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Research paper

Clinical and genetic characteristics of unusual G12P[11] rotavirus strains recovered from neonates: A study from Pune, Western India

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ABSTRACT

Rotavirus infections in neonates are generally nosocomial, and differ from pediatric infections both clinically and epidemiologically. These infections are predominantly asymptomatic and often associated with unusual strains. Globally, so far limited data is available on rotavirus infections in neonates admitted at Neonatal Intensive Care Unit. The aim of the present study is to determine the prevalence of rotavirus among neonates and to study their genetic characteristics. Stool specimens (n = 701) collected from neonates (n = 621) admitted during April 2016 to March 2018 mainly for prematurity, low birth weight and associated respiratory distress syndrome from two hospitals from Pune were tested for rotavirus, genotyped and representative strains were sequenced for the genes encoding outer capsid proteins, VP7 and VP4. Rotavirus was detected in 24.31% neonates. Majority of rotavirus infected neonates (98.68%) were asymptomatic. Peak rotavirus antigen detection (91.38%) occurred during the first 2 weeks of admission. Low, very low and normal birth weight neonates with gestational age ≥28 weeks had significantly higher rotavirus infection than those with extreme low birth weight with gestational age < 28 weeks. Rotaviral infections occurred almost evenly throughout the year without an apparent peak in colder months. Predominance of unusual G12P[11] strains (97.1%) was observed. Phylogenetic analysis of the partial VP7 coding gene revealed all G12 strains clustered in lineage III and shared 96.94%–100% (nucleotide) and 96.26%–100% (amino acid) identities among themselves, and 95.69%–98.98% (nucleotide) and 94.77%–98.98% (amino acid) with other lineage III G12 strains respectively. Similarly VP4 partial gene sequences of P[11] study strains shared 97.5%–100% (nucleotide and amino acid) identities among themselves and highest 93.34%–94.53% (nucleotide) and 93.57%–94.64% (amino acid) identity with vaccine strain 116E, G9P[11]. The study highlights high frequency of unusual G12P[11] strains among neonates for the first time in western India and reaffirms limited strain diversity in this population. The knowledge of neonatal strains is important for estimating the efficacies of rotavirus vaccines.

1. Introduction

Nosocomial infections represent a significant epidemiologic and economic problem worldwide (Gundeslioglu et al., 2016). Among enteric viruses, rotavirus (RV) is the most prevalent cause of nosocomial gastroenteritis (Ogilvie et al., 2012). Group A rotaviruses (RVA) are the most significant etiological agents of viral gastroenteritis in infants and young children. However, rotavirus infections (RVI) in neonates occur generally in nosocomial setting with substantial clinical and epidemiological difference with respect to older children. In neonates, RVI are predominantly asymptomatic or mild and are often associated with unusual rotavirus strains that are different from those circulating in older children (Cicirello et al., 1994; Haffejee, 1991; Kilgore et al., 1996; Linhares et al., 2002). RV nosocomial infections can occur throughout the year, although increased incidence in neonates is reported during winter months parallel with high RVI in children (Ray et al., 2007). Moreover, neonatal strains appear to be notably stable and can persist in particular settings for long periods of time in spite of hygiene measures (Koukou et al., 2015). Regardless of lack of associated disease, neonatal strains have gained importance as they confer

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protection against subsequent RV-associated diarrhea (Bishop et al., 1983; Bhan et al., 1993). A distinguishing feature of neonatal rotavirus infection is the limited spectrum of RV strains, compared with the high strain diversity observed among older children. In addition; these strains may provide opportunities for early discovery of novel RV strains that may later spread into the community. For instance, VP4 genotype P[6], known as neonatal RV strain with different G type combinations were detected in newborns years before they were established as a cause of diarrhea in older children (Hoshino et al., 2003).

The World Health Organization estimated that globally 215,000 (197,000–233,000) child deaths occurred during 2013 due to RV infections. India, Nigeria, Pakistan, and Democratic Republic of Congo accounted for approximately half (49%) of all estimated rotavirus deaths in 2013 (Tate et al., 2016). To overcome this disease burden, two oral live attenuated rotavirus vaccines, Rotarix™, the monovalent attenuated human RV vaccine (GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq® the pentavalent bovine-human reassortant vaccine (Merck & Co, Inc., Whitehouse Station, NJ) have been licensed in > 100 countries (http://www.view-hub.org/). However, differences in the protective efficacies of these vaccines against severe RV diarrhea in high income (> 85%), middle (72–83%) and low income (39–49%) countries have been predicted (Lopman et al., 2012). In addition to these two commercial and WHO prequalified vaccines, three other live attenuated orally administered vaccines have been licensed for national markets: ROTAVAC® was licensed for use in India in 2014; Rotavin-M1®, in Vietnam in 2007; and Lanzhou Lamb Rotavirus Vaccine, in China in 2000. ROTAVAC® manufactured by Bharat Biotech, is a monovalent human bovine rotavirus vaccine (116E). The 116E is a naturally occurring reassortant strain G9P[11], isolated from asymptomatic neonate at All India Institute for Medical Sciences (AIIMS), Delhi, India, and was regarded as well adapted to the neonatal gut and naturally attenuated. The vaccine development is based on the observation that neonatal RVI appear to elicit an immune response that affords significant protection against severe RV associated diarrhea (Glass et al., 2005). Based on similar observation another human neonatal vaccine strain RV3-BB, (G3P[6]) isolated from asymptomatic newborns in 1977 in Australia is under clinical development. Clinical testing with cell culture-adapted RV3 demonstrates that it is safe, attenuated and immunogenic in all age groups (Bines et al., 2018).

Although prevalence of RVs and their genotypes in children are well documented worldwide, very limited data are available for neonates admitted at Neonatal Intensive Care Unit (NICU) (Shim et al., 2012; Tai et al., 2012; Koukou et al., 2015). Recent studies have reported association of neonatal RVI with complications such as abdominal distention, bloody mucoid stools, feeding difficulty, encephalopathy, necrotizing enterocolitis (NEC) as against typical gastrointestinal symptoms such as diarrhea, vomiting, dehydration in older children (Ku et al., 2018; Shim et al., 2012; Tai et al., 2012). Epidemiological data regarding RVI within NICU populations are still inadequate. Moreover, RVs once introduced in hospital settings such as NICUs, can become a troublesome problem of nosocomial infections. Significant proportions of asymptomatic RVI shed virus for prolonged periods, thereby contaminating the environment and facilitating the spread of the virus (Koukou et al., 2015). Neonatal infections due to RV have rarely been surveyed and only few studies are reported from India and other countries (Bhan et al., 1993; Kilgore et al., 1996; Linhares et al., 2002; Ray et al., 2007; Tai et al., 2012; Koukou et al., 2015). The absence of an effective surveillance system has resulted in under-reporting of nosocomial RVI in NICUs. So far no such studies have been undertaken in western India where RV is the major enteric viral pathogen detected in children, both in sporadic cases and outbreaks of acute gastroenteritis. Keeping in view of the previous reports available in the area of research from India and other countries, the present study was designed to estimate the prevalence of RVs among neonates admitted at NICU, to value the associated clinical features and also to characterize RV strains in order to determine whether these strains are similar or distinct from those circulating in the community.

2. Methods

2.1. Study design

The surveillance was conducted during two consecutive years spanning from April 2016 to March 2018 at National Institute of Virology (NIV) and NICUs of two major tertiary care hospitals, KEM Hospital and Smt. Kashibai Navale Medical College and General Hospital, (SKNMC&GH) Pune, western India. KEM Hospital is located centrally in Pune while SKNMC&GH is ~ 13 km away from the city and patients belonging to middle and low socio economic status are referred to these hospitals, respectively. Neonates admitted are mostly born in the maternity ward of these hospitals and in few cases referred by other local hospitals. The study was approved by the institutional human ethics committee of NIV and both the hospitals.

2.2. Sample collection

Stool samples were collected randomly from neonates admitted at NICUs with the complications for prematurity e.g., low birth weight, associated respiratory distress, sepsis, asphyxia, etc. Written informed consents were obtained from parents/guardians. Additional 2 to 4 stool samples were collected from few neonates on every 7th day after collection of first sample to determine whether the frequency of neonatal RV infections increase with the duration of hospitalization. All the stool samples collected from the hospitals were transported to NIV on wet ice and stored at 4°C until RV testing.

2.3. Clinical information

Demographic and clinical data for gestational age, birth weight, gender, labor, referral facility, clinical manifestations on and during hospitalization, duration of stay of neonates were recorded in case reporting forms (CRF).

2.4. Testing for rotavirus antigen

The stool specimens were tested for RVA antigen using a commercial ELISA kit (Premier Rotaclone, Meridian Bioscience, Inc., Cincinnati, OH, USA) as per the manufacturer's instructions. All specimens were stored at −20°C for further testing. Neonates were considered as RV positive if at least one of the stool samples was tested positive.

2.5. G- and P-typing and nucleotide sequencing and phylogenetic analyses

The viral nucleic acids were extracted from 20% (w/v) suspensions of all RV positive stool specimens using spin columns (Qiagen, Hilden, Germany) as per manufacturer's instructions. All RV positive samples were genotyped using VP7 and VP4 genes based multiplex reverse transcription (RT)-PCR according to the methods previously described (Chitambar et al., 2014). The viral RNA was subjected to one step RT-PCR (Qiagen, Hilden, Germany) using the sets of outer primers: 9Con1-L/VP7-R deg.; Con3/Con2 and oligonucleotide primers that could amplify VP7 genotypes G1- G4, G8-G10 and G12 and VP4 genotypes P[4], P[6], P[8], P[9]; P[10] and P[11]. For samples originally non-typeable for P type, a new set of primers specific for P[11] was designed (Table 1). All PCR products were analyzed on 2% agarose gels by electrophoresis using Tris Acetate EDTA (TAE) buffer, pH 8.3, containing ethidium bromide (0.5 μg/ml) and visualized under UV illumination.

First round PCR amplicons of representative rotavirus strains (n = 20) were sequenced using BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). The sequences were read from automated DNA sequencer ABI-PRISM 3720 Genetic

Table 1
Primers specific for rotavirus P [11] type.

Name	Sequence (5′-3′)	Region (nt) [a]	Size (bp) [b]	Reference
2126F	GAGTTAGTTACTGATTCGCCCG	2126–2148	227	This study
2352R	GTCACATCCTCATACAAA	2335–2352		

[a] Nucleotide.
[b] Base pairs.

analyzer (Applied Biosystems) after purification using the DyeEx 2.0 spin columns (Qiagen, Hilden, Germany). Sequence identity was determined through RotaC, a web-based RVA genotyping tool (http://www.regatools.be/rota20/). Sequences were aligned and the most optimal nucleotide substitution models were determined in Mega version 6 software (Tamura et al., 2013). Maximum likelihood phylogenetic tree construction was performed using the General Time Reversible (GTR + G) and Tamura Nei (G + I) models for VP7 and VP4 respectively. The reliability of phylogenetic trees was tested by applying bootstrap test with 1000 bootstrap replications.

Non-typeable samples were further subjected to RT-PCR for VP6 gene using primers VP6F and VP6R to rule out the possibility of any false positivity of RVA by ELISA (Gomara et al., 2002).

2.5.1. Nucleotide sequence accession numbers

The nucleotide sequences of VP7 and VP4 genes of the study strains have been submitted in GenBank under the accession numbers MH559132-MH559151 and MH559152-MH559171, respectively.

2.6. Statistical analysis

The statistical analysis of demographic and clinical data was carried out using OpenEpi 3.01 (Dean et al., 2018). Chi-square test was used for analyzing 2×2 or mxn contingency tables. p-values < 0.05 were considered as statistically significant.

3. Results

A total of 701 stool specimens collected from 621 neonates admitted at NICUs of two local hospitals during April 2016 to March 2018 in Pune, were included in the study. Additional 2 to 4 stool samples were collected from 55 neonates on 7th day after collection of first sample. RV was detected in 151 (24.31%) neonates, of which 149 (98.68%) neonates were asymptomatic. RV positivity was significantly higher in

Table 3
Clinical characteristics of Rotavirus positive and negative neonatal cases on admission.

Clinical manifestations	Total n = 621	Rotavirus negative (%) n = 470	Rotavirus positive (%) n = 151	p value
Prematurity		311 (66.17)	108 (71.52)	0.222
Respiratory Distress Syndrome		286 (60.85)	85 (56.3)	0.320
Low birth Weight		375 (79.8)	127 (84.1)	0.241
Jaundice		98 (20.85)	36 (23.84)	0.437
Suspected Sepsis		46 (9.8)	16 (10.6)	0.773
Vital Unstable		30 (7.23)	4 (2.64)	0.079
Feeding intolerance		25 (5.32)	7 (4.63)	0.741
Birth Asphyxia		**27 (5.74)**	**2 (1.32)**	**0.025**
Hypoglycemia		**12 (2.55)**	**12 (7.94)**	**0.002**
Congenital Heart Defects		18 (3.82)	3 (1.99)	0.277
Seizures or tremor		13 (2.8)	5 (3.31)	0.728
Cold Stress		12 (2.55)	2 (1.32)	0.376
Vomiting		5 (1.06)	5 (3.31)	0.056
Abdominal distention		7 (1.49)	1 (0.66)	0.433
Fever		5 (1.06)	2 (1.32)	0.792
Diarrhea		5 (1.06)	2 (1.32)	0.792
Necrotizing enterocolitis (NEC)		5 (1.06)	0	–
Other Variables				
Meningomyelocele		3 (0.64)	1(0.66)	0.974
Hypoxic Ischemic Encephalopathy		1 (0.21)	1(0.66)	0.396
Tracheoesophageal fistula		5 (1.06)	0	–
Imperforate Anus		0	2 (1.32)	–
Duodenal atresia		0	1 (0.66)	–
Cystic Fibrosis		0	1 (0.66)	–
Twin to twin Transfusion Syndrome (TTTS)		0	1 (0.66)	–
Medullary Nephrocalcinosis		0	1 (0.66)	–
Anorectal Malformation		0	1 (0.66)	–
Gastrochisis		2 (0.425)	0	–
Jejunal atresia		2 (0.425)	0	–
Cleft lip		2 (0.425)	0	–
Down's Syndrome		2 (0.425)	0	–
Hirschsprung disease		1 (0.212)	0	–
Hydronephrosis		1 (0.212)	0	–
Omphalocele		1 (0.212)	0	–

A bold significe the clinical manifestation which are significantly associated with RVI (birth asphyxia and hypoglycemia).

Table 2
Demographic characteristics of Rotavirus positive and negative neonatal cases in NICUs Pune, western India (April 2016 –March 2018).

Demographics	Total n = 621	Rotavirus negative (%) n = 470	Rotavirus positive (%) n = 151	p value
Gender				
Male		262 (55.74)	94 (62.25)	0.159
Female		208 (44.25)	57 (37.75)	
Birth weight (g)				
Extreme LBW (≤1000 g)		59 (12.55)	8 (5.30)	0.017
VLBW (1001–1500 g)		130 (27.66)	42 (27.81)	
LBW (1501–2499 g)		186 (39.57)	77 (50.99)	
Normal birth weight (≥2500 g)		95 (20.21)	24 (15.9)	
Gestational age (wk)				
Extreme pre term (< 28 wk)		35 (7.44)	5 (3.31)	0.024
Very pre term (28 to < 32 wk)		120 (25.53)	34 (22.52)	
Moderate to late pre term(32 to < 37wk)		156 (33.2)	69 (45.7)	
Full term ≥37 (wk)		159 (33.9)	43 (28.48)	
Labour				
Normal vaginal delivery		174 (37.02)	42 (27.81)	0.039
Lower section cesarean section (LSCS)		296 (62.98)	109 (72.2)	
Referal facility				
Inborn		418 (88.94)	134 (88.74)	0.947
Outborn		52 (11.06)	17 (11.25)	

Table 4
Clinical characteristics associated with rotavirus infection in neonates during hospitalization.

	Total n = 621	Rotavirus negative (%) n = 470	Rotavirus positive (%) n = 151	p value
Fever		2 (0.42)	1(0.66)	0.715
Diarrhea		1 (0.21)	2 (1.32)	0.086
Vomiting		100 (21.3)	39 (25.83)	0.244
Feeding intolerance		114 (24.25)	30 (19.87)	0.267
Abdominal distention		38 (8.08)	13 (8.6)	0.838
Necrotizing enterocolitis (NEC)		14 (2.9)	4 (2.64)	0.834
Weight Loss		10 (2.13)	7 (4.63)	0.100
Culture proven Septicemia		64 (13.61)	16 (10.6)	0.335

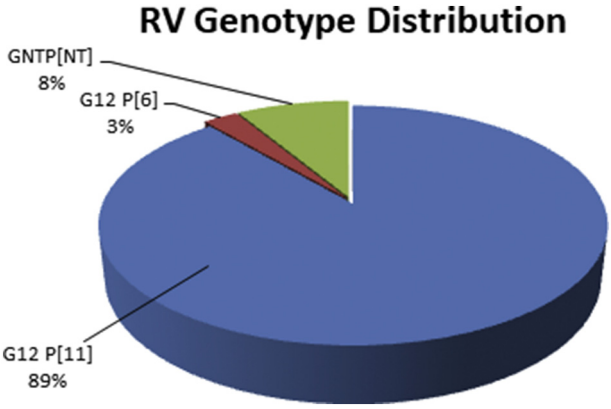

Fig. 1. Distribution of rotavirus genotypes in neonates at NICUs in Pune, western India (April 2016 –March 2018).

the year 2016–17 as compared to 2017–18 (29.61% vs. 18.28%, p = 0.0012).

Analysis of demographic characteristics of RV infected neonates is summarized in Table 2. Percentage of males with RVI did not differ significantly from females. Although RV antigen could be detected from day 1 to day 28 post admission, 91.38% of the cases occurred within the first 2 weeks on admission. The mean age of neonates at the day of sample collection was 6.8 days (range 1 to 28 days). Significantly lower RVI cases were observed in extreme preterm neonates compared with moderate to late, very and full term neonates (p = 0.024). Likewise RVI in neonates with extreme low birth weight (LBW) was significantly lower than low, very low and normal birth weight (p = 0.017). In general low, very low and normal birth weight neonates with gestational age ≥28 weeks had significantly higher RVI than those with extreme LBW with gestational age < 28 weeks. Also significantly more neonates born by lower segment cesarean section than normal vaginal delivery were found infected with RVI (p = 0.039).

Clinical profile of neonates on admission is shown in Table 3. Low birth weight (< 2500 g) and respiratory distress were the most frequent cause of admission. It was observed that, hypoglycemia and birth asphyxia were significantly higher in cases with RVI than without RVI.

Comparisons of the clinical characteristics of neonates with and without RVI during hospitalization revealed no significant differences in fever, diarrhea, vomiting, weight loss feeding intolerance, abdominal distention, Necrotizing enterocolitis (NEC) and Septicemia (Table 4). Similarly, length of hospital stay did not differ significantly between the two groups (p = 0.5136) (data not shown).

In the present surveillance, the multiplex PCR carried out for genotyping of RV strains (n = 151) showed amplification of VP7 and VP4 genes in 138 (91.4%) whereas 13 (8.6%) remained G/P non-typeable (Fig. 1). Moreover, 13 non-typeable strains did not show amplification in first round PCR. Since all non-typeable strains showed amplification for VP6 gene, it was confirmed that they were true positive strains.

Among 138 strains typed for both VP7 and VP4 genes, 20 (17.54%) strains initially were P non-typeable. These strains showed P[11] specificity only after inclusion of newly designed VP4 primers. Interestingly, unusual G12P[11] strain was detected in 134 of 138 (97.1%) strains with both G and P types and was observed as the most predominant type throughout the study. Only 4 stool specimens (2.9%) showed G12P[6] genotype specificity and all four G12P[6] strains were detected in May 2017.

There was no seasonality to RVI, with infections occurring almost evenly throughout the year without an apparent peak in colder months (p = 0.924) suggestive of a different circulation pattern than in children.

Phylogenetic analysis of the partial VP7 coding gene of G12 neonatal strains revealed that all the study strains clustered in lineage III (Fig. 2). The sequence analysis revealed 96.94%–100% (nucleotide) and 96.26%–100% (amino acid) identities among G12 study strains, and 95.69%–98.98% (nucleotide) and 94.77%–98.98% (amino acid) with other lineage III G12 strains, respectively. Similarly, VP4 partial gene sequences of P[11] study strains shared 97.5%–100% (nucleotide and amino acid) identities among themselves. Moreover, these strains shared the highest 93.34%–94.53% (nucleotide) and 93.57%–94.64% (amino acid) identity with vaccine strain 116E, G9P[11]. In addition, relatively lower identities i.e., 86.21–87.99%, 85.49–89.3% (nucleotide) and 86.79–88.93%, 86.43–90.36% (amino acid) were noted with human G10P[11] and bovine P[11] strains, respectively. The study strains and vaccine strain 116E, G9P[11] formed a distinct cluster in the phylogenetic tree far distant from the human G10P[11] and bovine G6P[11] strains (Fig. 3).

4. Discussion

Unusual Bovine-human reassortant G9P[11] rotavirus was first identified in an outbreak of asymptomatic RVI in neonates at All India Institute of Medical Sciences (AIIMS), New Delhi, in 1985 (Bhan et al., 1993). Subsequently, a multicenter surveillance study conducted in Delhi during 1986 to 1993 demonstrated RV infections in 43 to 78% of hospitalized infants between 4–6 days old, born at five of the six hospitals. The two novel RV strains, G9P[11] (116E) and G9P[6], were common cause of infections in the hospital nurseries (Cicirello et al., 1994; Das et al., 1994). Follow-up of infants infected as neonates with RV confirmed that subsequent rotavirus infections were associated with less severe diarrhea (Bhan et al., 1993). Neonatal isolate 116E with VP4 gene of bovine RV origin has been successfully developed as vaccine candidate. A similar pattern was observed in studies conducted in Bangalore and Mysore, southern India. The study showed I321- like G10P[11] strains exclusively associated with asymptomatic infections in neonates and estimated the prevalence of 39.3% in this group (Vethanayagam et al., 2004). The strain I321, with all genes of bovine rotavirus origin except NSP1 and NSP3 was also developed as a candidate vaccine, but later withdrawn because of poor immunogenicity (Kang, 2016). A study carried out in Vellore, south India from 1999 to 2000 showed presence of I321- like G10P[11] strains (N155) in both asymptomatic and symptomatic neonates (Gomara et al., 2004). Additional survey carried out at AIIMS, New Delhi, from 2005 to 2006 reported G12P[6], then an emerging strain in 16% of neonates (Ray et al.,

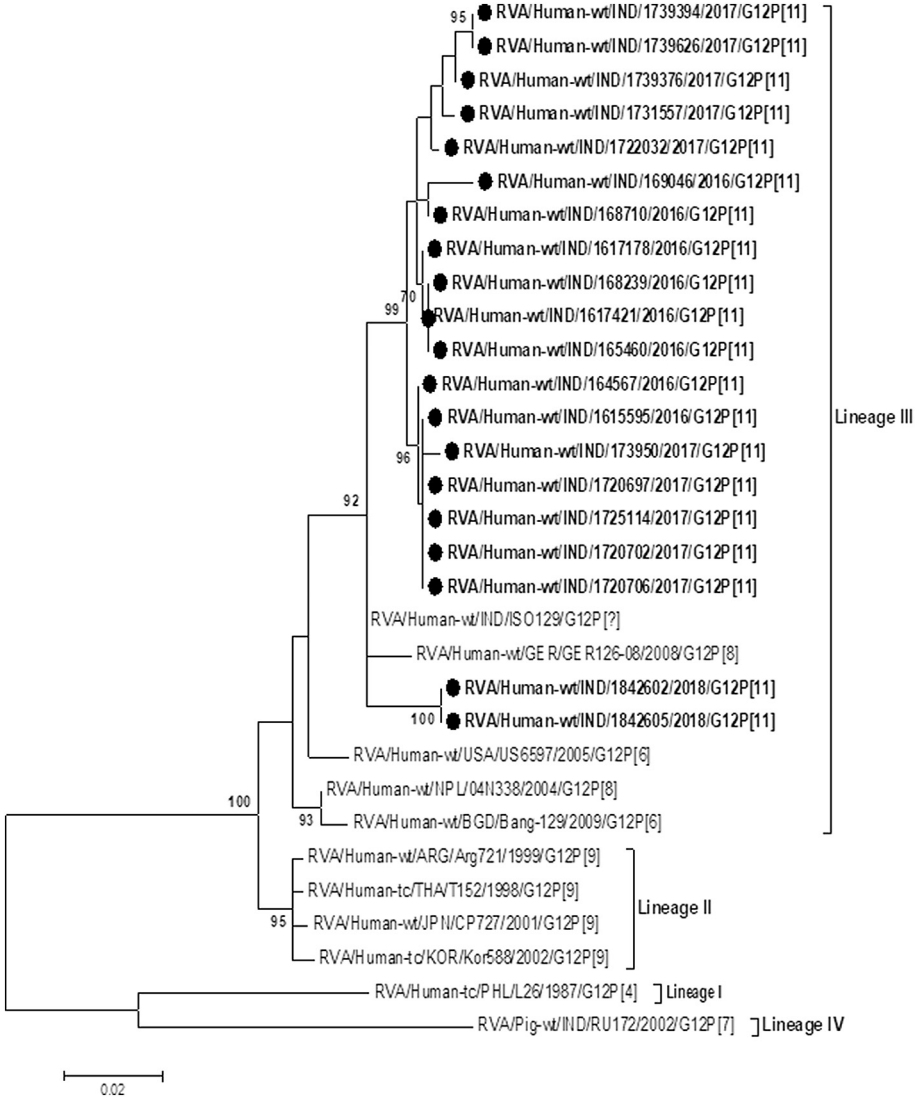

Fig. 2. Phylogenetic tree based on partial nucleotide sequences of the VP7 gene (nt 49–930) of G12 RVA strains. The neonatal G12P[11] rotavirus strains from the study (in boldface) are highlighted in filled circles. Phylogenetic analysis was performed using the maximum-likelihood method with General Time Reversible (GTR + G) as best nucleotide substitution model provided by MEGA v.6 software. Bootstrap values (1000 replicates) above 70% are shown. Scale bar indicates evolutionary distance (nucleotide substitutions per site).

2007). Present study showed RV in 24.31% neonates suggesting a large nosocomial RV burden in neonates admitted in NICUs in Pune, western India. The data obtained was consistent with earlier global reports which described high prevalence of RVI in neonates (Bhan et al., 1993; Kilgore et al., 1996; Shim et al., 2012; Koukou et al., 2015). However, in contrast to previous reports, the clinical spectrums i.e. fever; vomiting, diarrhea, feeding intolerance, abdominal distention, NEC and septicemia do not differ significantly between neonates with RVI and those without RVI.

G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] are the five major RV strains detected in children with acute gastroenteritis and under circulation worldwide. Since the begining of the new millennium, G12 strains have emerged as sixth major human RV genotype (Wylie et al., 2017). For G12, the origin is not clear, though pigs are suspected as a potential host reservoir for this genotype (Rahman et al., 2007). Based on phylogenetic and phylodynamic analyses, G12 genotype strains have been subdivided into four lineages (Matthijnssens et al., 2008). Lineage I contains only prototype G12P[4] strain L26 that was first strain detected in Philippines in 1987. Lineage II contains G12P[9] strains isolated in South America and Asia. Lineage IV contains RU172, the only one porcine G12P[7] strain known to date. The majority of the currently known G12 strains with P types [6], [8] cluster in a Lineage III, which is the largest. G12 strains detected in humans to date have been associated with the major P types P[4], P[8], P[6], P[9]. In this regard, it is noteworthy to first report high frequency of rare combination; G12P[11] in asymptomatic neonates in Pune, western India. The present study supports the hypothesis that unusual RV strains play a major role in neonatal infections in developing countries (Das et al., 1993; Kilgore et al., 1996). This is the first report describing nosocomial rotavirus circulating in western India. The strain appears to be stable and has an ability to persist in NICUs settings for a long period of time. It is interesting to note that unlike neonates, vast strain diversity was observed in children aged < 5 years admitted for acute gastroenteritis during the same period in Pune, India. Circulation of G1P[8], G1P[6], G2P[6], G3P[8], G9P[4], G12P[11], G10P[8], G3P[8]P[4] RV strains was observed and unexpectedly predominance of genotype G3P[8] was noted (unpublished data).

Rotavirus P[11] genotypes are the predominant bovine rotavirus strains found in India, and are the commonly detected in cattle worldwide (Fukai et al., 2002; Varshney et al., 2002). Zoonotic transmission and gene reassortments between human and animal rotaviruses contribute to diversity in rotaviruses infecting humans. Rotavirus P

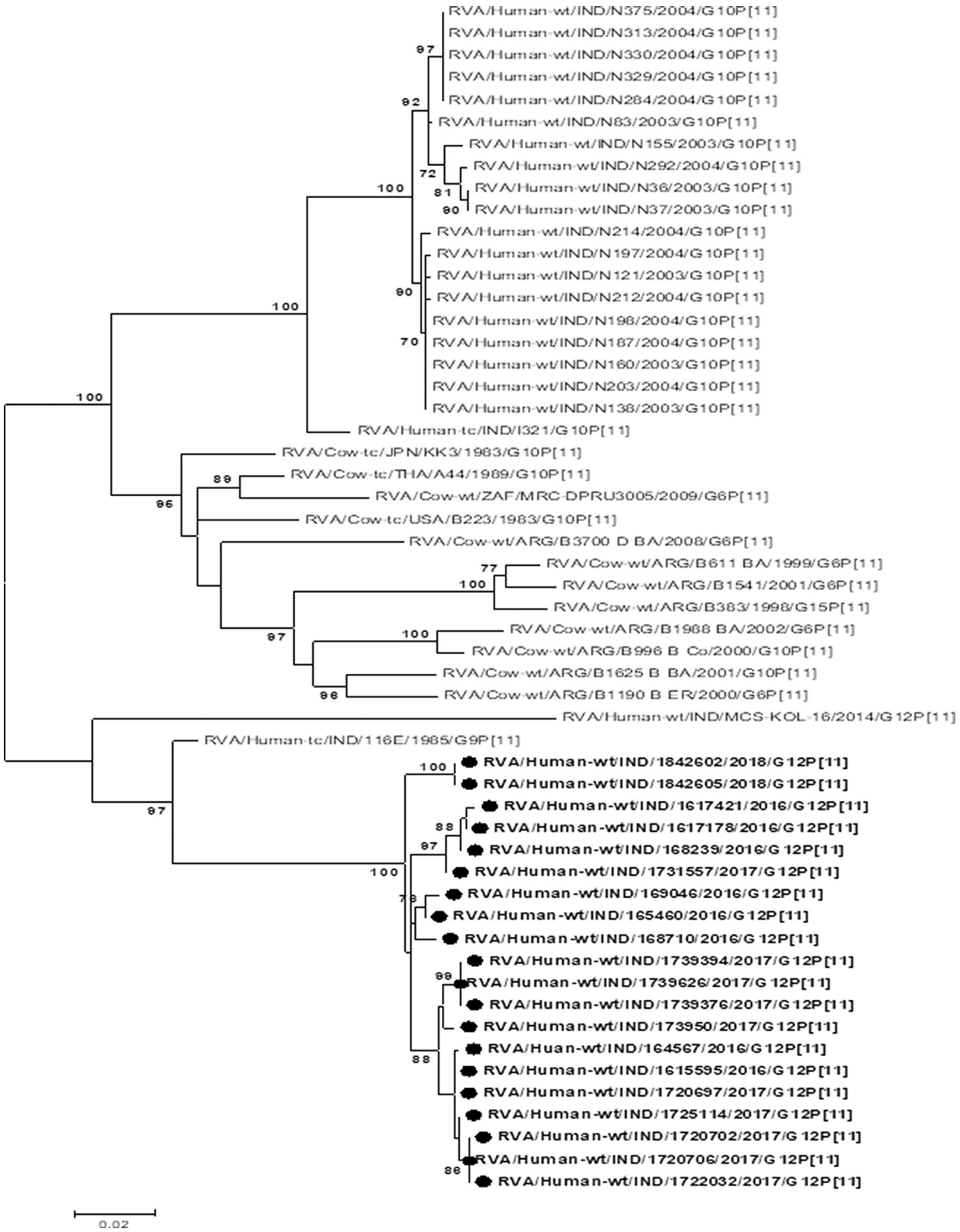

Fig. 3. Phylogenetic tree based on partial nucleotide sequences of the VP4 gene (nt 10–850) of P[11] RVA strains. The neonatal G12P[11] rotavirus strains from the study (in boldface) are highlighted in filled circles. Phylogenetic analysis was performed using the maximum-likelihood method with Tamura Nei (G + I) as best nucleotide substitution model provided by MEGA v.6 software. Bootstrap values (1000 replicates) above 70% are shown. Scale bar indicates evolutionary distance (nucleotide substitutions per site).

[11], in combination with multiple G types i.e., G9, G10, G12 have been associated with asymptomatic neonates suggesting that RV with P[11] specificity might have an enhanced capacity to replicate in the newborn gut. It is still not clear whether factors such as the prevalence of protecting maternal antibodies against common types and/or immature gut physiology contribute to the lack of diarrhea in neonates. The study supports the predilection of naturally occurring reassortant RVs with P [11] specificity for neonates. For 20 (17.54%) of 134 G12P[11] strains,

P-type could be determined only after designing a new set of typing primers. This finding is in concordance with reported previously by Chitambar et al., 2014 that point mutations at the primer binding site might decrease the affinity of primer binding and may result in the failure to type. This underscores an utmost need for regular revision of primers.

An important observation meriting particular attention was finding of G12P[6] strain in one of the hospitals in May 2017. All four G12P[6] strains clustered in May 2017 and surprisingly G12P[11] was not detected during this period from the same hospital. The strain was first detected in 7 day old term neonate who was transferred from maternity ward of the hospital to NICU due to complaint of mild diarrhea. However, the strain was replaced by more predominant and established G12P[11] and subsequently G12P[6] was not detected further.

Our observations are in concordance with those reported by Ray et al., 2007, that it is difficult to speculate the possible source of G12 and P[11] RV strains as well as the mechanism of their introduction into NICUs. Unfortunately we do not have any previous data on strains prevalence in neonates from western India. In our previous reports we have mentioned circulation of G12 in hospitalized children in Pune (Chitambar et al., 2014). Also P[11] type in combination with various G-types are common in Indian population or may have been introduced in local population through Rotavac vaccine. Introduction of G12 and P [11] strains in hospital setting by children admitted in pediatric ward or hospital staff or mothers admitted to maternity ward or by other mechanism could be speculated. It is possible that G12P[11] presently circulating could have resulted by reassortment between P[11] and G12 strains. Full genome analysis are needed to ascertain and to understand the origin of G12P[11] strains. The study showed a point-source nosocomial infection with a single G12P[11] strain prevailing for almost 2 years. Similar observations were reported in previous studies on neonatal RV infections which demonstrated that RV persisted for several years at hospital settings (Ray et al., 2007; Kang et al., 2018). It appears that crowding, sharing of barrier gowns by mothers while handling neonates at NICUs, low viral infecting dose, high contagiousness via fomites could contribute to further spread of the virus.

In summary, RV G12P[11] strain is prevalent at a high frequency in neonates in NICUs in western India. These findings support previous observations that newborns are sensitive hosts for novel strains, show limited strain diversity and play role as a reservoir for RV. Monitoring of early natural RV infections before immunization will be of importance for the ecological studies of rotavirus strains and also for improved estimation of vaccine efficacy. Differences in the efficacies of rotavirus vaccine in middle and low income countries may be related to early natural exposure of neonates before immunization. Nevertheless, continued surveillance studies on RVs in NICUs of divergent regions where nosocomial infections are frequent are essential. Approach will help in reporting of nosocomial viral infections in hospital databases and might be helpful in development of intervention strategies for better management and prevention of RVI which is a major global public health concern.

Author contributions

Conception and Design, V.G., S.R., M.B., A.B. and U·V; Performing the Experiments, Designing the Work, Acquisition, Analysis, and Interpretation of data, S.R.; Statistical Analysis A.W.; Writing the Original Manuscript, S.R.; Manuscript Revision and over all Execution: V.G.; S.R.; Resources, U.V. and M.B.

Conflict of interest statement

The authors have no conflict of interest.

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