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Molecular identification of rotavirus strains involved in gastroenteritis among children in federal capital territory, Abuja Nigeria

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ABSTRACT

This study was conducted to determine the prevalence and genetic profile of the rotaviruses involved in gastroenteritis among children in Federal Capital Territory, Abuja, Nigeria. Two hundred and five stool samples were collected from children (1-5yrs) presenting diarrhea at the paediatrics Departments of five hospitals. The stool samples were screened, using Enzyme Linked Immunosorbent Assay. Sixteen stool samples were found positive, representing 7.8% prevalence. Children within 1-2years had 4.39% while those within 3-5years recorded 3.41%.During dry season, the prevalence was more (4.87%) compared to the rainy season (2.93%). Identification of the rotavirus strains using reverse transcriptase-polymerase chain reaction (RT-PCR) revealed genotypes such as G1, G2, G8, P4, P8, and P6. Hospital and community based studies should be encouraged in order to have a more clear picture about the prevalence and the strains of the virus in circulation in the studied area.

Introduction

The genome of rotavirus consists of eleven unique double helix molecule of RNA which contains 18,555 nucleotide base pairs in total. Each gene codes for one protein, except 9 and 11 which each code for two proteins (Kuta *et al.*, 2014). The RNA is surrounded by a three layered icosahedral protein capsid.

The particle of rotavirus is about 70-76.5nm in diameter. When the layer is absent it measure about 55nm, within the inner capsid is the 37nm core, which contains the RNA genome and have a buoyant density of 1.36g/ml(Mishra *et al.*, 2010). The outer capsid shell comprises six viral proteins (VPs) that form the virus particles. These structural proteins are called VP1, VP2, VP3, VP4, VP6, and VP7.

The G-protein and the P-protein both induce neutralization antibodies in natural infection and for the bases for the G-P subtypes classification of rotavirus (Mishra *et al.*, 2010).In addition there are also six non structural proteins. These include NSP1, NSP2, NSP3, NSP4, NSP5, and NSP6. Which all participate in rotavirus replication or multiplication in the host cell (Robert,2004).

This study determined the genetic profiles of rotavirus stains involved in gastroenteritis in Federal Capital territory, Abuja, Nigeria.

Materials and methods

Description of sturdy area

The federal capital territory is the home of Abuja, the capital of Nigeria. The territory was formed in 1996 from parts of Nasarawa, Niger and Kogi states. it is the central region of the country. Unlike the states of Nigeria, which are headed by elected Governors, it is administered by the Federal Capital Territory Administration headed by a Minister.

The Territory is located just north of the confluence of the Niger and Benue rivers. It is bordered by the sates of Niger states to the West and North, Kaduna to thenorth East, Nasarawa to the east and south and kogi to the south west. The FCT lies between latitude 8.25° and 9.20°N of the equator and longitude 6.45° and 7.39°E of Greenwich meridian.

The federal capital territory has a land mass of approximately 8000 square kilometers of which the actual city occupies 250 square kilometers. It is situated within the savannah region with moderate climate condition. It has population of 1,405,201.The territory is currently made up of six area councils (2006 Census).

Determination of sample size

The sample sizefor this research was determined by the formula below.

$$n = \frac{t^2 \times p(1-p)}{m^2}$$ (Aminu *et al.*, 2009)

where:

n = Required Sample size

t = Confidence level at 95%

p = Prevalence of the disease

m=Margin of error at 5%

Sample Collection.

Two hundred and five stool samples were collected from children(1-5years) presenting diarrhea at the paediatric Departments in five hospitals located in parts of the Federal Capital Territory, Abuja. The samples were collected after seeking a written consent from the parent/guardian of the children. The stool samples were collected in sterile sample bottles and transported to the department of Microbiology, Faculty of Science, Ahmadu Bello University (ABU) Zaria and stored under frozen conditionfor further analysis.

Preparation of buffer

Five hundred milliliter (500ml) concentrate buffer was added to 500ml of distilled water and was mixed thoroughly to obtain a balanced solution of 1000ml.The solution was used as

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solvent to dissolve the frozen stool samples in preparation for ELISA screening.

Preparation of Stool Samples for Enzyme Linked Immunosorbent Assay (ELISA)

The two hundred and five frozen stool samples were thawed and each diluted to a ratio of 1:5 by adding 1gram of stool sample to 4ml of dilute buffer in each case. The mixture were mixed thoroughly and allowed to settle for 20 minutes and the supernatants collected wereused for ELISA screening (Dennehy and Penelope, 1990).

Enzyme Linked Immunosorbent Assay (ELISA)

Each of the 205 stool samples were screened as follows: One hundred microliter (100µl) of positive control reagent was introduced into well two and three of the 96 titre plates. Also 100 microliter of negative control reagent was introduced into wells four and five.From well six to the 96th well, 100µl of the supernatant phase of the stool sampleswere separately introduced and then incubated and at room temperature for 30minutes.The well were washed with dilution buffer solution(wash solution).Two(2) drops of reagent one (blue solution) was introduced into each of the wells except well one(blank).It was then incubated at room temperature for 5minutes, and then washed again. Two drops of chromogen was added to each well and was mixed thoroughly using taping strip holder. Finally two drops of stop solution was added into each well and was mixed. Visual observation and readings from spectrophotometric (model 065SPZ USA) and bichromatic (model B4482 taiwan) device was done and the results were recorded.(Valentine *et al.*,2012).

Extraction of Rotavirus RNA from Positive Stool Samples

In accordance with the methodology of Aminu*et al.* (2009); Dzikwi *et al.* (2009); and Valentine *et al*(2012).All Enzyme Linked Immunosorbent Assay (ELISA) positive stool samples were subjected to rotavirus RNA extraction as follows; Three hundred microliter (300µl) of lysis buffer was added to one hundred microliter (100µl) of each diluted positive stool sample. The mixtures were transferred into collection tubes and centrifuge at 12,000rpm for 2minutes. The flows through from the collection tubes were discarded. Three hundred micolitre RNA buffer was added to the column and centrifuged at 12,000rpm for 30seconds.The flow through was again discarded and Zymo spin column tubes were placed into the collection tubes. Three hundred microliter of RNA buffer was added to the column tubes again, spinned at 12,000rpm for 30seconds, and the flow through from the collection tubes was also discarded. The Zymo spin column tubes were further spinned at 12,000 rpm for one minute in an empty collection tubes in order to ensure complete removal of the buffer.

The Zymo-spin column tubes were placed into the RNase free tubes. Ten microliter (10µl) of the RNase free water was introduced into the column tubes and the mixtures were allowed to stand at room temperature for one minute. The mixture were then centrifuged at 12,000 rpm for one minute to elute the virus from the stool samples and were used immediately for the generation of cDNA forVP7 and VP4 genes of the rotavirus.

Generation of VP7 and VP4 cDNA

The cDNA was generated from the RNA extracted by reverse transcriptase polymerase chain reaction (RT-PCR) as described by Mishra *et al*. (2010).

Generation of VP7 complimentary DNA (cDNA)

In order to generate full length VP7 cDNA from the dsRNA extracted from the positive stool samples, 8 microliter (8µl) of the extracted dsRNA was introduced into 500ul PCR tubes. Then the primer pair (Beg9 and End9) at volume of 1µl each were introduced into the PCR tubes.The mixtures were heated to 95^{o}C for 5minutes to denature dsRNA and cooled on ice immediately for 2minutes.Reverse transcriptase master mix(RT-MM) was introduced into the mixture of rotavirus RNA and primer pair. Finally the mixtures were centrifuged at 10,000 rpm for one minute and incubated at 42^{o}C for 30minutes for the generation of cDNA.

Generation of VP4 cDNA

The VP4 cDNA was generated from the extracted dsRNA as follows: Eight (8) microliter of the extracted dsRNAwas introduced into 500 microlitre PCR tubes. Then, 1µl each of the primer pair (con2 and con3) were introduced into the PCR tubes. The mixture of rotavirus RNA and primer pair was heated to 95^{o}C for 5minutes to denature dsRNA and was cooled on ice immediately for 2minutes. Reverse transcriptase master mix (RT-MM) was introduced into mixture .Finally the mixtures were centrifuged at 10,000 rpm for a minute and incubated at 42^{o}C for 30minutes for the generation of cDNA (*Aminu et al., 2009 and Mishra et al.,2010).*

Preparation of agarose Gel

Twenty milliliter (20mil) Tris acetate buffer (TAE buffer) was mixed with 80ml of distilled water to make 100ml. Two (2) gram of agarose was weighed and dissolved into the solution. The mixture was heated at 85^{o}Cfor 2 minutes to ensure complete dissolution of the gel and was allowed to cool to 45^{o}C, after which 10µlethidium bromide was added to the gel.

Casting of the Gel

The gel was poured into the gel cassette tray with a comb in place. The gel was allowed to set at room temperature for 45minutes.The tray was submerged beneath TAE running buffer in an electrophoresis apparatus and the comb was removed.

Loading of the Gel

Ten microliter (10 µl) of cDNA was mixed with loading dye and loaded into separate wells of the gel. The first and the last wells of the gel were loaded with 1kb DNA ladder. The gel was electrophoresed at 100v for 45 minutes at room temperature. The electrophoretic apparatus was switched off and the gel was removed, it was viewed first under uv-light and the image captured using gel documentation machine. (Model 012VR Germany).

Amplification of VP7 cDNA

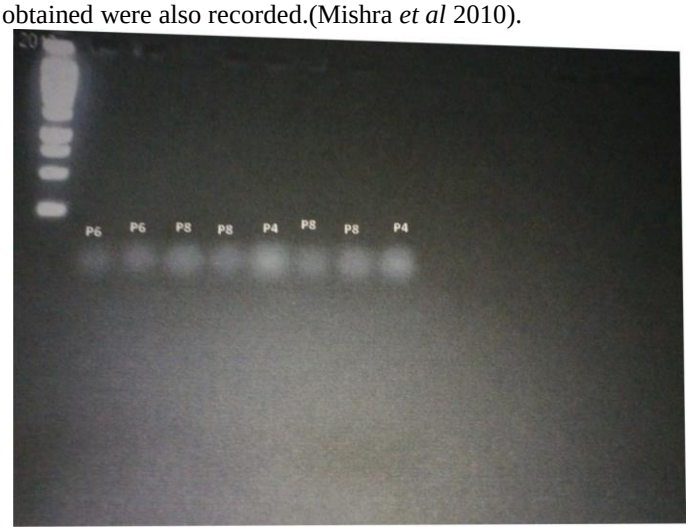
The amplification of vp7 cDNA was conducted using nested multiplex primers, to determine the specific genotypes i.e(G-types) on the basis of the migration pattern on agarose gel. Eight microliter (8 µl) of the vp7 cDNA was introduced into 500µl PCR tubes, 2µl of RNA water was added and mix with

the cDNA in PCR tubes. In addition to the mixture, 15µl of the PCR-master mix was introduced to the PCR tubes. Finally 1µl of the G-type specific primers and 2 µl of consensus primer such as Beg9 was added to the mixture. These were amplified in PCR machine(Tech gene model 32089 UK) programmed to run 30 cycles.

After the PCR, 2% agarose gel containing 10 microliter (10 µl) ethidium bromide was prepared and it was cast. The PCR product was mixed with loading dye was loaded in the wells and the gel was run for 45minutes to identify the bands and their sizes.

Amplification of vp4 cDNA

The procedure for the PCRof vp4 cDNA was similar to that of vp7 cDNA except that, the primers used specifically for determination of P-types were different from the ones used for VP7 cDNA (G-types). In the same vein the loading procedure was same as conducted in the case of vp7 cDNAand the result obtained were also recorded.(Mishra *et al* 2010).



Statistical Analysis

Chi square test was used to determine the relationship between the estimated P-values and the calculated chi square values

Results

Two hundred and five stool samples were screened and the prevalence was 7.8%. Children within 1-2 years old had 4.39% and those within 3-5 years recorded 3.41 %(Table1).During dry season, the infection rate was more (4.87%) compared to rainy season (2.93%) (Table2) and the genotypes identified include G1,G2,G8,P4 ,P6 and P8(Table3).

Table 1. Rotavirus infection according to age of the children

Age	Number of sample screened	Number of sample positive	Prevalence (%)
1 – 2	95	9	4.39
3 – 5	110	7	3.41
Total	205	16	7.80

□□ = 0.462

Table 2. Rotavirus infection according to Seasonal variation

Season	Number of sample screened	Number of sample positive	Prevalence (%)
Rainy	87	6	2.93
Dry	118	10	4.87
Total	205	16	7.80

□□ = 0.6

Discussion

The prevalence of rotavirus among children (1-5years) was investigated in Federal Capital territory Abuja, Nigeria. The result revealed the prevalence of 7.8%. Similar incidence has been reported in previous study by Kuta *et al*,(2013)in Niger state. There seems to be not much difference between the prevalence reported in the previous study and the present study, this makes the outcome of this report comparable with the previous.

Table 3. Rotavirus G –P genotypes identified in the studied area

G-types	frequency	Percentage(%)	P-types	frequency	Percentage %
G1	3	23	P4	2	25
G2	5	39	P6	2	25
G8	5	39	P8	4	50

The infection rates among the children differ according to their age groups. It was observed that children at the age of 1-2years had more infection rate compared to the children at the age of 3-5 years (Table1). Studies by Mishra *et al*.(2010), Valentine *et al.* (2012)have reported that infection with rotavirus has no respect for age. In this study, chi square test confirmed that age was not a factor, hence the outcome of this study corroborate the previous studies.

Similarly, infection rate was high during dry season, compared to rainy season (Table 2). Studies by Armah *et al*.(2003), Mishra *et al*.(2010),Valentine *et al*(2012) have reported similar incidences. Despite the difference observed in the infection rates in the two seasons, chi square test revealed that seasonal variation was not a factor. Therefore the findings in this study differ with the previous reports. Hence the result of this and the previous are not comparable.

The genotypes identified in this study include G1, G2,G8, P4, P6, and P8. Studies by Armah *et al.* (2003); Robert (2004), Glass *et al.* (2006), Mishra *et al*.(2010). Have reported Genotypes G1, G 2, G8, P4, P6 and P8 as the global most common genotypes. In this study, the emergence of global most popular genotypes in Nigeria could be attributed to cosmopolitan nature of the Federal Capital Territory.

The fact that diverse genotypes were isolated in patients stool in geographic locations like Nigeria, gives roomto speculate that the rotavirus have high adaptive capacity to withstand the harsh weather condition in a geographic location like Nigeria.

Conclusion

More enlightment campaign should be encouragedto reduce the burden of the disease. Further investigation should be carried out to ascertain the genetic combinations of the virus.

References

Aminu M, Esona MD, Geyer A, Steel AD (2009). Epidemiology of rotavirus nd astrovirus infections in children in North Western Nigeria. *Annals of African Medicine* 7(4), 168-174

Kuta F.A, Uba A, Nimzing L, Damisa D, and Adabara N.U (2013). Epidemiology and Molecular identification of Rotavirus strains Associated with Gastroenteritis in children in Niger State. *Nigerian journal of Technological research*8,(2), 68-71

Mishra V, Awasthi S, Nag VL, Tandon R (2010). Genetic diversity of group A rotavirus starins in patient aged 1-36 months admitted for acute watery diarrhea in Northern Indian. A hospital based sturdy. *Journal of clinical Microbiology and infectious Diseases* 16 (1), 4 -50

Robert RF (2014). Pathogenesis of Intestinal and systemic Rotavirus infection. *Journal ofvirology*78(19), 102013-10220

Armah GE, Steele AD, Binka FN, Esona ND, Asmah HR, Anto F, Brown D, Green J, Cutts F, Hall A (2013). Changing patterns of Rotavirus Genotype in Ghana: Emergence of Human

Rotavirus G9 asa major cause of Diarrhea in children. *Journal of clinical microbiology* 41(6), 2317-2322

Valentine NN, Achidi EA, Gonsu HK, Lyonga EE, Mathew DE, Krisztian B, Obama AMT(2012). Epidemiology of rotavirus diarrhea in children under 5years in Northern Cameroun. Pan African Medical Journal 11-73.