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Microbiological Assessment of Stool Specimens of Children with Diarrhoea in Benin City, Nigeria

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Authors' contributions

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

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ABSTRACT

Diarrhoeal disease is a public health problem for children worldwide especially in developing countries. This study aimed at assessing the microbial agents associated with childhood diarrhoea in Benin City, Nigeria. Faecal specimens were collected from patients and controls within 0-60 months of age and were analyzed by microbiological techniques. Viral agents were detected by immunochromatographic method, parasitic agents by microscopic method, while bacteria and fungi were isolated by cultural method. The overall results showed a prevalence of 208 (41.3%) for patients and 7 (5.7%) for control. Sex and age group did not show statistical significance (P>0.05) with respect to diarrhoea. However, maternal occupation and season of the year showed statistical significance with P=0.001 and P=0.029, respectively. Antibiotic susceptibility pattern showed that the bacterial isolates were most sensitive to Ceftriazone, Ofloxacin, Ceftazidine and Cefuroxime. Microbial associated diarrhoea is still a public health problem in Benin City, Nigeria, as seen in the 41.3% prevalence. Bacteria, viruses, fungi and parasites were found to be associated with the infection. Thus, comprehensive stool analysis is needed for determining the pathogen in patients with clinical symptoms of diarrhoea in this locality.

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1. INTRODUCTION

Diarrhoea is one of the main causes of morbidity and mortality in children younger than 5 years of age in developing countries where the average number of episodes of diarrhoea per child per year within this age group is 3.2 [1,2]. The World Health Organization (WHO) has estimated that 1.5 billion episodes of diarrhoea occur every year in developing countries, resulting in 3 million deaths [3]. Acute diarrhoea is one of the leading causes of morbidity and mortality in children in most developing countries. Despite the use of oral rehydration therapies, deaths due to diarrhoea in children aged less than 5 years are still estimated to be about two million per year [4].

In sub-Saharan Africa, mortality caused by acute diarrhoea varies from 1.9% of all deaths in The Gambia to 37% in Nigeria, with most of the deaths occurring during the first year of life [5]. Even though morbidity caused by diarrhoea is still high, mortality has been decreasing worldwide, mainly because of improved management [2,6,7,8]. Diarrhoea is most often a symptom of gastrointestinal infection caused by bacteria, viruses or parasites. Commonly, these pathogens are transmitted via the faecal-oral route, where the pathogens are excreted from the intestinal tract of a person or animal carrying the illness and are ingested by another [9]. Many of the risk factors for contracting diarrhoeal illness are associated with poor socioeconomic conditions, such as lacking access to safe water and sanitation, poor hygiene practices and unsafe human waste disposal [10-12].

In developing countries, some children experience many episodes of diarrhoea in their first year of life. Repeated and persistent diarrhoea in young children contributes to significant cognitive and growth impairment that can impact school performance and development [13,14]. In tropical regions, acute infective diarrhoea is widely prevalent and is an important factor contributing to morbidity and mortality, especially in children. Persistent diarrhoea with prolonged symptoms increases morbidity and mortality. Chronic diarrhoea is often associated with malabsorption of nutrients [15].

In most of the Nigerian hospitals, comprehensive routine analysis of stool specimens for patients with diarrhoea is not carried out, and as a result many important pathogens of interest are missed out and this situation affects data collation as regards diarrhoeal diseases. Moreover, in many developing countries including Nigeria, research works have mainly focused on bacterial and parasitic aetiologies of diarrhoea. Hence this work tends to provide a holistic approach regarding full microbiological studies of diarrhoea in children of 0-60 months of age in Benin, City, Nigeria.

2. PATIENTS AND METHODS

The study population consisted of infants and young children of 0– 60 months of age attending eight health centres in Benin City, Nigeria. A total of six hundred and twenty six (626) stool specimens comprising 504 diarrhoeic children with 273 males and 231 females were sampled. Also, 122 apparently healthy children without diarrhoea within the same age group consisting of 68 males and 54 females were included as control. A structured questionnaire was used to obtain demographic information from the parents or guardians of the children. Verbal informed consent was also obtained from the parents or guardians prior to specimen collection. This study was carried out between June 2011 and September 2012. Ethical approval was obtained from the state health ethics committee.

2.1 Specimen Collection and Processing

The specimens were collected into sterile wide mouth specimen containers and processed following standard microbiological techniques for viral, bacterial, fungal and parasitic agents.

2.2 Detection of Viral Agents

Rotavirus and adenovirus were detected using VIKIA Rota-Adeno rapid test device (Biomerieux, France). The manufacturer's instructions were strictly adhered to in the performance of the test. Similarly, norovirus was detected using RIDA QUICK Norovirus (N1403) rapid test device (R – Biopharm AG, Germany).

2.3 Isolation of Fungal Agents

Stool specimens were examined for the presence of fungal agents by microscopy and

cultural method [16,17]. Suspected yeast colonies from Sabouraud dextrose agar plates were subcultured onto Candida CHROMagar (BBL, Heidelberg, Germany) at 37°C for 48 hours. The Candida colonies were then classified into species according to the colour of the colony using a standard chart. Yeast count was determined by colony count and the count equal to or greater than 10^5 cfu/ml was considered as overgrowth [17].

2.4 Detection of Parasitic Agents

Stool specimens were examined for the presence of intestinal parasites (cysts, trophozoites, larval and ova) by direct microscopy using formal ether concentration method and modified Ziehl Nelseen method as described by Cheesbrough [18].

2.5 Isolation of Bacterial Agents

This was carried out as described by Cheesbrough [19]. Briefly, stool specimens were cultured into Mac Conkey agar, Desoxycholate citrate agar, Salmonella-shigella agar and Selenite F. broth. The plates were incubated at 37°C for 24 hours. Suspected colonies were identified by standard laboratory procedures. Analytical profile index (API 20E) made by BioMerieux, France, was used in bacterial identification. Briefly, a plastic strip holding 20 mini-test tubes was inoculated with a saline suspension of a pure culture and this was used to fill the tubes. Tubes containing ADH, LDC, ODC, H_2S, and URE were covered with mineral oil for anaerobic reaction to take place. The strips were covered and incubated in a humidity chamber for 18 to 24 hours at 37°C. The colour of the tubes were read and converted to a seven digit code. The code was fed into the manufacturer`s data base via touch-tone telephone and the system gives the identification, usually as genus and species or manually using a reference book giving a specific code for each genus and species. However, for *Escherichia coli* a further identification test was performed using serological method. Briefly, slide agglutination test using *E. coli* polyvalent antisera was performed on all suspected isolates and agglutination reaction means a positive result. The bacterial isolates were subjected to antimicrobial susceptibility test using the agar disc diffusion method. Sensitivity was determined according to the standard of the National Committee for Clinical Laboratory Standard (NCCLS) now called Clinical and Laboratory Standards Institute (CLSI) [20].

2.6 Statistical Analysis

To test if the isolation frequency of enteric agent is different between the patient and control groups, between the two sex groups, among the groups of different ages, different manners of breastfeeding, different occupations of the mothers, and different seasons, the data obtained were analyzed using chi-square analysis by means of a statistical software "INSTAT" which automatically calculate the statistical values and a P- value of less than 0.05 was taken as statistical significance.

3. RESULTS

In this study, 208 (41.3%) among the 504 children with clinical symptoms of diarrhoea were positive for at least one enteric agent, while the positive cases were 7 (5.7%) among the 122 apparently healthy children without diarrhoea in the control group. The distribution of infection in groups with different sex age and breastfeeding manners were not statistically significant (P>0.05) (Table 1). The effect of some variables on the prevalence of diarrhoea showed that occupation and season had significant association (P=0.001) and (P=0.029) respectively with diarrhoea, while level of education and source of water were not statistically significant (P>0.05) (Table 2). The distribution of microbial agents from diarrhoeal patients and control is showed in Table 3. The antibiotic susceptibility pattern of bacterial isolates is showed in Table 4.

4. DISCUSSION

Microbial agents associated with childhood diarrhea in Benin City, Nigeria were investigated in this study. A prevalence of 41.3% was found. This value is lower than those reported in Nigeria, other African countries and some parts of the world [4,21-28]. Similarly, it is higher than some other reports [29,30]. However, from the aforementioned studies in different parts of the world, the prevalence of microbial-associated diarrhoea differs from one locality to another. This observation is also in concordance with the reports of Thapar and Sanderson [31] who stated that the contribution of the various pathogens to diarrhoea may differ substantially between regions depending on local meteorological,

geographic and socioeconomic conditions. Other variations could be explained by the differences in the study population, endemicity of microbial agents and specific practices prevalent among the different populations.

The absence of significant difference in infection frequency among the sex and age groups could be attributed to the fact that risk factors associated with diarrhoea are environmental and socio-demographic and not biological [11]. In terms of age, infection was highest in the first year of life with 91 out of 208 infected, followed by the second year with 53 children infected and the third year of life with 42 infected; while the fourth and fifth year had 22 infected children. Although no significance was detected, there was a gradual decrease in the prevalence of diarrhoea from the first year of life to the fifth year. This is consistent with other reports [32,33] that diarrhoeal diseases decrease with increasing age as there is a progressive development of immunity against microbial agents.

The breast feeding status within the first six months of life showed that it was not statistically significant (P=0.169). However, infection was more in infants who were partially breastfed (47.9%) than those who were exclusively breastfed (29.2%). This is in concordance with other reports [34,35] which stated that breast

Table 1. Distribution of infection in groups with different sex, age and breastfeeding manners

Groups	Tested Number	Infected number (%)	P- value
Sex			0.888
Male	273	114 (54.8)	
Female	231	94 (45.2)	
Total	**504**	**208 (41.3)**	
Age (months)			0.622
6	97	42 (43.3)	
7-12	106	49 (46.2)	
13-18	70	28 (40.0)	
19-24	61	25 (41.0)	
25-30	53	22 (41.5)	
31-36	50	20 (40.0)	
37	67	22 (32.8)	
Total	**504**	**208 (41.3)**	
Breastfeeding status			0.169
Exclusively breastfed	24	7 (29.2)	
Partially breastfed	73	35 (47.9)	
Total	**97**	**42 (43.3)**	

Table 2. Effects of some variables on the prevalence of diarrhea

Demographics	No. examined	No. infected (%)	P-value
Occupation			0.001
Civil servants	129	52(40.3)	
Traders	168	88(52.4)	
Farmers	64	18(28.1)	
Artisans	143	59(41.3)	
Level of education			0.254
No formal education	28	9(32.1)	
Primary school	188	78(41. 5)	
Secondary school	221	99 (44.8)	
Tertiary institution	67	22(32.8)	
Source of water			0.890
Pipe borne	5	2 (40.0)	
Bore hole	477	198(41.5)	
Protected well	22	8(36.4)	
Season			0.029
Dry	213	76 (35.7)	
Rainy	291	132 (45.4)	

Table 3. Distribution of microbial agents from patients and control

Microbial agents	Patients frequency (%)	Control frequency (%)
Rotavirus	106 (33.4)	0
Adenovirus	42 (13.2)	0
Norovirus	20 (6.3)	0
Escherichia coli	61 (19.2)	0
Klebsiella oxytoca	16 (5.1)	0
Proteus mirabilis	13 (4.1)	0
Providencia alcalifaciens	6 (1.90)	0
Acinetobacter baumanni	4 (1.3)	0
Staphylococcus aureus	5 (1.6)	0
Shigella flexneri	4 (1.3)	0
Candida albicans	20 (6.3)	3 (42.9)
Candida krusei	9 (2.8)	2 (28.6)
Crytosporidium species	7 (2.2)	1 (14.3)
Cyclospora species	4 (1.3)	1 (14.3)
Total	**317 (100.0)**	**7 (100.1)**

Table 4. Antibiotic susceptibility pattern of bacterial isolates

Microorganism	No. tested	No. (%) sensitive to							
		CRO	OFX	CXM	AUG	CN	CAZ	AMX	AX
Escherichia coli	61	53(86.9)	52(85.2)	50(82.0)	38(62.3)	29(47.5)	52(85.2)	0	0
Klebsiella oxytoca	16	13(81.3)	13(81.3)	12(75.0)	10(62.5)	7(43.8)	12(75.2)	0	0
Proteus mirabilis	13	10(76.9)	10(76.9)	9(69.2)	6(46.2)	5(38.5)	10(76.9)	0	0
Providencia alcalifaciens	6	4(66.7)	4(66.7)	3(50.0)	2 (33.3)	2(33.3)	4(66.7)	0	0
Acinetobacter baumanni	4	3(75.0)	3(75.0)	3(75.0)	1(33.3)	1(33.3)	2(50.0)	0	0
Staphylococcus aureus	5	3(60.0)	3(60.0)	3(60.0)	1(20.0)	0	3(60.0)	0	0
Shigella flexneri	4	3(75.0)	2(50.0)	2(50.0)	0	0	2(50.0)	0	0
Total	**109**	**89(81.7)**	**87(79.8)**	**82(75.2)**	**58(53.2)**	**44(40.0)**	**85(78.0)**	0	0

Key: CRO= ceftriazone, OFX = ofloxacin, CXM = cefuroxime, AUG = amoxycillin-clavulanate, CN=gentamicin, CAZ = ceftazidine, AMX = amoxycillin, AX= ampicillin – cloxacillin

milk contains antibodies and white cells which impact passive immunity against diseases including diarrhoea.

The prevalence of paediatric diarrhoea with maternal socioeconomic status was statistically significant with respect to occupation (P = 0.001). Paediaric diarrhoea was highest among children whose mothers were traders (52.4%). This is consistent with the reports of Omokhodion et al. [36] that the children who often accompanied their mothers to the markets for selling their wares are constantly exposed to health hazards especially contaminated food and water as a result of the poor environmental conditions of Nigerian markets. This could be the reason for what was observed in this study. The frequency of distribution of microbial agents showed that rotavirus was the most predominant with 33.4%, similar with other reports [21,22,24,30].

The distribution of infection according to season was statistically significant (P = 0.029), that the microbial agents were more in the rainy season (45.4%) than that in the dry season (35.7%). This agrees with other reports [37-39] who stated that the water quality is likely to be particularly poor in the rainy season and diarrhoeal prevalence peaks in many regions of the developing world, because the precipitation run off tends to increase faecal contamination of unprotected surface water sources. A comparative assessment between microbial agents found in patients and control was seen with respect to *Candida* species and coccidian parasites. This may indicate that the controls were carriers of microbial agents (asymptomatic) due to faecal contamination of the environment. The antibiotic susceptibility pattern of bacterial isolates showed that Ceftriazone, Ofloxacin, Ceftazidine and Cefuroxime were the most active antibacterial

agents. The isolates were moderately sensitive to Amoxicillin-clavulanate and 40.4% of the isolates were sensitive to gentamicin.

5. CONCLUSION

Microbial associated diarrhoea had a prevalence of 41.3% in this study. Bacterial, viral, fungal and parasitic agents were found to be associated with diarrhoeal infections, as well as socioeconomic status and season of the year. Comprehensive analysis of stool specimens to accommodate the aforementioned agents is advocated and this will enhance proper diagnosis.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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