

Clinical Study of Acute Childhood Diarrhoea Caused by Bacterial Enteropathogens

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ABSTRACT

Objective: There are not a large number of studies in India which can enlighten us regarding acute childhood diarrhoea and far lesser in number when it comes to its bacterial enteropathogenesis. The present study is specially targeted to determine the prevalence of various bacterial enteropathogens causing acute childhood diarrhoea and to find out their respective pattern of clinical features.

Method: All children under 12 years of age enrolled between 1st June, 2012 and 31st July 2012, in the Outpatient department, Inpatient department of pediatrics section and casualty of hospital, who presented with acute diarrhoea. Data collected by mean of study questionnaire. Stool sample were processed for bacteriological analysis. In 280 samples bacteria were isolated with the help of microscopy, culture and biochemical reactions. The isolates obtained were tested for antimicrobial sensitivity over Mueller Hinton agar by Kirby Bauer-disk diffusion method.

Results: Out of 280 children frequency of diarrhoeagenic bacteria isolated from the samples showed that *Escherichia coli* was recorded as the predominant bacteria with 44.2% of prevalence followed by *Shigella*, *Salmonella*, *Klebsiella* and *Campylobacter* with 28.2%, 13.6%, 7.8% and 6.1% respectively. Patients falling in the age group of 1-3 years. were the major sufferers of

diarrhoea due to all etiologies except *Klebsiella* which mainly had impact on the patients below six months.

Fever and vomiting were predominant symptoms. All 100% patients with *Salmonella* as etiology presented with fever. The maximum number of patients had frequency of diarrhoea less than five times a day (52.5%). And among the patients who presented with frequency of more than 10 times a day *Salmonella* was a major causative agent found. Maximum patients presented with some dehydration (67.1%) and the patients who presented with severe dehydration *Salmonella* was identified as a major etiological agent.

Majority of isolated bacterial agents were resistant to Co-trimoxazole and *Shigella* being highly resistant enteropathogen isolated. *Salmonella* spp. were least resistant isolates. None of the isolates were resistant to Cefotaxime, Cefuroxime and Azetronam.

Conclusion: Results of study reveal that *Escherichia coli* is a predominant bacterial enteropathogen causing diarrhoea and *Salmonella* is a major contributor to the diarrhoea causing severe dehydration and to the clinical features like fever, vomiting and more than 10 times of frequency of stools. *Shigella* is among highly resistant isolates while *Salmonella* isolates had least resistance to majority of antibiotics.

Keywords: Infective diarrhoea, Diarrhoeagenic bacteria, Dehydration, Antimicrobial resistance

INTRODUCTION

Diarrhoea is defined as having loose or watery stools at least three times per day or more frequently than normal for an individual. More than 1 billion cases and at least 4 million deaths per year are attributed to diarrhoea worldwide [1]. In developing countries an estimated 1,000 million episodes occur each year in children below five years, causing 5 million deaths among them annually, out of which 80% occur in the first two years of life [2]. Fifteen countries contribute three quarters of childhood deaths due to diarrhoea in children under five years of age worldwide out of which India ranks first [3]. In India acute diarrhoeal diseases lead to 13% deaths in under five age group, during the year 2009, about 11.2 million cases with 1,762 deaths were reported [4]. According to a report by National Institute of Cholera and Enteric Disease, Kolkata, (India), crude death rate due to diarrhoea in rural India is 9.3 per 1,000 population and the diarrhoeal deaths account for 22% of total rural deaths among 0 to six years age group children [5]. According to annual Uttarakhand State Report 2009, out of 29 disease outbreaks, acute diarrhoeal disease and food poisoning contributed for 3 of them [6]. *Rotavirus* is the single most common pathogen which causes gastroenteritis in both developed and developing countries but the trend of bacterial enteropathogens vary largely between developed and developing countries [7]. Five types of

Escherichia coli are responsible for as much as 25% of all diarrhoeal diseases in developing countries. *Shigella* species are responsible for 10 to 15% of acute diarrhoeas in children less than 5 years of age [8]. Other bacterial enteropathogens are *Vibrio cholerae*, *Campylobacter jejuni*, *Aeromonas species*, *Bacteroides fragilis* and *Providencia alkalfaciens*. The trends of bacterial enteropathogens causing gastroenteritis keep on changing with change in standard of living and environmental hygiene [7]. So the periodic renewal of the knowledge about trends of the bacterial enteropathogens is very essential. An epidemiologic study of an infectious disease in a community is always considered to be an initial step toward the introduction of the proper interventions for controlling the disease because the features and the patterns of isolation of etiologic agents of the disease vary from place to place depending on the local meteorology, geography, and socio-economic elements [9].

This study was conducted in Veer Chandra Singh Garhwali Government Medical Science & Research Institute, Srikot, Srinagar, Pauri Garhwal Uttarakhand, (India), which is a tertiary health care centre. This study aims at bridging the gap between the large number of diarrhoea patients and scanty data and knowledge about prevalent bacterial enteropathogens in Uttarakhand state.

Since diarrhoea is a disease in which the prevalence of different etiological agents vary largely on the basis of geographical

region, socio-economic status, standard of living and environmental hygiene, henceforth the studies carried out in other places may not fit aptly in this hilly regions of Garhwal.

For earlier and the specific intervention for diarrhoea its very important to know the exact pathogen and its antimicrobial sensitivity in that particular area so that we can decrease the financial load on the patient as well as on health care. This study also takes an overview of the awareness-application gap for oral rehydration therapy among the population under study.

OBJECTIVES

- To find out various bacterial enteropathogens causing acute diarrhoea in children.
- To correlate the clinical presentation of acute diarrhoeal disease according to bacterial enteropathogens.
- To find the antibiotic resistance among the various bacterial isolates.

METHODOLOGY

This is a cross-sectional observational study including 280 individuals conducted at a tertiary health care centre from 1st June 2012 to 31st July 2012. The study was approved by Institutional Ethics Committee of the Institute patient with history of acute diarrhoea i.e. history of loose stools at least 3 times a day or increased frequency for the particular individual since less than 14 days of duration, patient should fall in the age group of 0-12 years of age group, He /she should have enrolled in out door patient department (OPD) or indoor patient department (IPD) of department of Pediatrics or Emergency department were enrolled for the study. The children with diarrhoea due to malnutrition and infective diarrhoea caused by other than bacteria were excluded from the study.

An informed consent was taken from parents or guardians after explaining them all prospects of study. A pre formed questionnaire was filled by asking some questions to the parents or guardians of the patient who included questions about the following:

- Particulars of the patient.
- A complete history of the duration, frequency, characteristics of diarrhoea and associated symptoms.
- Preadmission treatment.
- Awareness about oral rehydration therapy and whether patient started it or not.
- Presence of any other case of diarrhoea in the family or locality of the patient.
- Water source of the family and the method of drinking water storage.

A record sheet was filled by clinical examination of the patient which included.

- General physical examination, vitals.
- Assessment of nutrition.
- Assessment of dehydration according to the description in Nelson's Textbook of Pediatrics.

Sample Collection: Patient's attendant was given a sterile wide mouthed container and completely explained to collect the stool sample.

Laboratory Analysis: The stool samples were sent to microbiology laboratory of the institute without using any transport media within 24 hours of presentation of the patient.

Stool sample was subjected to microscopy, culture and antimicrobial susceptibility test.

Rota virus-latex agglutination method.

Parasites-iodine wet mount.

For *Escherichia coli* sample was cultured on Bloodagar, On MacConkey's medium, colonies are bright pink due to lactose fermentation. Growth is inhibited on selective media such as deoxycholatecitrate agar.

For *Salmonellae spp.* – identified by no change in colour of MacConkey agar due to non lactose fermenting nature of its colonies. Enrichment media used was Selenite-F Broth.

For *Shigella spp.*– colorless colonies on MacConkey agar. Deoxycholate citrate agar was used as a selective media

For *Vibrio cholera* - enrichment media used was Alkaline peptone water. Plating media used was TCBS medium.

For identification of *Klebsiella spp.* - identified by its large mucoid colonies when grown on blood agar.

The patients with stool samples showing presence of virus or parasites were excluded from the study.

Further the samples were subjected for antimicrobial susceptibility with the help of Mueller Hinton agar using Kirby bauer-disk diffusion method .

RESULTS

Epidemiological features: During two month study period, out of all the presented with acute diarrhoea, 280 children whose stool culture positive for bacteria were included in the study. The data collected by mean of structured study questionnaire revealed various epidemiological features of these children with acute diarrhoea and bacterial enteropathogens as etiological agent.

[Table/Fig-1] shows the pattern of age distribution among cases. Out of total studied cases maximum fall under the age group of 1-3 years i.e 100 (35.7%) followed by age group of 3-5 years (22.1%), least number of cases were seen in age group of 9-11 years (4.2%).

Age group	No. of children (%) n=280
<6 months	26 (9.2)
6 months- 1 year	23 (8.2)
1-3 years	100 (35.7)
3-5 years	62 (22.1)
5-7 years	28 (10)
7-9 years	18 (6.4)
9-11 years	12 (4.2)

[Table/Fig-1]: Age distribution

About 54.6% of all the cases were males and 45.4% were females. Other epidemiological characteristics are shown by [Table/Fig-2].

Epidemiological factor	No. (%) n=280	
Sex	Male	153 (54.6)
	Female	127 (45.4)
Residence	Rural	161 (57.5)
	Urban	119 (42.5)
Most frequently used water source	Gadhera (natural water spring)	46 (16.4)
	Water tank	95 (33.3)
	Old water tank	56 (20)
	Public hand pump	44 (15.7)
	Public tap	39 (13.9)

[Table/Fig-2]: Distribution of various epidemiological factors among the children presented with acute diarrhoea

Parents of 263 (93.9%) were aware of Oral Rehydration Solution (ORS) therapy but was applied by parents of only 173 children (61.8%).

Prevalence of Bacterial Enteropathogens

Escherichia coli was the most frequent enteropathogen isolated in diarrhoeal stool samples, being found in 124 samples (44.2%), 79 (28.2%) samples were positive for *Shigella*, 38 (13.6%) samples had shown *Salmonella* while *Klebsiella* and *Campylobacter* were positive in 22 (7.8%) and 17 (6.1%) samples. None of the sample was positive for *Vibrio* [Table/Fig-3].

Bacterial agent	No. (n=280)	Percentage age
<i>Escherichia coli</i>	124	44.2
<i>Shigella</i>	79	28.2
<i>Salmonella</i>	38	13.6
<i>Klebsiella</i>	22	7.8
<i>Campylobacter</i>	17	6.1
<i>Vibrio</i>	00	00

[Table/Fig-3]: Prevalence of various bacterial enteropathogens identified among children with acute diarrhoea

Age group	<i>E. coli</i> n=124 (%)	<i>Shigella</i> n=79 (%)	<i>Salmonella</i> n=38 (%)	<i>Klebsiella</i> n=22 (%)	<i>Campylo- bacter</i> n= 17 (%)
<6 months	14 (11.4)	--	--	12 (54.5)	--
6 months- 1 year	18 (14.5)	7 (8.9)	1 (2.6)	5 (22.7)	2 (11.8)
1-3 years	43 (34.7)	26 (32.9)	19 (50)	3 (13.7)	9 (52.9)
3-5 years	27 (21.7)	18 (22.7)	9 (23.7)	2 (9.1)	6 (35.3)
5-7 years	12 (9.6)	14 (17.7)	3 (7.9)	--	--
7-9 years	8 (6.5)	8 (10.2)	2 (5.3)	--	--
9-11 years	2 (1.6)	6 (7.6)	4 (10.5)	--	--

[Table/Fig-4]: Age related prevalence of various bacterial enteropathogens causing childhood diarrhoea

Clinical characteristic	n=280 (%)	<i>E.coli</i> n=124 (%)	<i>Shigella</i> n=79 (%)	<i>Salmonella</i> n=38 (%)	<i>Klebsiella</i> n=22 (%)	<i>Campylobacter</i> n=17 (%)	
Fever	251 (89.6)	116 (93.5)	72 (91.1)	38 (100)	16 (72.7)	9 (52.9)	
Vomiting	173 (61.8)	55 (44.4)	61 (77.2)	32 (84.2)	13 (59.1)	12 (70.6)	
Frequency of diarrhoea	<5 times /day	147 (52.5)	102 (82.3)	8 (10.1)	2 (5.3)	19 (86.3)	16 (94.1)
	5-10 times/day	117 (38.2)	22 (17.7)	73 (92.4)	15 (39.5)	3 (13.6)	1 (5.9)
	>10 times /day	23 (8.2)	--	2 (2.5)	21 (55.2)	--	--
Degree of dehydration	No dehydration	65 (23.5)	44 (35.5)	12 (15.2)	--	5 (22.7)	4 (23.5)
	Some dehydration	188 (67.1)	80 (64.5)	60 (75.9)	18 (47.4)	17 (77.3)	13 (76.5)
	Severe dehydration	27 (9.6)	--	7 (8.7)	20 (52.6)	--	--

[Table/Fig-5]: Clinical characteristics of children with acute diarrhoea and their presentation depending upon the various bacterial etiological agent

Duration	n=280 (%)	<i>E.coli</i> n=124 (%)	<i>Shigella</i> n=79 (%)	<i>Salmonella</i> n=38 (%)	<i>Klebsiella</i> n=22 (%)	<i>Campylobacter</i> n=17 (%)
≤ 1 day	27 (9.6)	9 (7.2)	4 (5.1)	5 (13.2)	4 (18.2)	5 (29.4)
1-3 days	35 (12.5)	10 (8.1)	13 (16.4)	7 (18.4)	3 (13.6)	2 (11.8)
3-5 days	114 (40.7)	52 (41.9)	37 (46.8)	17 (44.7)	4 (18.2)	4 (23.5)
5-7 days	64 (22.8)	25 (20.2)	19 (24.1)	7 (18.4)	10 (45.5)	3 (17.6)
>7 days	40 (14.2)	28 (22.5)	6 (7.6)	2 (5.3)	1 (4.5)	3 (17.6)

[Table/Fig-6]: Duration of illness among cases and its relation to various etiological agents causing childhood diarrhoea:

Antibiotic	<i>E.coli</i> n=124 (%)	<i>Shigella</i> n=79 (%)	<i>Salmonella</i> n=38 (%)	<i>Klebsiella</i> n=22 (%)	<i>Campylobacter</i> n=17 (%)	n=280(%)
Amoxicillin + clavulanic acid	12 (9.6)	14 (17.7)	2 (5.2)	1 (4.5)	1 (5.8)	30 (10.7)
Ampicillin+ sulbactam	22 (17.7)	10 (12.6)	3 (7.8)	2 (9.1)	2 (11.8)	45 (16.1)
Cefuroxime	--	--	--	--	--	--
Co-trimoxazole	37 (29.8)	54 (68.4)	9 (23.6)	14 (63.6)	9 (52.9)	123 (43.9)
Cefotaxime	--	--	--	--	--	--
Aztreonam	--	--	--	--	--	--
Piperacillin-tazobactam	8 (6.4)	5 (6.3)	7 (18.4)	8 (36.4)	1 (5.8)	24 (8.5)
Ciprofloxacin	4 (3.2)	9 (11.4)	10 (26.3)	7 (31.8)	3 (17.6)	25 (8.9)

[Table/Fig-7]: Antimicrobial resistance of bacterial specimens isolated from diarrhoeal stool

The age related distribution has shown that majority of the bacterial isolates have peak incidence between age 1-3 years being 34.7% for *E.coli*, 32.9% for *Shigella*, 50% for *Salmonella*, 52.9% for *Campylobacter* on the other hand, *Klebsiella* has peak incidence among infants (77.2%) [Table/Fig-4].

E.coli is the only agent distributed among all age groups showing 25.8% incidence among infants, 56.5% between 3-5 years and least in age group >5 years (17.7%) [Table/Fig-4].

Both *Shigella* and *Salmonella* had least incidence among infants (8.9 % and 2.1% respectively) and their incidence increases with age showing peak between age group 1-5 years (55.7% and 73.6% respectively).

Both *Klebsiella* and *Campylobacter* had shown no isolates in age group >5 years.

Clinical Characteristics

[Table/Fig-5,6].

Antibiotic Resistance

[Table/Fig-7].

DISCUSSION

Very few studies were conducted in India which focuses on pattern of bacteria as etiological agent in children presented with acute diarrhoea. This study covering a two month period was unique in its kind and was one of the very few studies which focused on epidemiological, microbiological and clinical aspects of childhood diarrhoea of bacterial etiology. The number of males suffering with diarrhoea were more in number than female with a ratio of 1:1.202 which is similar to the finding of the studies done over childhood diarrhoea [10-12].

Age distribution shows that children suffering from diarrhoea were 17.4% below 1 year of age, 57.85% were between 1 and 5 years of age and 20.6% of more than 5 years of age [Table/Fig-1]. The maximum sufferers were between 1 and 3 years of age. A similar pattern of age distribution has been found in earlier done studies [13,11].

The study reveals that diarrhoea is more common in children living in rural set up (57.5%) than the one living in urban set up (42.5%) [Table/Fig-2]. This can be attributed to poor quality of hygienic condition and sanitation practices in rural population which contributes high risk of infection especially among children. Other epidemiological factors tend to play a role in increased susceptibility of infection to enteropathogen among rural population. The main source of water available to rural people in present study area either natural springs or public handpumps, which are at more risk of contamination, resulting in outbreaks of diarrhoea.

Questionnaire also reveals that out of all 93.9% of the parents of patients under study were aware about the oral rehydration therapy while only 61.8% practiced it. An almost similar pattern has been found in a study [14,15]. The major reason behind this gap is considered lack of maternal knowledge towards diarrhoea and oral rehydration therapy [14].

In our study *Escherichia coli* was recorded as the predominant bacteria with 44.2% of prevalence followed by *Shigella*, *Salmonella*, *Klebsiella* and *Campylobacter* with 28.2%, 13.6%, 7.8% and 6.1% respectively [Table/Fig-3]. This pattern was similar to other studies done in India by workers like Joshi et al., and Subhash kumar et al., [16,17] which had shown higher incidence of diarrhoeagenic *E.coli*, supporting the well-documented role of *E.coli* spp. in diarrhoeal disease [18]. However, this pattern is in contrast to other areas of Africa and Malaysia [10], which had reported *E.coli* as least common etiological agent causing agent, which can be because of alteration in epidemiological features of host as well environment.

Of all the bacterial diarrhoeal cases 36.42% were from age group 1-3 years. This can be attributed to negligent attitude of parent's toward children of this age group and increased mobility of children due to their recently acquired walking ability. *Escherichia coli* has been found to be one of the causative agents at all age groups. *Klebsiella* mostly affected children of age group less than six months. *Shigella* and *Salmonella* are least likely to cause infantile diarrhoea but their incidence increases with age. These findings are consistent with those of African studies [19,20]. This might be so because *Salmonella* are least likely to adapt to domesticated cattles and all varieties of fowls and chickens. The reservoir of infection in animals constitute the principal source of diarrhoea caused by *Salmonella* spp [21]. *Campylobacter* and *Klebsiella* were the causative agent till the age of five years only [Table/Fig-4]. The higher incidence of *Campylobacter* among children do not match with findings shown by Gascón et al., and Lindblom et al., [22,23] who described low incidence among children but our findings matches to greater extent with work done by NguendoYongsi who had shown higher prevalence of *Campylobacter* among children <5 years [11].

Fever was found to be a predominant clinical feature present in 89.6% of patients of diarrhoea. All 100% patients who had diarrhoea due to *Salmonella* suffered from fever, followed by patients of diarrhoea due to *Escherichia coli* (93.5%), *Shigella* (91.1%), *Klebsiella* (72.7%) and *Campylobacter* (52.9%) [Table/Fig-5]. From the above finding it can be deciphered that fever is a common clinical feature in diarrhoea with *Salmonella* occupying the majority with all the cases presenting with fever.

Vomiting was found less common i.e., only in 61.8% of the diarrhoea patients. Again 84.2% of the patients with diarrhoea due to *Salmonella* showed vomiting as a clinical feature while in *Shigella* (77.2%), *Campylobacter* (70.6%), *Klebsiella* (59.1%) and *Escherichia*

coli (44.4%) lesser number of patients presented with vomiting. This above mentioned finding again denotes the more severe nature of diarrhoea caused due to *Salmonella* [Table/Fig-5].

Maximum number of the patients (52.5%) presented with frequency of diarrhoea less than 5 times a day. Least number of the patients (8.2%) presented with frequency of more than 10 times a day. And rest 38.2% lied in the frequency of in between 5 and 10 times a day. Among the patients who presented with the frequency of diarrhoea less than 5 times a day, maximum number (86.3%) was contributed by *Klebsiella*. *Shigella* contributed maximum number (92.4%) to those who presented with frequency of 5-10 times per day. And *Salmonella* was the major (55.2%) agent to those who presented with frequency of stools more than 10 times a day [Table/Fig-5].

Out of all 67.1% children presented with some dehydration followed by no dehydration (23.5%) and severe dehydration (9.6%). Out of 124 patients of *Escherichia coli* 80 patients presented with some dehydration and rest with no dehydration. While the patients with *Salmonella* as etiology suffered more with severe dehydration (52.6%) [Table/Fig-5].

Out of 280 patients maximum number of patients came after 3-5 days of illness. *Escherichia coli*, *Shigella* and *Salmonella* induced diarrhoea were mainly made the patient to come to the hospital after 3-5 days while in case of *Klebsiella* the maximum patients came after 5-7 days of illness which may be explained by the mildness of symptoms caused due to *Klebsiella* [Table/Fig-6].

[Table/Fig-7] shows the antimicrobial resistance pattern among all the pathogens that were isolated. The pattern of antibiotic susceptibility of various bacterial pathogens matches to greater extent to work done by Daniel R., and Diniz-Santos, et al., [24]. High resistance to Co-trimoxazole among all pathogens can be attributed to its injudicious use among the population because of its wide antimicrobial spectrum most likely to be used for various infections.

Considerable resistance rate has been found to Co-trimoxazole, Amoxiclav, ampicillin associated with sulbactam among *Shigella* specimens. Separate assessment of antimicrobial resistance patterns indicates that *Shigella* is more difficult to treat and requires careful consideration at the choice of antimicrobial therapy. Thus, use of these drugs is not recommended for the empirical treatment of acute bloody diarrhoea in children.

In very severe cases, with evidence of dissemination of disease, intravenous third generation Cephalosporins like cefotaxime and cefuroxime is the best choice for many, while other drugs can be adequate choices for the empirical treatment of severe acute diarrhoea in children.

Salmonella strains were found to be multi-susceptible, as high rates of resistance were not found against any of the drugs, except some resistance 26.3% was found to be against ciprofloxacin.

This resistance to ciprofloxacin among non-typhoidal *Salmonella* strains isolated from stool specimens has already been reported from several parts of the world [25,26].

Oplustil et al., did not isolate any strain of *Salmonella* resistant to fluoroquinolones from blood samples [27]. However, *Salmonella* spp. strains isolated from blood samples are recognized to be more susceptible than those from stools [28].

E. coli isolates were found to have a high rate of resistance to ampicillin, ampicillin + sulbactam and Co-trimoxazole, this can be explained as *E.coli* being so common agent of diarrhoea, its strains are most mostly exposed to different antibiotics injudiciously.

The knowledge of resistance pattern of common etiological agents in local area can help practitioners to choose an adequate antimicrobial drug to start empirical therapy in a patient with severe diarrhoea without knowledge of a specific pathogen. However, as soon as the

results of stool cultures are available, the therapy can be altered to a safer drug based on the antimicrobial susceptibility pattern.

CONCLUSION

As per the previous records Rotavirus caused majority of the childhood diarrhoea case which led to overlooking of bacterial diarrhoeal cases. This study was aimed at finding the prevalence of bacterial diarrhoea among childhood patients. *Escherichia coli* was found most common bacteria among total cases followed by *Shigella*, *Salmonella*, *Klebsiella* and *Campylobacter*. It was interesting to note that not only one but different types of bacteria were isolated from the stool sample. More studies should be carried out to prevent the disease in its primordial stage. Comparative studies related to causative agents like bacteria, viruses should be given more importance because they help in disease prevention strategies. Role of Information Education and Communication is very important regarding diarrhoeal and hence should be prioritized.

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