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Molecular Characterization of Human Rotavirus among Children with Acute Gastroenteritis in Ebonyi State

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ABSTRACT

Background: As the leading source of death for children under the age of five worldwide, diarrhea is second only to pneumonia. In Ebonyi State, it is a major contributor to emergency situations and elevated infant morbidity and mortality. When it comes to genotype-specific acute diarrhea in children, Group A Rotavirus is the most frequent viral etiologic agent, with different genotypes. The purpose of the research was to identify the most common rotavirus strains in circulation among children under 5 who were admitted to the hospital in Abakaliki with diarrhea.

Methods: A cross-sectional survey was conducted. In continuation of previous work by the same authors on prevalence and risk factors of rotavirus infection in children, RNA extraction was carried out, according to manufacturer's instructions using appropriate extraction kit, on 75 stool samples that were positive for rotavirus infection by ELISA procedure, followed by polymerase chain reaction and genotyping. The produced products of different sizes were then examined under ultraviolet (UV) illumination.

Results: The two most common G genotypes were G1 and G4 (42.5% and 38.4%, respectively). The most common P gene was P [8], which was followed by P [6] and P [4], both of which had a 5.5% prevalence. The two rotavirus types G1P [8] (11%) and G4P [8] (11%) made up the majority overall.

Conclusions: The most common rotavirus types in circulation were G1, G4, P8, G1P [8], and G4P [8].

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KEYWORDS:

Children, Diarrhea, Acute gastroenteritis, Rotavirus, Genotypes, Molecular Characterization.

INTRODUCTION

The definition of diarrhea is the passing of liquid or mushy stools more than three times in a 24-hour period a day[1]. Acute gastroenteritis continues to be the second-leading cause of mortality for children under the age of five, trailing pneumonia, despite global efforts in water, sanitation, and vaccination[2]. A high mortality rate of roughly 453,000 deaths per year is seen in cases affecting minors under the age of 5 [3], with the majority of these deaths taking place in Sub-Saharan Africa and Southeast Asia's developing nations. Every year, India alone reported almost 100,000 fatalities [4]. In Ebonyi, it is a major contributor to emergency cases as well as higher infant morbidity and mortality rates [5]. Rotavirus, with group A being the predominant strain, is the most frequent viral cause of diarrhea in children worldwide, accounting for a significant portion of outpatient appointments and hospitalizations [6]. It is the most involved viral agent, according to numerous studies conducted in Nigeria, where it has been linked to high rates of morbidity and mortality[3,7–14]. With 27 G genotypes (G1-G27) and 35 P genotypes (P[1]-P[35]) found in people [15–17], group A of the rotavirus causes the majority of the life-threatening infections. G-types 1-4 and P-types 4, 6, and 8 are frequently linked to human infections in 80% of instances of human diarrhea, respectively[18]. Multiple genotypes, including the G9, G12, G1P, G9P, G4P, and G3P, as well as the uncommon G12P [6], have been identified as the most common genotype combinations in Nigeria [8, 10, 11–13]. Location and season affect

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the specific rotaviral strains that cause the illness. Though the epidemiological basis of the genotype cycling phenomenon is undetermined, it, however, imposes difficulties in determining the appropriate composition and efficacy of rotavirus (RV) vaccines[19,20,21], especially when the efficacy is directly proportional to the prevalent genotypes in a particular region. Therefore, having a thorough understanding of the genetic diversity of rotavirus and keeping track of the constantly evolving strains will help contain the infection. The goal of this research was to identify the molecular diversity of rotavirus infection among children under the age of five in Abakaliki who had acute gastroenteritis.

METHODS

Ethical Considerations

The Alex Ekwueme Federal University Teaching Hospital, Abakaliki [AEFUTHA] Research and Ethics Committee (REC) office, which is located in the hospital, granted ethical approval for the index research work on April 26, 2018, with a Rec Protocol Number of 23/04/2018-24/04/2018. This research was conducted to advance a molecular aspect of earlier work by the same authors [22].

Study Design

Type, Location, and Period

It was cross-sectional research carried out in Abakaliki, the Nigerian state capital of Ebonyi. Alex Ekwueme Federal University Teaching Hospital in Ebonyi state was the study location. It was conducted over a four-month period, from November 2018 to March 2019.

Sampling and Sample Size

A total of 275 patients were recruited for the study. They were enrolled as they presented with acute watery diarrhea in the children's emergency rooms of the hospitals. The sample size was calculated based on the following formula. $N = z^2pq/d^2$

N = the desired sample size.

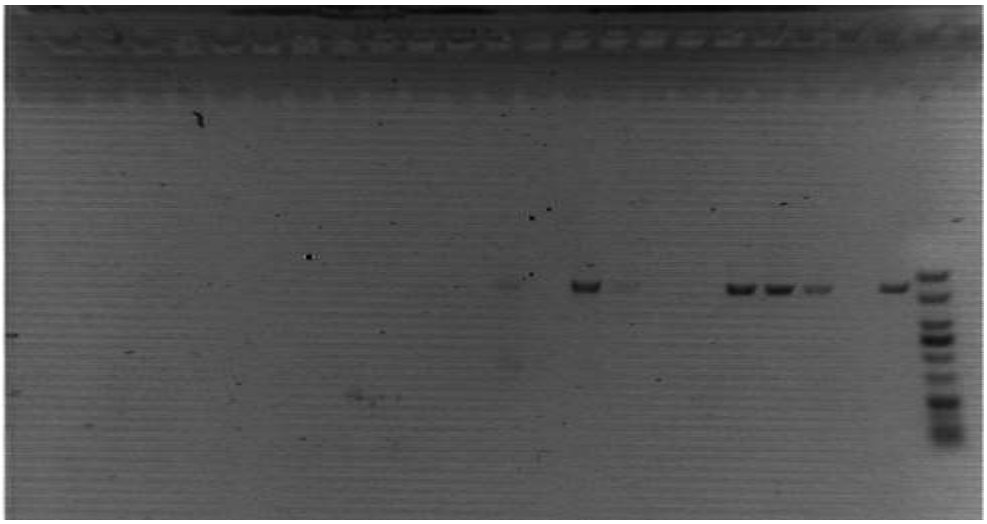

Fig. 1: VP7 1-20

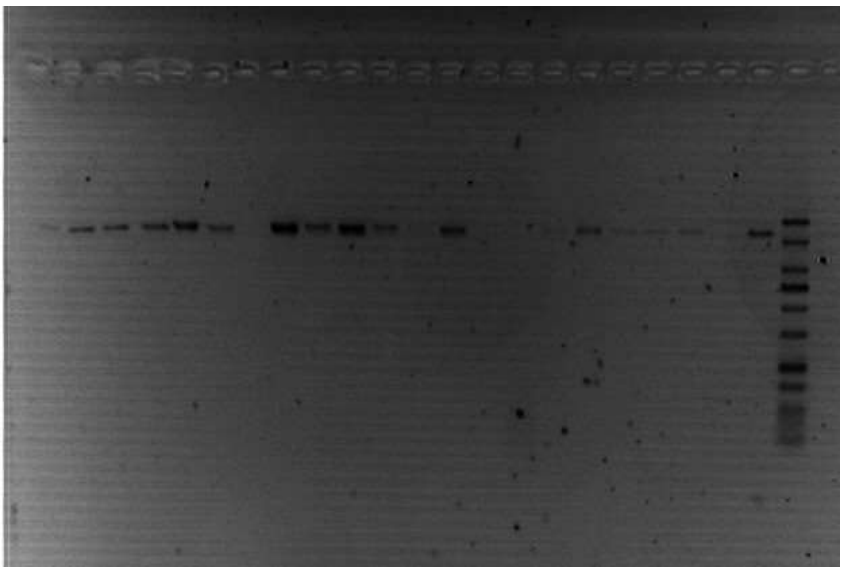

Fig 2: VP4 38-58

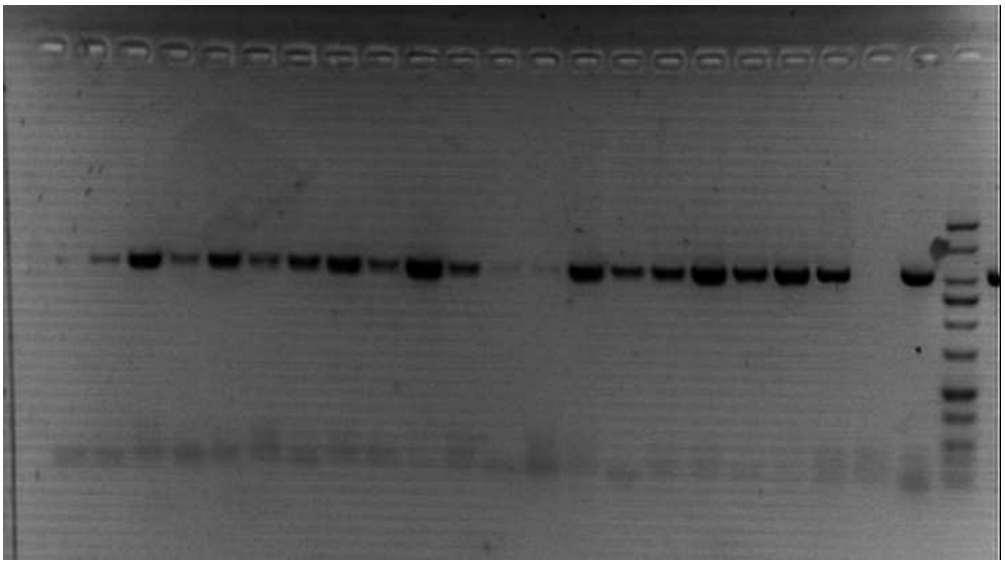

Fig 3: G1, G2, G8, G10 (1-20)

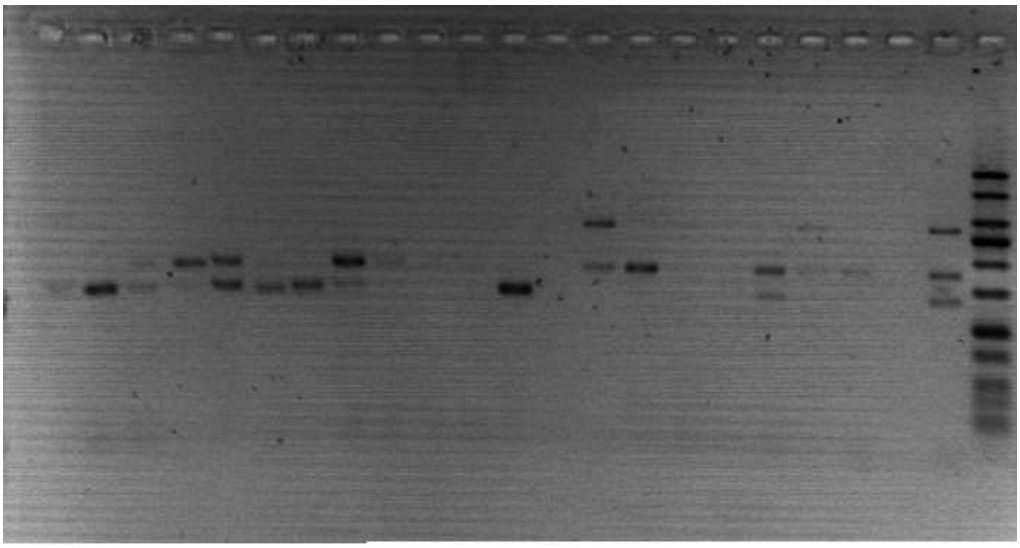

Fig 4: P4, P6, P8 (1-20)

Table 1: VP7 Consensus and G-type specific oligonucleotide primers for G genotyping25, 27, 30

Primer	Genotype	Sequence	nt Position	PCR Product
G-genotyping 1st amplification				
Vp7F	G1	ATG TAT GGT ATT GAA TAT ACC AC	51-71	
Vp7R	G1	AAC TTG CCA CCA TTT TTT CC	914-932	881
G-typing 2nd amplification				
Vp7R	G1	AAC TTG CCA CCA TTT TTT CC)	914 - 932	
aBT1	G1	CAA GTA CTC AAA TCA ATG ATG G	314- 335	618
aCT2	G2	CAA TGA TAT TAA CAC ATT TTC TGT G	411- 435	521
G3	G3	ACG AAC TCA ACA CGA GAG G	250- 259	682
aDT4	G4	CGT TTC TGG TGA GGA GTT G	480- 498	452
aAT8	G8	GTC ACA CCA TTT GTA AAT TCG	178- 198	754
aFT9	G9	CTT GAT GTG ACT AYaA AAT AC	757- 776	179
G10	G10	ATG TCA GAC TAC ARbA TAC TGG	666-687	266
G12	G12	GGT TAT GTA ATC CGA TGG ACG	548-567	396

R = Reverse, F = Forward, nt = nucleotide, Ya = C or T, Rb = A or G

Table 2 VP4 Consensus and P-Type Specific Oligonucleotide Primers for PGenotyping[25]

Primer	Genotype	Sequence	nt Position	PCR Product
P-typing 1st amplification				
Forward Con-3		TGG CTT CGC TCA TTT ATA GAC A		
	P [8]		11 - 32	
Reverse Con2		ATT TCG GAC CAT TTA TAA CC		876
	P [8]		868 – 887	
P-typing 2nd amplification				
Con 3	P [8]	TGG CTT CGC CAT TTT ATA GAC A	11-32	
2T-1	P [4]	CTA TTG TTA GAG GTT AGA GTC	474 – 494	483
3T-1	P [6]	TGT TGA TTA GTT GGA TTC AA	259 – 278	267
1T-1D	P [8]	TCT ACT TGG ATA ACG TGC	339 – 356	345
4T-1	P [9]	TGA GAC ATG CAA TTG GAC	385 – 402	391
5T-1	P [10]	ATC ATA GTT AGT AGT CGG	575 – 594	583
P [11]	P [11]	GTA AAC ATC CAG AAT GTG	305-325	312

Data analysis

Data management was done using SPSS Version 20.0 software. Associations were determined using Pearson's chi-square test.

Significant values were set at $P < 0.05$, with confidence intervals (CIs) of 95% where applicable.

RESULTS

Two hundred and seventy-five (275) participants, who met all the inclusion criteria were recruited, from whom 275 stool samples were collected and analyzed. Seventy-three (26.5%) were positive for rotavirus.

Rotavirus strain distribution

Sixty-five (89%) were G genotypes while P genotypes were 24(32.9%). Eight (11%) were neither G nor P (non-typeable). Sixty-three (86.3%) strains were detected as single G genotypes including G1 (31, 42.5%), G4 (28, 38.4%), and G12 (4, 5.5%) with G1 being the single most predominant G. Mixed G strains as G1+G4 (2, 2.7%) was equally detected. Table 3. Twenty-four (32.9%) single P genotypes were observed including P4 (4, 5.5%), P6 (4, 5.5%), and P8 (16, 21.9%) with P8 being the single most predominant P type. G and P combinations detected were twenty-four (32.9%). These included G1P8 (8, 33.3%), G4P 8 (8, 33.3%), G1P 6 (2, 8.3%), G1P4 (2, 8.3%), G4P 6 (2, 8.3%) and G4P 4 (2, 8.3%). Among them, G1P8 and G4P 8 were the most predominant combination strains. Table 3.

Table 3: Distribution of rotavirus strains circulating among under-five hospitalized for acute gastroenteritis in Abakaliki, Ebonyi state.

G genotypes	P genotypes No (%) of strains				**Total (%)**
	P8	P6	P4	P-Negative	
G1	8 (10.96)	2(2.74)	2(2.74)	19 (26.03)	31 (42.5)
G4	8(10.96)	2(2.74)	2(2.74)	16 (21.92)	28 (38.4)
G12	0 (0)	0 (0)	0 (0)	4 (5.47)	4 (5.5)
Mixed G	0	0	0	2 (2.74)	2 (2.7)
NT	0	0	0	8 (10.96)	8 (11)
G-negative	0	0	0	0	0(0)
Total	16(21.9)	4(5.5)	4(5.5)	49(67.1)	73(100)

NT = non-typeable.

DISCUSSION

The molecular epidemiology of pre-vaccine RVA strains that were prevalent in Abakaliki, Ebonyi state, Nigeria, is described in this research. The study[22] that described the risk factors for rotavirus-induced acute diarrhea in children under the age of five is built on this one. The current research

examines the rotavirus strains that are prevalent among children under the age of five in Abakliki. Infectious gastroenteritis caused by the rotavirus is very common in Abakaliki. Worldwide, dehydrating diarrhea continues to be a leading cause of morbidity and mortality in infants under the age of five [31, 32, 33, 34]. As a result, it is crucial to conduct continuous and sufficient surveillance before and after the introduction of a vaccine that will aid in epidemiologically determining dominant strains, vaccine efficacy and the detection of unusual strains in addition to defining disease burden [35-39]. The study found the following circulating genotypes and combination strains: G1, G4, G12, P[8], P[6], and P[4] and G1P[8], G4P[8, G1P[6, G4P[6, G1P[4, G4P[4, and G1G4 respectively. The circulating G and P genotypes displayed a varied distribution, with G1P[8] predominating. The findings were in line with studies carried out in Abuja, Nigeria, in 2019 [12, 40] as well as in some other parts of the country, including Enugu, Lagos, Ilorin, Sokoto, Benin, and Asaba (G1-G4, G9, G12, P6, P8, and G1P8; and G4P8) [8–10, 11, 41–43]. Similar socio-demographic characteristics, geographic settings, and environmental influences may be to blame for the parallels. The circulating genotypes identified in this study are comparable to reports of various strains discovered circulating in some African and global areas where G1P [8] was identified as the predominate strain[16,17, 44-50]. In Burkina Faso, earlier research had similarly demonstrated that G1P[8] was the most common strain circulating there within a brief period of time[51]. Despite being restricted to a specific area, these findings imply that the dominant genotype of RVA in Burkina Faso is susceptible to rapid change over a brief period of time. Other research has demonstrated that significant shifts in the genotype distribution of human rotaviruses occur constantly from one year to the next or from one location to another in Europe and Asia [52, 53]. According to reports from other parts of the West African sub-region [54, 55], G4 was primarily found in combination with P[8]. But in addition to the dominant G1P [8] and G4P [8], this research also found G4P [4] and G4P [6], which, despite having a relatively low prevalence, are distinct from the dominant strains reported in some other African countries [42]. Keep in mind that the components of the circulating predominant strains identified in this research are used to make the two most well-known and widely used rotavirus vaccines (Rotarix and Rotateq). (G1, G4, and G1P [8]). The overall picture from this index study and other studies indicates that the circulating strains are widely dispersed across various eras and regions of Nigeria and Africa, and by extension, the entire world [8, 20, 21, 56]. This emphasizes the rotavirus's dynamic nature and ability to reassort with numerous strains, including those from animals, resulting in the production of completely new novel strains[19,57]. Hence, it addresses the importance of continuous surveillance of this infection to unravel the unusual and emerging strains, which would impact adversely the efficacy of the vaccines for its eradication.

CONCLUSION

Rotavirus gastroenteritis is significantly prevalent (25,6%) in Abakaliki. There is a large amount of rotavirus diversity. The most common alleles of rotavirus are G1, G4, G12, P[8], P[6], and P[4]. The two most common G/P pairs in circulation were G1P and G4P[8]. Despite their low frequency, the following strains were also found: G1P [6], G1P [4], G4P [6], G4P [4], and G1G4.

LIMITATIONS OF STUDY

This research was a cross-sectional study that was conducted over the course of just 4 months in a hospital setting, covering just the urban center of the Ebonyi North zone of the state. Therefore, there is a strong possibility that this study group does not accurately reflect the prevalence of disease and circulating strains in the region. Therefore, a more thorough investigation that will cover a broader area and include outpatients in rural areas is required to get a better idea of the prevalence and circulating strains.

RECOMMENDATION

The study has provided information on the approaches needed to develop an effective rotavirus vaccine for this area. It is recommended that the identified strains (G1, G4, P [8], G1P [8], and G4P [8]) be included in the vaccine formulations for efficacy and adequate protection against rotavirus infections. Robust and ongoing tracking of the rotavirus strains that are presently circulating in this region is also essential before and after its introduction in order to evaluate the rotavirus vaccine's efficacy and keep an eye out for the emergence of unusual strains.

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