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Molecular Epidemiology of Viral Enteric Pathogens Associated with Acute Diarrhoea in Children under 5 years in Ibadan Nigeria

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ABSTRACT

Background: Diarrheal diseases are a major cause of death in children under five, responsible for approximately 9% of global child mortality. This study investigated the molecular epidemiology of viral enteric pathogens linked to acute diarrhea in children under five in Ibadan, Nigeria.

Method: 273 children with symptoms of acute diarrhea were enrolled. Fresh stool samples were collected in sterile, RNase-free containers, preserved at -80°C, and analyzed using molecular techniques, including nucleic acid extraction and Real-Time PCR amplification.

Results: The results showed Adenovirus as the most frequently detected virus (24.5%), followed by Norovirus Type 2 (5.9%) and Type 1 (2.9%). Mixed infections accounted for 2.2% of cases, while Rotavirus Group A, Sapporo Virus, and Astrovirus were not detected. Infections were most prevalent in children aged 0–1 year, particularly for Adenovirus (49.2%) and Norovirus Type 2 (68.8%). Statistically significant age-related differences were observed for Adenovirus, Norovirus Type 1, and mixed infections ($p = 0.0001$). Although males had slightly higher rates of Norovirus Types 1 and 2 and mixed infections, these differences were not statistically significant. Water source revealed a higher prevalence of Adenovirus in children using tap water (55.2%), while Norovirus Type 2 and mixed infections were more common among borehole water users (43.8% and 66.6%, respectively). Though not statistically significant, some associations approached significance.

Conclusion: The study highlights the persistent impact of environmental contamination and inadequate vaccine coverage, emphasizing strengthening routine immunization efforts for children under two years of age.

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Introduction

Diarrhea is defined as the frequent passing of loose or watery stools, typically three or more times within a 24-hour period. This condition can result in dehydration, loss of appetite, and significant weight loss [1]. It is particularly widespread in low-income regions where sanitation infrastructure is lacking, a common scenario in many African countries [1, 2]. Compounding the problem, these areas often have high rates of individuals living with Human Immunodeficiency Virus HIV, whose compromised immune systems make them more susceptible to opportunistic infections, including those that cause diarrhea. While global deaths from diarrhea have been decreasing, children under the age of five in Africa may experience up to twelve episodes each year, increasing their risk of malnutrition, mortality, and overall poor health outcomes [2]. The transmission of diarrhea pathogens typically occurs through the ingestion of contaminated food and water [1]. The microorganisms responsible for causing diarrhea generally fall into three main categories: viruses (such as

Rotavirus, Sapporo virus, Astrovirus, and Adenovirus), bacteria (including diarrhoeagenic *Escherichia coli*, *Shigella*, *Salmonella*, and *Campylobacter jejuni*), and parasites (such as *Entamoeba histolytica* and *Giardia lamblia*) [3]. Among these, viral agents are the leading cause of diarrhea across Africa. Notably, Rotavirus A is recognized as the primary cause of severe diarrhea in young children globally and has been linked to more than 50% of gastroenteritis cases in this age group [3,4].

Diarrhea diseases are a major contributor to child mortality, responsible for roughly 9% of all deaths in children under five worldwide. This burden is especially high in regions such as South Asia and Sub-Saharan Africa [4]. According to the World Health Organization (WHO), 1.7 billion cases of childhood diarrhea were recorded globally in 2017, resulting in around 370,000 deaths [3]. United Nation Children Fund UNICEF also reported that diarrhea claims the lives of about 2,195 children each day surpassing the mortality rates of Acquired Immune Deficiency Syndrome AIDS, malaria, and measles combined. Sub-Saharan Africa alone accounts for roughly one-third of these annual diarrhea episodes [4, 5]. The high incidence in the region is largely due to limited

access to clean drinking water, inadequate waste disposal systems, overcrowded living conditions, and poor hygiene practices [5]. Since the immune systems of young children are not fully developed, these harsh environmental factors further increase their susceptibility to diarrhea diseases [5,6]. Although comprehensive data on diarrhea incidence is limited, it is estimated that diarrhea-specific mortality among African children under five is approximately 106 deaths per 1,000 live births [7]. In Nigeria alone, over 315,000 annual deaths among preschool-aged children have been attributed to diarrhea diseases [7,8].

The study of the molecular epidemiology of viral pathogens linked to acute diarrhea in children under five plays an important role in public health and disease control [8]. This discipline explores the genetic makeup and molecular behavior of viruses causing gastrointestinal illnesses, especially in early childhood, with a focus on understanding how they spread, mutate, and affect population health [7]. The development of molecular diagnostic techniques has significantly advanced our knowledge of viral gastroenteritis [7, 8]. Tools such as PCR (polymerase chain reaction), genome sequencing, and genotyping enable the accurate detection and classification of viral strains [3]. By examining the genetic sequences of these viruses, researchers can trace transmission pathways, identify outbreaks quickly, and track the appearance of new, more dangerous or treatment-resistant strains [9]. The aim of this study is to determine the molecular epidemiology of viral enteric pathogens associated with acute diarrhoea in children under five years in Ibadan, Nigeria. The objectives are to determine the prevalence, characterize the diversity, socio-demographic distribution and relationship between of viral enteric pathogens in children under five years in Ibadan, Nigeria.

Materials And Methods

Study Design

This study utilized a molecular cross-sectional design to detect the molecular epidemiology of viral enteric pathogens associated with acute diarrhea in children under five in fecal samples collected from the study population. The sample size for this study was determined using Fisher's formula: $n = Z^2Pq/d^2$.

The number of samples studied was guided by the upper limit required, and gave 95% level of confidence at an expected prevalence of about 55% pilot study, after using the precise prevalence formula.

$N = z^2pq$

D^2 (Araoye, 2004)

Where N = the desired sample size (when population is greater than 10,000)

z = is a constant given as 1.96 (or more simply at 2.0) which corresponds to the 95% confidence level.

P = proportion of the target population that have the characteristic i.e 22% = 0.01

$q = 1.0 - p$

d = acceptable error 5%.

$$N = \frac{(1.96)^2 \times 0.22 \times (1 - 0.22)}{(0.05)^2}$$

$$N = \frac{(1.96)^2 \times 0.22 \times 0.78}{(0.05)^2}$$

$$N = \frac{3.8416 \times 0.01 \times 0.78}{0.0025}$$

$$N = 260$$

To make up for the sampling error or drop outs, a minimum of 273 sample was collected and used for the research.

Study Participants

Stool samples were obtained from 273 children aged 0 to 5 years, including hospitalized patients presenting with symptoms of acute gastroenteritis of no more than seven days' duration. The sampling was conducted at the University College Hospital (UCH) in Ibadan.

Sampling Technique

Children aged 0 to 5 years presenting with acute gastroenteritis (defined as the passage of three or more loose stools within 24 hours, lasting no longer than seven days) were recruited consecutively from the University College Hospital (UCH), Ibadan. Eligible participants were identified by attending clinicians, and parents or guardians were informed about the study's purpose and procedures.

Ethical Consideration

Ethical consideration was sorted for and obtained by Ethical and Research Committee of University College Hospital (UCH), Ibadan. Individual consent for collection of samples was requested through verbal and documented data of subject; by educating the subjects on the importance of the research and those that gave their consent was enlisted for the research (AD 13/479/045).

Inclusion Criteria

Children under five years with confirmed acute diarrhea cases was included in this study.

Exclusion Criteria

Patients with pathological conditions different from cases of acute diarrhea under the age of five was excluded.

Informed Consent

Parents and guardians were thoroughly informed about the study, including its objectives, procedures, potential risks and benefits, and the rights of participants. They were encouraged to ask questions and were assured that participation was entirely voluntary. Consent forms were provided, and written consent was obtained prior to the inclusion of their children in the study.

Socio Demographic and Clinical Data

Data were gathered from all participants whose parents or guardians provided informed consent. Socio-demographic and clinical information was collected using a structured questionnaire, which was completed by the parents or guardians of each child. The questionnaire comprised the following sections:

- **Demographic Data:** Age, gender, place of residence (urban or rural), and socio-economic status.
- **Environmental Factors:** Sources of drinking water, type of sanitation facilities, and hygiene practices.

Validity and Reliability

- **Validity:** The questionnaire was validated through a pilot study involving 25 participants and expert review.
- **Reliability:** Evaluated using the test-retest method, yielding a Cronbach's alpha between 0.75 and 0.88.

Administration Method

- **Mode:** Administered through face-to-face interviews by trained field personnel.
- **Completion:** Parents or guardians filled out the questionnaire with assistance from field staff to ensure clarity and accuracy.

Timing and Location of Data Collection

- **Period:** Data were collected between December 2024 and May 2025.
- **Location:** The study was carried out at University College Hospital (UCH), Ibadan.

Stool Sample Collection

- **Target Samples:** Stool specimens were obtained from children presenting with acute gastroenteritis, defined as a sudden onset of diarrhea (three or more loose stools within 24 hours).
- **Procedure:** Participants were informed through healthcare facilities, and written consent was obtained before sample collection.

Study Limitation

This study has a few limitations. First, it was hospital-based and may not fully reflect the burden of viral gastroenteritis in community settings. Second, the study relied on the detection of a limited panel of viral pathogens; other enteric viruses or co-existing bacterial and parasitic pathogens may have been missed. Third, seasonality was not specifically analyzed, which could affect viral prevalence patterns.

Sample Collection and Laboratory Diagnosis

Extraction of Nucleic Acid

Viral nucleic acids were extracted using the DaAn Gene DA0591 RNA/DNA extraction kit, following the manufacturer's protocol. Initially, the lysis solution was combined with carrier RNA (prepared and used immediately) and centrifuged to form the lysis working solution. Into a 1.5 mL centrifuge tube, 50 µL of proteinase K was added, followed by 200 µL of the specimen and 200 µL of the lysis working solution. The tube was securely capped, then vortexed for 15 seconds to ensure thorough mixing and centrifuged briefly at high speed for 10 seconds to minimize bubble formation during incubation. The mixture was incubated at 72°C for 10 minutes, with the eluent preheated concurrently [10].

250 µL of ethanol was added to the mixture, the tube was sealed and vortexed again for 15 seconds. The entire mixture was transferred to a spin column and centrifuged at 12,000g for 1 minute at room temperature. The spin column was placed in a new collection tube, and 500 µL of inhibitor remover was added. After another centrifugation at 12,000g for 1 minute, the spin column was transferred to a fresh collection tube. This step was repeated with 500 µL of deionized water, conducted twice to ensure complete deionization. The spin column and collection tube were allowed to sit at room temperature, then centrifuged at 14,000g for 3 minutes to eliminate residual ethanol [10].

The spin column was then placed into a new 1.5 mL centrifuge tube, left open at room temperature for 2 minutes. Subsequently, 50 µL of preheated eluent was carefully applied to the center of the spin column membrane, capped tightly, and centrifuged at 14,000g for 1 minute. The resulting eluate in the centrifuge tube represented the extracted nucleic acid, which was stored at -80°C until further use [10].

Detection of Viral Enteric Pathogen PCR protocols

Viral Enteric Pathogen detection was performed by Real Time Polymerase Chain Reaction (rt-PCR) using a set of commercially prepared kit (Rotavirus Group A/Norovirus type 1/Norovirus type 2 RNA and Sapporo virus type-V/human Astrovirus Classic Type/ Human Adenovirus RNA detection kit).

The sample was analyzed using the BioRad CFX96 real-time PCR system, a molecular diagnostic device manufactured in China. Negative control, Positive control was set, and test sample parameters to perform PCR experiment according to the operating instructions of the instrument, corresponding sample name were recorded. FAM channel was selected to detect Human Adenovirus, VIC channel was also selected to detect Astrovirus, ROX to detect Sapporo virus type-V and CY% to detect human Internal Control. The Reaction Volume per Well was set at 25µL. Recommended Setting of reaction procedure was set using manufacturers instruction [11].

Result Analysis

Once the reaction was completed, the data were automatically stored. Baseline parameters including the Start value, End value, and Threshold were adjusted based on the amplification plot, aligning with real-time observations. Typically, the Start value was set between 3 and 15, and the End value between 5 and 20. The amplification curve of the negative control was adjusted to remain flat or below the threshold line. Analysis commenced only after ensuring that all parameters complied with quality control standards.

Quality Control

- **Negative Control:** No amplification curves were observed in the FAM and VIC detection channels.
- **Positive Control:** Clear amplification curves were present in both FAM and VIC, ROX channels, with cycle threshold (Ct) values not exceeding 30.00 for each channel [11].

Data Analysis

The data was analyzed using SPSS software Version-23 (SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as numbers (n) and percentages (%).

Results

Socio-Demographic Characteristics of the Study Participants

A total of 273 stool samples were obtained from children experiencing diarrhea. The samples were analyzed using gene extraction and Real-Time RT-PCR Amplification techniques to identify the presence or absence of rotavirus A, adenovirus, Norovirus Type 1, Norovirus Type 2, Sapporo Virus Type-V and possible co-infections involving Norovirus Type 2 and Adenovirus viruses.

Table 1 summarizes the socio-demographic characteristics of children sampled for viral enteric pathogen analysis in Oyo State, Nigeria. Most samples (42.1%) came from infants aged 0–1 year, highlighting a focus on early childhood, with additional representation from children aged 2–5 years. There was a nearly even gender distribution, with slightly more females (52.4%). The majority of participants lived in urban areas (71.3%), and most attended crèche (63.2%), emphasizing the study's concentration on very young children. Hygiene practices were relatively good, with 79.8% of caregivers reporting regular handwashing with soap. Tap water was the most common water source (55.7%), followed by boreholes (25.6%), while fewer relied on wells, sachet, or bottled water.

Prevalence of Viral Enteric Pathogens among Children in Oyo State, Nigeria

Figure 1 presents a pie chart illustrating the prevalence of viral enteric pathogens among the study participants. The majority, 67%, tested negative for any infection, while 33% were found to have positive bacterial enteric pathogens.

Prevalence of co-infection of Viral Enteric Pathogens among

Children in Oyo State, Nigeria

Figure 2 displays a pie chart illustrating the prevalence of co-infections involving viral enteric pathogens among the study participants. Co-infections were identified in 2% of the participants.

Distribution of Viral Enteric Pathogens among Children in Oyo state, Nigeria

Table 2 presents the distribution of viral enteric pathogens among children among study participants. Rotavirus Group A, Sapporo Virus Type-V, and Astrovirus were not detected in any of the 273 samples. Norovirus Type 1 showed a low prevalence (2.9%), while Norovirus Type 2 was slightly more common (5.9%). Adenovirus was the most frequently identified virus, found in 24.5% of the children. Co-infection with Norovirus Type 2 and Adenovirus occurred in 2.2% of cases.

Age Distribution of Viral Enteric Pathogens among Children in Oyo State, Nigeria

Table 3 outlines the age-related distribution of viral enteric pathogens among study participants. Norovirus Type 1 showed no strong age trend, with cases fairly spread across all age groups. Norovirus Type 2 was most common in infants aged 0–1 year (68.8%), indicating higher susceptibility in this group. Adenovirus was also most prevalent in the 0–1 year group (49.2%), with lower but equal rates (25.4%) in the 2–3 and 4–5 years groups. Co-infection of Norovirus Type 2 and Adenovirus occurred primarily in the 0–1 year group (66.7%), with fewer cases in 2–3 years and none in 4–5 years. A p-value of 0.0001 for Adenovirus and Norovirus Type 1 suggests a significant association between age and infection.

Gender Distribution of Viral Enteric Pathogens among Children in Oyo State, Nigeria

Table 3 presents the gender distribution of viral enteric pathogens among study participants including Norovirus Types 1 and 2, Adenovirus, and their co-infection. Males had higher infection rates for Norovirus Type 1 (75%) and Type 2 (68.8%) compared to females, but the p-values (0.64 and 0.90) indicate no statistically significant gender differences. Adenovirus infections were more evenly distributed, with slightly more females (56.7%) affected, and a p-value of 0.43 also showing no significant gender difference. Co-infections were more common in males (83.3%), but again, the difference was not statistically significant (p = 0.36).

Distribution of Viral Enteric Pathogens among Children under five years Based on Residence in Oyo State, Nigeria

Table 3 shows the distribution of viral enteric pathogens among study participants based on their place of residence. Norovirus Type 1 was found only in urban and semi-urban areas (50% each), with none in rural areas; this distribution was statistically significant (p = 0.0001). Norovirus Type 2 was most common in urban areas (50%),

followed by semi-urban (43.8%) and rural (6.2%) areas, though the difference was not statistically significant (p = 0.25). Adenovirus was also most prevalent in urban areas (67.2%), with lower rates in semi-urban (29.8%) and rural (3%) areas, but the variation was not statistically significant (p = 0.82). Co-infection with Norovirus Type 2 and Adenovirus was highest in urban areas (50%), with significant differences by residence (p = 0.0001).

Distribution of Viral Enteric Pathogens among Children under Five Years Based on Educational Level in Oyo State, Nigeria

Table 3 summarizes the distribution of viral enteric pathogens among study participants based on educational level (Crèche, Nursery, and Primary). Norovirus Type 1 and Type 2 were most prevalent in crèche children, with 75% of cases for both viruses occurring in this group. Norovirus Type 1 showed a statistically significant difference across educational levels (p = 0.0001), while Norovirus Type 2 did not (p = 0.328). Adenovirus was also most common among crèche attendees (70.1%), though the difference was not statistically significant (p = 0.76). Co-infection with Norovirus Type 2 and Adenovirus was evenly split between crèche and nursery children (50% each), with none in primary, and this difference was statistically significant (p = 0.0001).

Distribution of Viral Enteric Pathogens among Children under Five Years Based on their sources of water in Oyo State, Nigeria

Table 4 details the distribution of viral enteric pathogens among study participants based on their water sources. Norovirus Type 1 was most common among users of borehole and tap water (37.5% each), though the association was not statistically significant (p = 0.086). Norovirus Type 2 was most prevalent in borehole water users (43.8%), but the distribution showed no statistical significance (p = 0.96). Adenovirus infections were highest among those using tap water (55.2%), with a near-significant p-value of 0.082. Co-infections were most associated with borehole water (66.6%), and this relationship was marginally significant (p = 0.054), suggesting a potential link between co-infection and borehole water use.

Distribution of Viral Enteric Pathogens among Children under Five Years Based on their Parents/Guardian Hygiene (regular hand washing) in Oyo State, Nigeria

Table 4 shows the distribution of viral enteric pathogens among study participants based on their parents’ or guardians’ hand-washing habits. Most cases of Norovirus Type 1, Type 2, Adenovirus, and co-infections occurred among children whose caregivers reported regular hand washing. However, the p-values for all pathogens (ranging from 0.25 to 0.63) indicate that these differences are not statistically significant. Thus, regular hand washing by caregivers was not significantly associated with reduced infection rates in this study.

Table 1: Socio-Demographic Characteristics of Participants

Factor	Frequency	Percentage (%)
Age		
0-1	115	42.1%
2-3	61	22.3%
4-5	97	35.5%
Gender		
Male	130	47.6%
Female	143	52.4%
Residence		
Urban	196	71.3%

Semi-urban	65	24.3%
Rural	12	4.5%
Educational Status		
Creche	189	63.2%
Nursery	78	26.8%
Primary	6	2.1%
Risk factors		
Regular Hand Washing		
No	55	20.1%
Yes	218	79.8%
Sources of water		
BH	70	25.6%
TW	152	55.7%
W	25	9.2%
BW	7	2.6%
SW	19	7.0%

Note: BH-Borehole water, W- Well Water, BW- Bottle Water, SW- Sachet Water, TW- Tap Water

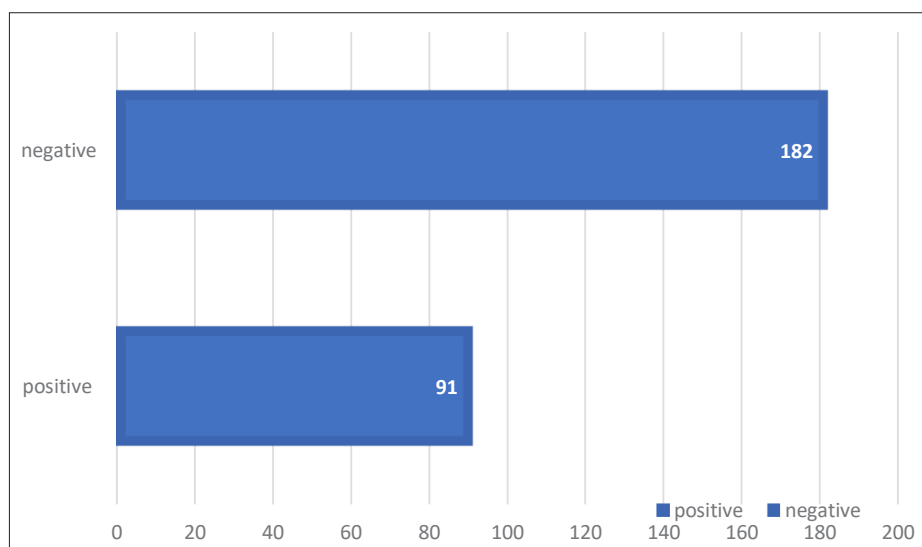


Figure 1: Prevalence of Viral Enteric Pathogens among Children in Oyo State, Nigeria

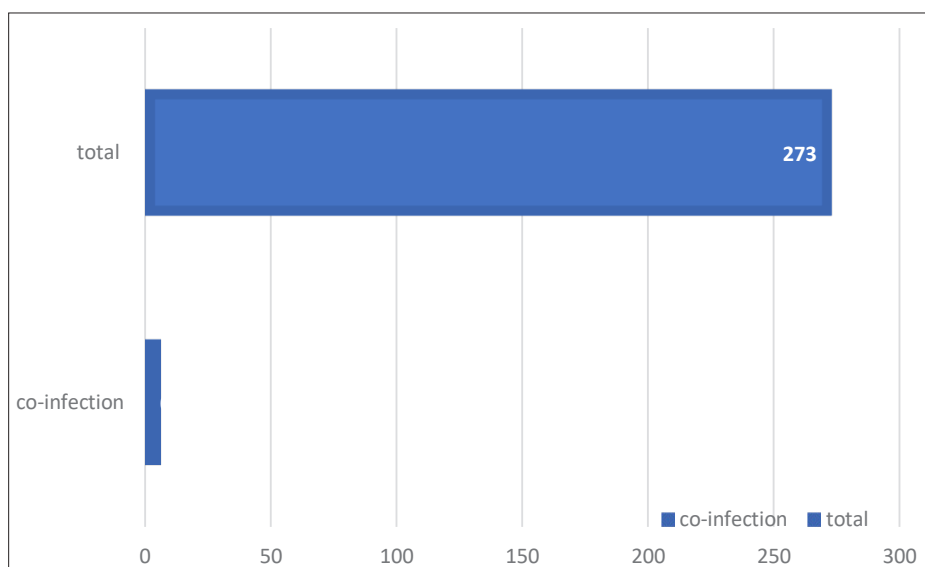


Figure 2: Prevalence of Co-Infection of Viral Enteric Pathogens among Children in Oyo State, Nigeria

Table 2: Distribution of Viral Enteric Pathogens among Children in Oyo state, Nigeria

	Rotavirus Group A	Norovirus Type 1	Norovirus Type 2	Sapporo Virus Type-V	Astrovirus	Adenovirus	Norovirus Type 2 and Adenovirus Co-Infection
Positive	0(0%)	8(2.9%)	16(5.9%)	0(0%)	0(0%)	67(24.5%)	6(2.2%)
Negative	273 (100%)	265(97.1%)	257(94.1%)	273(100%)	273 (100%)	206(75.5%)	267(97.8%)

Table 3: Distribution of Viral Enteric Pathogens among Children under Five Years Based on Sociodemographic Factors in Oyo State, Nigeria

Sociodemographic Factor	Category	Norovirus Type 1 (N=8)	Norovirus Type 2 (N=16)	Adenovirus (N=67)	Norovirus Type 2 & Adenovirus Co-Infection (N=6)
Age (Years)	0–1	3 (37.5%)	11 (68.8%)	33 (49.2%)	4 (66.7%)
	2–3	2 (25%)	2 (12.5%)	17 (25.4%)	2 (33.3%)
	4–5	3 (37.5%)	3 (18.7%)	17 (25.4%)	0 (0%)
	p-value	0.0001*	0.83	0.0001*	0.0001*
Gender	Female	2 (25%)	5 (31.2%)	38 (56.7%)	1 (16.7%)
	Male	6 (75%)	11 (68.8%)	29 (43.3%)	5 (83.3%)
	p-value	0.64	0.90	0.43	0.36
Residence	Urban	4 (50%)	8 (50%)	45 (67.2%)	3 (50%)
	Semi-urban	4 (50%)	7 (43.8%)	20 (29.8%)	2 (33.3%)
	Rural	0 (0%)	1 (6.2%)	2 (3%)	1 (16.7%)
	p-value	0.0001*	0.25	0.82	0.0001*
Educational Level	Creche	6 (75%)	12 (75%)	47 (70.1%)	3 (50%)
	Nursery	1 (12.5%)	3 (18.8%)	19 (28.4%)	3 (50%)
	Primary	1 (12.5%)	1 (6.2%)	1 (1.5%)	0 (0%)
	p-value	0.0001*	0.328	0.76	0.0001*

* Significant at $p < 0.05$

Table 4: Distribution of Viral Enteric Pathogens among Children under Five Years Based on Risk Factors in Oyo State, Nigeria

Risk Factor	Category	Norovirus Type 1 (N=8)	Norovirus Type 2 (N=16)	Adenovirus (N=67)	Norovirus Type 2 & Adenovirus Co-Infection (N=6)
Source of Water	BH	3(37.5%)	7(43.8%)	18(26.9%)	4(66.6%)
	TW	3(37.5%)	4(25%)	37(55.2%)	0(0%)
	W	2(25%)	3(18.8%)	4(6%)	1(16.7%)
	BW	0(0%)	0(0%)	3(4.5%)	0(0%)
	SW	0(0%)	2(12.4%)	5(7.4%)	1(16.7%)
	p-value	0.086	0.96	0.082	0.054
Parent/Guardian Hygiene (Hand Washing)	No	2(25%)	2(12.5%)	12(17.9%)	1(16.7%)
	Yes	6(75%)	14(87.5%)	55(82.1%)	5(83.3%)
	p-value	0.63	0.25	0.39	0.36

* Mean significant $p < 0.05$

Discussion

Viral enteric pathogens are a major cause of diarrheal illness among children under the age of five, particularly in low- and middle-income countries where sanitation and hygiene infrastructure may be inadequate [7]. These viruses are highly contagious and are primarily transmitted through the fecal-oral route, including contact with contaminated hands, surfaces, food, and drinking water [3]. Unlike bacterial infections, viral diarrheal diseases are generally self-limiting, but they can still lead to serious complications such as severe dehydration, especially in infants and malnourished children. Among the most significant viral agents is rotavirus, which has historically been the leading cause of severe diarrhea and hospitalizations in young children worldwide [5].

In socio-demographic profile of the study participants, the majority of samples (42.1%) were from children aged 0–1 year, with 4–5-year-olds comprising 35.5%, and 2–3-year-olds 22.3%. Females slightly outnumbered males at 52.4% and 47.6%, respectively.

Most children (71.3%) lived in urban areas, while 24.3% were from semi-urban and 4.5% from rural areas. In terms of education, 63.2% attended crèche, 26.8% nursery, and 2.1% primary school. A large proportion (79.8%) of parents or guardians reported regular handwashing with soap and water. Regarding water sources, 55.7% of children used tap water, 25.6% used borehole water, while smaller numbers relied on well water (9.2%), sachet water (7.0%), and bottled water (2.6%). The findings of this study however differ from that of Omosigho *et al.*, 2024 in whose study majority of the participants were within the age of 4-5 years [12].

Viral enteric pathogens are widely acknowledged as major contributors to acute diarrheal illness in children under the age of five [3]. These viruses account for a significant share of diarrheal disease cases due to their ease of transmission, persistent presence in the environment, and the heightened vulnerability of young children whose immune systems are not yet fully developed [13]. The data of this study reveals Norovirus Type 1 had a relatively low prevalence, with only 8 children (2.9%) testing positive, while Norovirus Type 2 was found in 16 children (5.9%), indicating it was slightly more prevalent. The most frequently detected virus in the study was Adenovirus, which was found in 67 children (24.5%). The findings of this study contradict the findings of Omosigho *et al.* 2024 whose study showed that rotavirus was more commonly associated with diarrhea (27%) in infants and young children less than 5 years old in Offa Kwara State, however both studies identified the presence of co-infection among participants [12]. The combined prevalence of norovirus (8.8%) shares a slight similarity with a study in South Korea by Ryoo, (2020) which stated that norovirus was the second most common viral cause of diarrhea in children, contributing to about 20% of pediatric gastroenteritis cases [14]. The findings of this study also contradict the findings of Zhang *et al.* 2016 whose study found a high prevalence of 20% of co-infection of rotavirus group A and norovirus GII among asymptomatic children and 4.4% prevalence of co-infections among symptomatic children [15]. The reason for this prevalence can be attributed to the poor hygienic living conditions of participants.

Age is a key determinant in the susceptibility, severity, and prevalence of viral enteric infections in children. Infants and young children, particularly those under two years of age, are at greatest risk of contracting viral pathogens that cause diarrhea, due to immature immune systems, increased environmental exposure, and changing feeding practices [16]. The highest prevalence in this study was recorded among the age group 0-1 year. This agrees with the findings of Arowolo *et al.* 2020 who reported a higher prevalence in children aged 1-2 years in Ogun [17]. Similarly, the findings of this study also agree with the findings, 2016 which states that norovirus is most commonly detected in children under three years, with peak cases among those aged 12 to 24 months [18]. However, the findings of this study contradict the findings of an Nsukka study by Chigor *et al.* 2023 whose study find no statistically significant correlation between age and co-infections of rotavirus, norovirus and adenovirus [19]. The higher prevalence recorded in children in this age group (0-1 year) in this study could be due to the poor hygiene of children caregivers.

Gender-based differences in the incidence and distribution of viral enteric pathogens among children with diarrhea have been increasingly observed in recent epidemiological studies [20]. In this study, gender was found not to be statistically significant in association with adenovirus ($p=0.43$) and norovirus type 1 and 2 ($p=0.64$ and $p=0.90$) in children, the distribution showed males

(75%) compared to females (25%) for norovirus 1. For Norovirus Type 2, males (68.8%) and females (31.2%), Adenovirus was more balanced, with females (56.7%) and males (43.3%). The findings of this study agree with previous findings conducted in Ilorin by Hasanpourghadi *et al.* 2021 and Abdel-Rahman *et al.* 2021 in Qatar, whose study also exhibited similar trend [21, 22]. The reason for the higher prevalence of viral enteric pathogens in male than females may be attributed to the fact that male and female immune systems are regulated differently due to hormonal variations (e.g., Testosterone vs. Estrogen). Estrogen is known to enhance immune response, which may make females more resilient to viral infections [9].

The distribution of viral enteric pathogens among children under five is significantly influenced by residential setting, which encompasses urban, semi-urban, and rural environments [23]. The data from this study reveal notable disparities in infection prevalence based on where children live. Urban areas reported the highest cases of all viral enteric infections detected, including Norovirus Types 1 and 2, Adenovirus, and co-infections of Norovirus Type 2 and Adenovirus followed by semi-urban, with the least prevalence in rural settings. For example, 67.2% of adenovirus infections were recorded among children in urban settings, while only 3% occurred in rural areas. While Norovirus Type 1 and co-infections showed statistically significant associations ($p = 0.82$ and $p = 0.25$), the distribution of Adenovirus and Norovirus Type 2 was not statistically significant ($p = 0.0001$) The findings of this study however contradict the findings of Balami, (2020) whose study observed an annual increase prevalence of diarrhea among children living Kintampo North Municipality, a town consisting majority of rural areas [24]. The findings of this study also align with the study of Ezech *et al.*, 2017 whose study believes that overcrowding, poor sanitation, and intermittent access to clean water of people living in urban area could prove to be a health challenge [25].

The high burden in urban settings may be attributed to overcrowding, poor sanitation, and intermittent access to clean water, which are common challenges in densely populated neighborhoods, particularly urban slums [25]. Potential confounding variables such as urban crowding, asymptomatic carriers, and shared childcare materials (toys, mats, surfaces) may have influenced transmission patterns. These factors are common in crèche and daycare settings and could facilitate fecal-oral spread, especially among children with immature immunity.

The findings of this study are consistent with findings by (2016), which highlight that urban environments with poor sanitation and hygiene facilitate the spread of multiple viral pathogens and co-infections, especially among young children [18].

The data also show a statistically significant association ($p = 0.0001$) for norovirus type 1 and co-infection of norovirus type 2 and adenovirus based on place of residence. This suggests that residential setting is a key determinant of infection risk for these particular pathogens. Such co-infections may result in more severe clinical outcomes, emphasizing the importance of environmental and infrastructural factors in viral transmission dynamics [26]. The reason for the increased prevalence of viral enteric pathogens among urban inhabitants could be attributed to the poor infrastructure and overcrowding.

The spread of viral enteric pathogens in children under five is strongly affected by the type of early learning setting they are

exposed to, which reflects their level of social exposure, hygiene supervision, and contact patterns [27]. Children attending crèche accounted for the highest proportion of viral infections across all categories 75% of norovirus type 1 cases, 75% of norovirus type 2 cases, 70.1% of adenovirus infections, and 50% of norovirus type 2 and adenovirus co-infections. This pattern highlights the heightened vulnerability of infants and toddlers in daycare settings, who are typically in the 0–2-year age range, a critical window for gastrointestinal infections. A statistically significant p-value (0.0001) for Norovirus Type 1 and co-infections involving Norovirus Type 2 and Adenovirus suggests that infection prevalence is meaningfully associated with educational level, especially among children attending crèche. These findings are consistent with studies of Cox, (2022) and Enserink et al. 2015 that show group childcare environments significantly increase the risk of viral gastroenteritis due to close contact, shared toys, inadequate hygiene practices, and the frequent handling of soiled materials [28, 29]. In contrast, children in nursery and primary school had markedly fewer infections. Nursery attendees comprised 18.8% of norovirus type 2 cases and 28.4% of adenovirus infections, while children in primary school represented a very small fraction, only 1.5% of adenovirus cases and none of the co-infections. These lower rates may reflect better hygiene behaviors, reduce hand-to-mouth activity, and increase immune maturity in older children. These findings align with the study of Collins and Shane, (2017) indicating that younger children are disproportionately affected by enteric viral infections, particularly in institutional settings like daycare centers and crèches [30]. the increase in prevalence of viral enteric pathogens among children in crèche may be due to the fact that at that age the children have underdeveloped immune system and also due to constant close contact.

The source and quality of drinking water play a central role in the transmission of viral enteric pathogens, particularly in young children whose immune systems are still developing. In many low- and middle-income countries, unsafe water sources are major contributors to diarrheal diseases caused by viruses such as rotavirus, norovirus, adenovirus, and astrovirus [31]. This study found that children who consumed tap water had the highest prevalence of viral enteric pathogens, predominantly Adenovirus, followed by those who drank borehole water. Bottled water was associated with the lowest infection rates. The p-values were 0.086 for Norovirus Type 1, 0.96 for Norovirus Type 2, 0.082 for Adenovirus, and 0.054 for co-infections, indicating no statistical relevance. The findings of this study align with the findings of Ndzinisa, (2021) whose study show that children who consume untreated or inadequately treated water such as from streams, wells, or poorly maintained taps face a significantly higher risk of viral diarrhea and this is especially true for rotavirus and norovirus, which are environmentally stable and require very low infectious doses to cause disease [32]. The findings of this study also agree with the findings of Potgieter et al., 2020 whose study identified several viral enteric pathogens such as adenovirus, rotavirus and norovirus among households, however the participants used river water as sources of drinking water which differs from this study [33].

The co-infection of viral pathogens notably norovirus and adenovirus were also observed in various water sources. This supports previous findings by Makimaa et al., 2020 that intermittent access to safe water increases vulnerability to infection and can facilitate the spread of multiple enteric viruses simultaneously [34]. The increased prevalence in this study may be due to poor maintenance of water sources infrastructures such as cracked

pipes, open storage tanks, and unprotected wells allow microbial and chemical contaminants to enter water systems.

Parental and caregiver hygiene practices play a critical role in determining children's exposure to viral enteric pathogens, particularly during early childhood when children are highly vulnerable to gastrointestinal infections. Poor hygiene among parents and guardians, especially inadequate handwashing after toileting or before food handling, significantly increases the risk of fecal-oral transmission of viruses such as rotavirus, norovirus, adenovirus, and astrovirus [35]. In this study, children whose parents or guardians consistently practice regular handwashing with soap and water exhibited a higher prevalence of viral infections, especially norovirus type 1, type 2, and adenovirus. The p-values were 0.63 for Norovirus Type 1, 0.25 for Norovirus Type 2, 0.39 for Adenovirus, and 0.36 for co-infections, showing no statistical significance. This finding contradicts with previous research by Oloruntoba et al., 2014 which indicated that poor caregiver hygiene is closely associated with higher incidence of pediatric diarrheal diseases, particularly in households with young children under five. Also, a study by wolf et al., 2022 show that handwashing with soap can reduce the risk of diarrheal diseases by up to 40%, highlighting its effectiveness as a simple yet powerful preventive measure [36]. The reason for increased prevalence among parents despite continuous practice of personal hygiene could be due to environmental contamination as Viruses like rotavirus and norovirus are extremely hardy and can survive on surfaces such as toys, floors, doorknobs, and tables for days.

Conclusion

This study aligns with global health efforts to reduce child mortality (SDG 3.2) and promote access to clean water and sanitation (SDGs 6.1 and 6.2). By identifying viral enteric pathogens and their associations with environmental and hygiene factors, the findings contribute to ongoing strategies for achieving these goals, particularly in low- and middle-income settings. The study provided insights into the burden, distribution, and transmission dynamics of key viral agents. Based on the findings, we recommend scaling up routine rotavirus vaccination coverage for children under two years of age, particularly in high-risk urban communities. Public health strategies should prioritize improving water infrastructure to ensure safe drinking water and promote targeted hygiene education, especially in early childcare centers where viral transmission is likely.

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