Asian Journal of Research in Infectious Diseases

1(2): 1-9, 2018; Article no.AJRID.44775

Prevalence of Some Enteropathogens among Diarrhoeic and Apparently Healthy Children in two Contiguous Petroleum Resources - Rich Areas of Niger Delta, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author IAA designed the study, performed the statistical analysis and also managed the analyses of the study. Author OI wrote the protocol, and the first draft of the manuscript. Author AEE managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRID/2018/v1i229766
Editor(s):
(1) Dr. Bobby Joseph, Professor, Department of Community Health, St. John's Medical College, Bangalore, India.
Reviewers:
(1) Nain Taara, University of Karachi, Pakistan.
(2) Hongying Zhang, China.
(3) Pooja Sarmah, Rajiv Gandhi University of Health Sciences, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/26951

Original Research Article

Received 08 August 2018
Accepted 12 October 2018
Published 31 October 2018

ABSTRACT

Aims: The study characterised some intestinal bacterial and parasitic infections involved in diarrhoea causation in contiguous communities between October 2013 and April 2014.
Study Design: A total of 150 freshly-voided diarrhoeic samples of children attending PHC, Eket and General Hospital, Ibeno, and 50 non–diarrhoeic samples (Controls) were collected. The controls were recruited from subjects outside the health facilities.
Methodology: Standard bacteriological procedures were used in the identification while antibiotic susceptibility test was done. Questionnaires were also administered.
Results: The mean prevalence rate recorded for enteropathogens among children was 73%. A high prevalence of enteropathogens was observed in the parameters examined. The bacterial

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pathogens considered were *Escherichia coli*, *Salmonella enteritidis*, *Shigella dysenteriae* and *Enterobacter* species. *Escherichia coli* were the most prevalent with the rates of 43.5% and 45% in Eket and Ibeno, respectively. Enteropathogenic *Escherichia coli* (EPEC) O26 and O111 were identified with an overall prevalence of 46.7%. Antibiotic susceptibility result shows that the organisms were most susceptible to Ciprofloxacin and resistant to Cotrimoxazole. The analyses of the questionnaires revealed that in the study area, children, whose parents have no formal education were more commonly infected with enteropathogens.

Conclusion: A systematic education of caregivers and children on personal hygiene is the best approach to reduce the intestinal pathogens.

Keywords: Enteropathogens; diarrhoea; antibiotics; susceptibility; children; Niger Delta; Nigeria.

1. INTRODUCTION

Intestinal pathogens are those organisms that cause intestinal diseases. Diarrhoea, the commonest infection caused by intestinal pathogens may result from the toxicity or invasion of the bowel by these pathogens. Infantile gastroenteritis is one of the major causes of severe morbidity and mortality among children in developing countries where mortality rate could be as high as 56% and exerts a high impact on health cost in industrialised countries [1].

Studies have shown that in Africa, diarrhoea has been estimated to be responsible for 25–75% of all infantile illnesses. In Nigeria, available reports indicate that annually more than 315,000 deaths of preschool going children are recorded as a result of diarrhoeal diseases [2]. Despite the public health implications and the enormous burden imposed on the Primary Health Care Delivery System by infantile diarrhoeal illnesses in the country, there is still negligence on the epidemiology and aetiology of infantile diarrhoea in many communities. The microbial quality of water in several rural Nigerian communities has been reported to be poor, unsafe and not acceptable for human consumption. The pathogens are variously incriminated in cases of diarrhoea, which in turn accounts for a substantial degree of morbidity and mortality in different age groups worldwide [3]. Based on this, it is necessary to have a fairly accurate picture of the situation to target interventions in an affected area.

In Nigeria, most rural communities rely on surface sources of water for drinking. These natural sources are likely to be polluted with domestic and industrial wastes. As a potential carrier of pathogenic microorganisms, water can endanger health and life. The pathogens most frequently transmitted through water are those which cause infection of the intestinal tract. The causative organisms of these pathogens are present in the faeces and urine of infected persons, and when discharged may gain entrance into a water body that ultimately serves as a source of drinking water in a community [4].

Infections by most of these organisms can be asymptomatic or can be treated with rehydration solutions. The use of antibiotics might shorten the duration of diarrhoea and limit the shedding of the organisms which otherwise might continue to pollute the environment and pose further risk of infection transmission. However, antimicrobial resistance is an evergrowing problem, and there is a need to monitor the susceptibility of common bacterial isolates to drugs used in the community to provide guidelines for the empirical treatment of bacterial infections [2]. In this study, the prevalence of some intestinal bacterial pathogens in diarrhoeal and non-diarrhoeal stools of children under-five years in Eket and Ibeno and the antimicrobial susceptibility of important bacterial isolates were determined.

2. METHODOLOGY

This work was carried out on fresh diarrhoeic faecal samples of 150 children and non-diarrhoeic faecal samples of 50 children which served as controls in Eket and Ibeno. The faecal samples were not contaminated with urine and were collected into clean, dry, transparent, leak-proof, wide-necked universal bottles. Sterile spoons were used to scoop a teaspoon amount of specimen, transferred into the universal bottles and labelled with the participant's name, age and sex. Mothers of the subjects selected for the study were given questionnaires to obtain information about their demographic data, all written in English and interpreted in their local dialect for those who were not educated. Samples were transported in improvised ice pack within 3 hours of collection to Public Health

Laboratory, University of Uyo, Nigeria for processing and examination.

The isolation of bacterial pathogens was done by culture method. The faecal samples were inoculated on the following media; MacConkey Agar (MCA), Deoxycholate Citrate Agar (DCA), Selenite Fluid (SF), Salmonella and Shigella Agar (SSA). All cultures were incubated aerobically at 37ºC for 24 hrs. Distinct colonies were Gram stained and sub-cultured onto a fresh sterile medium to ensure that further analysis was done with the fresh and pure culture. The isolates were maintained on nutrient agar slants and studies of the organisms were carried out from the pure slant cultures. Biochemical tests done for the identification of the isolates were; Motility, Indole, Citrate, Urease, Methyl Red, Voges-Proskauer, Kligler Iron Agar, Slide agglutination, and Antimicrobial sensitivity. Data generated from questionnaire administration were analysed using Statistical Product for Service Solutions (SPSS) Statistical Software, Version 20.

3. RESULTS

The male to female ratio of the sampled subjects in the study was 2:3. The prevalence of enteropathogens among children in the study areas was Eket, 74%, Ibeno, 72% and in the apparently healthy subjects (Control), 30% were infected with enteropathogens (Table 1). There was a statistically significant difference between the subjects in the study areas ($p>0.05$). The relationship between enteropathogens and the age of subjects showed that children in the age group 7 – 12 months in both Eket and Ibeno had the highest prevalence of 90%, respectively (Table 2). The prevalence of enteropathogens decreased with an increase in the age of participants, except in older children (≥ 2 years and above). Among the apparently healthy subjects, similar age-related pattern of infection was observed. The relationship between age of subjects and enteropathogens across the study areas showed no statistically significant difference ($p > 0.05$).

The distribution of enteropathogens among the children showed that children, whose mothers belong to housewife's group had the lowest prevalence of enteropathogens, (52.0%) and (50.0%) in Eket and Ibeno, respectively (Table 3). There was no statistically significant difference between the prevalence of enteropathogens in children and the occupational status of their mothers across the study areas ($p >0.05$). Children, whose parents had no formal education, were more commonly infected with enteropathogens as follows: Eket (92.0%), Ibeno (92.3%) and control (75.0%). The rate of prevalence decreased with increase in educational level across each of the study area. Children whose parents had tertiary education had the lowest prevalence of enteropathogens, (48.0%) and (33.3%) in Eket and Ibeno, respectively (Table 4). There was no statistically significant difference between enteropathogens prevalence in children and the various educational levels of their mothers across the study areas ($p >0.05$).

The relationship was established between the occurrence of enteropathogens and sources of drinking water used by subjects in the study areas (Table 6). The prevalence of enteropathogens, (90.0%) was highest in those who used stream water for drinking purpose in Eket, whereas in Ibeno (where no one among the examined subjects used stream water), borehole water had the highest prevalence of enteropathogens (82.5%). The relationship between water source used, and enteropathogens across the study areas showed the statistically significant difference ($p<0.05$).

The bacterial isolates with the highest prevalence were *Escherichia coli,* in Eket (43.5%) and Ibeno (45.0%) and *Salmonella enteritidis,* in Eket (26.1%) and Ibeno (35.0%), respectively (Table 6). EPEC O111 had the highest prevalence, 26.7% while EPEC O26 had the least, 20.0%. Subjects aged 7-12 months had the highest positive numbers in both EPEC O26 and 0111, with both having the prevalence of 50.0% and 62.5%, respectively. The overall prevalence of EPEC across the study areas was 46.7%. Findings showed that the rate of infection with EPEC serotypes decreases with increase in age of subjects (Table 7). In Eket, all isolates showed high sensitivity to Ciprofloxacin, Ceftriazone, Cefotaxime and Ceftazidime except with *E. coli* which showed a susceptibility of 8.7 % (Figs. 1 and 2).

In Ibeno, *E.coli* showed a high susceptibility to Ciprofloxacin, Ceftriazone, Ceftazidime and Cefotaxime. *Salmonella* showed a high susceptibility to Ciprofloxacin, Norfloxacin, Ceftazidime, Ceftriazone and Cefotaxime. *Shigella* showed a high susceptibility to Norfloxacin, Ciprofloxacin and Ceftriazone. *Enterobacter* showed a high susceptibility to only

Ciprofloxacin and Ceftriazone. Cotrimoxazole showed a high resistance to all the isolates except *E. coli* which showed susceptibility of 9.1% (Fig. 2). Fig. 3 shows the Susceptibility pattern of bacterial isolates to antibiotics in Control.

Table 1. Prevalence of enteropathogens among children in the study areas

Study Area	No. Examined	No. (%) Positive	Chi Square	P-value
Eket	Diarrhoeic participants (n = 100)	74(74.0)	30.01	> 0.001
	Apparently healthy subjects (Control) (n = 25)	9 (36.0)		
Ibeno	Diarrhoeic participants (n = 50)	36 (72.0)		
	Apparently healthy subjects (Control) (n=25)	6 (24.0)		
Total	200	125 (62.5)		

Table 2. Relationship between enteropathogens and age of participants

Age (Month)	Eket		Ibeno		Control	
	No. examined	No. +ve (%)	No. examined	No. +ve (%)	No. Examined	No. +ve (%)
≤ 6	20	13(65.0)	10	6(60.0)	10	2(20.0)
7-12	20	18(90.0)	10	9(90.0)	10	4(40.0)
13-18	20	15(75.0)	10	8(80.0)	10	3(30.0)
19-24	20	11(55.0)	10	4(40.0)	10	1(10.0)
≥ 24	20	17(85.0)	10	9(90.0)	10	5(50.0)
Total	100	74(74.0)	50	36(72.0)	50	15(30.0)

Table 3. Relationship between enteropathogens in children and occupational background of their mothers

Occupational Background	Eket		Ibeno		Control	
	No. examined	No. +ve (%)	No. Examined	No. +ve (%)	No. Examined	No. +ve (%)
House wife	25	13(52.0)	12	6(50.0)	12	3(25.0)
Self employed	25	23(92.0)	12	10(83.3)	12	5(41.7)
Civil Servant	25	21(84.0)	13	11(84.6)	13	5(38.5)
Unemployed	25	17(68.0)	13	9(69.2)	13	2(16.7)
Total	100	74(74.0)	50	36(72.0)	50	15(30.0)
	$\chi^2 = 3.189$; $P = 0.363$		$\chi^2 = 1.322$; $P = 0.724$		$\chi^2 = 1.880$; $P = 0.598$	

Table 4. Relationship between enteropathogens and educational background of their mothers

Educational Background	Eket		Ibeno		Control	
	No. Examined	No. +ve (%)	No. Examined	No. +ve (%)	No. Examined	No. +ve (%)
No formal Education	25	23(92.0)	13	12(92.3)	12	9(75.0)
Primary	25	21(84.0)	13	11(84.6)	12	4(33.0)
Secondary	25	18(72.0)	12	9(75.0)	13	1(7.7)
Tertiary	25	12(48.0)	12	4(33.3)	13	1(7.7)
Total	100	74(74.0)	50	36(72.0)	50	15(30.0)
	$\chi^2 = 3.730$; $P = 0.292$		$\chi^2 = 3.539$; $P = 0.3I6$		$\chi^2 = 12.457$; $P = 0.106$	

Table 5. Relationship between enteropathogens and source of drinking water

Source of water	Eket		Ibeno		Control	
	No. examined	No. +ve (%)	No. examined	No. +ve (%)	No. examined	No. +ve
Central Water Supply (piped)	10	4(40.0)	NA	NA	15	2(13.3)
Borehole	70	59(84.2)	40	33(82.5)	15	5(33.3)
Stream	10	9(90.0)	NA	NA	15	8(53.3)
Treated Table Water	10	2(20.0)	10	3(30.0)	5	0(0.0)
Total	**100**	**74(74.0)**	**50**	**36(772.0)**	**50**	**15(30.0)**
	$\chi^2 = 6.59; P = 0.03$		$\chi^2 = 16.65; P = 0.001$		$\chi^2 = 12.72; P = 0.001$	

Key: NA – Not Available, +ve – Positive, -ve – Negative, No. – Number, 0 – 'Absence of infection'

Table 6. Prevalence of bacterial isolates in diarrhoeic and control samples in the study areas

Bacterial Pathogens	Eket		Ibeno		Control	
	+ve No.	No. (%)	+ve No.	No. (%)	+ve No.	No. (%)
Escherichia coli	20	43.5	9	45.0	1	16.7
Salmonella enteritidis	12	26.1	7	35.0	4	66.6
Shigella dysenteriae	7	15.2	2	10.0	0	-
Enterobacter spp	3	6.5	2	10.0	0	-
Citrobacter spp	1	2.2	0	0	0	-
Klebsiella spp	2	4.3	0	0	1	16.7
Yersinia enterocolitica	1	2.2	0	0	0	0
Total	46	100	20	100	6	100

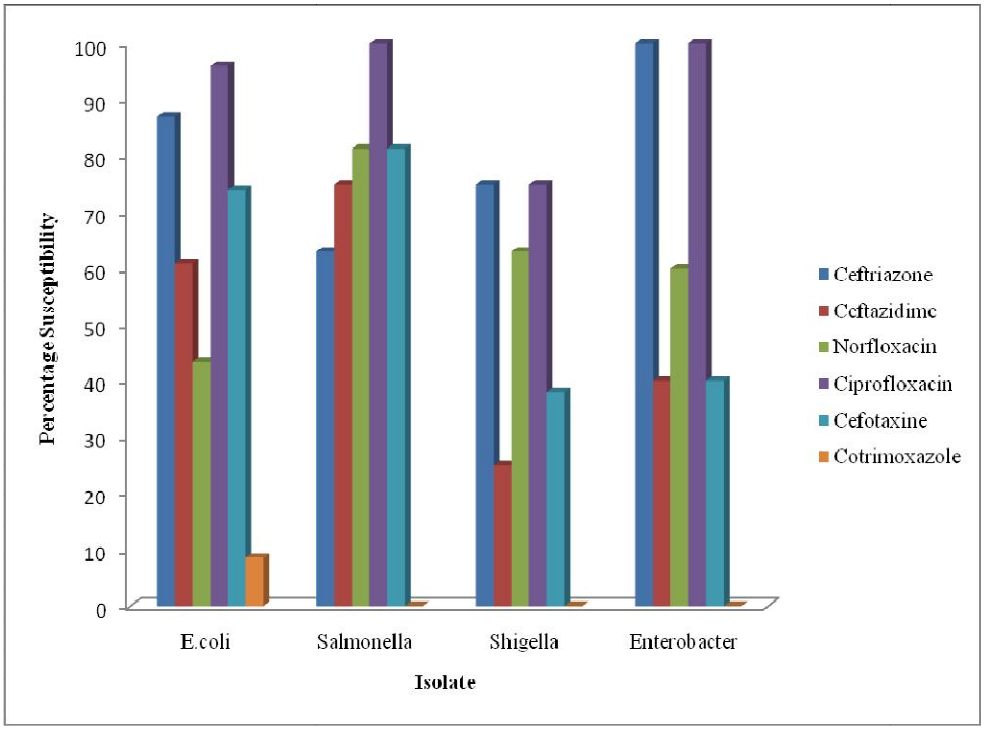

Fig. 1. Susceptibility pattern of bacterial isolates to antibiotics in Eket

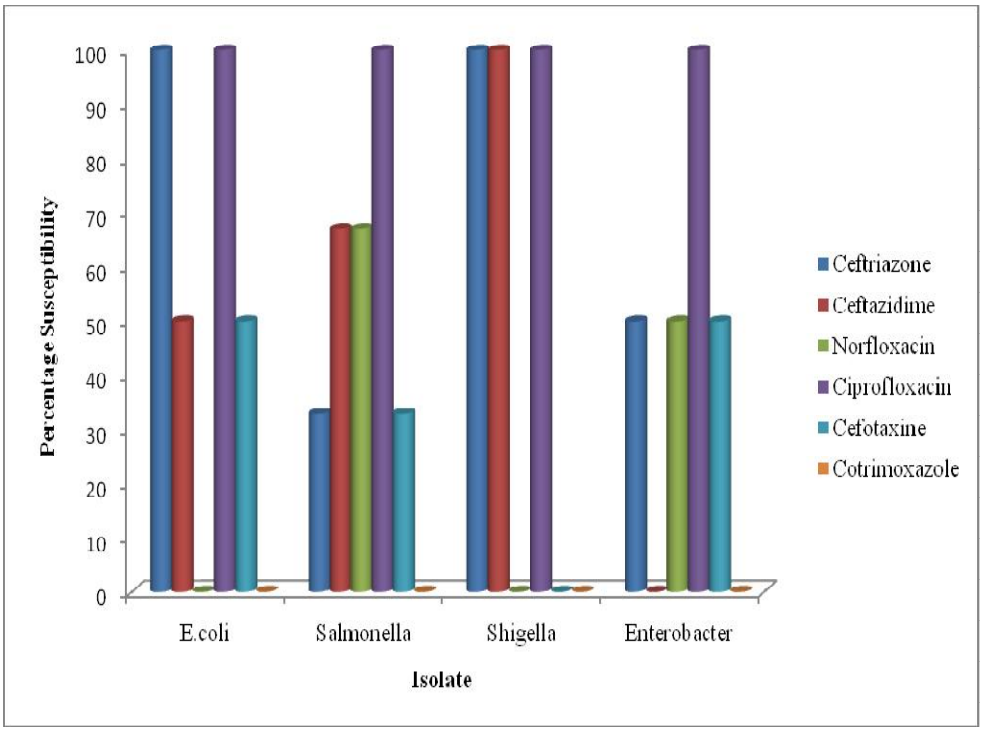

Fig. 2. Susceptibility pattern of bacterial isolates to antibiotics in Ibeno

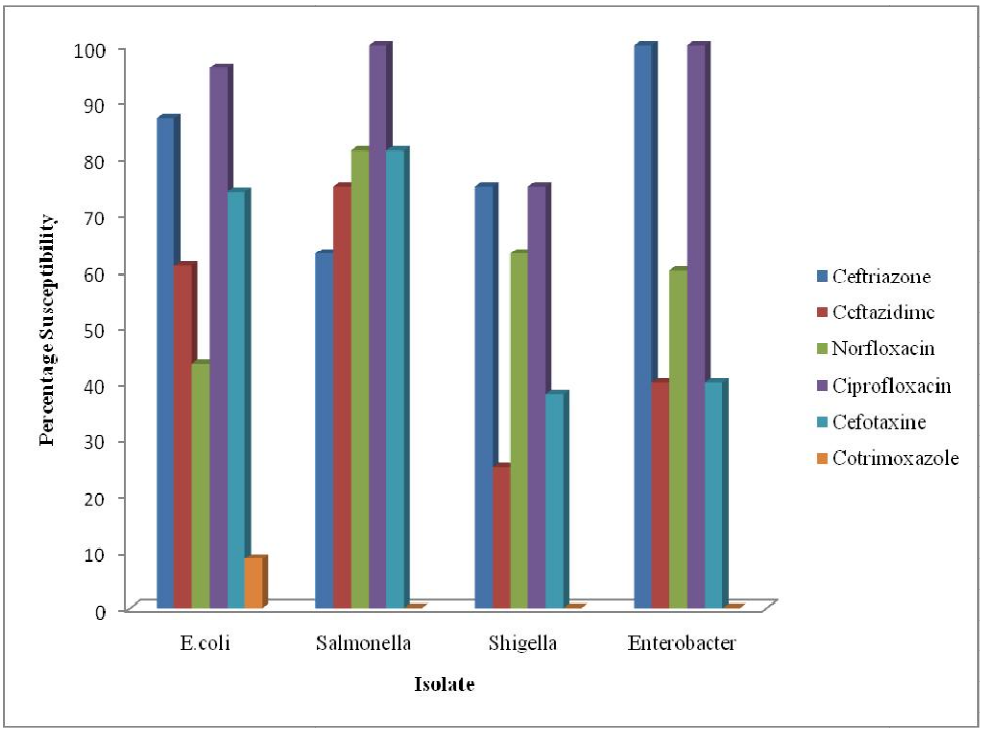

Fig. 3. Susceptibility pattern of bacterial isolates to antibiotics in Control

Table 7. Distribution of EPEC O26 and O111 in the study areas

Age group (Month)	Serotypes of EPEC		
		O26	O111
	No. Examined	No. (%)	No. (%)
≤ 6	5	1(33.3)	1(12.5)
7 – 12	14	3(50.0)	5(62.5)
13 – 18	6	1(16.7)	1(12.5)
19 – 24	3	0	1(12.5)
≥ 2 years	2	0	0
Total	30	6(20.0)	8(26.7)

4. DISCUSSION

Diarrhoeal disease of microbial origin remains one of the leading causes of severe morbidity and mortality among children in developing countries and has a high impact rate on health cost in industralised world [5]. The mean prevalence of enteropathogens in this study was 73%. This finding was comparable to the outcome of other studies [6,7]. Data obtained from the control samples revealed that a child who may be apparently healthy may act as an asymptomatic carrier harbouring a wide range of pathogenic microorganisms. Therefore, there should be growing concern among the public for regular medical check-ups for apparently healthy children.

The highest prevalence of enteropathogens was found in children between 7-12 months in Eket, Ibeno and control. This is the period when weaning practices begin in many parts of the world, including Nigeria. During the weaning period, mothers supplement breast milk with milk formulas and other foods, exposing their children to infections which may be caused by eating meals that were not hygienically prepared. Also, during this period a child learns how to crawl and toddle, along with picks and bites things from the dirty floor. These may be some of the reasons why infection is most prevalent among children in this age group [8].

The prevalence of enteropathogens in Eket and control was highest in those who used stream water for drinking purpose. Ibeno is a community surrounded by creeks, tributaries and an ocean, a coastal region which is not suitable for the existence of stream. Therefore, borehole water had the highest prevalence. The study also revealed that pipe-borne water in Ibeno had no pathogen because its potability is absolute as it is being treated and provided by Exxon Mobil. The high prevalence of enteropathogens among subjects who used stream water may be as a result of faecal contamination of the water bodies by infective pathogens.

The distribution of enteropathogens among the children in relation to their mothers' nature of work revealed that the children whose mothers were housewife had the lowest prevalence of pathogens, while the self-employed and civil servant groups had the highest prevalence of 92.0%, 83.3% for Eket and 84.0%, 84.6% for Ibeno. Mothers who are employees or businesswomen leave their children under the care of maids and nannies. Therefore, the major factor attributed to this high prevalence may be non- adherence to hygienic measures by them [9].

Children of mothers without formal education were the most infected with the prevalence of enteropathogens in Eket, Ibeno and control. However, there was no significant difference in the prevalence of enteropathogens isolated in relation to the mother's level of education in this study. This is comparable to the results recorded in another study [9]. Also, children who were exclusively breastfed were not infected. The absence of pathogens in exclusively breastfed children corroborates findings from previous studies regarding the protective role of colostrum contained in breast milk which contains a high level of Immunoglobulin against bacterial gastroenteritis [10]. Faulty weaning practices, interruption of breast milk with milk formulas, cereals, etc. and poor hygiene during food preparation may contribute to increased gastroenteritis in mixed fed and no breast milk groups.

The bacterial pathogens encountered in this study were *Escherichia coli*, *Salmonella*, *Shigella*, *Citrobacter*, *Enterobacter*, *Yersinia* and *Klebsiella* species. Although the spectrum of bacterial pathogens implicated in acute diarrhoea

varies from one region to the other, *Escherichia coli* and *Salmonella* were the commonest pathogens in Eket and Ibeno. This was in conformity with the findings made by Ethelberg et al. [5]. Both *Escherichia coli* and *Salmonella* species were reported to be very commonly associated with enteric diseases in developing countries and are more important to the epidemiology of diarrhoea in poorer areas [11]. The prevalence of *Shigella* species recorded in this study was in contrast to the findings of Ethelberg et al. [5]. The prevalence of *Escherichia coli* and *Enterobacter* species is generally an indication of faecal contamination of water as it is commonly found in the gastrointestinal tract and faeces of humans. Their presence provides direct evidence of faecal contamination probably resulting from the poor personal hygiene such as hands not properly washed after defaecation. The use of uncomposted manure and irrigation water are also potential sources for *Escherichia coli* and *Salmonella* [12]. Infection of the participants with *Salmonella* could be as a result of contamination of food by caregivers who may be asymptomatic carriers.

The distribution of EPEC serotypes O26 and O111 showed that children between 7-12 months had the highest prevalence of serotype O26 and O111 in Eket and Ibeno. This is a pathogenic microorganism which has been somehow ignored and is not routinely sought for in the medical laboratory as a pathogenic organism causing diarrhoea, but may cause deaths among children unnoticed. The overall prevalence of EPEC in this study was in conformity with the findings by Kandakai-Olukemi et al. [13]. The prevalence of EPEC in participants below 6 months of age was not as high as that of participants within 7-12 months. This could be due to the acceptance of exclusive breastfeeding by mothers in the study areas. Breast milk (colostrum) contains high level of immunoglobulin A (IgA) antibodies against the EPEC virulence factors and therefore confers protection to exclusively breastfed babies. This finding is in contrast to an earlier study [14]. One probable reason for this difference may be due to the number of children being exclusively breastfed in those studies. In Egypt, most of the mothers exclusively breastfeed their children till the age of 1 to 2 years [5].

The overall susceptibility profile of isolates to antibiotics showed that Ciprofloxacin had the highest susceptibility to all the isolates in the two study areas followed by Ceftriazone. The high susceptibility of Ciprofloxacin is comparable to data obtained in a study by Ogbonnaya et al. [15]. However, this finding is in contrast to the data obtained by another study [16], and this may depend on the prescription pattern in their country. The present finding has shown that some specific antimicrobial treatments may be required to supplement supportive anti-dehydration treatment which is the cornerstone of acute infant diarrhoea therapy. This calls for the selective use of antimicrobial agents. To drive home the findings of this study, diarrheal disease remains one of the top two causes of young children mortality in the developing world. This precarious situation can be diminished by improvements in water/ sanitation infrastructure and hygiene [17].

5. CONCLUSION

From the findings of this study adequate provision should be made by mothers before the weaning period, to ensure that a child adapts to the new brand of feeding. Although the study was based on the detection of enteropathogens in diarrhoeic children, the control specimens also showed a significant level of pathogens. Thus, asymptomatic cases pose a risk in spreading the pathogens in the community unnoticed. The menace caused by these pathogens in children less than five years of age is of public health importance and therefore cannot be overemphasised.

CONSENT AND ETHICAL CONSIDERATION

Informed consent was obtained from the caregivers of the children and ethical consideration was obtained from the State Ministry of Health, Akwa Ibom State, Nigeria. Ethical approval for this study was received from the PHC, Eket and General Hospital, Ibeno Management, and the parents/caregivers of the subjects selected for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26951