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Research Paper

Determinants of Rotavirus among diarrheal children (0-5yrs) attending some health facilities in Sokoto Town, Nigeria

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Abstract

The aim of this research was to identify and examine Rotaviruses at a molecular level and to explore connections between Rotavirus and certain influencing factors among children in specific hospitals in Sokoto State. A collection of 350 stool samples was gathered from children under the age of five who were experiencing diarrhea. These samples underwent analysis to detect the presence of Group A Rotavirus antigens, utilizing the CUSABIO Human Rotavirus (RV Ag) ELISA kit. Result reveals that, children who were fed with breast milk and solid foods had the highest prevalence (39.6%), while children fed with solid food recorded only 15.1% prevalence. Chi-square test revealed statistically significant relationship between Rotavirus diarrhea and type of food fed, $p<0.05$ (0.0204). Major sources of water of the families of infected children were tap water (54.75%) and well water (39.6%) respectively, while river and pond water were 3.8% and 1.9% respectively. Children who often had contact with animals have high prevalence (69.8%) than their counterparts (5.7%). Chi-square test revealed a significant association of Rotavirus diarrhea and child's contact with animals ($p<0.05$). The percentage positive was higher in children whose parents were civil servants (47.2%) and was lower in children of un-employed parents (28.3%). Children whose parents had primary level of educations experienced the highest number of rotavirus disease. There was a significant association between Rotavirus disease and type of food, source of water, contact with animals and level of education of the parents. Interventions including awareness creation, and routine screening of Rotavirus at hospitals will be helpful to protect the public health against the Rotavirus.

Introduction

On many occasions, children or infants are inflicted with an infection incited by Rotavirus, a virus type that spurs diarrhea (Pius et al., 2023). Rotavirus exists as non-enveloped, possessing double stranded type of RNA, and is a member in the Reoviridae (Dossouvi et al., 2023). Around the world about 128, 500 people are dying due to Rotavirus, and Sub-Saharan areas of the world contributed 70% of the cumulative deaths due to the virus (Gbebangi-Mauzemu et al., 2023). Circa 258 million incidences occur every year as a result of Rotavirus, therein 24 million of them cause hospitalizations (Abdou et al., 2023; Ali et al., 2023). More often Rotavirus is causing infections that modify the morphology of the cells in the duodenum and in turn fever, vomiting, lethargy, hypovolemia, dehydration, diarrhea and even death in children (Jampanil et al., 2023; Mir et al., 2023). Evidences have related that the Rotavirus infection can be distributed to other parts of the body such as blood, respiratory system, nervous system, lymphs, urinary system, and liver (Pius et al., 2023). The fecal-oral pathway plays a vital role in the transmission of Rotavirus. Thus, environment, sanitation, behaviors, and relations could positively or negatively affect the spread of Rotavirus (Ali et al., 2023; Islam et al., 2023; Jampanil et al., 2023; Mir et al., 2023).

Although, hospitals are major elements in the prevention of Rotavirus infection, there are difficulties (such as cost of testing the Rotavirus) that prevent them from routinely diagnosing the virus (Ali et al., 2023; Digwo et al., 2023). Likewise, cost, cultural biases, and other issues have constantly affected the vaccination as an effective tool in circumventing the effects of Rotavirus or other infectious problems in the state (Ali et al., 2023; Pius et al., 2023). In the same vein, the effectiveness of healthcare systems in according secondary or other higher levels of prevention services to children suffering from Rotavirus problems is militated due to factors such as scarcity of personnel, scarcity of healthcare facilities, and traditional medicines among others (Pius et al., 2023). Therefore, the effects of Rotavirus among children in the state such as Sokoto (with poor settings in rural dwellings) are multifold and could not be controlled effectively without knowing the associated factors or proper utilization of public health strategies. A study of this kind revealed the actual determinants inciting the Rotavirus diarrheal problems in children with a view to suggest better ways of managing issues at societal or public health level, and with the aim to lay foundation for policy makers to take attention and formulate better public health policies. Similarly, the study could encourage scholars to have a foundation for further studies aimed at improving public health at community level (Digwo et al., 2023; Nazari et al., 2023). The objective of this work was to survey determinants of Rotavirus among diarrheal children (0-5yrs) attending some health facilities in Sokoto Town, Nigeria and the link to some determinants of the Rotavirus.

Materials and Methods

Study Area

The current study was conducted in Sokoto state of Nigeria, situated in the extreme Northwestern region of the country, spanning between latitudes 12^0N and $13^0$58'N, and longitudes 4^0 8'E and $6^0$54'E, and. Encompassing an approximate land area of 32,000 square kilometers, Sokoto is home to approximately 5,307,154 individuals (2,706,649 male and 2,600,506 female), with 97.7 persons/km^2 (NIPC, 2021). Sokoto is bordered by the Republic of Niger to the north, by Kebbi state to the west and southwest, and by Zamfara state to the east. The exact location of the study was shown by the map in the Figure 1.

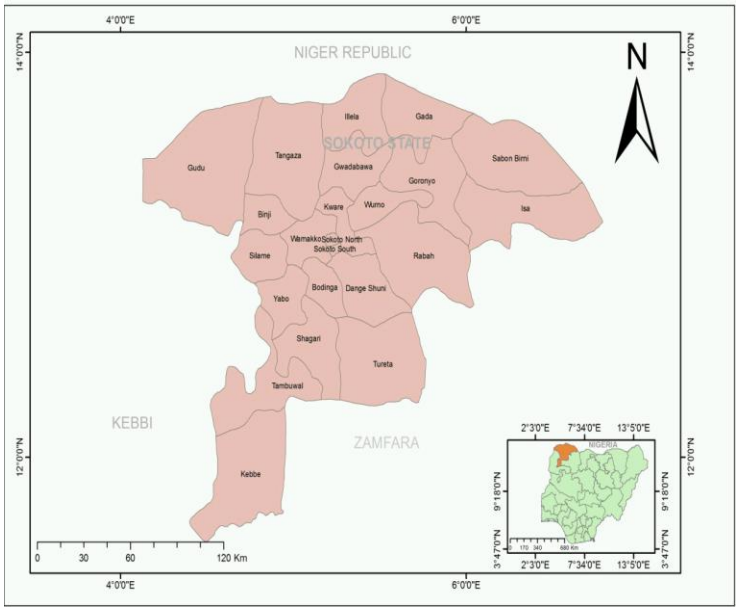

Figure 1. Map of the study location; Source: Sarkingobir et al. (2023)

Furthermore, this state comprises individuals from diverse educational, socio-economic and religious backgrounds, residing in the neighborhoods with varying sanitation levels. The primary sources of drinking water supply include tap water, boreholes, and wells. Healthcare facilities, often located tens of kilometers away, serve the populace, while pit latrines are prevalent in the majority of households. The predominant livelihoods involve cattle rearing and subsistence farming (Aminu et al., 2008). The study was carried in select hospitals within Sokoto Metropolis, such as Specialist Hospital (SH), and Usmanu Danfodiyo University Teaching Hospital (UDUTH) which cater to patients from both the capital city and surrounding areas within the state and neighboring states.

Inclusion Criteria

The study population comprised children under five years old who presented with diarrhea at SH and UDUTH between May 2019 and April 2020. The study only enrolled children whose parents provided signed informed consent forms. Those under five years old who didn't have any diarrhea symptoms were excluded from participation.

Sampling Design

The sample size was determined utilizing the equation: $n = t^2 \times p (1-p) / d^2$ (Sarmukaddam & Garad, 2004). The expected prevalence (denoted as 'p') was derived from prior research conducted by Alkali et al. (2016), revealing 25.5% occurrence rate of Rotavirus infection among animals and humans in Sokoto metropolis. In the above equation:

n = sample size required
t = confidence intervals at 95% (standard value of 1.96)
p = The approximate occurrence rate of Human Rotavirus infection detected among children in

Sokoto, Nigeria is 25.5% (Alkali et al., 2016).
d = desired absolute precision (5%)

Therefore:

$n = (1.96)^2 x\ 0.255(1-0.255)/0.05^2$

$n = 3.84x\ 0.255x0.75/0.0025$

$n = 0.73/0.0025$

$n = 292$

Using the above formula, the minimum required sample size for the study was determined to be 292. However, to enhance the likelihood of obtaining positive samples, a total of 350 samples were utilized. Among these, 200 samples were gathered from SH Sokoto, while 150 samples originated from UDUTH. The study employed a simple random sampling technique, ensuring that every child meeting the inclusion criteria had an equal opportunity to be selected from the study population.

Sample Collection, Transportation and Preservation

A case of diarrhea in children was characterized by the passage of three or more loose, liquid, or watery stools within a 24-hour period. Following examination by the physician at the pediatric outpatient clinic, 5ml samples of diarrheic stool were obtained from each child by using sterile universal containers. These containers were appropriately covered, labeled, and transported on ice to the Research Laboratory at the Usman Danfodiyo University Sokoto, where they were stored at -20°C for subsequent analysis. A ten percent fecal suspension was then prepared using phosphate-buffered saline and stored at 4°C.

Sample Processing

Detection of rotavirus

A commercially available ELISA kit for Human Rotavirus antigen (RV Ag) from CUSABIO BIOTECH CO., LTD. (www.cusabio.com) was employed to measure the concentrations of Human Rotavirus antigen (RV Ag) in fecal samples collected from the participants of the study. The procedure followed the instructions provided by the manufacturer.

Preparation of the wash buffer

In 9ml of distilled water, 1ml of the wash buffer concentrate was added. The crystals, if present in the concentrate was allowed to dissolve in a water bath at 37°C.

Test procedure

Micro plate well labeled ‘A1 was set as blank (without any solution). In other micro plate wells labeled ‘B, C, D, 50µl of Negative control was added to each respectively. Then 50µl of positive controls was added to microplate wells labelled E and F respectively. To the remaining 90 wells, 50 µl of the stool suspensions was then added to each well. To each well, 50µl of the Horseradish peroxidase (HRP) conjugate was added except the blank well. Then covered with adhesive strip provided and incubated at room temperature for 15 minutes. Each well was aspirated and washed

by adding 50µl to each well, the plate was washed off 10 times with 500µl of the wash buffer. After washing the plate, 50µl of Substrate A and 50µl Substrate B was added to each well and incubated at room temperature in the dark for 10 minutes. The reaction was stopped by adding 50µl of stop solution to each well. The blank well was taken as zero and the optical density of each well was determined within 10 minutes, using a microplate reader set to 450nm (Dorsey et al., 2017).

Determination of results

The result was read visually and spectrophotometrically and interpreted as follows:

A. Visual observation

Positive samples show blue to yellow colour before and after addition of stop reagent respectively, while negative samples remained colorless.

B. Photometric readings

Calculation of cut-off values: Cut-off value = the average value of OD negative + 0.1 (if OD negative<0.05, calculated as 0.05), the negative control OD value was less than 0.1, and the positive control OD value should be greater than 0.8. The average value of OD negative = OD of B +C+D/3 = 0.500+0.513+0.532/3 = 0.515+0.1 = 0.615.

Therefore, the results were interpreted as Positive: While OD sample ≥ Cut-off value and Negative: While OD sample < Cut-off value.

Statistical Analysis

The data obtained from this research were analyzed using IBM SPSS software, version 22 (IBM Corp, Armonk, NY, USA). Absolute and relative frequency (%) were used for descriptive statistics of categorical and ordinal variables. The Chi-square test for tables were used to compare categorical variable proportions. A p-value less than 0.05 was considered to be significant and associations were expressed in odds ratio (OR) and respective 95% confidence interval. When the p-value was less than 0.05, the mean between the two groups were considered significantly different.

Ethical Considerations

The ethical clearance was obtained from the ethical committee of Specialist Hospital Sokoto and Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Sokoto State before the commencement of the research work. Informed written consent was also obtained from the parents and/ or guardian of each child prior to inclusion in the study. A questionnaire was administered to obtain socio-demographic data from the parents or legal guardians of each child such as identification number, address, gender, age, and education level of the mother, breastfeeding, sanitation condition, child's contact with animals and drinking water source.

Results and Discussion

Table 1: presents prevalence of Rotavirus diarrhea in children with respect to their food type, children who were fed with breast milk and solid foods had the highest prevalence (39.6%) while children who were fed with solid food recorded only 15.1 prevalence. Chi-square analysis

indicated statistically significant relationship between Rotavirus diarrhea and type of food fed, $p<0.05$ (0.0204).

Family water sources and the distributions of Rotavirus diarrhea from children in Sokoto State are presented on Table 2 and had shown the summary of the rate of detection of rotavirus according to source of water. The major sources of water of families of infected children were Tap water (54.75%) and Well water (39.6%) respectively, while river and pond water were 3.8% and 1.9% respectively and there was a statistical association between rotavirus diarrhea and source of water ($p<0.05$).

Distributions of Rotavirus diarrhea among children that had contact with animals are presented in Table 3. The results of the distribution of rotavirus diarrhea in children in Sokoto according to child's contact with animals have shown that children who often had contact with animals have high prevalence (69.8%) and a lower prevalence was recorded in children that never had contact with animals (5.7%). Chi-square analysis indicated significant association of Rotavirus diarrhea and child's contact with animals ($p<0.05$).

Table 1: Prevalence of Rotavirus diarrhea in children with respect to their food type in Sokoto State.

Type of food	**No. Negative**	**No. Positives**	**Percentage positive**
Breast feeding only	76	15	28.3
Breast feeding and solid food	65	21	39.6
Solid food only	75	09	17.0
Others	81	08	15.1
Total	297	53	100

p-value=0.0204, X^2= 9.791, 3

Table 2: Family water sources and the distributions of Rotavirus diarrhea among children in Sokoto State.

Source of Water	**No. Negative**	**No. Positive**	**Percentage Positive**
Tap	126	29	54.7
Well	99	21	39.6
River	51	02	3.8
Pond	21	01	1.9
Total	297	53	100

p-value=0.0255, X^2= 9.308

Table 3: Distributions of Rotavirus diarrhea among children that had contact with animals in Sokoto State.

Contact with Animal	No. Negative	No. Positive	Percentage Positive
Often	147	37	69.8
Rare	80	13	24.5
Never	70	03	5.7
Total	297	53	100

P- value= 0.0051, X^2= 38.503

Distribution of Rotavirus diarrhea among children according to occupation of the parents in Sokoto state are presented in Table 4. The percentage positive was higher in children whose parents are civil servants (47.2%) and is lower in children of unemployed parents (28.3%). The results when subjected to statistical analysis did not show any statistically significant difference between the Rotavirus diarrhea and occupation of the parents (p>0.05; 0.4978).

Table 4: Distributions of Rotavirus diarrhea among children according to occupation of the parents in Sokoto State.

Occupation of the parents	No. Negatives	No. Positives	% Positives
Civil servant	123	25	47.2
Self-employed	97	13	24.5
Unemployed	77	15	28.3
Total	297	53	100

P-value=0.4978, X^2=1.395, 2

Distributions of Rotavirus diarrhea among children according to level of education of the parents in Sokoto State are presented in Table 5. Children whose parents had primary level of educations had the highest number of Rotavirus disease, while children whose parents had tertiary level of education had the lowest percentage of the disease. The results on the distribution of Rotavirus diarrhea in children in Sokoto State according to parent's level of education had shown an association between Rotavirus diarrhea among children and parents' level of education ($p<0.05$).

Table 5: Distributions of Rotavirus diarrhea among children according to level of education of the parents in Sokoto State.

Parents educational level	No. Negative	No. Positive	Percentage Positive
Formal	87	07	13.2
Primary	100	33	62.3
Secondary	50	10	18.9
Tertiary	60	03	5.7
Total	**297**	**53**	**100**

p- value= 0.0051, X^2= 0.1953

The Figure 2 shows the prevalence of Rotavirus diarrhea according to child's attendance of Day Care center. The results showed that most of the infected children attended Day Care Center (73.6%). While few of the infected children did not attend Day Care Center (26.4%). However, Chi-square analysis indicated nonsignificant association between Rotavirus diarrhea and attendance of Day Care Center (*p>0.05*).

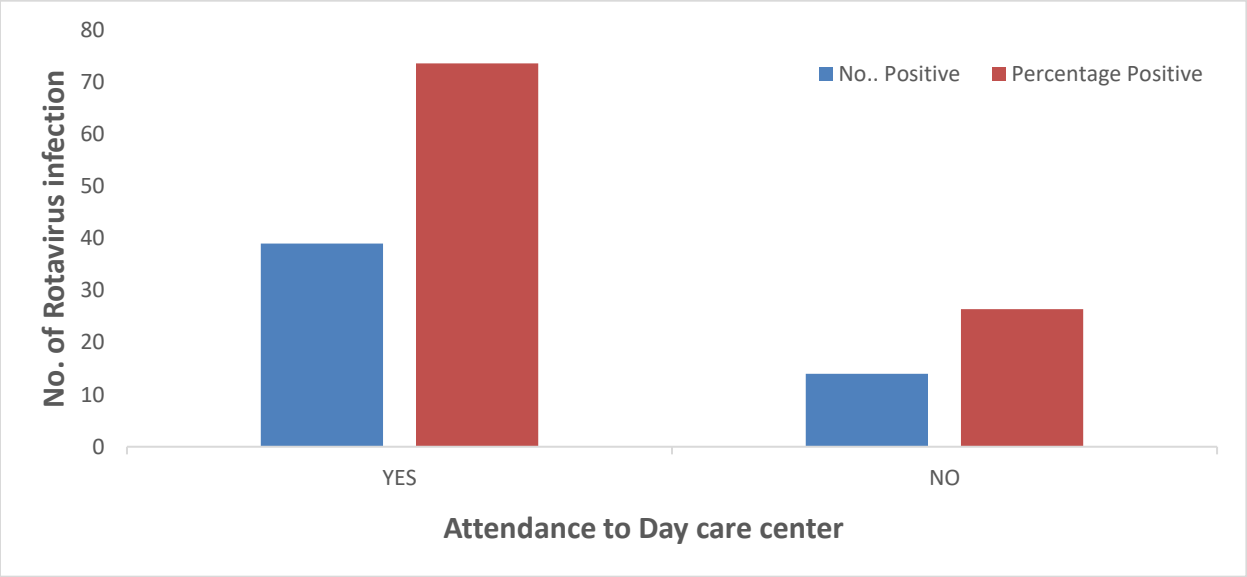

Figure 2: Prevalence of Rotavirus Diarrhea according to child's attendance to Day Care Center in Sokoto.

Type of child's food in relation to Rotavirus diarrhea showed a higher prevalence among children fed with breast milk and solid food (39.6%) and low prevalence was recorded among children fed exclusively with breast milk (15.1%). Statistical analysis indicated significant relationship between Rotavirus diarrhea and type of food (*p<0.05*). This may be due to the presence of Rotavirus antibodies in breast milk and other components that reduce the risk of Rotavirus

infection in babies that are fed exclusively with breast milk. A similar work reports that exclusive breastfeeding is significant. Therewith, breast milk provides the child with both nutritional and immunological support needed for growth and development (Musa et al., 2023). Thus, consumption of breast milk and solid foods that show highest association/prevalence of diarrhea due to Rotavirus might be due to contamination resulted from poor personal hygiene, poor hygiene, and poor preparation of the foods (Abdullahi et al., 2023; Garatsa et al., 2023). Meta-analysis performed in a study in Indonesia reveals that lack of exclusive breastfeeding is a risk factor of Rotavirus diarrhea (Pratama et al., 2023). The reason why children fed with breast milk and solid foods show more Rotavirus infection than others could be due to lack of exclusive breastfeeding. A study in Bangladesh reported that children 0-6 months old that were not exclusively breastfed experience more diseases including diarrhea (Abdulla et al., 2022). Berhanu et al. (2015) defined non-exclusive breastfeeding as a practice of feeding children (0-6 moths) with other food sources apart from milk, and it has a great impact on health, as well as developing child.

The results on the distribution of Rotavirus diarrhea in children in Sokoto state according to parent's level of education had shown that, there was an association between Rotavirus diarrhea among children and parents' level of education ($p<0.05$). Children whose parents attended secondary school and those not educated (western education) had children with highest prevalence of rotavirus excretion. This in accordance with the submitted findings elsewhere by Musa et al. (2023) who related those higher levels of education resulted in better health. Education people have lower occurrence of mortality and morbidity as well as disability. Educated people have better economy that positively help health and education implore people to know things and act better (Musa et al., 2023; Garba et al., 2023; Umar et al., 2023). Garatsa et al. (2023) showed that parental education improves determinants of diarrhea such as water, sanitation, and hygiene. Poor parental education risk children with Rotavirus diarrhea (Pratama et al., 2023).

Meanwhile, the result presented on the distribution of Rotavirus diarrhea according to occupation of the parents in Sokoto state did not show statistically significant difference between the Rotavirus diarrhea and occupation of the parents ($p>0.05$), but highest prevalence of Rotavirus excretion was recorded in children whose parents are farmers and business men and women (self-employed). These observations were not statistically significant ($p>0.05$) indicating that observed differences may be due to chance and not a certainty and this implies that rotavirus infects regardless of parent's demographic characteristics. Certainly, occupation of parents affects the health of their wards (Dikko et al., 2022). Occupation is a socioeconomic index that when at high level helps people to afford healthcare and make positive healthy promotion decisions as well. Likewise, lower occupation shows lower economic level and hinders health in many respects including the uptake of immunization (Dikko et al., 2022; Garatsa et al., 2023). High income is related to low prevalence of Rotavirus diarrhea, while low-income experience high infection (Nazari et al., 2023). However, the reason why children of civil servants are more affected with Rotavirus could be because, civil servants are more prone to engage in non-exclusive breastfeeding, a practice that increases infection. Likewise, the civil servants are more prone to knowledge of complementary feeding and inurn no-exclusive breastfeeding (Abdulla et al., 2022).

The relative frequency of infected children was higher in children attending school daycare centers in comparison with those children who do not attend daycare centers. This indicated a significant relationship between rotavirus infections and environment (Daycare Centre) or lower rate of breast feeding in these children ($p<0.05$). Therefore, this finding suggested that the rotavirus can be transmitted due to close contact with the infected children.

It was similarly observed that, higher prevalence was recorded in children who had close contact with animals and lower prevalence was recorded in children who rarely had contact with animals. Therefore, interspecies transmission and, more importantly exchange of genetic material between animal and human strains through gene assortment can lead to the emergence of novel Rotavirus Group A (Garba et al., 2023; Lenguiya et al., 2023). Living close to animals is reported factor associated with possibility of transmission of communicable diseases. In a study done by Youssef et al. (2023) living close to chicken and calves was associated with Rotavirus infection in children. This conforms with another work that showed animals could be source/reservoir of Rotavirus infection affecting humans in Thailand (Jampanil et al., 2023).

In current study, it was also found that tap water, and well water consumption was linked to Rotavirus diarrhea more often. This observation could be due to contamination of water. The risk of contamination of tap, and well water could be higher than that of pond and river water and in turn the difference in Rotavirus prevalence. In another work, it was reported that poor sanitation and poor water quality was recorded a risk of Rotavirus infection in India (Abdullahi et al., 2023; Mir et al., 2023). The reason why children from families with tap and well water sources show more Rotavirus positives than families with pond and river could be because tap water is supplied through pipes that are nowadays flowing via gutters or sewages or waste materials that due to cracks or relations the microbes and other harmful pollutants find their ways into the water been supplied to the public. Additionally, there were reports showing that municipal water supply system could not remove all the water pollutants (microbial or chemical in nature) the water due to either lack of machines or technical- knowhow (Ugwu etal., 2016; Wali et al., 2022). In this vein, many reports reiterated that, tap water are not been completely purified before releasing to the public domain (Madueke et al., 2014; Ugwu etal., 2016; Wali et al., 2022). Many of the wells in the state are mostly open, in turn allowing microbes to get into the water via the course of open defecation. Knowing fully that, open defecation is a major practice in rural areas in the state (Abdullahi et al., 2023).

Conclusion

There is a significant association between Rotavirus disease and type of food, source of water, contact with animals and level of education of the parents. A combination of determinants needs to be addressed to curtail the problems of Rotavirus in children at community level.

Recommendations

Considering the findings of this research work, the following recommendations are suggested:

i) Rotavirus detection in diarrhea cases should be included in routine laboratory tests for effective diagnosis and treatment of the infection.
ii) Parents/guardians and teachers in daycare centers should be enlighten on the mode of transmission of rotaviruses in their various places and how they can prevent children from rotavirus diarrhea.
iii) Sokoto state government, as a matter of urgency should include rotavirus vaccines in routine immunization to children fewer than 5 years of age and should provide a supportive care in the management of the disease in the state.
iv) Strain diversity is a factor in vaccine effectiveness in low- and middle-income countries and this demand intensive yearly surveillance of Rotavirus in the study area and other part of the country.

v) Environmental samples should also be used instead of clinical samples to compare and ascertain Rotavirus load in the study area.
vi) Human and animal samples of a given area should be included in characterization Rotaviruses to determine the true picture of interspecies transmission.
vii) The circulating strains identified in the present study should be considered in the production and implementations of vaccines in the study area.

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