ROTAVIRUS VP7 AND VP4 TYPES CIRCULATING IN PLATEAU STATE, NIGERIA.

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ABSTRACT

Objective:

To determine the rotavirus genotypes circulating in Plateau State using RT – PCR.

Materials and Methods:

Thirty one rotavirus isolates recovered from diarrhoeic stools in 1998 and 1999 were analyzed using nested multiplex RT – PCR technique for the determination of VP7 and VP4 genotypes

Results:

VP7 typing revealed the presence of the global epidemiologically important VP7 strains G1-G3 except genotype G4. 'Mosaic' rotaviruses G1/3 were also found. The VP4 types identified were P (6), P(8) and P(4). Mixed genotypes of P(4)/P(6) were also detected. The predominant strains circulating during 1988 and 1999 were P(6) G1, P(6) G3, P(8)G1 and P(8)G3. Some strains could not be typed by the primers used.

Conclusion:

The application of molecular techniques of PCR to detect and characterize rotavirus strains circulating, contributes to the knowledge and burden of rotavirus strain diversity even though some strains could not be typed. This provides fundamental data necessary for rotavirus vaccine design and implementation in Africa.

Key words: Rotavirus, genotypes, circulating in Plateau State, Nigeria.

INTRODUCTION :

Diarrhoeal disease is responsible for literally millions of deaths annually[3] Rotavirus is the major cause of severe diarrhoea among children under 5 years of age globally, causing 20-40% of hospitalizations for acute watery diarrhoea and is estimated to cause 600,000 to 870,00 deaths each year[6,13]. In developing countries, is estimated to cause 2.4 to 3 million deaths each year[3,13]. Previous studies, in Nigeria have associated rotavirus with childhood diarrhoea.[2,7,9].

Gastroenteritis is a major cause of childhood morbidity and mortality in Africa[3,12]. Improvements in water supplies and excreta disposal may reduce the transmission of enteric bacteria and parasites but are unlikely to reduce incidence of rotavirus diarrhoea. Vaccines are

therefore being developed as primary public health intervention to reduce the burden of diarrhoea caused by rotavirus[4,11]. The knowledge of the epidemiology of rotavirus infection and circulating strains is essential for the evaluation of these vaccines[13].

Rotavirus displays diverse and complex serotypes specificties[19]. Ten VP7 serotypes and 7 VP4 genotypes have so far been described and serotypes specific antibodies are known to play an important role in protection against rotavirus illnesses[17].

Successful human rotavirus vaccine would need to generate protection against the common circulating VP7 serotypes (G1 – G4) and probably G9. A comprehensive survey of the circulating VP7 serotypes and VP4 genotypes is required. The information on this is not well known in Africa particularly in Nigeria. This study reports the circulating rotavirus genotypes circulating in Plateau State, Nigeria.

MATERIALS AND METHODS:

Diarrhoeal faecal specimens collected between January, 1998 and April, 1999 from 672 young children less than 5 years of age were initially screened for rotavirus antigen using a commercial ELISA (IDE IA ^{Tm} Dako, UK). Further examination of the faecal specimens were carried out by polyacrylamide gel electrophoresis (PAGE) for diversity of strains and use of monoclonal antibody- based ELISA with panel epitope specific monoclonal to the VP6 subgroup and VP7 genotype antigen

Thirty-one (31) of the rotavirus isolates obtained were then selected for G and P typing. Rotavirus Vp7 G and VP4 P types were determined by the use of nested PCR requiring a cocktail of primers specific for the human Vp7 and VP4 genotypes agarose gel electrophoresis and ethidium bromide staining as described previously[8,10,18].

Briefly, rotavirus double stranded was extracted by phenol-chloroform method and purified using the Rnaid extraction method using Rnaid plus kit (Bio 101, Inc. la Jolla, Calif) supplied by the Southern Cross Biotechnology (Pty) Capetown, South Africa.

VP7 primers utilized were:

Beg^9(^{5}'GGCTTTAAAAGAGAATTTCCCTCTGG^3')and(^{5}'GGTCACATCACAATTCTA ATCTAA^3') with six type specific primers each located at different hypervariable region of the gene. VP4 Oligonucleotide primers used were Con^3 (^{5}'TGGCTTCGCCATTTTATGACA^3') and Con^2 (^{5}'ATTTTCGGACCATTAACC^3') with a cocktail of five type primers specific for the 876 base pair fragment.

The purified dsRNA specimens were used as templates for reverse transcription polymerase chain reaction. Amplification of the cDNA was done in a thermal DNA cycler (Perkin Elmer) for 30 cycles at 94^{0}C for 1 minute to denature cDNA, 42^{0}C for 2 minutes to anneal the primers and 72^{0}C for 3 minutes for the extension of strands.

RESULTS: Analysis of the 31 rotavirus isolates in 1998 and 1999 by RT PCR using primers Beg^9/End^9 yielded intense products of predicted size of 1062 base pairs (bp) for 21 (67.7) out of 31 tested isolates (figure1). When products of the first amplification were subjected to VP7 genotyping ds DNA products of about 749 bp and 374 bp corresponding to G1 and G3 were identified in 1998 (figure 1) while in 1999 no G1 was found. However G3 and G2 types were observed No G4 were identified.

When VP4 primers Con 3 and Con 2 were used, VP4 PCR products of predicted size of 876bp were observed (figure 2). VP4 genotype P(6) and P(8) were observed in 1998 while in 1999, P(6), P(8) and P(4) were detected. Most of viral isolates carried a P(6) VP4

genotype as determined by the RT – PCR analysis. The P(6) genotype was observed in 14 (45.2%) of typed specimen (figure 3). Mixed genotypes of P(4) and P(6) were also seen. High proportion of isolates remained non-typeable.

DISCUSSION:

The epidemiologically important serotypes were found circulating in Plateau state except G4. This is peculiar to studies conducted in Nigeria[1,14,16] Earlier studies in Maiduguri area in Northern Nigeria have reported similar findings of G1/G3 and 'mosaic' rotaviruses a large number of untyped strains[14]. Studies in other African countries have demonstrated the presence of genotypes G1 – G4[15,28] It is of interest that P(6) genotype was more prevalent than P(8) strains circulating in Plateau State. Seven VP4 genotypes have been described, however only P8 and P4 are believed to be in circulation with P (8) being the major circulating strain.

The epidemiological significance of P(6) rotaviruses will need to be defined through continued surveillance and typing of circulating strains, since rotavirus vaccine strategies are based on a polyvalent vaccine candidate encompassing the epidemiologically important group A rotavirus serotypes. These observations therefore raise the question as to the role of the P(6) rotavirus in asymptomatic infection. This would lead to important considerations for the genotype as a vaccine candidate. Mixed genotypes will require sequencing studies undertaken to determine whether these specimens have dual specificity or whether this was a result of primer non specificity.

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