Aetiology of Acute Infantile Diarrhoea in the south-Eastern Nigeria: An Assessment Of Microbiological And Antibiotic Sensitivity Profile

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Citation

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Abstract

An assessment of microbiological profile of enteric pathogens in pediatric stool specimens and antibiotic sensitivity pattern of implicated bacterial agents were performed in Abakaliki, Nigeria. Of the 150 children diagnosed with diarrhoea (cases), at least one enteropathogen was detected in 122(81.3%). Among the 50 children without diarrhoea (controls), 4(8.0%,) had enteropathogen. Rotavirus was detected in 35(23.3%) cases but none in the controls. Bacterial detected among cases included Escherichia coli (15.3%), Salmonella species (11.3%) and Klebsiella species (7.3%). Entamoeba histolytica and Giardia lamblia were detected in 3.3% and 2.7% of the cases respectively. Infants aged 0-12 months old recorded highest frequency of enteropathogens. Enterobacter species, Shigella species and Campylobacter species exhibited a 100% susceptibility to oflaxacin, gentamycin and ciprofloxacin. All bacterial isolates showed 100% resistance to septrin, while E. coli, Salmonella species, Campylobacter species exhibited 100% resistance to augmentin. Immunization/effective childcare programmes, improved personal and environmental hygiene and health-education recommended.

INTRODUCTION

Infections are the major cause of severe morbidity and mortality among children worldwide [1]. Diarrhoeal illness stands, as an important cause of infectious morbidity in children, exceeded only by respiratory tract infections and mortality is currently associated with cases that evolve without proper feeding or rehydration care, invasive diarrhoeas with extraintestinal or systemic involvement, or persistent diarrhoeas that occur especially in infants from low-level socioeconomic groups, who suffer previous deficiencies and develop severe nutritional consequences of enteric infection [2]. Diarrhoea defined as excessive and frequent evacuation of watery faeces, usually indicating gastrointestinal distress or disorder of 3-7 days duration, is a frequent illness in developing countries and contributes to the deaths of 4.6 million to 6 million children annually in Asia, Africa, and South America and it has been estimated that in the very poor countries of these regions each child suffers up to 15 to 19 episodes of diarrhoea per year [2,3].

Despite much progress in the understanding of pathogenesis and of management, diarrhoeal illness still remains one of the most important causes of global childhood mortality and morbidity largely because the aetiology and pathogenesis of persistent diarrhoea are usually multifactorial and sometimes can not be identified [4]. Reports from different parts of the world have implicated various pathogens such as parasites, bacteria and viruses with outbreak of infant diarrhoea disease in children [5,6,7,8,9,10]. However in the developing countries including Nigeria, infantile diarrhoea disease is grossly under-reported and the incidence under-estimated, this is attributed to poverty and ignorance among affected group who usually constitute up to 80% of the population of the area [11].

In Nigeria, available reports indicate that more than 315,000 deaths of preschool age children are recorded annually as a result of diarrhoea disease [12,13]. Nevertheless, despite the public health implications and enormous burden imposed on the primary health care delivery system by infantile diarrhoea illness in the country, there is still paucity of information on the epidemiology and aetiology of infantile diarrhoea in many regions including the South-eastern Nigeria. Since the pathogens responsible for diarrhoea infections employ ingenious mechanisms to establish disease, regional variation in the microbiological profile may

exist even in the same country [3,11]. The persistent seasonal outbreak of diarrhoeal disease among children in the South-eastern Nigeria, particularly in Abakaliki and its environs, and the rising trend of resistance to antimicrobial agents used for treating diarrhoeal infections in the area, necessitated this investigation. In Ebonyi State south-eastern Nigeria where this study was conducted, diarrhoea episode represents more than 80% of hospitalized paediatrics cases from December to April each year and about 10% during the other months, with mortality rate estimated to be 7.5% (Ogbu et al. 2006: unpublished observation). This study was the first systematic attempt to assess the microbiological spectrum and antibiotic sensitivity profile of the aetiology of infantile diarrhoea disease in the South-eastern Nigeria with the view to providing scientific information required for effective public health interventional efforts and adequate management of the disease. The public health implications of findings are discussed as they affect the primary health care delivery system in Nigeria and other developing countries with similar setting.

MATERIALS AND METHODS

STUDY AREA

The study was conducted from October 2005 through March 2006 which corresponds to the season of infantile diarrhoea disease outbreak in Abakaliki, one of the major cities in the south-eastern Nigeria and capital of Ebonyi State. The area lies approximately between longitude 8061611 E and latitude 602212611 N and is located on the lower belt of the Niger. The climate is tropical and the vegetation characteristic is predominantly the semi-tropical rain forest with an average annual rainfall of about 1600mm and average atmospheric temperature of 30oC. There are two distinct seasons; the wet and the dry seasons. The former occurs between April and October, while the latter takes place from November to March.

The area is traversed by a number of rivers which include Iyiudele River, Iyiokwu River, Ebonyi River and Okpuru River and form a confluence at the Southern part of the city. These rivers constitute the major sources of water supply especially to the suburbs and rural communities bordering the city. Water from these streams and rivers serve for drinking, washing, bathing and irrigation purposes for the medium and small-scale farming which is a major occupation of the people inhabiting the suburbs of Abakaliki where many varieties of the vegetables and fruits sold in the city are grown. The rivers and other water courses drain the city collecting both human and animal wastes from homes, hospitals, markets and industries and are often polluted with organic substances with higher concentrations during the dry season.

ETHICAL CONSIDERATION

Approval for the study was obtained from the Research Ethics Committees of Ebonyi State University Teaching Hospital (EBSUTH), Abakaliki. The approval was on the agreement that patient anonymity must be maintained, good laboratory practice/quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only.

STUDY POPULATION/SAMPLING TECHNIQUE

Infants aged 0-43 months with acute diarrhoea illness hospitalized in the three major hospitals in Abakaliki-namely, Ebonyi State University Teaching Hospital (EBSUTH), Federal Medical Centre (FMC) and Mile-Four Hospital (MFH), were enrolled into the study. Prior to enrolment, clinical diagnosis of acute diarrhoea was established by a paediatrician. A total of 150 infants with the acute diarrhoea illness were studied. About five to ten grams (or milliters) of faeces for etiologic studies was obtained from nylon diapers of each child with plastic spoons and collected in sterile plastic universal specimen containers and were submitted to the laboratory in insulated, refrigerated boxes for copromicrobiologic studies, which included investigation of bacteria, viruses, and parasites. The samples were examined within 8 hours of collection. To serve as control, stool specimens were obtained from 50 infants without any symptom of diarrhoea as confirmed by a paediatrician. The controls were individually matched with cases on gender, age and place of residence and were selected during immunization programme at the hospitals.

LABORATORY ANALYSIS

Standard parasitological and bacteriological techniques as well as the enzyme-linked immunosorbent assay ELISA for rotavirus screening were performed to identify implicating microorganisms.

Parasitology: The concentration method described previously [9,14] was used. Briefly, formalin (10%) was added to the specimen to a total volume of 20 ml. The specimen was then sifted through a gauze mesh, diluted 2:1 with phosphate-buffered saline (pH 7.2), and spun at 1,600 rpm for 2 min. The pellet was resuspended in 7 ml of phosphate-buffered saline plus 3 ml of ethyl acetate and

centrifuged for 2 min at 1,600 rpm. Two drops were placed on a glass slide and covered before being examined with a X10 objective lens for ova and a X20 objective lens for protozoan cysts after staining with a drop of 1% iodine in 2% potassium iodide.

Bacteriology: Isolation of bacterial agents from specimens was done by culture method as described previously [15], using the following media; MacConkey agar, Deoxycholate citrate agar (DCA), Salmonella Shigella agar (SSA) and Thiosulphate citrate bile-salt sucrose agar (TCBS). The stool specimens were inoculated on the sterile agar media in petri dishes, and then incubated at 37oC for 24 hours. Colonies formed were sub cultured on Nutrient agar and gram stained. Isolates were subjected to biochemical test for further identification as previously described [15]. Antibiotic sensitivity test was performed using the disk diffusion method earlier described [15,16]. Known local strains of Escherichia coli and Staphylococcus aureus obtained from the Bacteriology Laboratory of Ebonyi State University Teaching Hospital (EBSUTH), were used as the control organisms. The following antibiotics were employed for the sensitivity analysis; ofloxacin, gentamycin, ciprofloxacin, perflacin, ampicillin, augumentin, and septrin. Their selection was based on local prescribing policies and availability. They were sourced from government approved pharmacies.

Rotavirus investigation: A 10% stool suspension in phosphate-buffered saline (PBS) medium was prepared, and the suspensions were stored at -20°C. Rotavirus testing was performed using commercially available Enzygnost enzyme linked immunosorbent assay (ELISA) kits (Behringwerke, Marburg, Federal Republic of Germany). Manufacturer's instructions were strictly followed to determine rotavirus positive specimens.

TREATMENT STRATEGIES

As part of the treatment strategies adopted in this study, mothers of diarrhoea cases without signs of dehydration were educated on management of diarrhoea at home so that they could practice mixing and administering oral rehydration salts (ORS) solution. The reduced osmolarity ORS solution was used and this composed of 75mmol/litre of sodium; 65mmol/litre of chloride; 75mmol/litre of anhydrous glucose; 20mmol/litre of potassium; and 10mmol/litre of citrate. Mothers were taught to recognize the signs that indicate the child should be brought back to the health worker. Dehydrated children were treated with ORS solution (or with an intravenous electrolyte solution in cases of severe dehydration) and their mothers were asked to stay with their children to give oral rehydration therapy (ORT) and continue breast-feeding with the supervision and encouragement of a health worker. Mothers were taught how to give ORS solution, continue ORT at home, feed during and after diarrhoea, and recognize the signs indicating that a child should be brought back to the hospital. Mothers were also taught the importance and need for improved general sanitation, personal hygiene and giving the child safe drinking water.

Treatment was provided to diarrhoea cases with other problems, such as dysentery, which do not require admission. Antibiotics (e.g., Ofloxacin administered twice daily for 7 days) were used only as needed while antidiarrhoeal drugs were never administered. Before diarrhoea cases left the hospital, it was ensured that any other health problems or concerns have been attended to. The children were provided with zinc supplementation to be given by their mothers at the dosage of 20 mg per day of for 10–14 days (10 mg per day for infants under six months old).

RESULTS

Of the 150 children diagnosed with diarrhoea (cases), at least one enteropathogen was detected in 122(81.3%) of them, while among the 50 children without diarrhoea (controls), only 4(8.0%) had enteropathogen (Odd Ratio=50.1, 95% CI.,45.7-54.5; Relative Risk=10.2, 95% CI.,7.5-12.9). Among all samples tested, Rotavirus was the single most predominant pathogen and was detected in 35(23.3%) cases but non in the controls. The pathogens of bacterial origin occurring with the highest frequency among the cases included Escherichia coli (15.3%), Salmonella species (11.3%), Klebsiella species (7.3%) and Enterobacter species (6.0%), while among the controls, E. coli (4.0%) recorded the highest prevalence (Table 1).

Figure 1

Table 1: Prevalence of enteropathogens among children with diarrhoea (cases) and Children without diarrhoea in Abakaliki south-eastern Nigeria

Pathogenic Microorganisms	Children with diarrhoea (cases N=150) No.(%) infected	Children without diarrhoea (Control N=50) No.(%) infected
Rotavirus	35(23.3)	0(0.0)
Escherichia coli	23(15.3)	2(4.0)
Salmonella spp	17(11.3)	1(2.0)
Klebsiella spp	11(7.3)	0(0.0)
Enterobacter spp	9(6.0)	0(0.0)
Shigella spp	5(3.3)	0(0.0)
Yersina enterocolitica	4(2.7)	0(0.0)
Campylobacter jejuni	4(2.7)	0(0.0)
Entamoeba histolytica	5(3.3)	0(0.0)
Giardia lamblia	4(2.7)	0(0.0)
Trichuris trichiura	1(0.7)	1(2.0)

Entamoeba histolytica an enteric parasite was detected in 5(3.3%) of the cases but non in the controls. Other enteric parasites namely, Giardia lamblia and Trichuris trichiura were isolated from both the cases and the controls (Table 1).

The frequency of occurrence of the enteropathogen generally decreased with increase in the age of the infants with diarrhoea. Infants aged 0-12 months old recorded the highest frequency of enteropathogens followed by those aged 13-24 months old. Among these two age categories, Rotavirus (38.1% vs. 28.3%), E. coli (21.4% vs. 18.9%), Salmonella species (19.0% vs. 11.3%), Klebsiella species (14.3% vs. 9.4%) and Enterobacter species (11.9% vs. 7.5%) were the most predominant aetiological agents detected (Table 2).

Figure 2

Table 2: Age-related prevalence of enteropathogens among children with diarrhoea in Abakaliki south-eastern Nigeria

Enteropathogen	Age in months (number of cases)				
	0-12 (42)	13-24 (53)	> 36 (29)	25-36 (26)	Total (150)
	Number (%) of enteropathogen detected				
Rotavirus	16(38.1)	15(28.3)	4(13.8)	0(0)	35
Escherichia coli	9(21.4)	10(18.9)	1(13.8)	0(0)	23
Salmonella species	8(19.0)	6(11.3)	1(3.4)	2(7.7)	17
Klebsiella species	6(14.3)	5(9.4)	0(0)	0(0)	11
Enterobacter species	5(11.9)	4(7.5)	0(0)	0(0)	9
Shigella species	2(4.8)	2(3.8)	1(3.4)	0(0)	5
Yersina enterocolitica	3(7.1)	1(1.9)	0(0)	0(0)	4
Campylobacter jejuni	2(4.8)	2(3.8)	0(0)	0(0)	4
Entamoeba histolytica	2(4.8)	3(5.7)	0(0)	0(0)	5
Giardia lamblia	1(2.3)	3(5.7)	0(0)	0(0)	4
Trichuris trichiura	0(0)	1(1.9)	0(0)	0(0)	1

The antibiotic susceptibility patterns of the bacterial isolates are shown in Table 3. Results indicated that Enterobacter species, Shigella species and Campylobacter species exhibited a 100% susceptibility to oflaxacin, gentamycin and ciprofloxacin. All the bacterial isolates showed a 100% resistance to septrin, while E. coli, Salmonella species, Campylobacter species and Yersinia enterocolitica exhibited a 100% resistance to augmentin. Oflaxacin, gentamycin and ciprofloxacin appeared the more effective antibiotics as all the bacterial isolates showed a 75-100% susceptibility to them.

Figure 3

Table 3: Antibiotic Sensitivity Pattern of Bacterial Isolates from Children with Diarrhoea in Abakaliki South-Eastern Nigeria

Antibiotics	Percentage Susceptibility Of Bacterial Isolated						
	Escherichia coli	*Salmonella* Species	*Klebsiella* Species	*Enterobacter* Species	*Shigella* Species	*Campylobater jejuni*	*Yersina enterocolitica*
Ofloxacine	92.3	88.0	90.0	100.0	100.0	100.0	100.0
Gentamycine	88.5	88.9	81.8	100.0	100.0	100.0	100.0
Ciprofloxacine	88.5	83.3	81.8	100.0	100.0	100.0	75.0
Ampicillin	23.1	22.2	36.4	20.0	15.0	0.0	0.0
Augumentin	0.0	27.3	20.0	15.0	0.0	0.0	0.0
Septrin	0.0	0.0	0.0	0.0	0.0	0.0	0.0

DISCUSSION

Infantile acute gastroenteritis of microbial origin is said to still constitute a frequent problem, with high mortality rate in developing countries and high impact on health costs in industrialized countries [17]. In this present study, it was established that as high as 83.1% of cases of acute infantile diarrhoea in Abakaliki, south-eastern Nigeria was associated with enteropathogenic microoganismsms and was considerably higher than the frequency of the pathogens in the controls (8.0%). This was comparable to the outcome of studies in Calcutta, India [5], Sao Paulo, Brazil [18], and Santiago, Chile [19], were intestinal pathogens were respectively detected in 81.5%, 72.8% and 73.7% of children with acute diarrhoea and the frequency was significantly higher than in the controls. Our result suggests that other causes or pathogens beyond the scope of this investigation may have been responsible for the outstanding 16.9% diarrhoeic cases. Earlier similar studies conducted in Boston, USA; Copenhagen, Denmark; and Bahia, Brazil, indicated that possible aetiologies for infantile diarrhoea cases include currently unknown gastrointestinal infections, nongastrointestinal illnesses and dietary/environmental or other non-infectious factors [8,10,20].

Rotavirus was found to be the most common pathogenic agent detected in this investigation. A number of earlier studies in both developed and developing countries, have shown viruses, especially rotavirus to be a leading cause of gastroenteritis in children [92021]. And the recent estimates of diarrhoea-related childhood deaths gave an estimated 611,000 (range 454,000-705,000) rotavirus-related deaths [7]. However, other viruses that have been implicated in childhood diarrhoea include adenovirus, norovirus, sapovirus and calcivirus [9,20,21]. Although these other viruses were not investigated in this present study, the possibility that any of them might have also contributed to the diarrhoea in this study may not be completely ruled out. This finding underscores the importance of the integration of viral diagnosis in the assessment of the microbiological profile of aetiological agents of infantile diarrhoea especially in the developing countries. It was suggested that a combination of microscopic examination of stool samples with specific enzyme immuno-assays (EIA) to detect virus antigen in stool specimens may be suitable for routine diagnostics [22].

Although the spectrum of bacterial isolate implicated in acute childhood diarrhoea varies from one region to the other, E. coli and Salmonella species were the commonest bacteria pathogens isolated in this investigation and this was in conformity with findings from Sao Paulo Brazil [20] and Bissau, Guinea Bissau [23]. Both E. coli and Salmonella species are reported to be very commonly associated with enteric diseases in developing countries and are more important to the epidemiology of diarrhoea in poorer areas [24,25,26]. In this study, a number of the cases with the acute diarrhoea were from families of higher socioeconomic class, however, the great majority of them were from socioeconomically deprived group inhabiting low income areas and the suburbs where little basic amenities are available. Furthermore, in this latter group, malnutrition is a predominant feature and is known to play significant role in the severity of enteric infection [3]. These factors may have influenced the outcome of this investigation.

The occurrence of enteric infections of parasitic origin in the present study was relatively low, though E. histolytica and G. lamblia were isolated from 3.3% and 2.7% of the cases respectively. Similar studies in various parts of the world have consistently indicated that enteric parasites were less frequently associated with acute infant diarrhoea compared to pathogens of viral and bacterial origin [2,5,18].

Our result showed that the majority of the enteric pathogens were detected among the cases who were 24 months of age and less, which may be said to corresponds to the period when children's contact with environmental pathogens increases dramatically. This is in agreement with findings from previous studies in Brazil, Denmark and Turkey [9,10,27].

Because specific antimicrobial treatment may be required to supplement supportive anti-dehydration treatment which is the cornerstone of therapy of acute infant diarrhoea, selective use of antimicrobial agents therefore, cannot be overemphasized. This is vital especially in the developing countries, where inadequate health services, inadequate drug supplies, non-adherence to treatment strategies and dubious drug quality all favour the emergence of microbial resistance [28]. Therefore, it is worth noting that one outcome of the increased availability and usage of antimicrobial agents for symptomatic treatment of illness has been the emergence of antimicrobial resistance. This was clearly evident from this study where up to 100% resistance was observed in two to three antibiotics (septrin, augmentin and ampicillin) by E. coli, Salmonella species, Campylobacter jejuni, and Yersina enterocolitica. This is of particular concern in the developing world including Nigeria because fewer affordable, appropriate, and effective treatment options such as ciprofloxacin and oflaxacin are readily available in most rural communities. It has become increasingly important to monitor patterns of resistance as the antimicrobial susceptibility of bacterial pathogens which contribute significantly to the burden of infant diarrhoea is declining.

In conclusion, it is important to state that our inability to screen for other potential enteric pathogens particularly viruses (e.g., adenovirus, norovirus, sapovirus and calcivirus), the rather low population size of cases and the limited number of antibiotics used in the sensitivity test were among the limitations of this study. Also the investigation was conducted during the epidemic season and there is a need to perform similar study in the non epidemic season for the purpose of comparison. Nevertheless, the present study has provided insight into the burden of infantile diarrhoea in Nigeria. As a public health measure to reduce the disease burden, an integrated package of immunization services and other childcare programmes need to be implemented in addition to well-focused health-education messages to improve treatment-seeking behaviour for childhood diarrhoea as well as improved personal and environmental hygiene.

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References

1. Lawn JE, Cousens S, Zupan J, Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: when? Where? Why?.Lancet. 2005; 365:891-900.
2. Torres ME, Pirez MC, Schelotto F,Varela G, Parodi V, Allende F, et al. Etiology of children's diarrhea in Montevideo, Uruguay: associated pathogens and unusual isolates. J Clin Microbiol 2001;39:2134-9.
3. Thapar N, Sanderson IR. Diarrhoea in children: an interface between developing and developed countries. Lancet 2004; 363:641-53.
4. Garcia Cristia Y, Fragoso Arbelo T, Valdes-Dapena Vivanca M, Sagaro Gonzalez E, Gorrin Castellanos N. Intestinal microflora in children with acute and persistent diarrhea [Article in Spanish].G E N 1994; 48:236-44.
5. Chatterjee BD, Thawani G, Sanyal SN. Etiology of acute childhood diarrhoea in Calcutta. Trop Gastroenterol 1989; 10:158-66.
6. Parashar UD, Gibson CJ, Bresse JS, Glass RI. Rotavirus and severe childhood diarrhea. Emerg Infect Dis 2006;12: 304-6.
7. Ali MB, Ghenghesh KS, Aissa RB, Abuhelfaia A, Dufani M. Etiology of childhood diarrhea in Zliten, Libya. Saudi Med J 2005; 26:1759-65.
8. Vernacchio L, Vezina RM, Mitchell AA, Lesko SM, Plaut AG, Acheson DW. Diarrhea in American infants and young children in the community setting: incidence, clinical presentation and microbiology. Pediatr Infect Dis J 2006; 25:2-7.
9. Olesen B, Neimann J, Bottiger B, Ethelberg S, Schiellerup P, Helms M, et al. Etiology of diarrhea in young children in Denmark: a case-control study. J Clin Microbiol 2005; 43:3636-41.
10. Diniz-Santos DR, Santana JS, Barretto JR, Andrade MG, Silva LR. Epidemiological and microbiological aspects of acute bacterial diarrhea in children from Salvador, Bahia, Brazil. Braz J Infect Dis 2005; 9:77-83.
11. Synder JD, Merson MH. The Magnitude of the Global Problem of Acute Diarrhea Disease A Review of Active Surveillance Data. Bull of Health Org 1982; 60:605-15.
12. Babaniyi OA. Oral Dehydration of Children with Diarrhea in Nigeria, A 12 Year Renew Of Impact on Morbidity and Mortality from Diarrhea Disease and Diarrhea Treatment Practices. J Trop Paediat 1991; 37: 16-66.
13. Alabi SA, Audu RA, Ouedeji KS. Viral, Bacteria and Parasitic Agents Associated With Infantile Diarrhea In Lagos. Nig J Med Res 1998; 2:29-32.
14. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 1. UK: Cambridge University Press, 1998.
15. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part 2. UK: Cambridge University Press, 2000.
16. World Health Organization (WHO). Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Heath Importance in the Developing World. WHO/DCS/CSR/RMD/2003.6. Geneva: WHO, 2003.
17. Guarino A, Albano F, Berni Canani R. Acute infectious diarrhea in children. [Article in Italian]. Ann Ist Super Sanita 1998; 34:495-512.
18. Souza EC, Martinez MB, Taddei CR, Mukai L, Gilio AE, Racz ML, et al. Etiologic profile of acute diarrhea in children in Sao Paulo.[Article in Portuguese]. J Pediatr (Rio J) 2002;78:31-8.
19. Saelzer E, Munoz P, Pena A. Bacterial isolation in infants hospitalized for acute diarrhea.[Article in Spanish]. Rev Chil Pediatr 1989;60:328-33.
20. Ethelberg S, Olesen B, Neimann J. Risk factors for diarrhea among children in an industrialized country. Epidemiology 2006; 17:24-30.
21. Zurawska-Olszewska J, Krzeslowska I, Dlugosz G, Krasiukianis A. Etiology of acute diarrheas in children from the Lodz region. I. Occurrence of etiologic agents.[Article in Polish]. Med Dosw Mikrobiol 2002;54:129-36.
22. Liu C, Grillner L, Jonsson K. Identification of viral agents associated with diarrhea in young children during a winter season in Beijing, China. J Clin Virol 2006;35:69-72.
23. Valentiner-Branth P, Steinsland H, Fischer TK. Cohort study of Guinean children: incidence, pathogenicity, conferred protection, and attributable risk for enteropathogens during the first 2 years of life. J Clin Microbiol 2003; 41:4238-45.
24. Ansaruzzaman M, Albert MJ, Nahar S, Byun R, Katouli M, Kuhn I, et al. Clonal groups of enteropathogenic Escherichia coli isolated in case-control studies of diarrhea in Bangladesh. J Med Microbiol 2000;49:177-85.
25. Gomes TA, Rassi V, MacDonald KL, Ramos SR, Trabulsi LR, Vieira MA, et al. Enteropathogens associated with acute diarrheal disease in urban infants of Sao Paulo, Brazil. J Infect Dis 1991; 164:331- 7.
26. Notario R, Borda N, Gambande T, Sutich E. Species and serovars of enteropathogenic agents associated with acute diarrheal disease in Rosario, Argentina. Rev Inst Med Trop Sao Paulo 1996; 38:5-7.
27. Karadag A, Acikgoz ZC, Avci Z, Catal F, Gocer S, Gamberzade S, et al. Childhood diarrhoea in Ankara, Turkey: epidemiological and clinical features of rotavirus-positive versus rotavirus-negative cases. Scand J Infect Dis 2005; 37:269-75.
28. World Health Organization (WHO). Overcoming Antimicnbial Resistance. WHO Report on Infections Disease. WHO/CDS 2000.2.Geneva:

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