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Acute diarrhoea in hospitalized under-five children in Ilorin, Nigeria: Relationship between isolated enteropathogens and clinical outcome

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Abstract*: Background:* Acute diarrhoea due to different enteropathogens contributes significantly to childhood morbidity and mortality globally, despite the advances made in diarrhoea management via use of fluid therapy and zinc supplementation.
Aim: To determine the prevalence of bacterial and viral etiology among children hospitalized with acute diarrhoea and the relationship with clinical outcome.
Methods: A total of 135 children aged one to 59 months with acute diarrhoea were recruited in Ilorin, Nigeria. Stool specimens were investigated for three viruses (rotavirus, adenovirus and norovirus) and bacterial pathogens using immunochromatographic and conventional culture techniques respectively.
Results: One hundred and twenty-two (90.4%) study participants had stools that yielded enteropathogens, while 13 (9.6%) had no isolates. Sixty-one (45.2%) children had a single pathogen isolated, while 61 (45.2%) had co-infections. The prevalence of viruses was 17.0% with rotavirus, adenovirus and norovirus detected in 10.3%, 3.7% and 3.0% respectively. Bacterial pathogens were isolated in 28.2% with *Escherichia coli* (14.1%), *Klebsiella spp*. (8.2%), *Proteus spp.*(3.7%) and *Pseudomonas aeruginosa* (2.2%) detected as isolates. The duration of hospital stay, likewise the duration of diarrhoea were prolonged by the presence of an infectious aetiology and co-infections ($p < 0.001$ and $p = 0.04$ respectively). However, these clinical outcomes were not influenced by the type of bacteria/virus isolated nor the enteropathogens constituting co-infection ($p > 0.05$).
Conclusions: There was a high burden of infectious aetiology of childhood diarrhoea, with bacteria being the predominant enteropathogens isolated. The clinical outcomes of the children studied were influenced by the isolation of enteropathogens and the presence of multiple infections.

Keywords: Acute diarrhoea, children, enteropathogens, clinical outcome, Nigeria.

Introduction

Diarrhoea is defined as the passage of at least three loose or liquid stools per day.[1] Episodes of diarrhoea are classified as acute diarrhoea, dysentery or persistent diarrhea with acute diarrhoea lasting for less than fourteen days and accounting for most of the burden attributable to the disease.[1] It is currently the fourth leading cause of morbidity and mortality in children aged less than five years worldwide.[2–4] In 2015, 8.6% of the 5.8 million global deaths in under-five children was due to diarrhoea disease.[2–4] Indeed, it contributes 10% to under -5 mortality rates of India, Nigeria and Pakistan which together constitute the highest burden (40%) to global under-5 mortality.[5,6]

In Africa's most populous country, Nigeria, acute diarrhoea remains a public health concern. Children below five years old have an average of 3-5 diarrhoea episodes per annum with an estimated 150,000 deaths annually.[7] Mortality from childhood diarrhoea may occur from complications such as shock, electrolyte imbalance and sepsis.[4,8] In terms of morbidity, children in Nigeria and other developing countries suffer other direct consequences of diarrhoea such as malnutrition, diminished growth and impaired cognitive function.[8,9]

Diarrhoea disease of infectious origin could be due to different microbial agents, however viruses and bacteria are the most frequently isolated globally.[8,10–12] Furthermore, the contribution of these pathogens to childhood diarrhoea differ substantially between regions depending

on the local meteorological, geographic and socioeconomic conditions.[10] Global estimates show that rotavirus, adenovirus and norovirus contribute the highest viral-specific diarrhoea morbidity and mortality, while bacteria frequently implicated include *Escherichia coli*, *Shigella spp*., *Salmonella spp*. and *Campylobacter jejuni*.[4,10,13] These enteropathogens of diarrhoea mostly have a faeco-oral transmission mostly through contaminated water, food, hands and feeding utensils, although are less commonly spread via direct contact among persons.[8,11,12]

While there have been advances in the management of acute diarrhoea via the use of oral rehydration therapy and zinc supplementation, it still constitutes about 13% of all years lost due to ill-health, disability or early death (disability-adjusted life years) worldwide.[4] Consequently, this affects the health, school attendance and educational performance of children, with long term negative impact on the socio-economic development of countries. Therefore, exploring the relationship between other factors such as isolated enteropathogens and clinical outcome would not be amiss. This might give better insight into the clinical management of pathogen-associated diarrhoea.

Patients and Methods

This descriptive, cross-sectional study was conducted at the Emergency Paediatric Unit (EPU) of the University of Ilorin Teaching Hospital (UITH) located in Ilorin, Kwara State, North Central Nigeria between December 2015 and September 2016.Ethical approval was obtained from the Hospital Ethical Review Board (UITH/CAT/189/19^{A}/637). All parents / caregivers of eligible children were informed of the purpose of the study, expected procedures and potential risks and benefits following which a written consent was obtained prior sample collection.

A total of 135 children aged one to 59 months presenting with acute watery diarrhoea were recruited. Acute diarrhoea was defined as passage of loose or watery stools at least three times within a 24 hour period, developing over a few hours or days and lasting fewer than 14 days.[1]

The exclusion criteria were children with persistent diarrhoea (>14 days), dysentery, positive HIV status, severe malnutrition and presence of chronic gastrointestinal disorders e.g Crohn's disease. The minimum sample size required was determined using a formula for estimation of a single proportion as follows:

$$N = \frac{z^2 p(1-p)}{d^2}$$

Where z: standard normal variable at 95% confidence level (1.96); p: proportion about prevalence of diarrhoea[14]; d: 0.05 (5% margin of error). A purposive sampling was used until the desired sample size was attained. Socio-demographic and clinical details were obtained using a structured questionnaire that was pretested with appropriate modifications made prior commencement of the study.

Fresh stool samples were collected into clear, wide-mouth sterile universal bottles with proper identification labeled on each bottle and immediately processed or kept in a refrigerator at 4^0C until processing (within 6 – 8 hours of collection). Rotavirus, adenovirus and norovirus were detected by immunochromato graphic technique (ICT) using rapid enzyme immunoassay kits manufactured by Oxoid Inc., United Kingdom. Faecal samples were diluted to 10% suspension by putting approximately 100µl of liquid faeces in an appropriately labeled sterile container using transfer pipettes and adding 1ml of sample diluent from the immunoassay kit to be used. Briefly, 2 drops (100µl) each of diluted faecal samples, negative control and positive control were added to separate micro wells. Thereafter, 2 drops (100µl) of enzyme conjugate was added to each microwell and incubated at room temperature for 60 minutes. The contents of the microwells were then poured out and washed with diluted wash buffer. Two drops (100µl) of colour substrate was added to each microwell and incubated at room temperature for 10 minutes. Results were interpreted according to the manufacturer's instructions.

For bacteria isolation, each specimen was examined macroscopically for consistency, colour and presence/ absence of mucous and blood. The samples were then cultured on differential, selective and enrichment media namely MacConkey bile salt agar, Xylose Lysine Deoxycholate agar (XLD) and Selenite F broth. Each of the agar plates were labelled with numbers allocated to subjects after which a sterile wire loop was used to inoculate each faecal sample using streaking technique.[15] Each plate was incubated at 37^0C for 18-24hours and examined for possible bacteria growth. Agar plates with positive cultures had their representative colonies gram stained. Biochemical tests such as citrate, indole, urease, oxidase and triple-sugar-iron (TSI) tests were then carried out to identify bacterial isolates using standard microbiological techniques.[15]

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (IBM Corp., Armonk, NY, USA). Measures of central tendency, dispersion of quantitative variables, and proportion for the qualitative variables were presented. The independent t-test (t) and analysis of variance (ANOVA) test were used to identify significance when comparing less than three, and three or more continuous variables respectively. The Least Significant Difference (LSD) posthoc test was used when appropriate. The study examined the effect of the enteropathogens isolated on two diarrhoea- related outcomes – duration of hospital stay and duration of diarrhoea. Probability values $p < 0.05$ were accepted as statistically significant.

Results

A total of 135 fecal samples were analyzed from the subjects, comprising 85 males (63.0%) and 50 females (37.0%) with a male-female ratio of 1.7:1. The mean age of the subjects was 10.4 ± 2.8 months. Children aged one year and below comprised 74.1% of the study population (Table 1). One hundred and twenty two (90.4%) of the children with acute diarrhoea had stools that yielded pathogens, while 13 (9.6%) did not yield any organism. Bacteria were detected as a single infection in 38 (28.2%) children, while 23 (17.0%) had single viral aetiology. Multiple (bacteria and/or viral) enteropathogens occurred as co-infections in the remaining 61 (45.2%) children (Table 2). Of the bacteria isolated, *Escherichia coli* accounted for 14.1%, *Klebsiella spp.* 8.2%, *Proteus spp.* 3.7% and *Pseudomonas aeruginosa* 2.2%. No isolates of *Salmonella spp*. and *Shigella spp*. were detected. Rotavirus was the most frequently isolated among the viruses (10.3%). Adenovirus and norovirus were detected in 3.7% and 3.0% of the children respectively. Table 3 shows that 77.9% of the study population who had enteropathogens isolated in their stool was aged between 2-12 months, while children > 12 months of age constituted the remaining 22.1%.

Table 1: Age and gender distribution of the study population

Age group (months)	Subject Male n	Female n	Total n (%)
2 – 12	63	37	100 (74.1)
13 – 24	15	12	27 (20.0)
>24	7	1	8 (5.9)
Total	85 (63)	50 (37)	135 (100)

Table 2: Frequency distribution of enteropathogens in the study population

Enteropathogens	Subjects n =135 (%)
Children with isolated enteropathogens	122 (90.4)
Bacteria (single infection)	38 (28.2)
Escherichia coli	19 (14.1)
Klebsiella spp.	11 (8.2)
Proteus spp.	5 (3.7)
Pseudomonas aeruginosa	3 (2.2)
Salmonella spp.	0 (0.0)
Shigella spp.	0 (0.0)
Virus (single infection)	23 (17.0)
Rotavirus	14 (10.3)
Adenovirus	5 (3.7)
Norovirus	4 (3.0)
Co-infections	61 (45.2)
Bacteria-Virus	37 (27.4)
Bacteria-Bacteria	13 (9.7)
Virus-Virus	11 (8.1)

The mean duration of hospital stay of the subjects was 4.2 ± 1.3 days. As shown in Table 4,children with diarrhoea due to enteropathogens had a significantly longer duration of hospital stay when compared with those with no isolate (p<0.001), likewise children in whom multiple pathogens were isolated as against those with single bacterial or viral aetiology (p <0.001). However, individual pathogens among the bacteria / viruses isolated did not influence the duration of hospital stay (p =0.170 and p =0.117 respectively). Furthermore, children with co-infections had a comparable duration of hospital stay irrespective of the enteropathogen combination (p =0.096).

The mean duration of diarrhoea in the subjects was 7.0 ± 2.3 days. Table 5 shows that the subjects with no isolates in their stools had a significantly shorter diarrhoea duration when compared to those with isolated enteropathogens (p =0.04). Similarly, the duration of diarrhoea in children with single pathogens was significantly shorter than those with multiple infections (p =0.007). The diarrhoea duration was however not influenced by the type of pathogens among the bacteria or viruses isolated (p =0.820 and p =0.195 respectively). Subjects in whom bacteria and viruses co-infected had a comparable diarrhoea duration with those with co-infections consisting only bacteria or viruses (p =0.112).

Table 3: Age distribution of enteropathogens isolated in the study population

Age group (months)	Enteropathogens Bacteria n = 38	Viruses n = 23	Co-infections n = 61	Total n=122(%)
2 – 12	29	17	49	95 (77.9)
13 – 24	7	5	9	21 (17.2)
>24	2	1	3	6 (4.9)

Table 4: Mean duration of hospital stay based on enteropathogens isolated

Variable	Frequency	Duration of hospital stay (days) Mean ± SD	t	*p* value
Pathogen isolated				
Yes	122	5.0 ± 0.8	5.864	<0.001
No	13	3.4 ± 1.8	F	
Type of pathogen				
Bacteria	38	3.2 ± 1.5[a]	59.113	<0.001
Viruses	23	3.5 ± 1.3[a]		
Co-infection	61	6.4 ± 1.7[b]		
Bacteria				
Escherichia coli	19	3.1 ± 1.5	1.776	0.170
Klebsiella spp.	11	4.0 ± 1.0		
Proteus spp.	5	2.8 ± 1.4		
Pseudomonas aeruginosa	3	2.5 ± 0.7		
Virus				
Rotavirus	14	3.7 ± 1.3	2.393	0.117
Adenovius	5	3.5 ± 1.6		
Norovirus	4	2.0 ± 1.4		
Co-infections				
Bacteria-Virus	37	6.6 ± 2.1	2.438	0.096
Bacteria-Bacteria	13	5.8 ± 1.6		
Virus-Virus	11	5.2 ± 1.9		

t: Independent samples t test; F: ANOVA (Analysis of Variance) followed by LSD (Least Significant Difference) posthoc test; the different alphabets (a,b) connote a significant difference between the means.

Table 5: Mean duration of acute diarrhoea based on enteropathogens isolated

Variable	Frequency	Duration of diarrhoea (days) Mean ± SD	t	*p* value
Pathogen isolated				
Yes	122	7.1 ± 2.1	2.062	0.041
No	13	5.8 ± 2.7	F	
Type of pathogen				
Bacteria	38	7.3 ± 3.6[c]	5.247	0.007
Virus	23	6.7 ± 2.0[c]		
Co-infection	61	8.7 ± 2.6[d]		
Bacteria				
Escherichia coli	19	7.8 ± 3.8	0.307	0.820
Klebsiella spp.	11	8.1 ± 3.6		
Proteus spp.	5	8.5 ± 0.7		
Pseudomonas aeruginosa	3	6.2 ± 2.8		
Virus				
Rotavirus	14	6.8 ± 1.0	1.779	0.195
Adenovirus	5	6.4 ± 2.4		
Norovirus	4	5.1 ± 2.2		
Co-infections				
Bacteria-Virus	37	9.1 ± 2.8	2.273	0.112
Bacteria-Bacteria	13	8.2 ± 1.8		
Virus-Virus	11	7.4 ± 1.6		

t: Independent samples t test; F: ANOVA (Analysis of Variance) followed by LSD (Least Significant Difference) posthoc test; the different alphabets (c,d) connote a significant difference between the means.

Discussion

This study underscores the importance of bacteria and viruses as a cause of childhood diarrhoea as demonstrated by the high prevalence (90.4%) of isolated enteropathogens in the study population. This finding is consistent with a prevalence of 92.7% by an earlier report from India.[16] However, this is at variance with other studies that reported lower prevalences between 64.0 and 81.3% in Africa.[17–20] The high prevalence in this current study may be due to the exclusion of subjects with co-morbidities such as HIV infection and severe malnutrition, which increases the risk of diarrhoea. In addition, this study also investigated the presence of two other viral pathogens (norovirus and adenovirus) besides rotavirus in acute diarrhoea which might have increased the frequency of cases with known aetiology, thereby resulting in few children without identifiable stool enteropathogens (9.6%).

As with earlier studies conducted in Africa, bacteria were the predominant enteropathogens isolated in the subjects with a prevalence of 28.2%.[17,18,20] In contrast, researchers from China reported viruses as the prominent pathogens associated with acute diarrhoea.[21,22] Socio-economic factors such as poverty, lack of potable water, poor sanitation as well as malnutrition and poor hygiene in the developing world could have contributed in no small measure to the predominance of bacteria infection reported in the African region where this study was conducted.[23–25]

Escherichia coli was the leading cause of acute diarrhoea among the isolated bacteria pathogens in this study, which is similar to other African studies.[17,18,26,27] The 14.1% prevalence observed in this study is comparable to other studies emanating from Nigeria and Central Africa Republic,[19,20,28] although lower than findings in Burkina Faso and Bangladesh.[17,29] In contrast, prevalence rates as low as 4.7% have been reported in China.[30] These varying prevalences may be ascribed to differences in infrastructural and socioeconomic indices among the cities where the studies were conducted.

In this study, *Klebsiella spp*. was isolated in 8.2% of the children which is in conformity with previous Nigerian studies.[20,31] However, a prevalence of 34.5% was reported in an Indian study.[16] *Pseudomonas aeruginosa* was reported in 2.2% of the subjects, which is consistent with the prevalence of 2.7% detected by a similar study also conducted within the same geographical region (North central Nigeria).[31] This previous study reported an 8.4% prevalence rate of *Proteus spp*. which was slightly higher than the 3.7% found in the present study.[31]

Interestingly, there were no isolates of *Salmonella spp*. and *Shigella spp*. cultured in this study, a finding in concordance with an earlier study conducted in Enugu, Nigeria.[32] This is in contrast to other reports across Africa which detected these enteric pathogens at rates ranging from 2.2 – 10.0% and 2.5 – 8.0% respectively.[17–19,26] This may be attributed to the exclusion of children with dysentery, as a well known mechanism of action of *Salmonella spp*. and *Shigella spp*. is intestinal mucosal invasion causing bloody stools.[33] Furthermore, the growth of these aforementioned pathogens may have been suppressed by premedication with antibiotics prior hospital presentation as this could have been responsible for false negative results.[34]

The overall prevalence of viruses isolated in this study was 17.0% which is similar to an earlier Nigerian study,[35] although research has shown varying prevalence of viral pathogens across the globe. Rotavirus was the most frequently detected viral pathogen which lends further credence to its well documented role in childhood diarrhoea globally. The observed 10.3% prevalence in this study is in accordance with other reports from Nigeria[36] and Kenya[37] although somewhat lower than other reported prevalences of 15.6% to 56.0%.[38–41] The lower prevalence of rotavirus detected in this study may be due to seasonal factors, as the period of sample collection in this present study (December – September) excluded some months in the harmattan season which might have reduced the isolation rate. The documented peak period of Rotavirus infection is during the cool harmattan season and timing of collection of stool samples during this period would increase isolation rates of the pathogen.[42,43]

The proportion of norovirus in the subjects identified in

this study (3.0%) is in keeping with studies emanating from Nigeria and India.[39,44] Conversely, higher prevalences between 6.7% and 23.1% have been reported.[22,45,46] Adenovirus was detected in 3.7% of the study population which is in accordance with observations from studies conducted in Burkina Faso[17] and Iran[47], although a slightly lower prevalence of 2.0% was reported in a Brazilian study.[48] Against this background, it is noteworthy that both adenovirus and norovirus are becoming important causes of childhood diarrhoea in this clime.

This current study found a high proportion of co-infections involving enteropathogens of acute diarrhoea (45.2%). This is at variance with lower values of 10.0 to 40.1% documented across Africa and beyond.[16–19,21,22] The high occurrence of bacterial and/or viral co-infections in this study may be due to the fact that these pathogens are food-borne and therefore tend to be found in the same contaminated food or water increasing the likelihood of mixed infections in risk areas. As earlier noted, poor sanitary conditions as well as poor quality of food and drinking water may play a pivotal role to widespread transmission of these pathogens.[24,25] Therefore, the current non-recommendation of routine antibiotic use during acute diarrhoea episodes in this clime may need to be reviewed.

Against the background of paucity of published studies documenting the relationship between enteropathogens of childhood diarrhoea and clinical outcome for comparison, this current study found that the presence of an isolated pathogen, as well as multiple infections prolonged both the duration of diarrhoea and hospital stay. This could be attributed to the pathogenic mechanisms of these pathogens on the intestinal walls such as production of enterotoxins/cytotoxins, mucosal adherence, invasion and destruction resulting in net secretion with subsequent diarrhoea.[15,24,33,42] This effect could have been accentuated by the synergism of pathogenesis involving two or more enteropathogens, hence the worsening of clinical outcome variables in children with co-infections.

The duration of diarrhoea or hospital stay however did not differ irrespective of the type of bacteria / virus isolated, nor the enteropathogen combination constituting the co-infection. A plausible reason could be the similar pathogenic mechanisms of diarrhoea by some bacteria and viruses.[15,33,42] Furthermore, the duration of symptoms from these enteropathogens are usually self-limiting, lasting a few days to a week, less so with institution of appropriate therapy such as fluid therapy, zinc supplementation and antibiotics where necessary.[24,25] The paucity of published studies has not enabled the comparison of these findings, therefore more studies will be needed to disprove or otherwise the aforementioned observations.

The primary limitation of this study was that the isolated bacterial pathogens were not serotyped.

Conclusion

There is a high burden of infectious aetiology of childhood diarrhoea in this clime, with bacteria being the predominant enteropathogens isolated. Co-infections with two or more enteropathogens were also common. The clinical outcomes of the children studied were adversely influenced by the presence of enteropathogens, as well as co- infections. However, more research is needed to understand the effect of these enteropathogens on diarrhoea-related outcomes in childhood diarrhoea.

Authors' Contribution

Afolabi OF: Concept and design, literature search, data acquisition and analysis, statistical analysis, manuscript preparation, final approval of manuscript.

Saka AO: Definition of intellectual content, literature search, data analysis, manuscript editing and review, final approval of manuscript.

Ojuawo A: Definition of intellectual content, data analy sis, manuscript editing and review, final ap proval of manuscript.

*All authors take responsibility for the integrity of the work and are therefore designated as guarantors.

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