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Viral Gastroenteritis among Children under 5 Years in Dutsinma Local Government Area, Katsina State, North-West Nigeria, West Africa

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Abstract

Rotaviruses and adenoviruses conjointly form the leading cause of epidemic gastroenteritis in developed and developing countries. The prevalence of this array of human viral pathogens in under five years children of Dustin-ma Local Government Area of Katsina State, North-west Nigeria was investigated on diarrheal stool samples of 152 patients of acute viral gastroenteritis by Reverse Transcription Polymerase Chain Reaction (RT-PCR) as approved by the state committee on ethics after parental agreement. Analyses revealed prevalence of 54% (35/65) and 46% (30/65) for rotaviruses and adenoviruses respectively in 2013 and then 62.2% (51/82) for rotaviruses followed by 37.8% (31/82) for adenoviruses in 2014 with a total of 87 rotavirus positive samples (57.24%) while 61 samples (40.13%) were adenovirus positive. Only 4 samples (3%) were infected by other diarrheal viral pathogens.

Keywords

Rotavirus, Adenovirus, Viral Gastroenteritis, Children under Five Years, Nigeria

Subject Areas: **Virology**

1. Introduction

Viral gastroenteritis hereafter referred to as VGE is a common disorder among children worldwide. This heath condition is associated with high mortality and morbidity. More than 2 million children are lost to death annually due to diarrheal viral infections, which represents about 19% of death in this population [1]. In developing countries, the VGE's incidence rate is 2.1 to 3.8 per child from 11 to 48 months of age per year [1] [2]. In Nigeria,

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Diarrhea presents the highest morbidity rate and is the major cause of death during the first year of life [3]. Rotaviruses are double stranded RNA viruses as opposed to human adenoviruses known with double stranded DNA material [4]. While rotavirus has been reported as the leading cause of diarrhea affecting children below 5 years, adenovirus and other viral agents are currently increasingly reported [5]. Even though enteric adenovirus has been identified at 9% among children with diarrhea, it is established to have very low communicability between close contacts in the same house hold. VGE is usually self-limiting in immunocompetent individuals; however rare fatalities can occur in immunocompromised body systems. Up till 2006, at least 10 cases of laboratory acquired adenovirus infections were reported by Public health Agency of Canada. Meanwhile, it was pointed out that rotavirus was stable at low and high relative humidity and non-stable in the medium range of relative humidity of fecal samples [6]. The potential vehicular role of human hands was also reported. Rotaviral antigens are detectable in hand washings of persons who attend to patients with rotaviral gastroenteritis (GE). These viruses can survive for several days on contaminated non-porous inanimate surfaces [7]. Rotavirus transmission occurs fecal-orally and becomes a critical water quality issue when soil and water become contaminated with feces [8]. The burden of diarrheal disease has now imposed on research institutions the responsibility to disseminate this information and scientifically map out strategies to effectively prevent the spread of this viral disorder in children and abate the rates of mortality and morbidity of VGE in low-income settings of developing countries. It is in this light that we initiated a study to establish the immediate and collateral causes of the spread of VGE among children of less than 5 years [9] in Dutsin-ma local Government Area of Katsina State, North-West Nigeria, West Africa.

2. Materials and Method

This work was designed to retrospectively investigate 152 diarrheal patients [10] of less than 5 years of age who were diagnosed with enteric adenovirus and rotavirus pathogens in Dustin-ma Local Government's Medical center in Dustin-ma, Katsina State North-West Nigeria, West Africa. These specimen were collected and investigated for the presence of rotavirus and adenovirus antigens within the period from January 2013 to December 2014 in Dustin-ma Hospital and transported to the Department of Biological sciences in Ahmadu Bello University Zaria for analyses using molecular techniques of Polymerase Chain reaction (PCR) for amplification and Reverse Transcription-Polymerase Chain Reaction (RT-PCR) for investigation and electrophoresis.

2.1. Nucleic Acids Extraction from Stool Samples

The Nucleic acids from stool samples were isolated following the protocol made public by O'Neil and his colleagues and already used by Catriona Logan and her colleagues in 2006 [4]. RNA and DNA were co-extracted for the isolation of viral nucleic acids from stool samples. All specimens were extracted in a devoted class 2 laminar flow hood using exclusively assigned pipettes and aerosol-resistant pipette tips to ensure the integrity of our results. Fecal samples were prepared as 10% suspensions with STAR buffer, Roche Diagnostics GmbH, Mannheim, Germany. Equal Chloroform quantities of 0.1× volume were added, and following mixing, the samples were spun at 8000 × *g* for 10 min. Stool extract that formed the aqueous layer was removed to a fresh tube and stored at −80˚C,. The extraction was completed before freezing. Stool extracts of 200 µl were further purified with the QIAGEN QIAamp DNA Blood mini kit. Total nucleic acids were adsorbed from the spin columns using 50 µl of nuclease-free water. Extracted nucleic acids were stored at −80˚C. Stool samples were coextracted with a negative extraction control consisting of 200 µl of STAR buffer to which no stool had been added.

2.2. Molecular Techniques for Detection of Rotavirus and Adenovirus

Reverse Transcription RT-PCR and then electrophoresis were performed. Reverse Transcription was run on the final volume with the help of TaqMan reverse transcription kit (Applied Biosystems) on a GeneAmp 9700 thermocycler from Applied Biosystems following the methodology of Catriona Logan and her colleagues [4]. The reactions contained 1× RT buffer, 5.5 mM $MgCl_2$, 0.5 mM each deoxynucleoside triphosphate (dNTP), 2.5 µM random hexamer, 0.4 U/µl RNase inhibitor, 1.25 U/µl Multiscribe reverse transcriptase, and 17.5 µl of extracted nucleic acids. Extracted nucleic acids were collected from −80˚C storage and immediately added to

200-µl thin-walled PCR tubes containing the RT buffer, dNTPs, and $MgCl_2$. The reaction components were denatured by heating at 95˚C for 5 min and then quickly chilled on ice for 5 min. Random hexamer, RNase inhibitor, and Multiscribe reverse transcriptase were thereafter added to the mixture. Thermal cycling parameters for the RT reactions were as follows: 10 min at 25˚C, 30 min at 42˚C, 20 min at 48˚C, and 5 min at 95˚C. Completed RT reactions were stored at −20˚C.

2.3. Primers and Probes for RT-PCR

All primers and probes were imported from Applied Biosystems. RT reaction samples were analyzed by PCR, in duplicate wells for adenoviral and rotaviral targets on ABI 7000 sequence detector (Applied Biosystems) under universal temperature cycling conditions. RNase and DNase digestion of extracted stool samples were carried out separately to determine the source of nucleic acid responsible for PCR amplification, using RNase Cocktail and TURBO DNA-*free* (Ambion (Europe) Ltd at 37˚C for 30 min [4].

2.4. RT-PCR Amplification Reaction

Amplification carried on a GeneAmp PCR system 9700 (Applied Biosystems) under the initial DNA denaturation temperature 95˚C for 10 min; 45 amplification cycles with denaturation at 94˚C for 45 s, annealing at 50 to 55˚C for 45 s, and extension at 70˚C for 92 s; and a final incubation at 72˚C for 10 - 12 min [4].

2.5. Electrophoresis

Amplified PCR products were analyzed by electrophoresis on 2% ethidium bromide-stained agarose gels and viewed under Ultra-Violet illumination.

3. Results

In total, 87 samples (57.2%) were positive of rotavirus and 61 (40.1%) were adenovirus positive, only 4 samples (3%) were infected by other viral pathogens. Clinical laboratory data complementary information on one hand and demographic data on the other provided supplementary information regarding the measure of interest in hygiene standards and accessibility to potable source of water in affected communities. It was then derived that 84 patients (55.2%) use domestic water supplied conjointly by the dams of Dutsinma and Zobe located in the same Local Government area which is supplied to populations without treatment and 68 (44.8%) patients lived in remote villages with no drainage nor sewage network. The results of the investigation of rotavirus and adenovirus in stool samples of under five years VGE patients in Dutsinma Local Government Area of Katsina State in Nigeria are tabulated (**Table 1**).

4. Discussion

Our investigation depicted the rotavirus detection rate of 57.2% (87/152) of the samples while 61 samples 40.1% (61/152) were adenovirus positive. Viral gastroenteritis is an inflammation of the stomach and intestines [11] caused by one of any of the numerous viral pathogens which spreads through close contact [12] with carriers of the virus, or contaminated food or water [13]. In detailed studies, monthly surveillance has revealed highest frequency pick over a 2 year period between July and April with the 2 major pathogens of interest (rotavirus and adenovirus). Of 152 patients, 5.9% (9/152) were positive for rotavirus and 2% (3/152) with adenovirus for the month of April 2013. In April 2014 the frequency ascended to 7.2% (11/152) for rotavirus and 3.3% (5/152) with adenovirus. This may be assumably caused by drought severity in April that Dutsinma and Zobe dams serve both as drinking points for cattle and also supply sources for the communities, increasing viral proliferation among the populations benefiting from water poor supply structures available in Dutsinma Local Government [5]. In July 2013 the frequencies of rotavirus and adenovirus revealed 5.9% (9/152) and 2.6% (4/152) respectively, these data rose to 7.2% (11/152) for rotavirus and 4.6 % (7/152) with adenovirus in July 2014. These statistics might be justified by rain waters which fulfill vehicular functions for pathogens [5] [14] since most of these villages have no drainage nor sewage systems, the highest rain fall is observed in the savannah region of Nigeria in the months of July, August and September.

Table 1. Viral infections per month per village in Dutsinma Local Government Area, Katsina State, Nigeria.

Com		Karofi		Makera		Kuki		Shema		Tabawa		Wanruma		Dutsinma	
Yrs / Mths		2 × 13	2 × 14	2 × 13	2 × 14	2 × 13	2 × 14	2 × 13	2 × 14	2 × 13	2 × 14	2 × 13	2 × 14	2 × 13	2 × 14
Jan	Rota	1						1		1					1
	Adeno							1							1
Feb	Rota	1					1	1			1				1
	Adeno			1			1								
Mar	Rota	1			1				1						1
	Adeno			1			1		1			1			2
Apr	Rota	2	2		2	2			2		2	2		3	3
	Adeno	1		1		1	1		2			1		1	
May	Rota		1			1			1				1		1
	Adeno	1			1	1		1					1		1
Jun	Rota	1		1			1						1		1
	Adeno	1		1			1			1		1		1	
Jul	Rota	1	2	2	1	3	2	1		2	3		1		2
	Adeno	2		1		1	1	1	1	1	1		1	1	
Aug	Rota	1							1		1				1
	Adeno	1		1				1							
Sep	Rota			1		1		1	1			1			1
	Adeno	1			1				1						
Oct	Rota		1				1	1			1		1		1
	Adeno	1	1		1				1		1				2
Nov	Rota	1			1			1					1		1
	Adeno	1			1				1		1				1
Dec	Rota		1												
	Adeno				1		1								

Rota: rotavirus; Adeno: adenovirus; com: communities; Yrs: years; Mths: months.

5. Conclusion

These findings highlight the need for a better picture of the burden of children under 5 years VGE's diarrhea mortality and morbidity that will ultimately result in practical planning [13] for the prioritization of intervention policies for potable water supply to populations in low income settings in developing countries [15]. This can be achieved by setting up systems able to generate representative quality data on a regular basis using local co-variants from remote population of considered locations to restrict the spread of these pathogens.

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