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Enteropathogens Associated with Childhood Diarrheal Cases seen at a Tertiary Hospital in Nguru, Yobe State of Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors KOO and CUA designed the study, performed the statistical analysis, wrote the protocol, literature search and wrote the first draft of the manuscript. Authors MGN, MYB, HU, ZAB and UH managed the analyses of the study and author RTA, clinically reviewed the patients. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: Childhood diarrheal diseases are common clinical episodes seen among children under 5 years old in the developing countries of sub-Saharan Africa and Asia. Epidemiological information of enteropathogens associated with childhood diarrhea will provides clinical information to alliterate and enhance effective therapy management in our hospital.
Study Design: Retrospective analysis of enteropathogens associated with childhood diarrheal cases.
Place and Duration: The study was carried out in Federal Medical Centre, Nguru, over one year period from January to December, 2010.
Methodology: Fecal specimens were collected from patients presented with childhood diarrheal symptoms seen at the tertiary hospital at Nguru, Nigeria over the study period. Standard microbiological methods were employed in the enteropathogens detection. A total of 144 diarrheic fecal specimens were examined for existence of enteropathogens. The

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breakdowns of associated clinical diagnosis are as follows, gastroenteritis, 14 (9.7%), diarrhea, 80 (55.6%), dysentery, 31 (21.5%) and mucoid/bloody stool, 19 (13.2%).
Results: Of the 144 specimens analysed, enteropathogens were found in 89 (61.8%), 41 (46.1%) parasites and 48 (53.9%) bacterial cases respectively. Only two bacterial groups were identified, 43 (29.9%) were *Escherichia coli* and 5 (3.5%) belonged to *Shigella* spp. Among the parasites, *Enteamoeba histolytica* was the most prevalent (31 isolates, 21.5%), followed by *Ascaris lumbricoides* with 7 isolates (4.9%), *Taenia saginata* with 2 isolates (1.4%) and Hookworm with only 1 isolate (0.4%). Statistical significant difference was observed when the isolation frequency of enteropathogens was compared with the age-group and associated clinical diagnosis of the patients ($p<0.02$). Co-infections were observed in 16 (12.2%) cases, including 10 (62.5%) cases of *E. coli / E. histolytica* and one case (6.3%) of *A. lumbricoides* and *Shigella* spp.
Conclusion: The frequency of enteropathogens detected in this study was similar with those reported in other studies. In addition, it provides the epidemiological information on enteropathogens associated with childhood diarrhea in the studied region and serves as a guide to pediatricians towards empirical therapy.

Keywords: Diarrheal; non-diarrhea; aetiological agents; prevalence; Nguru, Nigeria.

1. INTRODUCTION

Diarrheal diseases are one of the leading cause of high morbidity and mortality, particularly among children under 5 years of age in developing countries of sub-Saharan Africa and Asia [1,2]. Studies have reported that approximately 1.87 million (approximately 19%) death are recorded globally due to the childhood diarrhea, mainly in Africa and Asia [3,4]. The high incidence level of the disease is common in areas associated with poor hygiene conditions, malnutrition, lack of safe drinking water and improper disposal of human and animals waste. The level varies with geographical location and socio-economic status of the population. In contrast, improvement in standard of personal hygiene, environmental condition and standard of living in the community would invariably leads to reduction in the incidence of diarrheal disease.

Aetiological agents of diarrheal diseases are known to be diverse, which includes bacterial, viral and parasites. The commonest enteropathogens include *Escherichia coli*, *Salmonella* spp., *Shigella*, *Entamoeba histolytica*, *Giardia lambia*, *Ascaris lumbricoides*, *Vibrio cholerae* and rotavirus. However, the frequency of enteropathogens and clinical presentations differs with geographical location, severity of diseases, level of personal and environment hygiene [5]. Clinical approach in the treatment of the cases will depends on adequate epidemiological information of childhood diarrheal in a peculiar environment, particularly as relates to aetiological agents and clinical presentations. The information obtained from such study will further formed an integral part of early preventive measures.

Nguru is a remote settlement in the Yobe state, one of the 6 administrative states in the northeastern Nigeria geo-political zone boarded with Chad and Niger. Located in the arid region, Nguru is a semi-cosmopolitan town with significant part encroached by Sahara desert. Inhabitants are mainly farmers, involved in rearing of ruminant animals like cattle, sheep goat and camel for economic purposes. Housing settlements pattern are mixture of old mud houses and modern architectural designs. The seasonal pattern can be divided into three, cold (October-January), hot humid (February-May) and rainy (July-September) seasons. Similar to the most African settlements, the improper disposal of human and animal wastes within the communities and the blocked drainage system in Nguru

encouraged the outbreak of diarrheal diseases, especially during the rainy seasons. However, the type of enteropathogens and associated clinical presentation, as well as the seasonal pattern and severity of the diseases in this region have not been studied. Based on this observation, we decided to examine the prevalence and aetiological spectrum of childhood diarrheal cases received in the Federal Medical Centre of Nguru, which is a major tertiary hospital (250 bed size) equipped to provide services in subspecialities of medicine to the local population and neighboring citizens of Chad and Niger.

2. MATERIALS AND METHODS

This retrospective study was conducted in the medical microbiology laboratory, Federal Medical Centre, Nguru in collaboration with the Pediatric department, Federal Medical Centre, Nguru. Criteria of inclusion, microbiological data of childhood diarrheal cases of patient aged less than 12 years were extracted and collated for the retrospective study analysis. Demographic information extracted from the laboratory request form, included, age, sex and associated clinical diagnosis and type of enteropathogens detected. The specimens collected from patients by the standard procedure as instructed by the laboratory staff, and were submitted immediately to the medical microbiology laboratory for isolation and identification of enteropathogens.

2.1 Parasitological Studies

The fecal specimens were examined for presence of ova, cysts and trophozoites of protozoan and helmthins, by formol-ether concentration and Lugol's iodine floatation method [6].

2.2 Bacteriological Studies

The fecal specimens were inoculated into Selenite F broth (Biotech, India), which was incubated at 37ºC for 24 hours. The Selenite F broth was subsequently subcultured by spread an aliquot of 0.1 mL onto blood agar (Biotech, India), MacConkey agar (Biotech, India) and Thiosulphate-citrate bile salt triple sugar (Biotech, India) agar plates and then incubated at 37ºC for 24-48 hours. All the plates were examined for presence of suspected colonies of Enterobactericae associated diarrhea The suspected colonies were identified and isolated by using the colonial morphology, gram reaction, motility test, and biochemical reaction –indole , glucose, sucrose and lactose ultilization tests [6].

2.3 Data Analysis

The demographic variables and identified enteropathogens were collated and analysed using SPSS version 16.0. The values were expressed in means and percentages. Comparison of demographic variables was determined by chi-square test. The level of significance of $p<0.05$ was employed.

3. RESULT

3.1 Information of Specimens

As shown in Table 1, of the 144 microbiological data of fecal specimens analysed over the study period, the mean age of the patients was 24.50 ± 0.88 months and the gender

distribution was 80 (55.5%) males and 64 (44.4%) female respectively. The age-group distribution of the patients was as follows: 12-48 months, 71 (49.3%); 60-96 months, 41 (28.5%); 108-144 month, 22 (15.3%) and <12 month, 10 (6.9%). The breakdown of associated clinical diagnosis as follows: diarrhea, 80 (31.6%); gastroenteritis, 14 (5.5%); dysentery, 31 (12.3%); and mucoid/bloody stool, 19 (7.5%).

Table 1. Demographic variables, clinical diagnosis and microbiological data of patient studied(%)

Variables	Frequency (%)
Mean age	34.0±0.78months
Sex Male	80(55.5)
Female	64(44.4)
Age-group	
<12month	10(6.9)
12-48	71(49.3)
60-96	41(28.5)
108-144	22(15.3)
Associated Clinical diagnosis	
Gastroenteritis	14(9.7)
Diarrhea	80(55.6)
Dysentery	31(21.5)
Mucoid/bloody	19(13.2)
Parasite	
Entamoeba histolytica	31(34.9)
Ascaris lumbricodes	7(8.0)
Taenia saginata	2(2.2)
Hookworn	1(1.1)
Bacterial pathogens	
Escherichia coli	43(48.3)
Shigella spp	5(5.6)

3.2 Identification of Enteropathogens

A total of 89 (61.8%) enteropathogens were identified and 55 (38.2%) cases yielded negative results. Of the 89 enteropathogens detected, 41 (46.1%) were parasites and 48 (53.9%) were bacterial pathogens. Among the parasites, *E. histolytica* accounted for 45 (17.8%), followed by *A. lumbricodes*, 9 (3.6%); *T. saginata*, 3 (1.2%) and Hookworm, 1 (0.4%). Among the bacterial pathogens, *E. coli* accounted for 65 (25.7%), *Shigella* spp. for 7 (2.8%) and *Salmonella* sp. for 1 (0.4%). The distribution of enteropathogens in accordance with age-group of the patients (Table 2), *E. histolytica, A. lumbricodes* and *E. coli* were identified in all the age-groups, and were particularly frequent for the group of 12-48 months. Similar pattern was observed in associated clinical diagnosis (Table 3). All the eneteropathogens were observed in the diarrheal cases, except the *Shigella* spp.. E. *histolytica* and *E. coli* were the most prevalent in diarrhea and dysentery cases. Two *Shigella* isolates were obtained in two cases of dysentery, and three in mucoid/bloody stool cases. Co-infection (parasitic and bacterial) was observed in 16 (12.2%) cases, with the combination of *E. histolytica* and *E. coli* in 10 (62.5%) cases, followed by *A. lumbricodes* and *E. coli*, 3 (18.8%); *T. saginata* and *E. coli*, 2 (12.5%); and *A. lumbricodes* and *Shigella* spp., 1 (6.3%).

Table 2. Distribution of enteropathogens according to age-group of patients

Age-group(mths)	Cases with Parasites					Cases with bacterial pathogens			
	E. histolytica	*A. lumbricoides*	*T. saginata*	Hookworm	Subtotal (%)	*E. coli*	*Shigella*	Subtotal (%)	Total (%)
<12	1	1	0	0	2(2.2)	5	0	5(5.6)	7(7.8)
12-48	14	2	2	1	19(20.3)	26	3	29(32.6)	41(53.9)*
60-96	9	3	0	0	12(13.5)	7	1	8(9.0)	18(22.5)
108-144	7	1	0	0	8(3.0)	5	1	6(6.7)	7(15.7)
Total(%)	31(34.9)	7(8.0)	2(2.2)	1(1.1)	41(46.1)	43(29.9)	5(3.5)	48(53.9)	89(100)

Significant statistical difference between age-group and enteropathogens (p<0.002)

Table 3. Distribution of enteropathogens according to clinical diagnosis

Clinical details	Cases with Parasites					Cases with bacterial pathogens		
	E. histolytica	*A. lumbricoides*	*T. saginata*	Hookworm	Total (%)	*E. coli*	*Shigella*	Total (%)
Gastroenteritis	3	0	0	0	3(3.3)	3	0	**3(3.3)**
Diarrhoea	16	4	2	1	23(25.8)	27	0	**27(30.3)***
Dysentry	10	1	0	0	11(12.4)	10	2	**12(13.5)**
Mucoid/bloody stool	2	2	0	0	4(4.5)	3	3	**6(6.7)**
Total (%)	31(34.9)	7(8.0)	2(2.2)	1(1.1)	41(46.1)	43(29.9)	5(3.5)	48(53.9)

**Significant statistical difference between enteropathogens and clinical diagnosis (p<0.001)*

4. DISCUSSION

In this study, the prevalence level of enteropathogens detected was 61.8%(89), 46.1% (41)parasites and 53.9%(48)bacterial pathogens. This level falls within the reported level range of 60% and 80% in similar studies carried out in Nigeria [7-10]. In Ghana, a prevalence level of 77.1% was reported in a study conducted in northern part of Ghana [11] and higher level greater than 50% in Asia and South America. [5,12-15]. Variations in the prevalence level of childhooddiarrheal studiescan be attributable to difference in geographical locations, studied population, severity of the diseases, seasonal variation and isolation techniques. Fifty-five (38.2%) diarrheic feacal specimens yielded noenteropathogens, this might due to fact implicating enteropathogens are routinely search for, or lack of basic laboratory for identification. In contrast, relatively high number of enteropathogens have been identified in studies that employed much sensitive methods like latex agglutination and ELISA methods.

Overall, a total of 7 enteropathogens were identified in this study, Escherichia coli and *Entamoeba histolytica*, were the most commonest, similar to findings reported in other studies in Nigeria and elsewhere [7,8,10,16,17]. Similar high frequency of *E. coli* isolates, and low number of *Shigella* spp isolates was also reported in studies conducted in Armristar, India(14) and Orissa, India(15). In most routine medical laboratories, lack of basic facilities limits possible identification of bacterial to species level/or serotypes. The drawback of this study, was non-serotyping of the *E. coli* isolates that could have epidemiological information on prevalent *E. coli* serotype associated with childhood diarrhea in the study region. Also, the number of enteropathogens detected were relatively few, compared with other studies, that might be due to geographical location, seasonal pattern, severity of diseases and methodology employed. In Asia and South America, relatively high number of pathogens (*Vibrios, Campylobacter spp, Shigella spp*) are reported mostly during raining [5,11]. The reason for such pattern cold be attributed to c contamination of source of water in the community due to improper disposal of human and animal wastes. Similarly, other studies have linkedassociation between domestic water source and risk of diarrhea [18], fecal contamination of water source [19] and the use of shallow well [20].

The infection route is facilitated viafeacal-oral route,, person-to-person(hand –to-mouth) contact, contact with formites, which mostly common with children. In this study, high prevalence of enteropathogenswere observed more among males and children within the age-group 12-48.This age-group have high possibility of contact with contaminated food and drinks, and soil due to their daily outdoors activities. Studies have reported close association between contamination of the source of water and risk of developing diarrhea [18-22]. Ova of animals enteropathogens such as *A. lumbricoides, T. saginata* and hookworm, can persist in the soil for long time and invariably responsible for contamination of water that could initiate diarrheic processes.

Shigellosis isa major public health problem responsible for high morbidity and mortality rate among children aged less than 5years in tropical countries [23-26]. In this study, 5Shigellaspp were isolated, 2 isolates in patients presenting with dysentery and 3 isolates in mucoid /bloody stool. *Shigella flexneri*, is mostly identified in cases of dysentery, particularly in patients with mucoid/bloody stool. However, serotyping of *Shigella* spp isolates provides a detailed clinical information on bacteria serotype associated with childhood diarrhea as reported in other studies [27,28]

In this study, we observed co- infection level of 12.2%, in which combination of *E. coli* and *E. histolyitca* accounted for 62.5%. Our level of 12.2% is low, when compared similar study in India that reported a level of 20.7%(29). Co- infection pattern denotes a clinical situation whereby more than one enteropathogen are involved in initiating the diarreal case .It is also a common pattern observable in childhood diarrheal cases , reported in developing countries. Such pattern revealed the possibility of multiple source of infection and associated enteropathogens spectrum. Although, the WHO recommendation of fluid and electrolytes replacement remains the standard approach, information on associated enteropathogens could assist in empirical therapy in reducing high morbidity and mortality rate that might be due to these enteropathogens negative impact.

5. CONCLUSION

Although the number of enteropathogens identified in this study were relatively low, but were similar to those reported in to other studied. However, it provided a baseline epidemiological information of enteropathogens and associated clinical conditions in childhood diarrheal cases. The limitation of the study are (i) as a retrospective study, there is high possibility of documentation error, and sampling problems (ii) the high negative result from 55 stools due to limited identification methods. Further studies at larger scale is being suggested in order to evaluate other contributory factors in the community and their effect on implicating enteropathogens.

COMPETING INTEREST

Authors have declared that no competing interest exists.

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