Circulation of rotavirus genotypes and their distribution among vaccinated and non-vaccinated children in Abuja, Nigeria

ABSTRACT

Introduction: Nigeria had planned to introduce the rotavirus vaccine in the National Immunisation Programme in 2014 but this has yet to be done. Nigeria has the continent's highest mortality due to diarrhoeal diseases with little information on specific prevalent genotypes. The main objectives of the study were to identify the predominant rotavirus genotypes and to examine the effects of existing local vaccination programmes on prevailing rotavirus genotypes and on preventing rotavirus diarrhoea.

Methodology: A one-year prospective descriptive study of children under 5 with acute diarrhoea was conducted from September 2012 to August 2013. Children with acute diarrhoea attending three government hospitals andone private hospital were recruited. Children without diarrhoea were also recruited as a control group.Rotavirus ELISA and RNA extraction were done with commercially available kits and positive samples were subjected to RT-PCR and electrophoresis to determine VP7 (G) and VP4 (P) genotypes.

Results:Stool samples were collected from 1242 (93.3%) participants, of whom 957 (77.0%) were ambulatory, 123 (9.9%) hospitalised and 160 (12.8%) controls without diarrhoea. Rotavirus-ELISA was positive among 123 (11.4%) children with diarrhoea. The predominant VP7 genotypes were G2 (n=33, 26.4%) followed by G9 (n=24, 19.2). The main VP4 (P) genotypes included P [4] (n=45, 36.0%) followed by P [6] (n=40, 32.0%). The predominant genotype combinations found were G2 P [4] (n=21, 16.8%), G3 P [6] and G1 P [6] (each n=16, 12.8%), and G12 P [8] (n=15, 12.0%). Very few mixed infections were found in only one government hospital 4 (6.4%). Among 94 unvaccinated children with rotavirus isolates that were genotyped, G2 P [4] (n=19, 20.2%) and G1 P [6] (n=16, 17.0%) were predominant. Among 12 vaccinated children, 2 isolates each (16.6%) were found of G3 P [6], G9 P [4], G12 P [8] and G2 P [NT] with no G1 isolates.

Conclusion:The emergence of new genotypes such as G 12 P [8] found in this study emphasizes the need for continued prospective monitoring of rotavirus at the molecular level to detect new threats to vaccine programmes in future.

Keywords: Rotavirus, genotypes, children, Abuja, Nigeria

Comment [BM1]: Research study is already ten years old. Now definitely these research findings supposed to be of no value / obsolete.

Comment [BM2]: Only three Govt hospitals and one private hospital were the sampling site which suggests wrong methodology as far as sampling is concerned for generalizing any research documents.

Comment [BM3]: 957 + 123 + 160 = 1240 , then how it is written for 1242, Pl explain

INTRODUCTION

Rotavirus infections are known to cause about 50% of diarrhoeal cases worldwide, it was estimated that about 800,000 children die each year from rotavirus disease and over two million are hospitalized (Tate et al., 2012).A significantly huge burden of the rotavirus disease is concentrated in low income countries particularly in sub-Saharan Africa.Rotavirus infection is estimated to cause the death of over 230,000 infants yearly in the African continent alone (Mwenda et al., 2010). Few research in Nigeria have focused on assessing the circulating genotypes, some of the few studies include the study of(Steele et al., 2002)which reports the predominance of G9, G3, G1, G8, and G2 genotypes in Plateau state.(Audu et al., 2002a)also reported the circulation of G1, G3, G4 and P [6] and P [4] genotypes in Lagos

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state.Throughout the course of this study, there was no single publication from Abuja that had been identified. The main aim of this study is to determine the circulating rotavirus genotypes and their distribution among vaccinated and unvaccinated children.

Comment [BM6]: Rewrite the sentences with clear objectives

Serotypes in Nigeria

A handful of publications described the serotypes/genotypes circulating in Nigerianchildren. In a recent study in Ile-Ife, southern Nigeria wherefew children were enrolled, the investigation revealed thatG1P [8] were prevalent among children <1 andthere was a high prevalence on the emergence of G12 P [8] (Japhet *et al.*, 2012).In addition, positive cases in the study were mixed G and P genotypes(Aminu *et al.*, 2010). Genotype diversity in another survey was described in two regions of Nigeria, Lagos state which is in southern Nigeria and Kwara statein the north-central. Children below the age of five were enrolled and results from the study revealed G1 as the most prevalent followed by G3 and G4.The rate of mixed G serotypes was relatively high. Mixed P serotype was also relatively high and concluded that the high rate of mixed type may be an implication to vaccine development (Audu *etal.*, 2002b) which also agrees with the finding of Japhet *etal* (2012). A study in Jos, Nigeria report that the circulation of novel G9 and G8 rotavirus strains,in addition to G2 and G4 which were isolated a in few samples from the study population. The report emphasised the global distribution of the G9 strains (Steele *etal.*, 2002). Rotavirus strains studied shows that G1, G2, G3and andare more frequently detected in diarrhoeic stool of children for example the study conducted in Lagos between 1996-1997 revealed that G1 is the most common serotype followed by G3, mixed G1/G2 was the first reported in Nigeria in this study. P [6the] was most reported followed by P [8] with P [4] being the least (Audu *etal.*, 2002a). Another study conducted in Zaria between 1997 and 1998 among children under the age of five revealed that G1 and G3 serotypes as the most predominant with G1/G3 being less common among the population surveyed (Pennap *etal.*, 2000), this study also showed a similar result as that of Audu*etal* (2002a). Adah in 1997 reported that G3 are predominant in southern Nigeria with some infections being high in the study. Limitations of the study highlighted that some non-typed but could be a result of the primers used to detect the serotypes in the study (Adah *etal.*, 1997).Serotypes in other regions in West Africa showed similarities with those of Nigeria. For example in Ghana, a study aimed at investigating genotype diversity showed that G3P[6] and G9P[8] are in relatively high proportion. Though a study in Nigeria investigated by Audu*et al* (2002b) incidentally found similar genotypes (Audu et al., 2002a). In another study in Ghana G2P [6], G3P [4] and G9P [8] made up half of the genotypes detected in Kassena-Nankana district G3 was found to be the most predominant strain,

Comment [BM7]: Sentence is confusing. Rewrite with suitable references

Comment [BM8]: Put comma after Ghana

Comment [BM9]: Put Comma after district

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followed by G2. These findings were similar in several investigations by some authors in Nigeria (Binka et al., 2003, Steele et al., 2002, Audu et al., 2002a, Adah et al., 1997).

METHODOLOGY

The study was aone-yearprospective survey of children with acute diarrhoea in Abuja, Nigeria. Children under the age of five were recruited in four hospitals. Demographic data and stool samples were collected from September 2012 – August 2013 from 1331 children with controls. Rotavirus ELISA and RNA extraction were done with commercially available kits and positive samples were subjected to RT-PCR and electrophoresis to determine VP7 (G) and VP4 (P) genotypes.The characterisation and genotyping for Rotavirus positive cases wereconductedat theUniversity of Liverpool, United Kingdom.

Comment [BM10]: Perspective or retrospective study??

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Comment [BM12]: 1242 written in abstract, here it is 1331, Which one is right? Kindly Explain

Comment [BM13]: Characterisation and genotyping done at UK why??

RESULTS AND DISCUSSION

The results are presented as follows:

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COMBINED G AND P TYPES

Table 1 showed the G and P combination in the study for all the hospitals surveyed in the different locations in Abuja, Nigeria. A total of 125 samples were genotyped for VP7 (G) and VP4 (P). A total of twelve combinations of G and P were identified in the study. G2 P [4] combination was found to be the most predominant 21 (16.8%) with the majority of it found in Nyanya General Hospital 10 (8.0%), the second highest was found in Zankli Medical Centre 06 (4.8%) with the lowest of same combination found in Gwagwalada Town clinic 02 (1.6%).The second highest combination in the study was G1 P [6] found in 16 samples, 16 (12.8%), this combination was found to be predominant in two hospitals with the highest recorded in Nyanya General Hospital 12 (9.6%) and the lowest in University of Abuja Teaching hospital 4 (3.2%). This is similarity the G3 P [6] with aoverall of 16 (9.6%) with most found in Zankli Medical Centre 07 (5.6 %) and Nyanya General Hospital 05 (4.0 %). G9 P [4] is next most predominant withan overall of 13 (10.4%) with the majority in Nyanya General Hospital. Other genotypes found in the study include G9 P [8the] with majority found in Nyanya General Hospital 04 (3.2%). The combinatwiththewtheith least number in the study is G3 P [8] with 01 (0.8%) in UATH, G2 P [8] 2 (1.6%) in UATH and ZMC and G9 P[6] 2 (1.6%) found in NyanyaGeneral Hospital only and G12 P [4] in Zankli Medical centre only.

G COMBINATION WITH NON-TYPABLE P

This G2 P [NT] combination was found in 4 samples out of 125 overall samples which constitute 3.2% of the specimens found in Nyanya General Hospital and Zankli Medical

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Centre. G9 P [NT] 03 (2.4%) was the second highest which was most predominant in Nyanya General Hospital also. Others include G2 P [NT] with an overall of 04 (3.2%), and the least of this group in the study is G3 P [NT] found in only Zankli Medical Centre 01 (0.8%) and G10 P [NT] in Nyanya General Hospital.

P COMBINATION WITH NON-TYPABLE G

Only two specimens in the study were found with this combination of [NT] P6 and [NT] P8 found in Nyanya General Hospital 01 (0.8%). Three specimens also in the overall study were not typed at all for both G and P.

DOUBLE COMBINATION OF GENOTYPES (G and P)

This was found in four specimens in the entire studies all found in Nyanya General Hospital, they are G2 P[4] P[8], G9 P[4] P[8], G2 G3 P[6], and G2 G12 P[8] in equal proportion of 1 (0.8%) respectively.

Table 1: Common Genotypes single and combined, by hospital

GENOTYPES	FREQUENCY BY HOSPITALS				PERCENTAGES N=125
G TYPES	**UATH**	**GTC**	**NGH**	**ZMC**	
G1	04	-	12	-	16 (12.8%)
G2	04	03	15	11	33 (26.4%)
G3	06	03	05	09	23 (18.4%)
G9	04	-	19	01	24 (19.2%)
G10	-	-	01	-	01 (0.8%)
G12	06	-	06	09	21 (16.8%)
Mixed	-	-	02	01	03 (2.4%)
nt	-	-	02	02	04 (3.2%)
TOTAL G's	24	06	62	33	125
P TYPES					
P4	06	05	22	12	45 (36.0%)
P6	08	01	22	09	40 (32.0%)
P8	10	-	08	05	23 (18.4%)
Mixed	-	-	02	02	04 (3.2%)
nt	-	-	08	05	13 (10.4%)
TOTAL P's	24	06	62	33	125
	UATH	**GTC**	**NGH**	**ZMC**	
G1 P[6]	04	-	12	-	16 (12.8%)
G2 P [4]	03	02	10	06	21 (16.8%)
G2 P [6]	-	01	01	02	04 (3.2%)
G2 P [8]	01	-	-	01	02 (1.6%)
G3 P [4]	01	03	-	01	05 (4.0%)
G3 P [6]	04	-	05	07	16 (12.8%)
G3 P [8]	01	-	00	-	01 (0.8%)
G9 P [4]	02	-	10	01	13 (10.4%)
G9 P [6]	-	-	02	-	02 (1.6%)
G9 P [8]	02	-	04	-	06 (4.8%)
G12 P [4]	-	-	-	04	04 (3.2%)
G12 P [8]	06	-	04	05	15 (12.0%)

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G2 P[nt]			02	02	04 (3.2%)
G3 P[nt]				01	01 (0.8%)
G9 P[nt]	-	-	03	-	03 (2.4%)
G10 P[nt]	-	-	01	-	01 (0.8%)
G12 P[nt]			02		02 (1.6%)
G[nt] P6	-	-	01	-	01 (0.8%)
G[nt] P8	-	-	-	01	01 (0.8%)
ntnt	-	-	01	02	03 (2.4%)
Mixed Infection	**UATH**	**GTC**	**NGH**	**ZMC**	
G2 P[4] P[8]	-	-	01	-	01 (0.8%)
G9 P[4] P[8]	-	-	01	-	01 (0.8%)
G2 G3 P[6]	-	-	01	-	01 (0.8%)
G2 G12 P[8]	-	-	01	-	01 (0.8%)
	24	**06**	**62**	**33**	**125**

Frequency of genotypes among vaccinated and unvaccinated children.

Frequency of genotypes among vaccinated and unvaccinated children.

The most predominant combination is the G2 P[4] with 21 (16.8%) which was found most among unvaccinated childrenin table 2. The second most predominant genotype among the unvaccinated children is the G3 P [6] and G1 P [6] with 16 (12.8%) respectively. The next most common combination is the G12 P [8] with 15 (12.0%) and also G9 P [4] which accounts for 13 specimens with 10.4%. The least combinations in the study include G3 P[8], G3 [NT], G10 P[NT], NT P[6], NT P[8] with 1 (0.8%) respectively. The majority of these combinations appear to be most predominant in unvaccinated children with the least among vaccinated children. The unusual combinations appear to be in children with unknown vaccination status which constitute a proportion of 1 (0.8%) in all respects. Overall, in the study 94 samples which constitute 75.2% appear among unvaccinated children, twelve samples with 9.6% appear to be among the vaccinated children and 19 (15.2%) constitute children with unknown vaccination status.

Table 2: Showing frequency of genotypes among vaccinated and unvaccinated children.

Genotypes	Vaccinated patients	Unvaccinated patients	Unknown	Total
G1 P[6]	-	16	-	**16 (12.8%)**
G2 P[4]	1	19	1	**21 (16.8%)**
G2 P[6]	1	3	-	**04 (3.2%)**
G2 P[8]	-	2	-	**02 (1.6%)**
G3 P[4]	1	3	1	**05 (4.0%)**
G3 P[6]	2	12	2	**16 (12.8%)**
G3 P[8]	-	1	-	**01 (0.8%)**
G9 P[4]	2	11	-	**13 (10.4%)**
G9 P[6]	-	2	-	**02 (1.6%)**
G9 P[8]	-	3	3	**06 (4.8%)**

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G12 P[4]	-	-	4	**04 (3.2%)**
G12 P[8]	2	12	1	**15 (12.0%)**
G2 [NT]	2	-	2	**04 (3.2%)**
G3 [NT]	-	-	1	**01 (0.8%)**
G9 [NT]	-	3	-	**03 (2.4%)**
G10 P[NT]	-	1	-	**01 (0.8%)**
G12 [NT]	-	2	-	**02 (1.6%)**
NT P[6]	1	-	-	**01 (0.8%)**
NT P[8]	-	1	-	**01 (0.8%)**
NT NT	-	3	-	**03 (2.4%)**
G2 P[4] P[8]	-	-	1	**1 (0.8%)**
G9 P[4] P[8]	-	-	1	**1 (0.8%)**
G2 G3 P[6]	-	-	1	**1 (0.8%)**
G2 G12 P[8]	-	-	1	**1 (0.8%)**
Total	**12 (9.6%)**	**94 (75.2%)**	**19 (15.2%)**	**125**

Estimate of vaccine effectiveness in the current study

The formula for calculating the vaccine effectiveness using the screening method according to Hatton 1990:
VE = (PPV – PCV / PPV [1-PCV])
VE= Vaccine effectiveness
PPV = Proportion of children vaccinated
PCV = Proportion of cases vaccinated

Table 3: 2x2 Table of rotavirus positivity (RV+ or RV-) among vaccinated and unvaccinated children in the study to estimate vaccine effectiveness in the study

CCS	**Outcome**			**Total**
Exposure		RV+	RV-	
	Vaccinated	17	304	321
	Unvaccinated	106	649	755
Total		123	953	1076

Using data from this study, PPV = 321/1076= 0.298

PCV = 17/123 = 0.138

VE = 64.3%

CONCLUSION AND RECOMMENDATIONS

Monovalent rotavirus vaccine RV1 (Rotarix) had been available on parental demand in one centre for more than a year, but not in the other clinics. Few of the parents had good knowledge about the availability of vaccines and only a quarter could remember if/how often

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their child might have been vaccinated. Vaccination cards were often not available for verification.In the clinic/hospital in which prior vaccination could be linked to hospital admission records, at least 8.4% of vaccinated 525 children were admitted over the next year with all causesof diarrhoea.A greater proportion of children with diarrhoea with less severe disease (judged by activity scores) had been vaccinated than the children with worse activity scores. A smaller proportion of children with rotavirus isolated from their stools had been vaccinated than those without rotavirus in their stools. Using the indirect estimate method of Poole, a vaccine protective efficacy of 64.3% was estimated.This is similar to results in prospective studies on several continents, including recent detailed studies in Malawi.

The P and G genotypes prevalent in Abuja were similar to reports from the past and recently reported studies in Nigeria and neighbouring Ghana; although past reports from northern Nigeria show a differing pattern.Looking at the rotavirus genotypes determined in 125 subjects, in the group of vaccinated children previously given the R1V vaccine, there were no G1 genotypes compared to 0 in the group of children of uncertain vaccination status. However, G1 was found in 16 (12.8%) isolates and was found most in unvaccinated children. This is indirect support for vaccine efficacy.There were no other major differences in genotypes and combinations between vaccinated and unvaccinated groups except the novel genotype G12 was found in 2 (12.5%) of vaccinated children. This genotype has recently emerged in several continents and may be more pathogenic. This requires further monitoring.The findings support the planned (but delayed) introduction of rotavirus vaccine in the whole of Nigeria but emphasise the need for adequate investment in quality assured surveillance and virological surveillance to monitor this. The emergence of new rotavirus genotypic combinations may pose a threat to vaccine efficacy in the future.

Comment [BM15]: Your conclusion and objective statement is contradictory and confusing.

Comment [BM16]: Whole Conclusion needs a major revision with only valid conclusive findings from the research. It should not be so elaborative and confusing

Comment [BM17]: Rewrite the references as per journal guidelines

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