International Journal of Applied Research 2017; 3(7): 385-391

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2017; 3(7): 385-391
www.allresearchjournal.com
Received: 15-05-2017
Accepted: 16-06-2017

Savak Jasmina S
Microbiology Department, T.N. Medical College and B.Y.L. Nair Charitable Hospital, Maharashtra, India

Vaidya Shashikant P
Quality Control Department, Haffkine Bio-pharmaceuticals Co. Ltd, Maharashtra, India

Deshpade Sunita D
Microbiology Department, T.N. Medical College and B. Y. L. Nair Charitable Hospital, Maharashtra, India

Kar Saraswathy
Microbiology Department, T.N. Medical College and B. Y. L. Nair Charitable Hospital, Maharashtra, India

Correspondence
Savak Jasmina S
Microbiology Department, T.N. Medical College and B.Y.L. Nair Charitable Hospital, Maharashtra, India

Isolation of common infective agents causing chronic diarrhoea in clinically diagnosed malnourished children

Savak Jasmina S, Vaidya Shashikant P, Deshpade Sunita D and Kar Saraswathy

Abstract
Diarrhoea is a common complaint in pediatrics which may arise from many different etiological factors and have a major impact on nutrition. Malnourished children develop diarrhoeal illnesses more frequent and severe than well-nourished children. Detection of etiological agents of infective diarrhoea is important for therapeutic aspects and for implementation of appropriate control strategies. The problem of antimicrobial resistance in microorganisms causing diarrhoeal diseases in both developed and developing countries continues to be alarming. An attempt is made to screen etiological agent of chronic diarrhoea in malnourished patients. Stool samples were subjected for macroscopic, microscopic and cultural examination to demonstrate pus cells, red blood cell, trophozoites, ova of parasites, fungi and bacteria including *E. coli* serotype O157:H7. **S**usceptibility pattern of the bacterial isolates to common antibiotics used in the treatment obtained from clinical samples was carried out by Kirby-Bauer disk diffusion method. Rotavirus antigen was detected by serological method using RIDASCREEN ®Rotavirus kit., which involves Enzyme immunoassay. In the study, comparison between acute and chronic diarrhoea cases revealed that *E. coli* is the commonest etiology in acute as well as chronic diarrhoea cases. Children less than 1 year were found to be predominant group associated with infective chronic diarrhoea more prone to severe malnutrition. Study concludes, chronic diarrhoea is more common in malnourished children with Grade II and III malnutrition. Bacterial infection is more common with chronic diarrhoea in malnourished children. *E. coli* Serotype EPEC was found to be associated with persistent diarrhoea in malnourished children, where as EHEC was found predominantly associated with acute diarrhoea. As per suggestive mechanisms of chronic infective diarrhoea in malnourished children, normal commensal *E. coli* can be one of the predominant etiological agent for chronic infective diarrhoea in malnourished children.

Keywords: diarrhoea, malnourished children, etiological factors, pediatrics, **S**usceptibility pattern

Introduction
Diarrhoea is a common complaint in Paediatrics. Evaluation of an infant or a child with diarrhoea requires an understanding of the pathophysiology of diarhhoea as well as a basic understanding of fluid absorption. Diarrhoea in Peadiatrics age group may arise from many different etiological factors, which may produce diarrhoeal stool through one or more different mechanisms. In most cases of Paediatrics, especially in the case of chronic diarrhoea the differential diagnosis varies with age. The clinician must correlate age of the patient, the nature of stool, the chronicity of the problem and other relevant historical factors in order to formulate an adequate differential diagnosis [1].

In India, diarrhoeal diseases is a major public health problem among children under the age of five years [2]. In health institution, up to a third of total Paediatric admission is due to diarrhoeal diseases and up to 17 per cent of all deaths in indoors Paediatrics patients are diarrhoeal related. The household survey conducted during 1994 shows that the morbidity rate in terms of diarrhoea episodes per year per child under five years is about 1.7. [2] From a global perspective, diarrhoea and dehydration represent an ever-greater problem [3]. The total number of Paediatric deaths secondary to diarrhoea and dehydration is estimated at 4 million per year [3]. In less developed countries diarrhoeal morbidity is especially severe. Black and co-workers found children under three year had diarrhoea for an average of 55 day/year in Bangladesh. There is an increased susceptibility as evidenced by a higher age-specific attack

rate for *Rotavirus, Salmonella, Campylobacter, Aromonas, Entero Hemorregic E. coli* and *Giardia* [3].

Diarrhoeal diseases have a major impact on nutrition. Malnourished children develop diarrhoeal illnesses more frequent and severe than well-nourished children [3]. Recurrent diarrhoeal diseases are responsible for growth flattering in children, which increases susceptibility to infection with enteropathogens. It includes young age, immune deficiency, measles, malnutrition, travel to an endemic area, lack of breast-feeding, exposure to unsanitary condition, ingestion of contaminated food or water, level of maternal education and day-care center attendance [3, 4].

In formulating a differential diagnosis in a child with diarrhoea, it is necessary to differentiate acute and chronic diarrhea [1]. The duration of acute diarrhoea is short, usually less than two weeks. It is often caused by an enteric infection [4-6]. Acute diarrhoea lasts for less than two weeks [4-6]. Which is nearly always be presumed to be of infective type [4, 6]. *E. coli* is probably very common cause of diarrhea [7]. The other common organisms found are *Salmonella, Shigella, Campylobacter, Cryptosporidium, and Giardia*. Cholera is rare in eastern countries, which is common in travelers.

Viruses particularly *Rotavirus* is common in children, *Norwalk virus* is rare [4, 6, 7]. Toxin and food poisoning is also a cause due to *Staphylococcus toxins*, Bacillus toxin etc. Diarrhoea is also common, as a side effect of drug in which *Clostridium deficille* is a common but potentially serious infection related to antibiotics use [8]. Parasite and worms also cause diarrhoea often present with weight loss, irritability, rashes and anal itching. The common parasites are Pinworms, Hook worms, Ascaris and Tapeworms. Amoebic dysentery due to *Entaemoeba histolytica* is an important cause of bloody diarrhoea, which requires appropriate and complete medical treatment [8].

Diarrhoea that lasts for 14 days or more than 14 days are considered to be of chronic type [4, 5, 6]. It is not uncommon for diarrhoea to persist. Diarrhoea due to some organisms may persist for year without significant long-term illness. More commonly diarrhoea will slowly ameliorate but the patient becomes a carrier. This is often an indication for treatment, especially in food handlers [8]. Certain infectious agents including *Salmonella, Clostridium defficile, Yersinia, Aromonas, Giardia, Cryptosporidium* and *Entero Pathogenic E. coli*, are known to cause episodes of diarrhoea lasting several weeks [7, 8]. Infection with multiple pathogens may also cause infectious diarrhoea that persists longer than 14 days [7, 8].

Diarrhoea also has significant impact on nutrition, episodes of diarrhoea leads to impaired appetite, intestinal villi atrophy and loss of nutrients through stool [9]. which is identified as a major determining factor leading to malnutrition [9]. Child with multiple episodes of diarrhoea suffers most severely from Protein-energy malnutrition [6]. Even a brief episode of diarrhoea leads to loss of 1 to 2 percent of body weight per day. If dirrhoea becomes unusually prolonged or is recurrent, the child becomes severely malnourished. Malnourished children are more prone to infections as well diarrhoea-malnutrition-diarrhoea cycle contributes to large majority of early childhood deaths either directly or indirectly [6]. About 3-20 % episodes of acute diarrhoea, which last for 2 or more weeks, are classified as chronic diarrhoea. This is responsible for about 45 % of diarrhoea related deaths. Majority of these occur in malnourished children [10]. In 20-25 %-hospitalized children malnutrition exist in clinical forms, which is more severe and accounts for at least 20 % of hospitalized deaths. Majority of them have predisposing factors, Respiratory, GI tract disease etc. [11]

In malnourished children local intestinal immunity tends to decrease [12]. More severe the malnutrition severe is the immunological disability. Hence brief episode of infection affects the local mesenteric lymph nodes, which further decreases the immunity and increases the chance of infection and reinfection by pathogens. Most of the times commensals over grow or get absorbed in gut wall and produce systemic infection such as septicemia. This is common in malnourished children with chronic infective diarrhoea and acts as important risk factor for mortality. Chronic diarrhoea has emerged as an important public health problem in developing countries, as it contributes to a substantial portion of the nutritional decline and mortality associated with diarrhoeal illness [12].

Detection of etiological agents of infective diarrhoea is important for therapeutic aspects and for implementation of appropriate control strategies [8]. In developing countries, the bacterial pathogens are most commonly associated with endemic forms of diarrhoea [8]. The emergence and widespread distribution of drug resistant enteric bacteria have imposed serious limitations on successful antibiotics treatment. Spontaneous acquisition of drug resistance among enteric pathogens is due to selective pressure of antibiotic therapy [8]. This problem of antimicrobial resistance in microorganisms causing diarrhoeal diseases in both developed and developing countries continues to be alarming [13]. These multiple drug resistance strains have caused major disease outbreaks with high mortality and morbidity in developing countries [13].

Keeping in mind the above facts, we have made an attempt to screen etiological agent of chronic diarrhoea in malnourished patients And to isolate common causative agent causing chronic diarrhoea in clinically diagnosed malnourished children.

Material and Methods

This prospective longitudinal study was undertaken at the Dept. of Microbiology, T.N. Medical College and B.Y.L. Nair Charitable Hospital, Mumbai. Selection of subjects for the study and control group was done on the basis of standard classification of malnourished and chronic diarrhoea. Study group included all types of infective chronic diarrhoea, except, anaerobic infection in pediatric group while chronic diarrhorea cases due to drugs or antibiotics, carbohydrates intolerance, tropical spruce, chronic pancreatitis were excluded. Acute diarrhoea cases in pediatric group were taken as control group. The detailed clinical and personal history of the subject was recorded which included age, sex, socio-economical status, educational history of parents. Patients recruited were either hospitalized or outdoor patients at B.Y.L. Nair Charitable Hospital and patient visiting the primary health centers at Cheetah camp and Shivaji nagar were included. The subjects in the present study were of age group of more than 1 month old up to 12yrs. Study group and Control group included 100 patients each.

Random collection of stool sample was done in a sterile wide mouth container before starting any empirical treatment, radiological examination [14] from clinically

diagnosed malnourished children with infective chronic diarrhoea. Each stool sample was immediately, within 30 minutes, transported to the Microbiology laboratory [14].

Each stool sample was subjected for macroscopic examination, which included Gross appearance, Color, Consistency, Odor, frank blood and presence of adult worm or its fragment [14].

Saline wet mount of each stool sample was carried out for detection of pus cells, red blood cell, trophozoites and ova of Parasites [31-33] odine wet mount was carried out for detection of cysts of parasites. [41, 15, 16].

Thin smear were prepared on a new, clean, dust and grease-free slide by means of nichrome loop. These smears were fixed to the slide and following staining method was carried out [16]. Gram's staining [33] was done to demonstrate pus cells, Gram positive and Gram Negative bacteria [16]. Modified Zeihl Neelsen acid-fast stain [33] used for detection of *Isospora belli*, *Cryptosporidium* and *Cylospora* [15, 16].

A loopful of stool sample was inoculated and streaked on culture media. All universal aseptic precautions were taken during processing of samples [16]. MacConkey's agar was used to differentiate lactose fermenters and non-lactose fermenters organisms. *E. coli* Hichrome agar was used selectively to identify *E. coli* serotype O157:H7 (EHEC) [16]. *Salmonella-Shigella* agar was used for isolation of *Salmonella spp.* and *Shigella* spp.[33]*Campylobacter* agar was incubated at 37 ºC (in the presence of 5-10 % CO2) for 48 hrs to isolate *Campylobacter* spp.[33]Sabourauds dextrose agar slants were incubated at 37 ºC and room temparature and was observed till 10 days [16].

The minimum battery of tests was performed for Identification of isolates obtained [16]. Enterobacteriacea Identification kit (RAPID KD 003 Hi 25$^{Tm)}$ was used for identification and speciation of *Salmonella*, *Shigella* and *Klebsiella* isolates. *E. coli* serotyping of EPEC was carried out by using antisera kit (Denka Seiken UK, Ltd.)

To study the susceptibility pattern of the bacterial isolates to common antibiotics used in the treatment [17] of the isolates obtained from clinical samples was carried out by Kirby-Bauer disk diffusion method according to NCCLS guidelines. Sterile Muller Hinton agar [17] was used for the study.

The antibiotics used for *E. coli*, *Klebsiella* spps, *Shigella* spps, *Salmonella* spps and *Proteus spps* are as follows*:* Ampicillin (10mcg), Amoxyclav (10mcg), Ceftriaxone (30mcg), chloramphenicol (30mcg), Cotrimoxazole (25mcg), Nalidixic acid (30mcg) and Ciprofloxacin (5mcg).The antibiotics used for *Vibrio chlorae* were as follows*:*Ampicillin (10mcg), Amoxyclav (10mcg), Ceftriaxone (30mcg), chloramphenicol (30mcg), Cotrimoxazole (25mcg), Nalidixic acid (30mcg), Ciprofloxacin (5mcg), Tetracycline (30mcg) and Furazolidone (50mcg). The antibiotics used for *Psuedomonas* spps. were as follows*:* Piperacillin (10 U), Cefotaxime (30mcg), Cefoperazone (75mcg), Gentamicin (10mcg), Amikacin (30mcg) and Ciprofloxacin (5mcg), The zone showing complete inhibition was measured and diameters of the zones to the nearest mm were recorded. It was compared with control tests. Control tests using standard strains of test organisms were carried out to monitor internal quality control.

Rotavirus antigen was detected by serological method using RIDASCREEN ®Rotavirus kit, which involves Enzyme immunoassay for the detection of rotavirus.

Results

Table 1: Gender wise distribution of patients in study and control group.

Gender	Groups		Total (%)
	Study (n1) (%)	Control (n2) (%)	
Female	43.00	36.00	39.50
Male	57.00	64.00	60.50
Total	100.00	100.00	100.00

* p value < 0.001 n1= 100 n2=100

Male preponderance was observed in both the groups. Females comprised of about 43 % in Study group and 36 % in Control group.

Table 2: Comparison of Age groups between study and Control groups

Age groups	Groups		Total (%)
	Study (n1) (%)	Control (n2) (%)	
a) > 1 yr	65.00	18.00	41.50
b) 1 to 2 yrs	2.00	15.00	8.50
c) 2 to 3 yrs	0.00	8.00	4.00
d) 3 to 4 yrs	12.00	14.00	13.00
e) 4 to 5 yrs	4.00	7.00	5.50
f) 5 to 6 yrs	9.00	13.00	11.00
g) 6 to 7 yrs	2.00	5.00	3.50
h) 7 to 8 yrs	4.00	5.00	4.50
i) 8 to 9 yrs	2.00	6.00	4.00
j) 10 & >	0.00	9.00	4.50
Total	100.00	100.00	100.00

* p value > 0.001, n1= 100, n2=100

The majority of children comprised of age group less than 1 yr of age in study group that is 65 % and in control group that is 18 %.

Table 3: Malnutrition grading among study group cases.

Gender	Malnutrition grades		Total (%)
	Grade II (%)	Grade III (%)	
Female	55.80	44.20	100.00
Male	40.40	59.60	100.00
Total	47.00	53.00	100.00

* p value < 0.001, n = 100

Grade II malnutrition was predominant (56 %) female children, where as Grade III was common in male children (60 %.)

Table 4: Distribution of age groups with relation to malnutrition grades in study group cases

Age groups	Malnutrition grades		Total
	Grade II	Grade III	
a) >1 yr	16 (24.60%)	49 (75.40%)	65 (100.00%)
b) 1 to 2 yrs	2 (100.00%)	0 (0.00%)	2 (100.00%)
d) 3 to 4 yrs	11 (91.70%)	1 (8.30%)	12 (100.00%)
e) 4 to 5 yrs	2 (50.00%)	2 (50.00%)	4 (100.00%)
f) 5 to 6 yrs	8 (88.90%)	1 (11.10%)	9 (100.00%)
g) 6 to 7 yrs	2 (100.00%)	0 (0.00%)	2 (100.00%)
h) 7 to 8 yrs	4 (100.00%)	0 (0.00%)	4 (100.00%)
i) 8 to 9 yrs	2 (100.00%)	0 (0.00%)	2 (100.00%)
Total	47 (47.00%)	53 (53.00%)	100 (100.00%)

* p value > 0.001, n =100

Grade III was more predominant than Grade II malnutrition in 'a' group i.e. less than 1 yr, which was 76 % and 25 % respectively.

Table 5: Comparison of malnutrition grades between study Cases and Controls

Malnutrition grade	Groups		Total
	Cases (n_1) %	Control (n_2) %	
Grade I	0 (0.00%)	83 (83.00%)	83 (41.50%)
Grade II	47 (47.00%)	17 (17.00%)	64 (32.00%)
Grade III	53 (53.00%)	0 (0.00%)	53 (26.50%)
Total	100 (100.00%)	100 (100.00%)	200 (100.00%)

*p value > 0.001, n_1 =100, n_2=100

As shown in Table V and Graph 5 Grade I malnutrition cases (83 %) were maximum followed by Grade II (17 %) mainly associated with Control group i.e. acute diarrhoea cases. Grade III malnutrition (53 %) was predominantly followed by Grade II malnutrition (47 %). in study group i.e. chronic diarrhoea cases.

Table 6: Number of Fungal isolates from study Cases and controls.

Fungi isolated	Groups		Total
	Study (n1)	Controls (n2)	
Candida albicans	2	0	1.00 %
No fungal isolate	98	100	99.00%
Total	100	100	100.00%

*p value > 0.001, n1 = 100, n2 = 100

Two isolates of *Candida albicans* were obtained from study group patients.

Table 7: Distribution of bacterial isolates from study cases and controls

Organisms isolated-Bacteria	Groups		Total
	Cases n_1	Controls n_2	
E. coli	65	77	(71.00)
Klebsiella pneumoniae	18	13	15.5%
Pseudomonas aruginosa	0	3	1.50%
Proteus vulgaris	1	0	0.50%
Staphylococcus aureus	0	2	1.00%
Shigella flexneri	4	2	3.00%
Salmonella paratyphi A	3	0	1.50%
Salmonella typhi	3	0	1.50%
Vibrio cholerae	0	3	1.50%
No bacterial isolate	6	0	3.00%
Total	100	100	100.00%

*p value > 0.001, n_1 = 100 n_2 = 100

Culture positivity was observed 100 % and 94 % in Control and Study group patients studied. *E. coli* remained the predominant isolate among Study and Control group patients i.e. 65 % and 77 %, followed by *Klebsiella pnuemoniae*, as 18 % and 13 % respectively. *Pseudomonas aruginosa* (3 %), *Staphylococcus aureus* (2 %), *Vibrio cholerae* (3 %) isolates were obtained only from Control group and *Salmonella typhi* (3 %) and *Salmonella paratyphi A* (3%) *Proteus vulgaris* (1 %) from Study group patients. *Shigella flexneri* was isolated from both Study and control group patient as 4 % and 2 % respectively.

Table 8: Distribution of EHEC and EPEC in study Cases and controls

E. coli serotype	Groups		Total
	Cases (n_1)	Control (n_2)	
EHEC	0	15	7.50%
EPEC	7	0	3.50%
No serotype detected	93	85	89.00%
Total	100	100	100.00%

* p value > 0.001, n_1 = 100, n_2 = 100

Total 142 *E. coli* isolates were obtained from Study and Control group cases. These were further typed by using special Hicrome agar and specific antisera, only 15 isolates from Control groups were confirmed as EHEC(O157:H7) and,7 isolates from study cases were identified as EPEC. Remaining 120 *E. coli* isolates may be of EIEC or ETEC or normal commensal. This typing was not possible due to unavailability of specific antisera.

Table 9: Parasites detected in Cases and controls

Organisms detected-Parasite	Groups		Total
	Cases n_1	Controls n_2	
Entaemoeba histolytica	2	0	1.00%
Hymenolepis nana	2	0	1.00%
Isospora belli	1	0	0.50%
No parasite detected	95	100	97.50%
Total	100	100	100%

* p value < 0.001, n_1 = 100, n_2 = 100

Five parasites were detected in Study group only i. e. 5 %

Table 10: Rotavirus Antigen detected in Cases and controls

Rotavirus Antigen	Groups		Total
	Cases n_1	Controls n_2	
Detected	4	14	9.00%
Not detected	96	86	91.00%
Total	100	100	100.00%

* p value > 0.001, n_1 = 100, n_2 = 100

Rotavirus antigen was detected in 4 % cases of Study group and 14 % cases of Control group.

Table 11: Antibiotics Sensitivity Pattern of Clinical isolates obtained from Cases and Controls

Organisms	Antibiotics used								
	Ampicillin	**Amocyclav**	**Ceftriazone**	**Chloram-phenicol**	**Cotrimaxazole**	**Nalidixic acid**	**Ciprofloxacin,**	**Tetracycline**	**Furazolidone**
	R	**R**	**R**	**R**	**R**	**R**	**R**	R	R
E.coli (n =142)	2 (2%)	19 (13%)	3 (2%)	28 (20%)	7 (5%)	13 (9%)	11 (8%)	N.D	N.D
Klebsiella (n = 31)	10 (32%)	12 (39%)	3 (10%)	12 (39%)	6 (19%)	10 (32%)	20 (65%)	N.D	N.D
Shigella spp (n = 6)	1 (17%)	2 (33%)	0 (0%)	3 (50%)	1 (17%)	0 (0%)	0 (0%)	N.D	N.D
Salmonella spp (n =6)	0 (0%)	1 (17%)	0 (0%)	3 (50%)	1 (17%)	0 (0%)	0 (0%)	N.D	N.D
Proteus spp (n = 1)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	N.D	N.D
Vibrio (n = 3)	0 (0%)	0 (0%)	0 (0%)	1 (33%)	0 (0%)	1 (33%)	0 (0%)	0 (0%)	3 (100%)
	Piperacillin	Cefotaxime	Ceftazidime	Cefoperazone	Gentamicin	Amikacin	Ciprofloxacin		
Pseudomonas spp (n = 3)	3 (100%)	2 (67%)	2 (67%)	3 (100%)	3 (100%)	2 (67%)	3 (100%)	N.D	N.D

Key: n: No of bacterial isolates R: resistant isolates N.D: Not done

Discussion

Diarrhoeal illness in children is one of the leading causes of morbidity and mortality in developing countries and is an important cause of morbidity in developed countries. Differential diagnosis between diarrhoeal cases is essential in case of children. Acute diarrhoea is always of infectious etiology and mostly self-limited and does not require much evaluation. Simultaneously persistent or chronic diarrhoea carries the high risk of mortality in children and always requires medical supervision and complete evaluation [1, 3, 8].

The present study included total of 200 paediatric patients suffering from diarrhoea that is 100 malnourished children with infective chronic diarrhoea cases and 100 children with acute diarrhoea.

Majority of the cases were of age group less than 1 yr which was 65 % and 12 % cases comprised of age group 3 to 4 years. Which was well correlated with David *et al*. study in 2001, which states that children less than 5 yrs old are particularly susceptible to infectious diarrhoeal disease for a variety of epidemiological and immunological reasons [8]. And male predominance was seen in malnourished children with chronic diarrhoea. Muzumdar *et al*. in 2000 in his study on 'The effect of birth interval on malnutrition in Bangladeshi infants and young children' documented that children were at higher risk of malnutrition if they were females [18]. Diarrhoea can develop in healthy individual, but when it is chronic diarrhoea and associated with malnutrition considered as dangerous web.

In the present study 53 % grade III and 47 % of grade II malnutrition were observed with chronic diarrhoea, which leads to cause early childhood deaths. Similar finding was also documented by Butta *et al*. in 1997 (Pakistan) and Roy *et al*. in 2000 (Bangladesh) reported that malnourished children are more prone to develop persistent diarrhoea, which corroborates with the present study findings [19, 20].

Present study shows that grade III malnutrition was more predominant in children less than 1 yr which is 49 % with infective chronic diarrhoea. Roy *et al*. has documented the similar findings that younger children (> 2yrs) had significantly higher level of severe malnutrition than the children aged 2 yrs or older children who had chronic diarrhoea which found to be concurrence with present study [20].

All stool samples were cultured on appropriate media and examined for the growth of aerobic bacterial and fungal pathogens. *E. coli* was predominant bacterial isolate obtained from malnourished children with infective chronic diarrhoea accounting for 65 %. This was followed by *Klebsiella pnuemoniae* 18 %, *Shigella flexneri 4 %, Salmonella typhi 3 %, Salmonella paratyphi A* 3 % and *Proteus vulgaris* 1 %.. Jindal *et al*. study from Amritsar reported, *E.coli*, *Salmonella typhimurium*, *Shigella* spp and *Campylobacter* spp. as a causative agent of chronic diarrhoea [21]. Lopez *et al*. reported that *E.coli* and *Shigella* spp. showed higher frequency in chronic dairrhoea.[45] These findings corroborates the present study.

Candida albicans was identified in 2 % cases from study group patients which correlates with Jindal *et al*. finding as 1.3 % *Candida albicans* reported from children with chronic diarrhoea [22]. In our study the *Candida albicans* was isolated from HIV positive children in 2 % cases which is concurrence with Lee et. al (Washington) study who found that yeast and fungal infections usually occur in immunocompromised patients with chronic diarrhoea. Copious yeast was present in their stool sample. Candida spp. may be pathogen in some condition [23].

E. histolytica (2 %), *H. nana* (2 %) and *Isospora belli* in 1 % of cases were detected from malnourished children with infective chronic diarrhoea in the present study. Bhandari *et al*. (India) found *G. lamblia* in a significantly higher proportion in persistent diarrhoea cases so as Lanate *et al*. found *Cryptosporidium* from chronic diarrhoea and Jindal *et al*. reported *Cryptosporidium*, *E. histolytica* and *G. lamblia* were detected from stool sample of children with chronic diarrhea [24, 25]. In our study *G. lamblia* and *Cryptosporidium* was not detected from any patient with persistent diarrhoea. In our study *Isospora belli* (1 %) was detected in HIV positive children with chronic diarrhoea, Smith *et al*. documented that *Isospora belli* is seen rarely in individuals with normal immune system but in immunocompromised patients,which leads to chronic high volume watery diarrhoea, persistent for months [8].

In the present study Rotavirus antigen was detected in 4 cases from study group and 14 cases from Control group cases which suggests Rotavirus infection is mostly associated with acute diarrhoea but can cause persistent

diarrhoea in malnourished children, which is well corroborates with Kapikian *et al*. and Turgeon *et al*. study, reported that Rotavirus is recognized increasingly as the major cause of severe diarrhoea in young children and is responsible for 20 % to 70 % of hospitalization for diarrhoea among children worldwide. The population most commonly affected by rotavirus infection is children between 6 to 4 months of age with underlying malnutrition. Symptoms are more severe and diarrhoea can persist for as long as > 14 days [26].

In the present study total 132 *E.coli* isolates were obtained from study and Control group cases. Efforts were taken to type all *E. coli* strain by using selective media or specific antisera. Hicrome agar was used to type EHEC (O157:H7) strains and EPEC were confirmed by using specific antisera. Confirmation of EIEC and ETEC requires specific antisera which was difficult to arrange for the present study due to the financial constraints. Hence, EIEC and ETEC typing was not done. Secondly as per literature survey there is no evidence documented regarding association of EIEC and ETEC especially with persistent diarrhoea cases. Text book of Paediatrics by Ghai *et al*., Nelsson *et al*., explains the etiology of chronic infective diarrhoea cases in malnourished children as common commensal *E.coli*, due to malnutrition and decreases immunity collectively leads to normal commensals to become pathogens to causes severe disease conditions in children. In the present study 15 % EHEC (*E. coli* O157: H7) was confirmed from acute diarrhoea cases only., similar finding were reported by Turgeon *et al*. from (Washington) in 2001 that *E. coli* O157:H7 is one of the common EHEC serotype which produces Shiga-like toxin causing acute grossly bloody diarrhoea [26]. In our study 7 *E. coli* were confirmed as EPEC serotype from study group cases, which correlates with the findings of Lopez *et al*. in 1991 who also reported EPEC incidences in children with persistent diarrrhoea [43].

Antibiotic Sensitivity testing of all bacterial isolates was carried out by using Standard antibiotics as per hospital policy. It was observed that more than 50 % bacterial isolates from study group were sensitive to most of the antibiotics used.

All cases from the present study were collected from peripheral primary health centers like Shivaji nagar and Chitah camp area. Major group of patients visit these centers are mostly illiterate and from very low socio-economic strata of the community. These factors contributes towards negligence of child's health and results in malnutrition and development of chronic diarrhoea. All cases studied were first time visiting to doctors at primary health centers. All stool samples were collected from patient before starting antibiotic therapy. Patients with repeated episodes of acute diarrhoea along with malnutrition lead to low immunity and damage of intestinal wall. In such conditions normal commensal *E. coli* can increase in number, colonises on the gut wall. These organisms can translocate to mesenteric lymph nodes and so to systemic circulation and can become fatal to patient. This vicious cycle continues and harm the patient. Repeated treatment with various antibiotics can develop resistance towards antibiotics and induced diarrhoea in the patients as side effect [4, 5, 6].

Conclusion

In the present study, comparison between acute and chronic diarrhoea cases revealed that *E.coli* is the commonest etiology in acute as well as chronic diarrhoea cases. Though, exact demarcation between types of *E. coli* was not observed but finding of this study suggest that multiple acute diarrhoeal episodes in children leads to develop malnourished condition which decreases the immunity in children and may supports to develop normal commensal flora as etiological agents for repeated prolong diarrhoeal condition in paediatric cases.Children less than 1 yr were found to be predominant group associated with infective chronic diarrhoea more prone to severe malnutrition. Grade III and grade II malnutrition was found to be predominant. study concludes, chronic diarrhoea is more common in malnourished children with Grade II and III malnutrition. Such malnutrition conditions are most commonly observed in early age of life. Study concludes, bacterial infection is more common with chronic diarrhoea in malnourished children. *E. coli* is the predominant etiological agent for chronic infective diarhoea in malnourished children. *E. coli* Serotype EPEC was found associated with persistent diarrhoea in malnourished children, where as EHEC was found predominantly associated with acute diarrhoea. As per suggestive mechanisms of chronic infective diarrhoea in malnourished children, normal commensal *E. coli* can be one of the predominant etiological agent for chronic infective diarrhoea in malnourished children.

Recommendations

More research work on chronic and acute diarrhoea in malnourished children with respect to its etiological agents, its control and treatment is needed in developing countries like India.

Acknowledgement

The authors are extremely thankful of Indian council Of Medical Research for providing financial support and to Microbiology

Department of T.N. Medical College and B.Y.L. Nair Charitable Hospital, India for providing research facilities to conduct this work.

References

1. Wylline H, Diarrhoea. Pediatrics Gastro-intestinal diseases, Saunders publication, Edn. 1999, 2.
2. Park K. Textbook of Preventive and Social Medicine. Banaradidas Bhanot Publishers, Edn. 2000, 16.
3. Laney DW, Cohen MB. Approach to the pediatric patient with diarrhea, In: Gastroenterology clinics of North America. 1993; 22(3):517-533.
4. Nelson. Textbook of pediatrics, Edn 17, Saunders publication. 2004, 1.
5. Harrison's Principals of Internal Medicine, Edn 16, Mc Graw hill publication. 2004, 1.
6. Ghai OP. Essential pediatrics, Edn 6, Dr.Ghai publication, Delhi. 2004.
7. Cohen MB, Giannella RA. Bacterial infections: Pathophysiology, clinical feature and treatment, Edn 1, Raven press, New York. 1991, 395.
8. Christina M. Surawic Z. Infectious diarrhea. In Gastroenterology clinics of North America. 2001; 30(3):1-194.
9. Patwari AK *et al*. Diarrhea and malnutrition interaction. I. J. of Pediatrics. 1999; 66(1s):124-34.
10. Bhandari N *et al*. Prognostic factors for persistent diarrhea managed in a community setting, In

Symposium: Gastroenterology. I.J. of Pediatrics. 2000; 67(10):739.
11. Jadha AR, Harshprasad L, Seth B, Sawant M, Persistent diarrhea. J. of General Medicine. 2002; 14(3):267-377.
12. Bhaskaran P, Banerjee R. Malnutrition and infection in children, Community and social pediatrics, Edn 1, Jaypee Brothers New Delhi. 2012.
13. Mamatha B. Screening of Medicinal plants used in Rural Indian folk Medicine for treatment of diarrhea. Reads. 2005, 685.
14. MacCkie, McCartney. Practical Medical Microbiology, Edn 14 Churchill Livingstone. 1997.
15. Chaterjee KD. Text book of Parasitology, Edn 12, Chaterjee Medical Publication. 1980.
16. Koneman EW. Text book Diagnostic Microbiology. Edn 5, Lippincot. 1995.
17. Reeves DS. Laboratory methods in antimicrobial chemotherapy, Edn 1, Churchill Livingstone. 1978.
18. Mazumdar *et al*. The effect of birth interval on malnutrition in Bangladesh infants, J. of Biosocial science. 2000; 32(3):289-300.
19. Butta ZA., Nizami SQ. Risk factors for mortality among hospitalized children with persistent diarrhea in Pakistan. J. of Tropical Pediatrics. 1990; 43(6):330-6.
20. Roy NC *et al*. Use of mid-upper arm circumference for evaluation of nutritional status of children and for identification of high risk group for malnutrition in rural Bangladesh. J. of Health, population and nutrition. 2000; 18(3):171-180.
21. Jindal *et al*. A study of infective etiology of chronic diarrhea in children in Amritsar, Rev. Gastroenterology. 1996; 16(3):214-21.
22. Lopez MD *et al*. Enteropathogenic agents isolated in persistent diarrhea, J. Diarrhoeal Dis. Rev. 1999; 9(4):315-17.
23. Scott DL, Surawicz CM. Infectious cause of chronic diarrhea. In: Gastroenterology clinics of North America. 2001; 30(3):202-203.
24. Bhandari N, Bahl R, Dhuri T, Kumar R. Role of Protozoa as risk factors for persistent diarrhea. Indian J. Of pediatrics. 1999; 66:21-26.
25. Lanate *et al*., Parasitic agent in children diarrhea and malnutrition in West Africa. J. Of Medicine. 1997; 16(1):36-39.
26. Turgeon DK, Fritsche TR. Laboratory approaches to infectious diarrhea. In Gastroenterology clinics of North America. 2001; 30(3):693-707.