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Rotavirus disease (gastroenteritis) in children with diarrhea (0-5 years): Determined prevalence in selected hospitals of Sokoto City, Nigeria

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ABSTRACT

Rotavirus is the most common cause of severe diarrhea among children under five years of age worldwide. This study was conducted to detect the prevalence of Rotaviruses in children attending selected hospitals in Sokoto State. Three hundred fifty stool samples were collected from diarrheic children under five years of age. Samples were analyzed for Group A rotavirus antigens using the CUSABIO Human Rotavirus (RV Ag) ELISA kit. Rotaviruses were detected in stool samples of 53/350 children, given a 15.1% prevalence, with the infection occurring throughout the study period. The percentage of Rotavirus infections was 52.8% and 47.2 % among males and females, respectively. The Rotavirus cases were significantly higher in children below two years of age 32/53 (60.4%) than children between 2-5 years of age 21/53 (39.6%). The result further showed that 35/53(66.1%) of the positive cases exhibited three major symptoms of the disease, while 13/53 (24.5%) exhibited diarrhea with either fever or vomiting, while 9.4%(5/53) showed only diarrhea. Vaccination and other related interventions, including routine diagnosis, should be provided to safeguard public health against the Rotavirus.

ABSTRAK

Rotavirus adalah penyebab paling umum diare parah pada anak di bawah lima tahun di seluruh dunia. Penelitian ini dilakukan untuk mendeteksi prevalensi Rotavirus pada anak-anak yang dirawat di rumah sakit tertentu di Negara Bagian Sokoto. Tiga ratus lima puluh sampel tinja dikumpulkan dari anak-anak di bawah usia lima tahun yang menderita diare. Sampel dianalisis untuk antigen rotavirus Grup A menggunakan kit ELISA CUSABIO Human Rotavirus (RV Ag). Rotavirus terdeteksi pada sampel tinja 53/350 anak, dengan prevalensi 15,1%, dengan infeksi terjadi sepanjang masa penelitian. Persentase infeksi Rotavirus masing-masing adalah 52,8% dan 47,2% pada laki-laki dan perempuan. Kasus Rotavirus secara signifikan lebih tinggi pada anak-anak di bawah usia dua tahun 32/53 (60,4%) dibandingkan anak-anak antara usia 2-5 tahun 21/53 (39,6%). Hasilnya lebih lanjut menunjukkan bahwa 35/53 (66,1%) dari kasus positif menunjukkan tiga gejala utama penyakit, sementara 13/53 (24,5%) menunjukkan diare disertai demam atau muntah, sedangkan 9,4% (5/53) hanya menunjukkan gejala penyakit yang sama. diare. Vaksinasi dan intervensi terkait lainnya, termasuk diagnosis rutin, harus diberikan untuk menjaga kesehatan masyarakat terhadap Rotavirus.

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1. Introduction

The Rotavirus is distinguished for being a segment possessing RNA virus type, lacking an envelope, and being a member of the family Reoviridae. This virus is famous for causing a scourge of severe gastroenteritis among younger children across all parts of the world [1-2]. It was related that children in Industrialized, developing, and underdeveloped nations are at risk of Rotavirus; that is, children at two years old are potential victims of the virus [3]. Every time and season of the year, the Rotavirus does not spare children from its infection, therefore becoming a public health thorn worldwide [3]. The initial two years of the life of children are of more concern because, in addition to that, the Rotavirus starts its striking invasion of many children



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(especially in poor settings or countries) and causes mortalities every year [4]. About 30-50% of diarrhea in children is due to Rotavirus, and the prevalence is still high [2, 4]. Poor settings such as poor hygiene and sanitation (eliciting fecal-oral transmission), contaminated food intake, contact with contaminated animate or inanimate objects, age, poor hygiene, poor water supply, and poor waste management practices are some of the resultant determinants leading to Rotavirus diarrhea [5, 6]. The symptoms of the disease manifest in forms such as fever, vomiting, abdominal complaints, nausea, watery stool, cough, and runny nose among the affected children [5].

Indeed, in the sub-saharan parts of the African continent, gastroenteritis is a huge burden on children, aggravated by poor politics, poor amenities, and poor economy. In addition to that, mortalities and morbidities are recorded. A survey of 15 African countries revealed that, out of every 25 million children born yearly in the sub-sahara regions, 4.3 million of them are joining their ancestors; in addition to that, 90% of the deaths are linked to diarrhea, and most of the factors of the diarrhea are emanating from infection [1]. Nigeria is categorized among the forefront countries with a Rotavirus infection rate; in addition to that, about 33,000 mortalities occur yearly among children [5].

However, the most important intervention towards the scourge of Rotavirus is vaccination, among other strategies. But, any intervention needs information for making policies and plans [4-5]. Therefore, there needs to be more information regarding the prevalence of Rotavirus in Nigeria and Sokoto (mainly). Likewise, the Rotavirus is misdiagnosed and mistreated in many hospitals because of difficulties [5]. Thus, this study aimed to detect and determine the Prevalence of Rotavirus disease (gastroenteritis) in Children with diarrhea (0-5 years) in selected hospitals in Sokoto metropolis.

2. Method

2.1. Study area

The study area is Sokoto State of Nigeria. The state is located at the extreme Northwestern Nigeria between longitudes 40 8'E and 60 54'E and between latitudes 120N and 13058'N. The state covers a total land area of about 32,000 square km; it has approximately 5,307,154 (Male- 2,706,649 and Female- 2,600,506) people, suggesting a population density of 97.7 persons per square km [7]. Sokoto forms boundaries with the Republic of Niger to the North, Kebbi State to the West and Southwest, and Zamfara State to the East.

Moreover, the state is an area with people of different religious, educational, and socio-economic backgrounds living in neighborhoods with distinctly different levels of sanitation. Tap, Borehole, and well water are the primary sources of drinking water supply in this area. Clinics and health care centers may be up to tens of kilometers away, and most houses have pit latrines. The main occupations here are cattle rearing and subsistence farming [8]. The research was conducted in some selected hospitals in Sokoto Metropolis, including Usmanu Danfodiyo University Teaching Hospital (UDUTH) and Specialist Hospital (SH), Sokoto. All these hospitals are located in the Sokoto metropolis, the capital of the state, receiving patients from different parts of the state and neighboring states.

2.1.1. Inclusion and exclusion criteria

Children under five years of age with complaints of diarrhea attending any of the two selected hospitals (SH and UDUTH) from May 2019 to April 2020 and whose parents had signed written informed consent forms were chosen as the study population. Children under five years of age without any complaints of diarrhea were excluded from the study.

2.1.2. 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 each child's parents and/or guardians before inclusion in the study. A questionnaire was administered to obtain socio-demographic data from each child's parents or legal guardians, 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. The clinical variables such as signs and symptoms, duration of diarrhea, vomiting, and fever were also registered.

2.2. Study design

The study is cross-sectional. Children of ages 0-5 years attending any of the two (2) selected hospitals mentioned above with complaints of diarrhea during twelve months (May 2019-April 2020) were considered.

2.3. Sample size determination

The size of samples was calculated using the formula [9]:

$$n = \frac{t^2 p(1-p)}{d^2} \quad (1)$$

Description:

n = Required sample size

t = Confidence intervals at 95% (standard value of 1.96)

p = Estimated prevalence of Human Rotavirus infection observed in children in Sokoto, Nigeria is 25.5% [1].

d = Desired absolute precision (5%)

The 'p' value (expected prevalence) was obtained from the previous work by [1], who reported a prevalence rate of 25.5% of rotavirus infection among humans and animals in the Sokoto metropolis. Therefore,

$$n = \frac{(1.96)^2 (0.255)(1-0.255)}{0.05^2} = \frac{(3.84)(0.255)(0.75)}{0.0025} = \frac{0.73}{0.0025} = 292$$

Based on this formula, the minimum sample size calculated for this study was 292, but this study used 350 samples to increase the probability of positive samples.

2.4. Sampling method

A simple random sampling technique was applied for this study where each child in the study population had an equal chance of sampling, provided the child met the criteria for inclusion. Only children under five who complained of diarrhea attended any of the two selected hospitals whose parents/guardians filled out the questionnaire and signed a written consent form were recruited for the study. Two hundred (200) samples were collected from Specialist Hospital Sokoto, and one hundred and fifty samples (150) from Usman Danfodiyo University Teaching Hospital Sokoto (UDUTH).

2.5. Sample collection, preservation, and transportation

Three hundred fifty stool samples from children 0- 5 years of age with diarrhea were collected in these two selected Hospitals after the physician examined each patient at the pediatric outpatient clinic. A diarrhea case was defined as a child passing three or more loose, liquid, or watery stools within 24 hours. Exactly 5mls of diarrheic stool samples were collected from each child using sterile wide-mouth universal containers, which were covered and labeled accordingly. They were transported on ice to a Research laboratory at the Faculty of Veterinary, Usman Danfodiyo University Sokoto, for analysis and stored at -20°C. 10% fecal suspension was prepared using phosphate buffer saline and was kept at 4°C.

2.6. Sample processing

2.6.1. Detection of rotavirus

A commercial Cusabio Human Rotavirus Antigen (RV Ag) Elisa kit (Cusabio Biotech Co., Ltd. www. Cusabio.com) was used to detect Human Rotavirus Antigen (RV Ag) concentrations in the fecal samples obtained from the study subjects. The test was conducted following the Manufacturer's instructions.

2.6.2. Preparation of the wash buffer

1 ml of the wash buffer concentrate was added to 9 ml of distilled water. If the crystals were present in the concentrate, they were allowed to dissolve beforehand by warming in a water bath at 37°C.

2.6.3. Test procedure

The microplate well labeled A1 was set as blank (without any solution). In a microplate, wells labeled B, C, D, and 50µl of Negative control were added to each. Then, 50µl of positive controls was added to microplate wells marked E and F, respectively. 50 µl of the stool suspensions were added to each well to the remaining 90 wells. Each well-added 50µl of the Horseradish Peroxidase (HRP) conjugate except the blank well. Then, it was covered with an 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 ten times with 500µl of the wash buffer. After washing the plate, 50µl of Substrate A and 50µl Substrate B were 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 [10].

2.7. Determination of results

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

2.7.1. Visual observation

Positive samples showed blue to yellow color before and after adding the stop reagent, respectively, while negative samples remained colorless.

2.7.2. 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, i.e. the average value of OD negative = $OD \text{ of } B + C + \frac{D}{3} = 0.500 + 0.513 + \frac{0.532}{3} = 0.515 + 0.1 = 0.615$.

Therefore, the results were interpreted as follows:

- a. Positive: While OD sample \geq cut-off value,
- b. Negative: While OD sample < cut-off value,

2.8. 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 was used to compare categorical variable proportions such as locations, months, sex, age, food type, and clinical symptoms between group A rotavirus-positive and group A rotavirus-negative Children. A p-value less than 0.05 was considered 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 was considered significantly different.

3. Results and Discussion

3.1. Results

A total number of 350 fecal samples were screened with a Cusabio Elisa Kit for the presence of rotavirus antigens; out of this number, 200 were collected from SH Sokoto and 150 from UDUTH Sokoto, all in Sokoto metropolis, a capital city of Sokoto state. Fifty-three (53) of the screened samples were

recorded positive. At the same time, two hundred and ninety-seven (297) were found negative, which accounted for a 15.1% overall detection rate of rotavirus diarrhea in Sokoto State. Thirty (30) positive cases were from SH Sokoto and Twenty-three (23) from UDUTH Sokoto, as shown in Table 1. The result of the distribution of rotavirus for Hospitals has not shown any statistically significant difference because the p -value > 0.05 (0.9999).

The sex distribution of Rotavirus disease among children in Sokoto is presented in Table 2. The result shows that males (52.8%) are more associated with rotavirus than females (47.2%). Chi-square analysis did not show any statistically significant differences between males and females because ' p ' > 0.05 . The result of Rotavirus diarrhea and associated symptoms of infected children in Sokoto shows that 66.1% of the positive cases exhibited three major disease symptoms. In comparison, 24.5% exhibited diarrhea with either fever or vomiting, while 9.4% had only diarrhea, as presented in Table 3. The rotavirus disease occurred throughout the study period (May 2019- April 2020). However, positive cases increased in June 2019, followed by July 2019. There was a statistically significant difference between the prevalence of rotavirus and monthly distribution because the ' p ' < 0.05 (0.0028), as shown in Figure 1.

Table 1. Detection rate of Rotavirus diarrhea in children from selected Hospitals in Sokoto State.

Hospital name	Negative	Positive	(%) Positive
SH, Sokoto	170	30	56.6
UDUTH, Sokoto	127	23	43.4
Total	297	53	100

p -value = 0.9999, $\chi^2 = 8.934$

Description:

UDUTH : Usmanu Danfodiyo University Teaching Hospital

SH : Specialist Hospital

Table 2. Sex distribution of Rotavirus diarrhea from children in Sokoto State.

Sex	Negative	Positive	(%) Positive
Male	150	28	52.8
Female	147	25	47.2
Total	297	53	100

p -value = 0.7679, $\chi^2 = 22.78; 4$

Table 2. Sex distribution of Rotavirus diarrhea from children in Sokoto State.

Symptoms	Negative	Positive	(%) Positive
Diarrhea, vomiting, and fever	45	35	66.1
Diarrhea, and vomiting	63	10	18.9
Diarrhea, fever	76	3	5.6
Diarrhea only	113	5	9.4
Total	297	53	100

p -value = 0.0001, $\chi^2 = 70.37$

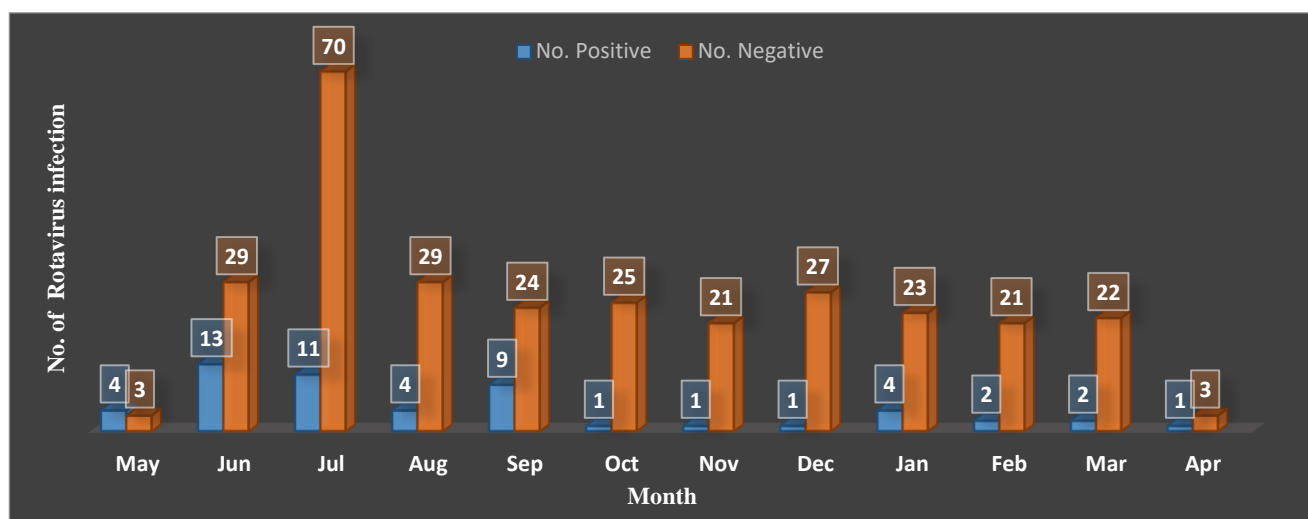


Figure 1. Monthly distribution of Rotavirus diarrhea among children in Sokoto State.

Figure 2 shows the age distribution of Rotavirus diarrhea among children in Sokoto, with children less than two years (13-24 months) of age having the highest prevalence of 37.7%, and the lowest prevalence was recorded in children that more than two years (49-60 Months) with 9.4%. Chi-square analysis indicated a significant association between rotavirus diarrhea and age ($p < 0.05$).

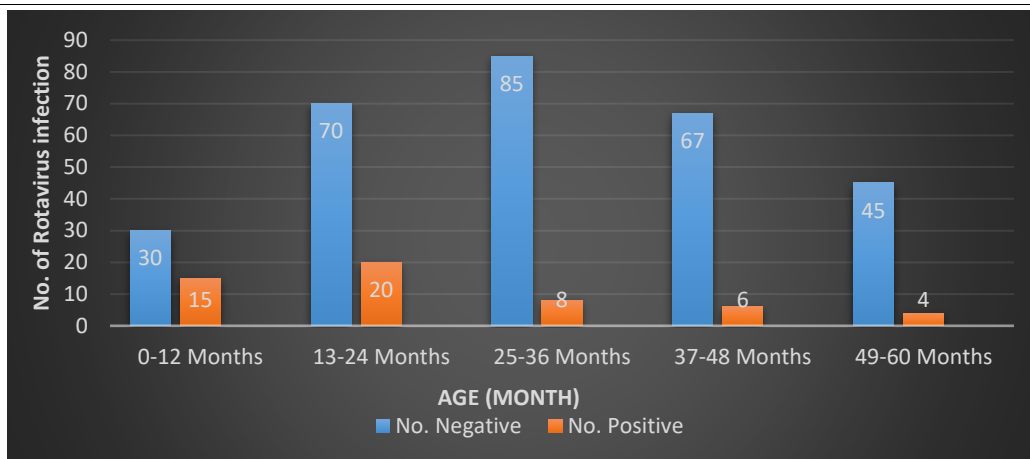


Figure 2. Age distribution of Rotavirus diarrhea among children in Sokoto State.

3.2. Discussion

Rotavirus is the most common cause of severe diarrhea among infants and young children worldwide. In developing nations, this virus is responsible for most diarrhea-related childhood deaths [11]. The prevalence of Rotavirus diarrhea from global surveillance networks and hospital-based studies ranges from 6% to 56%. In contrast, the hospital-based WHO global networks for surveillance of Rotavirus diarrhea report in the African region estimated the Rotavirus rate to range from 39-52% [12-13].

Among diarrhea-causing viruses, Rotavirus is the most important etiological agent worldwide and is implicated in severe dehydrating diarrhea requiring hospitalization [14]. The 2019 Institute for Health Metrics and Evaluation (IHME) statistics ranked Nigeria globally with the highest rotavirus mortality [15]. Close contact among humans, livestock, and other animals in developing countries makes the possibility of the emergence of virulent rotavirus strains very high due to gene re-assortments [16-17]. Therefore, interspecies transmission between humans and animals is standard and may be obtainable in the study area. The disease burden may increase due to the occupational and socio-economic status of the populace. Borehole and well water are the area's major sources of drinking water. Rotavirus diarrhea is important in developing countries like Nigeria but is not routinely diagnosed in most Nigerian hospitals. Despite recent studies, the existent data still need to be available for some African countries like Nigeria, which continues to be among the top 10 countries with the greatest burden of Rotavirus Infection [5]. Epidemiological studies of rotavirus infection reveal the greatest diversity of rotavirus strains in West African countries such as Nigeria [8]. The condition calls for a stronger need for strain surveillance of Rotavirus in Sokoto state. Vaccination status regarding the different strains of Rotavirus has not been established for many years in the study area, and therefore, the study has an impact on vaccine implementation. The study aimed to detect and determine the Prevalence of Rotavirus disease (gastroenteritis) in Children with diarrhea (0-5 years) in selected hospitals in Sokoto metropolis.

Therefore, in the present study, Rotavirus was detected in 53 stool samples of the children presented with cases of gastroenteritis out of 350, accounting for 15.1% prevalence, which is in line with global prevalence ranges of Rotavirus diarrhea but did not fall in the WHO African region range probably due to year to year variation, sample storage, and geographical factors. However, the result is relatively similar to the 15.6% recorded in Zaria [18], 18% in North-western State, Nigeria [8], 13.8% in Jos [19], and 14.0% in Namibia [20]. Furthermore, the result of the present study is lower to 25.5% recorded in Sokoto [1], 32.2% in Kaduna [21], 36.5% in Kano [22], 35.0% in Lagos [23] and 56.0% in Enugu [24]. Higher prevalence was reported in many African countries, such as 36% in Madagascar [25], 39.8% in Ghana [26], and 26.4% in South Africa [27]. Meanwhile, the result is higher than 11% reported in Jos, Nigeria [28] and 6% recorded in Kwara State, Nigeria [29].

A higher prevalence of Rotavirus was detected in males (52.8%) than in females (47.2 %) in this study. This finding follows other studies that detected prevalence levels of 64.2% in males and 35.8% in females [30], 52.9% in males and 47.1% prevalence was reported in Sokoto by [1], 33.0% in males and 31.4% in females was reported by [21] and 67.5% in males, 32.5% in females was shown in children less than five years of age in eastern Nepal by [31]. Chi-square analysis indicated no significant difference in the association between Rotavirus and sex ($p > 0.05$). Whether this the difference is due to sex susceptibility or by chance is, however, questionable and needs further investigation.

In the present study, age-wise distribution of Rotavirus diarrhea is more prevalent in children less than two years of age, with the highest prevalence between 13-24 months (37.7%) followed by 0-24 months (22.6%), which accounted for 60.4% more than 25-60 months of age that has 39.6% prevalence. This age distribution is comparable to the previous report of 74.7% prevalence of Rotavirus diarrhea in children less than two years of age in Sokoto [1], 90.9% of positive cases of Rotavirus in children under two years of age in Jos, Nigeria by [19], 70.9% prevalence in under two years was reported by [31]. 66.1% in less than two years and 39.9% in more than two years was reported by [31]. 80.2 % prevalence in Thailand was also reported by [31]. Statistically, there is a significant association between age and Rotavirus diarrhea ($p < 0.05$). The association is following the assumption that in under-developed areas, the early peak of Rotavirus diarrhea may result from early exposure to contaminated sources as well as over-crowded homes, more so since almost all humans experience at least one Rotavirus infection by three years of age and circulating Rotavirus antibodies remain detectable indefinitely [33].

In this study, diarrhea (100%), vomiting (83.75), and fever (92.5%) remain significant in Rotavirus infection based on the statistical analysis that shows $p < 0.05$. The same was observed in the study conducted by [34]. Indeed, Rotavirus infection has been associated with severe diarrhea episodes and vomiting, which often lead to severe dehydration in babies and young children [35]. However, the result of this study shows the prevalence of major symptoms (diarrhea, vomiting, and fever), and chi-square analysis indicates a significant association between Rotavirus and three major symptoms ($p < 0.05$) but no significant association between rotavirus diarrhea and other respiratory symptoms (upper and lower respiratory infections) and therefore, ($p > 0.05$). The results follow many reports that indicated the presence of fever in about 45%-84% of patients suffering from Rotavirus diarrhea [36-39].

4. Conclusion

Fifty-three samples were detected positive for Rotavirus antigens out of three hundred and fifty samples, and therefore, the prevalence is 15.1% (53/350). There is a significant association between Rotavirus disease and age. However, there is no significant association between Rotaviruses and sex. High numbers of rotaviruses were detected in Children with the three symptoms (diarrhea, vomiting, and fever), followed by those with either fever or vomiting, then those with diarrhea only.

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