PREVALENCE OF ENTEROPATHOGENS CAUSING DIARRHEA IN CHILDREN (0-5 YEARS) IN KEFFI LOCAL GOVERNMENT AREA OF NASARAWA STATE, NIGERIA

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B. E. Ablaku[1*], Z. M. Sabo[2] and A. Ani[2]

[1]Department of Engineering, Lower Benue River Basin Development Authority, Makurdi, Benue State, Nigeria

[2]Department of Medical Microbiology, University of Jos, Plateau State, Nigeria

*Corresponding author: striz86@gmail.com

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Abstract: A study on the prevalence of Enteropathogenic *Escherichia coli* (EPEC) and other associated Enteropathogens among children (0-5 years) in Keffi Local Government Area, Nasarawa state, Nigeria was carried out to determine the prevalence of EPEC and other associated enteropathogens causing diarrhoea in the study area. Two hundred and seventy (270) stool samples were examined. These stool samples were collected from Federal Medical Centre Keffi, General hospital Keffi, Primary Health Care (PHC) centres in Padan Sarki, Angwa Waje, Tsohon Kasuwa, Sabon Gari and Angwa Jaba. Biochemical test was used to identify bacteria isolates. Out of the 270 stool samples, EPEC was not isolated in any of the stool samples examined using polyvalent antisera (poly 2, poly 3 and poly 4) and monovalent antisera (O55, O111 and O114) agglutinating specific for EPEC (Oxoid UK). Other enteric bacteria tests include: *Salmonella* species 38(14.1%) and *Shigella* species 23(8.5%). From the microscopic examination of the stool samples examined, 8.6% cysts *Entamoeba* species and 3.1% eggs of *Ascaris lumbricoides*, were identified. Hence, there is need to improve personal and environmental hygiene in the study area.

Keywords: Amboi forest reserve, forest stocking, basal area, tree density

Introduction

Diarrheagenic *E. coli* are recognized as five major pathotypes; *Enterotoxigenic* (ETEC), *Enteropathogenic* (EPEC), *Enteroinvasive* (ETEC), Shiga-like toxin-producing (STEC), *Enterohaemorrhagic* (EHEC) and *Enteroaggregative* (EAEC) *E. coli* (Nataro and Kaper, 1998). Diffuse-adherent *E. coli* (DAEC), also known as Diarrhoea associated Haemolytic *E.coli* (DHEC) and Cytolethal distending toxin-producing *E.coli* (CDT-EC) are also described as diarrheagenic (Nataro and Kaper, 1998; Clarke 2001). Their epidemiology and diarrheagenic potential are however, not yet clear (Yoshio *et al*., 2017).

With a better knowledge of the determinants of disease, strategies to reduce the burden of diarrheal disease were launched worldwide. As a result, global estimations of the number of diarrheal related deaths in children under five years old, have shown a steady decline, from 4.6 million in the 1980s (Synder and Merson, 1982) to 3.3 million in the 1990s (Bern *et al*., 1992), 2.5 million in the year 2000 (Kosek *et al*., 2003), 1.35 million in 2008 (Black *et al*., 2008) and finally 0.8 million in 2010 (Liu *et al*., 2010).

Despite the above reports of an important death decline, diarrheal disease continues to be a health problem and remains the second most common infectious cause of mortality among children under five years of age especially in developing countries. Estimates are that diarrheal disease accounts for 11% in Africa and South East Asia and 12% in Eastern Mediterranean of the total deaths by region (Liu *et al*., 2010). Quite the opposite mortality in the more developed regions has been reduced to very low levels; only 4% in Europe and in the regions of North America (Liu *et al*., 2010; Yoshio *et al*., 2017).

World Health Organization (WHO) (2003) stated that, diarrhoea caused by *E. coli* is a health problem among children in the developing countries. There has been research priority on the diarrheal disease control program by the WHO. Ogunsanya *et al.* (1994) and Jindal *et al.* (1995) in their separate works also agreed that *E. coli* play an important role in the ethology of diarrhoea.

The relative importance of *Enteropathogenic E.coli* as a cause of sporadic diarrhoea in industrialized and developing countries need to be reassessed (Levine *et al*., 1984). It ranks second among the noticeable diseases in Nigeria (Ogunsanya *et al*., 1994). About 300 children die every day from dehydration and malnutrition caused by diarrhoea (WHO, 2009). Diarrhoea caused by EPEC and some *Enteropathogens* infection is one of the major health problems for children in many developing countries. Travellers to these developing countries are also susceptible to diarrhoea (Adachi *et al*., 2001; Ogata *et al*., 2002; Robin-Browne and Hartland, 2002). Routine Laboratory diagnosis does not involve determining prevalence of *Enteropathogens*, therefore, the need to assess the relative prevalence of *Enteropathogens* as a cause of sporadic diarrhoea in developing countries cannot be undermined (Levine *et al*., 1984). This study is aim at studying the prevalence of some Enteropathogenic *Escherichia coli* causing diarrhoea among children (0-5years old) in Keffi Local Government Area, Nasarawa State, Nigeria.

Materials and Method

This study was conducted in Keffi Local Government Area, Nasarawa State, Nigeria. Keffi is a major commercial city in the state. Keffi has an area of 138 kilometres square and a population of 92,664 according to 2006 census. Study population was Children between the ages of 0-5 years old, in the study area.

Stool samples was collected randomly, from children (0-5years old) in clean leak-proof containers from children attending General Hospital Keffi, and Primary Health Care (PHC) centres (Padan Sarki Keffi, Angwa Waje, Tsohon Kasuwa, Sabon Gari and Angwa Jaba) in Keffi LGA, Nasarawa State, Nigeria. Stool samples (45) were collected from each of the selected hospital and PHCs.

The stool samples were examined macroscopically for colour, consistency, blood, mucus and presence of adult worms. The stool samples were analysed by the for saline wet method and examined using 10× and 40× objectives (Jindal *et al*., 1995; Yoshio *et al*., 2017).

The stool samples were inoculated directly on MacConkey agar plates by streaking method and incubated in inverted position at temperature of 37^0C for 18-24 hours (Cheesbrough, 2000). The isolates were grouped into lactose fermenting and non-lactose fermenting colonies, which were

then characterized, based on the biochemical tests namely: Citrate Utilization Test, Indole Test, hydrogen sulphide production and gas production test (using triple sugar iron agar) (Cheesbrough, 2009).
The results obtained were subjected to analysis of variance at (P=0.05).

Results and Discussion

The age group of the study population was 0-60 months. Out of the 270 stool samples analysed, 75 bacteria isolates showed negative agglutination with EPEC antisera type II-IV (Table 1). Further examination of the 270 stool specimens using standard methods of bacteria stool culture showed presumptive identification of 38(14.1%) *Salmonella* species and 23(8.5%) *Shigella* species.
The results of other pathogenic bacteria 61(22.6%) and parasites' cysts and eggs 11(5.1%) detected in this study showed no significant difference among male and female children (P>0.05) in Table 2.
Table 3 relates bacterial and parasitic enteric pathogens identified with age of the study population. Of the total number 156 stool samples examined between age brackets of 0-12 months the following bacterial and parasitic enteric pathogens were identified; 0% EPEC, 15(9.5%) *Salmonella species*, 17(10.9%) *Shigella species.* 1(0.6%) cysts of *Entamoeba species* and 1 (0.6%) eggs of *Ascaris lumbricoides* were seen.
Between age brackets of 13-24 months 49 stools were analysed and the following bacterial and parasitic enteric pathogens were identified 0% EPEC, 8(16.3%) *Salmonella species*, 1(2.0%) *Shigella species.* 2(4.1%) cysts of *Entamoeba species* and 0(0.0%) eggs of *Ascaris lumbricoides* were seen.
Between the age brackets of 25-36 months old, 27 stool samples were examined and the following bacterial and parasitic enteric pathogens were identified: 0% EPEC, 7(25.9%) *Salmonella species*, 2(7.4%) *Shigella species.* 2 (7.4%) cysts of *Entamoeba species* were seen and 1 (3.7%) eggs of *Ascaris lumbricoides* were seen.
Between age brackets 37-48 months 25 stool samples were examined and the following bacterial and parasitic enteric pathogens were identified: 0% EPEC, 3 (12.0%) *Salmonella species*, 3(12.0%) *Shigella species.* 1 (4.0%) cysts of *Entamoeba species* and 0(0.0%) eggs of *Ascaris lumbricoides* were seen.
Stool samples (13) examined between age brackets 49-60 months old and the following bacterial and parasitic enteric pathogens were identified: 0% EPEC, 5 (38.5%) *Salmonella species*, 0(0.0%) *Shigella species.* 2 (15.4%) cysts of *Entamoeba species* and 1 (7.7%) eggs of *Ascaris lumbricoides* were present. There is no significant association age and bacterial and parasitic enteric pathogens (P>0.05).
Table 4 relates the bacterial and parasitic enteric pathogens identified with feeding pattern.
Stool samples (64) were examined from children who were only breast fed (BF). The following bacterial and parasitic enteric pathogens Enteropathogens were identified: 0% EPEC, 6(9.4%) *Salmonella species*, 6(9.4%) *Shigella species*, 0(0.0%) cysts of *Entamoeba species* and 0(0.0%) eggs of *A. lumbricoides* were seen.
Stool samples (119) were examined from children who were undergoing mixed feeding (MF). The following bacterial and parasitic enteric pathogens were identified: 0% EPEC, 14(11.8%) *Salmonella species*, 13(10.9%) *Shigella* species, 2 (1.7%) cysts of *Entamoeba species* and 1 (0.8%) eggs of *Ascaris lumbricoides* were seen.
Stool samples (87) were examined from children who were on family diets/formula (FD/F). The following bacterial and parasitic enteric pathogens were identified: 0% EPEC, 18(20.7%) *Salmonella species*, 4(4.6%) *Shigella species*, 6(6.9%) cysts of *Entamoeba species* and 2 (2.3%) eggs of *Ascaris lumbricoides* were present. The result is significant (P<0.05).

Table 1: Bacterial and parasitic enteric pathogens detected in stool samples of children (0 – 5 years) according to stool consistency

Consistency Stool	No. of stool samples Examined	EPEC (%)	*Salmonella* species (%)	*Shigella* species (%)	Cyst of (%) *Entamoeba species*	Egg of (%) *A. lumbricoides*	No. pathogens seen (%)
Watery	68	0.00	11(16.2)	7(10.3)	7(10.3)	2(2.9)	18(26.5)
Mucoid	95	0.00	6(6.3)	6(6.3)	1(1.1)	0(0.0)	46(48.2)
Semi-formed	60	0.00	17(28.3)	4(6.7)	0(0.0)	1(1.7)	29(48.3)
Rice Water	9	0.00	2(22.2)	3(33.3)	0(0.0)	0(0.0)	3(33.3)
Formed	38	0.00	2(5.3)	3(7.9)	0(0.0)	0(0.0)	27(71.1)
Total	**270**	**0.00**	**38(14.1)**	**23(8.5)**	**8(3.0)**	**3(1.1)**	**123(45.5)**

Bacteria enteric pathogens were significantly higher than parasitic enteric pathogens P<0.005

Table 2: Bacterial and parasitic enteric pathogens identified according to sex of children (0 – 5 years)

Gender	No. of stool Samples Examined	EPEC (%)	*Salmonella* species (%)	*Shigella* species (%)	Cyst of *Entamoeba species* (%)	Egg of *A. lumbricoides* (%)	No. pathogens seen (%)
Male	125	0	19(15.2)	13(10.4)	3(2.4%)	0(0.0%)	58(46.4)
Female	145	0	19(13.1)	10(6.9)	5(3.4%)	3(2.1%)	65(44.8)
Total	**270**	**0**	**38(14.1)**	**23(8.5)**	**8(3.0%)**	**3(1.1%)**	**123(45.5)**

Bacteria enteric pathogens were significantly higher than parasitic enteric pathogens P>0.05

Table 3: Bacterial and parasitic enteric pathogens identified according to age group of the children (0 – 5 years)

Age group	No. of stool Samples Examined	EPEC (%)	*Salmonella* species (%)	*Shigella* species (%)	Cyst of *Entamoeba species* (%)	Egg of *A. lumbricoides* (%)	No. pathogens seen (%)
0 – 12	156	0	15(9.5)	17(10.9)	1(0.6)	1(0.6)	78(50.0)
13 – 24	49	0	8(16.3)	1(2.0)	2(4.1)	0(0.0)	26(53.1)
25 – 36	27	0	7(25.9)	2(7.4)	2(7.4)	1(3.7)	9(33.3)
37 – 48	25	0	3(12.0)	3(12.0)	1(4.0)	0(0.0)	9(36.0)
49 – 60	13	0	5(38.5)	0(0.0)	2(15.4)	1(7.7)	1(7.7)
Total	**270**	**0**	**38(14.1)**	**23(8.5)**	**8(3.0)**	**3(1.1)**	**123(45.6)**

Bacteria enteric pathogens were significantly higher than parasitic enteric pathogens P>0.05

Table 4: Bacterial and parasitic enteric pathogens identified according to feeding pattern

Feeding Pattern	No. of stool Samples Examined	EPEC (%)	*Salmonella* species (%)	*Shigella* species (%)	Cyst of *Entamoeba species* (%)	Egg of *A. lumbricoides* (%)	No pathogens seen (%)
BF	64	0	6(9.4)	6(9.4)	0(0.0)	0(0.0)	35(54.7)
MF	119	0	14(11.8)	13(10.9)	2(1.7)	1(0.8)	56(47.1)
FD/F	87	0	18(20.7)	4(4.6)	6(6.9)	2(2.3)	32(36.8)
Total	**270**	**0**	**38(14.1)**	**23(8.5)**	**8(3.0)**	**3(1.1)**	**123(45.5)**

Bacteria enteric pathogens were significantly higher than parasitic enteric pathogens P>0.05
BF: Breast-feeding, MF: Mixed feeding, FD/F: Family diet/Formula, No.: Number

The result of this showed that other enterobacteriaceae and parasites are possibly responsible for diarrhoea among children (0-5 years) in the study area.

Table 1 showed the total number of bacterial and parasitic enteric pathogens isolated to be 147 (54.4%). EPEC 0%, *Salmonella species* 38(14.1%), *Shigella species* 23(8.5%), and 8 (3.0%) and 3 (1.1%) of cysts and eggs of *Entamoeba histolytica/dispar* and *Ascaris lumbricoides* respectively. This finding result is corroborated with the finding that a number of genera within the family Enterobacteriaceae (*Salmonella, Shigella*) are human intestinal pathogens and are related to gastroenteritis, diarrhoea (Todar, 2008).

Microscopic examination of the stool samples analysed showed that 8 (3.0%) and 3 (1.1%) of cysts and eggs of *Entamoeba histolytica/dispar* and *Ascaris lumbricoides* were seen (Table 2). This result had lower prevalence compared with report of previous study (Mingpu *et al*., 2017; Hebbelstrup *et al*., 2017).

The association between bacterial and parasitic enteric pathogens identified according to sex (Table 2) is not significant (P>0.05). The table revealed that females have the highest prevalence of cysts of parasites 5(3.4%). This might be due to suggestion given in the previous studies by Adamu *et al*., 2006 and Noor-Azian *et al*., 2007. It was discovered that female children assume the roles of mothers as they play with peers. They learn how to cook using disposed tins and cans, water and soil which might have been seeded with cysts and eggs of parasites (Yoshio *et al*., 2017).

Table 3 showed the association between bacterial and parasitic enteric pathogens identified according to age of the children. The result is not significant (P>0.05). It was revealed that there is a progressive increase in prevalence of the parasites from 0.6% in age bracket 0-12 months to 15.4% in age bracket 49-60 months. This means that as the children grew older, they were not closely monitored by their parents to ensure proper hygiene. Children play on soil or play ground and sand boxes which might have been seeded or contaminated with eggs and cysts of parasites.

From table 4, the association between bacterial and parasitic enteric pathogens identified according to feeding pattern of children is significant (P<0.05). This suggests that the family diets/formula are prepared and served under poor personal hygiene and environmental sanitation. Adamu *et al*. (2006) and Noor-Azian *et al*. (2007) documented that intestinal parasitic infections have a worldwide distribution with high prevalence found in people with low socio-economic status and poor living conditions as well as people in over-crowded areas with poor environmental sanitation, improper garbage disposal, unsafe water supply and unhygienic habits. These factors are the cause of a major proportion of the burden of diseases and deaths in developing countries (Adamu *et al*., 2006; Hebbelstrup *et al*., 2017).

Conclusion

Serotyping showed no reaction for EPEC serotypes using polyvalent and monovalent agglutination antisera specific for *E. coli.*

Other Enterobacteriaceae isolated were 38(14.1%) *Salmonella* species and 23(8.5%) *Shigella* species. Microscopic examination revealed cysts of *Entamoeba histolytica/dispar* 8 (3.0%) and eggs of *Ascaris lumbricoides* 3 (1.1%). Children who were in the age bracket 49-60 months had the highest prevalence of parasites 2 (15.4%) and 1 (7.7%) of cysts and eggs of *Entamoeba histolytica/dispar* and *Ascaris lumbricoides* respectively. Also, children who were on family diets/formula had the highest prevalence 6 (6.9%), 2 (2.3%) of cysts and eggs of *Entamoeba histolytica/dispar* and *Ascaris lumbricoides.*

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