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ORIGINAL ARTICLE

Diversity of Rotavirus Strain Circulated in Gwangju, Republic of Korea

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Abstract
Objectives: The introduction of new rotavirus vaccines into the public sphere makes it necessary to maintain constant surveillance and to heighten public awareness of the appearance of new rotavirus strains. We describe the molecular epidemiology of circulating rotavirus strains after vaccine introduction.
Methods: We collected a total of 1070 stool samples from children with gastroenteritis from January 2013 to June 2013. The antigenic prevalence of rotavirus group A was distinguished using enzyme immunoassay. The G and P genotypes of enzyme immunoassay-positive samples were determined with reverse transcription-polymerase chain reaction and nucleotide sequencing analysis.
Results: Of the 1070 samples collected, 277 (25.9%) tested positive for rotaviruses by enzyme-linked immunoabsorbent assay. The most prevalent circulating genotype G was G1 (51.3%), followed by G2 (34.7%) and G9 (10.8%). The predominant type of genotype P was P[8] (66.1%), followed by P[4] (31.4%). In this study, nine genotypes were found. G1P[8] was the most prevalent (51.8%), followed by G2P[4] (30.5%), G9P[8] (9.9%), and G2P[8] (4.0%). Several unusual combinations (G1P[4], G3P[9], G3P[8], G4P[6], and G9P[4]) were also identified.
Conclusion: Molecular epidemiological knowledge of rotaviruses is critical for the development of effective preventive measures, including vaccines. These data will help us monitor the effectiveness of current rotavirus vaccines.

1. Introduction

Rotaviruses (RVs) are the leading cause of acute and severe gastroenteritis, diarrhea, and malnutrition primarily in children younger than 5 years [1], and are responsible for approximately 600,000 deaths worldwide each year [2,3].

RVs belong to the family Reoviridae. The viral genome consists of 11 segments of double-stranded (ds) RNA genome that encodes six structural proteins

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(VP1−4, VP6, and VP7) and six nonstructural proteins (NSP1−6) [1]. So far, RVs are subdivided into eight groups (A−H) on the basis of the antigenic properties or the amino acid sequences of the inner capsid protein VP6 [4].

Based on the differences of *VP7* and *VP4* gene sequences, RVs are divided into genotypes G and P, respectively. To date, at least 27 G and 35 P genotypes have been reported from humans and a variety of mammalian and avian species [5]. Of these, 11 G genotypes and 12 P genotypes have been isolated from humans [6−8]. However, the most commonly isolated human RV genotypes are a small number of combined G/P genotypes, such as G1P[8], G2P[4], G3P[8], G4P [8], and G9P[8] [8,9]. Certain G and P types have been found to be highly prevalent in different areas around the world such as G5 types in Brazil and G10 types in India [10,11]. More recently, a surveillance program directed by the World Health Organization noted that in 2010 the predominant uncommon strains were G12P[8] and G12P[6] viruses in Southeast Asia; G2P[6], G3P[6], and G1P[6] viruses in sub-Saharan Africa; G1P[4] and G2P[8] viruses in the Western Pacific; and G9P[4] viruses in the Americas [9].

In South Korea, molecular epidemiological studies have shown that G2, G4, and G9 were the most isolated genotypes from 1998 to 2004. G1 was the most predominant genotype from years 1987 to 1999, and genotypes G2, G3, and G4 were also isolated during the same period [12]. G11, a rare strain, was also reported for the first time in South Korea in 2007 [13]. Recently, there have been changes in the frequency of genotype isolations from more common G/P combinations with the exceptions of G1P[8] and G3P[8] to the rare combinations of genotypes such as G4P[6] and G2P[8] [14]. These distinct changes in the prevalence of circulating RV strains suggest that surveillance studies are important for the successful vaccine development and efficacy testing.

Even with the ongoing vaccinations against RVs, RV infection with high morbidity and mortality occurs. This indicates that we should take into account the role of RV vaccines in the natural temporal variability in genotypes G and P. Two live attenuated RV vaccines were licensed and became commercially available in South Korea. RotaTeq (Merck & Company, Inc, Whitehouse Station, NJ, USA), launched in September 2007, is a vaccine that consists of five distinct bovine reassortants, and each of the five vaccine strains contains outer capsid proteins from a serotype of the human RVs (G1, G2, G3, G4, and P[8]). Rotarix (GlaxoSmithK-line, Rixensart, Belgium), licensed in June 2008, consists of a single attenuated G1P[8] strain of human RV.

The effect of RV vaccines on the natural pattern of circulating RV strains in human populations is unknown and difficult to predict. Continuing surveillance is needed to identify the spectrum of protection engendered by each vaccine and the effect that each vaccine may have on circulating strains. Documentation of long-term temporal changes in RV strain distributions requires a detailed analysis of targeted monotypes in circulation prior to and after vaccine introduction.

In this study, we report the distribution of RV genotypes G and P that have been circulated during the first half of 2013 in Gwangju, South Korea.

2. Materials and methods

2.1. Sample collection

From January 2013 to June 2013, a total of 1070 stool samples were collected from children who were hospitalized with acute gastroenteritis symptoms in eight hospitals, Gwangju, South Korea. Samples were kept at 4°C until they were transported to the laboratory for analysis. Clinical information on age and sex of patients, dates of disease onset and sample collection, symptoms, RV vaccination history, etc., was recorded as background data.

2.2. RV antigen detection

RV antigens were detected in stool supernatants using enzyme-linked immunoabsorbent assay (ELISA) with VP6 group-specific antibody (BioTracer Rotavirus ELISA kit; Biofocus, Uiwang, Korea) according to the manufacturer's instructions. Specimens with Optic Density (OD) absorbance values >0.4 at a 450-nm wavelength were considered to be positive.

2.3. Reverse transcription-polymerase chain reaction for genotyping

G and P genotyping was performed using reverse transcription-polymerase chain reaction (RT-PCR) on 277 RV ELISA positive samples. Fecal specimens were diluted 1:10 in phosphate-buffered saline. After a thorough mixing, each fecal suspension was centrifuged for 20 minutes at 1000 × *g*, 4°C.

RV ds RNA was extracted from 140 μL of 10% fecal suspensions using an RNA extraction Kit [QIAamp Viral RNA mini Kit (spin protocol); Qiagen, Inc., Hilden, Germany] in accordance with the manufacturer's instructions. The extracted RNA was denaturated at 95°C for 5 minutes. RT-PCR was performed for RV G and P genotypes using Accupower Hotstart RT/PCR premix kit (Bioneer, Daejeon, Korea). We amplified an 881-bp fragment of the *VP7* gene with the consensus forward primer VP7-F (5′-ATG TAT GGT ATT GAA TAT ACC AC-3′) and reverse primer VP7-R (5′-AAC TTG CCA CCA TTT TTT CC-3′) [15]. We also amplified an 876-bp fragment of the *VP4* gene with the consensus forward primer Con3 (5′-TGG CTT CGC CAT TTT ATA GAC A-3′) and the reverse primer Con2 (5′-ATT TCG GAC CAT TTA TAA CC-3′) [16]. The PCR reaction for the *VP7* and *VP4* gene amplification