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Hospital based surveillance and genetic characterization of rotavirus strains in children (<5 years) with acute gastroenteritis in Kolkata, India, revealed resurgence of G9 and G2 genotypes during 2011–2013

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ABSTRACT

Introduction: India accounts for an estimated 457,000–884,000 hospitalizations and 2 million outpatient visits for diarrhea. In spite of the huge burden of rotavirus (RV) disease, RV vaccines have not been introduced in national immunization programme of India. Therefore, continuous surveillance for prevalence and monitoring of the circulating genotypes is needed to assess the disease burden prior to introduction of vaccines in this region.

Methods: During January 2011 through December 2013, 830 and 1000 stool samples were collected from hospitalized and out-patient department (OPD) patients, respectively, in two hospitals in Kolkata, Eastern India. After primary screening, the G-P typing was done by multiplex semi-nested PCR using type specific primers followed by sequencing. Phylogenetic analysis for the VP7 gene of 25 representative strains was done.

Results: Among hospitalized and OPD patients, 53.4% and 47.5% cases were positive for rotaviruses, respectively. Unlike previous studies where G1 was predominant, in hospitalized cases G9 rotavirus strains were most prevalent (40%), followed by G2 (39.6%) whereas G1 and G12 occurred at 16.4% and 5.6% frequency. In OPD cases, the most prevalent strain was G2 (40.3%), followed by G1, G9 and G12 at 25.5%, 22.8%, 9.3%, respectively. Phylogenetically the G1, G2 and G9 strains from Kolkata did not cluster with corresponding genotypes of Rotarix, RotaTeq and Rotavac (116E) vaccine strains.

Conclusion: The study highlights the high prevalence of RV in children with gastroenteritis in Kolkata. The circulating genotypes have changed over the time with predominance of G9 and G2 strains during 2011-2013. The current G2, G9 and G1 Kolkata strains shared low amino acid homologies with current vaccine strains. Although there is substantial evidence for cross protection of vaccines against a variety of strains, still the strain variation should be monitored post vaccine introduction to determine if it has any impact on vaccine effectiveness.

1. Introduction

Rotavirus is the leading cause of diarrhea and is associated with 453,000 childhood deaths globally [2]. India accounts for an estimated 457,000–884,000 hospitalizations, 2 million outpatient visits for diarrhea, resulting in huge medical and health care costs [1]. Annually more than 334,000 deaths occurring in Indian children are attributed to diarrheal disease, of which about 98,000 deaths are due to rotavirus alone [3].

Group A rotavirus (RVA) is a double stranded RNA virus consisting of 11 segments. Two outer capsid proteins, VP7 (G genotype) and VP4 (P genotype), independently elicit a serotype-specific neutralizing immune responses that may play an important role in protection against recurrent infections [4]. These viruses are genetically diverse, and RVA VP4 and VP7 encoding genes have been classified into atleast 27 G genotypes (G1–27) and 37 P genotypes (P[1]–[37]), respectively, based on differences in their nucleotide sequences [5,6]. The segmented nature of rotavirus genome

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provides the mechanism for the generation of genetic diversity by the process of genetic reassortment, which may occur during co-infections of circulating human and animal strains [7–9].

Two rotavirus vaccines namely Rotarix® (RV1; monovalent G1P[8]; GlaxoSmithKline Biologicals, Rixensart, Belgium) and RotaTeq® (RV5; pentavalent G1, G2, G3, G4,P[8]; Merck Vaccines, Whitehouse Station, NJ, USA) are commercially available since 2006. Recently, another oral live attenuated vaccine candidate has been evaluated in phase III studies in India, and is derived from a G9P [11] human bovine reassortant strain 116E [10–12]. Large scale vaccine trials with Rotarix and RotaTeq have shown high efficacy in developed countries of Europe, Australia and USA though efficacy is lower (39–72%) in low income countries of Asia and Africa [13–15]. In spite of lower efficacy, these vaccines reduce a greater number of severe rotavirus gastroenteritis events in developing countries because of the great background rate of disease, resulting in the WHO's recommendations for introduction of RV vaccines in national immunization programs worldwide in 2009 [16]. However, RV vaccines have still not been introduced in national immunization programme of most South Asian and African countries, for several reasons including lack of disease burden data and economic feasibility.

During the past decade, several surveillance studies in hospitalized children have reported prevalence and strain diversity of RVA across India [18–22]. A multicenter hospital based study (2005–2009) in India, including Eastern India, estimated 40% hospitalization rates due to rotavirus [17,21]. The predominant strain circulating during 2005–2009 was G1P[8], followed by G2P[4]. G3, G4, G9 and G12 strains were observed at lower frequency (<10%) [17,21,22].

Most surveillance studies done in India were focussed on children hospitalized with acute gastroenteritis; however, the proportion of RVAs in cases of milder diarrhea and often reporting to outpatient departments (OPD) (some or no dehydration) remains largely unknown. The aim of this study was to analyse prevalence of rotavirus among children either hospitalized with severe diarrhea or seeking treatment for milder diarrhea in OPD (during January 2011–December 2013) and to compare the rotavirus genotypes among the two sets of patients.

2. Methods

2.1. Sample collection and screening

The study was conducted from January 2011 through December 2013 in ID-BG Hospital and B.C. Roy Memorial Hospital for Children in Kolkata, Eastern India. Stool samples of every fifth admitted patient (≤5 years of age) with acute watery diarrhea, vomiting and abdominal pain, were collected. The inclusion criteria for OPD patients included passing of three or more loose/watery stools within 24 h [23]. A total of 830 stool samples were collected from hospitalized patients and 1000 stool samples were collected from OPD patients. The consent of the guardian was obtained prior to enrolling a child. The study was approved by the Institutional Ethical Committee, National Institute of Cholera and Enteric Diseases. Preliminary screening of the stool samples for the presence of RVAs was performed using Rota-Adeno kit as per the manufacturer's instructions (VIKIA® Rota-Adeno, Biomerieux® sa).

2.2. Viral RNA extraction and genotyping

All the rotavirus positive samples, detected by VIKIA® Rota-Adeno kit, were confirmed for positivity by reverse transcription and PCR to avoid a false positive result. RVA double-stranded RNA was extracted from feces of positive samples by using a commercially available RNA extraction kit (QIAamp viral RNA Mini Kit, Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Complementary DNA was synthesized from the

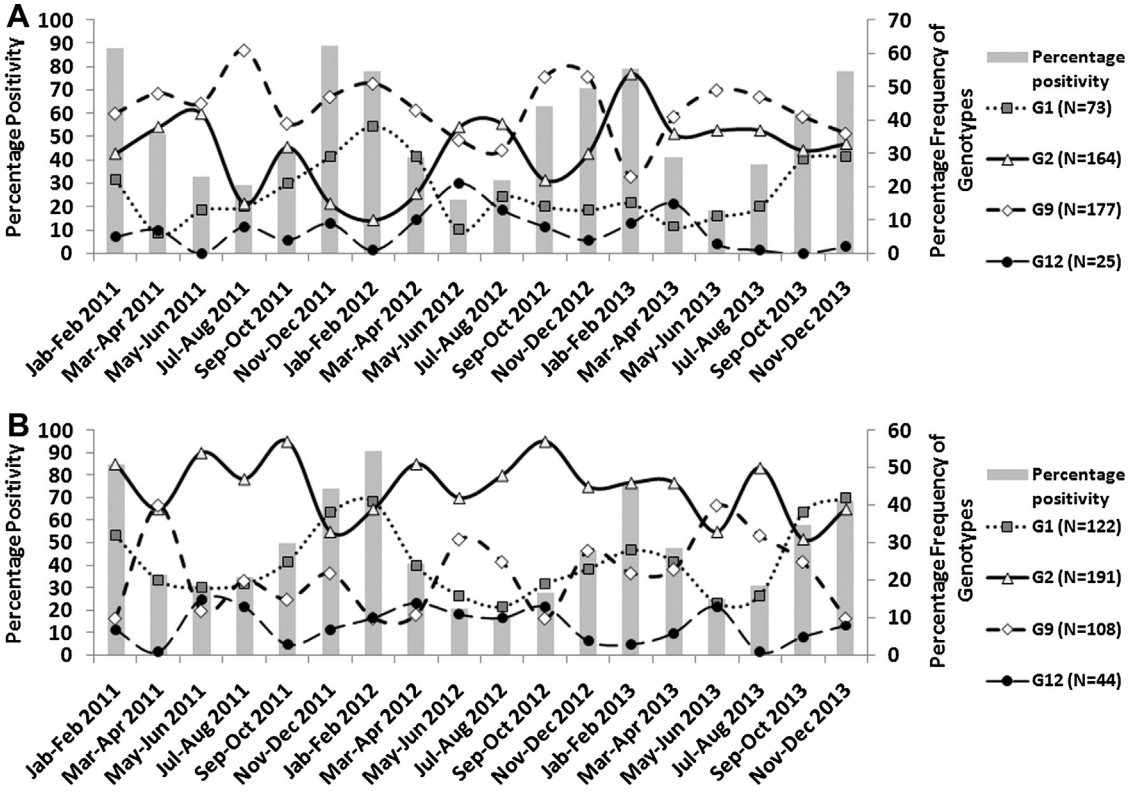

Fig. 1. Temporal distribution of rotavirus-positive cases in Kolkata during January 2011 through December 2013 in (A) hospitalized patients with severe diarrhea (<5 years) and (B) OPD patients (<5 years) with mild diarrhea. Lines represent month wise distribution of common genotypes (G1, G2, G9 and G12).