Hospital based surveillance and genetic characterization of rotavirus strains in children (<5 years) with acute gastroenteritis in Kolkata, India, revealed resurgence of G9 and G2 genotypes during 2011–2013

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ABSTRACT

Introduction: India accounts for an estimated 457,000–884,000 hospitalizations and 2 million outpatient visits for diarrhea. In spite of the huge burden of rotavirus (RV) disease, RV vaccines have not been introduced in national immunization programme of India. Therefore, continuous surveillance for prevalence and monitoring of the circulating genotypes is needed to assess the disease burden prior to introduction of vaccines in this region.

Methods: During January 2011 through December 2013, 830 and 1000 stool samples were collected from hospitalized and out-patient department (OPD) patients, respectively, in two hospitals in Kolkata, Eastern India. After primary screening, the G-P typing was done by multiplex semi-nested PCR using type specific primers followed by sequencing. Phylogenetic analysis for the VP7 gene of 25 representative strains was done.

Results: Among hospitalized and OPD patients, 53.4% and 47.5% cases were positive for rotaviruses, respectively. Unlike previous studies where G1 was predominant, in hospitalized cases G9 rotavirus strains were most prevalent (40%), followed by G2 (39.6%) whereas G1 and G12 occurred at 16.4% and 5.6% frequency. In OPD cases, the most prevalent strain was G2 (40.3%), followed by G1, G9 and G12 at 25.5%, 22.8%, 9.3%, respectively. Phylogenetically the G1, G2 and G9 strains from Kolkata did not cluster with corresponding genotypes of Rotarix, RotaTeq and Rotavac (116E) vaccine strains.

Conclusion: The study highlights the high prevalence of RV in children with gastroenteritis in Kolkata. The circulating genotypes have changed over the time with predominance of G9 and G2 strains during 2011-2013. The current G2, G9 and G1 Kolkata strains shared low amino acid homologies with current vaccine strains. Although there is substantial evidence for cross protection of vaccines against a variety of strains, still the strain variation should be monitored post vaccine introduction to determine if it has any impact on vaccine effectiveness.

1. Introduction

Rotavirus is the leading cause of diarrhea and is associated with 453,000 childhood deaths globally [2]. India accounts for an estimated 457,000–884,000 hospitalizations, 2 million outpatient visits for diarrhea, resulting in huge medical and health care costs [1]. Annually more than 334,000 deaths occurring in Indian children are attributed to diarrheal disease, of which about 98,000 deaths are due to rotavirus alone [3].

Group A rotavirus (RVA) is a double stranded RNA virus consisting of 11 segments. Two outer capsid proteins, VP7 (G genotype) and VP4 (P genotype), independently elicit a serotype-specific neutralizing immune responses that may play an important role in protection against recurrent infections [4]. These viruses are genetically diverse, and RVA VP4 and VP7 encoding genes have been classified into atleast 27 G genotypes (G1–27) and 37 P genotypes (P[1]–[37]), respectively, based on differences in their nucleotide sequences [5,6]. The segmented nature of rotavirus genome

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provides the mechanism for the generation of genetic diversity by the process of genetic reassortment, which may occur during co-infections of circulating human and animal strains [7–9].

Two rotavirus vaccines namely Rotarix® (RV1; monovalent G1P[8]; GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq® (RV5; pentavalent G1, G2, G3, G4,P[8]; Merck Vaccines, Whitehouse Station, NJ, USA) are commercially available since 2006. Recently, another oral live attenuated vaccine candidate has been evaluated in phase III studies in India, and is derived from a G9P [11] human bovine reassortant strain 116E [10–12]. Large scale vaccine trials with Rotarix and RotaTeq have shown high efficacy in developed countries of Europe, Australia and USA though efficacy is lower (39–72%) in low income countries of Asia and Africa [13–15]. In spite of lower efficacy, these vaccines reduce a greater number of severe rotavirus gastroenteritis events in developing countries because of the great background rate of disease, resulting in the WHO's recommendations for introduction of RV vaccines in national immunization programs worldwide in 2009 [16]. However, RV vaccines have still not been introduced in national immunization programme of most South Asian and African countries, for several reasons including lack of disease burden data and economic feasibility.

During the past decade, several surveillance studies in hospitalized children have reported prevalence and strain diversity of RVA across India [18–22]. A multicenter hospital based study (2005–2009) in India, including Eastern India, estimated 40% hospitalization rates due to rotavirus [17,21]. The predominant strain circulating during 2005–2009 was G1P[8], followed by G2P[4]. G3, G4, G9 and G12 strains were observed at lower frequency (<10%) [17,21,22].

Most surveillance studies done in India were focussed on children hospitalized with acute gastroenteritis; however, the proportion of RVAs in cases of milder diarrhea and often reporting to outpatient departments (OPD) (some or no dehydration) remains largely unknown. The aim of this study was to analyse prevalence of rotavirus among children either hospitalized with severe diarrhea or seeking treatment for milder diarrhea in OPD (during January 2011–December 2013) and to compare the rotavirus genotypes among the two sets of patients.

2. Methods

2.1. Sample collection and screening

The study was conducted from January 2011 through December 2013 in ID-BG Hospital and B.C. Roy Memorial Hospital for Children in Kolkata, Eastern India. Stool samples of every fifth admitted patient (≤5 years of age) with acute watery diarrhea, vomiting and abdominal pain, were collected. The inclusion criteria for OPD patients included passing of three or more loose/watery stools within 24 h [23]. A total of 830 stool samples were collected from hospitalized patients and 1000 stool samples were collected from OPD patients. The consent of the guardian was obtained prior to enrolling a child. The study was approved by the Institutional Ethical Committee, National Institute of Cholera and Enteric Diseases. Preliminary screening of the stool samples for the presence of RVAs was performed using Rota-Adeno kit as per the manufacturer's instructions (VIKIA® Rota-Adeno, Biomerieux® sa).

2.2. Viral RNA extraction and genotyping

All the rotavirus positive samples, detected by VIKIA® Rota-Adeno kit, were confirmed for positivity by reverse transcription and PCR to avoid a false positive result. RVA double-stranded RNA was extracted from feces of positive samples by using a commercially available RNA extraction kit (QIAamp viral RNA Mini Kit, Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Complementary DNA was synthesized from the
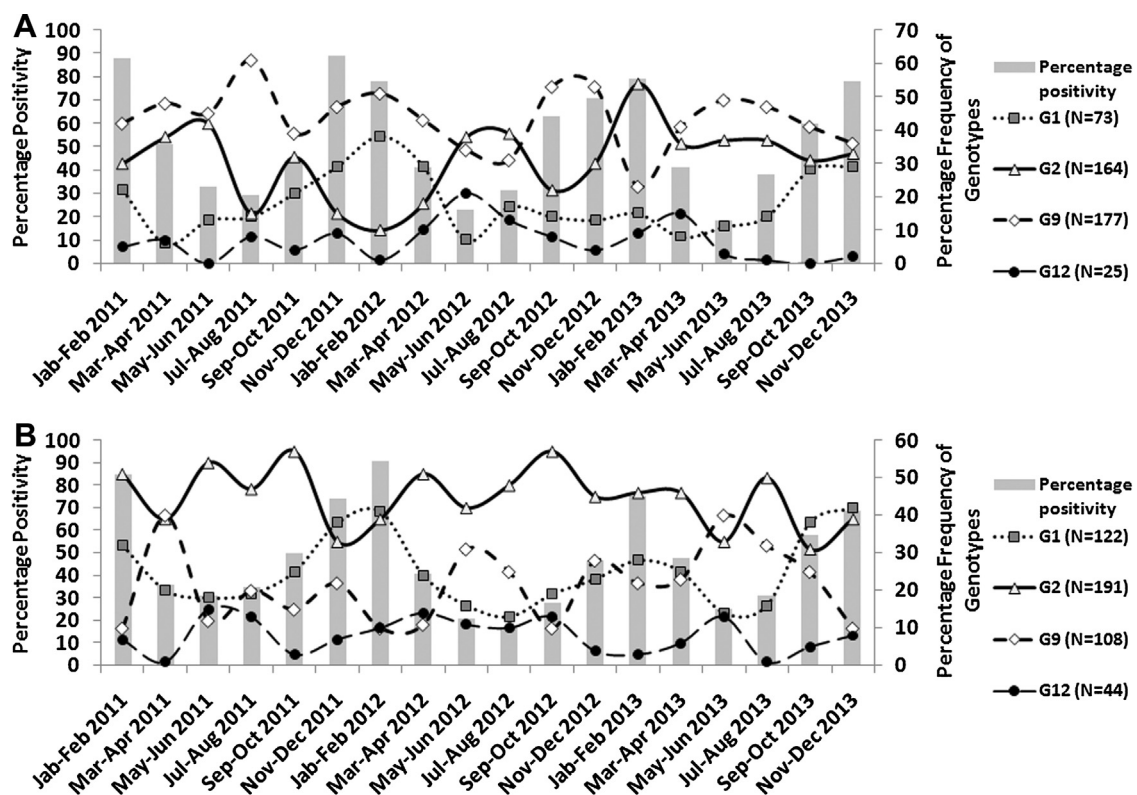

Fig. 1. Temporal distribution of rotavirus-positive cases in Kolkata during January 2011 through December 2013 in (A) hospitalized patients with severe diarrhea (<5 years) and (B) OPD patients (<5 years) with mild diarrhea. Lines represent month wise distribution of common genotypes (G1, G2, G9 and G12).
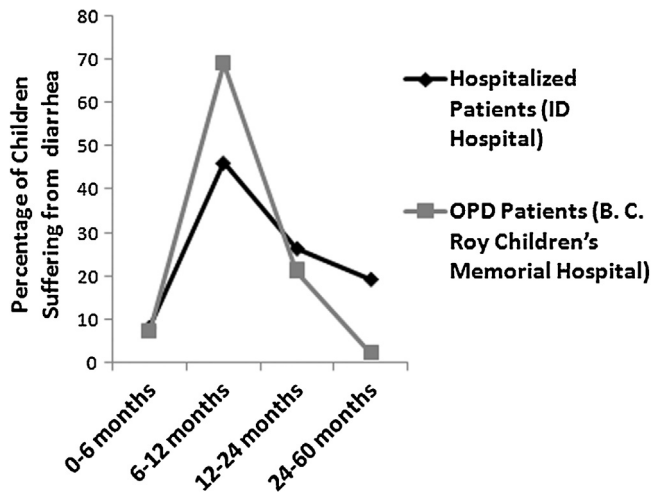
Fig. 2. Age-wise distribution of rotavirus-positive cases (0–5 years) among hospitalized and OPD patients.

Table 1
Samples tested by EIA and positive for rotavirus by hospitalization between January 2011 and December 2013.

	Hospitalized (patient <5 years) (%)	OPD patients (<5 years) (%)
Number of sample tested	830	1000
Number of rotavirus positive samples	443 (53.4%)	475 (47.5%)

2.3. Nucleotide sequencing and phylogenetic analysis

Nucleotide sequencing was carried out using the ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems, Foster City, California, USA) in an ABI Prism 3730 Genetic Analyzer (PE Applied Biosystems, Foster City, California, USA) as described previously [26]. Nucleotide and protein sequence BLAST search was performed using the National Centre for biotechnology Information (NCBI, National Institutes of Health, Bethesda, MD) Basic Local Alignment Search Tool (BLAST) server on GenBank database release 143.0 [27,28]. Pairwise sequence alignments were performed using LALIGN software (EMBnet, Swiss Institute of Bioinformatic, Switzerland), and multiple alignments were done with DDBJ software and CLUSTAL W. Amino acid sequences were deduced using the TRANSEQ software (Transeq Nucleotide to Protein Sequence Conversion Tool, EMBL-EBI, Cambridgeshire, UK). Phylogenetic tree was constructed using the MEGA (Molecular Evolutionary Genetics Analysis) program, version 5.2. Genetic

extracted viral RNA through reverse transcription in the presence of random hexamers. G and P genotyping was performed using VP7- and VP4-specific multiplex semi-nested RT-PCRs as described previously [24,25]. PCR products were purified with a QIAquick PCR purification kit (QiagenGmbH, Hilden, Germany).
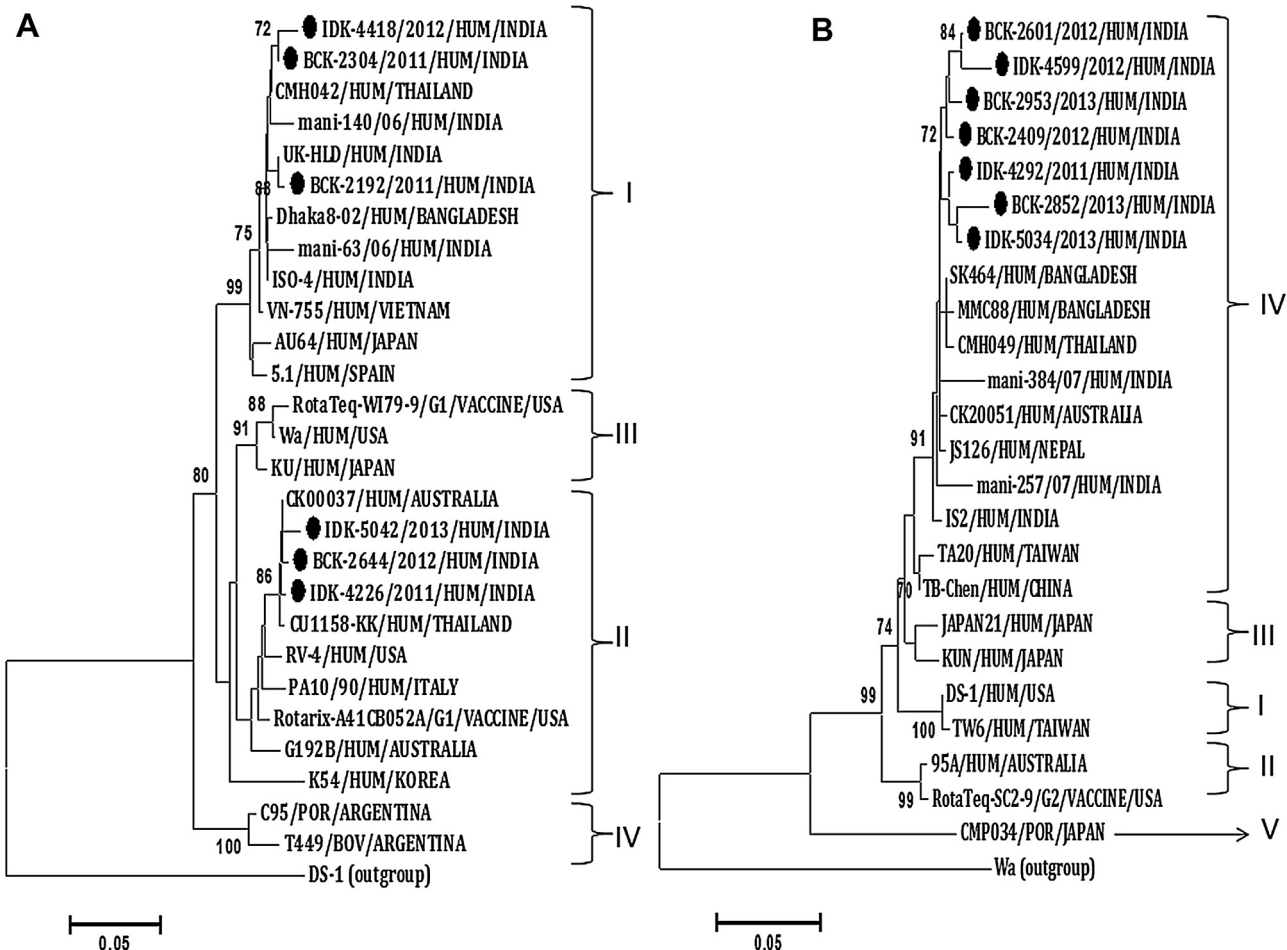

Fig. 3. Phylogenetic dendrograms based on nucleotide sequences of VP7 genes of (A) G1 and (B) G2 strains of Kolkata isolated during January 2011 through December 2013, with other known G1 and G2 strains, respectively. Scale bar, 0.05 substitutions per nucleotide. Bootstrap values less than 70% are not shown.

Table 2
Combinations of G and P types of rotavirus strains genotyped in (A) hospitalized and (B) OPD patients in Kolkata, India between January 2011 and December 2013.

(A) P-type	G-type						
	G1	G2	G4	G9	G12	Untypable	Total
P[4]	0	140	0	122	2	4	268
P[6]	6	6	1	0	4	0	17
P[8]	60	9	3	53	19	4	148
Untypable	4	6	0	0	0	0	10
Total	70	161	4	175	25	8	443
Percentage (%)	16.1	36.1	1.1	40.0	5.1	1.8	100

(B) P-type	G-type						
	G1	G2	G4	G9	G12	Untypable	Total
P[4]	10	173	0	72	9	0	264
P[6]	12	0	0	0	12	0	24
P[8]	100	18	0	36	23	0	177
Untypable	0	0	0	0	0	10	10
Total	122	191	0	108	44	10	475
Percentage (%)	25.5	40.3	0	22.8	9.3	2.1	100

distances were calculated using maximum likelihood statistical model and Jones–Taylor–Thornton (JTT) substitution model (at 1000 bootstrap replicates). Abbreviations used for sequence analysis are: hum, human; po/por, porcine; avi, avian; mur, murine; si/sim, simian; ovi, ovine; eq/equ, equine; bov, bovine; fel, feline; can, canine; lap/la, lapine; lam, lamb; tu/tur, turkey; ch/chi, chiken; cap, caprine; and out, outgroup. Lineage designation for

phylogenetic dendrograms of G1, G2, G9 and G12 strains were based on those reported in previous studies [29–42]. Complete nucleotide sequences of VP7 gene of the strains detected during this study were submitted to the GenBank database under the accession numbers: KF723263–KF723287 [KF723263–KF723268 (G1); KF723269–KF723275 (G2); KF723276–KF723283 (G9); KF723284–KF723287 (G12)].
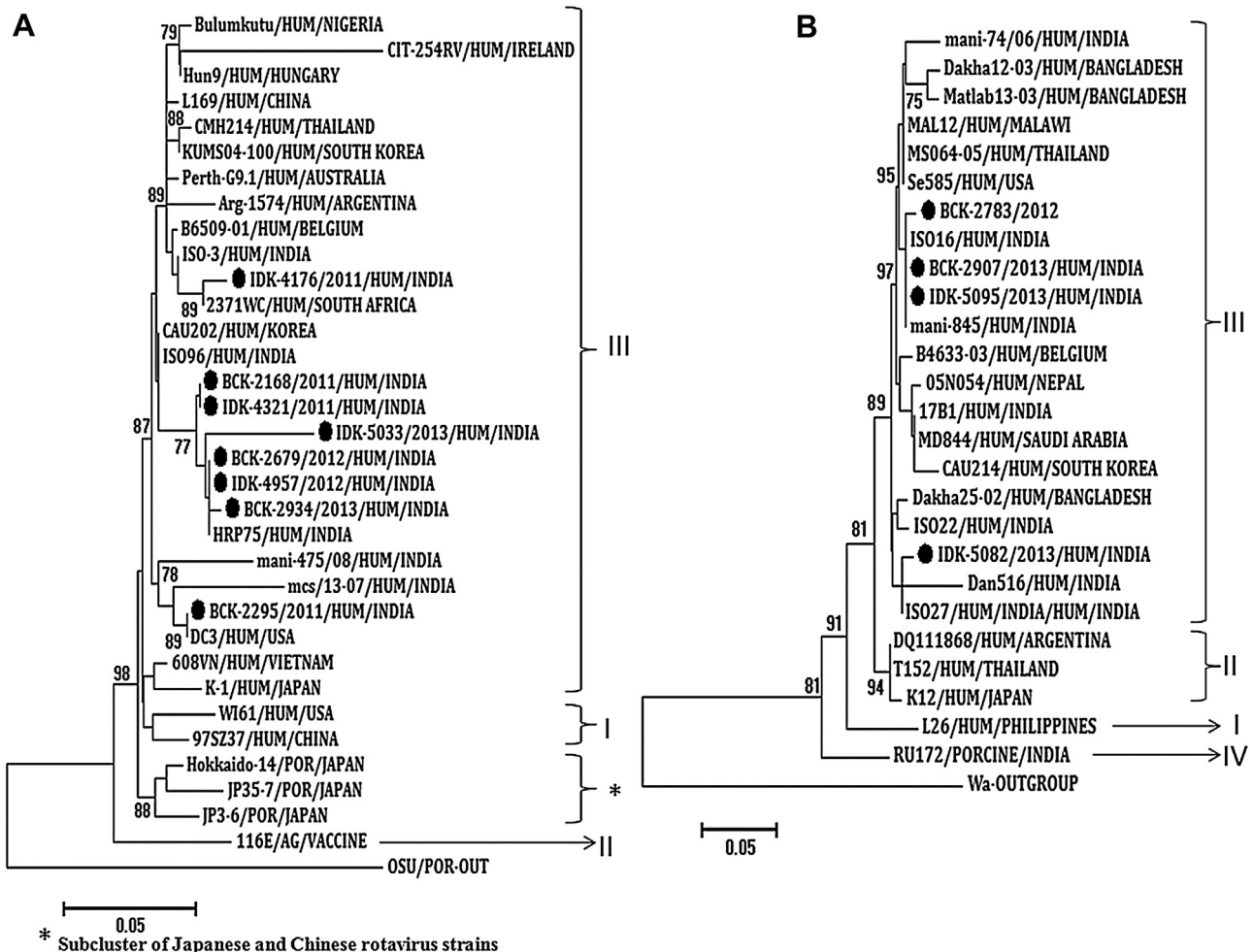

Fig. 4. Phylogenetic dendrograms based on nucleotide sequences of VP7 genes of (A) G9 and (B) G12 strains of Kolkata isolated during January 2011 through December 2013, with other known G9 and G12 strains, respectively. Scale bar, 0.05 substitutions per nucleotide. Bootstrap values less than 70% are not shown.

3. Results

3.1. Prevalence and epidemiology of RV in hospitalized and OPD patients

Among the 830 fecal samples from hospitalized children and 1000 samples from OPD cases, 443 (53.4%) and 475 (47.5%), respectively, were positive for RVAs (Table 1).

A distinct seasonal variation in rotavirus incidence was observed in both hospitalized and OPD cases, with low levels of positivity (10–25%) throughout the year (November–February: Winter season; March–June: Summer season; July–October: Rainy season), and the peak in incidence (70–80%) during winter season (December–February) (Fig. 1A and B). Monthwise genotype variation was also analyzed though no correlation between seasonality and increased frequency of particular genotype was observed (Fig. 1). In hospitalized children, G9 strains were observed at 25–55% frequency (Fig. 1A) whereas 10–45% incidence rate was observed in OPD children throughout the study period (Fig. 1B). G2 was observed at 10-55% frequency in hospitalized (Fig. 1A) and at 30–55% frequency among OPD children (Fig. 1B). G1 and G12 were observed at 10–40% and 0–20% frequency in both hospitalized and OPD children (Fig. 1A and B). In both the severe or mild diarrhea cases, the maximum number of rotavirus positivity was found in the age group of 6–12 months followed by 12–24months of children (Fig. 2).

3.2. Rotavirus genotypes among severe and mild gastroenteritis cases

Rotavirus genotypes were detected by multiplex semi-nested PCR method using G–P type specific primers and confirmed by full length sequencing of the VP7 genes and partial sequencing of the VP4 genes of strains representing different genotypes. Among 443 RVA positive samples from hospitalized children (<5 years), G9 in conjunction with P[4] and P[8], was most prevalent (40%), followed by G2P[4] (39.6%). G1P[8] and G12 genotype combined with P[8]/P[4]/P[6] were 16.4% and 5.6%, respectively. Other lesser common genotypes such as G1P[6], G2P[6], G2P[8], G4P[8] were observed at low frequencies (Table 2A).

Among 475 rotavirus positive cases from the OPD, the most prevalent strain was G2 in combination with P[4] (40.3%), followed by G1P[8] and G9 combined with P[4]/P[8] genotypes at 25.5% and 22.8%, respectively. G12 strains with either P[6] or P[8] genotypes occurred at 9.3%. Other uncommon strains like G1P[4], G1P[6], G2P[8] were also detected at low frequency (Table 2B).

3.3. Phylogenetic analysis of prevalent strains

The VP7 genes of 25 (G1 = 6, G2 = 7, G9 = 8 and G12 = 4 strains) representative rotavirus strains were analyzed based on the complete ORF (nt 49–nt 1026; 326 amino acids). Phylogenetic dendrograms based on nucleotide sequences were constructed and compared to previously reported G1, G2, G9 and G12 strains.

Kolkata G1 strains clustered in two subsets within two different lineages. One subset of G1 strains (BCK-2129/2011, BCK-2304/2011 and IDK-4418/2012) exhibited maximum similarities (>97%) with Thailand, India and Bangladesh G1 strains during BLAST analysis. Those strains remained in the same cluster within lineage I in phylogenetic dendrogram, though these were distant from the vaccine strains RotaTeq W179-9 and Rotarix A41CB052A (Fig. 3A). The other subset of G1 strains (IDK-4226/2011, BCK-2644/2012 and IDK-5042/2013) exhibited maximum similarities (>98%) with strains from Australia and Thailand. These G1 strains clustered with Rotarix vaccine strain within lineage II (Fig. 3A), while the VP7 (G1) of Rotateq vaccine strain clusters in lineage III (Fig. 3A).

Table 3
Amino acid identity (%) for VP7. Intragenotype similarities with Rotarix-G1, RotaTeq-G1, RotaTeq-G2 and 116E-G9.

Amino acid identities (%) between circulating and vaccine strains: VP7			
Strain name	Genotype/lineage	Rotarix-A41CB052A/G1	RotaTeq-WI79-9/G1
IDK-4226/2011	G1/II	96.9%	94.8%
IDK-5042/2013	G1/II	96.3%	94.2%
BCK-2644/2012	G1/II	95.4%	93.3%
BCK-2304/2011	G1/I	94.5%	93.3%
IDK-4418/2012	G1/I	92.6%	91.4%
BCK-2129/2011	G1/I	93.6%	92.3%
Strain name	Genotype/lineage		RotaTeq-SC2-9/G2
BCK-2409/2012	G2/IV		93.9%
IDK-4599/2012	G2/IV		92.6%
BCK-2601/2012	G2/IV		93.9%
BCK-2852/2013	G2/IV		91.7%
IDK-5034/2013	G2/IV		94.2%
BCK-2953/2013	G2/IV		92.6%
IDK-4292/2011	G2/IV		92.6%
Strain name	Genotype/lineage		116E/AG/VACCINE/G9
BCK-2679/2012	G9/III		91.4%
BCK-2934/2013	G9/III		90.8%
IDK-4957/2012	G9/III		92.6%
IDK-5033/2013	G9/III		90.5%
BCK-2168/2011	G9/III		92.6%
IDK-4321/2011	G9/III		91.4%
BCK-2295/2011	G9/III		92.3%
IDK-4176/2011	G9/III		89.9%

All G2 strains (BCK-2601/2012, BCK-2409/2012, BCK-2953/2013, BCK-2852/2013, IDK-4292/2011, IDK-4599/2012 and IDK-5034/2013) showed 98–99% nucleotide similarities with previously reported strains from India, Nepal and Bangladesh and clustered in lineage IV. The G2 strains from this study were distant to RotaTeq vaccine strains in lineage II (Fig. 3B).

Phylogenetic analysis showed all G9 strains from this study were in lineage III. Six of eight G9 strains (BCK-2168/2011, BCK-2679/2012, BCK-2934/2013, IDK-4321/2011, IDK-4957/2012 and IDK-5033/2013) revealed maximum identities (>96%) with previously reported human G9 strains from India and USA. These six G9 strains were in one subcluster, whereas, IDK-4176/2011 shared maximum homology with South African human G9 strain and BCK-2295/2011 was more similar with an American G9 strain. These two strains were placed in two other subclusters of lineage III (Fig. 4A). All the G9 strains from this study were found to be genetically distant from G9 vaccine strain 116E, which was in lineage II (Fig. 4A).

The current G12 strains shared close nucleotide similarity (>95%) with previously reported Indian human lineage III G12 strains. Sample IDK-5082/2013 formed distant subcluster, whereas other three (BCK-2783/2012, BCK-2907/2013 and IDK-5095/2013) formed another subcluster with Indian, Nepalese and Belgian G12 strains within lineage III (Fig. 4B).

3.4. Comparative analysis of VP7 protein of circulating Kolkata RV strains and the vaccine strains

The amino acid homology of the current circulating strains was compared to the vaccine strains. The lineage II G1 strains were similar (92–95%) to Rotarix-G1 strain which also clustered in lineage II (Fig. 3A), but lineage I G1 strains had 91–94% homology to either Rotarix-G1 or RotaTeq-G1 strains (Table 3). Amino acid homology of G2 strains with RotaTeq G2 was ~91%, whereas Kolkata G9 strains showed 89–92% amino acid homology with 116E-G9 vaccine strains (Table 3).

Table 4

Alignment of the amino acid residues defining the neutralization domains (designated as 7-1a, 7-1b and 7-2 [53]) of VP7 between- the G1 strains in Rotarix and RotaTeq and Kolkata G1 strains; G2 strain in RotaTeq and Kolkata G2 strains; and G9 strain in 116E and Kolkata G9 strains. Residues that differ are highlighted.

	VP7																													
	7-1a														7-1b						7-2								
	87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
Rotarix-A41CB052A/G1	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
RotaTeq-WI79-9/G1	T	T	N	G	D	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	S	L	S	M	N	G
IDK-4226/2011/G1	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
IDK-5042/2013/G1	T	T	N	G	E	W	K	E	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
BCK-2644/2012/G1	T	T	N	G	E	W	K	D	Q	S	V	V	D	K	Q	N	V	D	N	T	K	D	Q	N	L	S	M	N	G
BCK-2304/2011/G1	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
IDK-4418/2012/G1	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
BCK-2129/2011/G1	T	T	S	G	E	W	K	D	Q	N	V	V	D	R	Q	N	V	D	N	T	K	D	Q	N	L	S	T	N	G
RotaTeq-SC2-9/G2	A	N	S	D	E	W	E	N	Q	D	T	M	N	K	Q	D	V	S	N	S	R	D	N	T	S	D	I	S	G
BCK-2409/2012/G2	T	N	S	N	E	W	E	N	Q	D	T	M	N	K	Q	D	V	D	N	N	R	D	N	T	S	D	I	S	G
IDK-4599/2012/G2	T	N	S	N	E	W	E	N	Q	D	T	M	N	M	Q	D	V	D	N	N	R	D	N	T	S	D	I	S	G
BCK-2601/2012/G2	T	N	S	N	E	W	E	N	Q	D	T	M	N	K	Q	D	V	D	N	N	R	D	N	T	S	D	I	S	G
BCK-2852/2013/G2	T	N	S	N	E	W	E	N	Q	D	T	M	N	V	Q	D	V	D	N	N	R	D	N	T	S	D	I	S	G
IDK-5034/2013/G2	T	N	S	N	E	W	E	N	Q	D	T	M	N	K	Q	D	V	D	N	N	R	D	N	T	S	D	I	S	G
BCK-2953/2013/G2	T	N	S	N	E	W	E	N	Q	D	T	M	N	K	Q	D	V	D	N	N	R	D	N	T	S	D	I	S	G
IDK-4292/2011/G2	T	N	S	N	E	W	E	N	Q	D	T	M	N	C	Q	D	V	D	N	N	R	D	N	T	S	D	I	S	G

The VP7 trimer contains two structurally defined antigenic epitopes: 7-1 and 7-2. The 7-1 epitope spans the intersubunit boundary and is further subdivided into 7-1a and 7-1b [43]. A comparison of residues that constitute the 7-1a, 7-1b, and 7-2 epitopes of the Kolkata strains and the vaccine strains is presented in Table 4. Twenty nine amino acid residues of this antigenic epitope of the VP7 proteins of circulating G1, G2, and G9 RVA strains were compared with the Rotarix-G1, RotaTeq-G1, RotaTeq-G2, and 116E-G9 vaccine strains. Kolkata G1 strains showed mismatches in 94, 100, 123, 291 and 217 positions in 7-1a and 7-2 domains with Rotarix-G1and RotaTeq-G1strains. Kolkata G2 strains also showed mismatches in 4 positions, 87, 291, 213 and 242 in respect to RotaTeq-G2 strains. When VP7 protein of G9 strains were compared with 116E-G9 vaccine strain, it revealed that circulating lineage III G9 strains also differ from 116E strain within antigenic domain at 87, 94, 100, 291, 242, 145 and 221 positions (Table 4).

4. Discussion

In low income countries of Asia (India, Bangladesh, Pakistan, Vietnam, China) and Africa, high prevalence (30–40%) of RV has been reported among hospitalized children [17,44–49]. In this study, the incidence was higher in hospitalized children (53.4%) and out-patients (47.5%) than previous reports. The children seeking treatment in outpatient departments may constitute a major source for dissemination of virus. Unlike developed countries where one or two genotypes predominate in a season [54,55], a large number of genotypes was observed (G9, G2, G1, G12) at >15% frequency in Kolkata. This agrees with the previous reports from India and Bangladesh [17,44]. Although not demonstrated so far, emergence of new strains, which contributes to genetic diversity, may be one cause of lower vaccine efficacy in developing countries. Selective pressure resulting from population immunity may drive emergence of strains able to evade vaccine immunity [13].

Moreover for improving efficacy, mass vaccination of children through national immunization program is required, whereas in countries like India, currently only a small proportion of children are vaccinated. Considering the socio-economic structure, high cost of vaccines and the large diversity of strains in low income countries, successful implementation of RV vaccines is still an unfulfilled goal [17,25,50]. Thus to fulfill the lacunae of disease control by vaccination, continuous surveillance for RV is required to monitor incidence, circulating genotypes, emergence of new reassortant strains in population, which will also help in effective disease management and prevention of large scale outbreaks. In addition knowledge of currently circulating strains is needed prior to mass vaccination, for comparison and evaluation during post vaccination studies.

As Kolkata has a tropical climate, seasonality of rotavirus infection (Fig. 1) was found to be similar to that reported from some regions of Asian countries where low level of RV is detected throughout the year, but a peak is observed predominantly during winters [17,41,48,51,52]. In this study, most of the rotavirus positive children were from 6 to 12 months age groups (Fig. 2), suggesting that the post breast feeding age group is more prone to rotavirus infection.

In this study, G9 was the most common strain (40%) responsible for severe diarrhea related hospitalizations (Table 2). Previous studies during 2003–2009, showed that, in the eastern part of India, G1 (>50%) and G2 strains (~23–33%) were dominant, whereas G9 (2–10%) and G12 (8–17%) strains occurred at lower frequencies [19–21], and similar trends were reported in western, northern and southern parts of India [17,18,20–22]. During the current study period, G9 and G2 strains predominated, causing 75% and 62% of all RV infections among hospitalized and OPD cases, respectively. G1

genotypes were still observed at 16–25% (Table 2). Previously available two rotavirus vaccines have shown high effectiveness against several strains not in the vaccine including G9 and G12 in countries like USA [13,15], suggesting there is a heterotypic protection. Still in countries like India, where genotypic diversity is very high, strains like G9 and G12 should be included in the vaccine. The high prevalence of G9 observed in this study suggests that it may be valuable to have a vaccine that includes serotype G9 such as strain 116E, that is currently in the pipeline.

Nucleotide sequence based homology analysis with respect to previously reported G9 strains revealed close similarity of Kolkata G9 strains to previously reported lineage III strains from the Indian subcontinent (India, Bangladesh and Nepal) (Fig. 4A). The currently licensed vaccine from India (Rotavac) 116E, has G9P[11] genotype and the G9 strains from Kolkata showed low amino acid homology (89.9–92.6%) with 116E vaccine strain (Table 3), but the vaccine strain was derived from a non-symptomatic neonatal infection and was adapted to cell culture several years ago [10–12]. Similarly the circulating lineage II G1 and lineage IV G2 strains were also found to be distant from the current vaccine strains (Rotarix and RotaTeq). VP7 antigenic domain of Kolkata G1and G2 strains also revealed mismatches with that of vaccine strains (Table 4). Knowledge of currently circulating strains is needed prior to vaccination, for comparison and evaluation during post vaccination studies. Fluctuation of genotypes due to accumulation of point mutations (genetic drift) in the antigenic domain of VP7 gene is one potential reason for changes in circulating strains [53,54]. The amino acid analysis of the VP7 antigenic domains compared with vaccine strain was not done earlier in this region. The antigenic variation observed between circulating strains and vaccine strains may influence vaccine efficacy in these settings. Thus it will be important to monitor the impact of vaccine pressure on antigenic variation to determine whether particular changes will alter long-term vaccine effectiveness over time.

In addition to documenting the differences between circulating and vaccine strains, the study highlights the very high prevalence of RV in children reporting with severe diarrhea or milder disease. The circulating genotypes have changed over the time, with G9 and G2 genotypes being most predominant during 2011–2013. The study demonstrates the high burden of RV gastroenteritis, providing strong support to introduction of RV vaccines in the regions, where burden is high.

Conflict of interest

None.

Acknowledgements

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