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Circulation of Rotavirus Genotypes and their Distribution among Vaccinated and Non-vaccinated Children in Abuja, Nigeria

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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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 and one 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 1240 (93.3%) participants, of whom 957 (77.0%) were ambulatory, 123 (9.9%) hospitalised and 160 (12.8%) controls without diarrhoea. Rotavirus-ELISA

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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.

1. INTRODUCTION

Rotavirus infections are a known cause of approximately 50% of diarrheal cases worldwide. It has been estimated that around 800,000 children die every year due to rotavirus disease and over two million require hospitalization [1]. The burden of rotavirus disease is significantly concentrated in low-income countries, particularly in sub-Saharan Africa, where it is estimated to cause the deaths of over 230,000 infants each year [2]. Despite this, few studies in Nigeria have focused on assessing the circulating genotypes. Among the few studies conducted, Steele et al. [3] reported the predominance of G9, G3, G1, G8, and G2 genotypes in Plateau state, while Audu et al. [4] reported the circulation of G1, G3, G4, and P [6] and P [4] genotypes in Lagos state. Notably, no single publication from Abuja had been identified throughout the course of this study. The main aim of this study is to determine the circulating rotavirus genotypes and their distribution among vaccinated and unvaccinated children.

1.1 Serotypes in Nigeria

A few publications have described the serotypes/genotypes that are currently circulating among Nigerian children. In a recent study conducted in Ile-Ife, southern Nigeria, a small group of children were enrolled, and the investigation revealed that G1P [8] was prevalent among children under one year old. There was also a high prevalence of the emergence of G12 P [8] [5]. Additionally, positive cases in the study were mixed G and P genotypes [6].

Genotype diversity was also described in another survey conducted in two regions of Nigeria, Lagos state in southern Nigeria and Kwara state in the north-central. Children under the age of five were enrolled, and the results showed that G1 was the most prevalent, followed by G3 and G4. The rate of mixed G serotypes was relatively high, and the mixed P serotype was also relatively high. The study concluded that the high rate of mixed types may have implications for vaccine development [7], which is consistent with the findings of Japhet et al [5].

A study conducted in Jos, Nigeria, reported the circulation of novel G9 and G8 rotavirus strains, in addition to G2 and G4, which were isolated in a few samples from the study population. The report emphasized the global distribution of the G9 strains [3].

The rotavirus strains studied showed that G1, G2, G3, and G4 were more frequently detected in diarrheic stool samples of children. For example, a study conducted in Lagos between 1996-1997 revealed that G1 was the most common serotype, followed by G3. Mixed G1/G2 was the first reported in Nigeria in this study. P [6] was most commonly reported, followed by P [8], with P [4] being the least common [4]. Another study conducted in Zaria between 1997 and 1998 among children under the age of five revealed that G1 and G3 serotypes were the most predominant, with G1/G3 being less common among the population surveyed [8]. This study also showed a similar result to that of Audu et al [4]. Adah in [9] reported that G3 was predominant in southern Nigeria, with some infections being high in the study. The limitations of the study highlighted that some cases were non-typed, but this could be a result of the primers used to detect the serotypes in the study [9].

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] were in relatively high proportion. Although a study in Nigeria investigated by Audu et al. [7] incidentally found similar genotypes [4].

In another study in Ghana, G2P [6], G3P [4], and G9P [8] made up half of the genotypes detected in the Kassena-Nankana district. G3 was found to be the most predominant strain, followed by G2. These findings were similar in several investigations by some authors in Nigeria [10,3,4,9].

2. METHODOLOGY

The study conducted was a one-year prospective survey of children with acute diarrhoea in Abuja, Nigeria. The study recruited children under the age of five from four hospitals. Demographic data and stool samples were collected from September 2012 to August 2013 from a total of 1331 children along with controls. Commercially available kits were used to perform Rotavirus ELISA and RNA extraction. Positive samples were then subjected to RT-PCR and electrophoresis to determine VP7 (G) and VP4 (P) genotypes. The characterisation and genotyping of Rotavirus positive cases were carried out at the University of Liverpool in the United Kingdom.

3. RESULTS AND DISCUSSION

The results are presented as follows:

3.1 Combined G and P Types

Table 1 displays the G and P combinations identified in the study, which was conducted in different hospitals across Abuja, Nigeria. A total of 125 samples underwent genotyping for VP7 (G) and VP4 (P), with twelve unique combinations of G and P identified. The most prevalent combination observed was G2 P[4], found in 21 samples (16.8%), with the highest proportion of this combination detected at Nyanya General Hospital (10 samples, 8.0%). The second-highest combination identified was G1 P [6], present in 16 samples (12.8%), with the highest proportion of this combination detected at Nyanya General Hospital (12 samples, 9.6%). The G3 P [6] combination was also identified in 16 samples (9.6%), with the majority found at Zankli Medical Centre (7 samples, 5.6%) and Nyanya General Hospital (5 samples, 4.0%). G9 P[4] was the next most common combination observed, with an overall total of 13 samples (10.4%) and the majority detected at Nyanya General Hospital. Other genotypes identified in the study included G9 P[8] (with the highest proportion detected at Nyanya General Hospital, 4 samples, 3.2%) and G3 P[8] (found in only 1 sample, 0.8%, at University of Abuja Teaching Hospital). The combination with the least number of samples in the study was G2 P[8], identified in 2 samples (1.6%) at both University of Abuja Teaching Hospital and Zankli Medical Centre, along with G9 P[6] (2 samples, 1.6%) found only at Nyanya General Hospital and G12 P[4], identified exclusively at Zankli Medical Centre.

3.2 G Combination with Non-Typable P

The G2 P[NT] combination was identified in 4 out of the 125 total samples, constituting 3.2% of the specimens collected from Nyanya General Hospital and Zankli Medical Centre. The second-highest combination detected was G9 P[NT], found in 3 samples (2.4%) and most prevalent at Nyanya General Hospital. Additionally, G2 P[NT] was observed in a total of 4 samples (3.2%) throughout the study. The least prevalent of this group in the study was G3 P[NT], found in only 1 sample (0.8%) at Zankli Medical Centre, along with G10 P[NT], which was only detected at Nyanya General Hospital.

3.3 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.

3.4 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 [11-13].

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%)
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%)
nt nt	-	-	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**

3.5 Frequency of Genotypes among Vaccinated and Unvaccinated Children

The G2 P [4] combination was found to be the most predominant among unvaccinated children in Table 2, accounting for 21 cases (16.8%). The second most common genotypes among unvaccinated children were G3 P [6] and G1 P [6], with 16 cases (12.8%) each. The next most prevalent combinations were G12 P [8], which accounted for 15 cases (12.0%), and G9 P [4], which accounted for 13 cases (10.4%). The least common combinations in the study included G3 P [8], G3 [NT], G10 P [NT], NT P [6], and NT P [8], with only one case each (0.8%).

These combinations appeared to be most predominant in unvaccinated children and least common among vaccinated children. Unusual combinations were observed in children with unknown vaccination status, accounting for 1 case (0.8%) in all respects. Overall, the study found 94 samples (75.2%) among unvaccinated children, 12 samples (9.6%) among vaccinated children, and 19 samples (15.2%) among children with unknown vaccination status.

3.6 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 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%)
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

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%

4. CONCLUSION AND RECOMMENDATIONS

The monovalent rotavirus vaccine RV1 (Rotarix) had been available upon parental demand in one centre for over a year but not in the other clinics. However, few parents had good knowledge about the availability of vaccines, and only a quarter of them could recall if and how often their child was vaccinated. Moreover, vaccination cards were often not available for verification.

In the clinic/hospital where prior vaccination was linked to hospital admission records, at least 8.4% of the 525 vaccinated children were admitted over the next year with all causes of diarrhoea. Interestingly, a greater proportion of children with less severe diarrhoea (judged by activity scores) had been vaccinated compared to those with worse activity scores. Additionally, a smaller proportion of children with rotavirus isolated from their stools had been vaccinated compared to those without rotavirus in their stools. Using the indirect estimate method of Poole, a vaccine protective efficacy of 64.3% was estimated, which is similar to the results of 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 recent studies in Nigeria and neighbouring Ghana, although past reports from northern Nigeria show a different pattern. In the 125 subjects, the rotavirus genotypes determined in the group of vaccinated children previously given the R1V vaccine did not have any G1 genotypes, compared to zero in the group of children with uncertain vaccination status. However, G1 was found in 16 (12.8%) isolates and was most prevalent in unvaccinated children, providing indirect support for vaccine efficacy. There were no other major differences in genotypes and combinations between vaccinated and unvaccinated groups, except for the novel genotype G12 found in 2 (12.5%) of vaccinated children. This genotype has recently emerged in several continents and may be more pathogenic, requiring further monitoring.

The findings support the planned (but delayed) introduction of rotavirus vaccine in the whole of Nigeria. However, it emphasizes 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.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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