Vaccine 32S (2014) A62–A67

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Rotavirus diversity among diarrheal children in Delhi, India during 2007–2012

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ARTICLE INFO

Article history:

Keywords:
Rotavirus
Genotype
Non-typeables
Prevalence

ABSTRACT

Rotavirus is the leading cause of severe gastroenteritis in young children worldwide and is responsible for around 100,000 deaths in India annually. Vaccination against rotavirus (RV) is a high priority: 'ROTAVAC' an indigenous vaccine will soon be licensed in India. Surveillance to determine the impact of vaccines on emerging RV strains is required. In this study we compared the pattern of RV strains circulating in Delhi over a 5 year period with the strains over the past 12 years. The most commonly detected G genotypes were G1 (22.4%), G2 (17.2%), and G9 (25.2%) with P[4] (25.5%), P[6] (20%) and P[8] (16.9%) specificity. G12 genotype was found to be the fourth common G-type with 14.8% prevalence. Among the G–P combinations; G1P[8], G2P[4], G9P[8] and G12P[6] were detected at 7.2%, 7.2%, 5.2% and 10%, respectively. Of note, G9P[4] and G2P[6] that were rarely detected during 2000–2007 in Delhi, were observed quite frequently with prevalence of 6.5% and 3.4%, respectively. In total, 16 different G–P combinations were detected in the present study demonstrating the rich diversity of rotavirus strains in Delhi. Our data from the 12 year period indicate wide circulation of G1 and G9 genotypes in combination with P[8], G2 with P[4] and G12 with P[6] with high frequency of RV strains having rare G–P combinations in Delhi. Since the indigenous vaccine 'ROTAVAC' has a monovalent formulation, the impact of vaccines on strains and the effect of strain diversity on the efficacy of the vaccine should be monitored.

1. Introduction

Rotavirus is the most severe cause of diarrheal illness among infants and young children. Worldwide, nearly 453,000 children less than 5 years of age die each year due to rotavirus infection of which about 98,621 die in India each year [1]. Besides high mortality, rotavirus infection annually results in an estimated 457,000–884,000 hospitalizations and 2 million outpatient visits in children less than 5 years of age [2]. India spends approximately 41–72 million USD each year in medical costs treating rotavirus related diarrhea [2].

High rotavirus incidence, economic burden and loss of human life emphasize the need for inclusion of the rotavirus vaccine in the national immunization program. Two rotavirus vaccines, Rotateq® and Rotarix® have been licensed in several countries worldwide and are available in India. Although they have been highly successful in reducing rotavirus related hospital admissions in developed countries, their efficacy has been rather low in developing countries [3]. An indigenous Indian neonatal vaccine, ROTAVAC successfully completed the Phase III clinical trials and is expected to be licensed in India in early 2014. Once licensed, ROTAVAC would be a better alternative for inclusion in the national vaccination program and would also be beneficial for other developing countries due to low vaccine cost and large target population for vaccination.

Rotavirus vaccine efficacy depends largely on the 2 major outer viral proteins, VP7 (glycoprotein) and VP4 (protease sensitive protein) which are the prime targets for neutralizing antibodies and have been shown to generate protective immunity. They also form the basis of RV genotyping in which the VP7 protein defines the G-types and the VP4 defines the P-types [4]. At least, 27 G and 35 P genotypes have been identified in humans and animals [5]. The most common G genotypes reported worldwide are G1, G2, G3, G4 and G9 while P[4] and P[8] are most commonly found P

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http://dx.doi.org/10.1016/j.vaccine.2014.03.005

genotypes [6–9]. Certain G and P genotypes have also been found to be country specific. G5 were reported among rotavirus infected children in Brazil [10] while G6 and G8 have been found commonly in Africa [11,12]. Similarly, studies have reported genotype P[6] in several Asian and African countries [7,12–15]. Besides, the varying G and P types, reassortment due to co-infection of a human and an animal rotavirus strain results in the generation of novel strains [8,12,16], which may over time gain prominence. For future vaccine development and assessment of the vaccines already in use, vigilant rotavirus surveillance will determine the extent of rotavirus diversity within local populations. The aim of this 5 year study (2007–2012) was to identify rotavirus strain diversity and compare it with our previous genotyping data from an earlier study during 2000–2007 [17].

2. Materials and methods

2.1. Study samples

The fecal samples included in this study were collected at 2 Delhi hospitals: All India Institute of Medical Sciences (AIIMS), in South Delhi where we have pursued active rotavirus surveillance since August 2000 besides a gap during March 2003 to July 2004. To get better information of rotavirus strains circulating in Delhi, we chose another hospital located in Central Delhi, Kalawati Saran Children's Hospital (KSCH), with a dedicated unit for treating children with gastroenteritis and compared rotavirus genotype distribution with that found at AIIMS.

All children less than 5 years of age with acute watery diarrhea admitted at AIIMS during August 2007–July 2012 were enrolled in the study, while sample collection at KSCH was done during November 2009 to May 2010 for all diarrheal children falling under similar criteria as in AIIMS. The study was ethically approved by the AIIMS ethical committee. Written informed consent was obtained from parents/guardians of children followed by recording of clinical information and fecal sample collection. In total 756 children were enrolled, of which 513 and 243 were enrolled at AIIMS and KSCH, respectively. The fecal samples were stored in aliquots in −70 °C for further use in RV genotyping. To evaluate rotavirus strain diversity in Delhi over 12 years, genotyping data obtained during this present study (Aug 2007–July 2012) at AIIMS was compared with the genotyping data reported in our earlier study from the same collection site [17].

2.2. Rotavirus detection

A 10% supernatant of the fecal sample was used to detect rotavirus antigen by a commercial monoclonal antibody based enzyme immunoassay kit (Premier Rotaclone, Meridian Bioscience Inc., Cincinnati, OH, USA) [17].

2.3. RNA extraction

RNA extraction of rotavirus positive samples was taken from 10% fecal suspensions using Trizol method (Invitrogen Corp, Carlsbad, CA) following manufacturer's instructions and stored at −20 °C until further use [17].

2.4. Rotavirus genotyping

G and P genotyping of rotavirus positive samples was determined by multiplex RT-PCR (Qiagen One step RT-PCR kit) according to manufacturer's protocol. The primers used in the study have been described previously [17]. The amplified product was then analyzed on 2% agarose gel. Samples which did not react to any of G or P genotype specific primers were considered non-typeable.

3. Results

3.1. Prevalence of common G and P genotypes with emergence of G9P[4] rotavirus in Delhi

Of the 756 diarrheal specimens collected from two hospitals (AIIMS and KSCH), we found 290 (38.4%) positive for rotavirus. All 290 rotavirus positive samples were subjected to both G and P genotyping. We observed genotype G9 most frequently circulating in Delhi with a prevalence rate of 25.2% followed by G1 and G2 at 22.4% and 17.2%, respectively (Table 1). The previously reported [17] fast emerging genotype G12 had an overall prevalence of 14.8% throughout the study period. However, we seldom detected the G4 genotype (2.1%). Amongst the P genotypes, P[4] (25.5%) was most prevalent while P[6], P[8] and P[11] accounted for 20%, 16.9% and 2.1%, respectively (Table 1). Among the G–P combinations, we commonly detected 16 different rotavirus strains at varying frequencies. Among the globally common G–P combinations, G9P[8] was detected among 5.2% of the samples while both G1P[8] and G2P[4] showed 7.2% detection each. We detected 13 other unusual rotavirus strains of which, G12P[6] (10%), G9P[4] (6.5%) and G2P[6] (3.4%) were more frequent (Table 1). We also observed a high percentage of mixed infections: 6.9% of G mix and 14.5% of P mix. Besides mixed infections, nearly 11% and 21% of the total RV positives could not be G and P genotyped, respectively.

3.2. Comparison of rotavirus strain distribution at AIIMS and KSCH hospitals

At AIIMS, we found 35.9% (184/513) of samples positive for rotavirus antigen compared to 43.6% (106/243) of samples at KSCH. At both hospitals we found all G (G1/G2/G4/G9/G12) and major P (P[4]/[6]/[8]) genotypes, besides genotype P[11] which was found at AIIMS only (Fig. 1A and B). At KSCH we detected relatively high frequency of G1 (29.2%), G2 (19.8%) and G9 (32.1%) genotypes, while at AIIMS G1, G2, G9 and G12 had 19%, 15.8%, 21.2% and 21.2% detection rates, respectively. Among the G–P combinations, the common rotavirus strains at both the hospitals were G1P[8], G2P[4] and G9P[8] and in total constituted 19% and 20.7% of the total strains genotyped at AIIMS and KSCH, respectively (Fig. 1C). Among the unusual RV strains, we detected G2P[6] at KSCH only, and G9P[11] only at AIIMS. Although we found G12P[6] and G9P[4] at both hospitals, G12P[6] was more common at AIIMS (14.7%) than KSCH (1.9%) while G9P[4] was commonly found at KSCH (12.3%) than AIIMS (3.3%). We found nearly similar percentages of G and P mixes at both hospitals, however, G (15.8%) and P (25.5%) non-typeables at AIIMS were relatively more than G (4.8%) and P (13.2%) non-typeables at KSCH.

3.3. Temporal distribution of rotavirus strains detected at AIIMS during 2000 to 2012

The present rotavirus surveillance study (2007–2012) at AIIMS showed G12P[6], G2P[4], G9P[8] and G1P[8] to be the most prevalent strains with 14.7%, 8.7%, 5.4% and 4.9% detection rates, respectively (Fig. 2). These strains were also commonly detected at AIIMS during our earlier study (2000–2007) with prevalence rate for G12P[6], G2P[4], G9P[8] and G1P[8] being 11.4%, 14.8%, 4.9% and 19.4%, respectively, although G1P[8] and G2P[4] prevalence was relatively less during the present study. Among the other unusual G–P combinations, we found relatively similar percentages of rotavirus strains during the two study periods. Among the G genotypes, G12 and G9 were dominant during 2007–2012 with 21.2% and 20.6% prevalence respectively in comparison with 2000–2007 study which found G1 and G2 most common with 25.8% and 22.3% prevalence, respectively [17]. Among the P genotypes, we found