

ETIOLOGICAL AGENTS OF DIARRHEA IN CHILDREN AGED BELOW 5 YEARS OF AGE: A STUDY FROM A TERTIARY HEALTH CARE CENTER IN NORTH INDIA.

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ABSTRACT

Background: Diarrhea diseases remain the second leading cause of death among children under five years globally. The burden of diarrheal mortality is concentrated in low-resource settings. This study examined the causative agents of diarrhea in children under 5 years of age in sub himalayan areas of Kangra, Himachal Pradesh, India.

Material and Method: The present study was conducted in the Department of Microbiology of Dr. Rajendra Prasad Govt. Medical College and Hospital, Kangra at Tanda in collaboration with the Department of Pediatrics over a period of one year from September 2021 to August 2022. This study was carried out on stool samples of clinically diagnosed indoor cases of acute diarrhea in children under the age of five years received in the Department of Microbiology. It was a prospective descriptive hospital based study.

Results: A total of 103 stool samples of children under the age of five years were received to the department of Microbiology. Out of 103 participants, there were 47(46%) females and 56(54%) males. It was observed that Rotavirus was the most common causative agent in 16 (15.5%) cases followed by Adenovirus which was present in 14 (13.6%) samples, while 2 (1.9%) samples showed Astrovirus. Parasitic causes of diarrhea were found in 7 (6.79%) cases. Escherichia coli variant was observed in 24 (23.3%) samples and *Shigella sonnei* was observed in 3 (2.9%) samples.

Conclusion: We recommend that in cases of acute diarrhea in children, etiological agents should be identified for possible causes of diarrhea with various risk factors that could help in management. This will aid in establishing correct etiology and subsequent treatment of patients.

INTRODUCTION

Diarrhea is an amalgamation of two Greek words "Dia" and "rhien" meaning "through" and "to flow" respectively. Hippocrates (460-370 B.C) gave this clinical and epidemiological description of the existence of diarrhea[1]

Diarrhea as defined by the World Health Organization (WHO) is having three or more liquid or loose stools per day or having increased frequency of stools than in normal for that person(2) In developing countries diarrhea is a major public health problem. Diarrhea accounts for 19% of deaths under 5 years of age which is 1.87 million deaths globally.(3).

An estimated 3,00,000 children die each year in India making diarrhea as the 3rd major cause of death in children below 5 years of age. National institute of Cholera and Enteric Disease, Kolkata, reported crude death rate due to diarrhea in rural India as 9.3 per 1000 population and 22% of the total deaths among (0-6) years of age due to diarrheal disease(4).

Globally, acute gastroenteritis accounts for 10% of hospitalizations and 19% of deaths in children under 5 years[5] . It is said to be predominantly caused by viruses; with Rotavirus accounting for about 20% of fatal diarrhea globally, though they are also associated with bacteria and some protozoans(5-7).

In addition to rotavirus and DEC, other enteropathogens include Shigella spp., Salmonella spp., Vibrio cholerae and Campylobacter spp. may cause diarrhea.

The present study is a hospital based prospective descriptive study to understand the prevalence of various etiological agents associated with childhood diarrhea. The result obtained from this study will be helpful in identifying the prevalent cause of infection with diarrheal disease in children under 5 years, emerging antimicrobial resistance trends and formulate interventional strategies for effective management and control of diarrhea in under five children.

MATERIAL AND METHOD

The present study was conducted in the Department of Microbiology of Dr. Rajendra Prasad Govt. Medical College and Hospital, Kangra at Tanda in collaboration with the Department of Pediatrics over a period of one year from September 2021 to August 2022 after approval from the Institutional Ethics Committee and Protocol Review Committee. This study was carried out on stool samples of clinically diagnosed indoor cases of acute diarrhea in children under the age of five years received in the Department of Microbiology. It was a prospective descriptive hospital based study.

All stool samples were collected in a cylindrical, 25 ml screw capped, wide mouthed plastic leak proof container. Samples were transported to the Department of Microbiology within 30 minutes of sample collection. If delay longer than 2 hours is anticipated the specimens were collected in Cary Blair Transport Medium or any other suitable transport medium.

Stools were examined microscopically in saline and iodine preparation for detection of pus cells and parasites. Hanging drop preparation was prepared for detecting motility. Modified Ziehl Neelsen staining was done for detection of oocysts of Cryptosporidium. All samples were

plated on 5% sheep blood agar and MacConkey agar. Selective media such as XLD, TCBS, Sorbitol MacConkey agar, etc. were used whenever necessary and as per the results obtained in the microscopic findings. Detection was done on the basis of biochemical reactions and antimicrobial susceptibility was performed for bacterial isolates as per the standard guidelines.

Detection of common diarrhea causing viruses such as Rotavirus, Adenovirus, Astrovirus, Norovirus was done by antigen detection (ELISA) by using commercially available ELISA kits (Serazym). Detection was done according to the manufacturer's instructions.

RESULTS

A total of 103 stool samples of children under the age of five years, diagnosed with acute diarrhea were received from indoor patients from the Department of Pediatrics, Dr. Rajendra Prasad Government Medical College, Kangra at Tanda, Himachal Pradesh during the above mentioned period.

Out of 103 participants, there were 47(46%) females and 56(54%) males, with male to female ratio of 1:0.84.

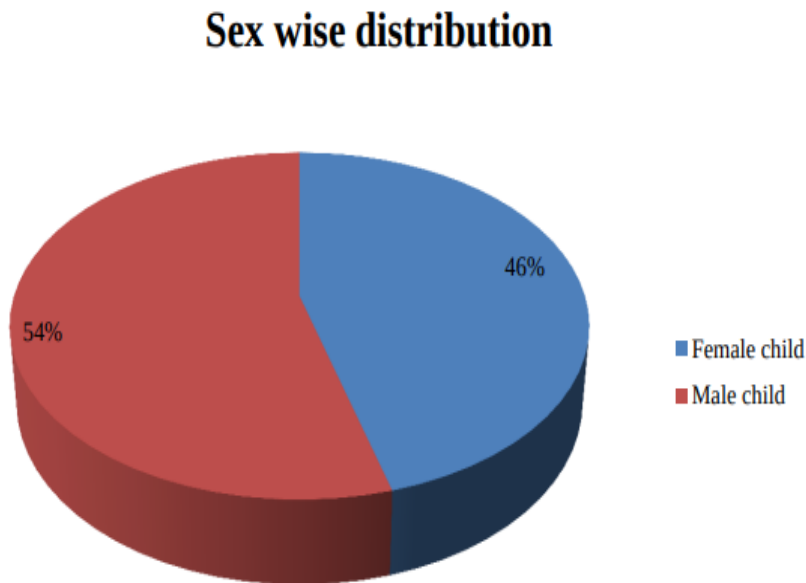


Figure 1: Age wise distribution

Out of the total 103 samples processed, 32 (31.06%) showed viral pathogens, 31 (30.9%) samples showed growth of bacterial organisms [7 (6.8%) were pathogens and rest 24 (23.3%) were probable pathogens] and 7 (6.79%) showed parasitic organisms.

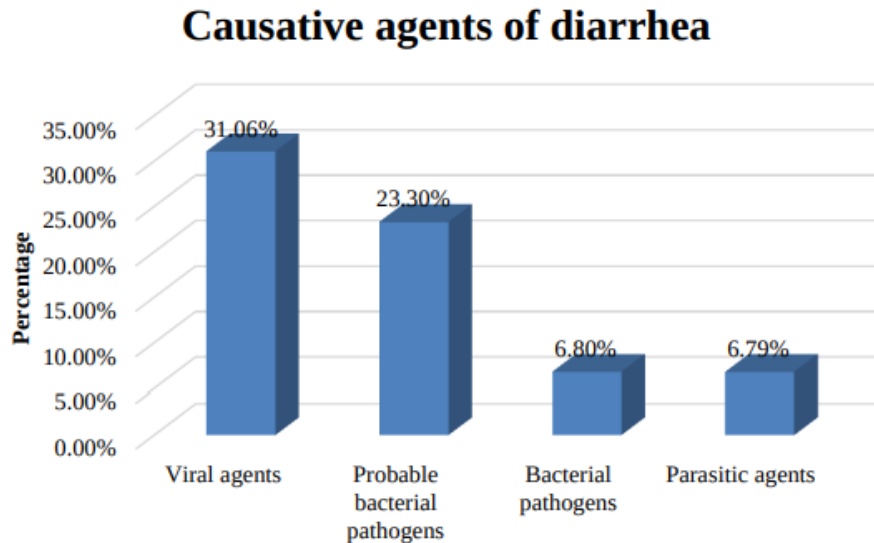


Figure 2: Distribution of diarrheal etiology

Parasitic causes of diarrhea were found in 7 (6.79%) cases. Cyst of *Giardia lamblia* and *Entamoeba histolytica* were seen in 3 (2.9%) stool samples each and fertilized egg of *Ascaris lumbricoides* was seen in 1 (0.97%).

Direct Microscopic Findings	Frequency (n)	Percentage (%)
Cyst of <i>Giardia lamblia</i>	3	2.9%
Cyst of <i>Entamoeba histolytica</i>	3	2.9%
Fertilized egg of <i>Ascaris lumbricoides</i>	1	0.97%
Presence of WBC	11	10.6%
Presence of RBC	4	3.9%

Table 1: Distribution of parasitic isolates.

Escherichia coli variant was observed in 24 (23.3%) samples and *Shigella sonnei* was observed in 3 (2.9%) samples. There were 1 (0.9%) samples in which *Salmonella typhimurium*, *Aeromonas hydrophila* and *Plesiomonas shigelloides* were seen.

Microbiological Culture	Frequency (n)	Percentage (%)
<i>Escherichia coli</i> variant	24	23.3%
<i>Shigella sonnei</i>	3	2.9%
<i>Salmonella typhimurium</i>	1	0.9%
<i>Aeromonas hydrophila</i>	1	0.9%
<i>Plesiomonas shigelloides</i>	1	0.9%
<i>Staphylococcus aureus</i>	1	0.9%
<i>Vibrio cholerae</i>	0	0%

Table 2: Distribution of Bacterial isolates

ELISA for detection of Adenovirus, Astrovirus, Norovirus and Rotavirus was done for all 103 cases. It was observed that Rotavirus was the most common causative agent in 16 (15.5%) cases followed by Adenovirus which was present in 14 (13.6%) samples, while 2 (1.9%) samples showed Astrovirus. However, there was no stool sample in which Norovirus was seen.

Viral pathogens	Frequency (n)	Percentage (%)
<i>Rotavirus</i>	16	15.5
<i>Adenovirus</i>	14	13.6
<i>Astrovirus</i>	2	1.9
<i>Norovirus</i>	0	0

Table 3: Distribution of viral isolates

It was observed that *E. coli* variant had maximum resistance to Ciprofloxacin which was observed in 13 (54.2%) followed by Ampicillin which was observed in 11 (45.8%) samples followed by Ceftriaxone resistance observed in 9 (37.5%) samples. Least resistance was seen against Ampicillin-Sulbactam in 4 (16.66%) and Amikacin in 5 (20.9%) samples.

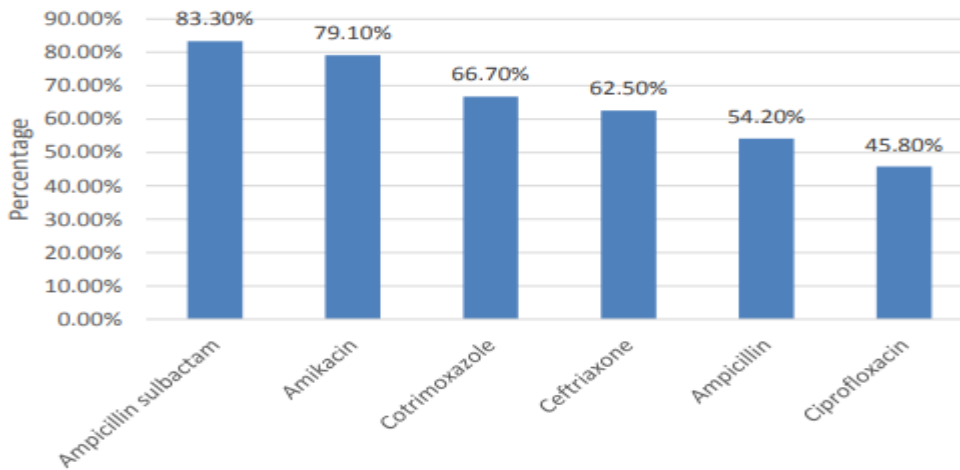


Figure 3: Antimicrobial susceptibility in *E. coli* variants.

There were 3 samples which showed *Shigella sonnei* of which 2 (66.66%) were resistant to Ciprofloxacin, Ampicillin and Cotrimoxazole and 1 (33.3%) was resistant to Ceftriaxone, Amikacin and Azithromycin.

Antibiotic susceptibility pattern of *Shigella sonnei*

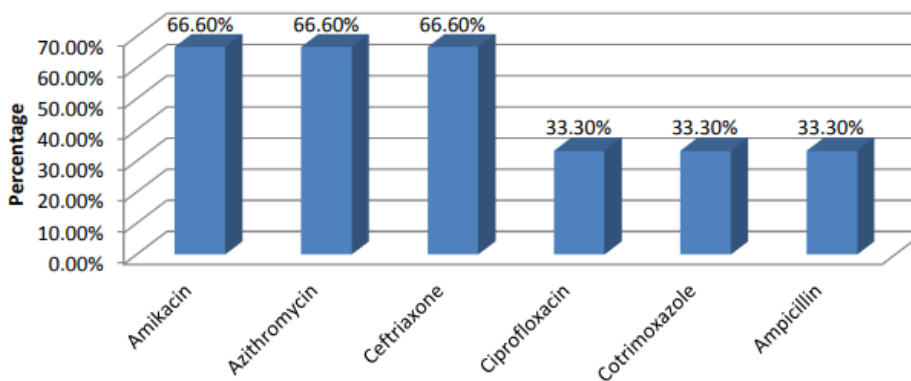


Figure 4: Antimicrobial susceptibility for *Shigella sonnei*

DISCUSSION

Diarrheal diseases, which is one of the leading reasons behind global mortality and morbidity, is more threatening for infants and young children. Childhood diarrhea is becoming an increasingly prevalent disease in developing countries like India.

In our study we observed that infectivity was higher in males (54.36%) than females (45.6%). The male to female ratio was 1:0.84. This observation is in concordance with a study done by Jarman AF et al(56.2 percent and 43.8 percent respectively)(8). Shah SM et al(9) in their study observed that the prevalence of diarrhea was marginally higher among girls than boys (53% vs 49%, respectively). No gender difference in the prevalence of diarrhea was observed in NFHS-1, NFHS-2, and NFHS-3(10-12).

A study conducted among under-5 children in the USA between 1997 and 2000 found a higher incidence rate of diarrhea among male children(13). Second, that in rural areas, which was predominant in our study, male children get more attention especially in relation to health problems and parents have better health seeking behavior in relation to male child's health and this could consequently lead to more male children in hospital.

In present study, cyst of *Giardia lamblia* and *Entamoeba histolytica* was seen in 3 (2.9%) stool samples each and egg of *Ascaris lumbricoides* was seen in 1 (0.97%). Uma Maheshwari et al(14) observed *Giardia lamblia* and *Entamoeba histolytica* trophozoites in 2 (3.3%) and 4 (6.6%) cases respectively.

In our study we found 24 probable pathogens (*E.coli* variant). Similarly, a study by Sharma B et al(15) showed that NLF *Escherichia coli* when processed on MALDI and multiplex PCR identified growth of diarrheagenic strains of *Escherichia coli*. Due to non-availability of multiplex PCR and Antisera for agglutination we gave the Non fermenter *Escherichia coli* as *Escherichia coli* variants.

Because of poor sanitation and lack of education about personal hygiene, diarrheagenic *E. coli* can be transmitted from patient to patient through feco-oral route.

It was observed that Rotavirus was the most common causative agent in 16 (15.5%) cases followed by Adenovirus which was present in 14 (13.6%) samples. 2 (1.9%) samples showed Astrovirus. However, there was no stool sample in which Norovirus was seen. Similarly in study by Verma S (16) it was observed that Rotavirus was the most common causative agent of diarrhea (52.5%) cases

Taneja N et al (17) in their study in Chandigarh observed that 50% of *Escherichia coli* in patients with acute diarrhea was resistant to Ciprofloxacin. Similarly, Aggarwal P et al(18) in their study to determine the role of *E. coli* in acute diarrhea among children aged less than five years reported 72.4% resistance towards ciprofloxacin.

CONCLUSION

We recommend that in cases of acute diarrhea in children, etiological agents should be identified for possible causes of diarrhea with various risk factors that could help in

management. Stool samples should be processed with direct microscopy, culture and ELISA for detection of agents. This will aid in establishing correct etiology and subsequent treatment of patients.

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