

DETECTION OF ENTERIC BACTERIAL PATHOGENS (*VIBRIO CHOLERA*E AND *ESCHERICHIA COLI* O157) IN CHILDHOOD DIARRHOEAL CASES

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Abstract: A hospital based cross sectional study was carried out in stool samples collected from cases of diarrhoea in children admitted to Oral Rehydration Therapy (ORT) ward, Kanti Children Hospital, Maharajung. A total of 204 stool samples collected from children below 15 years were processed at Department of Microbiology, Bir hospital during the study period, February 2004 to June 2004. The stool specimens were investigated for *Vibrio cholerae*, as well as *E. coli* O157. Bloody stools were more focused for isolation of *E. coli* O157. Out of 204 patients, 60.3% were male and 39.7% were female. The largest number of diarrhoeal patients belong to age group 0-5 years i.e. 112 (54.9%). *Vibrio cholerae* O1 was found in 86 (42.2%) cases. All *V. cholerae* O1 belong to Ogawa serovar and El Tor biotype. Out of 86 isolates, 52.3% were from male patient and 47.7% were from female patient. Highest incidence of *V. cholerae* O1 was found in age groups 5-10 (46.5%) Isolation of *V. cholerae* in 10 cases even in age group 0-2 was remarkable feature. Incidence of *V. cholerae* O1 was highest in the month of April (61.5%). *E. coli* O157 could not be detected in this study. Predominant or pure growth of sorbitol non fermenting (SNF) strains which were biochemically identified as *E. coli* but not agglutinated with *E.coli* antiserum were found in five cases. Tetracycline was 100 percent effective antibiotic followed by Norfloxacin and Ciprofloxacin to *V. cholerae* O1. In this study, patients suffering with cholera were mostly from Kalanki. People using municipal tap water were mostly affected (51.8%) Out of 30 isolates of processed, 22 isolates showed toxin production.

Key words: *Vibrio cholerae*; *E. coli* O157; Sorbitol non fermenting; Cholera toxin.

INTRODUCTION

Diarrhoea is one of the commonest problems found in pediatric clinics in any part of world. Diarrhoea and gastroenteritis comes the second position among top ten diseases admitted to hospital in the world. Each year 30,000 to 40,000 people die from diarrhoeal disease (Bista et al., 1993). The introduction of oral rehydration therapy and increasing access to clean water has caused a steady drop in mortality from diarrhea over the last two decades. However, acute diarrhoeal diseases (ADD) still cause approximately 3 millions deaths each year in the developing world in patients under five year of age. Small children are least likely to tolerate large fluid shifts, consequently at least 80% of these deaths occur in children below the age of 2 years. (Seear, 2000).

Nepal, being a developing country, diarrhoeal diseases are major problem. Though precise data on childhood mortality associated with diarrhoeal diseases in Nepal is not available, it has been estimated that approximately 25% of child death are associated with diarrhoeal diseases, particularly acute diarrhoea. (Bista, 2001). In Nepal, the incidence of diarrhoeal diseases rises sharply each year during the warm summer months, the small increase in the incident of food borne gastroenteritis occurs each April-May, followed immediately

by a sharp rise in the incidence of water borne gastroenteritis and cholera beginning with the monsoon rain in May. The epidemic tends to peak in July-August and subside by October. The case fatality rate (CFR) from acute diarrhoeal diseases is 1.95% in 1991, 1.39% in 1992, 0.13% in 1993, 0.17% in 1994, 2.56% in 1995, 1.43% in 1996, 0.9% in 1997, 2.05% in 1998, 4.59% in 1999, 2.91% in 2000, 2.79% in 2001 (EDCD, 2001).

Cholera is worldwide problem, especially in developing countries. It has been very rare in industrialized nations for the hundred years, however, the diseases is common today in other part of the world, including the Indian sub-continent and sub Sahara Africa. *Vibrio cholerae* is most dangerous vital pathogen, which has morbidity and mortality rate higher than other organisms (CDC, 1993). The total number of cases of cholera reported in Asian and African countries were nearly 1,50,000. According to reports from health authorities of Latin American countries, another 2,50,000 cases have occurred in Peru (WHO, 1991). Cholera is one of the most important causes of diarrhoeal diseases in Nepal also. In Nepal cholera was confirmed in 46%, 63% and 25% of faecal specimen processed in 1990, 1991 and 1992 respectively. (Bista, 2001)

E. coli O157:H7 is an emerging cause of food borne illness. It has been estimated that 73000 cases of infection and 61

deaths in the United States each year. Children below 5 years and the elderly people are more likely to develop serious complication. Since the first description of this illness in 1982, infections have been reported from more than 30 countries from six continents (CDC, 1997). *Escherichia coli* O157 is uncommon in Nepal, however, we cannot ignore it by studying the previous reports. This kind of study would help to assess the likelihood of human morbidity and mortality in future. Since the prevalence of *Vibrio cholerae* is higher in Nepal and its antibiotic resistance pattern is increasing day by day. The study was undertaken in order to know the incidence rate, clinical characteristics for rapid control, to control use of incomplete dosages of drugs, to initiate the responsible unit for avoiding unnecessary usage of drugs.

MATERIALS & METHODS

The study was carried out in infants and children with diarrhoea less than 15 years admitted at Kanti Children Hospital, Maharajgunj who passed watery stools with or without mucus or blood or loose stools with blood and/or mucus. During collection of samples, history of patient was noted according to questionnaire.

Then the stool samples were brought immediately to Microbiology Laboratory, Bir Hospital, Kathmandu. The samples were processed with standard laboratory techniques (Cheesbrough, 1998; Collee et. al, 1989; FDA Bacteriological Analytical Manual, 1995).

The stool samples were inspected macroscopically for its colour consistency whether formed, semi-formed or fluid, the presence of mucus, blood. After macroscopic examination, samples were cultured using appropriate media i.e. Thiosulphate Citrate Bile salt Sucrose (TCBS) agar for *Vibrio cholerae*, Sorbitol MacConkey agar (SMAC) for *E. coli* O157. Identification of the isolated organism was done using various biochemical & serological tests. Cholera toxin was detected using standard protocol (CDC, 2000).

RESULTS

There were altogether 204 stool samples processed for *Vibrio cholerae* and *Escherichia coli* O157. Out of 204 diarrhoeal patients 123 were male patients which constituted 60.29% and 81 were female patients which constituted 29.71% (Fig. 1). Majority of patient i.e. 112 (54.90%) belonged to the age group of 0-5, followed by age group 5-10, 60 (29.41%) and 10-15 32 (15.69%) (Fig. 2).

Vibrio cholerae was found in all age group. Out of total 86

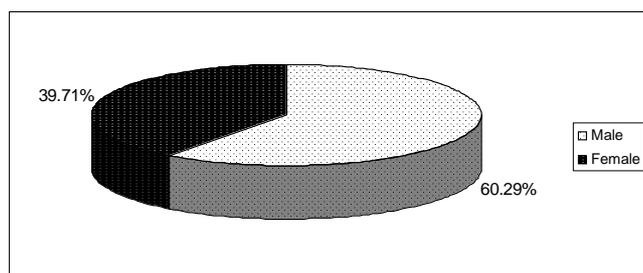


Figure 1: Gender-wise Distribution of Diarrhoeagenic Patients.

Figure 2: Age and Gender-Wise Distribution of Diarrhoeagenic Patients.

isolates of *Vibrio*, large number of *Vibrio cholerae* 40 (46.51%) was found in age group 5-10. Out of 86 *Vibrio cholerae*, 45 (52.33%) were from male patient and 41 (47.67%) were female patient (tab.1).

It was found that during five-month study period from February to June, the highest incidence was seen in the month of April (61.53%). In this year, *V. cholerae* was seen earlier i.e. in pre-rainy season. Highest incidence of *E. coli* was seen in month of March (5.26%) as compared to other months (fig. 3).

All isolates of *V. cholerae* O1 (100%) were found sensitive to Tetracycline followed by Norfloxacin (42.9%) and Ciprofloxacin (34.3%) (Fig. 4).

Out of 30 *V. cholerae* which were taken randomly, 22 (73.3%) isolates showed the toxin production (Tab. 2).

DISCUSSION

Diarrhoea is still the most common illness among children causing highest number of morbidity and mortality in the developing countries including Nepal. Due to lack of hygiene knowledge, unsafe drinking water and sanitation, illiteracy, lack of health education, under nutrition, incorrect feeding practice, wide spread faecal contamination of the environment, underlying disease, dense population in the region, gastric hypoacidity, immunodeficiency, superstition in rural areas are associated with the risk of children diarrhoea (WHO, 1993).

Although the diarrhoea is caused by numerous microflora, but in this study, we considered *Vibrio cholerae*, which is major pathogen as also suggested by Pokharel *et al.* (1997) in Kathmandu, Basak *et al.* (1992) in Calcutta and Shakya (1999) in Kathmandu. This study was also carried out with special interest to isolate Enterohaemorrhagic *Escherichia coli* O157 which is now recognized as an important human pathogen.

Out of 204 diarrhoeal children, 60.3% were male and 39.7% were female which shows the numbers of male patients were higher than the female patients (Fig. 1). This indicates that male are more susceptible than female. Similar results were observed by Aggrawal *et al.* (1989) in India, Huilan *et al.* in 1991, Aryal (1996), Shakya (1999), Gurung (1997), and Piya (2000) in Kathmandu. Diarrhoea occurs among all age group children. In this study, the diarrhoea was mostly seen in the age group 0-5, i.e. (54.9%) followed by age group 5-10, (29.4%) and (15.7%) in 10-15 age group (Fig. 2) The increment of

Table 1: Age & Gender-wise Distribution of Positive Results.

Age in yrs	<i>V. Cholerae</i>				<i>E. coli</i>				Total	%
	Male	Female	Total	%	Male	Female	Total	%		
0-5	12	12	24	27.90	3	2	5	100	29	31.86
5-10	24	16	40	46.51					40	43.95
10-15	9	13	22	13.95					12	13.18
Total	45	41	86		3	2	5		91	
%	52.337	47.67	42.16		60	40	2.45		44.60	

diarrhoea among the above age group might be due to lack of maternally acquire antibodies, the lack of active immunity in the infant, the introduction of food that may be contaminated with faecal bacteria, and direct contact with human or animal faeces, when infant starts to crawl. Most enteric pathogens stimulate at least partial immunity against repeated infection or illness, which helps to explain the declining incidence of disease in elder children and adults. Similar result was observed in the study conducted by Rajkarnikar (2000) where majority of patients (37.20%) belong to age group 0-5. In a study carried out in Kathmandu by Shakya (1999), reported higher number 60.85% of diarrhoeal children in the age group 0-3.

In this study, the incidence of cholera was found 42.2 percent. Similar result (41.7 percent) was reported by Ise *et al.*, in (1994). Out of 86 *V. cholerae*, 45(52.3%) were from male patients and 41(47.7%) were female patients (Tab.1). Large number of isolates 40 (46.5%) was found in age group 5-10. This might be due to unhygienic practices of children and their parents and also children of this age group were more likely to eat street foods. Cholera rarely occur in children under 30 years of age (Khan *et al.*, 1996). However in this study, 10 cases (11.6%) of cholera were observed in this age group, which is remarkable feature of our study.

All *Vibrio cholerae* O1 isolated in this study belong to Ogawa serovar and El Tor biovar. This was in agreement with study conducted in Delhi, India by Aggrawal *et al.* (1989) Ise *et al.* (1994) in Kathmandu and Shrestha and Shrestha (1989). However, the finding of Pokharel *et al.* (1997) was different that though Ogawa predominates, Hikojima is isolated and less often Inaba. The outbreak of cholera started suddenly in April. The incidence was highest in April (61.5%), and it gradually decreased onwards i.e. 56.4% in May, 47.9% in June (Fig. 3). Tetracycline seemed to be most effective antibiotic which was sensitive in 70 (100%) *V. cholerae* O1. Similar results were reported by Aryal (1996), Shakya (1999) and Gurung (1997).

Though there are wide varieties of strains of *E. coli*. We are only interested in *E. coli* O157 strains, since it causes severe haemolytic uremic syndrome and haemorrhagic colitis. And also, this kind of study was not done giving so much preference in Nepal. Since the intestine of the animals may be the reservoirs of these organisms for human infection and cattle particularly have been shown to harbor them Bettelheim

Table 2: Detection of Toxin Production.

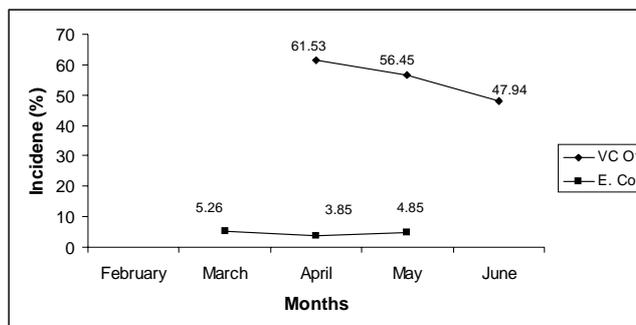


Figure 3: Monthly Occurrence of *Vibrio cholerae* O1.

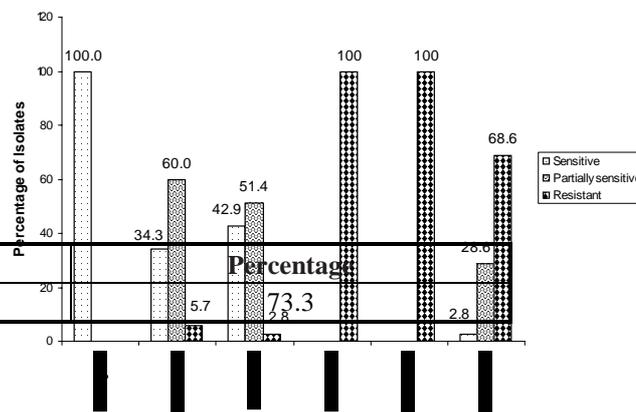


Figure 4: Antibiotic Sensitivity Pattern of *V. cholerae* O1.

(1996). This indicates the probabilities of wide dissemination of EHEC in our populations since majority of people living in this country are bucolic.

The stools with blood are more focused in our study to isolate *E. coli* O157. But we couldnot isolate this organism inspite of our special interest. Similarly, a study conducted by Gurung (1997), Karki *et al.* (2002), Shakya (1999) also reported no cases of *E. coli* O157. However in a study conducted by Aryal (1996), she found 1 (0.65%) cases of *E. coli* O157. Similarly Pokharel *et al.* (1997), found 2 cases of *E. coli* O157 during the study period from June 1995 to December 1996.

However in this study five sorbitol non-fermenting colonies which were biochemically identified as *E. coli* were isolated but they were not agglutinable with *E. coli* O157 antiserum. These organisms were considered as pathogenic *E. coli*. Such type of isolates (37%) were also found by Shrestha and

Shrestha. (1989) and considered as pathogenic *E. coli*. Similarly during February-March, 1994, four persons in Helena, Montana developed bloody diarrhoea and severe abdominal cramps. Stool cultures for *Salmonella*, *Shigella*, *Campylobacter*, and *Escherichia coli* O157:H7 were negative; however, sorbitol-negative *E. coli* colonies were identified in stools from all four patients. Isolates from three patients were identified at CDC as a rare serotype, *E. coli* O104:H21, which produced Shiga-like toxin II. (CDC/ 1995).

Vibrio cholerae produce an enterotoxin, which causes the intense diarrhoea which is so typical of the disease cholera. The detection of cholera toxin (CT) production is an important indicator of virulence. In this study, out of 30 *V. cholerae*, which were identified by biotyping and serotyping, 22 showed the toxin production. However, epidemic strain must produce enterotoxin. There might have low concentration of toxin produced, which couldn't be detected in the experiment. In a study conducted by Shrestha (1995), she found cholera toxin both on *V. cholerae* O139 (5) and *V. cholerae* O1 (1) by colony hybridization technique using oligonucleotide probe against ctx gene.

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