Original Research Article

Rotavirus in diarrhoeic children under-five attending a tertiary care hospital in Mumbai, India

Nikhil Khude¹, Lona Dash^{1*}, Sushma Save², Badhuli Samal¹, Jayanthi Shastri¹

¹Department of Microbiology, ²Department of Pediatrics, Topiwala National Medical College and B.Y.L. Nair Charitable Hospital, Mumbai, Maharashtra, India

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*Correspondence:

Dr. Lona Dash,
E-mail: lonadash@gmail.com

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ABSTRACT

Background: Diarrhoeal diseases are a leading cause of morbidity and mortality among children requiring hospitalization in developing countries. Rotavirus is the most common cause of acute diarrhoea among under-five children. Etiological diagnosis of diarrhoea would enable appropriate management of patients while limiting the spread of drug resistant pathogens. This study was undertaken to determine presence of rotavirus and other diarrhoeal pathogens in under-five diarrhoeic children attending a tertiary care hospital, and the related clinical presentations.

Methods: 120 stool samples of under-five children with acute diarrhoea, attending the OPD and indoor services of a tertiary care hospital, were studied over one year. Rotavirus antigen was detected using enzyme linked immunosorbent assay. Bacterial and parasitic pathogens were detected using standard microbiological techniques.

Results: Out of 120 stool samples tested, 36 were positive for Rota virus antigen. Bacterial isolates included *E. coli* (25), *Vibrio cholerae* (12) and *Aeromonas species* (1). Parasites were observed in nine samples and multiple pathogens in nine.

Conclusions: Rotavirus continues to be a major cause of childhood diarrhoea. As antibiotics have no role in the management of viral and parasitic diarrhoeas, etiological diagnosis is imperative for proper management of diarrhoea and prevention of indiscriminate use of antibiotics.

Keywords: Bacterial enteropathogens, Enzyme Linked Immunosorbent Assay, Infectious diarrhoea, Parasites, Rotavirus

INTRODUCTION

Diarrhoeal diseases continue to be a major concern for developing countries.¹ In India, about 248 million episodes of diarrhoea occur each year in children under 6 years, with an estimated 158,209 deaths per year.² Clinical manifestations of diarrhoea depend on the organism and host response to infection. The infectious causes of acute diarrhoea consist of viral, bacterial and parasitic agents. Among the viral agents, Rotavirus is an important cause of childhood diarrhoea. John et al estimated that 11.37 million episodes of rotavirus gastroenteritis occur each year in India.³ An accurate

diagnosis is critical for reducing the misuse of antimicrobial agents, the cost of treatment of diarrhoeal diseases and for preventing the development of drug resistant pathogens. This study was undertaken to determine presence of rotavirus and other diarrhoeal pathogens in under-five diarrhoeic children attending a tertiary care hospital, and the related clinical presentations.

METHODS

This prospective study was conducted from October 2010 to September 2011 at a tertiary care hospital after

approval by the Institutional Ethics committee. On obtaining informed consent from the parents/guardians, a single stool sample was collected from 120 under-five children, who presented with acute diarrhoea. These children, who either attended the outpatient's department (OPD) or were admitted in the hospital, were investigated for rotavirus and other enteric pathogens.

Detailed case history and demographic data were noted using a standardized case record form. According to the World Health Organization (WHO), diarrhoea is "the passage of three or more loose or liquid stools per day, or more frequently than is normal for the individual".⁴ Diarrhoea was further defined as acute if it lasted less than 2 weeks. The WHO criteria for dehydration were followed.⁴

Collection and transport of specimen

Parents were instructed to collect the stool specimen of the child in a sterile wide-mouthed screw-capped plastic or glass container and to submit it within 30 minutes to the laboratory.

Specimen processing

Freshly passed liquid stool samples were processed in the Microbiology Laboratory. One part was stored at -20°C for further studies. The second part was used for routine/microscopic examination for pus cells and for detection of parasites directly from specimen and also following Formol ether sedimentation technique and modified Acid-Fast staining of smears using cold Kinyoun technique (3% acid alcohol) for the detection of coccidian parasites.

Bacteriological culture was done on appropriate media by direct plating and after enrichment for *vibrio*, *salmonella*, *shigella* and *E. coli*.⁵⁻⁸ Cultures were identified by observing colony characteristics and standard identification procedures.⁷ All *V. cholerae* strains were serotyped by agglutination with *V. cholerae* polyvalent O1 antisera followed by Ogawa and Inaba antisera (Denka seiken, Japan). Antibiotic susceptibility test was performed for the bacterial enteropathogens by the Kirby Bauer Disc Diffusion Method (KBDDM), as per CLSI guidelines.⁹ Rotavirus antigen was detected in stool samples using ELISA as per manufacturer's instructions. A double antibody (sandwich) ELISA test (Rotavirus Antigen Detection Microwell ELISA, IVD Research Inc. Carlsbad, CA 92008 USA) was performed using a polyclonal anti-Rotavirus antibody to capture the Rotavirus antigen from the stool supernatant.

Data analysis

All collected data was analysed for number and percentage. For calculating statistical significance, P value <0.05 was considered.

RESULTS

General characteristics of children enrolled in the study

Out of the 120 children presenting with acute diarrhoea, only 13 cases were treated through the OPD and the remaining 107 were admitted in the hospital. Maximum children were in the age group of 13 to 24 months. The male:female ratio was 1:0.88 showing a slight male preponderance (Table 1).

Table 1: Age and gender-wise distribution of study population (n = 120).

Age (in months)	Male	Female	Total numbers
0-12	15	15	30
13-24	23	13	36
25-36	13	18	31
37-48	7	8	15
49-59	6	2	8
Total	64	56	120

Occurrence of enteric pathogens

A total of 74/120 stool samples (61.67%) yielded 83 enteropathogens. The distribution of pathogens is shown in Table 2.

Table 2: Detection of various Enteropathogens amongst study population (n = 120).

Enteropathogens	Number (%)
Rotavirus	36 (30.0)
Bacteria (Total)	38 (31.6)
<i>E. coli</i>	25 (20.8)
<i>Vibrio cholerae</i>	12 (10.0)
<i>Aeromonas</i>	1 (0.8)
Parasites (Total)	9 (7.5)
Protozoan	
<i>Entamoeba histolytica</i>	3 (2.5)
<i>Giardia lamblia</i>	2 (1.7)
Helminthic	
<i>Ascaris lumbricoides</i>	3 (2.5)
<i>Strongyloides stercoralis</i>	1 (0.8)
Total pathogens	83

Rotavirus was the predominant pathogen detected (36;30%). Among the bacterial enteropathogens, *E. coli* was the commonest (25;20.8%). *Vibrio cholerae* O1 El Tor was the next (12;10%) with 11 isolates identified as Ogawa and one as Hikojima. Enteric parasites were detected in nine cases (7.5%) (Table 2).

Modified acid-fast staining did not detect coccidian parasites in any of the samples. Among the 74 (61.67%) samples positive for enteropathogens, 65/74 (87.8%) had

a single pathogen and 9/74 (12.2%) had mixed infection (Table 3).

Table 3: Distribution of mixed infection cases (n = 9).

Enteropathogens detected	No. of cases
Rotavirus + <i>Vibrio cholerae</i>	1
Rotavirus + <i>E. coli</i>	4
Rotavirus + <i>Ascaris lumbricoides</i>	1
<i>Giardia lamblia</i> + <i>E. coli</i>	1
Trophozoite of <i>Entamoeba histolytica</i> + <i>E. coli</i>	1
Larva of <i>Strongyloides stercoralis</i> + <i>E. coli</i>	1
Total cases with mixed infection	9

Clinical features in Enteropathogen-positive cases

Among the 74 Enteropathogen-positive cases, only four were treated through the outpatient's department (OPD) of which *Entamoeba histolytica* and *E. coli* was detected in two cases each. Of the 36 rotavirus positive cases, 34 were hospitalized. The remaining two were initially treated through OPD but were subsequently hospitalized owing to dehydration. All rotavirus diarrhoea cases, cholera cases and the single case of *Aeromonas* diarrhoea were found to require hospitalization and accounted for 49/74 enteropathogen-positive diarrhoea cases. They presented mostly with dehydration (100%) and vomiting (13;26.5%) while cases with parasite-associated diarrhoea presented with abdominal pain (3) and fever (4), as shown in Table 4.

Table 4: Clinical features of cases with Enteropathogen-positive diarrhoea.

Enteropathogen detected	No. of cases	Dehydration			Abdominal pain	Vomiting	Fever
		Nil	Some	Severe			
Rotavirus	36	0	30	6	1	8	3
<i>E. coli</i>	25	16	8	1	2	5	5
Vibrionaceae (<i>V. cholerae</i> : 12, <i>Aeromonas spp</i> : 1)	13	1	9	4	0	4	0
Protozoa	5	4	1	0	2	1	2
Helminthes	4	3	1	0	1	1	2

Table 5: Macroscopic findings of Enteropathogen-positive stool samples (n=74).

Entero pathogens	No. of Stool samples positive	Mucus	Frank blood
Rotavirus	36	7	0
Bacteria			
<i>E coli</i>	25	6	1
<i>V. cholerae</i>	12	2	0
<i>Aeromonas spp.</i>	1	1	0
Parasites			
Protozoa	5	2	0
Helminthes	4	2	0

Of the 33 green liquid stool samples, 20 (60.6%) yielded pathogens. Rotavirus was the predominant enteropathogen associated with green liquid stools (13;39.4%) as compared to bacterial pathogens (4;12.1%), parasitic pathogens (2;6.1%) and one co-infection with bacteria and parasite (1;3.0%). This was statistically significant ($p < 0.01$), (p value was considered as 0.05). Conversely, of the 36 Rotavirus antigen positive stools, 13 (36.1%) were greenish in colour. Rotavirus was also detected in Rice watery/ white (10;27.8%), yellow (8;22.2%), and brown (5;13.9%) stools. Mucus was present in only seven of the samples and frank blood in none (Table 5). A total of 15;50% of the children in the

first year of life with acute diarrhoea had rotavirus antigen in stool samples. Thereafter, a gradual decline in positivity was observed with all samples in the fifth year showing negative results for Rotavirus antigen (Table 6).

Table 6: Rotavirus antigen positivity by age.

Age (in months)	Total Samples studied	No. of Rotavirus positive samples	Rotavirus positivity (%)
0-12	30	15	50
13-24	36	11	30.5
25-36	31	9	29
37-48	15	1	6.7
49-59	8	0	0
Total	120	36	100

Seasonal distribution of Rotavirus positive cases

Rotavirus antigen positive cases were detected throughout the year in the present study.

Outcome

Out of the 120 cases admitted with acute diarrhoea, the 12 cases of cholera and one of *Aeromonas* diarrhoea were transferred to an infectious disease hospital in the same

area and all the 120 cases were discharged following successful treatment.

DISCUSSION

Diarrhoeal diseases continue to be a major concern among under-five children in the developing countries. They are a leading cause of childhood morbidity and mortality besides contributing significantly to the problem of under-nutrition in infants and children.¹ Although diarrhoea is a clinical diagnosis, etiological diagnosis is imperative as antimicrobial agents are indicated in cases of bacterial and parasitic diarrhoeas only.¹⁰

A total of 120 children with acute diarrhoea were studied comprising of almost equal number of male and female children with slight male preponderance. A larger proportion of diarrhoea cases were in the age group of 0 to 36 months thereafter showing a decline. The World Health Organization (WHO) states that 'the incidence of diarrheal diseases varies greatly with the season and a child's age. The youngest children are most vulnerable: Incidence is highest in the first two years of life and declines as a child grows older.'¹¹ In humans, the tendency of rotaviral and enteropathogenic *E. coli* (EPEC) infections to affect young children is impressive. The explanations probably reside in age-related changes in gut mucosa, cell surface factors, microbial flora, environmental exposure and specific immune factors.^{10,12}

In the present study, overall enteropathogen positivity was 61.67% (74/120). This is in concordance with studies by Suwatano et al (60.9%) and Youssef et al (66.4%) from Thailand and Jordan respectively.^{13,14} Rotavirus was the predominant pathogen associated with acute diarrhoea (30%) in our series. This is comparable to findings of Youssef et al (32.5%) and Kelkar et al (28.2%) while being higher than that of Gupta et al (19.5%) and lower than that of Shariff et al (38.7%).¹⁴⁻¹⁷

In the present series, *E. coli* was the most frequently isolated bacterial pathogen (25/38;65.8%) which is in accordance with other studies.^{14,18} Next was *Vibrio cholera* 01 El Tor (12/38;31.6%). Eleven of these isolates being Ogawa and only one being Hikojima delineates the fact that the biotype El Tor and serotype Ogawa were prevalent in our region.

Of the parasitic enteropathogens, Nematelminthes class (4; 44.4 %) and protozoan parasites (5; 55.6 %) were detected in almost equal numbers. Among the helminthes, *Ascaris lumbricoides* was the commonest; this is in agreement to findings of Wabale et al showing predominance of *A. lumbricoides* in diarrhoeal stools in children.¹⁹ Among protozoan parasites, *Entamoeba histolytica* and *Giardia lamblia* were detected. It is well known that rotavirus and enterotoxigenic *E. coli* cause more than half of all cases of acute diarrhoea in children,

with rotavirus being the predominant etiological agent in children under five years of age.²⁰

Concomitant infection of rotavirus with bacterial agents and intestinal parasites was also observed. Mixed infection was observed in nine of 120 (7.5%) cases, this being a well-documented phenomenon in several countries.^{13,14} While Youssef et al did not detect pathogens in 33.6% cases, the present study failed to detect any pathogens in 38.33% samples; the various reasons for this may be a non-infectious etiology or a viral etiology that was not studied. Use of antibiotics prior to collection of stool sample may have resulted in a few culture negative reports.

Rotavirus detection declined with increase in age in the present study; while 50% positivity was noted in infants and 30.5% in children below 2 years we continued to detect cases in children between 2 to 3 years with declining positivity thereafter. Similar findings have been reported across various centres in India.^{15,16,21} Rotavirus is the most important cause of severe, life threatening gastroenteritis in children accounting for 20-50% of hospitalization for diarrhoea in children worldwide. Rapid acquisition of antibodies is known to take place in children between 6 and 24 months conferring immunity in the older age groups.¹⁵

A diagnosis of rotavirus infection provides the physician with useful information for patient management. Early institution of specific therapy prevents spread of infection, besides preventing the misuse of antibiotics. Sensitive enzyme immunoassays (EIA), available in kit form have made diagnosis easy and widely available.

Associated signs and symptoms of vomiting, fever and dehydration were broadly comparable to findings reported in other studies.¹⁶ While cholera and rotavirus diarrhoea were often associated with vomiting (25%) and always associated with dehydration, in contrast, parasite-associated diarrhoeas often presented with abdominal pain and fever. Rotavirus-associated diarrhoea cases generally tend to be more acute, with vomiting and dehydration and often require hospitalization.^{10,12} Lee et al have reported dehydration in 92% of the rotavirus positive cases in their series.²² In our series, the two OPD cases in which rotavirus antigen was detected had to be subsequently hospitalized. Thus, all the children with rotavirus diarrhoea required hospitalization as compared to 86% admission rate for rotavirus negative cases. In our setup, children with acute diarrhoea and dehydration are hospitalized and are treated with intravenous fluids alone; antibiotics are added in children presenting with toxic features, concomitant respiratory tract infection or protein energy malnutrition.

In the present study, it was observed that rotavirus was more often associated with greenish liquid stools (13;39.4%) compared to other pathogens. Conversely, greenish liquid stools also yielded rotavirus as the

predominant enteropathogen (13;36.1%). Other studies have also inferred that greenish liquid stool was a sensitive predictor of Rotavirus diarrhoea.¹⁶

Out of 25 *E. coli* strains, 20 were sensitive to amikacin, 14 to norfloxacin, 4 to ceftriaxone and two to trimethoprim-sulfamethoxazole (TMP-SMX) while all were uniformly resistant to nalidixic acid. Resistance to $\geq$ three antimicrobials was observed in 88% of the *E. coli*. Lanjewar et al noted that diarrhoea-causing *E. coli* (DEC) showed maximum susceptibility to amikacin (79.17%).²³

Antibiotics should be given only if diarrhoea is moderate or severe, as antibiotic treatment increases the likelihood of development of Haemolytic Uraemic Syndrome (HUS).^{1,11,15} TMP-SMX is the first-line drug to be used in enteric infections. However, in the present study, in-vitro susceptibility of TMP-SMX was very low; hence, an alternative antibiotic should be considered for empiric treatment when required. *V.cholerae* strains were found to be sensitive to ampicillin, tetracycline and chloramphenicol but resistant to TMP-SMX and furazolidone. WHO states, "In severe cases of cholera, antibiotics can reduce the volume and duration of diarrhoea and shorten the period during which cholera Vibrios are excreted. They can be given orally as soon as vomiting stops, usually within 3-4 hours after starting rehydration."²⁴ The patients who benefit most from antibiotics are those who are severely dehydrated. Indiscriminate use of antibiotics in mild cases can quickly use up supplies and hasten the development of antibiotic resistance among cholera Vibrios. For children, paediatric tablets or liquid preparations of TMP-SMX are recommended.^{1,24} A single dose of doxycycline has not yet been shown to be effective in children. Tetracycline, however, is effective in children but in some countries, is not available for paediatric use. Furazolidone, erythromycin and chloramphenicol are other effective alternatives for adults and children.²⁴ The choice of antibiotic should consider the local patterns of resistance to antibiotics. Antibiotic-resistant *Vibrio cholerae* 01 should be suspected if diarrhoea continues after 48 hours of antibiotic treatment.²⁴

The present study has certain limitations. Firstly, viruses known to be associated with childhood diarrhoea, like Norovirus, Astrovirus, Calicivirus and Norwalk agent were not studied. Secondly, characterization of *E. coli* could not be attempted due to resource constraints.

Bacterial and parasitic diarrhoeas can be prevented by improvements in hygiene and sanitation while Rotavirus diarrhoea can be prevented by adherence to stringent hand hygiene practices coupled with mass vaccination using a safe and effective vaccine.¹¹ ROTAVAC, the first indigenously developed rotavirus vaccine has obtained licensure in India in 2014. It has been shown to provide comparable protection against a wide variety of strains. There are additional oral rotavirus vaccines in the pipeline in India. Risk benefit analyses have shown that

rotavirus vaccine benefits greatly outweigh risk especially in high disease burden settings like India.²⁵

Out of the 120 cases admitted for diarrhoea in our study, 13 were transferred to an infectious disease hospital in the same area. All the children were discharged following recovery because of aggressive rehydration therapy and successful management. Thus, there were no deaths recorded in this series. This report, in concordance with the one from Nepal is encouraging when compared to the global mortality of 2.5 million deaths per year and 1,58,209 deaths per year in India due to diarrhoea.^{16,2}

CONCLUSION

The number of children presenting with acute diarrhoea, as also the maximum cases positive for rotavirus antigen was noted in the first three years of life. Hence, it is recommended to routinely screen cases of acute diarrhoea for the presence of rotavirus.

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