Original Article

# Molecular Characterisation and Clinical Correlates of Rotavirus in Children and Adults in a Tertiary Care Centre, Chennai, South India

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## Abstract

**Aims:** This study was undertaken to determine the rate of detection of rotavirus causing diarrhoea among children and adults, identify the common genotypes circulating and determine clinical correlates. **Settings and Design:** This is a cross-sectional study in a tertiary care centre. **Materials and Methods:** Stool samples were collected from adults and children, transported on ice, aliquoted and stored at − 80°C. Rotavirus antigen detection enzyme-linked immunosorbent assay was performed on all samples. Representative samples were typed by conventional hemi-nested VP7 and VP4 reverse transcription-polymerase chain reaction. **Statistical Analysis Used:** Test of proportion, Student's *t*-test and Chi-square test were used for statistical analysis. **Results:** A total of 444 stool samples were collected and tested over 14 months. Among these, 116 were paediatric with a rate of positivity of 36.21% and 328 were adults with rate of positivity of 20.73%. Among children under 5 years ($n$ = 90), the rate of positivity was 41.11%. Vesikari scale was used for clinical assessment. The mean ± standard deviation Vesikari score in rotavirus-infected children and rotavirus-uninfected children was 11.2 ± 3.2 and 8.9 ± 3.6, respectively, and the difference was statistically significant. Nineteen samples were genotyped in children < 5 years, 94.7% were of G1P[8] and 5.3% were of G9P[4] genotype. Genotyping of 14 adult samples, G1P[8](85.7%) was found as the predominant genotype, two samples (14.3%) were partially typed (G9PUT and G12PUT). **Conclusions:** The rate of positivity of rotavirus in children under 5 years was 41.11%. G1P[8] is the most common strain circulating across all age groups.

**Keywords:** Adults, G12, G1P[8], genotype, rotavirus, Vesikari

## Introduction

Acute gastroenteritis is the second most leading cause of death among under-five children globally.[1] Nearly one in five child deaths i.e., 1.5 million each year is due to diarrhoea and it kills more young children than AIDS, malaria and measles combined.[2] Diarrhoea due to infection is widespread throughout developing countries. Approximately 84% of child deaths due to diarrhoea were observed in Africa (46%) and South Asia (38%). In India, diarrhoeal deaths among children <5 years is 386,600 deaths.[3]

Rotavirus is the most common cause of vaccine-preventable severe diarrhoea.[4] Rotavirus causes an estimated 78,500 deaths, 872,000 hospitalisations and over 3.2 million hospital or clinic visits in children <5 years of age in India.[5] Rotavirus is not only limited to children, but can also infect adults. Very few studies document the burden of rotavirus across all age groups. There is very limited data on rotavirus gastroenteritis among adults. This study was undertaken to determine the rate of detection of rotavirus causing diarrhoea among children and adults. The study generated information on rotavirus genotypes prevalent in children and adults.

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## Materials and Methods

### Study duration and sample collection

Children and adults who were admitted with diarrhoea as a primary complaint were enrolled for the study. The study was conducted from February 2014 to March 2015 in a tertiary care centre in Chennai, during which a total of 1470 hospitalisations were documented with diarrhoea. Of these, 444 stool samples were collected from children and adults. The remaining patient samples could not be collected because of unwillingness on the part of patient to participate in the study or due to hospital-acquired diarrhoea or other reasons. The main objective was to determine the rate of detection of rotavirus in children and adults. No controls were included in the study.

### Criteria for the study

Infants >2 months of age, children and adults, having three or more loose or liquid stools per day (or) more frequently than is normal for the individual were included in the study. Among infants aged 1–2 months, stool samples were collected based on the caregiver's report of a recent change in the consistency or frequency of stools. The exclusion criterion was nosocomial diarrhoea (diarrhoea that occurs after 48 h of admission in hospital).

### Clinical correlation

We assessed the clinical severity using modified Vesikari scale (20-point scale) and a score of 0–5 was defined as mild, 6–10 as moderate and > 11 as severe.[6] A separate clinical pro forma was used to record the rotavirus vaccination history, duration, number of episodes of vomiting and diarrhoea, temperature, dehydration and mode of treatment. The information on stool culture for enteric pathogens (*Salmonella* and *Shigella*) and stool for ova/cyst for parasites was collected, and the details were documented in the pro forma.

### Sample collection

Stool samples were collected in a sterile container, transported in cold chain to the laboratory, divided into aliquots and stored at −80°C deep freezer. The samples were collected within a day or two of admission to exclude hospital-acquired diarrhoea.

### Screening of rotavirus

Stool samples from children and adults were screened for rotavirus antigen using commercially available antigen detection enzyme-linked immunosorbent assay (ELISA), Premier™ Rotaclone® (Meridian Bioscience, Inc., Cincinnati, USA) kit as per protocol. Readings were taken in a spectrophotometer at an absorbance value of 450 nm. The optical density values of the samples >0.15 were considered positive.

### Molecular characterisation

Genotyping was carried out on representative samples which tested positive for rotavirus antigen. The 20% faecal suspension was prepared using minimum essential medium (HiMedia Laboratories, Mumbai, India).

RNA was extracted using QIAmp Viral RNA Mini Kit (Qiagen, Germany). The positive controls for the assay were procured from Wellcome Trust Research Laboratory, Christian Medical College, Vellore. Complementary DNA (cDNA) was generated by reverse transcription in the presence of random primers using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). The cDNA was stored at −20°C until further testing.[7]

cDNA was obtained from the extracted RNA and used as a template for the VP7 first round polymerase chain reaction (PCR) with forward and reverse primers (Sigma Aldrich, USA); the VP7 gene was amplified. The first round amplified products were used as a template for the VP7 second round PCR targeting G genotype and it was detected by including individual primers (VP7R, G1, G2, G3, G4, G8, G9, G10 and G12).

The cDNA was used as a template for VP4 first round, and by using con2 and con3 primers, the VP4 gene was amplified. The first round amplicons were used as template for the VP4 second round PCR, and by using individual primers (con3, P[4], P[6], P[8], P[9], P[10] and P[11]), the respective *P* genotype was amplified.[8,9] The Master Mix comprises 10X PCR buffer, $MgCl_2$, Taq polymerase, deoxynucleotide triphosphates and nuclease-free water. The description of primers and cycling conditions is shown in Tables 1 and 2. Amplicons of both G and *P* types were resolved in 2% agarose gel electrophoresis in Tris acetic acid ethylenediaminetetraacetic acid buffer. Individual genotypes were identified on comparison with their respective base pair lengths by ultraviolet gel documentation system (Bio-Rad, Life Science, India).

### Statistical analysis

The data were analysed based on presentation of acute gastroenteritis in paediatrics and adults. Chi-square test of significance, Student's *t*-test and test of proportion were applied to analyse the severity of disease, and $P < 0.05$ was considered to be statistically significant. The statistical analysis was done using QuickCalcs, version 5 (GraphPad Software Inc., La Jolla, CA, USA).

### Ethics approval

Institutional Ethics Committee approval was obtained for the conduct of the study.

## Results

### Rotavirus infection in paediatrics

A total of 116 stool samples were collected from children aged >28 days to 18 years. The rotavirus ELISA positivity rate was found to be 36.2% ($n$ = 42). Among children, 53 samples were collected from males and 63 from females. The male-to-female ratio was 0.8:1.

We analysed the presentation of diarrhoea due to rotavirus in children under 5 years ($n$ = 90) and found rotavirus positivity rate to be 41.1% ($n$ = 37) [Figure 1]. The male-to-female ratio was 1:0.7. The rotavirus positivity rate was least among 0–5 months and highest among 12–23 months [Figure 2]. The

**Table 1: Primer sequences for VP7 and VP4 semi-nested polymerase chain reaction**

| PCR | Primer | Primer sequences | Product size |
|---|---|---|---|
| VP7 first round | VP7/F | 5' ATG TAT GGT ATT GAA TAT ACC AC 3' | 881 bp |
| | VP7/R | 5' AAC TTG CCA CCA TTT TTT CC 3' | |
| VP7 second round | G1 | 5' CAA GTA CTC AAA TCA ATG ATG G 3' | 618 bp |
| | G2 | 5' CAA TGA TATTAA CAC ATT TTCTGTG 3' | 521 bp |
| | G3 | 5' ACG AAC TCA ACA CGA GAG G 3' | 682 bp |
| | G4 | 5' CGT TTC TGG TGA GGA GTT G 3' | 452 bp |
| | G8 | 5' GTC ACA CCA TTT GTA AAT TCG 3' | 756 bp |
| | G9 | 5' CTT GAT GTG ACT AYA AAT AC 3' | 179 bp |
| | G10 | 5' ATG TCA GAC TAC ARA TAC TGG 3' | 266 bp |
| | G12 | 5' CCG ATG GAC GTA ACG TTG TA 3' | 396 bp |
| | VP7/R | 5' AAC TTG CCA CCA TTT TTT CC 3' | |
| VP4 first round | con3 | 5' TGG CTT CGC CAT TTT ATA GAC A 3' | 876 bp |
| | con2 | 5' ATT TCG GAC CAT TTA TAA CC 3' | |
| VP4 second round | P[4] | 5' CTA TTG TTA GAG GTT AGA GTC 3' | 483 bp |
| | P[6] | 5' TGT TGA TTA GTT GGA TTC AA 3' | 267 bp |
| | P[8] | 5' TCT ACT GGR TTR ACN TGC 3' | 345 bp |
| | P[9] | 5' TGA GAC ATG CAA TTG GAC 3' | 391 bp |
| | P[10] | 5' ATC ATA GTT AGT AGT CGG 3' | 583 bp |
| | P[11] | 5' GTA AAC ATC CAG AAT GTG 3' | 312 bp |
| | con3 | 5' TGG CTT CGC CAT TTT ATA GAC A 3' | |

PCR: Polymerase chain reaction

**Table 2: Cycling conditions of VP7 and VP4 semi-nested polymerase chain reaction**

| PCR | PCR amplification cycling condition |
|---|---|
| VP7 first round | Initial denaturation 94°C - 2 min<br>35 cycles of 94°C - 1 min, 52°C - 1 min, 72°C - 1 min<br>Final extension 72°C - 7 min |
| VP7 second round | Initial denaturation 94°C - 4 min<br>30 cycles of 94°C - 1 min, 42°C - 2 min, 72°C - 1 min<br>Final extension 72°C - 7 min |
| VP4 first round | Initial denaturation 94°C - 2 min<br>35 cycles of 94°C - 1 min, 50°C - 1 min, 72°C - 1 min<br>Final extension 72°C - 7 min |
| VP4 second round | Initial denaturation 94°C - 2 min<br>30 cycles of 94°C - 1 min, 45°C - 2 min, 72°C - 1 min<br>Final extension 72°C - 7 min |

PCR: Polymerase chain reaction

distribution of rotavirus-infected and rotavirus-uninfected children (0–5 years) by age, gender, area of residence and duration of hospital stay is shown in Table 3.

In our study, a variation in sex-wise distribution was observed, male children (67.6%) were affected more commonly than female children (32.4%), this was not statistically significant ($P = 0.2$).

In only two under 5 years' age children, a history of vaccination for rotavirus was elicited from the parents. None of the other 88 children received rotavirus vaccination.

In older children, >5–18 years of age ($n = 26$), the rotavirus positivity rate was found to be 19.2% ($n = 5$). In older children, the male-to-female ratio was 0.6:1.

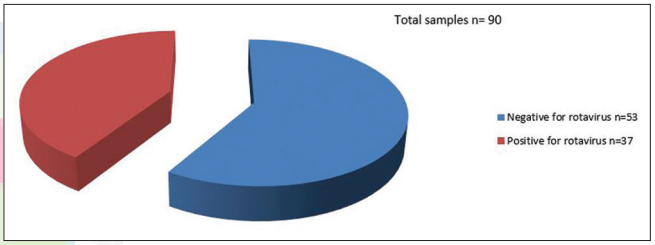


**Figure 1:** Proportion of rotavirus positives in hospitalised children under the age of 5 years.

## Clinical correlation by Vesikari scale

Modified Vesikari Clinical Severity Scoring Scale was applied to all children under 5 years and was analysed in whom data was available ($n = 72$). The classification of Vesikari scale among rotavirus-infected and rotavirus-uninfected children is shown in Figure 3. The mean ± standard deviation of Vesikari score in children tested positive and negative for rotavirus was 11.2 ± 3.2 and 8.9 ± 3.6, respectively. On application of Student's *t*-test, the severity of disease was found to be significantly higher among rotavirus-infected children when compared to rotavirus-uninfected children ($P = 0.01$). The clinical presentation and severity of acute gastroenteritis among rotavirus-infected and rotavirus-uninfected children and its statistical significance are shown in Table 4.

## Genotyping in children under 5 years of age

Genotyping was performed in 19 representative samples from children under 5 years of age. Among those samples, 94.7% ($n = 18$) were G1P[8] and 5.3% ($n = 1$) belong to G9P[4] genotype [Figure 4a]. G1P[8] is the predominant genotype circulating among children under 5 years of

age. The agarose gel photographs of amplified reverse transcription-PCR (RT-PCR) products of 'G' and 'P' types are shown in Figure 5a and b.

## Rotavirus infection in adults

Among the 328 stool samples tested from patients above 18 years of age, the rotavirus positivity rate was found to be 20.7% ($n$ = 68). A total of 163 stool samples were collected from males and 165 from females. The male-to-female ratio was 0.99:1. Rotavirus was detected in both adult males and females almost equally and no gender predomination was seen.

In the young adults group aged 19–40 years ($n$ = 132), rotavirus positivity rate was 18.2% ($n$ = 24). In the older adults group aged 41–65 years ($n$ = 160), rotavirus positivity rate was 23.1% ($n$ = 37).

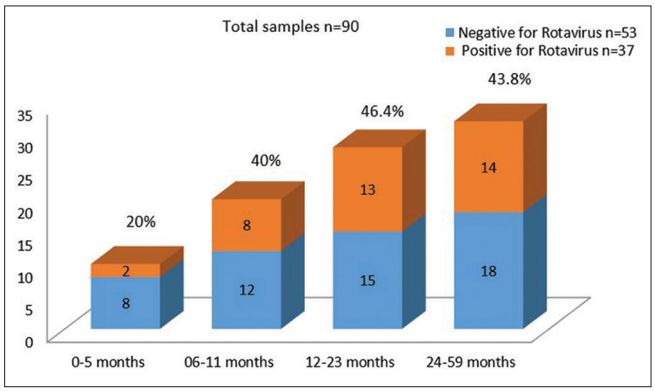


**Figure 2:** Age-wise distribution of rotavirus positivity among children under the age of 5 years.

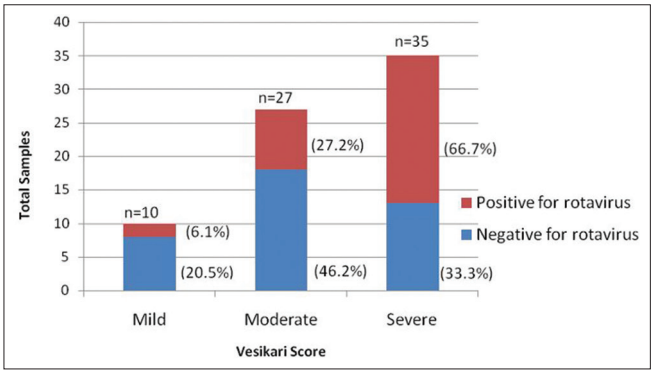


**Figure 3:** Comparison of clinical severity in children under the age of 5 years in rotavirus-positive and rotavirus-negative cases using modified Vesikari score.

**Table 3: Distribution of rotavirus-infected and rotavirus-uninfected children (0-5 years) by age, gender, area of residence and duration of hospital stay**

| Parameters | Rotavirus-infected children ($n$=37) (%) | Rotavirus-uninfected children ($n$=53) (%) | $P$ |
|---|---|---|---|
| Age, months (mean±SD) | 18.2±14.3 | 18.6±15.6 | 0.7[a] |
| Age (months) | | | |
| 0-5 | 2 (5.4) | 8 (15.1) | |
| 6-11 | 8 (21.6) | 12 (22.6) | |
| 12-23 | 13 (35.1) | 15 (28.3) | |
| 24-59 | 14 (37.9) | 18 (34) | |
| Gender | | | |
| Male | 25 (67.6) | 28 (52.8) | 0.2[b] |
| Female | 12 (32.4) | 25 (47.2) | |
| Area of residence | | | |
| Urban | 31 (83.8) | 38 (71.7) | 0.2[b] |
| Rural | 6 (16.2) | 15 (28.3) | |
| Hospital stay (mean±SD), days | 3.2±2.6 | 2.6±1.5 | 0.2[a] |

[a]$P$ value calculated using student $t$-test, [b]$P$ value calculated using Chi-square test. SD: Standard deviation

**Table 4: Clinical presentation and severity of acute gastroenteritis among rotavirus-infected and rotavirus-uninfected children**

| Parameters | Rotavirus-infected children ($n$=33) (%) | Rotavirus-uninfected children ($n$=39) (%) | $P$ |
|---|---|---|---|
| Vomiting | 24 (72.7) | 22 (56.4) | 0.15[a] |
| Vomiting >5 episodes/day | 14 (42.4) | 2 (6.1) | <0.01[a] |
| Dehydration | 23 (69.7) | 16 (41) | 0.02[a] |
| Vesikari score (mean±SD) | 11.2±3.2 | 8.9±3.6 | 0.01[b] |
| Disease severity by Vesikari score | | | |
| Mild | 2 | 8 | <0.01[c] |
| Moderate | 9 | 18 | |
| Severe | 22 | 13 | |

[a]$P$ value calculated using test of proportion, [b]$P$ value calculated using Student's $t$-test, [c]$P$ value calculated using Chi-square test. Clinical data were incomplete in 4 rotavirus-infected and 14 rotavirus-uninfected children, hence they were not included in clinical assessment. SD: Standard deviation

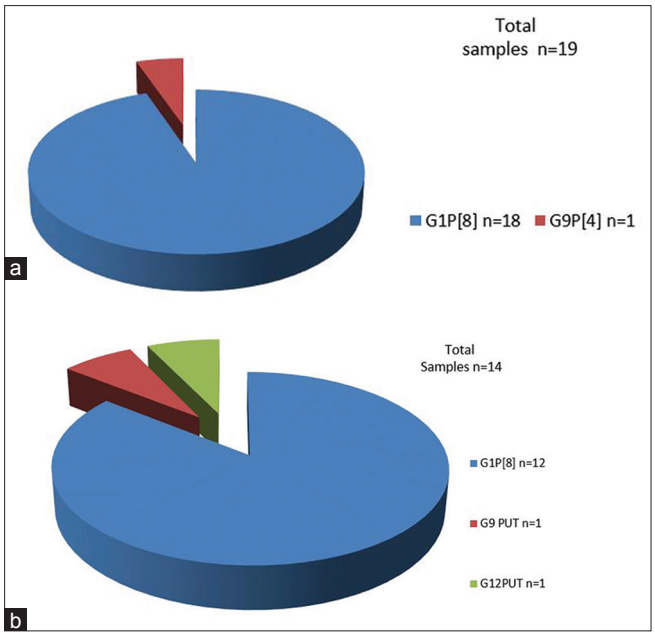


**Figure 4:** (a) Rotavirus genotypes among hospitalised children under the age of 5 years. (b) Rotavirus genotypes among hospitalised adults.

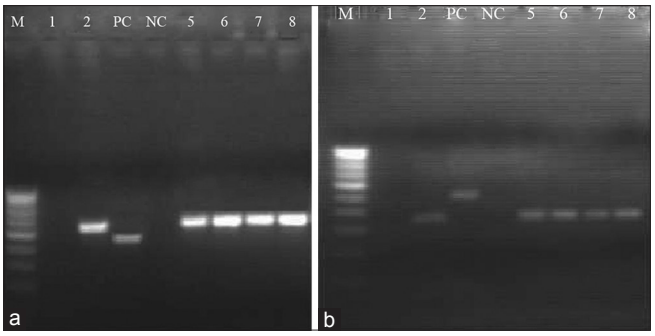


**Figure 5:** (a) Agarose gel photograph of amplified reverse transcription-polymerase chain reaction products of the G type. Lane M (100 bp molecular weight ladder), Lane 1 negative sample, Lanes 2, 5, 6, 7 and 8 G1, negative control and G2, positive control. (b) Agarose gel photograph of amplified reverse transcription-polymerase chain reaction products of the P type. Lane M (100 bp molecular weight ladder), Lane 1 negative sample, Lanes 2, 5, 6, 7 and 8 P[8], negative control and P[4], positive control. NC: Negative control, PC: Positive control.

Among the geriatric population, >65 years of age ($n$ = 36) rotavirus positivity rate was 19.4% ($n$ = 7). Age distribution of study participants is shown in Table 5.

**Table 5: Age-wise distribution, mean and standard deviation of the entire study participants**

| Age (years) | Rotavirus-infected children and adults ($n$=110) | | Rotavirus-uninfected children and adults ($n$=334) | |
|---|---|---|---|---|
| | $n$ (%) | Mean±SD | $n$ (%) | Mean±SD |
| 0-5 | 37 (33.6) | 18.2±14.3 | 53 (15.9) | 18.6±15.6 |
| 6-18 | 5 (4.5) | 11.4±4.3 | 21 (6.3) | 11.4±3.2 |
| 19-40 | 24 (21.9) | 28.9±6.0 | 108 (32.3) | 28.7±5.8 |
| 41-65 | 37 (33.6) | 51.4±5.4 | 123 (36.8) | 54.9±7.1 |
| >65 | 7 (6.4) | 74.9±9.0 | 29 (8.7) | 71.9±5.1 |

The mean + S.D was calculated in months for 0-5 years' age group. SD: Standard deviation

## Genotyping in adults

Genotyping was performed in 14 representative samples. These samples were previously tested positive for rotavirus antigen. Among those samples, 85.7% ($n$ = 12) were of G1P[8] genotype. G1P[8] is the predominant genotype circulating in adults. Two samples (14.3%) were partially typed and found to be G9 UT and G12 UT [Figure 4b].

## Seasonality of rotavirus infection

We analysed the occurrence of acute gastroenteritis and rate of positivity throughout the study period month wise. Rotavirus was detected with a similar detection rate throughout the year and there was no seasonality in the detection of rotavirus [Figure 6].

## Data on other enteric pathogens

Data on stool culture for enteric pathogens (*Salmonella* and *Shigella*) and stool for ova/cyst for parasites were available in 174 stool samples. Among these, the stool samples of six patients grew *Salmonella* spp. ($n$ = 4)/*Shigella* spp. ($n$ = 2). Cysts of *Entamoeba histolytica* ($n$ = 3), *Entamoeba coli* ($n$ = 2), *Giardia* spp. ($n$ = 1), ova of hookworm ($n$ = 1) and larva of *Strongyloides stercoralis* ($n$ = 1) were identified. Rotavirus co-infection was observed in four patients with *Salmonella* spp. ($n$ = 1), *Shigella* spp. ($n$ = 1), *E. histolytica* ($n$ = 1) and hookworm ($n$ = 1).

# Discussion

Rotaviruses are the leading cause of diarrhoeal disease worldwide and account for about 114 million cases.[10] India shares the highest burden of rotavirus infections among all countries.[11] The rotavirus positivity rate among children under 5 years of age was 41.1%. Detection rates of 26% in Vellore and 50% in Trichy have been reported previously.[12] Varying rates of positivity have been reported in recent multicentric studies ranging from 26.4% to 53.4%.[13,14] The Indian rotavirus surveillance (2005–2009) mean detection rate in South India was 44% and the overall Indian detection rate was 40%.[15] The worldwide rotavirus surveillance from over 100 countries had a median detection rate of 36%.[16] The rotavirus positivity rate among <6-month-old children was 20%. Previous studies have observed similar detection rates of 13.3%[17] and 18.8%.[13] Certain studies have documented gender predisposition in that male children are found to be infected with rotavirus more commonly than female children.[18] A similar finding was observed in our study but was not statistically significant. However, there are several reports that suggest no gender predisposition.[13] On application of modified Vesikari scale, the severity of acute gastroenteritis was higher among rotavirus-infected children when compared to rotavirus-uninfected children and was statistically significant.

In our study, we identified G1P[8] as the predominant genotype (94.7%) followed by G9P[4] (5.3%). The

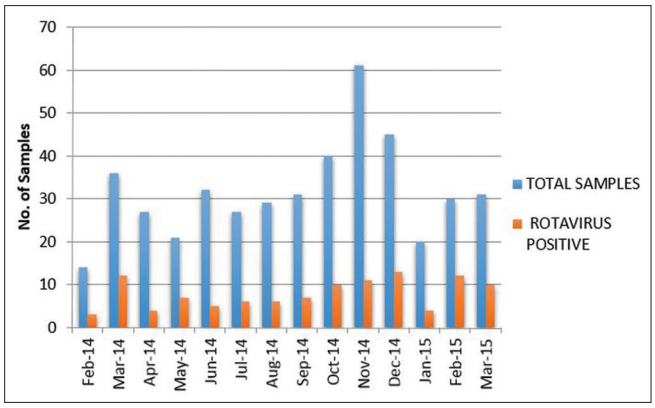


**Figure 6:** Distribution of stool samples from clinical suspect of rotavirus and its positivity by month.

most frequently observed rotavirus genotypes in global surveillance (2009–2012) were G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8].[16] Indian rotavirus surveillance have documented that G2P[4] and G1P[8] are the most common strains which constitutes around 50%. G12P[6], G12P[4] and G12P[8] were found to circulate in low level (7%).[17] In a recent multicentric study (2011–2012), G1P[8] strains predominated followed by G2P[4] and G9P[4].[13]

Rotavirus had varying detection rates in adults across different parts of the world as follows: In the USA, 2.2%; the United Kingdom, 4%; Nepal, 7%; China, 9% and Japan, 14%.[19-23] There is very limited data regarding rotavirus infection in adults in India. In an earlier study from South India, the rotavirus positivity rate was 3.8%.[24] In a previous study from West India, Group A rotavirus frequency of detection was found to decline from 18% in 2008 to 3.8% in 2012.[25] In our study, the rotavirus positivity rate among adults was found to be 20.7%. This is higher than previously reported studies from India. Conventional VP6 RT-PCR may be required to confirm the diagnosis for all the samples that tested positive by antigen detection ELISA.

We documented G1P[8] (85.7%) as the predominant strain circulating among adults. We also encountered partially typed strains, G9 PUT and G12 PUT. G1P[8] (47.8%) was the predominant strain in Vellore whereas G2P[4] (50%) was predominant among adolescents and adults in Pune. Few untypeable, partially typed and mixed genotypes were also observed.[24-26] G12 strains were first identified in India in 2003, and since then, they are found to circulate in low level among children under 5 years of age. Our study has detected the emergence of G12 strains in adults from India. However, G12 strains in adults have been identified earlier in Nepal,[21] the USA[19] and Japan.[27]

## Conclusion

The rate of positivity of rotavirus in children under 5 years was 41.11%. G1P[8] is the most common strain circulating across all age groups. Rotavirus is encountered throughout the year and therefore, public health programmes and preventive measures must take place throughout the year.

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## Conflicts of interest

There are no conflicts of interest.

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