Abstract

Rotaviruses, members of family *Reoviridae*, are the major etiologic agents of acute gastroenteritis in infants and young children, worldwide. These viruses are classified in seven antigenic groups (A-G) of which groups A, B and C are known to infect humans. A dual classification system based on the outer proteins VP4 (P) and VP7 (G) has identified 27G and 35P genotypes in group A rotaviruses. To date, more than 60 different G-P combinations have been identified. G1-G4, G9, P[4], and P[8] represent common types detected among children, worldwide. Five most common types (G1-G4, P[8]) have been targeted in the rotavirus vaccines. It is estimated that group A rotaviruses account for 527,000 deaths annually in children with majority of the deaths occurring in low income countries. These viruses are also known to cause gastroenteritis among adults in a variety of settings. Adults infected with such viruses could act as a source of infection to the susceptible individuals and affect the epidemiology of rotavirus disease in infants and young children. Infections with group B rotaviruses are also detected frequently in adolescents and adults.

At the time of initiation of this study, the data available on the rotavirus infections in adolescents and adults from India was limited to serological and molecular identification of group A and group B rotaviruses. In view of this, the present study was undertaken to understand the epidemiological and molecular diversities of rotavirus infections in adolescent and adult cases of acute gastroenteritis.

A total of 1591 fecal specimens were collected in 1993-1996 and 2004-2007 from adolescents and adults with acute gastroenteritis in Pune, India. Group A rotavirus was detected in 10.0% and 7.0% of the specimens collected from adolescents and adults respectively. Overall, the combined positivity values obtained for the two time points (1993-1996 and 2004-2007) were not different (p>0.05) in two groups examined in the study. Common rotavirus strains - G1P[8], G2P[4], G3P[8] and G4P[8] were detected in 53.1% of the specimens from 1993-1996, while the only prevalent strain
identified in 2004-2007 was G2P[4] (23.5%). Uncommon rotavirus strains increased from 7.8% (1993-1996) to 41.2% (2004-2007) while the prevalence of mixed rotavirus infections was significant (39% / 35%) at both time points. Though the proportion of nontypeable rotavirus strains was higher in 2004-2007 for both groups of patients, it was not statistically different from that of the 1993-1996 (P>0.05). Mixed infections detected by multiplex PCR were confirmed by sequencing two or more individual genotype-specific PCR products of the VP7 and VP4 genes from the specimens. The VP6 gene sequences of the nontypeable strains were most homologous to animal strains.

To detect rotaviruses in group A rotavirus antigen capture ELISA negative specimens, RNA PAGE was carried out on 704 specimens selected from the two time points (1993-1996 and 2004-2007). Group A and B rotaviruses were detected in 1.8% and 0.6% of the specimens respectively.

To study the inter and intragenotypic diversity between the group A rotavirus strains circulating at the two time points, sequencing and phylogenetic analysis of four genes encoding VP4, VP6, VP7 and NSP4 proteins was carried out. This was followed by analysis of linkage between the four genes studied.

The multiplex RT-PCR performed on 131 (n=84 from the 1990s and n=47 from the 2000s) rotavirus positive fecal specimens, detected single and mixed infections of VP7 (49.6% and 36.6%) and VP4 (43.8% and 56.2%) genotypes respectively. These included 43G1 (38.1%), 37G2 (32.7%), 8G3 (7.1%), 15G4 (13.3%), 10G9 (8.8%) and 73P[4] (43.2%), 69P[8] (40.8%) and 27P[6] (16.0%) specificities.

Sequencing and phylogenetic analysis of the VP7 gene amplicons revealed the presence of G1-IA (4.7%), G1-IB (69.8%) and G1-IC (25.5%) lineages within the G1 strains, G2-IBb1 (70.3%) and G2-IBb2 (29.7%) lineages within G2 strains, G3-3S1 (12.5%) and G3-3S4 (87.5%) lineages within G3 strains, G4-la (6.7%) and G4-lb (93.3%) lineages within G4 strains and G9-III lineage within G9 strains. The variability within the VP7 genotypes was also evident by 1.4-8.0% and 1.3-3.9% amino acid divergence detected.
respectively from the prototype strains and between the groups of strains at the two time points.

Phylogenetic analysis of the VP4 gene amplicons revealed circulation of P[4]-5 (95.9%) and P[4]-1 (4.1%) lineages in P[4]. P[8]-3 (85.5%), P[8]-2 (13.0%) and P[8]-4 (1.4%) lineages in P[8] and P[6]-1 (100%) lineages in P[6] genotypes. Amino acid divergence between the groups of strains with P[4] / P[8] / P[6] genotypes from both time points was 0.2-2.3%. Analysis of the linkage of VP4 (P[4] / P[8]) genotypes with VP7(G) genotypes showed significant difference (P<0.01) in their association with common and G nontypeable strains at both time points studied.

NSP4 and VP6 encoding genes of a total of 118 rotavirus strains recovered at the two time points 1993-1996 and 2004-2007 investigated in the study were characterized to determine their diversity and genetic linkage. Amplification of NSP4 and VP6 genes was noted in 82% and 89% of the strains respectively in RT-PCR. Sequencing and phylogenetic analysis of the VP6 genes showed distribution of VP6 genogroups in the lineages I-1 (1.4%), I-2 (50.7%) and II-4 (47.9%) in the 1990s and I-2 (73.5%) and II-4 (26.5%) in 2000s, indicating diversity in the genogroups at both time points. Amino acid divergence within the genogroup II strains from the 1990s and genogroup I strains from the 2000s was noteworthy (4.7-6.7%). Sequencing and phylogenetic analysis of the NSP4 genes showed almost equal distribution (45.0-55.0%) of genotypes A (E2) and B (E1) however, higher amino acid divergence was noted within the genotype B strains (upto 9.3%) than in genotype A strains (upto 2.9%) at the two-time points. Nearly 70% of the strains showed concordance in [NSP4-A (E2) - VP6 I (I2) or NSP4-B (E1) - VP6 II (I1)] in the NSP4 and VP6 genetic linkage. The discordance in the linkage noted in 29.7% of the strains was predominated by NSP4-B (E1) and VP6-I (I2) combination and appeared strikingly high in the infections caused by unusual and mixed rotavirus strains.

Eighty group A rotavirus strains typed for all four genes viz VP4(P), VP6(I), VP7(G) and NSP4(E) were analyzed to determine their genetic linkage. Of these 48% and 31% presented common genotype combinations (G1-P8-I1-E1, G2-P4-I2-E2, G3-P8-I1-E1 and G4-P8-I1-E1), 7.5% and 23.0%
presented unusual combinations (G2-P8-I2-E2, G9-P6-I1-E1, G9-P6-I1-E2, and G9-P6-I2-E1, G1-P[4]-I2-E1, G4-P[4]-I1-E2 and G9-P[4]-I1-E1), while 31.3% and 46.1% presented 21 different mixed (G and / or P) infections respectively in the 1990s and 2000s. A high frequency of discordance (52.2% - 69.2%) noted in the linkage of four genes highlighted occurrence of reassortment within the human group A rotavirus strains circulating among adolescents and adults.

Phylogenetic analysis of the VP4, VP6 and VP7 genes of the group B rotavirus strains classified them in the Indian-Bangladeshi lineage of G2 genotype described for group B rotavirus strains to date. All four strains showed 89.7-92.7% / 88.9-91.5%, 91.4 - 94.6% / 95.2 - 97.2% and 94.3-95.7% / 97.5-97.6% nucleotide / amino acid identities respectively with the VP4, VP7 and VP6 genes of the prototype strain, ADRV. With the recently reported strains, MMR-B1 (Myanmar) and IDH-084 and IC-008 (India) 96.7-99.1% / 92.8-97.3%, 95.4-99.5% / 96.6-99.2% and 98.1-99.8% / 100% nucleotide / amino acid identities were noted for these genes respectively.

To conclude, the present study reports molecular epidemiology of rotavirus infections among adolescents and adults for the first time from India. Identification of unusual strains and mixed infections of group A rotaviruses at a significant level among adolescent and adult cases of gastroenteritis indicates occurrence of a pool of strains that could affect the epidemiology of rotavirus infections in children. Intragenotypic diversity and discordance in the linkage noted in four important genes of group A rotaviruses emphasize the need for constant surveillance of rotavirus infections for better understanding of the evolution and transmission of group A rotaviruses in the community and evaluation of preset rotavirus vaccines. Also, the detection of group B rotavirus strains known to have potential to cause outbreaks of gastroenteritis warrants surveillance studies for this virus.