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Distribution of rotavirus genotypes in Dhaka, Bangladesh, 2012–2016: Re-emergence of G3P[8] after over a decade of interval

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ABSTRACT

Group A rotavirus causes a substantial proportion of diarrhoea related deaths worldwide among children under five years. We analyzed rotavirus prevalence and genotypes distribution among patients admitted with diarrhoea at icddr,b hospital in Dhaka during 2012–16. Stool specimens (n = 1110) were collected from diarrhoea patients and tested for RVA antigen using enzyme immunoassay. Rotavirus positive samples were G (VP7) and P (VP4) genotyped by RT-PCR and sanger sequencing. Data on clinical manifestations were collected from icddr,b hospital surveillance system. A total of 351 (32%) patients were positive for rotavirus antigen, about half of those were children under two years old. During the study period, G1P[8] (27%) was the most prevalent strain, followed by G12P[8] (15%) and G9[P4] (9%). Mixed G or P genotypes were identified in a substantial proportion (23%) with few strains of rare combinations such as G1P[4], G1P[6], G2P[6], G2P[8], G9P[6]. The genotypic fluctuation was noteworthy; G12P[8] was the major strain in 2012–14 but sharply decreased in 2015–16 when G1P[8] became the most common strain. G3P[8] re-emerged (17%) in 2016 after 11 years. Since the Government of Bangladesh has planned to include rotavirus vaccine in national immunization programme from 2018, our data will provide baseline information on rotavirus genotypes in the pre-vaccination era to observe the selection pressure on genotypes in the post vaccination epoch.

1. Introduction

Group A rotaviruses cause close to 215,000 diarrhoea-related deaths worldwide annually among children below age of five and the vast majority of mortality – more than 90 percent occurs in the low income countries in Asia and Africa. According to WHO estimation, 2700 of these deaths occur in Bangladesh [1]. An analysis using Pan American Health Organization's TRIVAC model reveals that the cost/disability adjusted life year (DALY) ratios due to rotavirus diarrhoea ranged from USD 58-142/DALY averted including GAVI subsidy and from USD 615-1514/DALY averted excluding the subsidy. This analysis also indicates that over ten years, rotavirus vaccination would prevent 4000 deaths, nearly 500,000 hospitalizations and 3 million outpatient visits for Bangladesh in the base scenario [2].

Rotaviruses are members of the Reoviridae family and holds a genome consists of 11 segments of double stranded RNA [3]. Genetic diversity of rotavirus has been described by a dual classification system based on two outer capsid proteins, VP7 (G-genotype) and VP4 (P-genotype) [4]. In addition, whole-genome based classification is used to assign genotypes to each strain. The nomenclature Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx represents the genotypes of VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP 5/6, respectively. Currently, there are 35 G, 50P, 26 I, 21 R, 19C, 19 M,30 A, 21 N, 21 T, 27 E and 21H types [4]. There are two major genotype constellations of human rotaviruses: Wa-like genogroup 1 (G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1) and DS1-like genogroup 2 (G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2) [5]. A third minor human genotype constellation; AU-1-like genogroup 3 (I3-R3-C3-M3-A3-N3-T3-E3-H3) is believed to have originated from cats or dogs [6]. The segmented rotavirus genome facilitates reassortment between strains, allowing both intra- and inter-genogroup reassortment.

Different G-types are being observed across the globe since the discovery of rotavirus in 1973. In humans, G1, G2, G3 and G4 genotypes were the most important genotypes until the early 1990s. Since then G9 genotype rapidly spread and became established in the human population as the fifth globally important human genotype. In the beginning of the 2000s, reports of the detection

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and augmented prevalence of G12 strains have appeared from Asia (Thailand, India, Korea, Japan, Bangladesh, Nepal, and Saudi Arabia) and the Americas (the United States, Argentina, and Brazil) [7,8]. Both genotypes G9 and G12 were reported as porcine origin. Other rotavirus genotypes which are believed to be of animal origin are being detected in low numbers or in geographically isolated regions in humans such as G5, G11 (porcine origin), G6, G10, G8 (bovine origin). Taken together, the emerging rotavirus genotypes in humans underline the close relationship between human and animal rotavirus strains [9].

Two licensed vaccines Rotarix® (GlaxoSmithKline) and RotaTeq® (Merck & Co. Inc.) have demonstrated high efficacy in high and middle income countries, however, the clinical trials failed to confer adequate efficacy (<60%) in low income countries [10]. In addition, several reports indicate that these oral live attenuated rotavirus vaccines successfully protect against the homotypic rotavirus strains; however, protection against heterotypic rotavirus strains (that are not included in the vaccines, G9 and G12 for example) still remains in query. A vaccine trial conducted in Bangladesh interprets that when classified as fully or partially homotypic versus fully heterotypic with respect to the G and P type of the vaccine, total effectiveness estimates were nearly similar, 48% and 43%, respectively [11]. Thus, the role of homotypic and heterotypic immunity to rotavirus and the target antigens in heterotypic immunity continues to be debated. Since vaccines may exert some selection pressure, a detailed image of global strain prevalence from the pre-rotavirus vaccine era is crucial to evaluate any potential changes in circulating strains observed after widespread introduction of rotavirus vaccines.

The Government of Bangladesh has decided to introduce rotavirus vaccine in the national immunization programme by 2018 [12]. Thus, the present study was designed to provide information on rotavirus diarrhoea and virus types prior to the introduction of vaccine. Here we provide evidence that: (a) rotavirus is responsible for about one third of the hospitalized diarrhoeal patients; (b) children less than two years old are more venerable age group for rotavirus diarrhoea; (c) diverse rotavirus genotypes are circulating; (d) increased rotavirus activity is observed in winter; and (e) G3 genotype emerged after a long interval.

2. Materials and methods

2.1. Study site

International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) has maintained the 'Diarrhoeal Disease Surveillance System (DDSS)' in Dhaka Hospital since 1979 [13]. For this study, fecal samples were collected from hospitalized patients of all age groups, admitted at icddr,b hospital due to acute diarrhoea between January 2012 and December 2016 under the DDSS. Informed consent were obtained from all parents or legal guardians of children and from adults before filling up the relative questionnaire and taking fecal samples for lab examinations.

2.2. Study population

The icddr,b hospital in Dhaka treats over 100,000 diarrhoea patients each year. Under the DDSS, systematic 2% subsamples of the total hospitalized diarrhoeal patients with or without vomiting are enrolled each year for diarrhoea etiology testing. For this study, we randomly selected 10% samples (n = 1110) by ID number of specimens from the total enrolled cases for rotavirus genotyping between 2012 and 2016.

2.3. Detection of group A rotavirus antigen by ELISA

All the selected samples for this study were tested for the presence of rotavirus VP6 antigen by using the ProSpect Rotavirus kit (Oxoid, UK) which utilizes a polyclonal antibody in a solid phase sandwich enzyme immunoassay according to manufacturer's instructions [14].

2.4. RNA extraction and RT-PCR

Viral RNA was extracted utilizing the QIAamp Viral RNA mini kit (Qiagen, Hilden,Germany) according to the manufacturer's instructions. In order to identify the VP7 and VP4 genotype, traditional reverse transcription PCR (RT-PCR) was performed with BegI, EndI and Con2, Con3 genotyping primers respectively described elsewhere [15,16]. The RT-PCR extracted RNA was denatured at 97 °C for 3 min and reverse transcription followed by polymerase chain reaction RT-PCR using the One Step RT-PCR kit (Qiagen). Concisely, the reaction was carried out with an initial reverse transcription step at 45 °C for 30 min, followed by 35 cycles of amplification (30 s at 94 °C, 30 s at 48 °C, 60 s at 72 °C), and a final extension of 7 min at 72 °C in a thermal cycler (Eppendorf AG, Hamburg, Germany). PCR products were run on a 1.5% agarose gel, stained with ethidium bromide and visualized under UV-light.

2.5. Sequence analysis

Nucleotide sequencing was carried out in an automated ABI3500 xL Genetic Analyzer (Applied Biosystem, Foster City, CA) and Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem), as per kit protocol. The electropherogram files were inspected using Chromas 2.23 (Technelysium). Sequence similarity searches were performed using the National Center for Biotechnology Information (NIH, Bethesda, MD, USA) Basic Local Alignment Search Tool (BLAST) server on Gen Bank database release 2.8.0. Sequences were aligned by using ClustalW located in BioEdit 7.0.5 [17].

2.6. Statistical analysis

Data were computed as number and percentage for categorical data and mean with 95% CI for continuous data. Employing chi-square test, a p-value less than 0.05 was considered significant and associations were expressed in 95% confidence interval (95% CI).

2.7. Phylogenetic analysis

Partial VP7 and VP4 nucleotide sequences of G3 strains were submitted to Genbank under accession number MH171725 and MH171726 and were used to construct phylogenetic tree using the neighbor-joining method. The bootstrap probability at a branching point was calculated with 1000 pseudo-replicate data sets. Genetic distances at the nucleotide level were calculated using the Kimura two-parameter method [18] by MEGA 7.0.18 software.

3. Results

3.1. Rotavirus antigen detection

During January 2012- December 2016, a total of 1110 fecal specimens were screened for group A rotavirus antigen by enzyme immunoassay and 351 (31.6%) were positive.

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