Surveillance and Molecular Characterization of Rotavirus Strains Circulating in Odisha, India after Introduction of Rotavac

Vishwanath Ghoshal1, Manas Kumar Nayak2, Namrata Misra3, Ranjith Kumar4, Samarasimha Reddy N4, Nirmal Kumar Mohakud2

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Abstract

Objective To know the rotavirus burden associated with acute gastroenteritis along with circulating genotypes among under-five children and to find out possible associations with different demographic and clinical predictors in a tertiary care teaching hospital in Bhubaneswar, Odisha.

Methods A prospective acute gastroenteritis surveillance conducted from February 2016 to June 2019 at a tertiary care pediatric hospital in Bhubaneswar has enrolled 850 children under five years of age. The stool samples were tested for VP6 antigen of rotavirus by enzyme immunoassay (EIA) and hemi-nested multiplex PCR to find out VP7 (G type) and VP4 (P type) genes. The data was presented using mean ± SD, median (IQR) along with frequencies and percentages.

Results Rotavirus positivity was found in 246 children (28.9%) with male: female ratio of 3:1. An increasing trend of rotaviral diarrheal cases was seen during the winter months. History of vomiting for 2 d, age group of 12–23 mo, and fever were significantly associated with rotavirus diarrhea having odd ratios of 1.80 (95% CI, 1.48, and 1.69, respectively). Among the genotypes, G3 and P8 were found to be most common in the present study.

Conclusion With the introduction of Rotavac in the state the overall rotaviral distribution has significantly changed. Children of 6–23 mo were the most affected age group in the study indicating the necessity of this vaccine in the early months of life.

Keywords Rotavirus • Under five • Vaccine • Diarrheal disease • Bhubaneswar • Acute gastroenteritis • G3P8 • Rotavac

Introduction

Rotavirus is the leading cause of childhood diarrhea [1, 2]. Belonging to the family Reoviridae, the virus has 9 species denoted by alphabets from A to I. Rotavirus-A is the cause of more than 90% of human cases while, rest of the species can be seen in pigs and birds. Rotavirus-A is further classified by binary system of rotavirus classification on account of the capsid proteins of outer layer namely VP4 (P type) and VP7 (G type) [3–6]. Diarrheal disease was fifth leading cause of mortality among 0–5 y children globally from 1990 to 2016 in 195 countries. Among these, rotavirus was the leading cause of mortality with 1,28,515 deaths [7]. In collaboration with UNICEF (United Nation Children’s Fund), the WHO had launched the Integrated Global Action Plan for the Prevention and Control of Pneumonia and Diarrhoea (GAPPD) to end all preventable childhood deaths by 2025 by preventing two leading cause of deaths in children viz. pneumonia and diarrhea. The specific goals for diarrhea was to reduce under-5 mortality to less than 1 per 1000 live births [8]. As, rotavirus plays a major role in majority of these deaths, the world has already started emphasizing on its prevention with the help of simple preventive measures like vaccination. Hence, WHO had emphasized to include such vaccines in all national immunization programs.
In India, as per the 2011 Indian birth cohort estimates, over 11 million episodes of acute gastroenteritis in under five children and around 78,000 deaths were caused due to rotavirus diarrhea [9]. According to a study done by ICMR Bhubaneswar, before the addition of rotavirus in National Immunization Schedule (NIS), the prevalence of rotavirus disease among childhood diarrhea cases was found to be 54%. The prevalent strains being G1P[8] followed by G2P[4] and the most commonly affected age group was found to be 7 to 12 mo [10]. Rotavac containing monovalent strain G9P[11] (also known as 116E) [11] live, attenuated, oral vaccine is manufactured by Bharat Biotech with an efficacy of 56.4% and 49% in the first and second year of life, respectively for severe non vaccine rotavirus gastroenteritis. Rotavac vaccine was introduced in NIS in India with the brand name Rotavac from April 2016 [10]. Before its introduction, the community was largely dependent on self-purchased vaccine.

Few studies from Bhubaneswar were in the pre-vaccination era and hence, the present study was done to determine the rotaviral characteristics of the circulating strains in the post-vaccination era. A genuine surveillance was necessary for detecting possible changes of circulating strains in this region. This study was conducted to know the rotavirus burden associated with acute gastroenteritis along with circulating genotypes among under five children and to find out possible associations with different demographic and clinical predictors in a tertiary care teaching hospital in Bhubaneswar, Odisha.

### Material and Methods

This prospective observational study was done in under-five children admitted for acute gastroenteritis (AGE) in the pediatrics ward of Kalinga Institute of Medical Sciences, Bhubaneswar from the month of February 2016 to June 2019. Children presenting with diarrhea for at least six hours were eligible for enrollment. Among the eligible candidates, the authors had enrolled children whose guardian or parent gave a written informed consent for the study. For all enrolled children, an attempt was made to collect stool sample within 48 h of admission. In this manner, the authors had collected stool samples of 850 participants. Children older than 5 y or with episodes of dysesthesia or those who were not falling under the AGE criteria (of more than 3 stool episodes in 24 h period) were excluded. Clinical assessment was done based on episodes of diarrhea, vomiting, fever and dehydration. Vesikari score was used for assessing the severity of diarrhea with mild being 0–5, moderate 6–10, severe 11–15 and 16–20 for very severe disease. Stool sample was collected from each enrolled child and stored in ice lined refrigerator room of the facility. The samples were delivered to CMC Vellore once a month.

At first, screening of these collected samples were done for identification of VP6 antigen. This was done with the help of enzyme immunoassay or EIA (Cincinnati, PremierTM Rotaclone, Meridian Biosciences Inc.). Manufacturer’s instruction was thoroughly followed in every process and positivity report was given for samples with OD value ≥ 0.150. These positive samples were sent for genotyping by PCR. For RNA (viral) extraction Qiagen, QIAcubeHT methods were used. These automated method utilizes 20% (W/V) stool suspension or 0.2 g of stool in 1 mL of minimum essential medium (MEM). This RNA (viral) was then converted to complementary DNA sequence and taken as a template, for the detection of genes with the help of heminested multiplex PCR technique using the required oligonucleotide primers [2, 12, 13]. In this way we had detected VP4 (P-type) and VP7 (G-type) in the given stool samples. Primers were included in PCR for identifying VP4 (P4, P6, P8, P9, P10, P11) and VP7 genotypes (G1, G2, G3, G4, G8, G9, G10, G12). A conventional VP6 PCR was used, in cases we were unable to detect any rotavirus genotype from stool.

In those stool samples, where we could not detect any genotypes by PCR, a conventional VP6 PCR was used to identify rotavirus positivity.

The data were analyzed by STATA-13 (StataCorp, USA). The mean ± SD, median (IQR) along with frequencies and percentages were used to present the data. Independent student t test was used to compare the means of categorical variables. Univariate logistic regression analysis was used to estimate the predictors of rotavirus diarrhea. The p value of

### Table 1  Distribution of rotavirus test positive cases by year of surveillance (2016 to 2019)

| Year | No. of children eligible for enrolment (from Diarrhea logbook) | No. of children enrolled N (%) | No. of children with stool-specimens available | Children with rotavirus-positive specimens by enzyme immunoassay |
|------|---------------------------------------------------------------|--------------------------------|-----------------------------------------------|---------------------------------------------------------------|
|      |                                                               |                                 |                                               | Number | Percentage |
| 2016 | 207                                                           | 192 (92.57)                     | 192                                            | 69     | 35.9        |
| 2017 | 352                                                           | 288 (81.82)                     | 288                                            | 47     | 16.3        |
| 2018 | 361                                                           | 268 (74.24)                     | 267                                            | 82     | 30.7        |
| 2019 | 145                                                           | 103 (71.03)                     | 103                                            | 48     | 46.6        |
| Total| 1065                                                          | 851 (79.91)                     | 850                                            | 246    | 28.9        |
< 0.05 was considered statistically significant. Ethical committee clearance was obtained from KIMS, Bhubaneswar and CMC Vellore before the commencement of the study.

**Results**

The present study had enrolled a total of 850 children. Around 246 children (28.9%) were found to be suffering from rotavirus diarrheal disease. The positivity percentage was highest in the year 2019 (46.6%) and lowest in the year 2017 (16.3%) (Table 1).

Figure 1 depicts the trend of diarrheal diseases throughout the study period. There was an accumulation of positive cases during the winter months, whereas the summer months were predominantly occupied with rotavirus-negative diarrhea cases. Monthwise distribution from 2016 to 19 shows that November to March accounts for majority of positive cases (186 cases; 75.6%) and the peak being the December to February.

The maximum number of rotavirus-positive cases were observed in the age group 12–23 mo with 107 (33.6%) cases followed by 6–11 mo with 70 (33.7%) cases. The overall percentage positivity in both age groups was almost similar in each group. In 0–5 y, 6 mo to 2 y were the most affected age group for rotavirus (177 out of 246 positive cases) (Fig. 2).

Among the present study participants, females were more commonly affected than males with nearly one in every three female children with diarrhea were found to be positive for rotavirus (32.1%). Observing demographic and clinical characteristics of the participants, it was found that age group of 0–5 mo, 12–23 mo, history of vomiting for 2 d and fever were significantly associated with rotavirus diarrhea (Table 2).

The Table 3 shows the overall genotypic distribution in rotavirus-positive cases among study participants. In the present study, G3P[8] was found to be more prevalent with more than half of the positive cases indicating the strain in their stool samples. The year-wise distribution also showed similar picture with G3P[8] being the most commonly encountered strain in stool analysis of rotavirus cases with 46.4% (32 out of 69 cases), 55.3% (26 out of 47 cases), 68.3% (56 out of 82 cases), 64.6% (31 out of 48 cases) in 2016–19, respectively. In 2016–17 and 2018–19, G1P[8] and the mixed variant were second most common strains associated with rotavirus cases. Over the period of time G1P[8] strains had declined and number of mixed genotypes had increased. It was found that G2P[4] and G9P[4] strains were also in increasing trend from the year 2016 to 2019.
Discussion

Episodes of Rotavirus diarrhea was found predominantly in the winter season in the present study. Similar observations were made by previous studies too. After which, many had considered using the term “winter diarrhea” for this disease [14–16]. The rotavirus detection rate in this study was found to be 28.9% which is similar to the findings of a study done in 7 sites (6 states) of India, with a range of rotavirus positivity from 23.5% to 49.1% [9]. The prevalence of rotavirus in diarrheal cases was identified as 54.8% which had indicated the high presence of wild virus in the Bhubaneswar city between September 2013 and May 2015 [10]. With no specific vaccine against rotavirus in the national program and with high burden of cases in the community the Government of India launched Rotavac in the country from Bhubaneswar. Now after three years of Rotavac...
immunization in UIP shedule there is a declining trend of rotaviral diarrheal cases.

Presence of vomiting and fever were found significantly associated with rotavirus diarrhea. Studies by Panicker et al., Konno et al., Bhandari et al. and Ansari et al. showed that fever and vomiting are often associated with rotavirus [17–20]. Among under-five children age group of 12–23 were significantly associated with rotavirus illness in the present study. An Indian Council of Medical Research (ICMR) study also found a higher prevalence of rotavirus disease among children aged 13–24 mo with 59.7% followed by 7–12 mo with 56.6%, which was similar to the present study which found 50.7% prevalence in both age groups [10, 21].

G3P[8] (58.9%) was found to be commonest strain causing rotavirus diarrhea in the present study which was followed by GP-mixed (17.5%) and G1P[8] (13.4%). In previous studies, G3P[8] was considered as the emerging strain. Many studies had found G1P[8] to be most prevalent in causing disease but now, the scenario has shifted to G3P[8] [3, 9]. In a joint study done by RMRC and ICMR Bhubaneswar during the pre-vaccination era, it was observed that before the inception of Rotavac, G1P[8] was the most common genotype (62.2%) circulating in the community [10]. This change or conversion may had come after the introduction of rotavirus vaccine (Rotavac) in the country in April 2016. This may also indicate a cross protection of G9P[11] vaccine, which needs further research and analysis. This changing pattern of genotype distribution suggests the continuous need for rotavirus disease surveillance and vaccine effectiveness studies for adequate management of rotavirus diarrhea in this region.

The absence of differentiation and further comparison of vaccine virus and wild G9 virus could have given us more in depth knowledge. The association between severity of side effects, like diarrhea with particular strains of rotavirus was also not assessed completely. Still, this study had found out the circulating pattern of rotavirus strains in Odisha. The present study was a part of large-scale surveillance of rotavirus across the country monitored by Christian Medical College, Vellore. The use of a single tool and analysis across all the sites could serve as a yardstick for future researches.

### Conclusions

The rotavirus positivity was found to be 29.2% and 6–23 mo age group being the most affected. The disease was mostly prevalent in winter months. The present study had found a change in genotypic distribution with G3P[8] being the prevalent strain. The vaccination status of these children with the identification and differentiation of wild and vaccine virus among them could have given more conclusive statement while considering the age of the participants. Nonetheless, the inception of vaccine did influence the overall frequency as well as distribution in the community. Hence, the use of such vaccine is highly recommended.

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### Authors’ Contributions

VG, NKM, SRN: Study design, manuscript development; MKN, NM: Data acquisition, management and manuscript development; VG, NKM, NM, RK, SRN: Data analysis, literature review and manuscript writing; All authors approved the final manuscript. NKM will act as guarantor of the study.

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| Genotypes | 2016 | 2017 | 2018 | 2019 | Total |
|-----------|------|------|------|------|-------|
| G10P[8]   | –    | 1    | –    | –    | 1 (0.4%) |
| G1P[6]    | 3 (75.0%) | –    | –    | 1 (25.0%) | 4 (1.6%) |
| G1P[8]    | 23 (69.7%) | 9 (27.3%) | 1 (3.0%) | –    | 33 (13.4%) |
| G2P[4]    | 3 (25.0%) | 2 (16.7%) | 2 (16.7%) | 5 (41.7%) | 12 (4.9%) |
| G3P[8]    | 32 (22.1%) | 26 (17.9%) | 56 (38.6%) | 31 (21.4%) | 145 (58.9%) |
| G9P[4]    | 1 (16.7%) | 1 (16.7%) | 1 (16.7%) | 3 (50.0%) | 6 (2.4%) |
| G9P[8]    | –    | –    | 1 (100.0%) | –    | 1 (0.4%) |
| Mixed     | 6 (14.0%) | 8 (18.6%) | 21 (48.8%) | 8 (18.6%) | 43 (17.5%) |
| UTUT      | 1 (100.0%) | –    | –    | –    | 1 (0.4%) |
| Total     | 246   | 246   | 246   | 246   | 246   |
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Compliance with Ethical Standards

Conflict of Interest None.

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