Sequence analysis of VP7 and VP4 genes of G1P[8] rotaviruses circulating among diarrhoeic children in Pune, India: A comparison with Rotarix and RotaTeq vaccine strains

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ABSTRACT

Background: The G1P[8] rotaviruses are a common cause of rotavirus diarrhoea among children in India. Two rotavirus vaccines licensed in India, Rotarix and RotaTeq, contain strains with G1 and P[8] genotypes. A comparative analysis of these genotypes in the live rotavirus vaccines with circulating rotavirus strains is essential for assessment of rotavirus diversity.

Methods: G1P[8] strains detected during rotavirus surveillance among diarrhoeic children hospitalized in Pune in 1992–1993 and 2006–2008, were included in the study. Amplification, sequencing and phylogenetic analysis of the VP7 and VP4 genes were carried out for identification of the G1 and P[8] lineages, respectively. Antigenic epitopes of VP7 and VP4 encoded proteins were compared to determine the differences between the G1P[8] strains from Pune and the vaccine strains.

Results: G1-Lineage 1, P[8]-Lineage 3 strains were predominant in Pune during 1992–1993 and 2006–2008. Strains of G1-Lineage 2, P[8]-Lineage 3 and G1-Lineage 1, P[8]-Lineage 4 were detected at low levels during 2006–2008. The G1-Lineage 1, P[8]-Lineage 3 strains showed up to eight amino acid changes, each in the VP7 and VP4 epitopes, with respect to the Rotarix vaccine strain (G1-Lineage 2, P[8]-Lineage 1) and the G1 (Lineage-3) and P[8] (Lineage 2) components of the RotaTeq vaccine. The G1-Lineage 2 strains were closer to both vaccine strains with no or only two amino acid substitutions in the VP7 epitopes. The divergent P[8]-Lineage 4 (OP354-like) strains showed fourteen and fifteen amino acid differences, with Rotarix and RotaTeq vaccine strains, respectively, in the VP4 epitopes.


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1. Introduction

Rotavirus is the most important cause of severe diarrhoeal illness in infants and young children, worldwide [1]. Annually, in India, rotaviral diarrhoea causes an estimated 122,000–153,000 deaths, 457,000–884,000 hospitalizations and 2,000,000 outpatient visits in children less than five years of age [2]. Rotaviruses, of the family Reoviridae, are triple-layered particles (TLPs) consisting of the outer capsid, inner capsid and core. The rotavirus genome consists of 11 dsRNA segments which code for the six structural (VP1–VP4, VP6, VP7) and five non-structural (NSP1–NSP5) proteins. The outer capsid proteins, VP7 and VP4, serve as viral attachment proteins and neutralization antigens [3]. VP4 is activated by proteolytic cleavage into two fragments—VP8* and VP5*. VP8* forms a globular attachment domain at the tip of the VP5* stalk [4]. A binary system classifies group A rotaviruses into 27 G and 37 P types [5,6], a classification initially based on neutralization specificities of VP7 (Glycoprotein) and VP4 (Protease sensitive protein). Globally, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8] genotype combinations of rotavirus strains are the most common cause of human infections [7]. Of these, G1P[8] strains are most predominant (37.7%) [7]. These strains exhibit diversity in the form of 11 G1 and 4 P[8] subgenotypic lineages [8,9]. According to a multi-centre hospital-based study carried out in India from 2005 to 2009, G1P[8] strains were highly prevalent [10].
Two rotavirus viruses, Rotarix and RotaTeq, are currently licensed in many countries including India. Rotarix is a monovalent vaccine containing the attenuated human G1P[8] rotavirus strain 89–12. RotaTeq is a pentavalent vaccine containing five human-bovine reassortant rotavirus strains, each representing one human genotype—W179-9 (G1), SC2-9 (G2), W179-9 (G3), BrB-9 (G4) and W179-4 (P[8]). Studies from different countries have revealed that the G1 and P[8] subgenotypic lineages included in these vaccines, prevalent at the time of vaccine development (1980s), are not predominant today [8,9,11–23].

Earlier, we have reported identification of different lineages within VP7 gene of G1 rotaviruses circulating in Pune, western India [24]. The study did not include analysis of the corresponding P[8] lineages of VP4 genes and the rotavirus vaccine strains, 89–12 (Rotarix G1P[8]), W179-9 (RotaTeq G1) and W179-4 (RotaTeq P[8]) were not compared due to the unavailability of their sequence data at the time. The aim of the present study was to assess the diversity of G1P[8] rotavirus strains circulating among children with diarrhoea in Pune during the two time periods, 1992–1993 and 2006–2008, and compare sequences with the G1 and P[8] components of vaccines.

2. Materials and methods

2.1. Specimen selection

A surveillance program for rotavirus disease and strains was carried out in children (<5 years), hospitalized for diarrhoea in Pune city during 1990s and 2000s [10,25] (Table 1). The G1P[8] rotavirus strains identified during the years 1992 (n = 8), 1993 (n = 11), 2006 (n = 21), 2007 (n = 29) and 2008 (n = 13) were selected in the present study for further characterization.

2.2. Amplification and sequencing of the VP7 and VP4 genes

Viral RNA was isolated from 30% (w/v) for solid and v/v for liquid stool suspensions of the selected G1P[8] rotavirus positive faecal specimens using TRIzol (Invitrogen, U.S.A.). Amplification of the complete VP7 gene (1062 bp) was carried out using the primers Beg9 and End9 [26] as described previously [24]. The partial VP4 gene (VP8* region: 10 to 729 bp) was amplified with primers con2 and con3 [27] using One-step RT-PCR kit (Qiagen, Germany). The PCR conditions involved an initial reverse transcription step of 30 min at 45 °C, followed by PCR activation at 95 °C for 15 min, 40 cycles of amplification (1 min at 94 °C, 1 min at 50 °C and 2.5 min at 70 °C) with a final extension of 7 min at 70 °C. The VP7 and VP8* amplicons were sequenced as reported previously [24].

Sequencing of the complete VP4 genes was carried out as described earlier [28] for six G1P[8] strains (NIV-0613158, NIV-06361, NIV-061060, NIV-0715880, NIV-075223, NIV-0833735) representing each of the two P[8] lineages (P[8]-3 and P[8]-4) identified in Pune on the basis of VP8* sequences.

2.3. Nucleotide sequence accession numbers

The VP7 sequences were submitted to GenBank under the accession numbers DQ886934-46, DQ886953-56, DQ886958, DQ886959, DQ886962, DQ886964-68, DQ886972, DQ875602, FJ948829-55, JN192054-55, JN192060-61, JN192063-64, JN192068-69, JN192071-75, JN192079, JN192082-83, JN192086, JN192089, JN192093-96, JN192098-99, JN192100-01, JN192112-13, JN192115-16, JN192119-26 and JN192128-31. The VP4 sequences were submitted under the accession numbers HQ881499 to HQ881575, EU984107 and HM467806-08.

2.4. Phylogenetic analysis

The VP7 and VP4 sequences of the G1P[8] reference strains [8,9] representing each of the 11 G1 and 4 P[8] subgenotypic lineages and the sequences of the Rotarix and RotaTeq vaccine strains were retrieved from GenBank. The sequences available in GenBank for G1P[8] strains from other cities [Kolkata (n = 8), Delhi (n = 3) and Manipur (n = 4)] included in the study were classified into lineages during comparative analysis.

Multiple sequence alignments were conducted using the ClustalW implementation in MEGA 5.05 [29]. Phylogenetic trees were constructed using the neighbour joining algorithm and Kimura 2-parameter model in MEGA 5.05. The statistical significance of the genetic relationships was estimated by bootstrap resampling analysis (1000 replications). Nucleotide and amino acid distances were calculated using Kimura 2-parameter model and P-distance model, respectively.

3. Results

3.1. Distribution of the G1P[8] rotavirus strains circulating in Pune into subgenotypic lineages

Phylogenetic analysis of the VP7 (Fig. 1(A)) and VP4 genes (Fig. 1(B)) showed clustering of the G1P[8] strains from Pune into G1-Lineage 1 or 2 and P[8]-Lineage 3 or 4 (Fig. 2). All the strains from the years 1992 (8/8, 100%) and 1993 (11/11, 100%) were placed into G1-Lineage 1, P[8]-Lineage 3. In the year 2006, the G1P[8] strains from Pune were distributed into G1-Lineage 1, P[8]-Lineage 3 (20/21, 95.2%) and G1-Lineage 2, P[8]-Lineage 3 (1/21, 4.8%). In 2007, while the G1-Lineage 1, P[8]-Lineage 3 strains continued to predominate (23/29, 79.3%), the prevalence of G1-Lineage 2, P[8]-Lineage 3 strains increased (5/29, 17.2%). In addition, one strain of G1-Lineage 1, P[8]-Lineage 4 (1/29, 3.5%) was detected. In 2008, the G1P[8] strains from Pune were distributed into G1-Lineage 1, P[8]-Lineage 3 (12/13, 92.3%) and G1-Lineage 1, P[8]-Lineage 4 (1/13, 7.7%).

Phylogenetic analysis of the G1P[8] strains from other cities in India (Fig. 1(A) and (B)) revealed circulation of the same subgenotypic lineages as in Pune. All G1P[8] strains from Kolkata (8/8, 2008–2009) and Delhi (3/3, 2000s) clustered into G1-Lineage 1, P[8]-Lineage 3. The G1P[8] strains from Manipur (2006–2007) were distributed into G1-Lineage 1, P[8]-Lineage 3 (2/4) and G1-Lineage 1, P[8]-Lineage 4 (2/4).

The Rotarix vaccine strain, 89–12, clustered into G1-Lineage 2, P[8]-Lineage 1. The WI79-9 (G1) strain of RotaTeq vaccine was placed in G1-Lineage 3 while the WI79-4 (P[8]) strain was classified in P[8]-Lineage 2 (Fig. 1(A) and (B)).

3.2. Comparative sequence analysis of VP7 and VP4 genes of rotavirus G1P[8] strains from Pune and rotavirus vaccines

The G1-Lineage 1 strains showed 92.8–95.2% nucleotide and 92.9–95.4% amino acid identity with the Lineage 2 of G1 Rotarix vaccine strain and 89.9–92.0% nucleotide and 92.0–94.4% amino acid identity with the Lineage 3 of the G1 strain in RotaTeq vaccine. The G1-Lineage 2 strains were closer to the Rotarix VP7 of the same lineage (97.3–97.5% nucleotide and 97.2–97.5% amino acid identity) than to the RotaTeq VP7 of Lineage 3 (92.1–92.2% nucleotide and 94.4–94.8% amino acid identity).

The VP8* of the P[8]-Lineage 3 strains were more similar to the RotaTeq P[8] (92.3–93.9% nucleotide and 92.9–95.8% amino acid identity) than to Rotarix VP8* (89.5–91.4% nucleotide and 90.8–93.3% amino acid identity). The divergent P[8]-Lineage 4 strains showed lower identities with both the vaccine strains (Table 2).
Fig. 1. Phylogenetic dendrogram of the nucleotide sequences representing G1P[8] rotavirus strains circulating in Pune, India. (A) VP7 (coding region: 49–1026 bp) and (B) VP4 (VP8* region: 10–729 bp) The strains from Pune are indicated by a solid triangle while the vaccine strains are indicated by a solid circle. Bootstrap values ≥70% are shown. The GenBank accession numbers for the VP7 gene sequences of the reference G1 rotavirus strains are AY631049 (Dhaka 8-02), DQ512979 (Thai-804), U26370 (Cos-70), K02033 (Wa), U26378 (Kor-64), DQ377573 (PA5/90), AB018697 (AU19), U26366 (Ban-59), U26373 (Egypt-7), DQ377574 (PA32/90), AF426162 (SW20/21), L24164 (C60). The GenBank accession numbers for the VP4 gene sequences of the reference P[8] rotavirus strains are L34161 (Wa), U30716 (F45), AJ605320 (Hun9), AJ302148 (OP354). The GenBank accession numbers for the sequences of the vaccine strains are JN849114 (Rotarix VP7), JN849113 (Rotarix VP4), GU565057 (RotaTeq G1 VP7), GU565044 (RotaTeq P[8] VP4). Representative strains from Kolkata, Delhi and Manipur have also been included in the dendrogram. The VP7 gene sequences for the strains from Kolkata are available under the accession numbers AB561878 (GRAVP7), AB553336 (GRAVP712), AB553329 (GRAVP730), AB551663 (GRAVP728), AB550709 (GRAVP727), AB550723 (GRAPV720), AB547709 (GRAVP718) while the VP4 sequences are available under the accession numbers AB561881 (GRAVP436), AB555332 (GRAVP432), AB555326 (GRAVP431), AB555325 (GRAVP430), AB551661 (GRAVP428), AB550708 (GRAVP427), AB544058 (GRAVP420), AB544056 (GRAVP418). The accession numbers for the VP7/VP4 sequences of the strains from Delhi are FJ827595/FJ827605 (Dan279), FJ827593/FJ861662 (Dan114), FJ827592/FJ827602 (Dan103) while those for the strains from Manipur are GQ229043/GQ240617 (mani-375/07), GQ229042/GQ240616 (mani-365/07), GQ229041/GQ240614 (mani-140/06), GQ229040/GQ240612 (mani-63/06).
Fig. 1. (Continued).
Table 1

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of faecal specimens collected</th>
<th>No. (%) of rotavirus positive specimens</th>
<th>No. of specimens subjected to genotyping</th>
<th>No. (%) of specimens positive for G1P[8] rotavirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992</td>
<td>144</td>
<td>32 (22.2)</td>
<td>16</td>
<td>8 (50.0)%</td>
</tr>
<tr>
<td>1993</td>
<td>335</td>
<td>104 (31.0)</td>
<td>38</td>
<td>11 (28.9)%</td>
</tr>
<tr>
<td>2006</td>
<td>301</td>
<td>96 (31.9)</td>
<td>96</td>
<td>27 (28.1)%</td>
</tr>
<tr>
<td>2007</td>
<td>311</td>
<td>137 (44.1)</td>
<td>137</td>
<td>64 (46.7)%</td>
</tr>
<tr>
<td>2008</td>
<td>231</td>
<td>65 (28.1)</td>
<td>65</td>
<td>13 (20.0)%</td>
</tr>
</tbody>
</table>

a Genotyping has been carried out by multiplex PCR.
b Number of specimens subjected to genotyping was considered as denominator for percentage calculation.

Fig. 2. Distribution of the G1P[8] rotaviruses circulating in Pune into subgenotypic lineages.

Both P[8] lineages showed higher amino acid divergence in VP8* region than in VP5* region (Table 2).

3.3. Comparison of the VP7 and VP4 antigenic epitopes of the G1P[8] rotavirus strains from Pune and rotavirus vaccines

The rotavirus VP7 protein consists of two antigenic epitopes: 7-1 (7-1a and 7-1b) and 7-2 encompassing 29 amino acid residues [30]. The G1-Lineage 1 strains from Pune showed 3–6 amino acid differences with the G1-Lineage 2 strain of Rotarix and 5–8 amino acid differences with the G1-Lineage 3 strain of RotaTeq vaccine (Table 3). The majority (92.1–100%) of the G1-Lineage 1 strains showed three and one amino acid differences, respectively, in epitopes 7-1a (N94S, S123N, K291R) and 7-2 (M217H) in comparison with both vaccine strains. All amino acid differences were common to the G1-Lineage 1 strains of both periods (1992–1993 and 2006–2008) with the exception of the substitution L148F in epitope 7-2 that was restricted to seven strains from the years 2006–2008. In addition, all G1-Lineage 1 strains had the substitutions D97E (epitope 7-1a) and S147N (epitope 7-2) when compared to the G1 strain of RotaTeq vaccine. Specific differences were noted at amino acid positions 125, 129 (epitope 7-1a), 212, 213 (epitope 7-1b) and 221 (epitope 7-2) in a few (1–3) of the G1-Lineage 1 strains on comparison with both vaccine strains.

The G1-Lineage 2 strains detected in Pune during 2006 and 2007 showed 100% sequence identity to the Rotarix vaccine strain (G1-Lineage 2) in the VP7 epitopes. With respect to the RotaTeq vaccine strain, the G1-Lineage 2 strains showed only two amino acid differences—D97E (epitope 7-1a) and S147N (epitope 7-2) (Table 3). Overall, the epitopes 7-1a and 7-2 were more prone to variations than epitope 7-1b among all G1 strains.

The VP4 protein of rotavirus consists of nine antigenic epitopes—four (8-1 to 8-4) in VP8* and five (5-1 to 5-5) in VP5*, which together include 37 amino acids [31,32]. The P[8]-Lineage 3 strains from Pune showed 5–8 amino acid differences with the P[8]-Lineage 1 strain of Rotarix and 2-5 amino acid differences with the P[8]-Lineage 2 strain of RotaTeq vaccine in the VP8* antigenic epitopes (Table 4A). These comprised S146G, S190N and N196G in epitope 8-1 and N113D, S125N, S131R, N135D in epitope 8-3 as compared with Rotarix vaccine strain. With regard to the P[8] strain of RotaTeq vaccine, the P[8]-Lineage 3 strains of this study showed three and one amino acid differences, respectively, in epitopes 8-1 (S146G, N190S, D196G) and 8-3 (N113D). Strain specific differences were noted at the amino acid positions 192, 193, 195 (epitope 8-1), and 114, 115, 116 (epitope 8-3) in a few (1-5) of the P[8]-Lineage 3 strains on comparison with both vaccine strains. Epitopes 8-2 and 8-4 were completely conserved. The amino acid substitutions in VP8* region were common to all P[8]-Lineage 3 strains at both time points (1992–1993 and 2006–2008).

To compare VP5* epitopes of the P[8]-Lineage 3 strains, we used complete VP4 sequences available for four P[8]-Lineage 3 strains, NIV-0613158, NIV-06361, NIV-061060, NIV-0715880 (Table 4B). These strains showed 1–2 amino acid differences (Y366D in all four strains, S388N in one strain, NIV-061060) with Rotarix and 2-3 amino acid differences (R384S, H386D in all four strains, S388N in NIV-061060) with RotaTeq in epitope 5-1. Epitopes 5-2 to 5-5 showed no variations (Table 4B).

P[8]-Lineage 4 strains, detected in Pune during 2007 and 2008, represented a highly divergent subgenotypic lineage and showed fourteen amino acid differences (twelve in VP8* and two in VP5*) with the Rotarix vaccine strain and fifteen amino acid differences (twelve in VP8* and three in VP5*) with the P[8] strain of

Table 2
Percent nucleotide and amino acid identities between the VP4 genes of G1P[8] rotavirus strains from Pune and rotavirus vaccines.

<table>
<thead>
<tr>
<th>Rotavirus strain/P[8] lineage</th>
<th>Percent nucleotide/amino acid identity with VP4 gene from vaccine strains</th>
<th>Rotarix</th>
<th>RotaTeq</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VP8*</td>
<td>VP5*</td>
<td>Complete VP4</td>
</tr>
<tr>
<td>NIV-06361/P[8]-3</td>
<td>90.9/92.1</td>
<td>88.6/95.1</td>
<td>89.4/94.2</td>
</tr>
<tr>
<td>NIV-061060/P[8]-3</td>
<td>91.0/92.9</td>
<td>89.2/95.3</td>
<td>89.7/94.4</td>
</tr>
<tr>
<td>NIV-0613158/P[8]-3</td>
<td>90.9/92.1</td>
<td>90.3/95.6</td>
<td>90.5/94.6</td>
</tr>
<tr>
<td>NIV-0715880/P[8]-3</td>
<td>90.2/91.2</td>
<td>89.2/95.6</td>
<td>89.5/94.2</td>
</tr>
<tr>
<td>NIV-07523/P[8]-4</td>
<td>87.0/87.0</td>
<td>88.4/94.7</td>
<td>88.0/92.5</td>
</tr>
<tr>
<td>NIV-083375/P[8]-4</td>
<td>86.8/87.0</td>
<td>88.6/94.9</td>
<td>88.1/92.6</td>
</tr>
</tbody>
</table>

a Six representative strains for which complete VP4 sequencing was done, are shown in the table.
### Table 3
Alignment of the amino acid residues in VP7 antigenic epitopes of the G1P[8] rotavirus strains circulating in Pune and rotavirus vaccine.

<table>
<thead>
<tr>
<th>Rotavirus strain / G1 lineage</th>
<th>Epitope 7-1a</th>
<th>Epitope 7-1b</th>
<th>Epitope 7-2</th>
</tr>
</thead>
</table>

The table indicates representative G1P[8] strains from children in Pune. The G1P[8] strains from adolescents and adults in Pune also showed the same lineage-specific amino acid substitutions (data not shown). Representative G1P[8] strains from Kolkata (GRAVP73, GRAVP731, GRAVP727, GRAVP718), Delhi (Dan279, Dan103) and Manipur (man-375/07, man-140/06) are also included for comparison. The amino acid differences between Rotarix and RotaTeq vaccine strains are shown in pink colour. The amino acid residues that differ from Rotarix are shown in yellow colour while those that differ from RotaTeq are in blue colour. The amino acid residues different from both Rotarix and RotaTeq are in green colour. The amino acid positions described previously [30] as neutralization escape mutation sites are shown in red colour.

RotaTeq vaccine (Table 4A and B). The variability between the [P8]-Lineage 4 and the vaccine strain was restricted to the epitopes 8-1, 8-2, 8-3 and 5-1 while the epitopes 8-4, 5-2 to 5-5 were completely conserved.

Comparison of the VP7 and VP4 epitopes of the G-Lineage1, [P8]-Lineage 3 strains reported from adolescents and adults in Pune [33,34], showed the same amino acid variations (data not shown) with respect to the vaccine strains as were noted in the present study (Tables 3 and 4) for the G-Lineage 1, [P8]-Lineage 3 strains from children in Pune.

Classification (Fig. 1(A) and (B)) and epitope analysis of the G1P[8] strains from Kolkata (2008–2009), Delhi (2000s) and Manipur (2006–2007) showed the same lineage-specific amino acid substitutions in the VP7 and VP4 epitopes, as noted for the strains from Pune (Tables 3 and 4). An earlier study of [P8] lineages of G1P[8] strains from Kolkata has described the circulation of [P8]-Lineages 3 and 4 during 2004–2005 [35]. These [P8]-Lineage 3 (ISO115, ISO114, ISO113, 2783) and [P8]-Lineage 4 (ISO117, ISO116, 47B3) strains also showed the same lineage-specific sequence variations in the VP8 epitopes (Table 4A).

### 4. Discussion

The World Health Organization has recommended inclusion of rotavirus vaccines in national immunization programs worldwide, especially in countries like India where diarrhoea is responsible for ≥10% mortality in children [36]. Two vaccines, Rotarix and RotaTeq are currently licensed for use against rotavirus. In India, Rotarix was launched in 2008 and RotaTeq in 2011. Both vaccines are available through the private sector. However, they have not been introduced into the national immunization program [37]. The Indian Academy of Paediatrics Committee on Immunization (IAPCOI) recommends administration of either of the vaccines to children with consent from the parents [38]. According to a nationally representative survey carried out during 2009–2010, 9.7% of sampled paediatricians in India reported routine administration of rotavirus vaccine [39]. However, given that the majority of childhood immunization is delivered by the public sector, data on rotavirus vaccine coverage in India is not currently available.

The mechanisms of protection against rotavirus after vaccination are not fully understood. This has resulted in the adoption of different approaches to the development of broadly protective vaccines. The RotaTeq vaccine (pentavalent) is based on the concept that genotype-specific neutralizing antibodies against the rotavirus outer capsid proteins VP7 and VP4 are the primary determinants of protection and thus includes VP7 and VP4 components of the major human rotavirus genotypes [40]. The Rotarix vaccine (monovalent G1P[8]), on the other hand, is based on the theory that protective immune response could be stimulated by B- or T-cell epitopes present on any rotaviral protein, and these epitopes may be conserved among different rotavirus VP7 and VP4 genotypes [40]. Both the vaccines have demonstrated efficacy against a range of genotypes in the developed countries [41–43].

The success of the rotavirus vaccines in India will depend on their ability to provide protection against the rotavirus strains prevalent in the country. G1P[8] rotavirus strains are predominant in India and are represented in both the current vaccines. In this study, we investigated the intragenotypic differences between the
Table 4
Alignment of the amino acid residues in (A) VP8* and (B) VP5* antigenic epitopes of the G1P[8] rotavirus strains circulating in Pune and rotavirus vaccines.

(A)

<table>
<thead>
<tr>
<th>Rotavirus strain / P[8] lineage</th>
<th>Epitope 5-1</th>
<th>5-2</th>
<th>5-3</th>
<th>5-4</th>
<th>5-5</th>
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<tbody>
<tr>
<td>G1-Lineage 1, P[8]-Lineage 3</td>
<td>384</td>
<td>386</td>
<td>388</td>
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<td>394</td>
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(B)

<table>
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<tr>
<th>Rotavirus strain / P[8] lineage</th>
<th>Epitope 5-1</th>
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<th>5-3</th>
<th>5-4</th>
<th>5-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1-Lineage 1, P[8]-Lineage 3</td>
<td>384</td>
<td>386</td>
<td>388</td>
<td>393</td>
<td>394</td>
</tr>
<tr>
<td>G1-Lineage 2</td>
<td></td>
<td></td>
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</tbody>
</table>

Table includes representative G1P[8] strains from children in Pune. The G1P[8] strains from adolescents and adults in Pune also showed the same lineage-specific amino acid substitutions (data not shown). Representative G1P[8] strains from Kolkata (GARVP432, GARVP431, GARVP427, GARVP418, ISO114, 47B3), Delhi (Dan279, Dan103) and Manipur (mani-375/07, mani-140/06) are also included for comparison. The amino acid differences between Rotarix and RotaTeq vaccine strains are shown in pink colour. The amino acid residues that differ from Rotarix are shown in yellow colour while those that differ from RotaTeq are in blue colour. The amino acid positions described previously [31, 32] as neutralization escape mutation sites are shown in red colour.

G1P[8] strains in India and the G1, P[8] components of Rotarix and RotaTeq vaccines, by comparison of the VP7 and VP4 sequences. We found that the G1P[8] rotavirus strains circulating in India belong to the G1-Lineage 1, P[8]-Lineage 3 (Figs. 1 and 2) and differ from the subgenotypic lineages of vaccine strains. On comparison with vaccine strains, the G1-Lineage 1, P[8]-Lineage 3 strains from India show amino acid variations at known neutralization escape mutation sites [30–32] within the VP7 and VP4 antigenic epitopes (Tables 3 and 4). Such amino acid variations between the different subgenotypic lineages warrant further investigation as they may ultimately affect vaccine efficacy, particularly if protection is mediated primarily by VP7 and VP4 genotype specific immune responses.

Antigenic differences have been reported previously between the G1-Lineage 2 and Lineage 3 strains which share 95.9–96.5% amino acid identity in VP7 protein and differ at the amino acid
positions 97 and 147 in the VP7 epitopes. Antisera raised against the G1-Lineage 3 strain, D, neutralized another strain (Wa) of the same lineage more efficiently than G1-Lineage 2 strains [44]. This raises questions of antigenic variability between the G1-Lineage 1 strains prevailing in India and G1-Lineages 2 (Rotarix) and 3 (RotaTeq) of rotavirus vaccine strains and the immune response induced by them.

A study conducted to examine the antigenic differences between the strain MX08-659 of P[8]-Lineage 3 and the Wa strain of P[8]-Lineage 1, has described the use of truncated recombinant VP8* peptides from each of these strains and suggested the presence of conserved epitopes in the VP8* variable region [45]. However, in the present study, comparison of the VP8* epitopes of the P[8]-Lineage 3 strains from India with the vaccine strains of P[8]-Lineage 1 (Rotarix) or Lineage 2 (RotaTeq) revealed amino acid differences (Table 4A and B) at known neutralization escape mutation sites [31,32].

Rotavirus strains belonging to the G1-Lineage 1, P[8]-Lineage 4 (Figs. 1 and 2) have been identified in India during the 2000s. The antigenic properties of the P[8]-Lineage 4 or OP354-like strains are not well understood. The P[8]-Lineage 4 strains are being increasingly detected worldwide [13,16,17,20,21,46–48] leading to speculation about the long term protective effect of the current vaccines against this divergent lineage.

The G1-Lineage 1, P[8]-Lineage 3 strains, indicating the same lineage-specific amino acid substitutions noted in the present study (Tables 3 and 4), are currently in circulation worldwide [8,9,11–23] including in Europe and America wherein the efficacy of rotavirus vaccines is high [41–43]. Thus, sequence differences in VP7 and VP4 encoding genes, between the circulating G1P[8]* strains and the G1, P[8] components of vaccine strains, do not seem to render any effect as yet on vaccine efficacy in these countries. In fact, Rotarix vaccine (monovalent G1P[8]) has been shown to be effective even against non-G1P[8] rotavirus strains [42,43]. Antibodies against immunorecessive neutralization epitopes in VP7 and VP4 which are conserved between genotypes and/or immune responses against internal or non-structural proteins are thought to be responsible for this apparent cross-protection [40]. Although both vaccines have shown substantial utility in Europe and America to date, it has been suggested that their long term use may result in selection of strains capable of escaping vaccine-induced immunity [49]. It is worth noting that, after the introduction of Rotarix vaccine in Belgium, the decrease of G1P[8] strains belonging to lineages closer to Rotarix was more than the decrease of G1P[8] strains distinctly related to Rotarix [50].

In conclusion, the present study describes differences between the G1P[8] rotavirus strains circulating in Pune, India and the G1 and P[8] components of the Rotarix and RotaTeq vaccines. In order to understand the significance of these differences and their influence if any, on vaccine efficacy, further investigation of the intragenotype antigenic variability and the protective mechanism of vaccines would be necessary. Any increase in use of the rotavirus vaccines in India, may have long term effects on strain evolution leading to emergence of novel strains. This warrants continuous monitoring of the subgenotypic lineages within the diverse rotavirus G1P[8] strains.

Conflict of interest statement

The authors have no conflicts of interest to report.

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