Canadian Journal of Pure and Applied Sciences
Vol. 12, No. 1, pp. 4381-4394, Feb 2018
Online ISSN: 1920-3853; Print ISSN: 1715-9997
Available online at www.cjpas.net

ONE-STEP DETECTION OF CONCOMITANT INFECTIONS, AND EPIDEMIOLOGIC PROFILE OF *ROTAVIRUS, ADENOVIRUS, ASTROVIRUS* AND *NOROVIRUS* DIARRHEA IN NIGERIAN INFANTS: IMPLICATIONS FOR DIARRHEA BURDEN

Michael Oluyemi Babalola
Epidemiology, Molecular Virology and Special Pathogens Research
Department of Microbiology, Adekunle Ajasin University, PMB. 001, Akungba Akoko, Ondo State, Nigeria

ABSTRACT

The etiology of infective diarrhea is polymicrobial, but erroneously attributable to teething in infants and young children in the community. In a resource limited setting, where diarrhea illness significantly contributes to hospitalization and mortality of children, we investigated the rapid simultaneous detection and epidemiologic profile of the four major enteric viruses of diarrhea, for prompt diagnosis, appropriate care / treatment, and prelude to instituting timely interventions to mitigate the burden of viral diarrhea disease in developing countries. In an hospital-based cross sectional descriptive study, convenience sampling was adopted to obtain 175 stool specimens after parental consent, from under -5 years children who sought treatment for acute diarrhea disease from August 2012 to July 2014 at a Tertiary teaching Hospital in Ado Ekiti, Nigeria. The specimens were tested for concurrent detection of concomitant infection by the target viruses, using the CerTest® 4th generation quadruple Immuno chromatographic assay, and the data generated were analyzed quantitatively. At least one viral agent was detected in 103/175 (59%) respondents. Single infection occurred in 87/103 (84.5%) and co-infections in 16/103 (15.5%), comprising 14/16 (87.5%) respondents with dual, and 2/16 (12.5%) with triple infections. The most prevalent virus was *Rotavirus* with a rate of 16% (28/175), *Adenovirus* was 12.6% (22/175), *Astrovirus* was 8.6% (15/175), while *Norovirus* was 12.6% (22/175). The prevalent age at infection by *Rotavirus* and *Adenovirus* were in children aged 0-6 months with 50% and 18.8% respectively, followed by *Norovirus* (27.3%) in children aged 19-24 months, while *Astrovirus* and *Norovirus* were 21.4% respectively in children aged 25-30 months. Coinfection by *rotavirus* + *adenovirus* was 6.3% in children 0-6 months; *rotavirus* + *norovirus* was 9.1% in children aged 19 - 24 months; *adenovirus* + *norovirus* was 3.6% in 13 - 18 months; *astrovirus* + *norovirus* was 7.1% in 25 - 30 months; and *rotavirus* + *astrovirus* + *norovirus* was 9.1% in children aged 13 - 18 months. The four viruses showed seasonal cluster in the dry months of Nov 2012 and 2013, followed by each of *Rotavirus*, *Adenovirus* and *Norovirus* in the months of December 2012 - February 2013, and December 2013 - February 2014. *Rotavirus* and *Adenovirus* slightly occurred year round with monthly peaks in Jan, Feb, and April. *Astrovirus* was detected consistently in the months of August - November 2012, and 2013, respectively. About 60% of acute gastroenteritis in hospitalized under-5 children were caused by at least one of the four major etiologies of viral diarrhea. Co-infection of children by the enteric viruses was high. A paradigm shift in preponderance from rotavirus to adenovirus or astrovirus is anticipated in the future. The concomitant infection by these viruses have significant implications for the burden of diarrhea disease, treatment outcomes, development of cocktail of viral diarrhea vaccines, implementation of immunization, and monitoring the success of vaccination in the children.

Keywords: Ado Ekiti, co-infection, diarrhea, rota+adeno+astronoro, infants.

INTRODUCTION

Acute infective gastroenteritis manifesting majorly as abdominal cramps, watery diarrhea, and vomiting is among the most common infections of humankind and its associated morbidity and mortality are greatest among children and the elderly (Wilhelmi *et al*., 2003). Diarrhea was reported to be the largest killer after pneumonia and neonatal deaths, and among infants and children below five years of age, an estimated 2.4 to 3.3 million deaths occur annually (Bryce *et al*., 2005; Post *et al*., 2011), while diarrhea accounted for 9.9% of the global 6.9 million deaths among children aged below 5 years (Liu *et al*., 2012; Fischer-Walker *et al*., 2013) in the year 2011.

Microbially induced gastroenteritis can be caused by any of the over 20 known enteropathogens (Levine, 1987; Lanjewar *et al*., 1994; Niyogi, 2005) including the viruses

Corresponding author e-mail: michael.babalola@aaua.edu.ng

(Castello *et al*., 2006). Enteric viral pathogens are the prominent etiologies of acute infective gastroenteritis, of which the four major clinically relevant groups include Rotavirus (Castello *et al*., 2006), Calicivirus (Noroviruses and Sapovirus), the fastidious Enteric Adenovirus types 40 & 41, and Astrovirus (Walter and Mitchell, 2000). The less common etiologies include Picornavirus (the Aichi virus), Coronavirus, Pestivirus, and Enterovirus 22 (Glass *et al*., 2001; Parashar and Glass, 2003). Each child practically experiences viral diarrhea irrespective of race and social-economic status within the first 5 years of life and this has the great economic burden for the system of public health services and the society (Patel *et al*., 2008). Transmission of these viruses from infected to uninfected human is majorly by contaminated food/water via the fecal-oral route. The main symptoms of viral gastroenteritis are watery diarrhea, vomiting, headache, fever, abdominal cramps, general malaise, low grade fever, nausea, and fatigue, that begin 1 to 2 days following infection by any of the viruses and depending on the respective virus, may last for 1 to 10 days, and may also be asymptomatic, thus contributing to spread of the viruses in the community (CDC, 2011).

Literature has it, that 130 million children develop diarrhea due to rotavirus annually (Wilhelmi *et al*., 2001), and is responsible for 20% of diarrheal deaths, and 6% of all diarrheal episodes in children aged below 5 years (Armah *et al*., 2003). In the developing countries, it was estimated that 500,000-870,000 deaths occur annually because of severe dehydrating diarrhea caused by human rotaviruses with 150,000-200,000 deaths occurring in Africa (Steele and Ivanoff, 2003; Tate *et al*., 2008). Rotavirus was discovered by Mebus *et al*. (1969) among Nebraska calves, and the wheel-like morphology was first described in humans by Bishop *et al*. (1973), when electron microscopy images reveal its presence in duodenal biopsies of children with acute gastroenteritis. On the basis of serological and genetic features, the genus Rotavirus of the Reoviridae virus family comprises eight approved groups A to H, and one candidate species designate *Rotavirus I* (Matthijnssens *et al*., 2012; Estes and Greenberg, 2012; Mihalov-Kovács *et al*., 2015). Among these, group A Rotavirus (ICTVdb No. 00.060.0.03.001) is recognized as the most common etiologic agent of severe dehydrating diarrhea in infants and young children in both developed and developing countries (Asmah *et al*., 2001). The Rotavirion (Fig. 1) consists of a triple-layered protein capsid of 100 nm in diameter, surrounding a genome of 11 segments of double-stranded RNA which encode 6 structural (VP1-VP4 and VP6, VP7) and 6 non-structural (NSP1-NSP6) proteins (Greenberg and Estes, 2009). The VP7 and VP4 gene segments code for two outer capsid proteins, a glycoprotein (G) and a protease - sensitive protein (P) that define the genotypes/serotypes and form the basis for classifying group A rotaviruses into G and P types (Estes and Kapikian, 2007).

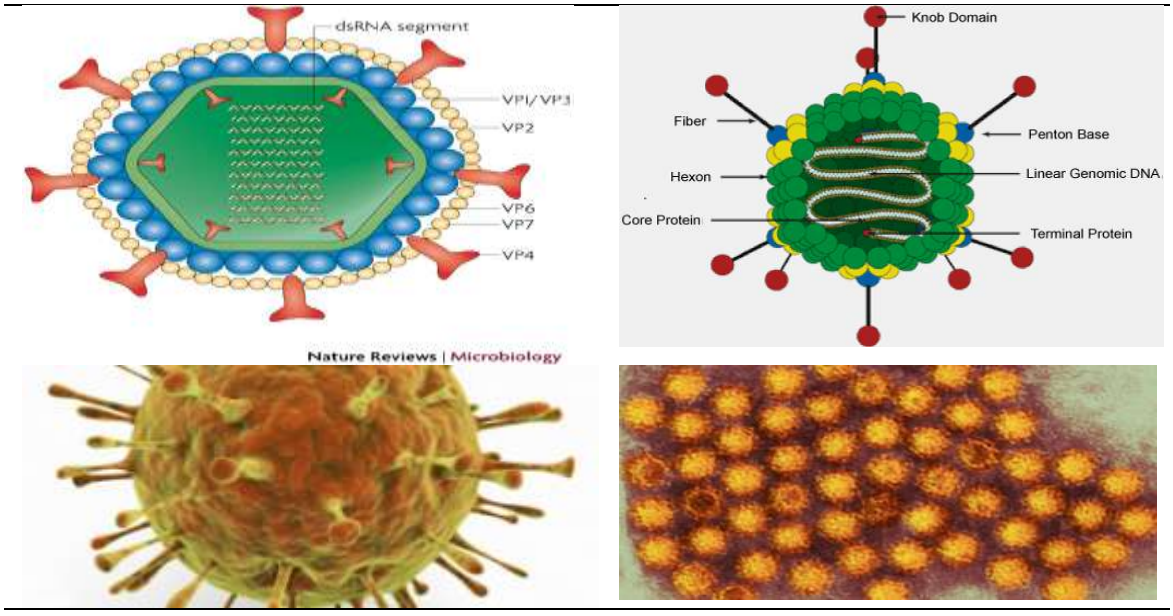

Fig. 1**.** (clockwise): Schematic diagram of the virion morphologies of Rotavirus, Adenovirus, Astrovirus and electron micrograph of Norovirus.

Human adenoviruses (HAdV) are major causes of clinical infections including respiratory diseases, gastroenteritis, and conjunctivitis (Avery *et al*., 1992; Van Heerden *et al*., 2003). Human *Adenovirus* belongs to the genus Mastadenovirus of the family Adenoviridae. The virion consists of non-enveloped, linear, dsDNA genome (26 -45

kb) encapsidated in an icosahedral protein shell (Fig. 1). There are 52 serotypes classified into six species, A-G (Sdiri- Loulizi *et al*., 2008; Walsh *et al*., 2009) of which species F serotypes 40 and 41 primarily infect the gut, contributing to 5%-20% of hospitalizations for childhood diarrhea (Girard *et al*., 2006). The incidence of infection was reported to be nearly 3 times greater in developing countries than developed ones (Mohammad *et al*., 2013).

In preceding studies to date in Nigeria, Nimzig *et al*. (2000) indicated a prevalence of 3.8% among diarrheic children in Jos, Audu *et al*. (2002) reported a prevalence of 3% among diarrheic under-5 years children in Lagos, Aminu *et al*. (2007) reported a rate of 8% among diarrheic children in Northwestern Nigeria, while Babalola *et al*. (2015) reported a rate of 18% among under-5 children in Owo, Southwest Nigeria. Astroviruses are non-enveloped viruses in the family Astroviridae, detected from the fecal samples of symptomatic and asymptomatic infantile diarrhea cases, and named by Madeley and Cosgrove (1975). The virion is 28–35nm in diameter with an icosahedral capsid enclosing a positive sense, single stranded RNA genome. Under the electron microscope, the capsid have a characteristic star–like morphology formed by five or six pointed surface structure from which it derived the Greek word "astron" (Fig. 1). Astroviridae of the genus Mamastro viruses infect mammals, while genus Avastro viruses infect and causes gastroenteritis in avian species.

There are at least eight human serotypes (HAstV-1 to HastV-8) while the prototype species is HAstV-1 (Walter and Mitchell, 2003; Fauquet *et al*., 2005). Astroviruses infect human of all ages but children up to seven years of age, the elderly and the immuno compromised are the most susceptible (Moser and Schultz-Cherry, 2005) accounting for about 2%-16% of diarrhea in hospitalized children, and 5%- 17% of diarrhea in the community (Hermann *et al*., 1991). Among the few studies in Nigeria, a prevalence of 16% was reported among children in Nassarawa state (Kuta *et al*., 2014), while Ayolabi *et al*. (2012) reported 40.4% in Lagos.

Norovirus prototype strain was first identified as the cause of a gastroenteritis outbreak in Norwalk, Ohio, in 1968, hence the common name, ''Norwalk-like virus'' (Adler and Zickl, 1969). Noroviruses belong to the family Caliciviridae, classified into five genogroups (GI through GV), which were further divided into at least 34 genotypes. Genotypes GI, GII and GIV infect and cause gastroenteritis in humans, while the GIII and GV typically infect animals. It is a small (Fig. 1), non-enveloped, positive sense, single stranded RNA virus that requires special cell systems for *in vitro* replication (Phillips *et al*., 2010). Norovirus gastroenteritis is characterized by vomiting (in 64% of adults, and 81% of children) with a seasonal pattern of increased infection rates during the winter (Najafi *et al*., 2012), hence the acronym ''winter vomiting disease''. Previous studies have reported noroviruses as the second most frequent etiologic agents of viral gastroenteritis in children (van Maarseveen *et al*., 2010; Koopmans *et al*., 2003), causing annual global mortality of 200,000 children in developing countries (Koopmans, 2008; Jakab *et al*., 2005).

Studies have indicated that immuno suppressed patients as a result of infection or development, constitute an important group for prevention of gastrointestinal infections, and the incidence of infection- related post-transplant viral diarrhea were reported to be up to 40% (Aggarwal *et al*., 1998; Liakopoulou *et al*., 2005). Hence, considering the importance of gastroenteritis prevention in immuno compromised individuals, the diagnosis of the etiology at the early stages of the disease is extremely important to reduce morbidity and mortality (Ribeiro *et al*., 2015). Interestingly, in developing countries like Nigeria where rotavirus and other enteric viral infections, morbidity, and mortality are high, no single study have provided a concurrent diagnosis, and the prevalence of these four important clinically relevant enteric viruses for which vaccines have not been successfully developed or implemented. This study was conducted to simultaneously detect these viruses, to determine the baseline prevalence, and map the pattern of infection by *rotavirus, Adenovirus, Astrovirus* and *Noroviruses* in real time, among under -5 children hospitalized of acute diarrhea disease in Ado Ekiti, Nigeria.

MATERIALS AND METHODS

Study Design

An hospital based, prospective, cross-sectional, descriptive study, aimed at detecting and determining the prevalence of group-A Rotavirus, Adenovirus, Astrovirus, and Norovirus, or co-infections of these viruses in cases of diarrhea among under-5 year children hospitalized of acute gastroenteritis in Ado Ekiti, Nigeria.

Study Centre

This study was conducted at the Neonatal Intensive Care Unit and the Children Emergency Wards of the Ekiti state University Teaching Hospital Ado Ekiti. The centre was selected on the basis of availability of the specified population, ability of the facility to handle severe disease and those that needed admission.

Study Population

Infants and young children aged between 0 and 5 years who presented at outpatient clinics or were admitted in wards, at the specified hospital for acute diarrheal illness, from August 2012 to July 2014 were the study population.

Inclusion Criteria

Children below 5 years of age that presented with acute diarrhea, having experienced an evacuation of loose watery stools, 3 or more times in a 24-hour period as reported by the parents or caregiver were enrolled in this study. Only the children with diarrhea that lasted for ≤ 9 days were included since studies have shown that the incubation period for most enteric virus illness is less than 48hrs and usually will last for 5-9 days (WHO, 2002).

Exclusion Criteria

Children that were more than 5 years of age, or with bloody diarrhea, or prospective participants whose parents declined consent were regarded as ineligible and excluded from the study.

Ethical considerations

This study devolved from approval by the Ethical Committee of the Ekiti State University Teaching Hospital, after thorough review of the application and proposals. Verbal Informed consent was obtained from the parent or guardian of the participants before sample collection.

Demographics and Clinical Factors

At the time of recruitment and admission of the participants to the hospital, the severity of diarrhea and demographics were determined. The parents were interviewed using a structured questionnaire to obtain demographic information and clinical factors that were associated with diarrhea. These included parents education/employment, the participants' age (months), the gender, sources of drinking water, feeding practices, duration of diarrhea before hospitalization, type of diarrhea or stool characteristics, frequency of diarrhea, severity of diarrhea such as vomiting, dehydration requiring Intravenous rehydration, increase in body temperature, antibiotic administration, and manifestation of Upper Respiratory Tract Infection (URTI).

Collection and Processing of Samples

By convenience sampling, stool specimens were collected by visiting the hospital 2 to 3 times a week, from under-5 children presenting in the hospital as outpatient or admitted for acute diarrheal illness one to 3 days after the onset of the disease. At each of the health facility, the children who satisfied the study inclusion criteria were asked to produce stool specimen by natural evacuation after obtaining consent from their parents / guardian. In one diarrhea episode, one fresh stool specimen was collected per child in clean, screw-capped tubes, labeled, and immediately stored frozen, prior to transportation on ice to the Virology department, University of Ibadan Nigeria, for further analysis.

Detection of Rotavirus, Adenovirus, Astrovirus and Norovirus by Lateral- flow EIA

The 4th Generation Certest® Rota+Adeno+ Astro+Noro / Combo cassette (CerTest® Biotech, Spain) is based on the principle of a qualitative immuno chromatographic assay for the determination of Rotavirus, Adenovirus, Astrovirus and Norovirus genogroups I and II, in stool samples. The stool specimens were tested for the presence of any of *rotavirus*, *adenovirus*, *astrovirus* and *norovirus*, or combinations of the viral antigens using the Combo cassette according to Certest Biotech instructions (CERTEST Biotech, 2013) and methods of Babalola *et al*. (2015).

Presentation of Data and Statistical Analysis

Data obtained in the study were recorded in Microsoft Excel, and analyzed using descriptive statistics as shown in percentages, mean, median and ratios. Inferential analysis and Infection proportions were tested for statistical significance by the use of chi-square (χ^2) test and used to compare groups. Differences were considered statistically significant if $P \leq .05$.

RESULTS

Demograhics of rotavirus, adenovirus, astrovirus and norovirus diarrhea among under -5 years children in Ado Ekiti, Nigeria

One hundred and seventy five (175) stool samples were collected from eligible children aged less than five years who were enrolled in this study. Ninety five (95/175:54%) respondents were Males while 80 (80/175:46%) were females, representing a male: female ratio of 1.2:1 (Table 2).

Serological detection of rotavirus, adenovirus, astrovirus and norovirus pathogens.

In a qualitative one-step detection of the presence of the four viral agents of diarrhea in all the 175 samples using the rapid Enzyme Immunochromatographic Combo cassette (CERTEST Biotech, 2013), there were distinct red line in the test windows and/or green lines in the control window indicating optimal performance of the assay, although the intensity of the color was directly proportional to the viral load in the respective samples tested (Fig. 2). The performance of the kit were validated to share no cross reactivity with other gastrointestinal pathogens found in feces, as well as 99% sensitive and specific, respectively (CERTEST Biotech, 2013).

Fig. 2a: Rotavirus.

Fig. 2b: Rotavirus.

Fig. 2c: Adenovirus.

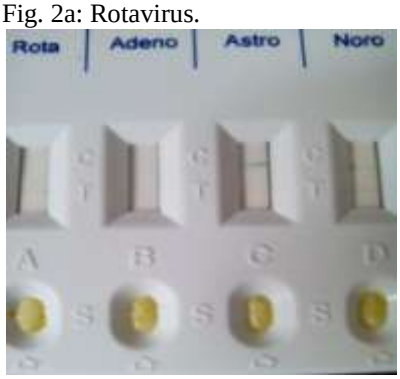

Fig. 2d: Rotavirus + Norovirus.


Fig. 2e: Adenovirus + Norovirus.

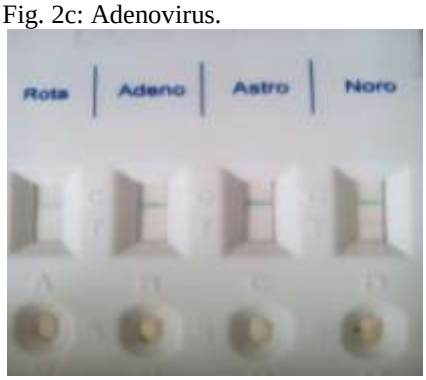

Fig. 2f: Norovirus.

Fig. 2. Representative results of Viral Assay of the fecal samples using the Certest® Combo cassette.

Overall prevalence of the enteric viruses in children with acute gastroenteritis

Of all the 175 fecal samples tested, at least one viral agent was detected in 103/ 175 (59%) of the respondents. Single infection occurred in 87/103 (84.5%) and co-infections in 16/103 (15.5%), including 14/16 (87.5%) respondents with dual, and 2/16 (12.5%) with triple infections.

No quadruple infection was detected in the study respondents. The most prevalent etiology was *Rotavirus* with a rate of 16% (28/175) and a male: female ratio of 1:1.3; *Adenovirus* was 12.6% (22/175) with a male: female ratio of 1:1; *Astrovirus* was 8.6% (15/175) with a male: female ratio of 1:1.2; while *Norovirus* was 12.6% (22/175) with a male: female ratio of 1:1.2.

Generally, coinfection (Fig. 2) by *Rotavirus* +*Adenovirus* was detected in 5/175 (2.9%); *Rotavirus* + *Norovirus* was found in 3/175 (1.7%); Adenovirus + *Norovirus* in 3/175 (1.7%); *Astrovirus* + *Norovirus* in 3/175 (1.7%); while *Rotavirus* + *Astrovirus* + *Norovirus* were detected in 2/175 (1.1%) of the study respondents (Table 1).

Table 1. Occurence of enteric viral etiologies of diarrhea among under-5 children in Ado Ekiti, Nigeria.

		Type of infection								
		Monoinfection (%)				**Coinfection (%)**				
Age group (months)	**Total sample tested**	***Rotavirus* positive**	***Adenovirus* positive**	***Astrovirus* positive**	***Norovirus* positive**	**Rota+ Adeno**	**Rota +Noro**	**Adeno + Noro**	**Astro+ Noro**	**Rota +Astro +Noro**
0-6	16	8 (50)	3 (18.8)	00 (00)	2 (12.5)	1(6.3)	00(00)	00(00)	00(00)	00(00)
7-12	64	11(17.2)	7 (11)	4 (6.3)	5 (7.8)	2 (3.1)	00(00)	1 (1.6)	2 (3.1)	00(00)
13-18	56	7 (12.5)	8 (14.3)	4 (7.1)	6 (10.7)	1 (1.8)	1(1.8)	2 (3.6)	00(00)	2 (9.1)
19-24	22	1(4.5)	4 (18.2)	4 (18.2)	6 (27.3)	1 (4.5)	2 (9.1)	00(00)	00(00)	00(00)
25-30	14	1(7.1)	00 (00)	3 (21.4)	3 (21.4)	00(00)	00(00)	00(00)	1 (7.1)	00(00)
31-36	02	00	00	00	00	00	00	00	00	00
>36	01	00	00	00	00	00	00	00	00	00
Total	175	28 (16)	22 (12.6)	15 (8.6)	22(12.6)	5 (2.9)	3 (1.7)	3 (1.7)	3 (1.7)	2 (1.1)

At least one viral agent was detected in 103/ 175 (59%) of the respondents. Single infection occurred in 87/103 (84.5%) and co-infections in 16/103 (15.5%), comprising 14/16 (87.5%) respondents with dual, and 2/16 (12.5%) with triple infections.

Monthly distribution of samples collected and detection rates of the viruses

Figure 3 shows the monthly distribution of the samples and the detection rates of each viral agent. The highest monthly rates of samples collected were in August, October, November, and December, 2012, as well as March and October, 2013. The monthly rates remained constant for the months of April to September 2013, increased in October and fell to a constant rate from Feb to May 2014.

All the four viruses comprising the *Rotavirus*, *Adenovirus*, *Astrovirus*, and *Norovirus*, clustered in the month of Nov 2012 and 2013 respectively, followed by *Rotavirus*, *Adenovirus* and *Norovirus* in the months of December 2012 to February 2013, and December 2013 to February 2014 respectively.

Rotavirus and *Adenovirus* slightly occurred year round with monthly peaks in Jan, Feb, and April. *Rotavirus* was detected consistently in the months of October 2012 to June 2013, and October 2013 to June 2014, while *Adenovirus* was detected consistently in the months of Nov. 2012 to June 2013, and Nov. 2013 to June 2014 respectively.

Astrovirus was detected consistently in the months of August to November 2012, and 2013 respectively, while *Norovirus* was found consistently in the months of September 2012 to Feb. 2013, and from October 2013 to Feb 2014.

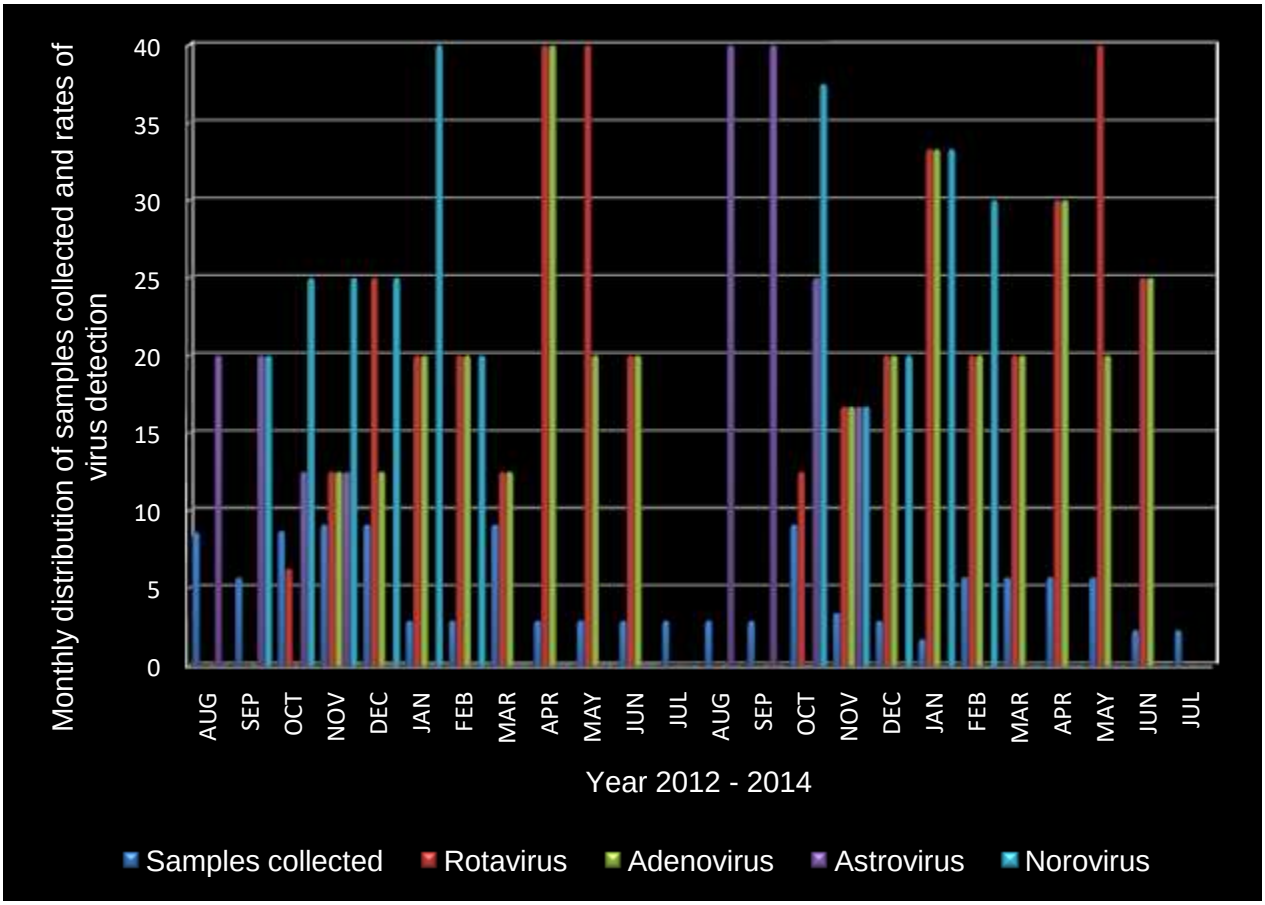

Fig. 3. Monthly distribution of samples collected and detection of *rotavirus, adenovirus, astrovirus* and *norovirus* pathogens among under 5 years children hospitalized of acute gastroenteritis from August 2012 to July 2014 at a Teaching Hospital in Nigeria.

Detection rates by Gender, and Age group Prevalence of monoinfection by Rotavirus, Adenovirus, Astrovirus, and Norovirus.

Monoinfection by *Rotavirus* was 16% (28/175) of which 6.3% were males and 9.7% were females. *Adenovirus* was 12.6% (22/175) where 6.3% were males and females respectively. *Astrovirus* was 8.6% (15/175) of which 4% were males and 4.6% were females, while *Norovirus* was 12.6% (22/175) of which 5.7% were males and 6.9% were females (Fig. 4).

The prevalent age at infection by *Rotavirus* (50%) and Adenovirus (18.8%) were in children aged 0-6 months, followed by *Norovirus* (27.3%) in children aged 19-24 months, while in children aged 25-30 months, *Astrovirus* and *Norovirus* were 21.4% respectively.

Coinfection by *rotavirus* + *adenovirus* was 6.3% in children 0 - 6 months; *rotavirus* + *norovirus* was 9.1% in children aged 19 - 24 months; *adenovirus* + *norovirus* (3.6%) in 13 - 18 months; *astrovirus* + *norovirus* (7.1%) in 25 - 30 months; and *rotavirus* + *astrovirus* + *norovirus* was 9.1% in children aged 13 - 18 months (Table 2).

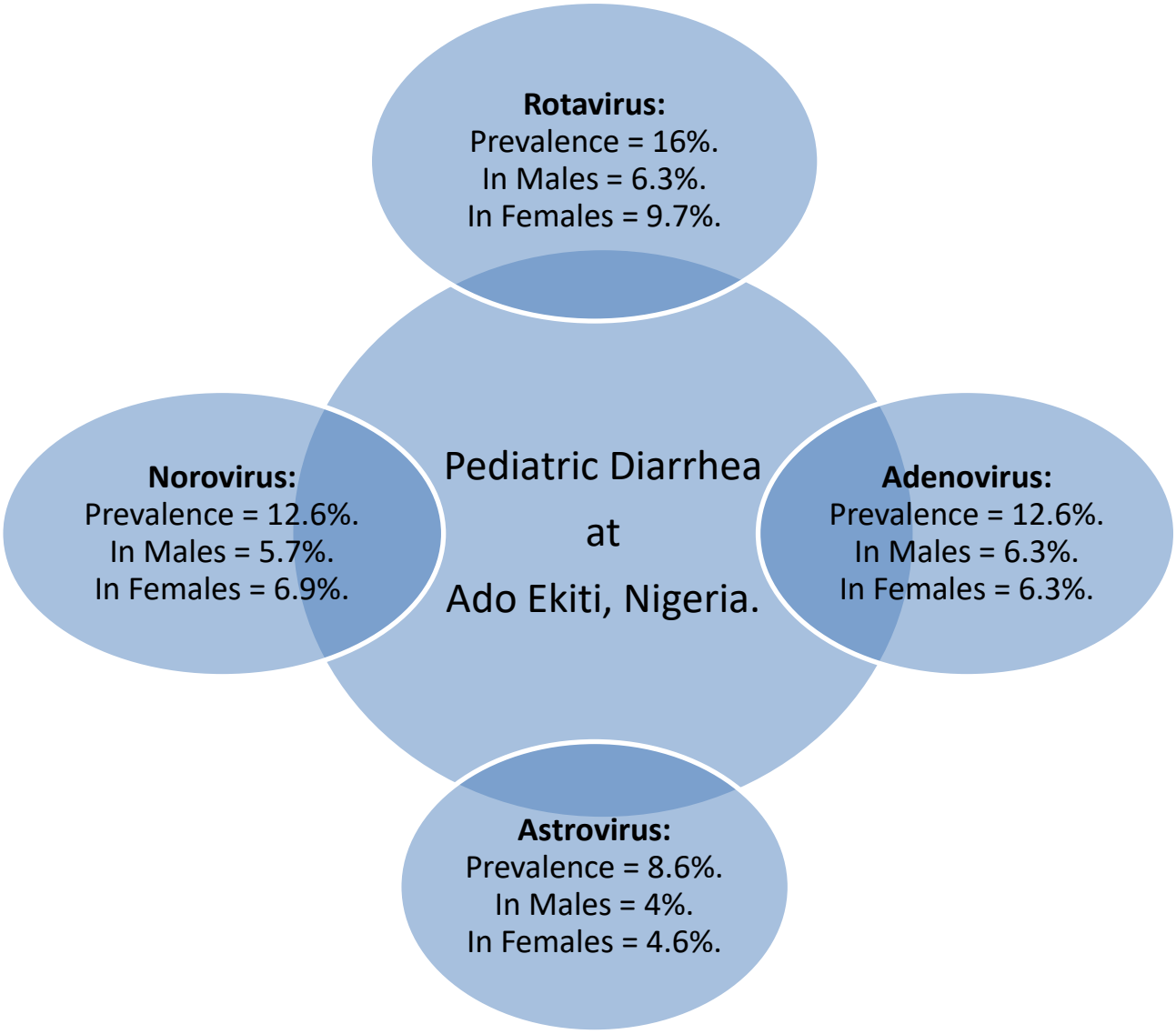

Fig. 4. Venn diagram showing the major viral etiologies, and Epidemiologic profile of Pediatric diarrhea among children hospitalized of acute gastroenteritis from August 2012 to July 2014 in Ado Ekiti Nigeria.

Table 2. Detection rates by Gender, and Age group prevalence of monoinfection by *Rotavirus*, *Adenovirus*, *Astrovirus and Norovirus* pathogens among children hospitalized of acute diarrhea from August 2012 to July 2014 in Ado Ekiti, Nigeria.

Age group (months)	***Rotavirus* (%)**		***Adenovirus* (%)**		***Astrovirus* (%)**		***Norovirus* (%)**	
	Number positive in Males	**Number positive in females**	**Number positive in Males**	**Number positive in females**	**Number positive in Males**	**Number positive in females**	**Number positive in Males**	**Number positive in females**
0-6	03/10 (30)	05/6 (83.3)	02/10 (20)	01/6 (16.7)	00/10 (00)	00/6 (00)	01/10 (10)	01/6 (16.7)
Prevalence	(50)		(18.8)		(6.3)		(12.5)	
7-12	05/36 (13.9)	06/28 (21.4)	03/36 (8.3)	04/28 (14.3)	01/36 (2.8)	03/28 (10.7)	02/36 (5.6)	03/28 (10.7)
Prevalence	(17.2)		(11)		(6.3)		(7.8)	
13-18	03/28 (10.7)	04/28 (14.3)	04/28 (14.3)	04/28 (14.3)	02/28 (7.1)	02/28 (7.1)	03/28 (10.7)	03/28 (10.7)
Prevalence	(12.5)		(14.3)		(7.1)		(10.7)	
19-24	00/14 (00)	01/8 (12.5)	02/14 (14.3)	02/8 (25)	02/14 (14.3)	02/8 (28)	03/14 (21.4)	03/8 (37.5)
Prevalence	(4.6)		(18.2)		(18.2)		(27.3)	
25-30	00/6 (00)	01/8 (12.5)	00/6 (00)	00/8 (00)	02/6 (33.3)	01/8 (12.5)	01/6 (16.7)	02/8 (28)
Prevalence	(7.1)		(00)		(21.4)		(21.4)	
31-36	00/1 (00)	00/1 (00)	00/1 (00)	00/1 (00)	00/1 (00)	00/1 (00)	00/1 (00)	00/1 (00)
Prevalence	(00)		(00)		(00)		(00)	
>36	00	00/1 (00)	00	00/1 (00)	00	00/1 (00)	00	00/1 (00)
Prevalence	(00)		(00)		(00)		(00)	
TOTAL	**11/95 (11.6)**	**17/80 (21.3)**	**11/95 (11.6)**	**11/80 (13.8)**	**7/95 (7.4)**	**8/80 (10)**	**10/95 (10.5)**	**12/80 (15)**

Seasonal association of monoinfection by rotavirus, adenovirus, astrovirus and norovirus among the study participants from August 2012 to July 2014 in Ado Ekiti Nigeria.

All the four viruses comprising the *Rotavirus*, *Adenovirus*, *Astrovirus*, and *Norovirus*, showed seasonal cluster in the dry season of November 2012 and 2013, followed by each of *Rotavirus*, *Adenovirus* and *Norovirus* in the dry months of December 2012 to February 2013, and December 2013 to February 2014. *Rotavirus* and *Adenovirus* slightly occurred year round with monthly peaks in Jan, Feb and April. *Rotavirus* was detected consistently in the months of October 2012 to June 2013, and October 2013 to June 2014, while *Adenovirus* was detected consistently in the months of Nov 2012 to June 2013, and Nov. 2013 to June 2014. *Astrovirus* was detected consistently in the months of August to November 2012, and August to November 2013. *Norovirus* was found consistently in the dry season of September 2012 to Feb. 2013, and October 2013 to Feb. 2014 (Fig. 3). None of the viruses was detected in the months of July in the 2 year period.

DISCUSSION

The etiology of a large proportion of diarrhea cases remain unknown as it was estimated that no causative agent can be identified in up to 40% of sporadic cases or in gastroenteritis outbreaks. However, the detection of novel or unexpected viruses is the first step in identifying the chemotherapy or prophylactics that could potentially close the diagnostic gap and pave the way for the development of more comprehensive preventive measures, and better treatments. The laboratory investigations of the major enteric viruses are traditionally laborious, technical, and time consuming for the needed rapid point of care diagnosis in developing countries. However, the ultimate goal of research is to generate translational findings for improved benefit to mankind particularly in the area of healthcare. Recently, Muhsen and Levine posited that some viruses, such as adenovirus have not been completely documented as a cause of severe diarrhea in developing countries (Muhsen and Levine, 2012). This may be attributed to the laborious methods and paucity of available technologies in investigating the enteric adenovirus serotypes. Many workers perceive the fastidious adenoviruses as a

formidable area of research and are therefore fast to neglect or conclude their non-association with diarrhea. However, the advent of rapid methods, with sufficient evidence in this study, in- tandem with the previous report of Babalola *et al*. (2015), Adenoviruses and astroviruses are becoming established etiologies of severe diarrhea in infants and young children of developing countries like Nigeria. Therefore, considering the sensitivity and specificity of the 4th generation rapid immunochromatographic kit, it was handy, reliable, economical, and amenable for use in the prompt diagnosis of the four enteric viral agents of diarrhea, to forestall the common empirical treatment with several antibiotics, antibiotic resistance, treatment failures, increased cost of healthcare, and mortality. Interestingly, this is the first single report in Nigeria and the second in Africa that focused concurrent investigation in real-time on the occurrence and prevalence of these four etiologies of viral diarrhea in the African children. At least one viral agent was detected in 59% of the respondents indicating a higher rate compared to 32.2% in a similar investigation in Tanzania (Moyo *et al*., 2007).

The results showed single infection in 87/103 (84.5%) and co-infections in 16/103 (15.5%), comprising 14/16 (87.5%) participants with dual, and 2/16 (12.5%) with triple infections. No quadruple infection in a single child was detected in the participants as previously suggested (CERTEST Biotech, 2013). Similar single studies in developed countries have reported various levels of prevalence of these enteric viruses.

In a London report by Cubitt *et al*. (1996), 28% of children with gastroenteritis were infected by rotavirus, 6% adenovirus, 3% astrovirus, and 3% calicivirus. Bon (1999) submitted that group-A rotavirus was 61%, calicivirus was 14%, astrovirus was 6%, while enteric adenovirus was 3% in children that sought treatment for gastroenteritis between 1995 and 1998 in France. Lee *et al*. (2007) reported that in Korean Children, rotavirus, norovirus, adenovirus and astrovirus were detected in 25.7%, 13.7%, 3.0%, and 1.1%, respectively.

In the African continent, Mona *et al*. (2013) found in Egypt that Rotavirus, norovirus, and adenovirus were 39%, 16.2% and 6.8% respectively, of the children with gastroenteritis. In this study, *Rotavirus, Adenovirus, Astrovirus,* and *Norovirus* were 16%, 12.6%, 8.6%, and 12.6% respectively, indicating unexpectedly lower rotavirus rate atypical of a developing country, but higher prevalence in Adenovirus and Astrovirus infections typical of the postulations of Mohammad (2013) in this prevaccine era. The ratings of these viruses as agents of gastroenteritis on the basis of incidence differ from worker to worker, and region to region. While Zlateva *et al*. (2005) claimed that Adenoviruses are second only to rotavirus as the most important causative agent of acute infantile gastroenteritis, Aminu *et al*. (2008) identified Astroviruses as one of the most frequent causes of infantile gastroenteritis, second in incidence only to rotavirus in Northern Nigeria (Aminu *et al*., 2008). Contrary to the foregoings, Iritani *et al*. (2003) in Japan, and Junquera *et al*. (2009) in Spain, reported in separate studies that noroviruses were the second most frequent etiologic agents of viral gastroenteritis in children. However, it is interesting to emphasize that in this present study, monoinfection by Adenovirus, or by norovirus were detected at similar rates (12.6%) among the respondents in Nigeria.

Rotavirus generally occurred in children aged 0-30 months with an overall prevalence of 16%, predominated in children aged 0—6 months with an age group prevalence of 50% and a significant higher rate in the females than the males. This rate contrasts the previous report of 25.8% reported by (Ayolabi *et al*., 2013) in Lagos, comparable to the 19.2% in Benin City Nigeria (Iyoha and Abiodun, 2015), but higher than the 13.8% in females of Jos Northcentral Nigeria (Junaid *et al*., 2011), and the 14.3% reported by Adah *et al*. (1997).

As no gender specific mutation is known regarding rotavirus infection, the observed difference in Jos may be attributed to socioeconomic dispositions, and differential cultural preferences for male children in the two geographical regions. Outside Nigeria, the observed rate in this study was lower than 25.5% reported in Ghana (Armah *et al*., 2002), 27.9% in Abidjan (Akoua-Koffi *et al*., 2007), and 34.3% in China (Lintao *et al*., 2013). Consistent with the recent report of Babalola *et al*. (2015), the seasonality of adenovirus (November to June), and norovirus (September to February) were similar, but the rate of adenovirus diarrhea (12.6%) was lower than the 18% previously reported, while norovirus was higher (12.6%) in Ekiti state than the neighboring Ondo state (8%), but lower than the 25.5% reported in Osun state (Japhet *et al*., 2012) and the 37.3% (Ayolabi *et al*., 2010) in Lagos state Nigeria.

In the Nigerian scientific ecosystem, this is the third report in the Southwest, of astrovirus diarrhea occurring at a prevalence of 8.6%. This rate is higher than 5% in the Northwest (Aminu *et al*., 2008), similar to 8.6% in Niger state (Kuta *et al*., 2014b) and lower than the 16% in Nassarawa, Northcentral Nigeria (Kuta *et al*., 2014a), and the reported 40.4% in Lagos (Ayolabi *et al*., 2012). The detection of astrovirus in this study corroborates the submission of Finkbeiner (Finkbeiner, 2009) that astroviruses may cause a large fraction of severe diarrhea cases than recognized, consequent upon his identification of two novel astroviruses Astv-MLB1 and Astv-VA1, confirmed with a further discovery of a variant Astv-

VA1/NMO-C responsible for severe encephalitis in an 18 months infant in the UK (Brown *et al*., 2015). The manifestation of similar sequelae is highly probable in the susceptible respondents in this study.

The observed rate of coinfection by the viruses is low but certainly considerable, regarding their contribution to the duration, pathogenesis, and clinical outcome of diarrhea. The simultaneous presence of rotavirus + adenovirus was 6.3% in children 0 - 6 months, rotavirus + norovirus was 9.1% in children aged 19 - 24, adenovirus + norovirus (3.6%) in 13 - 18 months, astrovirus + norovirus (7.1%) in 25 - 30 months, and rotavirus + astrovirus + norovirus was 9.1% in children aged 13 - 18 months. As the pathogens involved in a co-infection can interact synergistically, for example via the host's immune system, with the presence of one enhancing the abundance and/or virulence of the other, resulting in even greater pathogenesis (Griffiths *et al*., 2011), the increasing detection of more than one enteric virus in a single respondent calls for concern.

The interspecific pathogen interactions can alter the pathogen dynamics, host health, and the success or failure of control strategies (Lello *et al*., 2008), particularly vaccination. Therefore, given the rudimentary immune status of the respondents and the probable malnutrition in children, it is hereby postulated in tandem with the submission of Bhavnani *et al*. (2012), that the observed synergistic effects of coinfection contributed to the severity of the disease, clinical outcome of the infection, and the overall diarrhea disease burden.

CONCLUSION

With the current knowledge on the etiologies of viral diarrhea diseases, this is the first real-time concurrent investigation of the four viral diarrhea pathogens in Nigeria. This study demonstrated that close to 60% of acute gastroenteritis in hospitalized under-5 children were caused by at least one of the four major etiologies of viral diarrhea, and should therefore be put into perspective by caregivers during admission of children for treatment of diarrhea. Co-infection of the children by enteric viruses is highlighted and the predominance of these viruses has significant implications for the burden of diarrhea disease, treatment outcomes, development of cocktail of vaccines, and implementation of intervention measures in the Nigerian children.

ACKNOWLEDGEMENT

I gratefully acknowledge the supports and contributions of Prof. O. D Olaleye and Prof. (Mrs.) Georgina Odaibo of the Department of Virology, College of Medicine, University of Ibadan, Nigeria. All the children who participated in this study, and the parents who consented to the study are highly appreciated. Deep gratitude to the Hospital Management for the study approval, as we acknowledge the use of the Pediatric laboratory facilitated by Dr. I.O Oluwayemi, Dr. Ogundare, Dr. Mohammed, Mrs. Olaolorun, Mrs. Bukola Olakanmi-Falade and other Nurses at the Children Emergency Ward of the Ekiti state University Teaching Hospital, Ado Ekiti Nigeria.

REFERENCES

Adah, MI., Rohwedder, A., Olaleye, OD. and Werchau, H. 1997. Nigerian rotavirus serotypes could not be typed by PCR due to nucleotide mutation at the 3' end of the primer binding site. Archives of Virology. 142:1881-1887.

Adler, JL. and Zickl, R.. 1969. Winter vomiting disease. J Infect Dis. 119: 668-673.

Aggarwal, V., Williams, MD. and Beath, SV. 1998. Gastrointestinal problems in the immunosuppressed patient. Arch Dis Child. 78: 5-8 [PMID; 9534668 DOI: 10.1136'adc.78.1.5].

Akoua-Koffi, CV, Akran, I., Peenze *et al*. 2007. Epidemiological and virological aspects Rotavirus diarrhoea in Abidjan, Cˆoted'Ivoire (1997–2000). Bulletin de la Societe de Pathologie Exotique. 100 (4): 246-249.

Aminu, M., Ahmad, AA., Umoh, JU., de Beer, MC., Esona, MD. and Steele, AD. 2007. Adenovirus Infection in Children with Diarrhea Disease in Northwestern Nigeria. Ann African Med. 6 (4):168 -173.

Aminu, M., Esona, MD., Geyer, A. and Steele AD. 2008. Epidemiology of Rotavirus and Astrovirus Infections in Children in Northwestern Nigeria. Annals of African Medicine. 7(4):168-174.

Armah, GE., Essel, EK., Asmah, RH., Anto FR. and Binka, FN. 2002. Detection of human group C rotavirus in Ghanaian children. Ghana Medical Journal. 34:3-8.

Armah, RH., Green, J., Armah, GE., Gallimore, CI., Gray, JJ., Iturriza-Gomara M., Anto, F. and O'Duro, A. 2003. Changing patterns of rotavirus genotypes in Ghana: Emergence of human rotavirus G9 as a major cause of diarrhea in children. J. Clin. Microbiol. 41:2317-2322.

Asmah, RH., Jon, G., Armah, GE., Gallimore, CI., Gray, JJ., Iturriza-Gómara, M., Anto, F., Oduro, A., Binka, NF., Brown, DWG. and Cutts F. 2001. Rotavirus G and P Genotypes in Rural Ghana. Journal of Clinical Microbiology. 39 (5):1981-1984.

Audu, R., Omilabu, AS., Peenze, I. and Steele, AD. 2002. Isolation and identification of adenovirus recovered from the stool of children with diarrhea in Lagos, Nigeria. Afr J Health Sci. 9 (1-2):105-111.

Avery, RM., Shelton, A.P, Beards, GM., Omotade, OO., Oyejide, OC. and Olaleye, DO. 1992. Viral agents associated with infantile gastroenteritis in Nigeria: relative prevalence of adenovirus serotypes 40 and 41, astrovirus, and rotavirus serotypes 1 to 4. J. Diarrhoeal Dis Res. 10 (2):105-108.

Ayolabi, CI,, Ojo, DA., Armah, GE., Akpan, I. and Mafiana, CF. 2010. Detection and partial characterization of norovirus among children with acute gastroenteritis in Lagos, Nigeria. Int. J. Med. Med. Sci. 2(7): 216-221.

Ayolabi, CI., Ojo, DA. and Akpan, I. 2012. Astrovirus Infection in Children in Lagos, Nigeria. African Journal of Infectious Diseases. 6(1):1-4.

Ayolabi, CI., Ojo, DA. and Armah, GE. 2013. Electropherotypes and G-Types of Group A Rotaviruses Detected in Children with Diarrhea in Lagos, Nigeria. ISRN Virology. http://dx.doi.org/10.5402/2013/179871.

Babalola, MO., Odaibo, GN., Olaleye, OD. and Alonge, AO. 2015. Enteric Adenovirus and Norovirus Gastroenteritis among Under-5 years Children in Owo, Ondo State, Nigeria. British Journal of Medicine & Medical Research. 9(8):1-9.

Bhavnani, D., Goldstick, JE., Cevallos, W., Trueba, G. and Eisenberg, JN. 2012. Synergistic effects between rotavirus and coinfecting pathogens on diarrheal disease: evidence from a community-based study in northwestern Ecuador. Am J Epidemiology. 10:387-395.

Bishop, RF., Davidson, GP., Holmes, IH. and Ruck, BJ. 1973. Virus particles in epithelial cells of duodenal mucosa from children with acute nonbacterial gastroenteritis. Lancet. 8:2 (7841):1281- 1283.

Bon, F., Fascia, P., Dauvergne, M., *et al*. 1999. Prevalence of group A rotavirus, human calicivirus, astrovirus, and adenovirus type 40 and 41 infections among children with acute gastroenteritis in Dijon, France. J Clin Microbiology. 37:3055-3058.

Brown, RJ., Morfopoulou, S., Hubb, J., Emmeth, AW., Ip, W., Shah, D., Brooks, T., Pave, MS., Anderson, G., Virasami, A., Tong, CW., Clark, AD., Plagnol, V., Jacque, ST., Qasim, W., Hubank, M. and Judith, B. 2015. Astrovirus VA1/HMO-C: An increasingly Recognised Neurotropic Pathogen in Immunocompromised Patients. Clinical Infectious Dis. DOI;10.1093/cid/ciu940.

Bryce, J., Boschi-Pinto, C., Shibuya, K. and Black, RE. 2005. WHO estimates of the causes of death in children. Lancet. 365: 1147-52.

Castello, AA., Arguelles, MH., Rota, RP., Olthoff, A., Jiang, B., Glass, RI., Gentsch, JR. and Glikmann, G. 2006. Molecular epidemiology of group Arotavirus diarrhea among children in Buenos Aires, Argentina, from 1999 to 2003 and emergence of the infrequent genotype G12. J Clin Microbiology. 44 (6): 2046-2050.

CDC. 2011. Updated Norovirus outbreak management and disease prevention guidelines MMWR Recomm Rep. 60 (RR-3):1-18.

CERTEST Biotech SL. 2013. Certest Rota+Adeno+Astro+Noro One Step Combo Card test for Coloured Chromatographic Immunoassay for the simultaneous qualitative detection of Rotavirus, Adenovirus, Astrovirus and Norovirus in stool samples. Certest Biotech S.L. Industrial Rio Gallego, Zaragoza, Spain, www.certest.es, IURG86V rev 01:1-17.

Cubitt, WD. 1996. Historical background and classification of caliciviruses and astroviruses. Arch Virol. Suppl. 12:225-235.

Estes, MK. and Kapikian, AZ. 2007. Rotaviruses. In: Fields Virology. (5th ed.). Eds. Knipe, DM, Howley, PM. and Griffin, DE, Philadelphia: Lippincott Williams and Wilkins. 1917- 1974.

Estes, MK. and Greenberg, HB. 2013. Rotaviruses. In: Fields Virology. (6th ed.). Eds. Knipe, DM., Howley, PM. *et al*. Wolter Kluwer Health/Lippincott Williams & Wilkins. Philadelphia, PA. 1347-1401.

Fauquet, CM., Mayo, MA., Maniloff, J., Desselberger, U. and Ball, LA. 2005. Virus Taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses. Elservier Academic Press, London, U.K.

Finkbeiner, RS. 2009. Identification and characterization of novel astroviruses. All Theses and Dissertations (ETDs). Washing University Open Scholarship. Paper 110.

Fischer-Walker, CL., Rudan, I., Liu, L., Nair, H., Theodoratou, E. *et al*. 2013. Global burden of childhood pneumonia and diarrhea. Lancet. 381:1405-16.

Girard, MP., Steele, D., Chaignat, CL. and Kieny, MP. 2006. A review of vaccine research and development: human enteric infections. Vaccine. 24:2732-50.

Glass, RI., Bresee, J., Jiang, B., Gentsch, J., Ando, T., Fankhauser, R., Noel, J., Parashar, U., Rosen, B. and Monroe, SS. 2001. Gastroenteritis viruses: an overview. Novartis Foundation Symposium. 238:5-19. discussion 19-25.

Greenberg, HB. and Estes, MK. 2009. Rotaviruses: from pathogenesis to vaccination. Gastroenterology. 136:1939-1951.

Griffiths, EC., Pedersen, AB., Fenton, A. and Petchey, OL. 2011. The nature and consequences of coinfection in humans. Journal Infection. 10: 200-206.

Hermann, JE., Taylor, DN., Echeverria, P. and Blacklow, NR. 1991. Astroviruses as a cause of gastroenteritis in children. New England J. of Medicine. 324:1757-1760.

Iritani, N., Seto, Y., Kubo, H. *et al.* 2003. Prevalence of Norwalk like virus infections in cases of viral gastroenteritis among children in Osaka City, Japan. J Clin Microbiology. 41:1756-1759.

Iyoha, O. and Abiodun, PO. 2015. Human rotavirus genotypes causing acute watery diarrhea among under-five children in Benin City, Nigeria. Niger J Clin Pract. 18:48-51.

Jakab, F., Peterfai, J., Meleg, E., Banyai, K. and Mitchell, DK. 2005. Comparison of clinical characteristics between astrovirus and rotavirus infections diagnosed in 1997 to 2002 in Hungary. Acta Paediatr. 94:667-71.

Japhet, MO., Adesina, OA., Famurewa, O., Svensson, L. and Nordgren, J. 2012. Molecular Epidemiology of rotavirus and norovirus in Ile-Ife, Nigeria: High prevalence of G12P[8] rotavirus strains and detection of a rare norovirus genotype. Journal of Med Virology. 84:1489-1496.

Junaid, SA., Umeh, C., Atanda, OO. and Banda, JM. 2011. Incidence of rotavirus infection in children with gastroenteritis attending Jos University Teaching Hospital, Nigeria. Virology Journal. 8:233. http://www.virologyj.com/content/8/1/233

Junquera, CG., de Baranda, CS., Mialdea, OG. *et al*. 2009. Prevalence and clinical characteristics of norovirus gastroenteritis among hospitalized children in Spain. Pediatr Infect Dis J. 28:604-607.

Koopmans, M. 2008. Progress in understanding norovirus epidemiology. Curr. Opin Infect Dis. 21:544-552.

Koopmans, M., Vennema, H., Heersma, H., van Strien, E., van Duynhoveny, BD., Reacher, M. and Lopman, B. 2003. European consortium on foodborne viruses early identification of common source foodborne virus outbreaks in Europe. Emerg Infect Dis. 9:1136-1142.

Kuta, FA., Damisa, D., Adabara, NU. and Abdulsalam, R. 2014[a]. Prevalence of Astrovirus Infection in Children in Nasarawa State, Nigeria. Global Advanced Research Journal of Microbiology. 3(6): 102-105.

Kuta, FA., Adabara, NU. and Abdulsalam, R. 2014[b]. Epidemiology of Astrovirus infection among children (0 – 5years) in Niger state, Nigeria. Ife Journal of Science. 16(2):331-334.

Lanjewar, DN., Surve, KV., Maniar, JK. and Hira, SK. 1994. Parasitic diarrhea with AIDS. International Conference on AIDS. 10:158 (Abstract).

Lee, JI., Chung, JY., Han, TH. *et al*. 2007. Detection of human bocavirus in children hospitalized because of acute gastroenteritis. Journal of Infectious Diseases. 196:994-997.

Lello, J., Norman, RA., Boag, B., Hudson, PJ. and Fenton, A. 2008. Pathogen interactions population cycles, and phase shifts. Am Nat. 10:176-182.

Levine, MM. 1987. *Escherichia coli* that cause diarrhoea: Enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. Journal of Infectious Diseases. 155: 377-389.

Liakopoulou, E., Mutton, K., Carrington, D., Robinson, S., Steward, CG., Goulden, NJ., Cornish, JM. and Marks, DI. 2005. Rotavirus as significant cause of prolonged diarrhoeal illness and morbidity following allogenic bone marrow transplantation. Bone Marrow Transplant. 36: 691-694 [PMID: 16113671 DOI:10.1038/sj.bmt.1705127].

Lintao, S., Jintang, S., Lihua, S., Shuai, C., Haihong, L. and Lixian, M. 2013. Epidemiology and clinical features of rotavirus and norovirus infection among children in Ji'nan, China, Virology Journal. 10: 302. http://www.virologyj.com/content/10/1/302.

Liu, L., Johnson, HL., Cousens, S., Perin, J., Scott, S. *et al*. 2012. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 379:2151-61.

Madeley, CR. and Cosgrove, BP. 1975. Viruses in infantile gastroenteritis. Lancet. 2:124.

Matthijnssens, J., Otto, PH., Ciarlet, M., Desselberger, U., Van Ranst, M. and Johne, R. 2012. VP6- sequence-based cutoff values as a criterion for rotavirus species demarcation. Arch Virology. 157:1177-1182.

Mebus, CA., Underdahl, NR., Rhodes, MB. and Twiehaus, MJ. 1969. Calf diarrhea (Scours): reproduced with a virus from a field outbreak. Univ Nebraska Res Bull. 233:1-16.

Mihalov-Kovács, E., Gellért, Á., Marton, S., Farkas, SL., Fehér, E., Oldal, M., Jakab, F., Martella, V. and Bányai, K. 2015. Candidate new rotavirus species in sheltered dogs, Hungary. Emerg Infect Dis. 21:660-663.

Mohammad, M., Amini, E. and Shirazi, PT. 2013. Frequency of rotavirus and adenovirus gastroenteritis Among children in Shiraz, Iran. Iran Red Cres Med J. 15(8):729-33. doi: 10.5812.

Mona, ZZ., El-Sahn, SF. and Galal, ZA. 2013. Coinfection of Rotavirus Group A, Norovirus and Adenovirus in Egyptian Children with Gastroenteritis. Life Sci Journal. 10(2):848-852.

Moser, LA. and Schultz-Cherry, S. 2005. Pathogenesis of astrovirus infection. Review. Viral Immunology. 18:4-10.

Moyo, SJ., Gro, N., Kirsti, V., Matee, MI., Kitundu, J., Maselle, SY., Langeland, N. and Helge, M. 2007. Prevalence of enteropathogenic viruses and molecular characterization of group A rotavirus among children with diarrhea in Dar es Salaam Tanzania. BMC Public Health. **7**:359. doi:10.1186/1471-2458-7-359.

Muhsen, K. and Levine, MM. 2012. A systematic review and meta-analysis of the association between *Giardia lamblia* and endemic pediatric diarrhea in developing countries. Clin Infect Dis. 55 Suppl 4:S271-93.

Najafi, A., Kargar, M. and Jafarpour, T. 2012. Burden and Typing of Rotavirus Group A in Children with Acute Gastroenteritis in Shiraz, Southern Iran. Iran Red Crescent Medical Journal. 14 (9):531-540.

Nimzing, L., Geyer, A. and Sebata, T. 2000. Epidemiology of adenoviruses and rotaviruses identified in young children in Jos, Nigeria. South Afr J Epidemiol Infect. 15: 35-37.

Niyogi, SK. 2005. Shigellosis. Journal of Microbiology. 43:133-143.

Parashar, UD. and Glass RI. 2003. Viral causes of gastroenteritis. In: Viral gastroenteritis. Eds. Desselberger, U. and Gray, J. Amsterdam, Elsevier. 9:9-21.

Patel, MM., Widdowson, MA., Glass, RI. Akazawa, K., Vinje, J. and Parashar, UD. 2008. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerging Infectious Disease. 14:1224-31.

Phillips, G., Tam, CC. and Conti, S. 2010. Community incidence of norovirus-associated infectious intestinal disease in England: improved estimates using viral load for norovirus diagnosis. American Journal of Epidemiology. 171:1014-22.

Post, GB., Atherholt, TB. and Cohn, PD. 2011. Health and aesthetic aspects of drinking water. In: Water quality & treatment. (6th edi.). Ed. Edzwald, JK. Denver, Colorado: American Water Works Association. 1-100.

Ribeiro, J., Ferreira, D., Arrabalde, C., Almeida, S., Baldaque, I. and Sousa, H. 2015. Prevalence of Adenovirus and rotavirus infection in immunocompromised patients with acute gastroenteritis in Portugal. World J. Virology. 4(4):372-376.

Sdiri- Loulizi, K., Gharbikhelifi, H. and de Rougemont, A. 2008. Acute infant gastroenteritis associated with human enteric viruses in Tunisia. J Clinical Microbiol. 43: 1462-1464.

Steele, AD. and Ivanoff, B. 2003. Rotavirus strains circulating in Africa during 1996-1999; emergence of G9 strains and P[6]. Vaccine. 21:361-367.

Tate, JE., Burton, AH., Boschi-Pinto, C., Steele, AD., Duque, J. *et al*. 2012. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect Dis. 12:136-41.

Van Heerden, J., Ehlers, MM., Van Zyl, WB. and Grabow, WOK. 2003. Incidence of adenoviruses in raw and treated water. Water Research. 37(15):3704 -3708.

van Maarseveen, NM., Wessels, E., de Brouwer, CS., Vossen, AC. and Claas, EC. 2010. Diagnosis of viral gastroenteritis by simultaneous detection of Adenovirus group F, Astrovirus, Rotavirus group A, Norovirus genogroups I and II, and Sapovirus in two internally controlled multiplex real-time PCR assays. J Clin Virol. 49:205-10.

Walsh, MP., Chintakuntlawar, A., Robinson, CM. *et al***.** 2009. Evidence of molecular evolution driven by recombination events influencing tropism in a novel

human adenovirus that causes epidemic keratoconjunctivitis. PLoS ONE. 4: e5635.

Walter, JE. and Mitchell, DK. 2000. Role of Astroviruses in childhood diarrhea. Current Opinion in Pediatrics. 12(3):275-279.

Walter, JE. and Mitchell, K. 2003. Astrovirus infection in children. Current Opinion Infect. Diseases. 16:247-253.

Wilhelmi, I., Colomina, J., Martin, RD., Romall, E. and Sanchez-Fauquier, A. 2001. New immunochromatographic methods for rapid detection of rotaviruses in stool compared with standard enzyme immunoassay and latex agglutination techniques. Eur. J. Clin. Microbiol. Infect. Dis. 20:741-743.

Wilhelmi, I., Roman, E. and Sanchez-Fauquier A. 2003. Viruses causing gastroenteritis. Clinical Microbiology and Infection. 9(4):247-262.

World Health Organization. 2002. Generic protocol for (i) a hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children and (ii) a community-based survey on utilization of health care services for gastroenteritis in children. Field test version. Geneva. Document WHO/V&B/02.15.

Zlateva, KT., Maes, P., Rahman, M. and Van Ranst, M. 2005. Chromatography paper strips sampling of enteric adenoviruses type 40 and 41 positive stool specimens. Virology Journal. 2:6-10.

Received: May 16, 2017; Revised: Dec 3, 2017; Accepted: Dec 5, 2017