Original Research Article

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Prevalence and Molecular characterization of the Human Rotavirus strains detected in children suffering from acute gastroenteritis at Wardha

Nilesh Shyam Chavan[1]*, Dr. Nilima Tankhiwale[2]

[1]PhD in Medical Microbiology, J. N. Medical College, Wardha (Sawangi)
[2]Professor & Head, Department of Microbiology, J. N. Medical College, Wardha (Sawangi)
*Corresponding author: *nilesh.chavan005@gmail.com*

Abstract

Introduction: Human rotavirus infection remains a significant clinical problem through the world, infecting almost every child below 5 year age. **Material and Method:** Clinical presentations of acute gastroenteritis cases during the study period were analyzed. Rotavirus diarrhea was confirmed with ELISA. ELISA rotavirus positive samples were tested for molecular characterization (G & P typing), done by conventional polymerase chain reaction. **Results:** Between July 2010 and May 2013, among the 341 enrolled children below 5 years, a total of 40 were positive for rotavirus by the ELISA test. The prevalence of rotavirus diarrhoea 11.7%.The most common genotype wereG1 (57.5) followed by G2 (20%), G3 (10%), G4 (2.5%) and G9 (2.5%).The P type identified were P[4] (27.5%), P[8](47.5%), P [6](20%). The most common age of rotavirus diarrhea was 12-17 months. The predominant symptoms were watery diarrhea, fever, and vomiting. **Conclusion:** The prevalence of rotavirus diarrhea among children less than 5 years of age was   11.7%. and circulating stains of rotavirus was G1, G2,G3,G4 & G9.

Keywords: Rotavirus, Diarrhoea, Acute gastroenteritis.

Introduction

Diarrhoeal diseases account for approximately 17% of the 10.4 million deaths among children aged below 5 years globally [1]. Among children less than 5 years of age, rotavirus infection is the leading cause of moderate to severe acute gastroenteritis ,with account of 5-71% prevalence in various part of India.[2]48% of children experienced at least one episode of rotaviraldiarrhoea by age 3.[10]

Rotavirus is currently has 27 G type and 35 p type on based on the variability in the genes encoding VP7 and VP4 outer capsid proteins.[3]The most common G type reported from India are G1,G2, G3, G4, G8, G9, G10,G12 and P type are P(4) and P(8) P(6) ,P(9).P(10),P(11).[4]

The incidence and distribution of G and P genotype that cause disease in humans may vary by geographical location and by year. The major human G type are G1,G2,G3,G4and G9 which combined with the P type P[8],P [4] and P[6] ,account for 805 of rotavirus associated gastroenteritis episodes worldwide (Gentsch et al. 2005. Santos et al 2005).

Thus the aim of this study was to estimate the prevalence of rotavirus diarrhoea among children less than 5 years of age and to determine the circulating stains of rotavirus in and around Wardha.

Materials and Methods

Between July2010 and May 2013, 341 stool samples were collected from children below 5 years age presenting with acute gastroenteritis at Acharya Vinoba Bhave, hospital, Wardha.

Fresh Stool sample is collected within 24 hours of admission to hospital in a clean sterile bottle. Stool samples were examined macroscopically and microscopically and processed for bacteriological and parasitic infection. Rotavirus antigen was detected from the stool sample using commercially available ELISA kit from IVD Research INC, Caslsbad, CA USA. All ELISA positive samples were subjected for genotyping by using conventional PCR (Table.1).

Viral RNA was extracted from stool sample using Trizol reagent (Invitrogen Life Technologies).

Table: 1 Primers which are used for Genotyping

Primers(Polarity)	Type	Position(nt)	Sequence(5'-3)'	Size of amplicon (bp)
9con1(+)	VP7	37-56	TAGCTCCTTTTAATGTATGG	
9con2(-)	VP7	922-941	GTATAAAATACTTGCCACCA	905
9T-1(-)	G1	176-195	TCTTGTCAAAGCAAATAATG	159
9T-2(-)	G2	262-281	GTTAGAAATGATTCTCAACT	245
9T-3(-)	G3	484-503	GTCCAGTTGCAGTGTTAG	467
9T-4(-)	G4	423-440	GGGTCGATGGAAATTCT	404
9T-9B(-)	G9	131-147	TATAAAGTCCATTGCAC	111
G12R(-)	G12	471-490	AGTACAGTACCAAATTTCAT	454
Con3(+)	VP4	11-32	TGGCTTCGCTCATTTATAGAC	
Con2(-)	VP4	868-887	ATTTCGGACCATTTATAACC	877
1T-1	P8	339-356	TCTACTTGGATAACGTGC	346
2T-1	P4	474-494	CTATGGTTAGAGGTTAGAGTC	484
3T-1	P6	259-278	TGTTGATTAGTTGGATTCAA	268
ND-2	P11	116-133	AGCGAACTCTCACCAATCTG	123

Results

Between July 2010 and May 2013, among the 341 enrolled children below 5 years, a total of 40 were positive for rotavirus by the ELISA test. The prevalence of rotavirus diarrhoea 11.7%. The most common genotype were G1 (57.5) followed by G2 (20%), G3 (10%), G4 (2.5%) and G9 (2.5%). The P type identified were P [4] (27.5%), P[8](47.5%), P [6] (20%). Two samples found untype (Tables 1, 2). The most common age of rotavirus diarrhea was 12-17 months. The predominant symptoms were watery diarrhea, fever, and vomiting.

Table: 2 G& P genotype of rotavirus detected in stool samples from Children having Age.

|	P4	P6	P8
G1	07	06	10
G2	01	00	05
G3	02	00	02
G4	00	00	01
G1 G2 (Mixed)	00	02	03
G9	01	00	00
Untype	02		

Table :3 Monthly distribution of number of cases of age with no of positive rotavirus cases

Sr.No	Month and Year	No. of Diarrhoea Cases	No of cases positive for Rotavirus
1.	July 2010 to Dec 2010	07	02
2.	Jan 2011 to July 2011	35	04
3.	August 2011 to Dec 2011	39	04
4.	Jan 2012 to July 2012	133	16
5.	August 2012 to Dec 2012	55	03
6.	Jan 2013 to July 2013	72	11

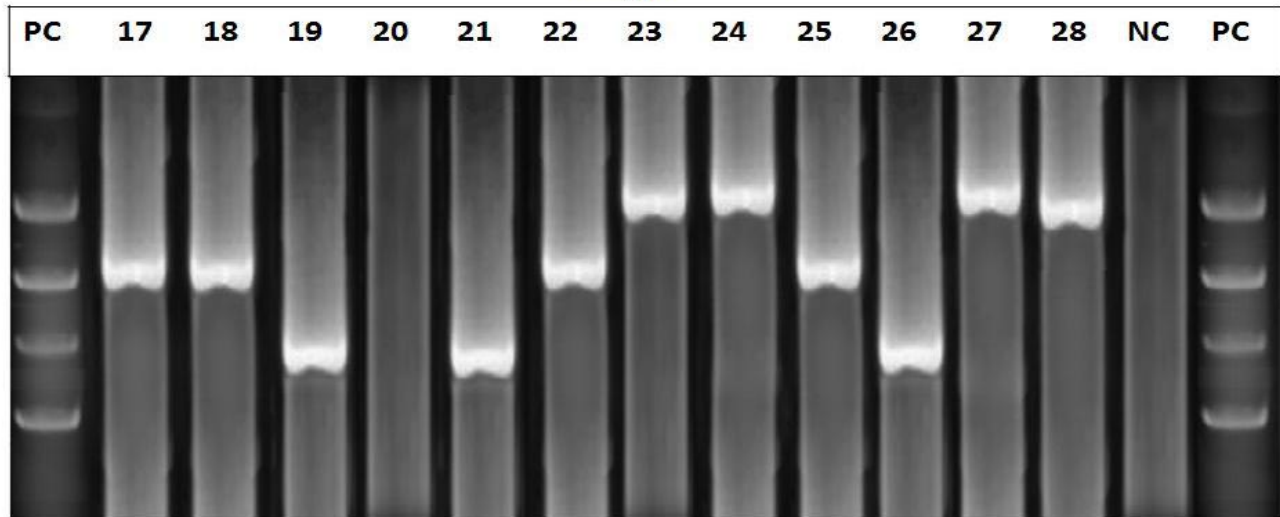**Figure: 1 A B C D shows the agarose gel picture of the common G and P type.**
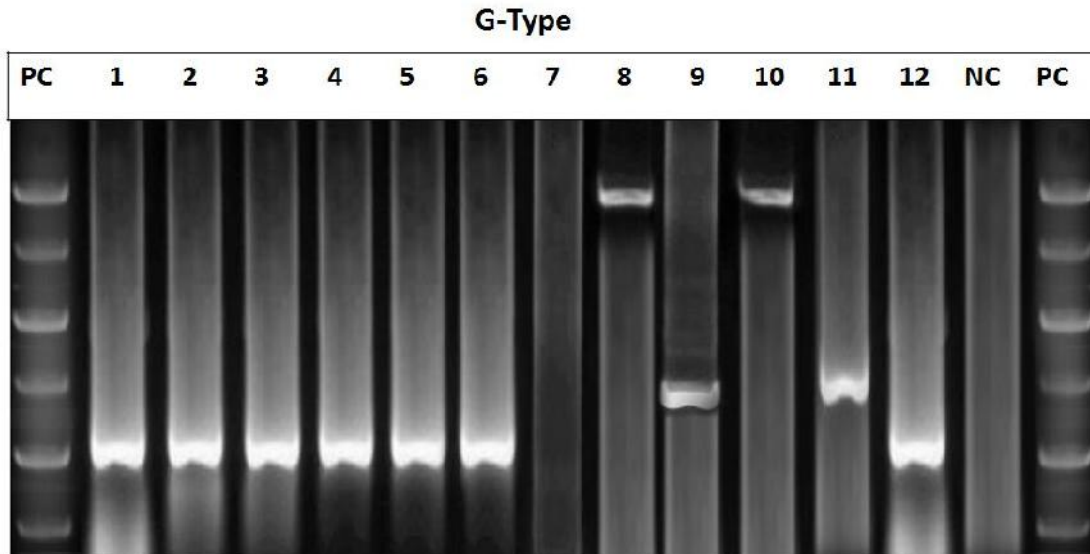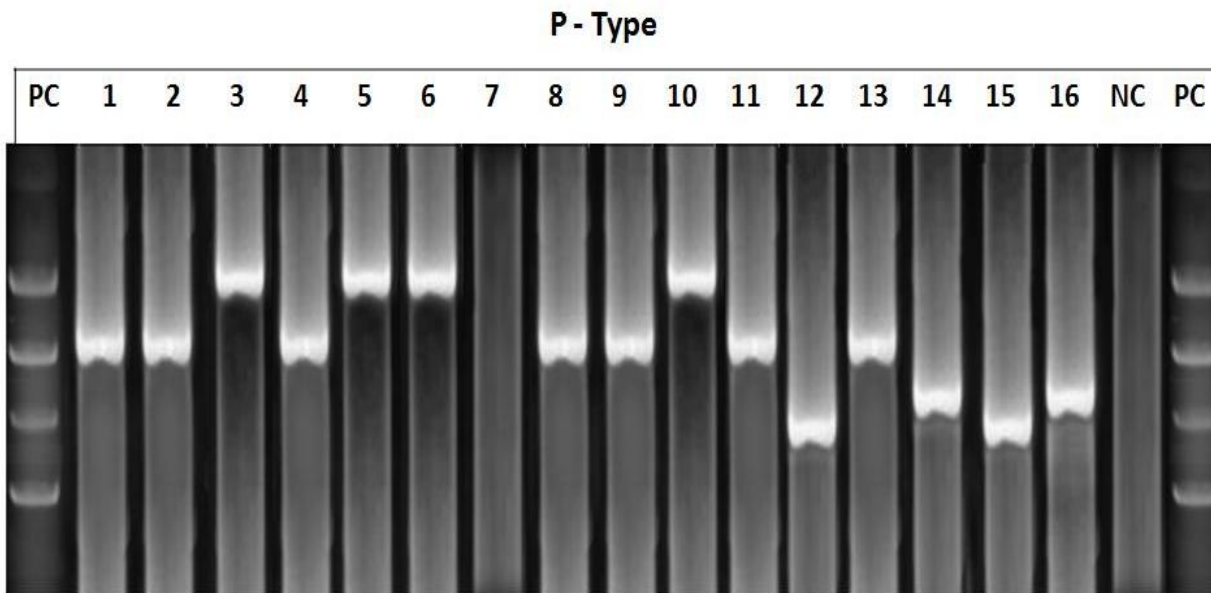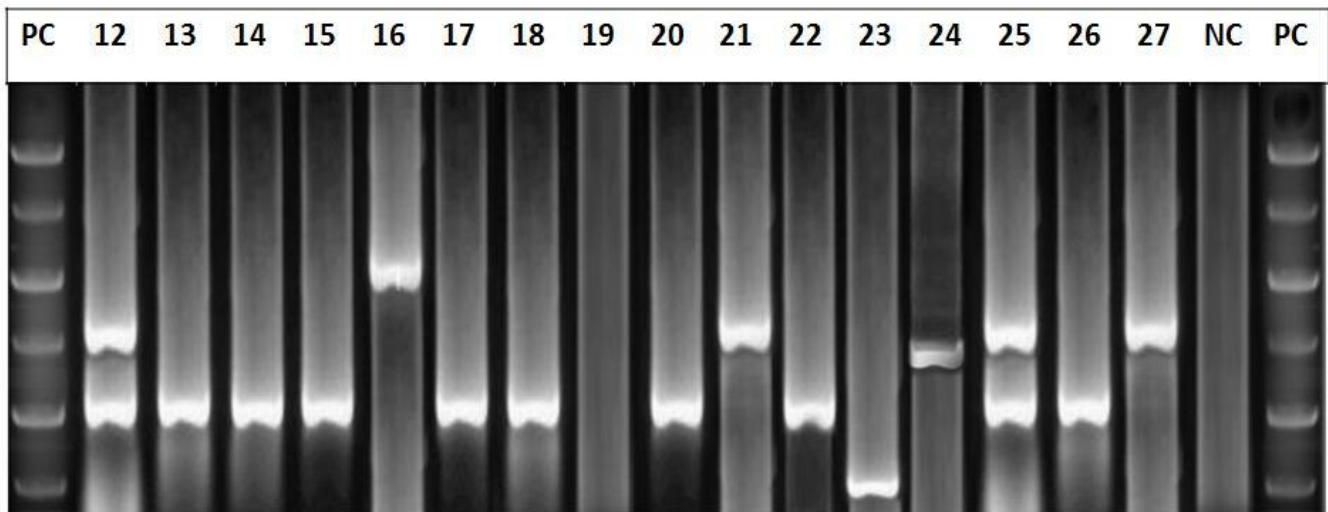

G-Type

PC- Positive control and NC –Negative control

P - Type

PC- Positive control and NC –Negative control

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P-Type

PC- Positive control and NC –Negative control

G-Type

PC- Positive control and NC –Negative control

Discussion

The prevalence (22.55%) of human rotavirus was recorded among children with acute diarrhea in Chennai.[5] Similarly in Vellore, only 18% prevalence was detected. 11.2 % in Mysore[6.]The result of present study indicates the human rotavirus are circulating among infants/children of Wardha, India.

In our study showed, genotype were G1 (57.5) followed by G2 (20%), G3 (10%), G4 (2.5%) and G9 (2.5%).Chakravarti et al 2010 found similar result of genotype. They observed G1 (60%), followed by G2 (16%) ,G3 (3%) and G9 (8%).

About the P type identified in our study were P[4] (27.5%), P[8](47.5%), P [6](20%). This findings are in concordance with Chakravarti et al . They showed P [8] (40%) followed by P[4] (26%) and P(6) [17%].

In most of studies from India the most common G types reported were G1, G2 and P type were P [4] and P [8]. A significant number of children also had mixed rotavirus infections.[11]

The present study 12.5 % of specimens showed mixed infections and 5% of the total samples remained untypeable.

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