



Intragenotypic diversity in the VP4 encoding genes of rotavirus strains circulating in adolescent and adult cases of acute gastroenteritis in Pune, Western India: 1993 to 1996 and 2004 to 2007

Vaishali S. Tatte and D. Shobha D. Chitambar *

Enteric Viruses Group, National Institute of Virology, Pune, India.

Abstract

Genetic variability of rotaviruses circulating in Pune, India at the two time points was determined by characterizing VP4 genes in 131 rotavirus strains detected in adolescent and adult cases of acute gastroenteritis. The multiplex RT-PCR classified the VP4 genes in 73 P[4] (43.2%), 69P[8] (40.8%) and 27 P[6] (16.0%) genotypes. Sequencing and phylogenetic analysis revealed increase in the prevalence of P[4]-5 and P[8]-2 and decline of P[8]-3 lineages in 2000s as compared to those identified in 1990s (92.8% Vs 100%, 4.2% Vs 33.3% and 93.7% Vs 66.7%, respectively). The P[4]-1 and P[8]-4 lineages circulated at low levels (7.1% / 2.1%) while presence of only P[6]-1 (100%) lineage was detected at both time. The strains with different VP4 genotype specificities displayed 0.2 to 2.3% amino acid divergence. A significant difference ($P < 0.01$) in their association with common and nontypeable G strains was noted between the two time points studied. This is the first report to describe the intragenotypic diversity in the rotavirus VP4 genes from adolescent and adult patients of acute gastroenteritis from India. VP4 being one of the major protective antigens, monitoring the mutations in this protein would be crucial to understand the evolutionary changes in rotaviruses and devise more effective vaccine strategies in developing countries.

Keywords: Group A rotavirus, VP4 genotypes, G and P typing, genetic diversity.

INTRODUCTION

Rotaviruses are well-recognized causative agents of severe gastroenteritis among infants and children, worldwide (Parashar et al., 2006). Taxonomically, these viruses belong to the family Reoviridae whereas in morphological analysis they appear as icosahedral particles consisting of 11 segments of double stranded (ds) RNA encased within a triple layered capsid composed of VP4, VP6, VP7 and VP2 proteins. The outermost layer has two proteins: the spike protein VP4 (P-protease sensitive) and the coat protein VP7 (G-glycoprotein) (Estes, 2001). Based on these proteins 27 G and 35 P types have been identified globally (Matthijnssens et al., 2008; Matthijnssens et al., 2011).

More than sixty different G-P combinations have been found in the rotavirus strains circulating worldwide. Of

these 12G and 15P genotypes have been detected in humans (Patel et al., 2011). The most commonly found G and P types are G1, G2, G3, G4, G9, P[4] and P[8], respectively (Santos and Hoshino, 2005; Matthijnssens et al., 2008). Of these most of the VP7 genotypes but only one VP4 genotype have been included in currently licensed rotavirus vaccines. It is expected that the implementation of the vaccination programs reduce significantly the hospitalizations and mortality related to rotavirus infection. However, existence of vast variations in the rotavirus strains and genotypes causing gastrointestinal infections is evident from the rotavirus surveillance studies conducted worldwide including India (Santos and Hoshino, 2005; Kang et al., 2009; Tatte et al., 2010a; Pietsch et al., 2011). Intragenotypic diversity

in VP7, NSP4 and VP6 encoding genes of such strains has been also reported from India (Arora et al., 2009; Tatte et al., 2010b). However, variations within VP4 genotypes circulating in the Indian population have been rarely reported (Samajdar et al., 2008).

A recent molecular epidemiology study carried out in adolescent and adults has reported an increase of mixed infections and increasing trend of genetic reassortment among rotavirus strains (Tatte et al., 2010a). Since adults frequently infected with rotavirus could act as reservoir of infection in early ages, more studies on the diversity within the rotavirus genotypes are necessary. The present study describes the assessment of temporal variations in the VP4 encoding genes of rotavirus strains recovered from adolescents and adults.

MATERIALS AND METHODS

Specimens

In earlier studies conducted at the two time points (1993 to 1996 and 2004 to 2007) in Pune, India for rotavirus surveillance in adolescent (9 to 18 years) and adult (>18 years) cases of acute gastroenteritis, a total of 131 fecal specimens were detected positive for group A rotavirus by ELISA (n = 118) (Tatte et al., 2010a) and RNA PAGE (n = 13). These consisted 26 (18/8) and 105(66/39) specimens respectively from adolescents and adults from the years, 1993 to 1996 (n = 84) and 2004 to 2007 (n = 47). Typing of rotavirus VP7 genes by multiplex PCR showed multiple G genotypes in the rotavirus strains. In the present study VP4 genes of these strains were scrutinized to classify them into lineages and sublineages. These also included VP4 genes from rotavirus strains (n = 31) detected in mixed infections reported earlier (Tatte et al., 2010a).

RNA extraction, RT-PCR and genotyping

Rotavirus ds RNA was extracted from 10% stool suspension prepared in phosphate buffered saline containing calcium chloride (PBS-CaCl₂) of pH 7.2, using Trizol® LS reagent (Invitrogen, Carlsbad, CA, USA), as per the manufacturer's protocol. The VP4 genes of rotavirus strains were subjected to RT-PCR followed by multiplex PCR for genotyping according to the method described earlier (Gouvea et al., 1990; Gentsch et al., 1992; Ituriza-Gomara et al., 2004) using a modified thermal cycling program (Chitambar et al., 2008). All of the PCR products, were analyzed by electrophoresis using 1X Tris Acetate EDTA (TAE) buffer, pH 8.3 on 2% agarose gels, containing ethidium bromide (0.5 ug/ml) and visualized under UV transilluminator.

Nucleotide sequencing and phylogenetic analysis

The multiplex PCR products obtained for different VP4 genotypes (P[8] – 345 bp, P[4] – 483 bp and P[6] – 267 bp) were purified on minicolumns (QIAquick, Qiagen, Hilden, Germany) and sequencing was carried out using ABI-PRISM Big Dye Terminator Cycle Sequencing kit v3.1 (Applied Biosystems, Foster city, CA) and a ABI-PRISM 310 Genetic Analyzer (Applied Biosystems Foster city, CA). The sequences were aligned with the corresponding sequences of rotavirus strains available in the GenBank by using Clustal W (Thompson et al., 1994). The phylogenetic analysis was carried out in MEGA 4 by using Kimura –2 parameter and

neighbour-joining method (Tamura et al., 2007). The reliability of different phylogenetic groupings was confirmed by using bootstrap test (1000 bootstrap replications) available in MEGA 4.

Accession numbers

Nucleotide sequences of the VP4 genes of this study have been deposited in the GenBank sequence under the accession numbers HQ260459-HQ260577 and FJ6323188-FJ623237.

Statistical analysis

The proportions across two different periods were compared using the Chi-square test with Yates's correction. P-values less than 0.05 were considered statistically significant.

RESULTS

P-typing of rotavirus strains

In a multiplex RT-PCR performed for amplification of VP4 genes of 131 rotavirus strains, a total of 169 amplicons were obtained indicating 74 single and 95 mixed rotavirus infections. Based on the electrophoretic mobilities of the PCR products obtained in multiplex PCR, VP4 genes were classified into 73 P[4] (43.2%), 69 P[8] (40.8%) and 27 P[6] (16.0%) genotypes. All of these VP4 genotypes were found to be in combination with different G genotypes (Table 1).

Nucleotide sequencing

All of the multiplex PCR products were sequenced to confirm the presence of P[4] / P[8] / P[6] genotypes of VP4 in single or mixed infections. The sequences were compared with those available in GenBank.

Phylogenetic analysis of P[4] strains

Phylogenetic analysis of the 73 P[4] strains recovered from 10 adolescents and 63 adults at two time points 1993 to 1996 (n = 41) and 2004 to 2007 (n = 32) showed clustering of the strains in two distinct lineages P[4]-5 and P[4]-1 of the five lineages known to date for this genotype (Figure 1).

All of the 70 strains placed in P[4]-5 lineage showed 95.3 to 99.5% / 94.8 to 100% and 95.9 to 100% / 96.1 to 100% nucleotide / amino acid identities respectively with the reference strains-TB-Chen, RMC/G66, 107EIB, Py04ASR42, Py05ASR60, KO-2, VN594, SC-185 and TW569 and within themselves (Figure 1). These strains were predominantly associated with mixed infections (n = 24, 34.3%) followed by G2 strains (n = 21, 30.0%), G non-typeable strains (n = 18, 25.7%) and unusual G types [G1 (n = 3, 4.3%), G3 (n = 1, 1.4%), G4 (n = 1, 1.4%) and G9 (n = 2, 2.9%)]. All three strains classified

Table 1. Distribution of rotavirus VP4 genotype combinations in different G types identified in 1993 to 1996 and 2004 to 2007.

G-P Types (%)	P[4]		P[8]		P[6]	
	No. positive (%)					
	1993-1996	2004-2007	1993-1996	2004-2007	1993-1996	2004-2007
Common n = 47 (27.8)	n =17(40.5)	n = 4 (12.9.0)	n = 25(52.1)	n = 1(4.8)	n = 0(0.0)	n = 0(0.0)
G1 (38.3)	0(0.0)	0(0.0)	17(35.4)	1(4.8)	0(0.0)	0(0.0)
G2 (44.7)	17(40.5)	4(12.9)	0(0.0)	0(0)	0(0.0)	0(0.0)
G3 (10.6)	0(0.0)	0(0.0)	5(10.4)	0(0)	0(0.0)	0(0.0)
G4 (6.4)	0(0.0)	0(0.0)	3(6.3)	0(0)	0(0.0)	0(0.0)
Unusual n = 12(7.1)	n = 0 (0.0)	n = 7(22.6)	n = 1(2.1)	n = 0(0.0)	n = 4(16.7)	n = 0(0)
G1 (25.0)	0(0.0)	3(9.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
G2 (8.3)	0(0.0)	0(0.0)	1(2.1)	0(0.0)	0(0.0)	0(0.0)
G3 (8.3)	0(0.0)	1(3.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
G4 (8.3)	0(0.0)	1(3.2)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
G9 (50.0)	0(0.0)	2(6.5)	0(0.0)	0(0.0)	4(16.7)	0(0.0)
Mixed n = 67(39.6)	n =21(50.0)	n = 6(19.4)	n =17(35.4)	n = 5(23.8)	n =17(70.8)	n =1(33.3)
G1 (31.3)	6(14.3)	1(3.2)	7(14.6)	1(4.8)	6(25.0)	0(0.0)
G1, G2 (13.4)	3(7.1)	1(3.2)	2(4.2)	1(4.8)	2(8.3)	0(0.0)
G1 G4 (1.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(4.2)	0(0.0)
G2 (5.9)	1(2.4)	1(3.2)	0(0.0)	1(4.8)	1(4.2)	0(0.0)
G2, G4 (4.4)	1(2.4)	1(3.2)	0(0.0)	1 4.8)	0(0.0)	0(0.0)
G2, G3 (4.4)	1(2.4)	0(0.0)	1(2.1)	0(0.0)	1(4.2)	0(0.0)
G3 (4.4)	1(2.4)	0(0.0)	1(2.1)	0(0.0)	1(4.2)	0(0.0)
G4 (14.9)	4(9.5)	0(0.0)	4(8.3)	0(0.0)	2(8.3)	0(0.0)
G9 (12.0)	2(4.8)	2(6.4)	0(0.0)	1(4.8)	2(8.3)	1(33.3)
G1, G2, G4 (7.5)	2(4.8)	0(0.0)	2(4.2)	0(0.0)	1(4.2)	0(0.0)
Nontypeable n = 43 (25.4)	n = 4(9.5)	n = 14(45.1)	n = 5(10.4)	n = 15(71.4)	n = 3(12.5)	n =2(66.7)
GNT (100)	4(9.5)	14(45.1)	5(10.4)	15(71.4)	3(12.5)	2(66.7)
Total	42	31	48	21	24	3

in the lineage P[4]-1 were closer to each other and with the prototype strain, DS-1 in nucleotide (96.6 to 100%) and amino acid (97.7 to 100%) sequences.

Differences within all P[4] strains by 5.4 to 6.4% / 3.7 to 6.0% and 2.5 / 2.3% in their nucleotide / amino acid sequences from the prototype strain, DS-1 of lineage P[4]-1 were observed during the 1990s and 2000s. The alignment of the partial deduced amino acid sequences (11-153 aa) showed variations at positions 32(S→N), 89(N→D), 99(N→S), and 120(I→V) as compared to the prototype strain, DS-1. Multiple strain specific changes were also shared by these strains at positions 35(V→I), 49(N→D), 113(T→A), 130(V→I) and 133(N→S).

Phylogenetic analysis of P[8] strains

The nucleotide sequence analysis of the 69 P[8] strains recovered from adolescents (n = 14) and adults (n = 55)

at two time points, 1993 to 1996 (n = 48) and 2004 to 2007 (n = 21) showed clustering of the strains in three distinct evolutionary lineages P[8]-2, P[8]-3 and P[8]-4 of a total of four lineages described to date for this genotype (Figure 2).

All of the 59 strains classified in P[8]-3 lineage showed 97.5 to 100% / 96.1 to 100% and 96.0 to 100% / 94.7 to 100% nucleotide / amino acid identities respectively with the reference strains- Kagawa/90-544, Kagawa/90-513, Hun 9, OP351, 27B3, Dhaka 25-02, ISO22, PA 25/03, 90-551 and CAU and within themselves. All these strains were predominantly associated with commonly detected rotavirus G genotypes [(25/59, 42.4 %); G1 (17/25, 68.0%), G3 (5/25, 20.0%) and G4 (3/25, 12.0%)] followed by mixed infections (20/59, 33.9%) and G-nontypeable strains (13/59, 22.0%). Only one of the strains (939961) showed unusual combination with rotavirus genotype G2 (1/59, 1.7%).

Four of the 9 strains grouped in lineage P[8]-2 were

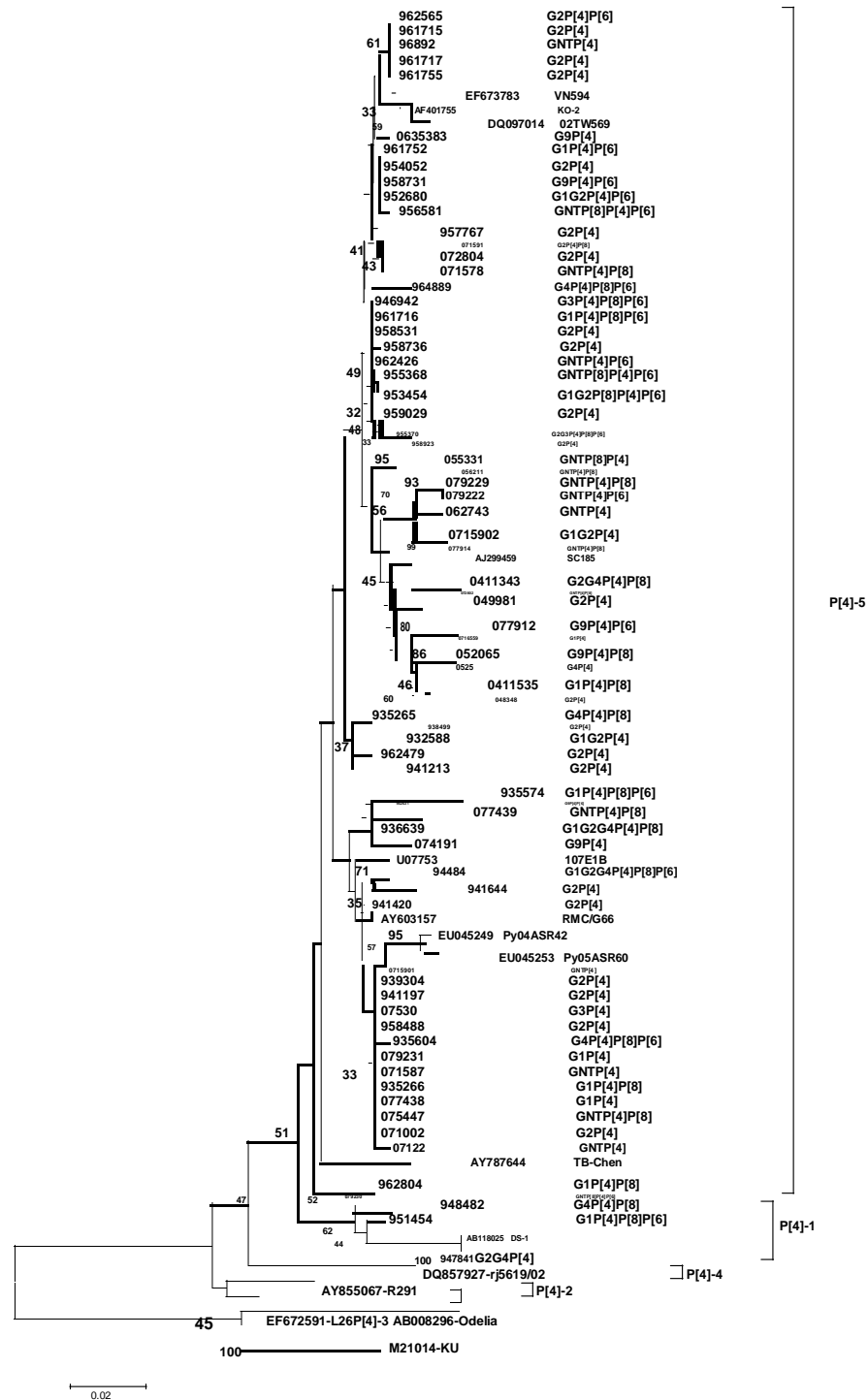


Figure 1. Phylogenetic tree based on partial nucleotide sequences of the VP4 (P[4]) gene (nt 43 to 456) of rotavirus strains recovered from adolescents and adults in 1993 to 1996 and 2004 to 2007. The strains of the present study are identified by the specimen number, followed by the genotypes identified in multiplex PCR. The reference strains are indicated by accession numbers followed by the strain names.

closer to the prototype strain, F45 with 95.7 to 96.8% and 96.7 to 98.9%, nucleotide and amino acid identities respectively. Within these strains nucleotide and amino acid identities were noted to be 96.4 to 100% and 97.8 to

100% respectively. Remaining five strains showed 100% identity with the KU strain at nucleotide and amino acid level. Majority of the strains (7/9) from this lineage were associated with non-typeable G genotypes.

1996 and 2004 to 2007 differed respectively by 6.6 to 8.2% / 4.7 to 6.4% and 3.0% / 2.2% in their nucleotide / amino acid sequences from the prototype strain, Wa of lineage P[8]-1 and from each other. The alignment of the partial deduced amino acid sequences (11-103aa) showed variations at positions 19(H→Y), 35(I →V), 38(S→G), 64(M→I) and 78(N→T) as compared to the prototype strain, Wa. These strains also showed strain specific changes at 20(T→I), 71(P→S), 75(T→K) and 77(P→L) positions.

Phylogenetic analysis of P[6] strains

Phylogenetic analysis of 27 P[6] strains recovered from adolescents (n = 3) and adults (n = 24) at two time points 1993 to 1996 (n = 24) and 2004 to 2007 (n = 3), showed clustering of all of the strains in lineage P[6]-I (Figure 3). All strains showed 94.1 to 100% and 93.9 to 100% nucleotide and amino acid identities respectively with the reference strains, M37, ST-3, SC2, MtA5, RV176, US1205 and Se585. Within these strains 96.1 to 100% nucleotide and 95.5 to 100% amino acid identity was noted. Nucleotide and amino acid divergence respectively of 0.4 to 2.3% and 0.2 to 1.6% was detected between the strains from 1993 to 1996 and 2007. The alignment of the partial deduced amino acid sequences (11-80 aa) showed variations at positions 30(T→S), 73(S→N) and 78(S→N) as compared to the prototype strain, M37.

DISCUSSION

Epidemiological surveys carried out in the late 1970s for the rotavirus strain surveillance used monoclonal antibodies. Though these studies established circulation of G1 to G4 serotypes in Europe, North America and Australia they also reported predominance of nontypeable rotavirus strains in South America, Africa and Asia (Desselberger et al., 2001). A complete assessment highlighting complex picture of the virus emerged with the availability of typing methods for both VP7 (G) and VP4 (P) genes (Gouvea et al., 1990; Gentsch et al., 1992). Rotaviruses identified to have different G and / or P types have indicated that a diverse gene pool contributes to human infections (Desselberger et al., 2001). Further, studies on genetic and antigenic variations in the rotavirus strains of the same genotypes have revealed intragenotypic diversity and rotavirus gene classification on the basis of lineages and sub lineages (Gouvea et al., 1999; Araujo et al., 2007; Espinola et al., 2008). Circulation of different lineages within the P[4], P[8] and P[6] genotypes of rotavirus VP4 genes isolated from UK, Hungary, Brazil and Paraguay (Itturiza-Gomara et al., 2001; Banyai et al., 2004b; Araujo et al., 2007; Espinola et al., 2008) has been reported. However, such

data from India is limited to rotavirus strains recovered from children (Samajdar et al., 2008; Zade et al., 2009). This study analyzes partial sequences of VP4 genes of rotavirus strains recovered from adolescents and adults at two different time points (1993 to 1996 and 2004 to 2007).

Varying frequencies of P[4] genotypes have been reported during rotavirus surveillance conducted in Brazil (21 to 26%), Sao Paulo (1%), India (33%), Korea (15.4%), Tunisia (13%) and Bangladesh (43%) (Araujo et al., 2002; Mascarenhas et al., 2002; Carmona et al., 2006; Volotao et al., 2006; Samajdar et al., 2006; Araujo et al., 2007; Chouikha et al., 2007; Le VP et al., 2008; Paul et al., 2010). Occurrence of five (P[4]-1 to P[4]-5) different lineages of P[4] genotype has been described on the basis of analysis of >100 strains circulating worldwide (Espinola et al., 2008). Three lineages P[4]-1, P[4]-4 and P[4]-5 have been described to combine with either of the G2, G3 and G9 types of VP7 gene. The remaining two lineages, P[4]-2 and P[4]-3 were found in association with G8 and G12 genotypes, respectively (Espinola et al., 2008). As against this, in the present study, P[4]-5 lineage clustered almost in equal proportion with the strains of G2 specificity and mixed / unusual infections / nontypeable G types. These results differed from the recent findings on clustering of G2P[4] strains in the P[4]-2 lineage from Thailand (Khamrin et al., 2010). The P[4] - 1 lineage described in the present study clustered only mixed G and / or P infections (3/73). Interestingly, a comparison between the two time points, 1993 to 1996 and 2004 to 2007 showed a significant difference (P<0.01) in the frequencies of association of P[4] with common and nontypeable G strains (Table 1).

Genotype P[8] has been found to have undergone genetic and antigenic drift (Jin et al., 1996) with cocirculation of four distinct P[8] lineages, globally (Gouvea et al., 1999; Cunliffe et al., 2001; Arista et al., 2005, 2006; Ansaldi et al., 2007; Espinola et al., 2008). Several studies have revealed cocirculation of mainly P[8]-1 and P[8]-2 lineages in the late 80's and early 90's and predominant circulation of P[8]-3 lineage in the 2000s (Cunliffe et al., 2001; Itturiza-Gomara et al., 2001; Arista et al., 2005; Araujo et al., 2007; Espinola et al., 2008; Le VP et al., 2010; Tort et al., 2010). In the present study P[8]-3 predominated at both time points (93.7% / 66.7%), thus indicating circulation of this sublineage in Pune, India since 1990s. Cocirculation of P[8]-2 and / or P[8]-4 was also noted at both time points with increase in the circulation of P[8]-2 lineage from 4.2% in 1990s to 33.3% in 2000s. Although P[8]-3 lineage grouped majority of the strains of present study, its association with G genotypes differed with time. In the 1990s it was predominantly associated with common G types (G1, G3 and G4) (25/45, 55.6%) followed by unusual and mixed infections with G and / or P types (16/45, 35.5%) and G nontypeable strains (4/45, 8.9%). On the contrary, strains (n =14) from 2000s associated predominantly with

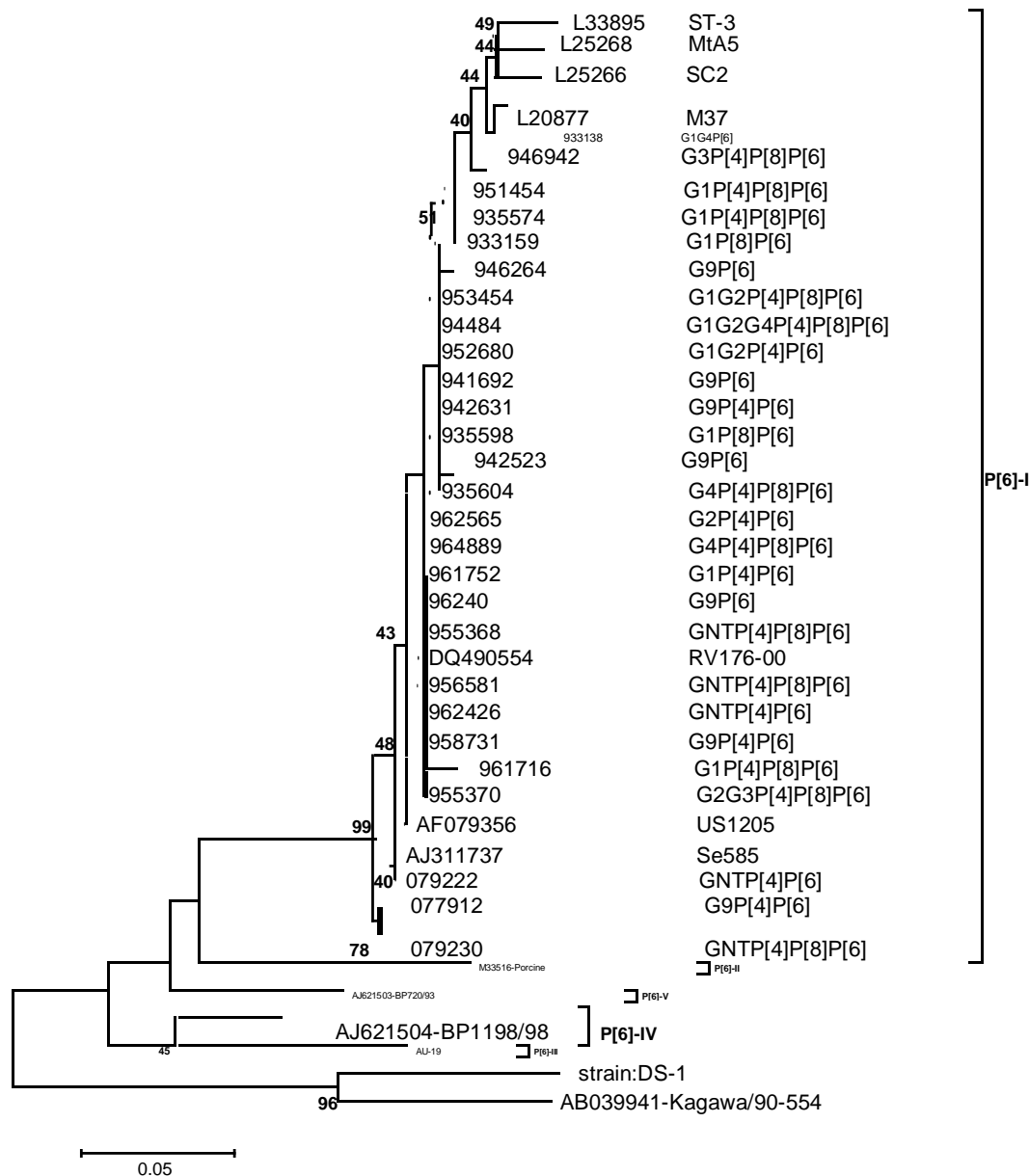


Figure 3. Phylogenetic tree based on partial nucleotide sequences of the VP4 (P[6]) gene (nt 40 to 249) of rotavirus strains recovered from adolescents and adults in 1993 to 1996 and 2004 to 2007. The strains of the present study are identified by the specimen number, followed by the genotypes identified in multiplex PCR. The reference strains are indicated by accession numbers followed by the strain names.

nontypeable G types (9/14, 64.3%) followed by mixed G and / or P types (5/14, 35.7%). These results differed from the findings documenting association of P[8]-3 lineage predominantly with common strains (Min et al., 2004; Araujo et al., 2007; Espinola et al., 2008; Samajdar et al., 2008; Le et al., 2010). It was also interesting to note that the common strains (G1, G3 and G4) from the 1990s of this study clustered in the P[8]-3 lineage and that they differed from the contemporary strains of the same types that clustered in P[8]-1 or P[8]-2 lineages (Iturriza-Gomara et al., 2001; Arista et al., 2005; Espinola

et al., 2008). The existence of constraints on the G9 strains circulating worldwide to belong to the P[8]-3 lineage (Iturriza-Gomara et al., 2001; Banyai et al., 2004a) was also noted in this study.

Circulation of P[8]-2 lineage reported at different time points has displayed clustering of rotavirus strains with different G types (G1-G4, G5, G8 and G9) (Iturriza-Gomara et al., 2001; Araujo et al., 2007; Espinola et al., 2008; Samajdar et al., 2008). The rotavirus strains detected in children from Vietnam, Brazil and Bangladesh have shown association of P[8]-2 lineage with common

(G1, G2, and G9) and mixed G strains (Araujo et al., 2007; Nguyen et al., 2008; Dey et al., 2009). In the present study this lineage clustered G non-typeable strains predominantly. However, it is possible that the nontypeable strains could be commonly circulating strains that remained undetected due to less viral load or genomic variations.

OP354 like lineage designated as P[8]-4 has been described to group G1, G3, G4, G9, G1G9, G1G4 and G3G9 strains from different countries (Cunliffe et al., 2001; Banyai et al., 2004a; Nguyen et al., 2008; Samajdar et al., 2008; Nagashima et al., 2010). The association of this lineage with triple P (VP4) type rotavirus infection (G1, P4, P8, P6) was noted for the first time in this study. A significant difference ($P < 0.01$) in the frequencies of association of P[8] with common and nontypeable G strains was evident between the two time points examined in this study (Table 1).

The P[6] genotype previously thought to be restricted to asymptomatic infections has also been identified in combination with G1-G4, G5, G8, G9, G11 and G12 genotypes detected in neonates and children with diarrhea (Adah et al., 1997; Pager et al., 2000; Cunliffe et al., 2002; Mascarenhas et al., 2002; Steele and Ivanoff, 2003; Page and Steele, 2004; Mascarenhas et al., 2006; 2007; Ahmed et al., 2007; Nguyen et al., 2007; Le et al., 2008; Banyai et al., 2009; Mukherjee et al., 2009; Shim et al., 2010). However, no data is available from adolescents and adults. Interestingly, majority of the strains with P[6] genotype in the present study were detected in the mixed infections and placed in lineage P[6]-I (M37 like).

In summary, the findings of this study highlight the intragenotypic diversity and temporal variations in rotavirus VP4 genes. Although it is known that homotypic and heterotypic immune response could be elicited by VP4, antigenic differences in various P[6] lineages identified in human and porcine species have been reported to generate differential neutralizing antibody response (Gorziglia et al., 1990; Li and Gorziglia, 1993; Nakagomi et al., 1999). In this context, it will be important to monitor alterations in the epitopes of P[8] and P[4] rotavirus strains grouped in different lineages and evaluate corresponding antibody responses as VP4 is a major protective antigen. Currently available rotavirus vaccines introduced mainly for the pediatric population constitute VP4 of P[8] genotype. In view of this, vigilance on the intragenotypic diversity in VP4 genes would provide a key to understand the evolution and genetic / antigenic differences in the rotavirus genotypes and assess the need for improvement in rotavirus vaccines.

REFERENCES

- Adah MI, Rohwedder A, Olaleya OD, Durojaiye OA, Werchau H (1997). Further characterization of field strains of rotavirus from Nigeria VP4 genotype P6 most frequently identified among symptomatically infected children. *J. Trop. Pediatr.*, 43: 267-274.
- Ahmed K, Anh DD, Nakagomi O (2007). Rotavirus G5P[6] in child with diarrhea, Vietnam. *Emerg. Infect. Dis.*, 13: 1232-1235.
- Ansaldi F, Pastorino B, Valle L, Durando P, Sticchi L, Tucci P, Biasci P, Lai P, Gasparini R, Icardi G, Paediatric Leghorn Group (2007). Molecular characterization of a new variant of rotavirus P[8]G9 predominant in a sentinel-based survey in Central Italy. *J. Clin. Microbiol.*, 45: 1011-1015.
- Araujo IT, Assis RM, Fialho AM, Mascarenhas JD, Heinemann MB, Leite JP (2007). Brazilian P[8], G1, P[8], G5, P[8], G9, and P[4], G2 rotavirus strains: nucleotide sequence and Phylogenetic analysis. *J. Med. Virol.*, 79: 995-1001.
- Araujo IT, Fialho AM, de Assis RM, Rocha M, Galvao M, Cruz CM, Ferreira MS, Leite JP (2002). Rotavirus strain diversity in Rio de Janeiro Brazil: characterization of VP4 and VP7 genotypes in hospitalized children. *J. Trop. Pediatr.*, 48: 214-218.
- Arista S, Giammanco GM, De Grazia S, Colomba C, Martella V (2005). Genetic variability among serotype G4 Italian human rotaviruses. *J. Clin. Microbiol.*, 43: 1420-1425.
- Arista S, Giammanco GM, De Grazia S, Ramirez S, Blundo C, Colombo C, Cascia A, Martella, V (2006). Heterogeneity and temporal dynamics of evolution G1 human rotaviruses in a settled population. *J. Virol.*, 80: 10724-10733.
- Arora R, Chhabra P, Chitambar SD (2009). Genetic diversity of genotype G1 rotaviruses co-circulating in western India. *Virus. Res.*, 146: 36-40.
- Bányai K, Esona MD, Kerin TK, Hull JJ, Mijatovic S, Vascónes N, Torres C, de Filippis AM, Foytich KR, Gentsch JR (2009). Molecular characterization of a rare, human-porcine reassortant rotavirus strain, G11P[6], from Ecuador. *Arch. Virol.*, 154: 1823-1829.
- Bányai K, Gentsch JR, Schipp R, Jakab F, Bene J, Melegh B, Glass RI, Szucs G (2004a). Molecular epidemiology of human P[8], G9 rotaviruses in Hungary between 1998 and 2001. *J. Med. Microbiol.*, 53: 791-801.
- Bányai K, Martella V, Jakab F, Melegh B, Szucs G (2004b). Sequencing and phylogenetic analysis of human genotype P[6] rotavirus strains detected in Hungary provided evidence for genetic heterogeneity within the P[6] VP4 gene. *J. Clin. Microbiol.*, 42: 4338-4343.
- Carmona RCC, Timenetsky MCST, Morillo SG, Richtzenhain L (2006). Human rotavirus serotypes G9, Sao Paulo, Brazil, 1996-2003. *Emerging. Infect. Dis.*, 12: 963-968.
- Chitambar SD, Tette V, Dhongde R, Kalrao V (2008). High frequency of rotavirus viremia in children with acute gastroenteritis :discordance of strains detected in stool and sera. *J. Med. Virol.*, 80: 2169-2176.
- Chouikha A, Fodha I, Noomen S, Bouzid L, Mastouri M, Peenze I, de Beer M, Dewar J, Geyer A, Sfar T, Gueddiche N, Messaadi F, Trabelsi A, Boujaafar N, Steele AD (2007). Group A rotavirus strains circulating in the eastern center of Tunisia during a ten-year period (1995-2004). *J. Med. Virol.*, 79: 1002-1008.
- Cunliffe NA, Gondwe JS, Graham SM, Thindwa BD, Dove W, Broadhead RL, Molyneux ME, Hart CA (2001). Rotavirus strain diversity in Blantyre, Malawi, from 1997 to 1999. *J. Clin. Microbiol.*, 39: 836-843.
- Cunliffe NA, Rogerson S, Dove W, Thindwa BDM, Greensill J, Kirkwood CD, Broadhead RI, Hart CA (2002). Detection and characterization of rotaviruses in hospitalized neonates in Blantyre, Malawi. *J. Clin. Microbiol.*, 40: 1534-1537.

- Desselberger U, Iturriza-Gómara M, Gray J (2001). Rotavirus epidemiology and surveillance. *Novartis. Found. Symp.*, 238: 125-147.
- Dey SK, Hayakawa Y, Rahman M, Islam R, Mizuguchi M, Okitsu S, Ushijima H (2009). G2 Strain of Rotavirus among Infants and Children, Bangladesh. *Emerg. Infect. Dis.*, 15: 91-94.
- Espinola EE, Amarilla A, Arbiza J, Parra GI (2008). Sequence and phylogenetic analysis of the VP4 gene of human rotaviruses in Paraguay. *Arch. Virol.*, 153: 1067-1073.
- Estes MK (2001). Rotavirus and their replication. In *Fields Virology*, 4th eds. Knipe DM, Griffin DE, Lamb RA, Martin MA, Roizman B, Status SE, Philadelphia, Lippincott Williams and Wilkins, pp. 1747-1785.
- Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, Das BK, Bhan MK (1992). Identification of group A rotavirus gene 4 types by polymerase chain reaction. *J. Clin. Microbiol.*, 30: 1365-1373.
- Gorziglia M, Larralde G, Kapikian AZ, Chanock RM (1990). Antigenic relationships among human rotaviruses as determined by outer capsid protein VP4. *Proc. Natl. Acad. Sci.*, 87: 7155-7159.
- Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, Fang ZY (1990). Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *J. Clin. Microbiol.*, 28: 276-282.
- Gouvea V, Lima RC, Linhares RE, Clark HF, Nosawa CM, Santos N (1999). Identification of two lineages (WA-like and F45-like) within the major rotavirus genotype P[8]. *Virus Res.*, 59:141-147.
- Iturriza-Gomara M, Isherwood B, Desselberger U, Gray J (2001). Reassortment in vivo: Driving force for diversity of human rotavirus strains isolated in the United Kingdom between 1995 and 1999. *J. Virol.*, 75: 3696-3705.
- Iturriza-Gomara M, Kang G, Gray J (2004). Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. *J. Clin. Virol.*, 31: 259-265.
- Jin Q, Ward RL, Knowlton DR, Gabbay YB, Linhares AC, Rappaport R, Woods PA, Glass RI, Gentsch JR (1996). Divergence of VP7 genes of G1 rotaviruses isolated from infants vaccinated with reassortant rhesus rotaviruses. *Arch. Virol.*, 141: 2057-2076.
- Kang G, Arora R, Chitambar S, Deshpande J, Gupta MD, Kulkarni M, Naik TN, Mukherji D, Venkatasubramanian S, Gentsch JR, Glass RI, Parashar UD, Indian Rotavirus Strain Surveillance Network (2009). Multicenter, hospital-based surveillance of rotavirus disease and strains among Indian children. *J. Infect. Dis.*, 200: S147-153.
- Khamrin P, Maneekarn N, Malasao R, Nguyen TA, Ishida S, Okitsu S, Ushijima H (2010). Genotypic linkages of VP4, VP6, NSP4, NSP5 genes of rotaviruses circulating among children with acute gastroenteritis in Thailand. *Infect. Genet. Evol.*, 10: 467-472.
- Le VP, Chung YC, Kim K, Chung SI, Lim I, Kim W (2010). Genetic variation of prevalent G1P[8] human rotaviruses in South Korea. *J. Med. Virol.*, 82: 886-896.
- Le VP, Kim JY, Cho SL, Nam SW, Lim I, Lee HJ, Kim K, Chung SI, Song W, Lee KM, Rhee MS, Lee JS, Kim W (2008). Detection of unusual rotavirus genotypes G8P[8] and G12P[6] in South Korea. *J. Med. Virol.*, 80: 175-182.
- Li B, Gorziglia M (1993). VP4 serotype of the Gottfried strain of porcine rotavirus. *J. Clin. Microbiol.*, 31: 3075-3077.
- Mascarenhas JDP, Linhares AC, Gabbay YB, Lima CS, Guerra SFS, Soares LS, Oliveira DS, Lima JC, Macedo O, Leite JPG (2007). Molecular characterization of VP4 and NSP4 genes from rotavirus strains infecting neonates and young children in Belém, Brazil. *Virus Res.*, 126: 149-158.
- Mascarenhas JDP, Linhares AC, Bayma APG, Lima JC, Sousa MS, Araujo IT, Heinemann MB, Gusmao RHP, Gabbay YB, Leite JPG (2006). Molecular analysis of VP4, VP7 and NSP4 genes of P[6]G2 rotavirus genotype strains recovered from neonates admitted to hospital in Belém, Br. *J. Med. Virol.*, 78: 281-289.
- Mascarenhas JDP, Linhares AC, Gabbay YB, Leite JRP (2002). Detection and characterization of rotavirus G and P types from children participating in a rotavirus vaccine trial in Belém, Brazil. *Mem. Inst. Oswaldo. Cruz. Rio de Janeiro*, 97: 113-117.
- Matthijnssens J, Ciarlet M, Heiman E, Arijis I, Delbeke T, McDonald SM, Palombo EA, Iturriza-Gomara M, Maes P, Patton JT, Rahman M, Ranst MV (2008). Full genome-based classification of rotaviruses reveals a common origin between human Wa-like and porcine rotavirus strains and human DS-1 like and bovine rotavirus strains. *J. Virol.*, 82: 204-3219.
- Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Bányai K, Brister JR, Buesa J, Esona MD, Estes MK, Gentsch JR, Iturriza-Gómara M, Johne R, Kirkwood CD, Martella V, Mertens PP, Nakagomi O, Parreño V, Rahman M, Ruggeri FM, Saif LJ, Santos N, Steyer A, Taniguchi K, Patton JT, Desselberger U, Van Ranst M (2011). Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Arch. Virol.*, 156: 1397-1413.
- Min BS, Noh YJ, Shin JH, Baek SY, Kim JO, Kyung M, Ryu SR, Kim BG, Kim DK, Lee SH, Min HK, Ahn BY, Park SN (2004). Surveillance Study (2000 to 2001) of G and P Type Human Rotaviruses Circulating in South Korea. *J. Clin. Microbiol.*, 42: 4297-4299.
- Mukherjee A, Dutta D, Ghosh S, Bagchi P, Chattopadhyay S, Nagashima S, Kobayashi N, Dutta P, Krishnan T, Naik TN, Chawla-Sarkar M (2009). Full genomic analysis of a human group A rotavirus G9P[6] strain from Eastern India provides evidence for porcine-to-human interspecies transmission. *Arch. Virol.*, 154: 733-746.
- Nagashima S, Kobayashi N, Paul SK, Ghosh S, Sarkar MC, Hossain MA, Krishnan T (2010). Identification of P[8]b subtype in OP354-like human rotavirus strains by a modified RT-PCR method. *Jpn. J. Infect. Dis.*, 63: 208-211.
- Nakagomi T, Horie Y, Koshimura Y, Greenberg HB, Nakagomi O (1999). Isolation of a human rotavirus strain with a super-short RNA pattern and a new P2 subtype analysis. *J. Clin. Microbiol.*, 37: 1213-1216.
- Nguyen TA, Hoang LP, Pham LD, Hoang KT, Okitsu S, Mizuguchi M, Ushijima H (2008). Use of sequence analysis of the VP4 gene to classify recent Vietnamese rotavirus isolates. *Clin. Microbiol. Infect.*, 14: 235-241.
- Nguyen TA, Khamrin P, Trinh QD, Pham LD, Hoang LP, Hoang KT, Yagyu F, Okitsu S, Ushijima H (2007). Sequence analysis of Vietnamese P[6] rotavirus strains suggests evidence of interspecies transmission. *J. Med. Virol.*, 79: 1959-1965.
- Page NA, Steele AD (2004). Antigenic and genetic characterization of serotype G2 human rotavirus strains from the African continent. *J. Clin. Microbiol.*, 42: 595-600.
- Pager CT, Alexander JJ, Steele AD (2000). South African G4P[6] asymptomatic and symptomatic neonatal rotavirus strains differ in their NSP4, VP8*, and VP7 genes. *J. Med. Virol.*, 62: 208-216.
- Parashar UD, Gibson CJ, Bresse JS, Glass RI (2006). Rotavirus and severe childhood diarrhea. *Emerg. Infect. Dis.*, 12: 304-306.
- Patel MM, Steele D, Gentsch JR, Wecker J, Glass RI, Parashar UD (2011). Real-world Impact of Rotavirus Vaccination. *Pediatric. Infect. Dis.*, 30: S1-S5.
- Paul SK, Ahmed MU, Hossain MA, Mahmud MC, Bhuiyan MR, Saha SK, Tabassum S (2010). Molecular characterization of group A human rotavirus among hospitalized children and adults in Bangladesh: finding of emerging G12 strains. *Mymensingh. Med. J.*, 19: 16-26.
- Pietsch C, Schuster V, Liebert UG (2011). A hospital based study on inter- and intragenotypic diversity of human rotavirus A VP4 and VP7 gene segments, Germany. *J. Clin. Virol.*, 50: 136-141.
- Samajdar S, Varghese V, Barman P, Ghosh S, Mitra U, Dutta P, Bhattacharya SK, Narasimham MV, Panda P, Krishnan T, Kobayashi N, Naik TN (2006). Changing pattern of human group A rotaviruses: emergence of G12 as an important pathogen among children in eastern India. *J. Clin. Virol.*, 36: 183-188.
- Samajdar S, Ghosh S, Dutta D, Chawla-Sarkar M, Kobayashi N, Naik TN (2008). Human group A rotavirus P[8] Hun9-like and rare OP354-like strains are circulating among diarrhoeic children in Eastern India. *Arch. Virol.*, 153: 1933-1936.
- Santos N, Hoshino Y (2005). Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev. Med. Virol.*, 15: 29-56.
- Shim SY, Jung YC, Le VP, Son DW, Ryoo E, Shim JO, Lim I, Kim W (2010). Genetic Variation of G4P[6] Rotaviruses: Evidence for novel strains circulating between the hospital and community. *J. Med. Virol.*, 82: 700-706.
- Steele AD, Ivanoff B (2003). Rotavirus strains circulating in Africa during 1996-1999; emergence of G9 strains and P[6] strains. *Vaccine*, 17:

- 361-367.
- Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, 24: 1596-1599.
- Tatte VS, Gentsch JR, Chitambar SD (2010a). Characterization of group A rotavirus infections in adolescents and adults from Pune, India: 1993-1996 and 2004-2007. *J. Med. Virol.*, 82: 519-527.
- Tatte VS, Rawal KN, Chitambar SD (2010b). Sequence and phylogenetic analysis of the VP6 and NSP4 genes of human rotavirus strains: Evidence of discordance in their genetic linkage. *Infect. Gen. Evol.*, 10: 940-949.
- Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic. Acid. Res.*, 22: 4673-4680.
- Tort LF, Volotao EM, de Mendonca MC, da Silva ME, Siqueira AA, Assis RM, Moratoria G, Cristina J, Leite JP (2010). Phylogenetic analysis of human P[8]G9 rotavirus strains circulating in Brazil reveals the presence of a novel genetic variant. *J. Clin. Virol.*, 47: 345-355.
- Volotao EM, Soares CC, Maranhao AG, Rocha LN, Hoshino Y, Santos N (2006). Rotavirus surveillance in the city of Rio de Janeiro-Brazil during 2000-2004: detection of unusual strains with G8P[4] or G10P[9] specificities. *J. Med. Virol.*, 78: 263-272.
- Zade JK, Chhabra P, Chitambar SD (2009). Characterization of VP7 and VP4 genes of rotavirus strains: 1990-1994 and 2000-2002. *Epidemiol. Infect.*, 137: 936-942.