated overnight at 37°C. A loopful from APW was inoculated onto ABA and a loopful from SFb onto SSA. Both media were then incubated for 18-24 hours at 37°C. We identified suspected colonies from all the media using standard microbiological procedures and the API 20E System (bioMerieux, France) wherever appropriate.[6,7] All microbiological media were Oxoid products (Oxoid Ltd, Basingstoke, Hampshire, England). Strains identified biochemically as *Salmonella* and *Shigella* were then serotyped in the Institute Pasteur, Tunisia. Rotavirus was identified with a monoclonal latex agglutination test (bioMerieux, France). We detected *Cryptosporidium* in stools using the modified Ziehl-Neelsen technique and *Entamoeba histolytica/ dispar* and *Giardia lamblia* by the direct wet mount and flotation techniques.[8] Bacterial isolates were tested for their susceptibility to antibiotics using the disk diffusion method.[9] *Escherichia coli* ATCC 25922 was used as a reference strain. The following antibiotics (Oxoid, England) were tested: ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cefoxitin (CEF), chloramphenicol (C), doxycycline (DOC), gentamicin (CN), nalidixic acid (NA), norfloxacin (NOR), trimethoprim-sulfamethoxazole (TMP-SMZ). We asked owners of pharmacies in Zliten what types of antimicrobial drugs were available for sale during the last 3 years prior to the commencement of the present study and whether a medical prescription was required for the purchase or not. For statistical analysis, the Epi-Info software version 6.04 (CDC, Atlanta, USA) was employed. *P*-values were calculated using Chi-squares test or Fisher's exact test wherever appropriate. A *p*-value <0.05 was considered statistically significant.

Results. Of the 169 stool specimens examined, we detected a single agent in 44.4% with the predominance of rotavirus in 26.6%, *Salmonella* in 13.6%, and *Cryptosporidium* in 13% of patients **(Table 1)**. Multiple agents (2 or more) were found in 12.4% stools; being mainly rotavirus plus *Salmonella* (4.1%) or rotavirus plus *Cryptosporidium* (2.4%) **(Table 2)**. Serotyping of isolated *Salmonella* resulted in 21 being *Salmonella enterica (S. enterica)* serotype heidelberg and 3 *Salmonella enteritidis (S. enteritidis)*. Both serotypes were isolated from one patient. Serotyping of *Shigella* resulted in 6 being *Shigella flexnerii (Sh flexnerii)* type 4 and 3 *Shigella boydii (Sh boydii)*. No significant differences were found in the isolation rates of different pathogens in relation to the gender of children. However, significant differences were found in isolation rates of rotavirus *(p*<0.02/OR=6.21), and *Entamoeba histolytica/dispar* (*E. histolytica/dispar*) (*p*<0.000004/ OR=8.88) were isolated more significantly from children aged >1 year **(Table 3)**. Single agents were detected at nearly equal rates throughout the year being 40% (10/25) in spring, 45% (28/62) in summer, 45% (23/51) in autumn, and 45% (14/31) in winter **(Table 4)**. Multiple agents were isolated significantly from patients in winter than in spring (*p*<0.03/OR=5.48), summer (*p*<0.002/OR=6.90), and autumn (*p*<0.02/OR=4.36). Rotavirus was significantly isolated in winter than in summer (*p*<0.05/OR=2.63) and *Salmonella* in winter (*p*<0.001/ OR=10.43), and autumn (*p*<0.005/OR=7.32) than in summer. *Cryptosporidium* was also isolated significantly from patients in winter than in summer (*p*<0.03/OR=8.25) and in autumn (*p*<0.00006/OR=27.50). On the other hand, *Aeromonas* was detected significantly in summer than in autumn (*p*<0.02/OR=undefined). Also, *E. histolytica* was detected significantly in summer than in spring (*p*<0.02/ OR=undefined).

Of the 148 children aged between a few days to <2 years, 10 (6.8%) were breast-fed, 99 (66.9%) artificially fed, and 39 (26.3%) were on mixed feeding (breast plus artificial). Although,

Table 1 - Causative agents of diarrhea in 169 diarrheic children in Zliten.

Agent(s)	N (%) positive
Single	75 (44.4)
Multiple	21 (12.4)
Rotavirus	45 (26.6)
Salmonella	23 (13.6)
Shigella	6 (3.6)
Aeromonas	9 (5.5)
Cryptosporidium	13 (13)*
Entamoeba histolytica/dispar	20 (11.8)
Giardia lamblia	2 (1.2)
Not-identifiable	73 (43)

*Specimens examined = 100

Table 2 - Multiple agents of diarrhea in 169 diarrheic children in Zliten.

Agent(s)	N (%) positive
Rotavirus + *Salmonella*	7 (4.1)
Rotavirus + *Cryptosporidium*	4 (2.4)
Salmonella + *Entamoeba histolytica/dispar*	2 (1.2)
Salmonella + *Cryptosporidium*	2 (1.2)
Shigella + *Aeromonas* + *Entamoeba histolytica/dispar*	1 (0.6)
Shigella + *Entamoeba histolytica/dispar*	3 (1.8)
Cryptosporidium + *Entamoeba histolytica/dispar*	1 (0.6)
Giardia lamblia + *Entamoeba histolytica/dispar*	1 (0.6)
Total	**21 (12.4)**

Table 3 - Age distribution of causative agents of diarrhea from 169 diarrheic children in Zliten.

Agent(s)	N (%) positive	
	≤12 months (n=124)	>12 months (n=45)
Single	55 (44)	20 (44)
Multiple	16 (13)	5 (11)
Rotavirus	42 (34)	3 (7)
Salmonella	22 (18)	1 (2)
Shigella	0 (0.0)	6 (13)
Aeromonas	3 (2)	6 (13)
Cryptosporidium	13 (16.5)*	0 (0.0)**
Entamoeba histolytica/dispar	6 (5)	14 (31)
Giardia lamblia	1 (1)	1 (2)
Not-identifiable	53 (43)	20 (44)

*Specimens examined = 79
**Specimens examined = 21

Table 4 - Seasonal distribution of causative agents of diarrhea from 169 diarrheic children in Zliten.

Agent(s)	N (%) positive			
	Spring (n=25)	Summer (n=62)	Autumn (n=51)	Winter (n=31)
Single	10 (40)	28 (45)	23 (45)	14 (45)
Multiple	2 (8)	4 (6.5)	5 (10)	10 (32)
Rotavirus	8 (32)	12 (19)	13 (25.5)	12 (39)
Salmonella	3 (12)	2 (3)	10 (20)	8 (26)
Shigella	1 (4)	3 (5)	2 (4)	0 (0.0)
Aeromonas	2 (8)	7 (11)	0 (0.0)	0 (0.0)
Cryptosporidium	0 (0.0)*	1 (6)**	1 (2)	11 (35.5)
Entamoeba histolytica dispar	0 (0.0)	11 (18)	7 (14)	2 (6.5)
Giardia lamblia	0 (0.0)	1 (2)	0 (0.0)	1 (3)
Not-identifiable	13 (52)	30 (48)	23 (45)	7 (23)

*Specimens examined = 2
**Specimens examined = 16

Table 5 - Distribution of causative agents of diarrhea in 169 diarrheic children according to source of infection.

Agent (s)	N (%) positive in children with infection	
	Hospital-acquired (n=14)	Community-acquired (n=155)
Single	3 (21)	72 (47)
Multiple	5 (36)	16 (10)
Rotavirus	5 (36)	40 (26)
Salmonella	5 (36)	18 (12)
Shigella	1 (7)	5 (3)
Aeromonas	0 (0.0)	9 (6)
Cryptosporidium	1 (20)*	12 (13)**
Entamoeba histolytica/dispar	1 (7)	19 (12)
Giardia lamblia	1 (7)	1 (1)
Not-identifiable	6 (43)	67 (43)

*Specimens examined = 5
**Specimens examined = 95

enteropathogens were isolated at higher rates from non-breast fed children, the differences were not statistically significant. Fourteen (8.3%) patients acquired their diarrhea in the hospital (symptoms appeared 72 hours after admission). Multiple agents (mainly rotavirus plus another agent) and *Salmonella* were detected in 36% (5/14) each from these children compared with 10% (16/155), and 12% (18/155) from children with community acquired diarrhea. Isolation of multiple agents and *Salmonella* from children with hospital-acquired diarrhea were statistically more significant ($p<0.02$/ OR=4.83 and $p<0.03$/OR=4.28) than from out patients with diarrhea **(Table 5).**

According to type of water consumed, 43.2% (73/ 169) of patients had treated water at home. Although, single pathogens, rotavirus, *Shigella, Aeromonas, E. histolytica/dispar,* and *G. lamblia* were isolated at higher rates from children with non-treated than from children with treated water at home, the differences were not statistically significant. However, nearly all parents stated that on certain occasions, during shortages of water particularly in summer, they used water from other sources not known whether treated or not. Eighty (47.3%) and 89 (53.3%) patients had diarrhea before and after using antimicrobial agents. With the exception of *Shigella* and *Aeromonas,* all other pathogens were isolated at higher rates from children that used antimicrobials before having diarrhea. However, the differences were not statistically significant. We observed fever (82%), vomiting (70%), abdominal pain (34%), dysentery (14%), convulsions (9%), and dehydration (73%) of the diarrheic children. We saw blood (15%), mucus (70%), and WBCs (39%) in the stools of patients. *Shigella* and *E. histolytica/dispar* were strongly associated with fever (100% and 95%), convulsions (50% and 30%), and blood in the stool (83% and 60%), while rotavirus and *Salmonella* were strongly associated with dehydration (98% and 91%). None of the patients had traveled abroad during the last 30 days of stool collection. Of 38 isolated gram-negative enteric pathogens (23 *Salmonella*, 6 *Shigella* and 9 *Aeromonas spp*) 100% were resistant to AMP, 94.7% to AMC, 63.2% to CEF, 68.4% to C and DOC, 47.4% to CN, 28.9% to TMP-SMZ, and all (100%) were susceptible to NOR. *Salmonella* isolates showed very high resistant rates being 87% to CEF, 78.3% to CN, 91.3% to DOC and 95.7% to C. Information on the availability of antimicrobial drugs in the 3 years prior to the present study were obtained from 5 pharmacies. Three of them, namely; NA, NOR (also ciprofloxacin) and TMP-SMZ were not available even in the pharmacy of Central Hospital in Zliten. Some of the antibiotics that were available can also be obtained in other formulations, for example CN and tetracycline eye drops. Owners of all pharmacies interviewed responded that a prescription was not required to obtain antibiotics.

Discussion. In the present work, we detected enteropathogens in 56.8% of patients studied. Recent studies from North African and Middle Eastern countries reported prevalence rates of enteropathogens between 46% and 61%.[5,10] We should mention that the list of enteropathogens investigated by these studies are not the same, which may also explain the differences in detection rates. Rotavirus was the most common enteropathogen associated with childhood diarrhea in Zliten being detected in nearly 27% of cases. Previous studies from the 2 major cities reported rates of Tripoli (31.9%) and Benghazi (21%), in Libya for rotavirus in children with diarrhea.[4,5] Using methods similar to ours, rotavirus as the major cause of diarrhea in children has also been reported recently from Bahrain (13.9%), Jordan (32.5%), and from Saudi Arabia (34.6%).[10-12] Several studies reported that the frequency of isolation of *Salmonella* in developing countries is low.[13-15] However, non-typhoidal *Salmonella* are well documented as the major bacterial cause of children diarrhea in Tripoli.[5,16] *Salmonella enterica* serotypes were detected in nearly 14% (23/169) of patients being the major bacterial pathogen in the present study. This finding confirmed the importance of *Salmonella* in childhood diarrhea in Libya even in a small semi-urban city like Zliten. El-Ghodban et al,[17] examined 21 *Salmonella* strains (16 from children with diarrhea and 5 from controls) isolated in Tripoli, and reported the detection of 7 serotypes with the predominance of serotype *Salmonella saintpaul*. The interesting finding of the present study was the detection of only 2 serotypes; *S. enterica* serotype *enteritidis* and *S. heidelberg* with the latter accounting for 78% (20/23). Urio et al,[15] reported the isolation of *Salmonella* from 3% of children with diarrhea in Botswana with the detection of only 2 serotypes, *Salmonella typhimurium* and *Salmonella paratyphi*. Another study on *Salmonella* associated childhood diarrhea in Calcutta, India reported 4 different serotypes with 70% being *Salmonella typhimurium.*[18] These differences in the number and types of serotypes of *S. enterica* cannot be explained easily, however, geographical, social and living-style differences between different communities may play a role.

In the present investigations, we isolated *Shigella* from 5% of diarrheic children with predominance of *Sh flexnerii* type 4. Ghenghesh et al,[19] isolated *Shigella* from 5.7% of diarrheic children in Tripoli with *Sh flexneri* type 2 predominating. They also reported the isolation of *Sh sonnei* but not *Sh boydii*, which was detected in this current study. Studies from the Middle Eastern region reported rates of

2.6%-17.1%.[10,12-20] Protozoans are a common cause of gastrointestinal infections in developing countries. Among the protozoans examined in the present study *Cryptosporidium* was the most common at 13%. A recent study from Saudi Arabia, on the prevalence of enteric protozoa among school children, reported the predominance of *Cryptosporidium* and found that 32% of 63 examined children with diarrhea to be excreting *Cryptosporidium* oocysts.[21] Others reported rates of 6.7% (Jordan) and 10% (Kuwait) in diarrheic children.[22,23]

An interesting finding of the present study is the detection of *G. lamblia* at a low rate of 1.2%. Rates ranging between 0.8-29% for *G. lamblia* in stools from diarrheic children were reported from the Middle East and North African region.[10,12,21,24-26] *Entamoeba histolytica,* the causative agent of amoebic dysentery, characterized by the presence of blood in the stool, is identical morphologically to the non-pathogenic *E. dispar*. The latter is the new species name for what was previously called 'non-invasive' or 'non-pathogenic' *E. histolytica.*[27] Of the 20 children with entamoeba, 12 had *E. histolytica/ dispar* as a single agent. We should mention that of these 12 children, 8 were positive for blood in their stools (results not shown). Al-Braiken et al,[21] reported the detection of *E. histolytica/dispar* in 14% Saudi children with diarrhea, a rate that is closely similar to ours (11.8%). Others from the North African and Middle Eastern countries reported rates ranging between 5-34.3%.[12,24,26] It is generally accepted that the vast majority of episodes of diarrhea in developed countries are caused by viral pathogens and exhibit distinct winter seasonality, while in developing countries, particularly those with poor hygiene and sanitation, bacteria and parasites predominate, and typically peak during summer months.[28-31] With the exception of multiple agents, that were significantly more prevalent in winter, no clear cut seasonal variation in the detection rates of the enteric agents examined was observed, with some of the agents (rotavirus) being detected significantly more in winter than in summer but not in other seasons and others (*Aeromonas*) in summer than in autumn but not in winter or spring. Previous studies from Libya reported similar findings.[5] Libya is a Mediterranean country characterized by hot summer, warm autumn and spring and mild winter. This may explain the lack of seasonal variation in the detection rates of enteric pathogens observed in the present and previous studies from Libya. Within the hospital setting, pediatric wards face both community and nosocomial (hospital-acquired) diarrhea. The latter may result in aggravating the underlying disease and delay hospital discharge.[32] Nosocomial transmission of non-typhoid *Salmonella* among children has been reported previously from Tripoli and Benghazi.[16,33] The significant isolation of *Salmonella* from children with hospital-acquired diarrhea in Zliten confirms the role played by non-typhoid *Salmonella* as an important cause of nosocomial diarrhea in Libyan hospitals. Although, hand washing is considered the most important method in preventing transmission of infection by the fecal-oral route, compliance of healthcare workers with standard precautions is variable and often poor.[34,35] In addition, the high rate of the *Salmonella* carrier state among health care workers in some Libyan hospitals may contribute to this problem.[16]

Naficy et al,[36] reported that 56% of Egyptian children with rotavirus diarrhea were dehydrated. In Zliten we found a very strong association (98%) of dehydrated children with rotavirus diarrhea. This observation supports the view that rotavirus may be responsible for a large part of the morbidity and mortality associated with this syndrome in Libya and gives more weight to the need of a rotavirus vaccine to protect the pediatric population in the country.[37] However, a number of questions had to be answered before embarking on such a project that include, among others, what are the groups, serotypes, genotypes and electrophoretic types of the strains that circulate in the country.

Recently, high rates of resistance to commonly used, cheap oral antibiotics among enteric pathogens has been reported from several developing countries.[15,38,39] The ease of which antimicrobial agents can be obtained in these countries has been blamed for this problem.[2] Such an ease has been clearly shown in Zliten were all the interviewed pharmacists stated that the need for a medical prescription to obtain an antibiotic is not required. More than 75% of our *Salmonella* isolates showed resistance to 6 commonly used antimicrobial agents in Libya. Others reported more than 40% of non-typhoid *Salmonella* from children in Tripoli to be resistant to 7 antibiotics.[17] The emergence of such high resistance rates among enteric bacteria in Libya as well as in other developing countries is a major setback in the control of infectious diarrhea in such countries. With the exception of the 2 isolates that were sensitive to CN all the remaining *S. heidelberg* isolates showed similar antibiogram. This may suggest the occurrence and spread of a predominant *S. heidelberg* antimicrobial-resistant clone in the Zliten area. However, the use of other methods (for example, IS200-PCR, pulsed-field gel electrophoresis) may be required to support such a suggestion. Although, resistance to fluoroquinolones among enteric bacteria has been reported from different parts of the world. All (100%) our bacterial pathogens were susceptible to NOR. Other reports from Libya reported similar findings.[17,33] Fluoroquinolones have been introduced into Libya

in the last decade and still not commonly used in human medicine in this country. This may be due, among other reasons, to their high price and lack of availability in pharmacies particularly in rural areas and small cities, as in the case with Zliten.

In conclusion, the present study is the first of its kind to be carried out in Libya that included the search for viral, bacterial and parasitic agents in children with diarrhea. Rotavirus, non-typhoid *Salmonella* and *Cryptosporidium* were found to be the most important enteric agents associated with childhood diarrhea in Zliten. The isolated bacterial pathogens showed high resistant rates, particularly among the *Salmonella*, to the commonly used antimicrobial agents. The ease of which these drugs are obtained in Zliten may play a role in such a resistance. Most of the children examined in the present work were dehydrated, which warrants the need for an educational program targeting mothers on the benefits of using oral rehydration solution in the treatment of diarrhea. The finding of a very strong association of dehydrated children in Zliten with rotavirus diarrhea adds more weight to the need of introducing a rotavirus vaccine to the vaccination program in Libya.

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References

1. Kosek M, Bern C, Guerrant RL. The global burden of diarroeal disease, as estimated from studies published between 1992 and 2000. ***Bull World Health Organ*** 2003; 81: 197-204.
2. Sack RB, Rahman M, Yunus M, Khan EH. Antimicrobial resistance in organisms causing diarrheal disease. ***Clin Infect Dis*** 1997; 24: 102-105.
3. Hohmann EL. Nontyphoidal Salmonellosis. ***Clin Infect Dis*** 2001; 32: 263-269.
4. Giasuddin ASM, Boryswick G, Abusedra A. Rotavirus-associated diarrhoeal disease in Libyan infants up to one year of age. ***Journal of the Islamic Academy of Sciences*** 1990; 3: 218-220.
5. Ghenghesh KS, Abeid SS, Bara F, Bukris B. Etiology of childhood diarrhea in Tripoli-Libya. ***Jamahiriya Medical Journal*** 2001; 1: 23-29.
6. Collee JG, Duguid JP, Fraser AG, Marmion BP, editors. Practical Medical Microbiology. 3th ed. Edinburgh: Churchill, Livingstone; 1989.
7. Carnhan AM, Behram S, Joseph SW. Aerokey II: a flexible key for identifying clinical Aeromonas species. ***J Clin Microbiol*** 1991; 29: 2843-2849.
8. Cheesbrough M, editor. Medical Laboratory Manual for Tropical Countries. England (UK): Thetford Press Ltd; 1984.
9. National Committee for Clinical Laboratory Standards (NCCLS). Performance Standards for Antimicrobial Disk Diffusion Susceptibility Tests for Bacteria that Grow Aerobically. Approved standard M2-A5. National Committee for Clinical Laboratory Standards. Villanova (PA); 1993.
10. El-Sheikh SM, El-Assouli M. Prevalence of viral, bacterial and parasitic enteropathogens among young children with acute diarrhea in Jeddah, Saudi Arabia. ***J Health Popul Nutr*** 2001; 19: 25-30.
11. Ismaeel AY, Jamsheer AE, Yousif AQ, Al-Otaibi MA, Botta GA. Causative pathogens of severe diarrhea in children. ***Saudi Med J*** 2002; 23: 1064-1069.
12. Youssef M, Shurman A, Bougnoux M, Rawashdeh R, Bretagne S, Strockbine N. Bacterial, viral and parasitic enteric pathogens associated with acute diarrhea in hospitalized children from northern Jordan. ***FEMS Immunol Med Microbiol*** 2000; 28: 257-263.
13. Georges MC, Wachsmuth IK, Meunier DMV, Nebout N, Didier F, Siopathis MR, et al. Parasitic, bacterial, and viral enteric pathogens associated with diarrhea in the Central African Republic. ***J Clin Microbiol*** 1984; 19: 571-575.
14. World Health Organization (WHO). The Treatment of Diarrhea. A Manual for Physicians and Other Senior Health Care Workers. Document WHO/CDR/95.3, 10/95. Geneva: WHO; 1995.
15. Urio EM, Collison EK, Gashe BA, Sebunya TK, Mpuchane S. Shigella and Salmonella strains isolated from children under 5 years in Gaborone, Botswana, and their antibiotic susceptibility patterns. ***Trop Med Int Health*** 2001; 6: 55-59.
16. El Nageh MM. Salmonella isolations from human faces in Tripoli, Libya. ***Trans R Soc Trop Med Hyg*** 1988; 82: 324-326.
17. El-Ghodban A, Ghenghesh KS, Marialigeti K, Abeid S. Serotypes, virulence factors, antibiotic susceptibility, beta-lactamase activity and plasmid analysis of Salmonella from children with diarrhea in Tripoli (Libya). ***Acta Microbiol Immunol Hung*** 2002; 49: 433-444.
18. Saha MR, Saha D, Dutta P, Mitra U, Bhattacharya SK. Isolation of Salmonella enterica serotypes from children with diarrhea in Calcutta, India. ***J Health Popul Nutr*** 2001; 19: 301-305.
19. Ghenghesh K, Bara F, Bukris B, Abeid S. Shigella-associated diarrhea in children in Tripoli-Libya. ***Saudi Med J*** 1997; 18: 557-559.
20. Al-Jurayyan NA, Al-Rashed AM, Al-Nasser MN, Al-Mugeiren MM, Al-Mazyad AS. Childhood bacterial diarrhoea in a regional hospital in Saudi Arabia: clinico-aetiological features. ***J Trop Med Hyg*** 1994; 97: 87-90.
21. Al-Braiken FA, Amin A, Beeching NJ, Hommel M, Hart CA. Detection of Cryptosporidium amongst diarrhoeic and asymptomatic children in Jeddah, Saudi Arabia. ***Ann Trop Med Parasitol*** 2003; 97: 505-510.
22. Nimri LF, Hijazi SS. Cryptosporidium. A cause of gastroenteritis in preschool children in Jordan. ***J Clin Gastroenterol*** 1994; 19: 288-291.
23. Iqbal J, Hira PR, Al-Ali F, Philip R. Cryptosporidiosis in Kuwaiti children: seasonality and endemicity. ***Clin Microbiol Infect*** 2001; 7: 261-266.
24. Al-Eissa YA, Assuhaimi SA, Abdullah AM, AboBakr AM, Al-Husain MA, Al-Nasser MN, et al. Prevalence of intestinal parasites in Saudi children: a community-based study. ***J Trop Pediatr*** 1995; 41: 47-49.
25. Moalla H, Fendri C. Etiology of acute diarrhea in children. ***Tunis Med*** 1994; 72: 25-28.
26. Habbari K, Tifnouti A, Bitton G, Mandil A. Intestinal parasitosis and environmental pollution: 1343 pediatric cases in Beni-Mellal, Morocco. ***Tunis Med*** 2000; 78: 109-114.
27. Petri WA, Singh U. Diagnosis and management of amebiasis. ***Clin Infect Dis*** 1999; 29: 1117-1125.
28. Bass D, Greenberg H. Group A rotavirus. In: Blaser M, Smith P, Ravdin J, editors. Infections of the gastrointestinal Tract. New York: Raven Press; 1995. p. 967.
29. Pang XL, Joensuu J, Vesikari T. Human calcivirus-associated sporadic gastroenteritis in Finnish children less than two years of age followed prospectively during a rotavirus vaccine trail. ***Pediatr Infect Dis J*** 1999; 18: 420-426.
30. Alam M, Akhtar YN, Ali SS. Seasonal variations in bacterial pathogens isolated from stool samples in Karachi, Pakistan. ***J Pak Med Assoc*** 2003; 53: 125-129.

31. Farthing MJ. Giardia lamblia. In: Blaser M, Smith P, Ravdin J, editors. Infections of the gastrointestinal Tract. New York: Raven Press; 1995. p. 1081.
32. Ratner AJ, Neu N, Jakob K, Grumet S, Adachi A, Della-Latta P, et al. Nosocomial rotavirus in a pediatric hospital. ***Infect Control Hosp Epidemiol*** 2001; 22: 299-301.
33. Taher IAA, Tobgi RS. Emergence of multi-drug resistant salmonellae isolated from neonates in Benghazi-Libya. ***J Bahrain Med Soc*** 1997; 9: 165-167.
34. Pittet D, Hugonnet S, Harbarth S, Mourouga P, Sauvan V, Touveneau S, et al. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. Infection control programme. ***Lancet*** 2000; 356: 1307-1312.
35. Ros SP, Cabrera-Ros BL. Poor compliance with universal precautions: a universal phenomenon? ***Pediatr Emerg Care*** 1990; 6: 183-185.
36. Naficy AB, Abu-Elyazeed R, Holmes JL, Rao MR, Savarino SJ, Kim Y, et al. Epidemiology of rotavirus diarrhea in Egyptian children and implications for disease control. ***Am J Epidemiol*** 1999; 150: 770-777.
37. Ghenghesh KS, Kreasta M, El-bakoush M, Tobgi R. Rotavirus-associated gastroenteritis. ***Jamahiriya Medical Journal*** 2002; 2: 12-17.
38. Shapiro RL, Kumar L, Phillips-Howard P, Wells JG, Adcock P, Brooks J, et al. Antimicrobial-resistant bacterial diarrhea in rural western Kenya. ***J Infect Dis*** 2001; 183: 1701-1704.
39. Rahman M, Islam H, Ahmed D, Sack RB. Emergence of multidrug-resistant Salmonella Gloucester and Salmonella Typhimurium in Bangladesh. ***J Health Popul Nutr*** 2001; 19: 191-198.