DISCUSSION
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Acute diarrhoeal diseases are an acknowledged major health problem, severely affecting the children from developing countries, but important to all countries of the world. This disease is perhaps the biggest child killer in developing countries, still 3.5 million children mainly in developing countries die every year due to diarrhoeal diseases. In India alone about 1 million children die of diarrhoeal disease every year.

In several developing countries 40 percent of hospital admissions are accounted for by acute diarrhoeal diseases, with a case fatality rate of 2.5 to 9.5 percent (Singh, 1983). In our pediatric out patient clinic acute diarrhoea constitute nearly 22% of the total attendance. In last one year (June, 90 to May, 91) 726 patients out of total 2995 admitted patients were suffering from acute diarrhoea, constituting 24.2% of total pediatric admissions. Out of 726 admitted patients 43 patients died (5.92%). Thus, acute diarrhoeal diseases constitute considerable disease burden in hospitals.

Most cases of acute diarrhoea in young children are due to infections with a variety of organisms. The recent recognition of role of certain viruses and bacteria now makes it possible to identity the causative agents in over two thirds of diarrhoea patients.
The yield of pathogens in stool specimens depend upon the facilities for isolation and identification of micro-organisms. There are a large number of published studies on aetiological agents of acute diarrhoea in children in India, but a small number of these include almost all recently recognised pathogens.

Various Indian investigators, who have included bacterial, viral and parasitic agents especially rotavirus and enterotoxigenic E. Coli in their study have reported 55-80 percent isolation rates of various enteropathogens (Bhat et al, 1985 - 72.3%, Sen et al, 1985 - 81.4%, and 62.1%, Bhan et al, 1987 - 72.3%). Mohandas et al (1987) isolated at least one enteropathogen in 55% cases of acute diarrhoea. In this study authors included investigation for other viruses also but did not include identification of enterotoxigenic E. Coli. Stoll et al (1982) in an extensive study in Bangladesh isolated at least one enteropathogen in 66% of their patients attending a hospital. In the present study overall isolation rate of micro-organisms was 65.2%. But due to non-availability of antisera for serotyping and limitation of facilities, tests of enterotoxigenicity were not carried out.

Rotavirus is the best known viral agent causing acute gastroenteritis. Rotavirus diarrhoea is a significant source of infant and childhood morbidity in tropical and non-tropical settings (WHO, 1989). The importance of rotaviruses in pediatric gastroenteritis has been well
documented in developed countries (Davidson et al, 1974, Kapiakian, 1976; Rodriguez et al, 1977; Brandt et al, 1984 and Ellis et al, 1984 etc.).

In the present study rotavirus was detected in 18% children suffering from acute diarrhoea using ELISA technique. The results obtained in the present study are in agreement with many studies conducted in India. Samantray et al (1982) and Bhan et al (1987) reported prevalence of rotavirus in an urban slum community in to be 21.2% and 20.3% respectively. Mohandas et al (1987) in their study in an outpatient clinic in Vellore observed 19% acute diarrhoeas were due to rotavirus. Bhat et al (1985) and Sen et al (1985) have reported similar incidence of rotavirus in hospitalized children (18.3%, 15.9% and 16.3% respectively). Various authors have reported higher detection rates of rotavirus in patients with acute diarrhoea requiring hospitalisation (Black et al, 1981; Samantray et al, 1982). This is because rotavirus disease has not greater potential to cause dehydration (Black et al, 1981).

Rotavirus diarrhoea is predominantly a disease of children under two years of age (Rodriguez et al, 1977; Black et al, 1982; Samantray et al, 1982 and Brandt et al, 1983). In the present study 32 out of 34 rotavirus positive patients were under 2 years of age, no rotavirus was detected after 3 years of age. The peak incidence of
rotavirus diarrhoea was during 7-12 month. Thus age distribution of rotavirus in the present study is consistent with previous studies. Antibody surveys from many areas of world have also revealed that most children acquire antibody against rotavirus by the end of third year of life (Kapikian, 1975, Jesudoss et al, 1978).

In the present study Shigella was isolated from 15 patients out of 189 patients studied giving a prevalence of 7.9%. Shigella is a constantly occurring organisms in most of the studies. The isolation rate of Shigella in the present study was consistent with the findings of Feldman et al (1970), Sanyal et al (1977), Agarwal et al (1981), Gupta et al (1985). However, isolation rates of Shigella in the present study are less in comparison to studies by Bhat et al (1985) and Santhanakrishnan (1987) who reported prevalence rates of Shigella to be 20.6% and 22% respectively in their studies. The difference in isolation rate varies according to epidemiological setting of study.

The isolation rate of Shigella in the present study was lowest below 6 months of age. The isolation rates were higher after infancy, with peak during 2 to 3 years of age. Santhanakrishnan (1987) have reported that children under 2 years are predominant victims, 80% cases in his study were children under 2 years of age. Various other studies have reported Shigellosis to be common during weaning period. But number of shigella in present study
is very small to elaborate any valid association regarding age distribution.

Stoll et al (1982) in their study found lowest incidence of *Shigella* during infancy and attributed it to probable protective effect of breast feeding or children in infancy has less exposure.

Salmonella as a causative agent of acute diarrhoea has remained relatively of less importance (Sanyal, 1981). Nontyphoid *Salmonella* like *S. typhimurium*, *S. newport* etc. have been implicated in both endemic and epidemic situations (Paul et al, 1981; Fule et al, 1985). In the present study isolation of *Salmonella* was low 0.16% which is consistent with many Indian studies (Bhan et al, 1987 - 2.5%, Sen et al, 1985 - 0.9%; Mohandas et al, 1987 - 3%).

Sanyal et al (1977) in their study could not isolate *Salmonella* from any case of acute diarrhoea. *S. typhimurium* was the commonest serotype in various studies (Saxena et al, 1981; Fule et al, 1985 and Bhat et al, 1985).

Out of 3 Salmonella isolates 2 were present below 6 months of age and 1 in 13-24 months age group. The number of *Salmonella* is too small to make any valid association with the age distribution.

With the development of practical methods of isolation of *C. jejuni*, from faeces, this organism has been gaining increasing recognition as an important enteric pathogen (Nayyar et al, 1983). In the present study Campylobacter was isolated from 5.8% cases of acute
diarrhoea. Many studies from developing and developed world have reported the isolation rates between 4 to 15 percent (Pai et al, 1979; Blaser et al, 1980; Nayyar et al, 1983; Bhan et al, 1987; Nair et al, 1985 and Mohandas et al, 1987). In the present study isolation rate of Campylobacter in acute diarrhoeal children is lower than many Indian studies (Nayyar et al, 1983 - 10.3%; Bhan et al, 1987 - 10.2%; Mohandas et al, 1987 - 13%). Whereas many other authors have reported relatively lower isolation rates (Khatua et al, 1984-2%; Bhat et al, 1985-3.2%; and Sen et al, 1985 - 2.6%).

In the present study difference between isolation rates of C. jejuni from patients with diarrhoea and from controls is not significant. This is in agreement with most of the studies from India and Bangladesh. Studies by Blaser et al (1980) from Bangladesh reported almost equal isolation rates from diarrhoeal and healthy children (12% and 14%). Rajan et al (1982) have shown higher excretion rate of C. jejuni from healthy children and adults in southern India. This high excretion rate of C. jejuni in nondiarrhoeal individuals makes it difficult to interpret exact significance of C. jejuni in causation of diarrhoea.

In the present study isolation rate of C. jejuni was maximum during 7-24 months of age group and isolation rates were lower before 6 months and after 2 years. In various studies where C. jejuni has been found to be more

_E. coli_ forms a part of normal flora of the gut but producing no disease. Normally several intricate mechanisms confine _E. coli_ to the large intestine and prevent spread to the proximal bowel. As a result of breakdown of natural defences, a complex series of interactions between external environment, the host and organisms leads to extensive colonization of small gut by _E. coli_ predisposing individual to syndrome of diarrhoea (Harris, 1976). Large scale proliferation of these organisms is demonstrated as pure or predominant growth on primary culture of stool.

In the present study _E. coli_ was cultured as a pure or predominant growth in 21.8%. Children with acute diarrhoea and in 11% non-diarrhoeal controls. Agarwal et al (1980) reported _E. coli_ as a predominant growth in 60.6% cases of acute diarrhoea in young children out of which only 21.2% were typable. Sarkar et al (1980) documented _E. coli_ in pure or predominant growth in 37% cases of acute diarrhoea out of which 58.8% were typable strains. Paul et al (1980) isolated _E. coli_ in a pure culture in 30.6% cases and 22.6% controls. In the present study isolation of _E. coli_ as predominant growth culture was lower in comparison to previous studies. This may be because many patients might have taken antibiotics before first contact in our out patient clinic.
To day it has became an acceptable practice to distinguish *E. coli* that cause diarrhoea into three types - Enterotoxigenic *E. coli*, enteropathogenic *E. coli* and enteroinvasive *E. coli* (Merson and Black, 1981). In the last two decade most of the studies on etiology of acute diarrhoea have included test for enterotoxigenicity for *E. coli*. In the present study due to certain constrains like non-availability of the typing antisera, and lack of laboratory facilities for testing enterotoxigenicity, we could not screen for *E. coli* strains as EPEC and ETEC. Hence our results regarding incidence in the present study is not comparable with the studies conducted in last two decades.

Isolation rate of *Entamoebahistolytica* and *Giardia lamblia* remained low in present study. *G. lamblia* and *E. histolytica* were isolated in 3.2% and 2.7% cases respectively. Only in the presence of trophozoites in stools examination, these organisms were considered possible diarrhoeal pathogen. Lower isolation of *G. lamblia* and *E. histolytica* in present study are consistent with studies of Sen et al (1981) & Bhan et al (1987). Mohandas et al (1987) reported 5% prevalence of *G. lamblia* in acute diarrhoea in young children. In present study *G. lamblia* and *E. histolytica* were not isolated in infancy, isolation rate was relatively higher after two years of age. However, sample size is very small to consider any association with age distribution.
Studies from Bangladesh have documented that diarrhoea associated with these two organisms is more common in older children suggesting short lived transplacental immunity, protection due to breast feeding or decreased exposure in infancy (Stoll et al, 1982).

The importance of breast feeding for the health of young children has been recognised for long time. The breast feeding has a major beneficial effect upon infection, malnutrition and unregulated fertility. The beneficial effect of breast feeding has been demonstrated by many extensive studies. Cunningham et al (1977) have shown that breast fed babies are less prone to development of enteral and other infections. Fallot et al (1980) have demonstrated that the incidence of breast feeding in hospitalised infants was 11% compared to expected figures of 25.2% in community in the same group. This shows that breast fed infants were less prone to development of serious illnesses.

Kumar et al (1981) in a longitudinal study have clearly demonstrated lower morbidity among breast fed infants in a community. Bhatia et al (1981) observed in hospital based study that bottle fed babies dominated the diarrhoeal group as compared to non diarrhoeal group. Mittal et al (1983) in their hospital based study also demonstrated that prevalence of bottle feeding was significantly higher in diarrhoeal group when compared with non-diarrhoeal group.

In the present study we observed that prevalence
of breast feeding was apparently higher in non-diarrhoeal group as compared to diarrhoeal group in children under two years. But this difference failed to reach the level of statistical significance (p > 0.05). However, in infants below 6 months the number of exclusively breast fed babies was significantly (p < 0.01) higher in non-diarrhoea group than that of diarrhoeal group. Thus it may be interpreted that breast feeding provides protection against diarrhoeal diseases. The protective role of breast feeding is discernible in younger infants in the present study.

In the present study an attempt was made to see the correlation between micro-organisms isolated in stool examination of exclusively breast fed and bottle fed (partly or solely) children of diarrhoeal group. It was observed that all the major micro-organisms i.e. rotavirus \textit{E. coli}, \textit{Shigella}, \textit{Salmonella} and \textit{Campylobacter} were isolated in both the groups. Isolation rates of \textit{Shigella} and \textit{Campylobacter} are apparently higher in bottle fed infants, but this difference was not statistically significant. \textit{E. coli} and rotavirus were isolated in almost similar proportion from bottle fed and breast fed patients.

It has been well shown in various studies that incidence of diarrhoeal disease is lower in breast fed infants. This decrease is attributed mainly to prevention of \textit{bacterial} diarrhoeas. Fallot et al (1980) failed to find any bacterial pathogens among hospitalised diarrhoeal infants who had been breast fed in a nontropical setting.
However, Cushing et al (1982) failed to find any protection against toxigenic *E. coli* among breast fed infants. It has been suggested that protection against *E. coli* infection may depend upon presence of specific *E. coli* antibodies in breast milk of the mother. Mittal et al (1983) in their hospital based study observed that there was no difference in enteropathogenic organisms isolated from differently fed children. *E. coli, Salmonella* and *Shigella* were isolated frequently in exclusively breast fed infants. The authors attributed their findings to heavy environmental contamination prevailing in our setting.

Weinberg et al (1984) have shown that incidence of rotavirus infection, its average age of occurrence and severity among differently fed infants are largely comparable. Samantray et al (1982) also observed that breast feeding provides only partial protection. In the present study no difference was observed in incidence of rotavirus detection in bottle fed (partly or solely) and breast fed patients. Thus our study is in agreement with study of Weinberg et al (1984).

It is noteworthy that in exclusively breast fed infants bacterial enteropathogens like *E. coli*, and *Shigella* and *Salmonella* were frequently isolated. This could be because in hot climatic conditions even young infants are often offered water, which is not boiled. And usually water in our set up is contaminated. Many parents use bottle for this purpose, which is another source of
heavy contamination. Thus the protective effects of breast feeding are probably overwhelmed by environmental condition in very poor socio-economic areas.

It has been suggested that determining factor in high prevalence of diarrhoeal disease in developing areas of the world would appear to be heavily contaminated environment, particularly water and weaning foods given to infants, and breast feeding in these areas can provide a significant but not absolute protection against diarrhoeal disease (Mittal, 1986).

Diarrhoea and malnutrition are commonly associated. Diarrhoea may affect nutritional status in several ways, decreased food intake (Molla et al (1986), withholding food as a measure to control diarrhoea, loss of micro and macro-nutrients, and catabolic response. On the other hand malnutrition also leads to increased prevalence of diarrhoeal disease. Ghai and Jaiswal (1970) have shown increased attack rate of diarrhoeal disease in malnourished children. However, study from Bangladesh has failed to show increase in attack rate (Chen et al, 1981). In a prospective study Chen et al (1981) suggested that the impact of diarrhoea in predisposing and exacerbating malnutrition may be most important of the bidirectional effect. The prominent effect of the malnutrition on diarrhoea may be through disease duration and mortality rather than through disease incidence.

In our study there is preponderance of malnourished children. 67.2% children in the present study are under
80% of the expected reference weight for age. A nutritional anthropometric study conducted in rural areas of Jhansi, from where most of our patients belonged, revealed that 82% preschool children were below Harvard standard of weight for height (Verma et al, 1980).

In the present study we analysed micro-organisms isolated in stool examination according to nutritional status. The isolation rates of various aetiological agents in normal, mild and severely malnourished children were 56.4%, 65.9% and 68.6% respectively. This difference in proportion of isolated organisms between normal and severely malnourished children (11.4% Vs 8.6%). But the difference is not statistically significant.

Samantray et al (1984) in their study reported no difference in rotavirus detection in normal nourished and malnourished children. Stoll in his extensive study documented that there was no significant difference nutritional status between children with shigellosis and those without. Mittal et al (1981) reported that isolation rate of bacterial enteropathogen from severely malnourished children were significantly higher as compared to normal nourished children. More definitive pathogens like Shigella Salmonella and Klebsiella were isolated more frequently from severely malnourished children.
The clinical features of acute diarrhoeal disease have been well documented. There is considerable overlap in the clinical features of diarrhoea attributed to different micro-organisms, and simultaneously there is variation in clinical features attributed to same organism. Clinically acute diarrhoea tend to present either as acute watery diarrhoea, with semifomed or semiliquid stools, or as dysentry i.e. blood in stools with or without mucous. It may be accompanied by vomiting, pain in abdomen, tenesmus, fever, concurrent respiratory symptoms etc.

In the present study watery diarrhoea was presenting symptom in 48.7% of the total patients studied. Invasive diarrhoea defined as presence of blood in stools with or without mucous was the chief complaint in 13.8% of total patients with acute diarrhoea. Stoll et al (1982) in their extensive study in Bangladesh reported 65% of acute diarrhoeas were watery and 20% were invasive in nature. Commonest organism in causation of watery diarrhoea are rotavirus, ETEC and V. cholerae (Stoll et al, 1982; Black et al, 1981). Jain et al (1983) and Mohandas et al (1989) in their study reported incidence of acute watery diarrhoeas to be 50.6% and 47% and of invasive diarrhoea 17.5% and 7% respectively.

Vomiting is an important complaint which may be associated with acute diarrhoea. 37.2% patients in present study had vomiting. Stoll et al (1982) reported vomiting in 58% of total patients attending hospital. Larger
proportion vomiting in their study could be due to high prevalence of ETEC and *V. cholerae*. Mohandas et al (1987) reported vomiting in 37.6% there is high degree of association in vomiting and rotavirus illness. ETEC and *V. cholerae* diarrhoea are also reported to be associated with more frequent vomiting (Stoll et al, 1982).

In the present study fever was observed in 40.7% cases. The presence of fever was more frequently associated with Shigella (*p < 0.05*). Various studies have reported concurrent upper respiratory tract infection with acute diarrhoea. The percentage of U.R.I. is quite variable in different studies. Some western studies have reported higher incidence of URI with rotavirus diarrhoea (Lewis et al, 1979). However many Indian studies have failed to find such association (Samantaray et al, 1982; Mohandas et al, 1987). In present study evidence of upper respiratory tract infection was observed in 39.1% of total patients.

Dehydration is universally known most important consequence. In present study 46% (87 out of 189) patients were dehydrated. 22.2% had mild, 14.8% moderate and 9% had severe dehydration. 56(29.6%) patients were admitted in pediatric ward for management.

Clinical illness attributed to rotavirus is typically described as watery diarrhoea generally with prominent vomiting and not uncommonly with fever and respiratory symptoms. In the present study watery stools
and vomiting were present in 82.3% and 70.6% cases respectively. A high degree of association between rotavirus aetiology and watery diarrhoea (p ≤ 0.01) and with vomiting (p ≤ 0.01) was found. The more frequent occurrence of watery diarrhoea and vomitings with rotavirus have been well documented in literature (Shepherd et al, 1975; Rodriguez et al, 1977; Black et al, 1981; Stoll et al, 1982 and Mohandas et al, 1987).

Blood in stools is rare in rotavirus diarrhoea mucous in stool may be present in upto 25% cases (WHO Scientific group). In present study blood was not observed in any stool sample however 17.6% children were having mucous in their stool.

Fever in association with rotavirus have been found to be more common in some studies (Stoll et al, 1982). However, earlier reports from west (Rodriguez et al, 1977) did not show any such significant association. We in present study could not find significant association (p ≥ 0.05) of rotaviral illness with URI. Lewis et al (1979) documented significant association of URI with rotavirus diarrhoea. However, some Indian studies (Samantray et al, 1982; Mohandas et al, 1987) did not find any significant association. In present study also does not show significant association (p ≥ 0.05) between rotavirus illness and upper respiratory tract infection.

In the present study patients with Shigella infection presented with both, watery diarrhoea and
dysentery. Presence of blood in stool (66.7%), mucous (73.4%) and pain in abdomen (46.6%) was present more frequently in *Shigella* infection. A statistically significant association of these features with shigellosis was observed. Fever was also present more frequently with *Shigella* infection. Presence of vomiting was relatively infrequent. Thus the presence of blood and mucous in stools and pain in abdomen indicate towards *shigella* aetiology. Though the sample size of study is small the results are in agreement with study carried out in Bangladesh (Stoll et al, 1982). *Shigella* produces diarrhoea by two mechanisms by toxin production affecting small bowel resulting in watery diarrhoea and by tissue invasion of colon resulting in classical dysentery. The patients with watery diarrhoea have more vomiting and more severe dehydration as compared to patients who present with dysentery (Stoll et al, 1982; Dutta et al, 1989).

Prolapse rectum have been reported to be common in shigellosis. Santhanakrishanan et al (1987) reported prolapse rectum in nearly half of the patients with shigellosis. In the present study we observed prolapse rectum in 20.0% patients. Serious complications like secondary sepsis which increase the morbidity and mortality in shigellosis (Alam et al, 1984) were not observed in our study.
Campylobacter was isolated in 11 patients with acute diarrhoea in present study. 6 patients (54.5%) had watery diarrhoea, 3 (27.3%) patients had invasive diarrhoea. Mucoid stools were present in 36.4% and pain in abdomen was present in 4 patients (36.4%). In half of our patients watery diarrhoea was present. Nayyar et al (1983) have also reported watery diarrhoea in nearly half of their patients and invasive features in one fourth patients. Our study is in agreement with the study conducted by Nayyar et al (1983). A high association with watery diarrhoea and campylobacteriosis was reported by Bhattacharya et al (1985) from Calcutta. Studies from Bangladesh (Blaser et al, 1980 and Stoll et al, 1982) have documented more frequent association of watery diarrhoea with campylobacteriosis. Reports from developed countries, however, have reported dysenteric syndrome more frequently associated with campylobacter diarrhoea (Butzler, 1981). Stoll et al (1982) in their study in Bangladesh documented that watery diarrhoea mucoid stools, pain in abdomen were more frequently present. However, in the present study the number of C. jejuni is too small to elaborate an association with many clinical features.