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Brief Communication

Detection and characterisation of rotaviruses from children less than 5 years hospitalised with acute gastroenteritis in Nagercoil

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Abstract

Group A rotavirus continues to be the major cause of severe gastroenteritis in young children in developing countries. In this study, we report the prevalence and genotype of rotaviruses identified from children <5 years of age hospitalised with acute gastroenteritis from Nagercoil, Tamil Nadu from 2007-2010. From the 139 children included in the study, 71 samples (51%) were positive by ELISA and 65 samples were positive by PCR-based methods. G1P[8] (44.6%) was the most commonly identified genotype. In addition, we report detection of rotavirus in two of three CSF samples from children with seizures.

Key words: *Cerebrospinal fluid, genotyping, Nagercoil, rotavirus*

Introduction

Rotaviruses are responsible for at least 36% of all diarrhoea admissions and for an estimated 37% of diarrhoea deaths, translating to 453,000 deaths in children less than 5 years of age across the world, with India alone contributing to 22% of deaths due to rotavirus.[1,2] Rotavirus is an 11-segmented double stranded RNA (dsRNA) virus and is classified into G and P genotypes based on the variability in the genes encoding VP7 and VP4 outer capsid proteins, respectively. Currently, 27 G types and 35 P types have been identified.[3] Recent data from the Indian Rotavirus Strain Surveillance Network have emphasised the need for region-specific genotyping information to study rotavirus epidemiology and to monitor strain variation after vaccine introduction.[4] The objective of this study was to detect and characterise rotavirus strains among children less than 5 years of age presenting with acute gastroenteritis requiring hospitalisation in Nagercoil. In addition, cerebrospinal fluid (CSF) from three children with acute gastroenteritis and seizures were also tested for rotavirus.

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Material and Methods

Between September 2007 and January 2010, 139 stool samples were collected from children less than 5 years presenting with acute diarrhoea and/or vomiting at the Moses Mathias Hospital in Nagercoil, southern India and were screened for rotavirus. In addition, cerebrospinal fluid (CSF) was collected from three children with gastroenteritis and seizures. The samples were stored at 4°C on collection and transported periodically on ice to the testing laboratory. The samples were stored at −70°C for characterisation of rotavirus strains. The study was approved by the Institutional Review Board.

All stool samples were screened for rotavirus using a commercial enzyme immunoassay for the detection of VP6 antigen (Dako Rota IDEIA, Ely, UK). Viral RNA was extracted from 30% fecal suspensions using the Trizol reagent (Invitrogen, Life Technologies, United Kingdom). Complementary DNA (cDNA) was generated by random priming and VP6 PCR was performed on all samples using previously published primers[5] to allow comparison between ELISA and PCR detection of rotavirus. All samples found to be positive by VP6 PCR were further characterised by genotyping PCR to identify the G and P genotypes using the primers and protocols used in the Indian rotavirus surveillance network.[4,6] Samples that were ELISA positive, but negative in the VP6 PCR were not retested by either assay or further characterised. The three CSF samples were also subjected to VP6 and genotyping PCRs.

Results

Rotavirus was detected by ELISA in 71 of 139 children, accounting for 51% of samples tested. Of these, 60 samples were confirmed as positive by VP6 PCR. Additionally, five samples that were negative by ELISA were positive by VP6 PCR. Of the 65 samples positive by VP6 PCR,

the most common genotype was G1P[8], accounting for 44.6% of all positive samples. The other genotypes were G2P[4] (18.4%), G12P[8] (12.3%) and G12P[6] (9.2%). Partial genotyping was obtained for five samples, and five samples (7.7%) remained untypable [Figures 1a-b and 2].

Two of three CSF samples were positive for rotavirus by VP6 PCR. One sample was from a child with G2P[4] rotavirus gastroenteritis and Reye's Syndrome. The other child presented with emesis, persistent pulmonary hypertension of newborn and pneumonia and the stool sample was negative for rotavirus. Although VP6 was positive in the CSF of these two children, the rotavirus in the CSF could not be genotyped by routine VP7 and VP4 typing techniques, and sample volume did not permit alternative approaches.

Discussion

Rotavirus was detected in 51% of the samples by ELISA and in 47% of samples tested by PCR for the VP6 gene. The samples that were initially positive by ELISA but were negative by VP6 PCR, were borderline positives; even though they were antigen positive, it is possible that with low initial viral content, RNA may have degraded by the time the samples reached the testing laboratory. This study demonstrates the high rate of rotavirus gastroenteritis in hospitalised children who generally have more severe disease. ELISA could be a simple and valuable diagnostic tool in regional hospitals where advanced molecular methods may not be available. Having regional data available may be useful in promoting the reduction of the indiscriminate use of antibiotics to treat children with gastroenteritis.

In this study, G1P[8] and G2P[4] account for 63% of all rotavirus genotypes. G2P[4] was the predominant strain in 2007 and G1P[8] in 2008 [Table 1]. In a previously published study, the dominant strain over the same 2-year period was G2P[4] in south India, while in north India G1P[8] was most frequently identified.[4,7] It is interesting to note that in 2007, G12P[6] and G12P[8] were each detected in 14.2% of the cases tested. There is limited data on the prevalence of the P[6] VP4 genotype in southern India. The fact that P[6] was seen with G12, a newly emerged G genotype during that period indicates that these strains may have been newly introduced in this region when compared to north India. There was a reduction in the prevalence of G12P[6] in the subsequent 2 years, while G12P[8] continued as a predominant genotype in 2009.

There have been a number of reports of extra-intestinal manifestations, such as respiratory infections and seizures, in children with rotavirus infections in recent years.[8,9] In this study, rotavirus was detected in the CSF of two children, including one with documented rotavirus shedding in stool. The samples could not be genotyped, possibly due to a very low viral load in CSF. Although the numbers of samples tested were very small, these and other similar data from elsewhere reinforce the need to testing for rotavirus as an extra-intestinal pathogen in children.[10]

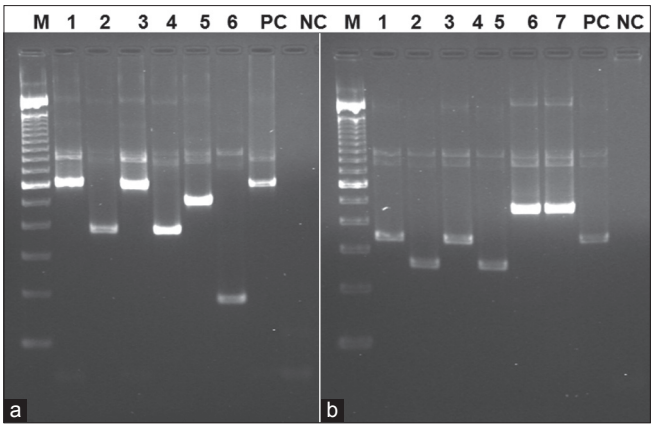

Figure 1: (a) and (b) show the agarose gel pictures of the common G and *P* types (A and B respectively)-M (100 bp molecular weight ladder)- G1P[8], G12P[6], G1P[8], G12P[6], G2P[4], G9P[4] AND Positive CONTROL (PC) G1P[8]

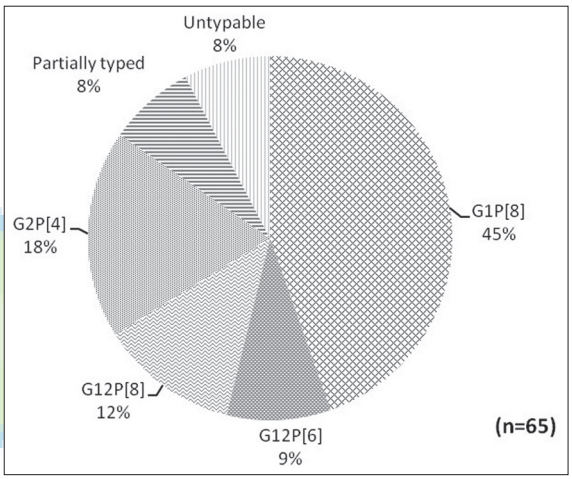

Figure 2: Distribution of rotavirus genotypes in Nagercoil

Table 1: Year wise distribution of common rotavirus genotypes in Nagercoil [*n* (%)]

Year	Stool samples tested (%rotavirus positive)	G1P[8] (%)	G2[P4] (%)	G12P[6] (%)	G12P[8] (%)
2007	45 (47)	1 (4.7)	**11 (52)**	3 (14.2)	3 (14.2)
2008	60 (47)	**23 (79.3)**	1 (3.4)	2 (6.8)	0 (0)
2009	34 (47)	4 (28.5)	0 (0)	1 (7.1)	**5 (35.7)**

Most common- bold

Summary

This study demonstrates that rotavirus is an important cause of severe gastroenteritis in children in southern India. Continued monitoring and surveillance of rotavirus

strains will be valuable, especially with the availability of two vaccines against rotavirus in a country with high strain diversity. The wider use of ELISA for detection of rotavirus may help establish a diagnosis in acute gastroenteritis and thus prevent indiscriminate use of antibiotics.

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