Bacterial Agents Associated With Acute Diarrhoea In Under-5 Children In Enugu, Nigeria.

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Abstract: Background: *Diarrhoea continues to be the scourge of children across the world. Despite efforts by the World Health Organisation and other agencies, much still remains to be done in combating diarrhoeal diseases in the developing world, including Nigeria.*
Objectives: *To determine the bacterial agents of diarrhoea in under-5 children and their antibiotic sensitivity patterns.*
Subjects and Method: *One hundred and seven-four children below five years of age with diarrhoea and a similar number as controls were recruited for the study between June 2005 and May 2006 at the University of Nigeria Teaching Hospital, Enugu, Nigeria. Their socio-demographic factors such as age, sex, breastfeeding status, parents' occupation/education, were obtained.*
Their stool samples were examined macroscopically and microscopically. Stool cultures and sensitivity tests were performed.
Results: *Enteropathogenic E. coli was identified in 41.4% of the stool samples. There was no statistically significant difference in breastfeeding between the subjects and controls (p>0.05). The age and sex distribution of the subjects with positive stool culture were not statistically significant (p>0.05) when compared with those with negative cultures. Blood in stool was significantly associated with positive stool culture (p=0.0112).*
*   Drugs that can be useful in the empirical treatment of diarrhoea due to enteropathogenic Escherichia coli are gentamycin, amoxicillin-clavulanate, ciprofloxacin and cefuroxime.*
Conclusion: *Enteropathogenic E. coli is a significant bacterial agent in diarrhoea in childhood. Improvement in breastfeeding, socioeconomic and nutritional status will lead to a reduction in the incidence of diarrhoea due to bacterial pathogens.*
Key Words: *Acute diarrhoea; Under-5; Sociodemographic factors; pathogens.*

I.   Introduction

Diarrhoea has been defined as a change in bowel habit from normal with an increase in stool volume and/or fluidity with or without an increase in stool frequency.[1]

Diarrhoeal diseases are one of the leading causes of morbidity and mortality in children worldwide. The problem is serious in tropical countries where it is responsible for more than half the deaths in children under one year of age, and over 1.3 million deaths in children below 5 years of age.[2,3]

Diarrhoea cuts across all ages.[4] Young children between the ages of 6-24 months, and those of lower socioeconomic status are the most commonly and severely affected.[4] Breastfeeding provides significant protection against diarrhoea in infancy.[5] Malnutrition predisposes to high transmission of diarrhoea.[6] Bacterial pathogens are important causes of diarrhoea in children, particularly in developing countries where standards of personal and community hygiene are low.[7]

II.   Subjects And Methods

The study was carried out at the University of Nigeria Teaching Hospital Enugu, Nigeria, a referral tertiary health facility. One hundred and seventy-four under-5 children with diarrhoea and a similar number of children as controls were recruited consecutively as they presented to the health facility from June 2005 to May 2006. The controls were matched for age, sex, socioeconomic and nutritional status.

A pretested questionnaire was used to obtain information on the age, sex, anthropometry, breastfeeding status, occupational/educational status of the parents.

The social class was determined using classification of social class as proposed by Oyedeji.[8] This classification used the parental occupation and educational attainment to determine the social class.
The nutritional status was determined using the modified Wellcome classification.[9]

Approval was obtained from Hospital Ethical Committee. Informed written consent was obtained from the parent or caregiver.

Clinical examination was performed on the subjects and controls. Five randomly selected stool samples were examined by the researcher and the Laboratory Scientist independently. The results were compared to ensure reliability. The Kappa score was 0.80.

Stool samples were collected from the patients. Samples were collected in clear wide-mouth universal containers and sent immediately to a registered medical laboratory scientist or kept in an insulated box with ice-packs until processing (within 6-8 hours of collection).

All stool specimens were analyzed following the standard methods of stool examination.[10] These included the following:

1. **Macroscopy:** Gross appearance of the stool was noted especially consistency, presence of mucus and blood.
2. **Microscopy:** A small portion of each stool was suspended in saline and then examined microscopically for pus cells and red blood cells.
3. **Bacteriology:** All stool samples in liquid form were inoculated directly, whereas formed stools from the controls were first suspended in sterile normal saline solution and then inoculated.

Primary isolation of the bacteria was performed using MacConkey agar and Desoxycholate citrate agar media and selenite-f enrichment medium. Each plate was subsequently incubated at 37° C for 18-24 hours, and then Gram-stained.

All isolates were characterized biochemical following conventional procedures.[10] The antimicrobial susceptibility testing was done using disc-diffusion method.[11]

Analysis of data was carried out using the Statistical Package for the Social Sciences software. The patients were grouped according to age, sex, nutritional status, socioeconomic status, breastfeeding status, bacterial isolates, etc. The Chi-squared and Fisher's exact probability tests were used where appropriate.[12]

A probability (p) value of <0.05 was considered significant. Results were presented in tables.

III. Results

Between 1st June, 2005 and 31st May, 2006, a total of 392 children were seen. Of these, 19 could not be matched for age and sex, while 25 were rejected due to incomplete data in their questionnaires. Ultimately, 348 children aged 2 to 59 months were studied with 174 serving as subjects and 174 as controls.

Age distribution of the children according to bacterial isolates

Table 1 shows the age distribution of children with acute diarrhoea according to bacterial isolates. These comprised children with positive stool culture, and those with negative stool culture. Among those with positive culture, the age group 2-24 months accounted for 63% of the total bacterial isolates.

TABLE I: AGE DISTRIBUTION OF CHILDREN WITH ACUTE DIARRHOEA

Age (months)	Subjects with Positive Stool Culture No %	Subjects with Negative Stool Culture No %	Total No %	p-value
2 – 12	30 (41.7)	47 (46.0)	77 (44.2)	0.718
13 – 24	16 (22.2)	22 (21.6)	38 (21.8)	0.918
25 – 36	13 (18.1)	20 (19.6)	33 (19.0)	0.797
37 – 48	7 (9.7)	6 (5.9)	13 (7.5)	0.342
> 48	6 (8.3)	7 (6.9)	13 (7.5)	0.716
Total	**72 (100.0)**	**102 (100.0)**	**174 (100.0)**	

Sex distribution of children with acute diarrhoea

More males had a positive stool isolates compared to the females, though the difference is insignificant (χ^2 =0.019, p=0.88), table II.

Stool frequency in children with acute diarrhoea

Table II shows the distribution of stool frequency in children with acute diarrhoea. 43% of those with positive culture had stool frequency of five or less, and 57.0% had stool frequency of six or more. Among those with negative culture, 45.1% had stool frequency of five or less, and 54.9% had stool frequency of six or more. When the two groups are compared, the difference was not statistically significant (χ^2=0.071, p=0.789).

Antibiotics treatment prior to hospitalization in children with diarrhoea

Table II shows the distribution of antibiotics treatment prior to hospitalization in children with acute diarrhoea. It shows that of those with positive stool culture, 34.7% received antibiotics and 65.3% did not receive antibiotics. Among the negative group, 40.2% received antibiotics and 59.8% did not receive antibiotics. When the two groups are compared, the difference is not statistically significant (χ^2 =0.537, p=0.464).

TABLE II: SEX, STOOL FREQUENCY AND ANTIBIOTICS USE IN CHILDREN WITH DIARRHOEA

	Subjects With Positive Stool Culture	Subjects With Negative Stool Culture	Total	
	No %	**No %**	**No %**	**p-value**
SEX				
Male	43 (59.7)	62 (60.8)	105 (60.3)	
				0.888
Female	29 (40.3)	40 (39.2)	69 (39.7)	
Total	**72 (100.0)**	**102 (100.0)**	**174 (100.0)**	
STOOL FREQUENCY				
3 -5	31 (43.0)	46 (45.1)	77 (44.2)	
				0.789
≥ 6	41 (57.0)	56 (54.9)	97 (55.8)	
Total	**72 (100.0)**	**102 (100.0)**	**174 (100.0)**	
ANTIBIOTICS USE				
Yes	25 (34.7)	41(40.2)	66(37.9)	
				0.464
No	47 (65.3)	61(59.1)	108 (62.1)	
Total	**72 (100.0)**	**102 (100.0)**	**174 (100.0)**	

Distribution of fever in children with acute diarrhoea

Table III shows the distribution of fever in children with diarrhoea. Among those who had positive stool culture, 72.2% had fever, while 27.8% did not have fever. In the group with negative culture, 72.5% had fever, while 27.5% did not have fever. When the two groups are compared, the result is not statistically significant (χ^2 =0.0023, p=0.962).

Distribution of mucus in stool in children with diarrhoea

Table III shows the distribution of mucus in the stools of children with acute diarrhoea. From the children with positive stool culture, 36.1% had mucus in their stools, and 63.9% did not have mucus in stool. Among the group with negative stool culture, 47.1% had mucus in the stool, while 52.9% did not have mucus in the stool. When the two groups of children are compared, the difference is not statistically significant (χ^2 =2.070, p=0.150).

Distribution of blood in stool in children with diarrhoea

Table III shows the distribution of blood in the stools of children with acute diarrhoea. Bloody stool was observed in 6.9% of the subjects with positive culture, and absent in 93.1%. All (100%) of the children with negative culture had no blood in stool. When those with positive culture are compared with their counterparts, the difference was statistically significant (p=0.0112).

TABLE III: DISTRIBUTION OF FEVER AND MUCUS/ BLOOD IN STOOL IN CHILDREN WITH ACUTE DIARRHOEA

	Subjects with positive stool culture No %	Subjects with negative stool culture No %	Total No %	p-value
Fever				
Present	52 (72.2)	74 (72.5)	126 (72.4)	
				0.962
Absent	20 (27.8)	28 (27.5)	48 (27.6)	
Total	**72 (100.0)**	**102 (100.0)**	**174 (100.**00)	
Mucus				
Present	26 (36.1)	48 (47.1)	74 (42.5)	
				0.150
Absent	46 (63.9)	54 (52.9)	100 (57.5)	
Total	**72 (100.0)**	**102 (100.0)**	**174 (100.0)**	
Blood				
Present	5 (6.9)	0	5 (2.9)	
				0.0112
Absent	67 (93.1)	102 (100.0)	169 (97.1)	
Total	**72 (100.0)**	**102(100.0)**	**174 (100.0)**	

Isolation of Enteropathogens

A total of 72 (41.4%) of the diarrhoeal stools contained recognizable enteropathogens compared with only 2 (1.2%) in the control group. The bacterial agent isolated from the study in both the subjects and controls was enteropathogenic strains of *E. coli*.

TABLE IV: ISOLATION OF ENTEROPATHOGENS

Bacteria	Subjects % (n = 174)	Controls % (n = 174)
Enteropathogenic *E. coli*	72 (41.4)	2 (1.2)
Shigella	0	0
Salmonella	0	0
Campylobacter	0	0

Antibiotic sensitivity pattern

Table V shows the antibiotic sensitivity pattern of enteropathogenic *E. coli* isolates. Fifty-three (73.6%) of the 72 bacterial isolates tested were susceptible to gentamycin, 47 (65.3%) to amoxicillin-clavulanate, 44(61.1%) to ciprofloxacin, and cefuroxime, 39(54.2%) and 34(47.2%) to cotrimoxazole and amoxicillin, respectively. The resistance to chloramphenicol was 72.2%, and ampicillin and tetracycline, which recorded 100% resistance each. Nineteen (26.4%) were resistant to gentamycin, 25 (34.7%) to amoxicillin-clavulanate and 28 (38.9%) resistance was to ciprofloxacin and cefuroxime.

TABLE V: ANTIBIOTICS SENSITIVITY PATTERN OF ENTEROPATHOGENIC *E. COLI*

Antibiotics	Sensitivity n (%)	Resistance n (%)
Gentamycin	53 (73.6)	19 (26.4)
Amoxicillin-clavulanate	47 (65.3)	25 (34.7)
Ciprofloxacin	44 (61.1)	28 (38.9)
Cefuroxime	44 (61.1)	28 (38.9)
Cotrimoxazole	39 (54.2)	33 (45.8)
Amoxicillin	34 (47.2)	38 (52.8)
Cephalexin	26 (36.1)	46 (63.9)
Ofloxacin	25 (34.7)	47 (65.3)
Chloramphenicol	20 (72.8)	52 (72.2)
Ampicillin	0	72 (100.0)
Tetracycline	0	72 (100.0)

IV.   Discussion

Acute diarrhoea is one of the leading causes of morbidity and mortality in children worldwide, especially in tropical countries where it is responsible for more than half the deaths in children under one year of age.[2] In this study, the highest frequency of culture positive diarrhoeal cases were among the subjects in the age group 2-12 months, and the 13-24 months age groups, and this is similar to reports by other authors.[4,7,13,14] This may be explained by the fact that at this stage, the children have started to move about and they put contaminated objects into their mouth.

The age range 37-59 months had the lowest frequency of bacterial isolates in this study. This is probably because of less tendency to put contaminated objects into the mouth-a finding similar to that of Oni and colleagues.[15]

There was a higher percentage of stool positivity among males than females. This was in agreement with the study of Coker et al[16] in Lagos, and Jinadu et al[4] in Ondo State. This may be due to the fact that males are generally more active than females, and therefore are more likely to pick particles from the ground and put into their mouth.

Eighty-two (47.1%) of the total population of the subjects in the present study were in the lower social class group (class IV-V). Oyedeji[8] in a study of the socio-economic and cultural background of children in Ilesha, Nigeria reported that 65% of their own patients were in the lower social class (IV-V). The preponderance of the subjects from the lower socio-economic class in the present study therefore is in agreement with the pattern observed in Ilesha.[8] This group is more likely to have poor water supply, poor personal hygiene, improper food preparation, poor weaning practices and overcrowding which predispose to high rate of transmission of diarrhoea.[17]

The highest case of diarrhoea was among those who used tap water (40.9%), followed by water tanker(26.4%), well water (12.6%) and borehole (9.2%). The relatively low case of diarrhoea in those who used well water agrees with work done in Mali by Plate et al.[18]

Also among the subjects with positive culture, 34.7% received antibiotics and 65.3% did not receive antibiotics. The increased frequency of diarrhoea among those who took antibiotics may be as a result of antibiotics-associated diarrhoea. Similarly, the smaller percentage of stool culture in those who took antibiotics may be due to the fact that antibiotics can inhibit the growth of bacteria and give a false-negative result.[19] From this study, the rate of use of antibiotics was between 59.8%-65.3%. This is almost similar to a study by Oni et al,[15] who found a rate of 54.5%.

In the study, there was a higher rate of diarrhoea with positive stool culture among children who were not breast-fed compared to those who were breast-fed. This finding is in keeping with a previous study.[5] It is known that breastfeeding provides a significant protection against diarrhoeal disease.[5] Breastfed children however, can have diarrhoea usually from lack of exclusive breastfeeding, or when they put contaminated objects into their mouth. This may have accounted for the high rate of diarrhoea even among the breastfed children, as in a previous study.[15]Fever was found in 72.2% of the subjects with positive stool culture and 72.5% of the subjects with negative culture. This is similar to previous studies,[20,21] which recorded 73.6% and 74.5% respectively. Mucus was seen in 36.1% of those with positive stool culture. This is similar to the study by Alabi et al.[20]

Bloody stool was seen in 6.9% of the subjects. This is also similar to a previous study,[14] which noted that 10% of diarrhoea cases had blood in stool. Stool frequency of 3-5 times was found in 43% of the subjects with positive stool culture, and a frequency of six times or more was seen in 57.0% of the subjects with positive culture. There was no statistical significant difference between these and their counterparts with negative stool culture.

Bacterial pathogen was identified in 72 (41.4%) of the subjects, with as high as 58.6% yielding no recognizable enteropathogens. The particular strain of the bacteria identified was the enteropathogenic strain of *E. coli*. This is in consonance with previous studies[22-24] which observed that enteropathogenic strain of *E. coli* was the commonest cause of diarrhoea in children less than 5 years of age. The high rate of antibiotic use could have suppressed the growth of other bacteria as it is known that premedication with antimicrobial agents can give rise to false-negative laboratory tests.[23-24] Again other enteropathogenic agents other than bacteria could have been responsible for the diarrhoea in the remaining 61.5% of the subjects.

The antibiotics sensitivity pattern shows high sensitivity to gentamycin, amoxicillin-clavulanate, ciprofloxacin and cefuroxime. The sensitive to gentamycin here is similar to a previous study,[20] which recorded a high sensitivity to gentamycin. Workers at Kenya,[25] Uganda,[26] and also in Nigeria,[27-30] have found a high resistance to ampicillin which is in agreement with findings in this study.

The high rate of isolation of *E. coli* in Enugu is a pointer to the fact that this organism still plays a significant role in acute diarrhoea. Preventive and control measures to reduce the incidence of diarrhoea should be adopted. These include improvement in personal and environmental hygiene. Sewage and refuse disposal methods should be improved upon. There is need to provide safe potable water.

Finally, the use of oral rehydration therapy in the management of acute diarrhoeal disease should be encouraged. This has helped in the prevention and correction of dehydration due to diarrhoea.

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