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 size for this research was determined by the formula below.

\[ n = \frac{t^2 \times p(1-p)}{n^2} \]

((Aminu et al., 2009))

where:
- \( n \) = Required Sample size
- \( t \) = Confidence level at 95%
- \( p \) = Prevalence of the disease
- \( n \) = 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 condition for 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...
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 1 gram of stool sample to 4 ml of dilute buffer in each case. The mixture were mixed thoroughly and allowed to settle for 20 minutes and the supernatants collected were reused for ELISA screening (Denneny and Penelope, 1990).

Enzyme Linked Immunosorbent Assay (ELISA)

Each of the 205 stool samples were screened as follows: One hundred microliter (100 µl) 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, 100 µl of the supernatant phase of the stool samples were separately introduced and then incubated and at room temperature for 30 minutes. 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 5 minutes, and then washed again. Two drops of chromogen solution were added to each well and mixed thoroughly using tapping strip holder. Finally two drops of stop solution was added into well one (blank). It was then incubated at room temperature for 45 minutes. The mixture were heated to 95°C for 5 minutes to denature dsRNA and cooled on ice immediately for 2 minutes. 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°C for 30 minutes 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 dsRNA was introduced into 500 microlitre PCR tubes. Then, 1 µl each of the primer pair (con2 and con3) were introduced into the PCR tubes. The mixture of rotavirus VP4 and primer pair was heated to 95°C for 5 minutes to denature dsRNA and was cooled on ice immediately for 2 minutes. 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°C for 30 minutes for the generation of cDNA (Aminu et al., 2009 and Mishra et al., 2010).

Preparation of agarose Gel

Twenty milliliter (20 ml) Tris acetate buffer (TAE buffer) was mixed with 80 ml of distilled water to make 100 ml. Two (2) grams of agarose was weighed and dissolved into the solution. The mixture was heated at 85°C for 2 minutes to ensure complete dissolution of the gel and was allowed to cool to 45°C, after which 10 µl ethidium 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 45 minutes. 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 1 kb DNA ladder. The gel was electrophoresed at 100 v 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 02VR 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 500 µl PCR tubes, 2 µl of RNA water was added and mix with
the cDNA in PCR tubes. In addition to the mixture, 15 µl of the PCR-master mix was introduced to the PCR tubes. Finally 1 µl 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 45 minutes to identify the bands and their sizes.

Amplification of vp4 cDNA

The procedure for the PCR of 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 cDNA and 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%(Table 1). During dry season, the infection rate was more (4.87%) compared to rainy season (2.93%) (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, G2, 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 the cosmopolitan nature of the Federal Capital Territory.

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

Conclusion

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

References

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