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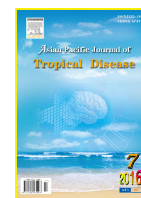

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Genetic diversity of rotavirus strains in children with diarrhea in Lagos, Nigeria

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ABSTRACT

Objective: To describe the genetic diversity of rotavirus strains circulating in Lagos, Nigeria.
Methods: A total of 302 stool samples were obtained from diarrheic children on admission to four hospitals in Lagos and screened for rotavirus antigen by enzyme immunoassay, while rotavirus VP4 and VP7 genotypes were determined by multiplex semi-nested RT-PCR using recognized primers and methods.
Results: Overall, the globally important genotypes, G1, G2, G3, G4 and G9, associated with P[4], P[6] and P[8] were detected. A rotavirus carrying G12 specificity was also detected at low frequency. The different rotavirus strains found in this study were G[2]P[6], G[2]P[8], G[1]P[8], G[1]P[6], G[3]P[6], G[9]P[6], G[2]P[4], G[4]P[4], G[12]P[8] and some mixed infections.
Conclusions: The study highlights the wide diversity of rotavirus strains and the potential emergence of unusual rotavirus in this region. It is therefore important to continue the epidemiological studies to monitor rotavirus strains associated with gastroenteritis in hospitals before and after the introduction of rotavirus vaccine.

1. Introduction

Rotavirus is a non-enveloped virus with icosahedral symmetry belonging to the family Reoviridae. The virion consists of three layers of proteins with the viral genome consisting of 11 segments of double-stranded RNA, which encodes six structural proteins, namely, viral proteins (VP) 1–4, VP6 and VP7 and six non-structural proteins, non-structural proteins 1–6[1,2]. The outer capsid is composed of two independent neutralization antigens, namely VP4 which determines P-genotype and VP7 which is denoted as G-serotype. VP7, the glycoprotein or G-protein encoded by gene 7, 8 or 9 depending on the strain, and VP4, the protease-cleaved or P-protein encoded by gene segment 4, determine the serotype specificity and form the basis of the binary classification (G and P types) of rotaviruses[3]. Both G and P proteins induce neutralizing antibodies and may be involved in protective immunity[4]. The most common G serotypes are G1, G2, G3, G4, and G9, in which G1 is the most prevalent one and G9 emerges fastest worldwide. At least 27 G genotypes and 35 P genotypes have been identified in human rotaviruses whereof genotypes G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] are responsible for 80%–90% of the childhood rotavirus disease burden globally[5].

The VP6 protein located in the inner capsid of the virus contains the antigenic determinants which classify them into seven serogroups of A to G. Groups A, B and C rotaviruses have been found both in humans and animals while groups D, E and F have been found only in animals. Group A rotaviruses have clearly been established as causing severe diarrhoeal disease in the young. Group B includes those viruses associated with animal epidemics of severe diarrhea primarily in adults in China. Group C viruses have been found in sporadic cases and outbreaks of diarrhoea in piglets and children[6].

Human group A rotaviruses are the most frequently identified etiologic agent in children hospitalized with acute, severe and dehydrating diarrhea worldwide[7]. It is estimated that 400 000–600 000 children, most of who live in developing countries, die from rotavirus infections each year. Children in sub-Saharan Africa account for over 40% of these deaths[8-10].

The epidemiology of rotaviruses has been reported in some studies in Nigeria[11-14]. However, to update our knowledge of the diversity of rotavirus strains infecting young children in Nigeria, prior to the official introduction of rotavirus vaccines, it is imperative to conduct rotavirus surveillance to determine the emergence of new or unusual

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The study protocol was performed according to the Helsinki declaration and approved by the Lagos State Hospital Management Board and the local ethical committees of the hospitals. Informed written consent was obtained from the parents/guardians of the children recruited in this study.

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genotypes that may have resulted from possible reassortment of co-circulating strains of different genotypes which may have implications on the existing vaccines.

2. Materials and methods

2.1. Subjects and sample collection

A total of 302 samples were collected from children less than 5 years of age presenting with acute diarrhoea, who attended Massey Street Children Hospital, Orile Agege General Hospital, Surulere General Hospital and Ikorodu General Hospital during the period August 2007–July 2008 in Lagos State, Nigeria. A diarrhea case in this study was defined as a child passing loose, watery or bloody stool three or more times in the past 24 hours. Basic demographic data, histories of the illness and clinical information about the children were obtained from their caregivers using questionnaires. The samples were collected and stored frozen at -20 °C in the Department of Microbiology, University of Lagos before transported to Noguchi Memorial Institute of Medical Research, University of Ghana, Legon, Ghana for analyses.

2.2. Virus detection

Ten percent suspension of all the diarrhea stool samples in phosphate buffered saline were tested for the presence of group A rotavirus antigen using commercially available enzyme immunoassay kit (Rotavirus IDEIATM, Dako, UK). The test was carried out according to the manufacturer's instructions.

2.3. VP7 and VP4 typing by RT-PCR

A total of 58 samples which were rotavirus antigen positive by EIA were further analyzed by PCR genotyping. Briefly, rotavirus double stranded RNA was extracted from these samples and purified using the RNaid kit (Bio 101 system, Qbiogene Carlsbad, USA). The extracts were used as templates for RT-PCR, *i.e.* the first and second round amplifications using random primers. For G-typing, a full-length 1 (1062 bp) gene segment 9, encoding the VP7 glycoprotein of human group A rotaviruses, was amplified with the forward primer Beg9 (5'GGCTTTAAAAGAGAGAATTTCCGTCTGG3') and the reverse primer End9 (5'GGTCACATCATACAATTCTAATCTAAG3'). This was followed by semi-nested PCR using G-serotype specific primers: aAT8 (5'GTCACACCATTTGTAAATTCG3'; nt 178–198), aBT1 (5'CAAGTACTCAAATCAATGATGG3'; nt 314–335), aCT2 (5'CAATGATATTAACACATTTTCTGTG3'; nt 411–435), aDT4 (5'CGTTTCTGGTGAGGAGTTG3'; nt 480–498), aET3 (5'CGTTTGAAGAAGTTGCAACAG3'; nt 689–709), aFT9 (5'CTAGATGTAACTACAACTAC3'; nt 757–776) and a G12 specific pair of primers [5'TCGTCATGCTGCCATTTA3'; nt 173–190 (forward) and 5'GTCCAGTCGGGATCAGTT3'; nt 327–344 (reverse)], to identify G types[15-17]. In a similar manner, PCR for P typing was carried out in two steps, *i.e.* first and second amplifications, as described by Armah *et al.*[18]. Briefly, a DNA fragment of 876 bp was amplified from gene segment 4, encoding VP4 protein using primers Con2 (5'ATTTCGGACCATTTATAACC3') in the forward direction and Con3 (5'TGGCTTCGCCATTTTATAGACA3') in the reverse. The VP4 genotypes of rotavirus strains were then determined for samples with successfully amplified full length gene segment 4 (876 bp fragment) using rotavirus specific primers 1T-1 (5'ACTTGGATAACGTGC3') KU(P8), 2T-1(5'CTATTGTTAGAGGTTAGAGTC3') RV5(4), 3T-1(5'TGTTGATTAGTTGGATTCAA3') 1076 (P6), 4T-1 (5'TGAGACATGCAATTGGAC3', K8(P9), 5T-1 (5'ATCATAGTTAGTAGTCGC3') 69M (P10) and consensus primer Con2[19]. All PCR products were electrophoresed at 100 V for 60 min in 2% agarose gels containing 2 µL ethidium bromide, viewed under UV illuminator and documented using the AlphaDigitDoc™ RT imaging system (Alpha Innotech Corporation, USA). The characteristic of G and P genotypes amplicons was determined against the 100 bp molecular size DNA marker (Promega).

3. Results

Three hundred and two stool specimens were analyzed for rotavirus, in which 78 (25.8%) tested positive for rotavirus antigen by EIA. Among these positive samples, 58 were typed using RT-PCR.

3.1. Distribution of G type

Ninety percent of the rotavirus isolates were assigned VP7 G-type specificity while 10% showed amplification failure. Six different rotavirus VP7 serotypes including the G9 and G12 were detected. In the study, genotypes G1 and G2 occurred predominantly with an equal proportion of 31.0% for each, followed by G3 (8.6%), G4 (5.2%), G9 (5.2%) and G12 (1.7%). Mixed infection (G1 + G2) and (G1 + G3) were also detected in 3 (5.2%) and 1 (1.7%) of the samples, respectively, while 6 (10.4%) were nontypeable (Table 1).

3.2. Distribution of P type

Out of the 58 amplified samples, 56 (96.6%) could be assigned to VP4 genotype. The VP4 genotypes detected during this study included three of the recognized human rotavirus VP4 alleles, P[4]. P[6] and P[8] as well as strains with dual P types P[4 + 6], P[4 + 8], P[6 + 8] and mixed infection of the three VP4 alleles P [4 + 6 + 8]. The most predominant P type was P[6] in 25 (43.1%) isolates, followed by P[8] in 9 (15.5%) and P[4] in 5 (8.6%). Seventeen (29.4%) were mixed P genotypes and 2 (3.5%) could not be assigned VP4 genotype (Table 1).

3.3. G and P type combination

Twenty distinct strains were identified with the predominant combinations being strains with genotypes G[2]P[6], G[1]P[8] and G[1]P[6] and each having same proportions (14%). These were followed by G[4]P[4], G[3]P[6], G[9]P[6] and G[2]P[4], occurring in the proportions of 6%, 4%, 4% and 4%, respectively. It is important to note that all the three samples that were identified as G4 were also typed as P4 genotype. The following unusual genotypes: G[4]P[4] (6%), G[12]P[8] (2%), G[2]P[8] (2%) and so many mixed infections were also observed (Table 1).

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