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Microbial Agents and Associated Factors of Persistent Diarrhoea in Children Less Than 5 Years of Age in Edo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author PEI designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors NOE, PEI managed the literature searches, analyses of the study and performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Persistent diarrhoea is a major health problem for children in developing countries.
Aims: This study was carried out to assess the microbial agents of persistent diarrhoea as well as other associated factors of infection.
Methodology: Faecal and blood samples were collected from children less than 5 years of age with persistent diarrhoea in Edo State, Nigeria. The faecal specimens were processed using standard microbiological methods while the blood specimens were processed for malaria parasites using staining technique.
Results: The overall results showed a prevalence of 42(25.1%). Single infection was 37(22.2%) while mixed infection had 5(3.0%). Rotavirus was the most prevalent microbial agent (23.4%), followed by *cryptosporidium species* (14.9%). Sex and age did not show statistical significance

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(P>0.05). The effects of associated factors of infection on diarrhoeal patients was not statistically significant (P>0.05), although, undernourished, low socioeconomic status and not breastfeeding children had higher rates of infection than their counterparts.
Conclusion: Persistent diarrhoea is not uncommon in this locality since the prevalence rate was 25.1% Thus, preventive measures should be put in place to address the associated factors of persistent diarrhoea in order to minimize the reoccurrence of the infection.

Keywords: Persistent diarrhoea; microbial agents; associated factors; faecal specimens; blood specimens.

1. INTRODUCTION

Persistent diarrhoea is operationally defined as an episode of acute infectious diarrhoea that lasts for 14 days or longer [1]. The World Health Organisation (WHO) reported that persistent diarrhoea comprises about 3 to 20% of diarrhoea episodes and up to 50% of diarrhoea-related deaths [2]. However with the recent improvements in rehydration therapy programme, a decline has been observed in deaths associated with acute watery diarrhoea and thus the problem of persistent diarrhoea has become more concerning. The contribution of persistent diarrhoea to overall diarrhoea mortality is increasing, and is often associated with malnutrition [3].

Persistent diarrhoea imposes an economic burden on low socioeconomic status countries with its devastating effects on nutritional status, growth and development of children [4,5]. Children living in poor areas with poor hygiene and sanitary conditions as well as those with poor nutrition are most likely to be at risk of developing diarrhoea. Previous antibiotic use and irrational use of antibiotics are also considered to be risk factors for persistent diarrhoea [6]. Children with Human Immunodefiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) are also at risk of diarhoeal infection [7- 9].

In developing countries, about 10% of diarrhoeal cases in children become persistent, especially among those less than three years. The episode may begin acutely either as watery diarrhoea or dysentery. This diarrhoea causes substantial weight loss in most patients and may be responsible for about one-third to half of all diarrhoea-related deaths [10]. WHO estimates that persistent diarrhoea accounts for about 10% of diarrhoea episodes and 35% of deaths in under 5 children. However, community studies show that for every 100 children below 4 years of age, 7 cases of persistent diarrhoea occur every year [11]. In Nigeria, studies on diarrhoea have mainly focused on acute diarrhoea with little information on persistent diarrhoea, resulting in paucity of information as regards persistent diarrhoea in the country. This study was undertaken to identify the microbial agents and other associated factors of persistent diarrhoea in children less than 5 years of age in Edo State, Nigeria.

2. PATIENTS AND METHODS

The study population was made up of children less than 5 years of age with clinical symptoms of persistent diarrhoea and who have had continuous episodes of diarrhoea over a period of two weeks and beyond, but less than four weeks. This was to ascertain the microbial agents and other associated factors that could be responsible for the prolonged diarrhoea. Patients were drawn from six different health centers (Iwogban, Ogbeson, Ugbekun, Evbomodu, Aduwawa and Eyaen primary health centres) in Edo State, Nigeria. These health centres are located at the outskirts of Benin City which is the capital of Edo State. The centres were used for this study because they are closer to the people of the community and also they are where cases of illness are first attended to before referral is made to secondary or tertiary health centre when the need arises. A total of one hundred and sixty seven (167), children with persistent diarrhoea made up of 92 males and 75 females were recruited for this study. A structured questionnaire was used to obtain demographic information from parents or guardians of the children. Verbal informed consent was obtained from the parents or guardians prior to specimen collection. The study was carried out between January 2012 and March, 2013. Ethical approval was obtained from the state health ethics committee.

2.1 Specimen Collection and Processing

Stool specimens were collected into sterile wide mouth specimen containers and processed using microbiological techniques. Blood samples were

also collected from the children to determine malaria parasites.

2.1.1 Detection of viral agents

This was carried out using immunochromatographic technique. Rotavirus and adenovirus was detected using VIKIA Rota-Adeno rapid test device (BioMerieux, France). Briefly, 2 drops of liquid stool specimen was added to the specimen dilution buffer and was vigorously shaken for homogeneity, and 2 drops of the diluted sample was placed into the sample well of the test device and was allowed to stay for 10 minutes. Results were interpreted according to the manufacturer's instructions. Norovirus was detected by RIDA QUICK Norovirus (N1403) (R-Biopharm AG, Germany). Briefly, 1000 ul of sample dilution buffer was taken into a separate labeled test tube and 100 ml of liquid stool was added and shaken for homogeneity. This was allowed to settle for 2 minutes and 250 µl of the supernatant was placed in a clean labeled test tube and 6 drops of conjugate 1 was added to the test-tube and shaken to mixed properly. The mixture was emptied into the sample well of the test device and incubated for 10 minutes at room temperature. 4 drops of conjugate 2 was added to the reaction window of the test device and incubated for 1minute at room temperature. 10 drops of wash buffer was added to the reaction window and was allowed to stay until the buffer was completely absorbed, and 6 drops of substrate was added to the reaction window and allowed to stay for 3 minutes the results were interpreted following the manufacturer's instructions.

2.1.2 Isolation of bacterial and fungal agents

This was carried out using microbiological techniques as described by Chessbrough [12]. Briefly, stool specimens were inoculated into appropriate media and incubated at 37°C for 24 hours. Bacterial identification was carried out using Analytical Profile Index (API 20E) by BioMerieux, France. The manufacture`s instruction was strictly followed. Similarly, yeasts identification was carried out using Candida nCHROMagar (BBL, Heldelberg, Germany). The yeasts coloies produced colours which were compared against a standard chart of Candida.

2.1.3 Detection of parasitic agents

This was carried out by microscopic techniques using formol ether concentration method, modified Ziehl Nelseen method, Geimsa and Fields stain methods [13]. Briefly, wet preparations were made after concentrating the stool and read using X10 and X40 objectives while dry preparations were read using X100 objective after appropriate staining for parasites has been made.

2.2 Weight Measurement

The children's weights were taken using scale measurement in kilograms. Weight for age z-scores (WFA) for boys and girls respectively from birth to 5 years (WHO child growth standards) were used to classify children as being malnourished or nourished. Those with weight for age less than minus two standard deviations were classified as malnourished and greater than minus two standard deviations as nourished [14].

2.3 Statistical Analysis

This was carried out to test the effects of sex, age, nutritional status, family socioeconomic status, malaria parasites and breastfeeding status on the prevalence of persistent diarrhoea among the studied population. Data obtained were analyzed for Chi-square analysis using the statistical software "INSTAT' which automatically calculate the imputed values to produce the desired result. A P-value of less than 0.05 was considered as statistically significant.

3. RESULTS

A total of 167 children with persistent diarrhoea were investigated for microbial agents and other associated factors of infection. The overall results showed a prevalence of 42(25.1%). Single infection was 37(22.2%) while mixed infection had 5(3.0%) (Table 1). Sex and age distribution of infection were not statistically significant with sex (P=0.229) and age (P=0.280) (Table 2). The frequency distribution of microbial agents showed rotavirus as the most prevalent with 11(23.4%) followed by *cryptosporidium species* with 7(14.9) (Table 3). The effects of associated factors of infection on patients with diarrhoea did not show statistical significance with nutritional status (P=0.168), family socioecononomic status (P=0.187), malaria parasites (P=1.000) and breastfeeding status (P=0.131). Though, regardless of statistical significance in the aforementioned associated factors, infection was higher in the undernourished, low socioeconomic status, and not breastfeeding children than their counterparts (Table 4).

Table 1. Characteristics of patients with persistent diarrhoea in Edo State

Characteristics	No infected	Percentage
Patients with at least one microbial agent.	42	25.1
Patients with single microbial agent.	37	22.2
Patients with mixed microbial agents.	5	3.0

Table 2. Sex and age distribution of 167 children with persistent diarrhoea in Edo State

Characteristics	No. tested	No. infected (%)	P-value
Sex			0.229
Male	92	27(29.3)	
Female	75	15(20.0)	
Total	**167**	**42(25.1)**	
Age (months)			0.280
1- 6	1	0	
7-12	42	12(28.6)	
13-18	62	21(33.9)	
19-24	33	6(18.2)	
25-30	19	2(10.5)	
31-36	8	1(12.5)	
>37	2	0	
Total	**167**	**42(25.1)**	

Table 3. Frequency distribution of microbial agents of persistent diarrhoea in Edo State

Microbial agents	No. Present	Percentage
Rotavirus	11	23.4
Adenovirus	5	10.6
Escherichia coli	5	10.6
Providencia alcalifaciens	3	6.4
Staphylococcus aureus	4	8.5
Candida albicans	5	10.6
Candida krusei	3	6.4
Cryptosporidium species	7	14.9
Cyclospora species	4	8.5
Total	**47**	**99.9**

Table 4. Associated factors of persistent diarrhoea in Edo State

Characteristics	No. Examined	No Infected (%)	P-value
Nutritional status			0.168
Nourished	52	9(17.3)	
Undernourished	115	33(28.7)	
Family socioeconomic status			0.187
Low	103	30(29.1)	
Middle/high	64	12(18.8)	
Malaria parasites			1.000
Present	58	14(24.1)	
Absent	109	28(25.7)	
Breastfeeding status(1-12 months)			0.131
Currently breastfeeding	62	11(17.7)	
Ceased breastfeeding	105	31(29.5)	

4. DISCUSSION

Persistent diarrhoea was investigated in children under 5 years of age in Edo State, Nigeria. Microbial associated diarrhoea had a prevalence of 25.1% with 22.2% single infections and 3.0% mixed infection. It has been suggested that

sequential infection may be the cause of persistent diarrhoea rather than single infection [1,15]. This may be the reason for the prevalence rate observed in this study.

As regards gender, males had 29.3% infection while females had 20.0%, and this was not statistically significant (P>0.05). A biological explanation may be related to the facts that boys during infancy have to build a larger muscle mass than girls, and as a result have increased demands for micronutrients, and are therefore more at risk of negative balance including lack of vitamin A and Zinc. Thus, this vulnerability might increase the risk of diarrhoea [16]. Another reason could be attributed to the fact that boys are usually more active than girls, and engage in a lot of playful activities in the surroundings which may be contaminated with infectious agents.

The age group distribution of infection did not show statistical significance (P>0.05), however, infection was highest in 13-18 months (33.9%), followed by 7-12 months with 28.6%. This could be attributed to the fact that children within 13-18 months must have been fully weaned, and may be on solid food which may likely be contaminated during preparation. Similarly, for 7-12 months, mixed feeding could be the cause of the prevalence recorded in this study, as exclusive breastfeeding stopped at 6 months.

The frequency distribution of microbial agents showed rotavirus to be the most prevalent (23.4%), followed by *cryptosporidium species* with 14.9%. The reason(s) for this may be due to the fact that in this locality viral diagnostic tests are not done routinely. Also for the parasitic infections, Ziehl Nelseen (ZN) stain is not usually done on stool specimens with diarrhoea in many laboratories. The reasons for the aforementioned challenges are due to the poor attention given to health care matters. In many developing countries, patients are diagnosed based on the available diagnostic materials which do not give a wide coverage of infectious agents and thus reoccurrence of illness may set in.

The effects of associated factors of infection on diarrhoeal patients was not statistically significant (P>0.05). Nutritional status showed that children who were undernourished had a higher prevalence (28.7%), as against 17.3% in the nourished children. This goes to show that nutritional status contributes to persistent diarrhoea. This is consistent with other studies [16-18]. As regards socioeconomic status children from low income families had a higher prevalence (29.1%), as compared to middle and high income with 18.8%. This agrees with other reports [16,19,20]. Co-infection of malaria with diarrhoea showed that 58 children had malaria, out of which 14 (24. 1%) were also infected with microbial agents. The co-morbidity of malaria with diarrhoea found in this study could be attributed to the fact that malaria infection is prevalent in the tropics. In terms of breastfeeding status within the first year of life (i.e 1-12 months), it was observed that children who were currently breastfeeding had lower infection (17.7%) as against those who have stopped breastfeeding (29.5%). This finding is consistent with other studies [16,21-23].

5. CONCLUSION

A prevalence of 25.1% microbial associated diarrhoea was found in this study. Rotavirus was the most prevalent microbial agent, followed by *cryptosporidium species*. Nutritional status, family socioeconomic status, malaria and breastfeeding, though not statistically significant, could be seen to show some predisposing factors for acquiring persistent diarrhoea in this locality. The issue of inadequate diagnostic materials is also a concern as regards persistent diarrhoea and addressing these challenges will help to reduce the reoccurrence of persistent diarrhoea among the studied population.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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