Original Article

Burden and Molecular Epidemiology of *Rotavirus* Causing Diarrhea among Under-Five Children: A Hospital-based Study from Eastern India

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Abstract

Background: *Rotavirus* (RVA) causes severe gastroenteritis in under-five children, and there are many diverse strains of the virus that are localized to different parts of the world. **Objectives:** To study the burden and molecular epidemiology of RVA causing gastroenteritis among children from Eastern India. **Materials and Methods:** This hospital-based cross-sectional study included children under-five with gastroenteritis. Demographic and clinical parameters were recorded in a predesigned pro forma. Stool samples collected from these children were initially screened for RVA VP6 antigen by enzyme immunoassay (EIA). Each EIA-positive sample was then subjected to RNA extraction, followed by reverse transcription, and heminested multiplex polymerase chain reaction for genotyping of RVA strains. **Results:** Of 320 included children, RVA was detected in 30.62% (98/320) cases by EIA. The highest incidence for RVA-positive cases (34.61%) was observed among children in the age group of 24–36 months, followed by 0–12 months (33.04%). Of the 97 completely typed samples, single genotype was detected in 85 (87.62%) samples with either G (VP7) or *P* (VP4) types. However, mixed genotypes were detected in 12 (11.21%) samples. G3P[8] (44.09%) was the most common genotype, followed by G1P[8] (32.65%), G2[P4] (5.10%), G1[P6] (3.06%), and G9[P4] (1.02%). **Conclusions:** The present study found RVA positivity in 30.62% of children with gastroenteritis, with the highest burden among 24–36 months old. The predominant genotypes were G1, G3, and P[8]. Further large-scale/multicentric studies should be conducted to document the diversity of circulating RVA genotypes in this region for giving inputs for vaccination strategy.

Keywords: Diarrhea, genotyping, G-type, molecular epidemiology, P-type, *Rotavirus*

Introduction

Rotavirus (RVA) is the leading cause of diarrheal death in children under 5 years of age.[1] Virtually, every child around the globe experiences RVA diarrhea by the age of 3–5 years. Majority of the RVA-associated gastroenteritis in developing countries is caused due to Group A RVA; India accounts for an estimated 457,000–884,000 hospitalizations and over 2 million outpatient visits for diarrhea.[1] Group A RVA is a double-stranded RNA virus consisting of 11 segments. Rotaviruses are nonenveloped, icosahedral, triple-layered particles; it contains two outer capsid proteins, VP7 (G genotype) and VP4 (P genotype), which independently elicit serotype-specific neutralizing immune responses that may play an important role in protection against recurrent infections. These VP7 and VP4 encoding genes of RVA are classified into 27 G genotypes (G1–G27) and 37 P genotypes (P[1]–P[37]), respectively.

The World Health Organization (WHO) has recommended the inclusion of RVA vaccination of all infants in the

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national immunization program.[2] At present, three globally licensed vaccines against RVA gastroenteritis, Rotarix (GlaxoSmithKline Biologicals), RotaTeq (Merck and Co., Inc.), and Rotavac (Bharat Biotech Ltd.), are available in India.[3] Rotavac is an indigenously developed vaccine which is WHO prequalified, which is being introduced in a phased manner into the national immunization program by the Government of India, from 2016.[4]

The enormous diversity of RVA is mainly because of point mutations, genetic reassortment, or introduction of animal viral strains to human beings.[5] With Odisha state being one of the early introductory regions for Rotavac vaccine, it is important to monitor the circulating RVA genotypes to detect changes or the emergence of new strains. Therefore, RVA surveillance is needed to monitor the prevalence and possible changes of the different G and *P* types circulating in the region.

This study was conducted to estimate the burden of RVA-associated gastroenteritis and identify the circulating RVA strains among children under 5 years of age, immediately after RVA vaccine introduction at a tertiary care teaching hospital in Bhubaneswar, Odisha.

Materials and Methods

Study setting, sample collection, and participants

In this cross-sectional study, children <5 years (0–59 months) of age admitted with acute gastroenteritis (defined by >3 unformed stools in any 24 h period of <5 days duration) to the Pediatrics Ward of Kalinga Institute of Medical Sciences (KIMS), Bhubaneswar, Odisha (from February 2016 to May 2017), were included. Being a hospital-based study, nonprobability [consecutive] sampling method was used for recruiting the cases. Stool samples were collected after taking consent from parents or legal guardians. Children with the following conditions were excluded: diarrhea is not the primary reason for admission, diarrhea developed postadmission, history of diarrhea for >5 days, and parents not willing to participate in the study. The hospital has a catchment area mostly from the following four districts of Odisha state: Khurdha, Cuttack, Puri, and Nayagarh. After collection, stool samples were placed in vaccine carriers with ice packs and transported to the referral laboratory at Christian Medical College (CMC), Vellore, once every month. Institutional Ethics Committee approval was obtained from KIMS Hospital and CMC, Vellore. Demographic and clinical details (age, sex, living area, sources of drinking water, maternal education, and clinical severity score) were recorded in a predesigned format. Vesikari clinical severity score was calculated using the table.[6]

Laboratory procedures

Stool samples received at CMC, Vellore, were stored at −70° C until further testing. The samples initially underwent screening for RVA VP6 antigen by enzyme immunoassay or enzyme immunoassay (EIA) (Premier™ Rotaclone, Meridian BioScience Inc., Cincinnati, Ohio, USA). The assay was performed as per the manufacturer's instructions. As recommended by the manufacturer, samples with an OD value of ≥0.150 were reported as positive. For samples positive by EIA, genotyping polymerase chain reaction (PCR) was performed to determine the RVA genotype.

Viral RNA extraction was performed from 20% (W/V) stool suspension (0.2 g stool in 1 ml MEM) using automated method (QIAcube HT, QIAGEN). By reverse transcription, the viral RNA was converted to complementary DNA (cDNA) using random primers (Invitrogen) and 200U/μl of Moloney murine leukemia virus reverse transcriptase enzyme (Superscript II MMLV-RT, Invitrogen). The cDNA was used as a template for genotyping in a heminested multiplex PCR for the detection of VP7 (G type) and VP4 (P type) genes, using published oligonucleotide primers.[7-10] Primers to identify VP7 genotypes G1, G2, G3, G4, G8, G9, G10, and G12 and VP4 genotypes P[4], P[6], P[8], P[9], P[10], and P[11] were included in the PCR reaction. The genotype identification was based on band sizes of the products. For samples, which were negative by genotyping PCR, a VP6 conventional PCR was performed to rule out RVA positivity. Sanger sequencing was used to further evaluate strains that could not be typed using standard PCR-based methods.

Statistical analysis

Descriptive analysis was used to report demographic characteristics of the participants. These data were entered into Excel 2003 (Microsoft) and were analyzed to evaluate the overall proportion of various G and *P* types in RVA-positive specimens. Chi-square test was used for the determination of differences in the proportions of RVA positivity according to gender, age groups, and hospital stay. Student's *t*-test was used for comparing the means of disease severity by Vesikari score. The level of significance was set at $P < 0.05$. Statistical analysis was conducted using SPSS software version 21, SPSS Inc., Chicago, Illinois, USA.

Results

Of the 320 diarrheal samples, 98 (30.62%) samples were EIA positive for RVA. There was no missing data as children were hospitalized and discharged as per the criteria. Overall, stool samples from 29.29% of males and 32.68% of females were positive for RVA. Age-wise distribution showed that majority of the RVA-associated gastroenteritis was found in the 24–36 months' age group (34.61%). The prevalence of RVA-associated gastroenteritis was slightly higher in rural areas (32.33%) compared to urban areas (30.34%). Sociodemographic data showed that majority of diarrheal episodes due to RVA were in the population who used shared community water (35.41%), compared to those using covered well (20%) or tap water at home (25.31%). RVA gastroenteritis was also associated with education level of mothers, children of mothers with basic education having higher proportion of diarrheal episodes (36.58%), compared to children whose mothers had university level education (24%), although the difference was not statistically significant [Table 1]. The

burden was high during the winter season (November–February) [Figure 1].

Genotype distribution

A total of 97 of the 98 EIA-positive samples were completely typed, whereas one sample was untyped. Of these 97 completely typed samples, 85 (87.62%) had single G and P types, whereas 12 (11.21%) were of mixed type (with more than one G and/or P type). Molecular typing of RVA genotypes showed that the most common G-Type was G3 (41.12%), followed by G1 (33.64%), G2 (4.67%), and G9 (0.93%). The predominant P-Type was P[8] (78.50%), followed by P[4] (6.54%) and P[6] (4.67%) [Figure 2]. Of the RVA G and *P* types identified in the study, G3P[8] (44.9%) was the most common genotype, followed by G1P[8] (32.65%), G2P[P4] (5.10%), G1[P6] (3.06%), and G9[P4] (1.02%). Genotypes G3P[8] and G1P[8] comprised a major proportion (77.6%) of the genotyped samples [Table 2].

Table 1: Sociodemographic profile of children hospitalized with diarrhea at Kalinga Institute of Medical Science, Bhubaneswar, Odisha, India

Variables	*n*	*Rotavirus* positive	Percentage
Gender			
Male	198	58	29.29
Female	122	40	32.68
Total	320	98	30.62
Age (months)			
0-12	115	38	33.04
>12-24	122	37	30.32
>24-36	052	18	34.61
>36-48	013	03	23.07
>48-60	010	02	20
Location			
Rural	167	054	32.33
Urban	145	044	30.34
Source of drinking water			
Cover well	010	02	20
Open well	007	02	28.57
Shared community	096	34	35.41
Tap to home	158	40	25.31
Maternal literacy			
Yes	305	96	31.47
No	007	02	28.57
Education level of mother			
None	007	02	28.57
Basic	041	15	36.58
High school	129	46	36.65
Higher secondary	110	29	26.36
University	025	06	24

Comparison of clinical characteristics of *Rotavirus* and non-*Rotavirus* diarrhea groups

The mean duration of hospital stay was longer in RVA diarrhea (3.83 ± 1.77) compared to non-RVA diarrhea (3.43 ± 1.32), and this difference was statistically significant. The mean Vesikari scores of RVA diarrhea group (13.86 ± 1.89) were higher compared to the non-RVA diarrhea group (13.19 ± 1.92), and this difference was also statistically significant. Around 96% (94/98) of the RVA diarrhea were of severe to very severe category, compared to 92% (204/222) of the non-RVA diarrhea [Table 3].

Discussion

In the present study, RVA diarrhea was high among children of 24–36 months of age, occurring mostly during winter season. Diversity of RVA genotypes was noted, with G3P[8] and G1P[8] being the most predominant circulating strains in the study area.

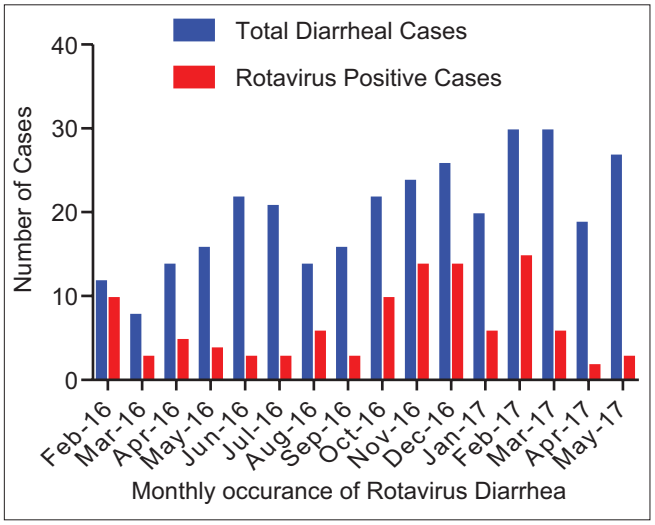

Figure 1: Frequency of diarrheal and *Rotavirus*-positive cases in children <5 years of age from February 2016 to May 2017 admitted at KIMS, Odisha

Table 2: Distribution of *Rotavirus* genotypes across age groups

Genotypes	Total	0-12 (months)	12-24 (months)	24-36 (months)	36-48 (months)	48-60 (months)
G1[P8]	32	15	11	05	01	00
G1P[6]	03		01	00	00	00
G2P[4]	05	02	02	01	00	00
G3P[8]	44	17	17	08	01	01
G9P[4]	01	00	01	00	00	00
Mixed	12	03	05	02	01	01
Untyped	01	00	00	01	00	00

Table 3: Comparison of clinical characteristics between *Rotavirus* and nonrotaviral diarrheal groups

Variable	*Rotavirus* positive (*n*=98)	*Rotavirus* negative (*n*=222)	*P*
Gender			
Male	58	140	0.707[a]
Female	40	82	
Age, mean±SD (months)	18.22±10.59	18.77±11.80	0.69[b]
Duration of hospital stay, mean±SD (days)	3.83±1.77	3.43±1.32	0.02[b]
Vesikari score, mean±SD	13.86±1.89	13.19±1.92	0.004[b]
Disease severity by Vesikari score (a twenty point scoring system)			
Mild (1-5)	0	0	0.08[a]
Moderate (6-10)	4	18	
Severe (11-15)	78	184	
Very severe (16-20)	16	20	

[a]Statistical significance based on Chi-square test, [b]Statistical significance based on Student's *t*-test for comparing means. SD: Standard deviation

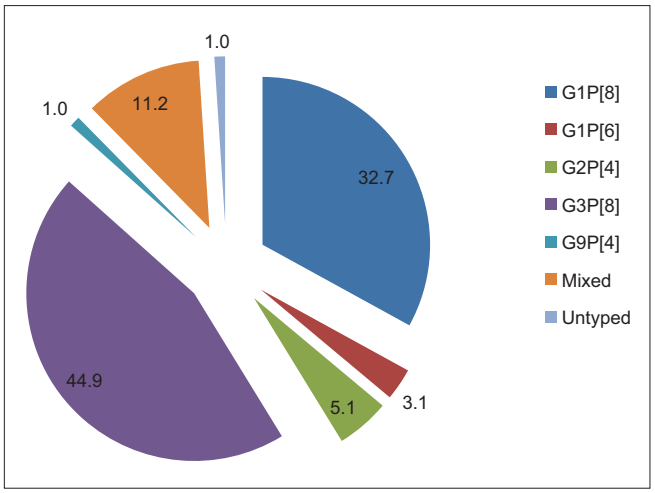

Figure 2: Distribution of *Rotavirus* genotypes in children <5 years admitted with diarrhea at KIMS, Odisha

In the developing counties of Asia, a high burden of RVA (30%–40%) has been reported among children hospitalized with diarrhea.[11,12] In this study, 30.62% of cases were positive for RVA, which is similar to the previous study from Eastern India.[13] Earlier reports from India, Japan, Malaysia, and Taiwan showed comparatively lower disease burden (20%–25%), but studies from Thailand, Myanmar, and Vietnam (31.1%–56%) showed a higher burden of RVA-associated gastroenteritis.[14]

In this study, a higher burden (34.61%) of RVA infection was found in the age group of 24–36 months in contrast to other studies where the burden was high within the first 2 years and more so after 7 months of age.[15-17] In Odisha, RVA vaccine was introduced in the routine immunization program in March 2016 (children <1 year being eligible to take vaccine). However, as the study population was not uniformly vaccinated for RVA vaccine, a large portion of 24–36-month-old children were unvaccinated compared to children of <24 months of age, which could be one of the reasons for a higher burden in this age group. The vaccination at an early age can be beneficial to prevent majority of cases of RVA disease. Previous reports have shown that children <4 months of age are initially protected to some extent by maternal antibodies against RVA, which could be one of the reasons for peaking of the disease after 7 months of age.[18] In the present study, RVA positivity was more in <7-month-old children compared to those <7 months old.

RVA infections are predominately observed in winter; however, seasonality may vary from year to year.[18] In the present study, RVA diarrhea was observed throughout the year, but peak in the number of cases was observed between November and February. Similar patterns of seasonal variations have been reported in previous studies.[19]

Studies on RVA epidemiology demonstrate the differences in strain circulation over time.[8,20] A large number of G-type genotypes (G3, G1, G2, and G9) were observed in the present study unlike the developed countries where one or two genotypes were reported predominant in a season.[7] The most prevalent RVA strains causing childhood diarrhea globally are G1–G4 and G9.[21] In the present study, G3 (44.12%) was the most common strain responsible for severe diarrhea-related hospitalizations, whereas previous studies from India suggested G1 and G2 stains as the most dominant.[22-24] In the present study, the major P-type was P[8] (78.50%), followed by P[4] and P[6]; previous studies also suggested similar trend where P[8] or P[6] were the leading strains.[17,25,26] The most common genotype combinations were found to be G3P[8], followed by G1P[8], G2P[P4], G1P[P6], and G3P[P6]. This pattern is in agreement with findings from other studies.[27,28]

Table 4 shows the list of most common RVA G and *P* type distribution in different parts of India, compared to the present study.[13,29-31] Globally, including India, the most common genotype is G1[P8] unlike the present study where G3[P8] was found commonly.[8] Previous studies from Eastern India conducted during 2005–2008 had found G1[P8] as the dominant genotype, but those conducted during 2011–2013 found G9[P4] as the most common genotype. One study from Odisha conducted during 2003–2005 found G2[P4] and G2[P8] as the most common genotypes, whereas another study from the same region during 2013–2015 found G1[P8] as the most common genotype. This changing pattern of genotype distribution suggests continuous need for RVA disease surveillance and vaccine effectiveness studies for adequate management of RVA diarrhea in this region.

The limitations of the present study include the following. We did not compare RVA vaccinated and nonvaccinated children for various clinical parameters as well as RVA genotype predominance pattern. The study does not provide information about the transmission pattern of RVA genotypes and vaccine effectiveness in this region.

Table 4: Studies from Eastern India showing genotype distribution pattern of *Rotavirus* in comparison to the current study

Study sites	Study period	Common *Rotavirus* genotypes	References
Kolkata	January 2011-December 2013	G9[P4] and G9[P8] 40%, G2P[4] 39.6%, G12[P8] 16.4%, G12[P4] 5.6%	28
Odisha	September 2013-May 2015	G1[P8] 62.16%, G1[P11] 9.45, G3[P8] 8.10%, G9[P8] 6.75%, G2[P4] 4.05%, G2[P6] 4.25%, G9[P4] 2.70%, G9[P11] 1.35%, G1[P6] 1.35%	12
Eastern India	June 2004-April 2005	G1[P8] and G1[P4] 29.9%, G2[P4] 20.4%, G1[P8] 11.6%, G1[P4] 9.5%, G12[P6] 8.2%, G12[P8] 7.5%	29
Manipur, North Eastern India	December 2005-March 2008	G1[P8] 36%, G2[P4] 22%, G12[P6] 8%, G9[P6] 3%	30
Current Study	February 2016-May 2017	G3P[8] 44.9%, G1P[8] 32.65%, G2P[P4] 5.10%, G1[P6] 3.06%, G9[P4] 1.02%	

Conclusions

In the present study, RVA gastroenteritis prevalence was 30.66% in under-five children in Bhubaneswar city, with most cases occurring in winter. There was diversity in identified RVA genotypes, with G3P[8] and G1P[8] being more common.

Ethics approval and consent to participate

Parents or legal guardians of all the study children provided written informed consent, and this study was approved by the Ethical Committee of Kalinga Institute of Medical Sciences (KIMS), KIIT University, and the Institutional Review Board of CMC, Vellore.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the parents and legal guardians have given consent for images and other clinical information to be reported in the journal. The parents and legal guardians understand that the names and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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