A Perspective Study on the Etiology of Diarrhea in Children Less than 12 Years of age attending Kanti Children's Hospital

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Abstract

Introduction: Diarrhea is the most common illness among children causing highest number of mortality and morbidity in the developing countries. **Objective:** This study was conducted to determine the etiological agents of diarrhea in children less than 12 years of age. **Methods:** The study was carried out in Tribhuvan University Teaching Hospital, Health Research Laboratory and stool specimen were collected from Kanti Children's Hospital between February 2007 and August 2007. The specimens were processed by standard microbiological methods, serological diagnosis for the complete identification of bacterial isolate and use of Rotaclone for diagnosis for Rotavirus. **Results**: A total 500 specimen were processed and 312 (62.4%) cases were identified with enteropathogens. Out of 500, 165 (33%) showed significant bacterial growth, 110 (22%) single or multiple parasitic infestation, and 167 (21.4%) Rotavirus. Among the bacterial isolate, *Escherichia. coli* (20.2%) were highest in number, *Entamoeba. histolytica* (10%), among the protozoa and *Ascaris lumbricoide* (1.4%) among the helminth. Age group 0-2 years showed most number of cases. The prevalence of Rotavirus infection was more in Inpatient (65.4%) than Outpatient (34.6%) (P>0.05). Ethnicgroup wise distribution showed that Gurung/Magar/ Rai/Tamang were highly infected (34.6%) (P>0.05). **Conclusion:** The results showed that bacteria were the major etiological agents of diarrhea in children than parasites and Rotavirus. *E. coli* among the bacteria, *E. histolytica* among the parasite and Rotavirus constituted the major causative agents identified. The age group 0-2years was the most vulnerable group where most of the enteropathogens were detected.

Key words: Bacteria, Children Diarrhea, Parasites, Rotavirus,

Introduction

Diarrhea is the clinical syndrome of diverse etiology, associated with passage of more than three loose watery stools in a 24 hours period and often accompanied by other clinical sign and symptoms including vomiting, fever, dehydration and electrolyte disturbances.[1]

Every year 10 million children less than 5 years of age die in the world more than 99% of these deaths are in developing countries and 70% are caused by infectious diseases. Nepal, being a developing country, faces the similar health problem as other developing countries. Diarrhea and gastroenteritis comes in second position among top ten diseases admitted to hospital[2].

Three kinds of clinical feature of diarrhea have been defined, each reflecting a different pathogenesis and requiring different approaches to treatment. These are acute watery diarrhea (AWD), dysentery, persistent diarrhea[3]. Acute Watery Diarrhea (AWD) lasts less than 14 days (most episodes last less than 7 days), and the most significant etiological agents are rotavirus, enterotoxigenic *Escherichia coli* (ETEC), *Shigella* spp, *Campylobacter jejuni and Cryptosporidium* spp. In some area *Vibrio cholerae* O1, *Salmonella* spp and

enteropathogenic *E. coli* (EPEC) are also important. Dysentery is defined as diarrhea with visible blood in faeces. The most frequent cause is *Shigella* spp especially *Shigella flexneri* and *Shigella dysenteriae type* 1 causing "bacillary dysentery". [4] Other causes include *Campylobacter jejuni* especially in infants and infrequently *Escherichia coli* or *Salmonella* spp, *Entamoeba histolytica* can cause serious dysentery in young children known as "amoebic dysentery". Persistent diarrhea begins acutely but is of unusually long duration (at least 14 days). There is no single microbial cause, although *Shigella* spp*, Salmonella* spp*, enteroaggregative Escherichia coli* and *Cryptosporidium* spp may play a greater role than other agents.

In Nepal, due to lower socio-economic status and poor hygienic condition of the people, intestinal parasitosis is very much prevalent and intestinal pathogens are important causative agents of diarrhea and are one of the major public health problems of the country[5].

Rotavirus is one of the etiological agents of childhood diarrhea and the cause of infant death in developing countries. Five million children under the age of 2 years die from diarrheal disease in developing countries each year and rotavirus infections account for about 20% of these deaths[6].

Low birth weight, malnutrition and Vitamin A deficiencies, water pollution etc are some of the predisposing factors for diarrhea[7]. Vitamin A deficiency predisposes children to infection by suppression of humoral and cell mediated immunity as well as changes in the gastric epithelium. Infection in turn reduces the absorption of vitamin A further depleting vitamin A stores and making the child more susceptible to infections. Zinc and vitamin A supplements seem to reduce the duration of diarrhea[8] .Other debilitating conditions, prior antibiotic therapy or, when the host immunity is compromised by prior viral infection are the risk factors that pose likely to infection. Such as severe, prolonged cryptococcosis has been associated with AIDS, and corticosteroide use.

Enteropathogens infect another person directly or indirectly through contaminated food and fluids or via direct contact with the infected faeces. In case of parasites, such as *Entamoeba histolytica*, *Giardia lamblia*, *Ascaris lumbricoides*, *Trichuris trichiura,* infected human are the reservoir and contaminated food and water are the chief sources of infection. Hookworm infection occurs through direct penetration of skin, usually through feet by larvae[9]. Transmission of rotavirus also most often occurs through faeco-oral route. Usually this occurs from poor hand washing or from ingestion of contaminated food or water. Fomites or inanimate objects such as utensils, towel, toys, book etc contain infectious discharge from a patient and they are capable of harboring and transferring the infectious agent to the healthy person. Unclean hands or fingers are the most common medium by which pathogenic agents are transferred to food from skin, bowel etc in cholera and dysentery[10].

Materials and Method

The study was carried out in Health Research Laboratory, Institute of Medicine, Kathmandu between February to August 2007. A total of 500 stool specimens from children below 12 years with clinical diagnosis of acute diarrhea were collected from Oral Rehydration Treatment (ORT) ward and Outpatient department (OPD) of Kanti Children's Hospital. Doctor's prescription were strictly observed to determine whether the case was diarrheal or not and random sampling was performed. After obtaining consent from parent/ guardian, a questionnaire of each subject was filed prior to sample collection.

The collected stool specimens were processed according to the standard laboratory procedure. Macroscopic examination was performed for the presence of blood, mucus and adult or larvae of helminthic parasites. Microscopic examination was done by using saline and iodine preparation. Culturing on differential, selective and enrichment medium was performed for isolation of *E. coli*, *Salmonella* spp, *Shigella* spp and *Vibrio* spp. and their subsequent identification was performed by biochemical methods. The primary media used were Salmonella-Shigella Agar and Mac Conkey Agar, TCBS Agar, and Alkaline peptone water, Selenite-F broth as enrichment medium. Furthermore, complete identification of these bacterial isolate was performed by serological methods. Serotyping was carried out with the use of monoclonal antigen raised against *Shigella* spp (Becton, Dickenson and Company), *Vibrio* spp (Denka Seiken), Salmonella spp (Tydal). Antibiotic susceptibility test for the bacterial isolate was determined by Modified Kirby-Bauer disc diffusion method as per NCCLS standard. For the qualitative detection of Rotavirus antigen, Microplate Enzyme immunoassay technique (Premier™ Rotaclone) was performed. Samples were cryopreserved at -70°C till the test was conducted. Purity plate and quality control was maintained throughout the experimental procedure to maintain the aseptic condition.

Results

Among the total subjects (n=500) enrolled for the study, 230 (46%) were inpatient admitted in ORT ward and 270 (54%) were attended in OPD, of which 313 (62.6%) were male and 187 (37.4%) were female.

Distribution of enteropathogens showed that potential enteric pathogen were identified in 312 (62.4%) cases. 165 (62.4%) significant bacterial growth, 110 (22%) showed parasites, with single or multiple infestation, and 107 (21.4%) were rotavirus (Table 1).

Table 1: Showing Distribution of Enteropathogens in total Processed Sample.

	Frequency	Percentage
Either of pathogen positive	312	62.4%
Bacteria	165	33%
Parasites	110	22%
Protozoa	93	18.6%
Helminth	17	3.4%
Rotavirus	107	21.4%
Total sample processed	**500**	**100%**

Among the total bacterial isolate *E. coli* was highest in number 101 (20.2%), *E. histolytica* 50 (10%) among the protozoan and *A. lumbricoides* 7 (1.4%) among the helminth (Table 2).

Ethnic group wise distribution of enteropathogens showed that Gurung/Magar/Rai/Tamang were highly infected (34.6% i.e. 108 out of 126) (Table 4).The association of presence of enteropathogens between different ethnic groups was found statistically significant (P>0.05).

Table 2: Showing Distribution of Etiological Agents among Bacteria and Parasites.

Etiological agents	Frequency	Percentage (N=500)
Bacteria	**165**	**33.0%**
Escherichia coli	101	20.2%
Shigella spp	35	7.0%
Vibrio cholerae	21	4.2%
Salmonella spp	8	1.6%
Parasites	**110**	**22.0%**
Entamoeba histolytica	50	10%
Giardia lamblia	32	6.4%
Cyclospora cayetanensis	11	2.2%
Ascaris lumbricoides	7	1.4%
Trichuris trichiura	5	1.0%
Hymenolepis nana	3	0.6%
Hookworm	2	0.4%

Age and gender wise distribution of bacterial enteropathogens showed the highest number of bacterial, parasitic and Rotavirus infection in age group 0-2 years (Table 3).

Table 3: Showing Age wise Distribution of Enteropathogens.

Age group in years	Total positive cases for bacterial enteropathogens Percent (N=165)	Total positive cases for parasitic enteropathogens Percent (N=110)	Total positive cases for Rotavirus, Percent (N=107)
0-2	76 (46.1%)	30 (27.7%)	67 (62.5%)
2-4	54 (21.2%)	27 (24.4%)	23 (21.4%)
4-6	27 (16.4%)	17 (15.3%)	11 (10.3%)
6-8	10 (6.1%)	19 (17.2%)	2 (2.0%)
8-10	10 (6.1%)	11 (10.0%)	3 (2.9%)
10-12	7 (4.2%)	6 (5.4%)	1 (0.9%)
Total	**165 (100%)**	**110(100%)**	**107(100%)**

Table 4: Showing Ethnic Group wise Distribution of Enteropathogens.

Ethnic group	Total number	Pathogen positive	Percentage (N=312)
Brahmin	128	72	23.1%
Kchetriya	110	64	20.5%
Newar	58	28	9.0%
Gurung/Magar/Rai/Tamang	126	108	34.6%
Mandal/ Jha/ Yadav	33	22	7.0%
Minority	45	18	5.8%
Total	**500**	**312**	**100%**

The highest number of specimen were collected in the month of June (117) and least in the month of August (23). The showed that the highest bacterial pathogen was detected most in the month of August and July, the most parasites in the month of August while Rotavirus showed the most infection in the month of February (Table 5).

A total of 230 inpatient, 70 (65.4%) showed rotavirus positive and out of 270 patients from outpatient department, 37 (34.6%) showed rotavirus positive (Figure 1).The association of presence of enteropathogens in Indoor patients and Outdoor patients in Rotavirus infection was found statistically significant (P>0.05).

Distribution of serotypes of *Shigella* spp, *Vibrio cholera, Salmonella* spp is shown in Table 6.

The antibiotic susceptibility pattern of bacterial isolate showed that *Shigella* spp was found to be most susceptible to Ciprofloxacin (94.3%), Norfloxacin (85.7%), Gentamicin (71.4%). *Salmonella* spp was found most susceptible to Ciprofloxacin and Gentamicin (75.0% each), *Vibrio* spp was found 100% susceptible to Ciprofloxacin and Gentamicin, 90.5% susceptible to Tetracycline (Table 7).

Table 5: Showing Month wise Distribution of Enteropathogens.

Months	Bacterial enteropathogens (Percentage)	Parasitic enteropathogens (Percentage)	Rotavirus (Percentage)
February	22 (30.1%)	8 (10.8%)	27 (36.9%)
March	16 (34.7%)	7 (15.1%)	17 (36.9%)
April	17 (29.7%)	10 (17.4%)	12 (21.0%)
May	21 (24.0%)	16 (18.2%)	17 (19.5%)
June	36 (30.6%)	37 (31.4%)	17 (14.5%)
July	43 (44.2%)	22 (22.3%)	14 (14.4%)
August	11 (47.6%)	10 (43.1%)	3 (13.0%)
Total	**165 (33.0%)**	**110 (22.0%)**	**107 (21.4%)**

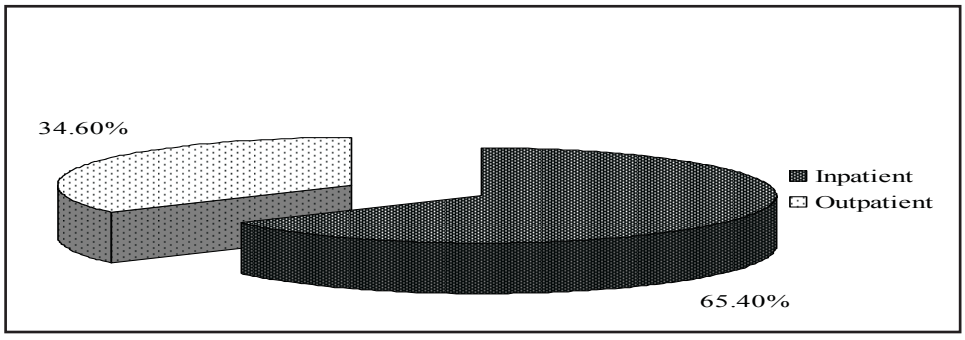

Fig 1: Showing Distribution of Rotavirus on the basis of Hospital Registration.

Table 6: Showing Distribution of Serotypes of *Shigella* spp, *Vibrio cholerae, Salmonella* spp.

Bacterial enteropathogens	Number	Percentage
***Shigella spp* (N=35)**		
Shigella dysenteriae	21	60%
Shigella flexneri	10	28.5%
Shigella boydii	3	8.6%
Shigella sonnei	1	2.9%
***Vibrio cholerae* (N=21)**		
Inaba O1	5	23.8%
Ogawa O1	16	76.2%
***Salmonella spp* (N=8)**		
Salmonella typhi	5	62.5%
Salmonella paratyphi A	2	25%
Salmonella paratyphi B	1	12.5%

Table 7: Showing Antibiotic Susceptibility Pattern of Bacterial Isolates.

Antibiotics	*Shigella* spp (N=35)		*Salmonella* spp (N=8)		*Vibrio* spp *(N=21)*	
	S	R	S	R	S	R
Ciprofloxacin	33 (94.3%)	2 (5.7%)	6 (75.0%)	2 (25%)	21 (100%)	0 (0%)
Gentamicin	25 (71.4%)	10 (28.6%)	6 (75.0%)	2 (25%)	21 (100%)	0 (0%)
Cotrimoxazole	20 (57.1%)	15 (42.9%)	3 (37.5%)	5 (62.5%)	0 (0%)	21 (100%)
Ampicillin	16 (45.7%)	19 (54.3%)	3 (37.5%)	5 (62.5%)	X	X
Norfloxacin	30 (85.7%)	5 (14.3%)	X	X	X	X
Nalidixic acid	24 (68.6%)	11 (31.4%)	X	X	0 (0%)	21 (100%)
Tetracycline	X	X	X	X	19 (90.5%)	2 (0.5%)
Erythromycin	X	X	X	X	8 (39.0%)	13 (61%)
Chloramphenicol	X	X	3 (37.5%)	5 (62.5%)	X	X

Note S=susceptible, R= resistant

Discussion

Diarrheal disease occupied the second place among the top ten diseases in Nepal[2]. The identification of etiological agents of diarrhea is extremely important, for it helps in precise diagnosis and proper management of patient in time. With a view to determine the common etiological agents of diarrhea, the study was conducted in children less than 12 years of age at Kanti Children's Hospital from February to August, 2007.

The isolation of bacterial enteropathogens accounted 33%, which is quite higher than in other study[8,9]. Such difference may be due to the reason that, only non-lactose fermenter had been considered and *E. coli* which is the major causative agent of infantile diarrhea had not been considered. While in this study, *E. coli* was also considered which rose up the percentage of isolation of bacterial enteropathogens.

Our study accounted for 22% parasites among total enteropathogens isolated. This result differ in accordance with the findings a study[11,12,13] where intestinal parasite was found about 70-78%. These differences in the rate of parasitic infection may be due to various factors like geographic distribution, seasonal variation, and climatic condition, different time of study period, socio-economic status, and degree of sanitary conditions etc of the subjects being studied. However, among all these the most important factor that must be credited is the National Intervention Program which gives anti parasitic drug along with the Vitamin A in children below 5 years of age in each 6 month period.

Altogether, 107 (21.4%) cases were found infected with rotavirus which is similar to other hospital based study at Kanti Children's Hospital [14]. The study showed that the highest rate of rotavirus infection was seen in hospitalized patients than the OPD patients. The chi-square test too showed that the hospital admission was statistically significant with rotavirus infection which is similar to similar study [15].

The frequency distribution showed that the highest number of enteropathogens was found in age group 0-2

years. Usually this relates to the development and status of the immune system which varies with age. It may also be associated with changes in normal flora coincidental to developmental changes in the human. Four million children under five years of age die annually from diarrhea, 80 percent of these deaths occur in the age of first two years of life [10].

Nepal is country inhabited by the population of diverse ethnic groups. The castes generally defined as Bahun, Kchetriya, Newar, Rai, Tamang, Limbu, Minorities etc. These castes define social status and influence many aspect of life including religious and cultural practice[16]. Ethnic group wise distribution of enteropathogens showed that Gurung/Magar/Rai/ Tamang were highly infected (34.6% i.e. 108 out of 126) which was found statistically significant at 5 % level of significance. But another study[17] found highest parasitic prevalence in Dalit (minorities), which may be attributed to the differences in socioeconomic status among the group, dominance of particular ethnic group in certain geographical area, illiteracy, lack of health education, cultural and religious factors etc.

The highest bacterial pathogen was detected most in the month of August and July and least in the month of May. The higher prevalence of enteropathogens in the month of June, July August may be due to favourable condition for transmission and proliferation of the etiological agents in the rainy season. Reports [2,4] further supports the fact that in Nepal, the incidence of diarrheal diseases rises sharply each year during the early warm summer and rainy season with the epidemic peak in July-August each year.

The rotavirus infection peaks during winter season. Rotavirus is known as a causative agent of winter diarrhea[18]. The exact cause of increasing rotavirus infection is not known but may be due to meteorological factor, in particular low temperature and low indoor relative humidity[19]. Since our study was carried in from February to August; the highest rotavirus infection was seen in February.

The antibiotic susceptibility pattern of the bacterial isolate showed that *Shigella* spp, was found to be most susceptible to Ciprofloxacin (94.3%), Norfloxacin (85.7%), Gentamicin (71.4%), Nalidixic acid (68.6%), Cotrimoxazole (57.1%). *Salmonella* spp was found most susceptible to Ciprofloxacin and Gentamicin (75.0% each), followed by Cotrimoxazole, Ampicillin, Chloramphenicol (37.5% each). *Vibrio* spp was found 100% susceptible to Ciprofloxacin, Gentamicin, 90.5% susceptible to Tetracycline and 39.0% susceptible to Erythromycin. Antibiotics eliminate pathogens and limit their carriage and systemic effects; however, in most diarrheal illnesses they do not alter the disease substantially, and in some, such as infection with *E coli* O157:H7, they make matters worse[20]. Their main use remains in the management of dysentery.

Although there are numerous projects and activities related to diarrheal disease control program like regular deworming, health education, toilet use, parasite control project, school health program etc. were being applied on the part of government, these attempts have remained to be ineffective[21]. The program focusing in health/ sanitary education regarding the etiology of diarrhea and its prevention as well as community intervention directed to vulnerable group should be conducted.

Conclusion

The children visiting the Hospital were found to be highly infected with bacterial etiological agents than parasitic and viral agents. *E. coli* among the bacteria, *E. histolytica* among the parasite and Rotavirus were the major causative agents identified. Age group 0-2 years showed the most number of enteropathogens.

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