Research article

Genotypic determination of human group A rotaviruses from Goa and Meghalaya states, India

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A B S T R A C T

Introduction: Rotavirus is the leading cause of diarrhoea in young children in India, responsible for an estimated 21357 mean numbers of deaths in 2010. Various genotypes of rotaviruses evolved due to mutational changes have been recognized. In this study, we determined the genotypes of rotaviruses involved in diarrhea in Goa and Meghalaya states of India.
Methods: The dsRNA of rotaviruses was extracted from stool samples and detected by Ribonucleic Acid-Polyacrylamide gel electrophoresis (RNA PAGE) and Reverse transcription-polymerase Chain Reaction (RT-PCR) targeting the partial VP7 gene. The full length VP7 and partial VP4 genes of rotavirus strains were amplified by RT-PCR followed by nucleotide sequencing. The RotaC classification tool was used to determine the genotypes.
Results: The positivity of rotavirus by PAGE and RT-PCR was observed to be 43.10% and 39.65% in Goa and 38% and 36% in Meghalaya, respectively. Though long electrophoretic profile was appeared to be the most pre-dominant rotavirus type in circulation in these two states, 96% of long and 84.61% short electropherotype profiles could be detected by RT-PCR. The dsRNA of rotavirus extracted from 36 samples could be transcribed and amplified by beg9end9 primers for G genotyping, while, 41 by con3con2 primers for P genotyping. G1P[8] and G1P[6] genotypes were commonly circulated in Goa and G1P[8] and G1P[4] genotypes in Meghalaya. On nucleotide analysis, 6 samples from Goa showed G1 genotype specificity, while, 3 showed P[8] specificity indicating the G1P[8] rotavirus circulating in Goa. In Meghalaya state, 3 strains showed P[8] and 2 showed P[4] genotype specificity. The majority of the G and P genotypes were closely related to each other and G1 genotypes appeared in two separate clusters, while, P[8] and P[4] appeared in the respective clusters.
Conclusion: The circulation of G1P[8], G1P[6] genotypes in Goa and the presence of G1P[8] and G1P[4] geno-types in Meghalaya was observed.

1. Introduction

Rotaviruses have been recognized as a common cause of infantile diarrhoea and severe gastroenteritis in children (Midgley et al., 2012). It causes an estimated 11.37 million episodes of acute gastroenteritis (AGE) in children < 5 years annually in India, resulting in total direct costs of Indian Rupee (INR) 10.37 billion per year (John et al., 2014). In 2011, it is estimated that there were 78,000 deaths due to rotavirus associated AGE in India, with the majority (75.6%, 59,000) in first 2 years in the life (John et al., 2014). As per the Global Burden of Disease Study, in India

21357.6 (13150.8–33967.0) deaths in children less than 5 yr occur due to rotavirus infection (Troeger et al., 2017). The first indigenous oral rotavirus vaccine (Rotavac) was introduced by the Indian Government into the Universal Immunization Programme (UIP) from 2016 in a phased manner. The vaccine is currently available in 28 states and union territories in India. Rotavac vaccine is an oral monovalent G9P[11] (11E6) vaccine, derived from a naturally attenuated human neonatal strain, which contains bovine strain segment (natural human-bovine recombinant) (Bhandari et al., 2014). Nair et al. (2019) described the protocol for surveillance to monitor the performance of rotavirus

vaccine. The efficacy of this vaccine was well studied in children <2 yrs of age, where, it was 55.1% (95% CI 39.9 to 66.4) for three doses of the vaccine given at 6, 10 and 14 weeks of age (Mehendale et al., 2016). Though the Rotavac vaccine is readily available with remarkable RV preventable results, the diarrhoeal episode due to rotavirus A (RVA) in children are still frequently recorded, particularly in winters from all the parts of the country.

Rotaviruses are classified into 10 groups (or species) as A–J, both animals and humans are infected with A–C, while, groups D–G are detected only in animals. Group H–J has been detected recently from animal sources based on the VP6 sequence analysis. Among all, group A rotaviruses are considered as an important viral diarrhoeal agent and the most prevalent cause of gastroenteritis in humans and animals worldwide. It can be further classified as P and G genotypes based on the sequence comparison of the VP4 and VP7 segments, respectively. Many new G and P genotypes of rotaviruses have emerged recently with different combinations. However, G1, G3, G4, G9 are more commonly associated with P[8], G2 with P[4] and G12 predominantly with either P [8] or P[6] rotavirus strains of human origin (Matthijnssens et al., 2009). Prevalence of childhood rotavirus has been reported to vary from 6-45% in different parts of India (Jain et al., 2001a) with the prevalent strains of G1, G2, G3 and G4 and P[8], P[6] and P[4], however, some other G types such as G6, G8, G9, G10, and G12 have also been identified as the cause of diarrhea (Ramani and Kang, 2007). The rotavirus strains prevalent globally differ than the strains prevalent in India with the varied rate of a frequency distribution. The common worldwide strains, G1P[8], G2P[4], G3P[8], and G4P[8] were underrepresented among Indian children (33%), whereas, strains of P-type [6] (G1P[6], G2P[6], G3P[6], G4P[6], and G9P[6] which primarily infect asymptomatic newborns but are rare in children with diarrhoea are common in India (43%) (Broor et al., 2003). A significant number of children also had mixed rotavirus infections. Several uncommon genotypes G1P[7], G9P[8], G5P[8], G3P [11], G12P[6], G2G9P[6] and G9G2P[11] were also observed (Chakraborty et al., 2016; Arun et al., 2019). Overall, G1P[8], G2P[4], G9P[8], G12P[8] and G12P[6] were identified as important G-P combinations. Further, wide geographical area, different agro-climatic conditions and extensive biodiversity of humans and animals thought to be responsible for the circulation of a wide range of genotypes in different parts of the country. Goa and Meghalaya are among the most popular tourist places in India where the movement of humans takes place from India as well as from all over the world, which may facilitate or expose the local population to a wide variety of rotavirus strains. Knowledge of molecular epidemiology and genotypic diversity of rotaviruses in circulation is important in order to modify existing rotavirus vaccine as well as for the development of suitable and effective vaccine/diagnostics to combat rotaviral diarrhea. Therefore, in this study, we determined different G and P genotypes of group A rotaviruses from Goa and Meghalaya, India.

2. Materials and methods

2.1. Collection of fecal samples

Inform consent was taken from the hospital in-charge and guardian of each child suffered from diarrhea with or without hospitalization, before collecting the specimen. Random sampling was carried out during 2012–2016 where diarrhoeal stool samples from unvaccinated children below 5 yr of age showing symptoms of loose, watery diarrhea with or without blood and/or mucous, presence or absence of dehydration and mild fever were collected aseptically during winter (November to February). A total of 158 diarrhoeal fecal samples (100 from Meghalaya and 58 from Goa) from children were collected from private and Government hospitals located in Goa and Meghalaya. These samples (5–15 g or ml) were collected individually in sterile wide-mouth containers, properly labeled and stored at -20°C till further processing.

2.2. Detection of rotavirus by RNA-PAGE and RT-PCR

About 10-20 stored stool samples were selected at each time for extraction of dsRNA of rotavirus followed by its detection by Ribonucleic Acid-Polyacrylamide gel electrophoresis (RNA PAGE) and Reverse transcription-polymerase Chain Reaction (RT-PCR). Double-stranded RNA was extracted from each fecal samples (100–200 μl) following the

Table 1. Primer sequences used for the rotavirus A gene amplification of different structural genes using RT-PCR assay. The primers used in multiplex genotyping PCR assay also listed.

Sl. No.	Gene/Genotype	Primer name	Primer details (positions)	Annealing temperature (°C)	Product size (bp)	Reference
1	VP7	Rota 1 – F Rota 2 – R	GATCCGAATGGTTGTGTAATCCAAT (nt 531-550) AATTCGCTACGTTTTCTCTTGG (nt 824-808)	55	304	Husain et al. (1995)
2	VP4	Con 3 – F Con 2 – R	TGGCTTCGCTCATTTATAGACA (nt 11-32) ATTTCGGACCATTTATAACC (nt 868-887)	53	876	Gouvea et al. (1990)
3	VP7	Beg 9 – F End 9 – R	GGC TTT AAA AGA GAG AAT TTC CGT CTG G (nt 1-28) GGT CAC ATC ATA CAA TTC TAA TCT AAG (nt 1062-1036)	52.5	1062	Gouvea et al. (1990)
6	G1	9T1-1	9T1-1- TCTTGTCAAAGCAAATAATG (nt 176 to 195)	42	158	Das et al. (1994)
7	G2	9T1-2	GTFAGAAATGAYTTCTCCACT (nt 262 to 281)	42	244	Das et al. (1994)
8	G3	9T-3P	GTCCAGTTIGCAGTGTTAGC (nt 484 to 503)	42	466	Das et al. (1994)
9	G4	9T-4	GGGTCGATGGAAAATTCT (nt 423 to 440)	42	403	Das et al. (1994)
10	G5	FT5	CATGTACTCGTTGTTACGTC (nt 779-760)	55	780	Gouvea et al. (1994a)
11	G6	DT6	CTAGTTCCTGTGTAGAATC (nt 499-481)	55	500	Gouvea et al. (1994a)
12	G8	HT8	CGGTTCCGGATTAGACAC (nt 273-256)	55	274	Gouvea et al. (1994a)
13	G9	9T-9B	TATAAAGTCCATFGCAC (nt 131 to 147)	42	110	Das et al. (1994)
14	G10	ET10	TTCAGCCGTTGCGACTTC (nt 714-697)	55	715	Gouvea et al. (1994a)
15	G11	BT11	GTCATCAGCAATCTGAGTTGC (nt 336-316)	55	337	Gouvea et al. (1994a)
16	[P1]	pNCDV	CGAACGCGGGGGTGGTAGTTG (nt 269-289)	55	622	Gouvea et al. (1994b)
17	[P4]	2T-1	CTATTGTTAGAGGTTAGAGTC (nt 474 to 494)	48	483	Gentsch et al. (1992)
18	[P5]	pUK	GCCAGGTGTCGCATCAGAG (nt 336-354)	55	555	Gouvea et al. (1994b)
19	[P6]	3T-1	TGTTGATTAGTTGGATTCAA (nt 259 to 278)	48	423	Gentsch et al. (1992)
20	[P7]	POSU	CTTTATCGGTGGAGAATACGTCAC (nt 389-412)	55	502	Gouvea et al. (1994b)
21	[P8]	1T-1	TCTACTTGGATAACGTGC (nt 339 to 356)	48	345	Gentsch et al. (1992)
22	[P9]	4T-1	TGAGACATGCAATTGGAC (nt 385 to 402)	48	391	Gentsch et al. (1992)
23	[P11]	pB223	GGAACGTATTCTAATCCGGTG (nt 574-594)	55	314	Gouvea et al. (1994b)

manufactures instructions using an RNA extraction kit (Qiagen Ltd.) and subjected to RNA-PAGE (7.5% resolving gel and 5.0% stacking gel). The gel was resolved by electrophoresis at 100 V at room temperature using 1X Tris-glycine running buffer (5 mM Tris, 50 mM glycine) in the discontinuous buffer system till it reached the bottom followed by silver staining (Herring et al., 1982). For detection of group A rotaviruses, reverse transcription and polymerase chain reaction (RT-PCR) were carried out to partially amplify the gene segment 8 or 9 (VP7 gene) of all RVA using forward primer Rota 1 and the reverse primer Rota 2 (Husain et al., 1995). The amplified products were then analyzed by electrophoresis on 1.5% agarose gel containing 2 μl ethidium bromide (10 mg/ml) in Tris-borate buffer, visualized with a UV transilluminator and photographed by gel documentation system.

2.3. G and P genotyping by RT-PCR

For G- genotyping, full-length VP7 (nt 1,062 bp) gene was amplified followed by two multiplex semi-nested PCRs using several sets of G genotype-specific primers (first multiplex- 9T1-1, 9T1-2, 9T-3P, 9T-4, 9T-9B; second multiplex - FT5, DT6, HT8, ET10, BT11) (Table 1) (Gouvea et al., 1990, 1994a; Das et al., 1994). For P- genotyping, the partial-length VP4 (nt 11–887) gene was amplified followed by two multiplex semi-nested PCRs using several sets of P genotype-specific primers (third multiplex-1T-1, 2T-1, 3T-1, 4T-1; fourth multiplex –pUK, POSU, pNCDV, pB223) (Table 1) (Gentsch et al., 1992; Gouvea et al., 1994b). The amplified products were analyzed by electrophoresis on 1.5% agarose gel as described earlier. Six positive PCR products of VP7 genes and 8 of VP4 genes were sequenced using commercial services (Genomics Corp-Xcelris Ahmedabad, India). The sequences obtained were analysed by the Basic Local Alignment Search Tool (BLAST) in NCBI and RotaC classification tool program proposed by the *Rotavirus Classification Working Group* (RCWG) to identify the genotypes (Maes et al., 2009).

2.4. Sequencing and sequence analysis

Multiple sequence alignment of the partial VP7 and VP4 genes of human rotaviruses of the present study were carried out with already reported sequences available in GenBank, NCBI using BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences of the isolates obtained were edited using the bioedit program in the DNA Star software. Multiple sequence alignment and phylogenetic tree of the partial VP7 and VP4 genes were constructed using the Megalign program (MEGA 7 software) (Kumar et al., 2016). The DNA sequences were aligned using the Clustal W method. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei (1987) after finding the best

suitable model in Mega 7 software. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method (Tamura, 1992) and are in the units of the number of base substitutions per site. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. Partial nucleotide sequences of the VP7 and VP4 genes of group A rotaviruses of the present study were deposited in GenBank under the accession nos. KP793014, KP793015, KP793016, KP793017, KP793018, KP793019, KP793020, KP793021, KP793022, KP793023, KP793024, KP793025, KP793026, and KP793027.

3. Results and discussion

3.1. Detection of rotavirus by RNA-PAGE and RT-PCR

Screening of the dsRNA (extracted from fecal samples) by RNA-PAGE yielded a typical 11 banded pattern of rotavirus due to its segmented genome (Figure 1). Among 158 samples examined by PAGE, 63 (39.87%) samples yielded typical rotavirus electrophoretic migration profiles, of which 15.82% were from Goa and 24.05% from Meghalaya. However, the positivity of rotavirus by PAGE was 43.10% in Goa and 38% in Meghalaya. Variations in the electropherotype pattern were observed where the long pattern was observed in 50 strains, while, short patterns in 13 strains. Thus, rotaviruses of the long electrophoretic profiles were the most predominant circulating strains in these two states. A long pattern seems to be dominant in the majority of the earlier studies (Broor et al., 1993; Dubal et al., 2013). More than 11 bands or mixed infections have also been reported earlier (Minakshi, 1999). The finding of extra viral RNA segments suggested the possibility of simultaneous or sequential infection by more than one electropherotype or a modification in the length of RNA segments during infection (Minakshi, 1999).

The standardized reverse transcription-polymerase chain reaction (RT-PCR) assay yielded expected products of the size of 304 bp on amplification of the VP7 gene using rota1 and rota2 primers (Figure 2). The positivity of rotavirus by RT-PCR in Goa was found to be 39.65%, while, 36.0% in Meghalaya (Table 2). The results of the RT-PCR also showed a similar trend as that of PAGE. Earlier studies also reported 38–40% rotavirus positivity during 2012–2016 (Girish Kumar et al., 2016; Banerjee et al., 2018). It was also observed that 96% of long and 84.61% short electropherotype profile samples were found to be positive by RT-PCR by rota1-rota2 primers. Additionally, 2 samples from Meghalaya and 1 from Goa which were negative by PAGE could be detected positive by RT-PCR targeting the partial VP7 gene. Thus, overall 62/158 (39.24%) stool samples were found to be positive for group A rotaviruses by RT-PCR.

In the present study, two simultaneous methods used for detection of rotavirus revealed interesting results where 3 samples which were
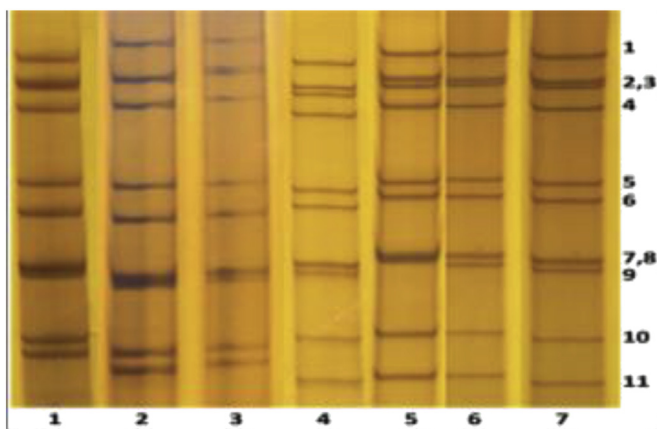

Figure 1. RNA-PAGE showing 11 bands of dsRNA of rotavirus. Lane 1, 3 & 6: Positive fecal samples.
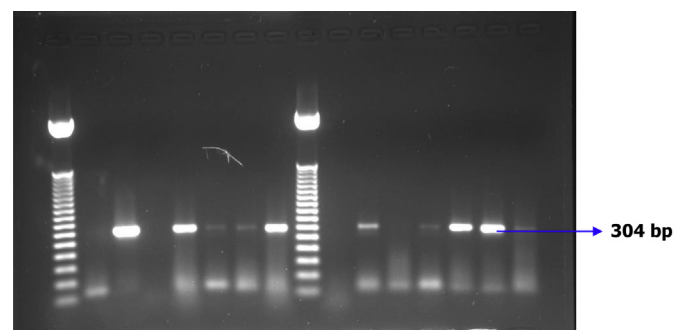

Figure 2. Detection of rotavirus by RT-PCR showing positive fecal samples using Rota 1 and Rota 2 primers. Lane 1 & 9: DNA ladder (50 bp). See supplementary material for full image.

Table 2. Details of the samples analysed for detection and genotypic characterization of rotavirus.

| State | No. of samples tested | No. of samples positive by PAGE | Electrophoretic pattern | No. of samples amplified by RT-PCR | | | G genotypes identified (no. of strains) | P genotypes identified (no. of strains) |
				Rota1 Rota2 (VP7 gene)	Beg9End9 (VP7 gene)	Con3Con2 (VP4 gene)		
Goa	58	25	Long = 19	18	12	13	G1(5), G3(2), G9(1)	P[8](7), P[6](5)
			Short = 6	5	4	4	G1(2)	P[8](2), P[4](1)
Meghalaya	100	38	Long = 31	30	18	20	G1(8), G12(3), G9(1)	P[4] (3), P[8](9), P[6](6)
			Short = 7	6	2	4	G1(2), G2(2)	P[4](3)
Total	158	63	63	59	36	41		

negative by PAGE could be detected by RT-PCR though only 59 samples (out of 63 PAGE positive samples) could be detected by RT-PCR (Table 2). Similar observations of simultaneous utilization of two or more tests for maximizing detection abilities have been reported earlier (Husain et al., 1995; Rawat et al., 2014). The low positivity by RT-PCR as compared to PAGE was also reported earlier both in humans and bovine samples (Wani et al., 2004). The presence of non-specific inhibitors in the faecal samples that were carried through the extraction procedure might be responsible for low detection rates by PCR (Xu et al., 1990; Wani et al., 2004). These inhibitors could be precipitated by ethanol and purified using CF11 cellulose (Xu et al., 1990; Parashar et al., 2003).

In the present study, overall 39.87% and 39.24% samples were found to be positive for rotavirus by PAGE and RT-PCR, respectively. An earlier study from Western India reported 47% of samples from unvaccinated children collected from Pune and 11.9% samples from Aurangabad to be positive for rotavirus (Jain et al., 2016; Maher et al., 2016). Higher positivity was observed in Goa than Meghalaya. Many diarrhoeal episodes and outbreaks have been reported in winter (Sherchand and Haruki, 2004). The colder climatic conditions of Meghalaya, located in North-Eastern part of India may favor the rotaviral episodes in children. A number of studies carried out in different parts of India revealed varied prevalence rates of rotavirus from 5 to 71% in hospitalized children less than 5 yr of age with acute gastroenteritis (Jain et al., 2001a; Broor et al., 2003; Kelkar et al., 1999). Previously reported region-wise prevalence of rotaviral diarrhoea varied from 6-45% in North India (Broor et al., 1993; Jain et al., 2001a); 16–22% from South India (Bhat et al., 1985; Brown et al., 1988); 38–41% from East India (Jain et al., 2001b; Banerjee et al., 2018) and 28–30% in West India (Kelkar et al., 1999; Jain et al., 2001b).

3.2. G and P genotyping of rotaviruses

All the samples positive by PAGE and RT-PCR were subjected to G and P genotyping by amplifying full-length VP7 and partial-length VP4 gene

using Beg9End9 and Con3Con2 primers giving the expected PCR product of 1,062 and 876 bp, respectively (Figure 3a, b). The dsRNA of rotavirus extracted from 36 samples could be transcribed and amplified by beg9end9 primers for further G genotyping, while, 41 by con3con2 primers for P genotyping. These differences were confirmed in the previous report (Mirza et al., 2018) where either more or less number of VP7 genes than VP4 and *vice versa* could be amplified. All the positive samples (n = 63) were subjected to the nested PCR for genotypic determination where 32P and 26G genotypes could be identified. In Goa state, the G1P [8] (4 strains), G1P[6] (3 strains) and G3P[8] (2 strains) were found to be the most prevalent strains followed by the G9P[8] (single strain). Similar trend was also noted for Meghalaya state, where, G1P[8] (6 strains) and G1P[4] (4 strains) could be identified. Other genotypes such as G2 and G12 among G type and P[6] and P[8] among P types have also been identified from these areas.

Six PCR products positive for the VP7 gene and 8 for the VP4 gene were randomly selected for nucleotide sequencing. From the blast results, it was observed that the 6 samples from Goa showed G1 genotype specificity, while, 3 samples showed P[8] specificity indicating the G1P [8] rotavirus was in circulation in Goa. From the present findings, it was noted that the G1P[8] rotavirus circulated in Goa and was a cause of diarrhea in children and infants. The G1P[8] was the predominant rotavirus genotype (56.3% and 62.7%) observed during the surveillance studies during 2012–2016 (Girish Kumar et al., 2016; Giri et al., 2019). In Meghalaya state, 3 strains showed P[8] and 2 showed P[4] genotypes specificity, while, the G genotype was not be typable. These data are consistent with the studies from various countries including Saudi Arabia and Ghana, which indicate that genotype G1P[8] is the most commonly reported and severe diarrhea associated strain followed by G2P[4] and G9P[8] genotypes, respectively (Almalki, 2018; Damanka et al., 2019). In contrast, the proportion of G3 strains (P[8] and P[6]) was significantly higher with the P[8] as the dominant genotype (80.3%) followed by P[6] (14.6%) among patients admitted to the hospital with severe dehydrating diarrhea (Athiyyah et al., 2019) was observed in Indonesia. Limited
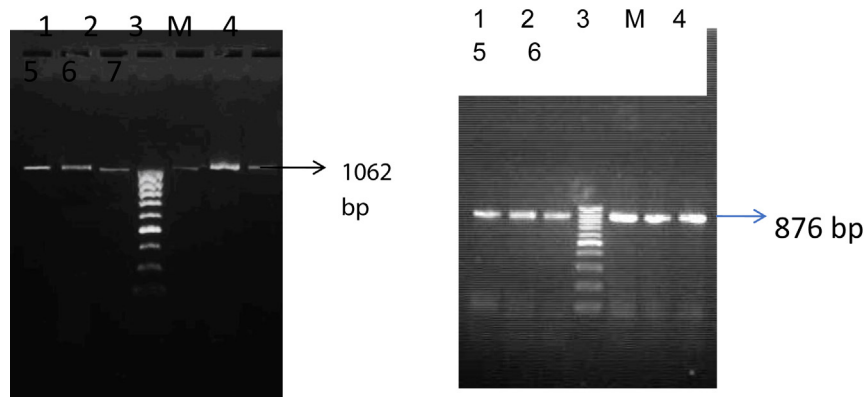

Figure 3. a. PCR for full length amplification of VP7 gene (1062 bp). Lane M: DNA ladder (100 bp); Lane 1–6: PCR products (1062 bp) of VP7 gene. See supplementary material for full image. b. Amplification of partial length of VP4 gene (876 bp) Lane M: DNA ladder (100 bp); Lane 1–6: PCR products (876 bp) of VP4 gene. See supplementary material for full image.
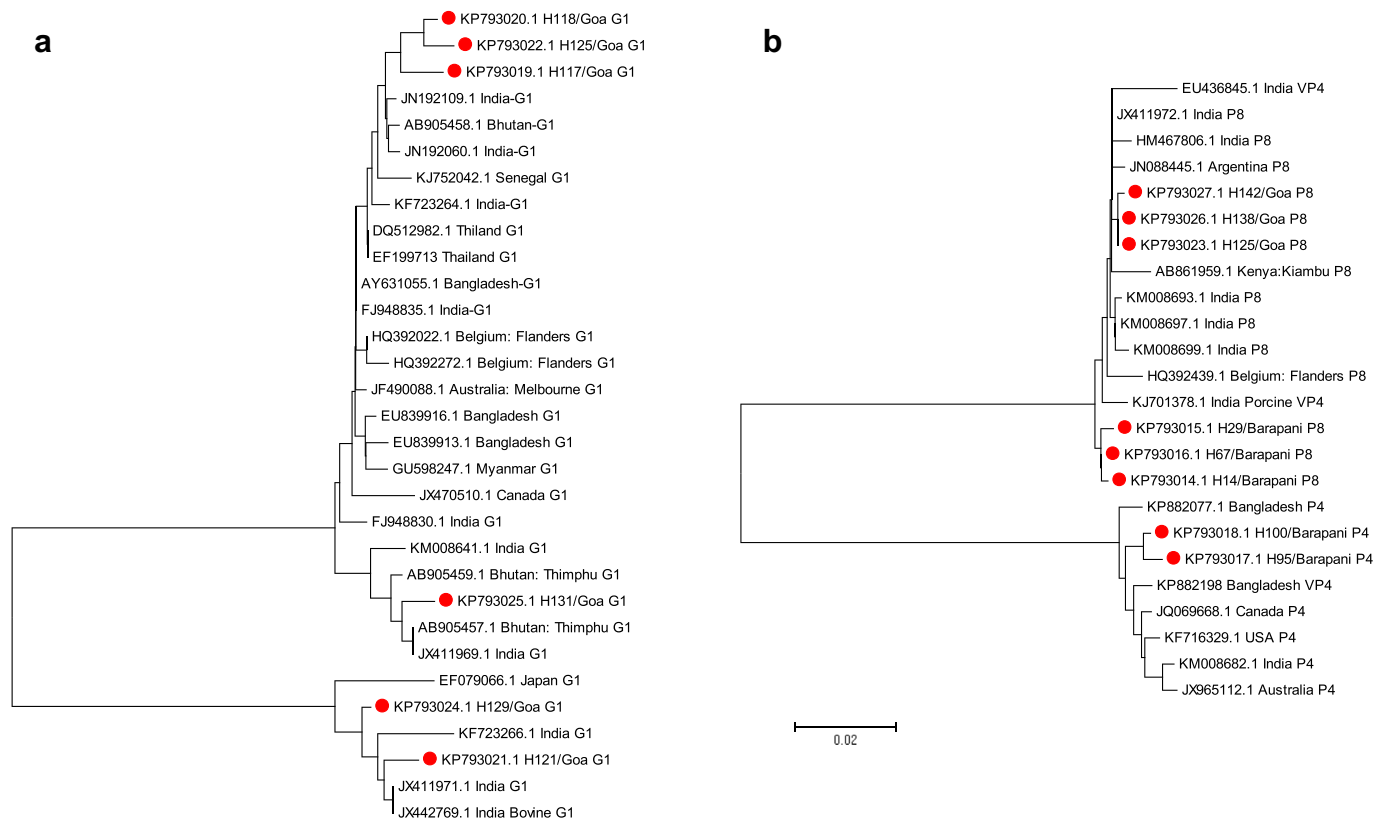
Figure 4. a. Phylogenetic tree of the all G genotypes. The optimal tree with the sum of branch length = 0.15134252 is shown. The analysis involved 31 nucleotide sequences. There were a total of 932 positions in the final dataset. b. Phylogenetic tree of the all P genotypes. The optimal tree with the sum of branch length = 0.23718660 is shown. The analysis involved 24 nucleotide sequences. There were a total of 778 positions in the final dataset.

studies on rotaviral genotypes in Eastern India revealed G1 (57%) as the most predominant type followed by mixed types (20%), G2 (13%) and G4 (9%) (NICED, Annual Report, 2002–03), while, G1P[8] genotype was frequently isolated from Western India (Chitamber et al., 2012; Jain et al., 2016; Maher et al., 2016). However, shift in dominating strains from the G1P[8] to G3P[8] was observed in 2016 from Eastern part of India (Banerjee et al., 2018). National *Rotavirus* surveillance in India also showed that the G1P[8] strain was the one among the two most common strains from December 2005 to November 2007 (Kang et al., 2009).

3.3. Sequencing and sequence analysis

Partial nucleotide sequences of the VP7 and VP4 genes of group A rotaviruses were deposited in GenBank. All the G genotype sequences obtained in the present study separated in two clusters (Figure 4a) where three sequences (KP793019, KP793020, and KP793022) formed a close sub-cluster and were closely related to the other sequences from India (JN192109.1, JN192060.1, and KF723264.1) Bhutan (AB 905458.1) and Senegal (KJ 752042.1). Another one partial sequence (KF793025.1) reported in this study was closely related to the two sequences from Bhutan (AB905459.1, AB905457.1) and one from India (JX411969.1). The other two sequences of the VP7 gene (KP793021, KP793024) which formed a separate cluster were observed to be closely related to other Indian rotavirus sequences (KF732366.1, JX411971.1, JX442769.1). Interestingly, it was observed that JX442769.1 sequence was of bovine origin. The comparative analysis demonstrated that G1 (n = 6) exhibited ≥92% identity with other Indian and worldwide G1 genotypes with the maximum genotypes showing close relatedness (≥98% identity). However, in the present study, G1 genotypes showed a very close identity (≥99%) with the G1 genotypes reported from India, Thailand, Bangladesh, and Senegal.

In the present study, the P genotype sequences also formed the two separate groups. However, 3 sequences each from strains detected from Goa (KP793026.1, KP793023.1, KP793027.1) and Meghalaya (KP793015.1, KP793016.1, KP793014.1) were observed in one cluster and indicated close relatedness among genotype P[8]. The other two sequences (KP793018.1, KP793017.1) of the genotype P[4] rotavirus detected from Meghalaya were closely related and formed a separate cluster. The comparative analysis demonstrated that all the three P[8] genotypes detected from Goa exhibited close relatedness with each other, while, ≥99% similar to P[8] genotypes from Meghalaya. All the P[4] and P[8] genotypes also showed close relatedness (≥98%) with the same genotypes submitted from various countries (Figure 4b). However, there was a great diversity of ≥15% between P[4] and P[8] genotypes.

4. Conclusions

The study observed the circulation of G1P[8], G1P[6] and G3P[8] in Goa and the presence of G1P[8] and G1P[4] genotypes in Meghalaya. Nucleotide sequences of G1, P[8] and P[4] genotypes revealed high homology to previously reported similar genotypes especially from India and Bangladesh. However, nucleotide diversity within the same and different genotypes needs to be studied and explored continuously. Therefore, the current rotavirus vaccines though it is currently effective, their efficacies need to be evaluated and continuous monitoring of rotavirus genotypes in diarrhoeal episodes in children of these two tourist places needs to be continued because of frequent movement of human population throughout the year from all over the world. Further, a majority of the rural human population is living in close proximity with their livestock which may favor the transmission of different types of rotaviruses between them.

Declarations

Author contribution statement

Abhay Raorane: Conceived and designed the experiments; Performed the experiments.

Zunjar Dubal: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sandeep Ghatak: Conceived and designed the experiments.

Michael Mawlong, B. Susngi, Virendra Gaonkar: Contributed reagents, materials, analysis tools or data.

Eknath Chakurkar: Analyzed and interpreted the data.

Sukhadeo Barbuddhe: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflicts of interest.

Additional information

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