Emergence of Novel Human Group A Rotavirus G12 Strains in India

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Rotaviruses are the major cause of acute gastroenteritis in infants and young of a wide variety of mammalian and avian species (11). In developing countries, approximately 130 million infants are infected with rotaviruses and there are 800,000 annual deaths (5, 12). In group A rotaviruses, 15 VP7 G serotypes and 21 VP4 P genotypes have been reported for humans, animals, and birds (17). Though 10 G types and 10 P types from humans have been reported (15), clinically and epidemiologically the most important strains belong to the G1 to G4 serotypes with P[8] and P[4] genotypes (6, 14). On the other hand, some unusual types (G5, G8, and G9) and rare combinations of G and P types from different countries have also been reported (1, 2, 3, 4, 13, 16, 20). The human rotavirus G12 strains (L26 and L27) were first detected from Philippines in 1990 (19). After more than a decade, in 2002, human G12 strains Se585 (9) and T152 (15) from the United States and Thailand, respectively, were reported. In this study, we report the detection of three rare G12 strains of human rotaviruses in India.

As part of a routine surveillance study for diarrheal diseases, stool samples were collected from children below 4 years of age from B. C. Roy Children’s Hospital and also from patients of all age groups admitted to the Infectious Diseases Hospital, Calcutta, India, in 2001. Stool samples were also collected from children with diarrhea from Assam Medical College, Dibrugarh, Assam, India; Capital Hospital and Municipality Hospital, Bhubaneswar, Orissa, eastern India; and Post-Graduate Institute of Medical Education and Research, Chandigarh, northern India. A total of 454 samples were screened for rotaviruses by RNA electrophoresis as described earlier by Herrick et al. (10). The fecal specimens were processed for extraction of rotavirus double-stranded RNA suitable for reverse transcription and amplification of the VP4 and VP7 genes as described previously (4). G and P typing of positive samples was carried out by nested and multiplex PCR with consensus and type-specific primers as described previously (4, 7, 8, 18, 21). The amplified products were purified with either the QIAquick gel extraction kit or the PCR purification kit (Qiagen, GmBH) in accordance with the manufacturer’s instructions. Direct sequencing was carried out by using ABI PRISM Big-Dye terminator cycle sequencing kits (Applied Biosystems) with an automated DNA sequencer, the ABI PRISM 310 genetic analyzer (Applied Biosystems). The sequencing of the VP7 genes of all three isolates was repeated three times to eliminate sequencing error. Nucleotide sequences of the VP7 genes of group A rotaviruses deposited at the GenBank database along with the VP7 gene sequences of ISO 1, ISO 2, and ISO 5 were analyzed for the construction of a phylogenetic tree by the Bootstrap neighbor-joining tree method, with a random number generator seed of 500 and 5,000 bootstrap trials, using the CLUSTAL X software program.

In 2001, a total of 454 stool samples were screened for rotaviruses by RNA electrophoresis; there were 326, 104, 98, and 26 samples from diarrheic patients in Calcutta, Dibrugarh, Bhubaneswar, and Chandigarh, respectively. Out of 161 rotavirus-positive samples, 126 samples were available in adequate quantity for VP7 genotyping. Reverse transcription-PCR results showed that the G types or P types of 21 samples could not be determined (data not shown). The full-length VP7 gene was amplified in the first PCR; however, there was no visible DNA band after the second round of multiplex PCR. A few such untypeable strains were selected for VP7 gene sequencing. After the sequencing, the partial sequences (32 to 1,034 bp) from three strains (ISO 1, ISO 2, and ISO 5) showed maximum homology with that from human prototype G12 strain L26 (19). The VP7 gene of group A rotaviruses is 1,065 nucleotides long and codes for a 326-amino-acid protein and showed maximum identity with that from the L26 rotavirus strain (Table 1). Low identities at the nucleotide and amino acid levels were observed (65 to 75% at the nucleotide level and 67 to 81% at the amino acid level) compared with all...
sequences from G1 to G15 prototype strains other than L26 (Table 1). These three G12 strains (ISO-1, ISO-2, and ISO-5) were collected from children less than 8 months of age admitted to the B. C. Roy Children’s Hospital between January and April 2001. ISO-1 and ISO-2 rotaviruses were long-electropherotype strains, whereas ISO-5 was a short-electropherotype strain. The VP4 genotype of ISO-2 and ISO-5 was P[6]; on the other hand, ISO-1 was untypeable.

To understand the potential implication of these findings for the epidemiology of rotavirus infection, we sequenced the VP7 genes of some untypeable strains. Sequencing three untypeable rotavirus strains resolved them as G12. G12 was first detected in Philippines in 1990 (19) and in the United States (9) and Thailand (15) in 2002. So this is the first report of the isolation of G12 strains in India. The percentages of homology at the nucleotide (90%) and amino acid (92%) levels with prototype G12 strain L26 were low. Similarly, the percentages of homology with other G1 to G15 type-specific strains of group A rotaviruses were very low. On the other hand, ISO-1 was untypeable.

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REFERENCES


