Microbiology Research Journal International

24(4): 1-15, 2018; Article no.MRJI.39741
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Prevalence of Enteropathogenic *Escherichia coli* in Children under 5 Years with Diarrhoea in Yola

Umar Abdullahi[1*] and Abana Abubakar Umar[2] and Jaafaru M. Inuwa[3]

[1]Department of Laboratory Technology, Modibbo Adama University of Technology, Yola, P.M.B 2076, Adamawa State, Nigeria.
[2]Adamawa State Ministry of Health, P.M.B 2078, Adamawa State, Nigeria.
[3]Department of Microbiology, Modibbo Adama University of Technology, Yola, P,M.B 2076, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AAU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author UA managed the analyses of the study. Author JMI supervised the research. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2018/39741
Editor(s):
(1) Dr. Giuseppe Blaiotta, Professor, Department of Agriculture, Division of "Grape and Wine Sciences", University of Naples Federico II, Via Universita' 100 – Palazzo Mascabruno, 80055 Portici, Italy.
Reviewers:
(1) Imoro Abubakari Zarouk, University for Development Studies, Ghana.
(2) M. I. O. Raji, Usmanu Danfodiyo University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/25792

Original Research Article

Received 1st January 2018
Accepted 5th March 2018
Published 4th August 2018

ABSTRACT

Diarrhoeal diseases constitute one of the most important causes of illness and death all over the world. In Nigeria, the epidemiology of diarrhoeagenic bacteria and the virulence of the various *E. coli* pathotypes have not been well studied. This study aimed to survey the prevalence of enteropathogenic *Escherichia coli* from children less than 5 years with diarrhoea in Yola metropolis in Adamawa State. A total of 200 stool samples were collected from children attending Health Facilities in Yola from May – December 2014. The samples collected comprised of 150 from patients with diarrhoea and 50 from patients without diarrhoea as control subjects. The samples were screened and examined for the prevalence of some organisms that could cause diarrhoea. The study shows that the prevalence of diarrhoea caused by bacteria is 21% and implicated *E. coli* with the highest prevalence of 10.5% followed by *Salmonella species 5%, Shigella species* 4%, and *Vibrio cholera* 1.*5%*. Most of these pathogens were isolated in female 11% as compared to males 10%, but the difference was not statistically significant. The isolated *E. coli* were found to be resistant to

**Corresponding author: E-mail: umarabdullahi@mautech.edu.ng;*

nitrofurantoin, ceftriaxone, gentamicin, augmentin, chloramphenicol, and ampicillin. *E. coli* strains were characterised by serotyping and genotyping to detect virulence genes (*eaeA* and *bfpA*). Only one of the isolates tested positive for *eae A* gene. These research findings show that, though there are a number of causative bacterial agents of diarrhoeal diseases. *E. coli* remains one of the primary cause's. *Salmonella* and *Shigella* were found important bacterial pathogens among paediatric patient in the selected health facilities in Yola. Improving the sanitary awareness through primary health education, careful surveillance, monitoring incidence and spread of diarrhoeal diseases, may help to reduce the disease burden in children.

Keywords: EPEC; serotyping; genotyping; pathotypes and E. coli.

1. INTRODUCTION

The microorganism *Bacterium coli commune* was discovered in 1885 by a German Pediatrician, Theodor Escherich during his work on bacteria in stools of infants with enteritis [1]. The bacterium has been recognised as an essential cause of food and water-related diseases since its discovery and is now known as *Escherichia coli* (*E. coli*). This bacterium belongs to the coliform group of microorganisms, which are a standard part of the standard facultative anaerobic microflora of the intestinal tracts of most mammals, including humans. This flagellated gut flora is mainly found in the colon [2]. *Escherichia coli* belongs to the genus *Escherichia* which in turn is part of the tribe Escherichia belonging to the family Enterobacteriaceae. The genus *Escherichia* contains four other species namely *E. hermanii, E. fergusonii, E. vulneris*, and *E. blattae*. *Escherichia blattae* were isolated from cockroaches, whereas *E. hermanii, E. fergusonii,* and *E. vulneris* were isolated from both intestinal and extra-intestinal human sources [2].

As a commensal, *E. coli* can be found in intestinal microflora of a variety of animals including humans. Not all the strains are harmless, and some can cause debilitating and sometimes fatal diseases in humans as well as mammals and birds [3]. Pathogenic strains are divided into intestinal pathogens causing diarrhoea also known as diarrhoeagenic *E. coli* (DEC) and extraintestinal *E. coli* (ExPEC) causing a variety of infections in both humans and animals including urinary tract infections (UTI), meningitis and septicemia [4]. Among the intestinal pathogens there are six well-described categories/pathotypes which are enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC) and diffusely adherent *E. coli* (DAEC) [5].

EPEC was the first pathotype of *E. coli* to be described which causes either watery or bloody diarrhoea [6]. Large outbreaks of infant diarrhoea in the United Kingdom led Bray (1945) to describe a group of serologically distinct *E. coli* strains that were isolated from children with diarrhoea but not from healthy children. EPEC strains are defined by the characteristic attaching and effacing effect that they elicit on interaction with epithelial cells and by the fact that they do not produce Shiga toxins [7]. EPEC infections are acquired primarily through ingestion of contaminated food or water [8]. Person-to-person and animal-to-human transmission is through the oral-faecal route [9]. Infections caused by EPEC are difficult to differentiate from those with other causes; symptoms include watery diarrhoea sometimes accompanied by low-grade fever and vomiting [10].

1. However, EPEC infection may be severe; vomiting may make oral rehydration severe, and life-threatening dehydration may ensue. Furthermore, a disease caused by EPEC may be protracted, resulting in weight loss, malnutrition, and death [11]. The primary objective of this study is to identify virulence factor in EPEC isolates

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted from April-December 2014 in Yola, which is the capital city of Adamawa State, Nigeria. Located on the Benue River, it has a total population of 395,871 inhabitants comprising of 208,193 males and 187,678 females [12].

Fig. 1. The map of Adamawa state, Nigeria

2.2 Study Population

A total of 200 stool samples were collected from infant and young children under 5 years attending the Emergency Paediatric Unit and Out Patient Department of 2 hospitals (Federal Medical Centre, Yola and Specialist Hospital, Yola) and 2 clinics (Valli and Triump Clinic, Yola).

One hundred and fifty (150) samples were collected from infants with diarrhoea and fifty (50) as control samples were collected from infants without diarrhoea. Diarrhoea was characterised by the occurrence of three or more loose, liquid or watery stools or at least one bloody loose stool in 24 hours period. Control subjects were children with other infection and no history of diarrhoea for at least 2 weeks and had not taken antibiotics for 2 weeks.

2.3 Study Size

A sample size of two hundred (200) was used based on the formula by Daniel [13] shown below for determining an adequate sample size to estimate the population prevalence with a good precision

$$n = \frac{Z^2 P (1 - P)}{d^2}$$

Where n = required sample size, Z is the confidence level at 95 % (standard value 1.96), P = 15% expected prevalence obtained from a recent study in Maiduguri [14] and d= show the margin of error at 5% (standard value 0.05).

2.4 Ethical Clearance

Ethical approval for the study was obtained from Adamawa State Ministry of Health, Yola. Informed consent was obtained from the patient, hospital authorities, and clinicians involved in the management of the patients. A designed questionnaire was used to obtained information from parents.

2.5 Sample Collection

Samples were collected as a stool or rectal swab. Stool samples were collected from patients in clear, transparent, sterilized wide-mouthed bottle. In the case of the rectal swab, a cotton swab was inserted into the rectum, rotated gently, and removed.

The highest number of samples (68) was collected from Triumph Clinic, Yola. Federal Medical Center, Yola has the least number of samples collected with 27 diarrhoeagenic samples and 10 control samples. The details of samples collected from the various hospitals/clinics are shown in Table 1.

Samples collected were categorised into age groups from 0 to 60 months of which the youngest child was 1 month, and the oldest was 59 months. Most of the samples were collected from Age group 0 – 12 months while the least

number of samples were collected from age group 37 – 48 months.

2.5.1 Processing of Samples

The samples were processed according to the guidelines provided by Cheesbrough [15] for the laboratory diagnosis of enteric pathogens. These included: macroscopy, microscopy, culture, biochemical testing and antimicrobial sensitivity testing.

2.5.2 Macroscopy

The samples were observed for colour, consistency, the presence of blood, mucus or pus and presence of the worm.

2.6 Identification of Parasites (Microscopy)

Identification of parasites was carried out according to Cheesbrough [15].

2.6.1 Culture

Samples collected were inoculated onto MacConkey agar, Salmonella-Shigella agar and peptone water and Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar incubated at 37^{o}C for 24 hours [15]. After 24 hours, the plates were observed for growth (Appendix i)

2.6.2 Biochemical Testing

All lactose fermenting colonies on MacConkey plate was subjected to the following biochemical test: Gram staining, oxidase test, Simmon's citrate utilization, methyl red test, Voges Proskauer test, lysine decarboxylation, H_2S/indole production, phenylalanine decarboxylation, urea hydrolysis, Kligler iron agar test and motility test [15].

2.6.3 Serotyping

The *E. coli* isolates were subjected to slide agglutination test with sera against 12 classical serogroups of EPEC which are O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142 and 158. Eight vials of Polyvalent antisera each containing a mixture of different monovalent antisera was used according to the instructions of the manufacturer (Denka Seiken Ltd., Tokyo, Japan). Polyvalent 1-3 contained the 12 serogroups of EPEC. The bacterial culture was suspended in 3 ml physiological saline and heated to 100°C for 1 hour. It was then centrifuged at 900 g for 20 minutes and the supernatant was discarded.

The precipitate was suspended in 0.5 ml physiological saline which was then used as the antigenic mixture. A drop of the antigenic mixture was then mixed with 1 drop of the specific O polyvalent antisera on a glass slide for 1 min and observed for agglutination. A drop each of the O polyvalent with physiological saline was used as a control. Strong agglutination observed within 1 minute was regarded as positive [16-17].

2.6.3 Genotyping (Detection of Virulence Genes)

The Enteropathogenic *E. coli* strains were confirmed using Polymerase Chain Reaction (PCR) by the detection of virulence genes (*eaeA* and *bfpA*) known to cause infantile diarrhoea. The detection of these virulence genes categorized EPEC strains into typical EPEC that possesses both the *eaeA* and *bfpA* and atypical EPEC that possesses only the *eaeA*.

2.6.4 DNA Extraction

DNA extraction was done according to the protocol of DNAlab (2015).

Table 1. Number and percentage of samples collected from each hospital

Hospitals	Diarrhea	Controls	Total	Percentage
Specialist Hospital, Jimeta	31	12	43	21.5
Triumph Clinic, Jimeta	53	15	68	34
Federal Medical Centre Yola	27	10	37	18.5
Valla Clinic, Yola	39	13	52	26
Total	150	50	200	100

Table 2 PCR of selected genes of EPEC

Category	Target	Sequence (5´–3´)	Annealing temp. (°C)	No. of cycles	Fragment size (bp)	Reference
EPEC	*eaeA*	F, CATTATGGAACGGCAGAGGT R, ATCTTCTGCGTACTGCGTTCA	55 (1 min)	35	790	Beaudry et al. (1996)
EPEC	*bfpA*	F, AATGGTGCTTGCGCTTGCTGC R, GCCGCTTTATCCAACCTGGTA	60 (1 min)	30	326	Gunzburg et al. (1995)

2.6.5 Polymerase Chain Reaction

The primers for the virulence genes shown in Table 2 were diluted based on manufacturer's instruction. PCR was performed using PCR PreMix and a 20 µl reaction mixture containing dH_2O 16 µl, Primer 2 µl, and template 2 µl was added. Amplification reactions were performed in a thermal cycler and for all amplification reactions; the mixture was heated at 94^{o}C for 5 min prior to thermocycling. The mixture was held at 57^{o}C for 7 min after the final cycle before cooling at 4^{o}C.

2.6.6 Agarose Gel Electrophoresis

Amplified products were analysed by using 2% agarose gel electrophoresis and visualized by staining with ethidium bromide (Appendix ii).

2.6.7 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out according to CLSI (2014).

3. RESULTS

3.1 Macroscopy

The appearance of the stool samples collected varies from child to child depending on the case (Table 3). Most stool samples collected from children with diarrhoea were watery compared to control samples which have more of loose samples. 4 samples had blood in them and 10 samples had mucous. No cases of worm were seen in the stool. 3 cases had Rice water stools with mucous flake.

3.2 Identification of Parasites (Microscopy)

Out of the 200 stool samples examined, 12 (6%) had intestinal parasites detected in them. A total of four parasite species were observed from the diarrhoea stool samples as shown in Table 4. Mixed infection was not observed. *Gardia lambia* was the most predominant (2.5%) out of the 6%, followed by *Entamoeba histolytica* (2%).

3.3 Isolation of Bacteria (Culture)

Different bacteria were isolated from stools of children with diarrhoea and from control samples. Pathogenic bacteria were isolated from 33 samples (22%) with diarrhea, 9 samples (18%) of control samples. Other bacteria not concerned with this study were isolated from 64 stool samples. No growth was observed in 94 samples. The number of bacteria isolated from stool samples is shown in Table 5. Age group 13 – 24 months had the highest percentage, (25%), while age group 37 – 48 month has the least percentage of samples which is 13.64%. Table 6 shows the number of bacterial isolates based on gender. 20.41% of the male samples had bacterial isolates while 21.57% of female samples had bacterial isolates.

3.4 Biochemical Test

The bacterial isolates were identified using biochemical test and a total of 21 samples were suspected to be *E. coli* which had the highest number of occurrence followed by *Salmonella* spp. with 10 isolates. Other organisms identified were *Shigella* spp. which has 8 isolates and *Vibrio cholerae* which has 3 isolates (Table 7).

3.5 Serotyping

A total of 21 isolates comprising of 18 diarrhoeagenic and 3 control samples identified as *E. coli* by biochemical test were serotyped using 8 vials of polyvalent antisera labelled as polyvalent 1-8. Isolates positive for polyvalent 1, polyvalent 2 and polyvalent 3 are suspected to belong to EPEC serogroup. 2 isolates were positive for Polyvalent 1 while 3 isolates were positive for Polyvalent 2 making a total of 5 isolates which were suspected to belong to the EPEC serogroup as shown in Table 8. The details of these 5 isolates are in shown in Table 9. Seven isolates were not positive for any of the polyvalent.

3.6 Genotyping

A total of 5 isolates which reacted positively for polyvalent 1 and 2 suspected to belong to EPEC serogroup were genotyped to detect for virulence genes *eaeA* and *bfpA*. Only 1 isolate (labeled 2) obtained from a male with watery diarrhoeagenic samples was detected positive for the *eaeA* but negative for *bfpA* gene as shown in plate1. The positive sample has a DNA fragment of 790bp and belongs to the atypical EPEC since it is negative for *bfpA gene*.

3.7 Antimicrobial Susceptibility Testing

A total of 21 *E. coli* isolates were tested for antimicrobial sensitivity pattern shown in Table 10. Eighteen (85.7%) of the 21 *E. coli* isolates

tested were susceptible to cefuroxime, 17 (81%) to gentamycin, 16 (76.2) to amoxicillin-clavulanate, 15 (71.4%) to ciprofloxacin, 11 (52.3%) to ceftazidime and 10 (47.6%) to ofloxacin. A total 14 (66.7%) isolates were found to be resistance to amoxicillin, 11 (52.4%) were resistant to ofloxacin and 9 (42.9%) were resistant to ceftazidime. A Multi-resistance was observed in 8 (38.1%) isolates.

4. DISCUSSIONS

Diarrhea due to bacterial infections is an important cause of morbidity and mortality in infants and young children in most developing countries including Nigeria [18] but the incidence and severity may vary depending on the location and time [19].

Table 3. Macroscopic appearance of stool samples

Colour	Diarrhoeagenic samples	Control samples	Consistency	Diarrhoeagenic samples	Control samples
Brownish	5	18	Formed	15	23
Yellowish	39	16	Semi-Formed	36	12
Greenish	47	11	Unformed	40	15
Black	9	3	Watery	45	0
Pale	0	2	Bloody	4	0
			Mucus	7	0
			Rice Watery	3	0
Total	150	50		150	50

Table 4. Percentage of occurrence of intestinal parasites

Parasites	No. seen	Percentage (%)
Entamoeba histolytica	4	2
Strongyloides stercoralis	1	0.5
Ascaris lumbricoides	2	1
Gardia lamblia	5	2.5
Total	12	6

Table 5. Distribution of bacterial isolates based on age groups

Age group (Months)	Diarrheagenic sample		Control samples		Total samples	
	No. Cultured	No of bacteria Isolated (%)	No. cultured	No of bacteria Isolated (%)	No. cultured	No of bacteria Isolated (%)
0 -12	48	9 (6.00%)	12	2 (4.00%)	60	11(5.50%)
13- 24	34	10 (6.67%)	6	0 (0.00%)	40	10 (5.00%)
25- 36	32	7 (4.67%)	8	2 (4.00%)	40	9 (4.50%)
37-48	12	2 (1.33%)	10	1 (2.00%)	22	3 (1.50%)
49-60	24	5 (3.33%)	14	4 (8.00%)	38	9 (4.50%)
Total	150	33 (22 %)	50	9 (18%)	200	42 (21%)

Table 6. Distribution of bacterial isolates according to gender

Sex	No. cultured	Bacterial Isolates	Percentage (%)	Chi Square	P value
Male	98	20	20.41	0.026	0.871
Female	102	22	21.57		
Total	200	42	21		

During the study period, more cases of diarrhoea were recorded in the two private clinics, namely Truimph and Valli clinic, than the two Government-owned hospitals (Specialist and

Federal Medical Center). This may be as a result of the strike action by medical officers in government-owned hospital during the study period which resulted in people going to the only available option which is the private hospitals. Age group 0 – 12 months has the highest number of samples collected which is 60 (30%) while age group 37 – 48 has the least number collected 22 (11%). However, the statistical association was observed (X^2 = 1.056 $p<0.05$) between age group and the presence of the bacterial isolates with age group 13 - 24 months having the highest occurrence of bacterial isolates as 25% and the least is age group 37 – 48 which has 13.6%. This follows the same trend with a study done in Abuja by Ifeanyi et al. [20] which shows that diarrhea is statistically associated with age and majority of the cases occurring in children between 7 months and 2 years of age. The reason for high incidence of bacteria isolates in age group 7 – 24 months could be due to the fact that children within this age group on their own cannot differentiate between what to eat and what not to eat; they have not learnt the rudiment of adherence to aseptic or hygienic practice; they can barely express themselves. Most diarrhoea occur during the first 2 years of life due to combined effects of declining levels of maternally acquired antibodies, the lack of active immunity in the infant, the introduction of food that may be contaminated with faecal bacteria and direct contact with human or animals faeces when the infant start to grow. Most enteric pathogens stimulate at least partial immunity against repeated infection or illness, which helps to explain the declining incidence of disease in older children and adults [21].

The age range 37-59 months had the lowest frequency of bacterial isolates in this study.

This is probably because of fewer tendencies to put contaminated objects into the mouth-a finding similar to that of Oni et al. [22]. Only 7% of the samples were infected with more than one pathogen. The physical appearance of the sample is very important when categorizing diarrhoea.

However, the appearance must be differentiated from a normal liquid sample from exclusively breastfed infants who may pass several soft, semi-liquid stools each day. Most bacterial pathogen where isolated from the watery and loose sample than form formed and semi formed stool samples.

In this study, the occurrence of bacterial aetiology of diarrhoea was 21% which followed the same trend as research done in Enugu and Onitsha [23]. The result was low compared to report in Kano State which was found to be 40.67% [24] and in Kaduna 44% [25]. *E. coli* had the highest prevalence rate of 10.5%, followed by *Salmonella* species, 5%, *Shigella* species, 4%, and *Vibrio cholera*, 1.5%. Majority of the previous studies found in literature in most countries also implicated *E. coli* as the predominant bacterial agent on diarrhoeal diseases [26-27]. Several studies in Nigeria have reported the prevalence of *E. coli* ranging from 7.5 – 15.5%. An exception to this report, however, was obtained by some workers in Saudi Arabia, who reported that Shigella had the highest incidence among other bacterial pathogens causing diarrhoea disease in the country [27]. These finding shows that, though there are a number of bacterial causative agents of diarrhoeal diseases but *E. coli* still remain one of the major causes.

No organism was isolated in 94 (47%) of the children this may be due to the fact that the study did not investigate all possible disease causing agents. Intestinal pathogens were isolated from 12 (6%) of the children. *Gardia lambia* was the most predominant (2.5%) out of the 6%, followed by *Entamoeba histolytica* (2%). These are risk factors for opportunistic infection in children. Most of these children whom samples were collected are female that is 102 (51%), compared to 98 (49%) who were male. Most of these pathogens were isolated in females 22 (11%) as compared to males 20 (10%) but the difference was not statistically significant (X^2 = 0.026 $p < 0.05$). This is in contrast to the work of Abdullahi et al. [28] where they reported that male children were more infected (22.33%) than female children (18.33%), although the difference was not statistically significant (X^2 = 0.531, $p>0.05$).

This categorization is necessary as different sample appearance is associated with different causative agents. Due to indiscriminate use of antibiotics by unqualified personnel and laypersons, resistance to an antibiotic is now common both in a healthy person and unhealthy person [29].

Resistance transfer in *EPEC* is often plasmid mediated. The antibiotic susceptibility testing from several studies conducted showed a high

level of resistant to most of the commonly used antibiotics. Ampicillin and Septrin are widely used to treat diarrhea because of their low cost and ready availability [30]. In this study, the Antimicrobial susceptibility pattern showed that *E. coli* isolates were most resistant to ampicillin, ofloxacin and ceftazidime. A similar study in Iran [31] showed high resistant of *E. coli* to ampicillin. The isolates were, however, susceptible to cefuroxime, gentamycin, ciprofloxacin and amoxicillin/clavulanate indicating that they are the effective antimicrobials against *E. coli* isolates. EPEC are among the most important pathogens infecting children less than 2 years of age in the developing world, but the prevalence may vary depending on differences in study population, age group, diagnostic criteria and diagnostic tools used [32]. Over the last several decades, the significance of EPEC infection has declined in published literature. The decline might be due to interventions, particularly breastfeeding promotion, or to the overestimation of these organisms in earlier studies that used O- or O:H typing compared to the recent ones, in which molecular methods and/or adherence assays were used for EPEC diagnosis [33-34]. The prevalence rate of enteropathogenic *E. coli* was 0.5% which is lower compared to other studies in Nigeria and outside the country. Bukar et al. [35] reported 15% in Maiduguri, Adebola et al. [36] 15% in Abuja, Ome and Nonye [37] 19.5% in Aba respectively. Iman et al. [38] reported 7.5% in Cairo and Addy et al. [39] 14.8% in Kumasi. The confirmed EPEC was obtained from a diarrheagenic sample of a one-year male child. The EPEC isolate had the virulent gene of *eaeA* which indicated that the *E. coli* detected belongs to the atypical EPEC (aEPEC). Typical EPEC (tEPEC) isolate was not identified in this study.

Many studies have found a significant association of EPEC with infant diarrhea compared to control samples [40-42]. Healthy carriage of enteric pathogens, in general, is very common in developing countries. Colonization rather than illness may result from an interplay of multiple factors including host susceptibility (related to the child's age, breastfeeding, nutritional and immunological status), bacterial factors (different virulence genes), and environmental factors (poor hygiene and high fecal contamination) [43].

Table 7. Bacterial Isolates in Stool Samples

Isolates	No. of samples positive
Shigella spp.	8
Salmonella spp.	10
Escherichia coli	21
Vibrio cholerae	3
Total	42

The pathogenic potential of EPEC has long been controversial. However, epidemiological studies clearly have demonstrated that aEPEC is important in endemic diarrhea in children as well as diarrhea outbreaks [44-45]. Recent data suggest that aEPEC are more prevalent than tEPEC in both developing and developed countries [46-48]. Case-control studies from many countries have shown that EPEC primarily causes disease in children less than two years of age [49-50]. The correlation between EPEC infection and diarrhoea has been shown to be strongest for infants < 6 months old, while EPEC is frequently present without causing symptoms among children older than 6 months to 2 years [51]. Atypical EPEC has been implicated as the causative agent in some outbreaks [52-53], whereas in other studies atypical EPEC has not been recovered more frequently from diarrhoeal cases than from controls (54). In some studies, the rate of isolation of atypical EPEC from children without diarrhoea was twice that of cases with diarrhea [55-56].

Table 8. Positive samples for EPEC serogroup

Polyvalent Antisera	Mixture of monovalent antisera	No. Positive	% Positive
Polyvalent 1	O1, O26, O86a, O111, O119, O127a, O128	2	15.4
Polyvalent 2	O44, O55, O125, O126, O146, 166	3	23
Polyvalent 3	O18, O114, O142, O151, O157, O158	1	7.7
Polyvalent 4	O6, O27, O78, O148, O159, O168	2	15.4
Polyvalent 5	O20, O25, O63, O153, O167	1	7.7
Polyvalent 6	O8, O15, O115, O169	1	7.7
Polyvalent 7	O28ac, O112ac, O124, O136, O144	2	15.4
Polyvalent 8	O29, O143, O152, O164	1	7.7
Total		13	

Table 9. Antibiotics susceptibility pattern of isolated *Escherichia coli*

Antibiotics	Disk	Susceptible		Resistant	
	Conc. (µg)	No	%	No	%
Ampicillin	10	7	33.3	14	66.7
Ciprofloxacin	5	15	71.4	6	28.6
Ofloxacin	5	10	47.6	11	52.4
Ceftazidime	30	11	52.3	9	42.9
Gentamycin	10	17	81	3	14.3
Cefuroxime	30	18	85.7	2	9.5
Amoxicillin/clavulanate	30	16	76.2	5	23.8

Table 10: Details of isolates suspected to belong to the EPEC Serogroup

Samples code	Sex	Age	Appearance	Sample source
2	M	1 years	Watery stool	Diarrheagenic
85	F	2 years	Loose stool	Diarrheagenic
124	F	8 months	Semi formed	Control
128	F	3 years	Loose stool	Diarrheagenic
173	F	14 months	Watery stool	Diarrheagenic

There have also been reports of atypical EPEC associated with both endemic diarrhea [57-58] and outbreaks [59-60].

5. CONCLUSION

The finding shows that, though there are a number of causative bacterial agents of diarrhoeal diseases, *E. coli* remains one of the major causes with *Salmonella* and *Shigella* being important bacterial pathogens among pediatric patient in the selected study hospitals in Yola. EPEC strains are not a common cause of diarrhoea in children with diarrhoea in Yola. Atypical EPEC is more prevalent compared to the typical EPEC which was not isolated in the study. Detection of the *eaeA* and *bfpA* genes is a useful method for the identification of and differentiation between typical and atypical EPECstrains.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bell C. Approach to the control of entero-haemorrhagic *Escherichia coli* (EHEC). International Journal of Food Microbiology. 2002;78:197-216.
2. Wilshaw GA, Cheasty T, Smith HR. *Escherichia coli*. In: Lund BM, Baird-Parker TC, Gould GW. (Eds.), The microbiological safety and quality of food II. Aspen Publishers Inc. Gaithersburg, Maryland. 2002;1136-1177.
3. Belanger L, Garenaux A, Harel J, Boulianne M, Nadeau E, Dozois CM. *Escherichia coli* from animal reservoirs as potential source of human extraintestinal pathogenic *E. coli*. FEMS Immunology and Medical Microbiology. 2011;62:1–10.
4. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. Nature Reviews Microbiology. 2004;2:123-140.
5. Nataro JP, Kaper JB. Diarrhoeagenic *Escherichia coli*. Clinical Microbiology Review. 1998;11:142-201.
6. Benenson AS. Control of communicable diseases manual. 6th ed. United Book Press; Baltimore. 2005;140–50.
7. Chen HD, Frankel G. Enteropathogenic *Escherichia coli*: Unravelling pathogenesis. FEMS Microbiology Review. 2005;29:83-98.
8. Nataro JP, Kaper JB. Diarrhoeagenic *Escherichia coli*. Clinical Microbiology Review. 1998;11:142-201.
9. Benenson AS. Control of communicable diseases manual. 6th ed. United Book Press; Baltimore. 2005;140–50.
10. Gomes T, Rassi V, Macdonald KL. Enteropathogens associated acute diarrhoeal disease in urban infants in Sao

Paulo, Brazil. Journal Infectious Diseases. 1991;164:331-337.
11. Sarah S. Principles and practice of pediatric infectious diseases. Churchill Livingstone; Philadelphia. 2008;3:797.
12. Nigeria Population Commission. Population and Housing Census; 2006.
13. Daniel WW. Biostatistics: A foundation for analysis in the health sciences. 7th edition. New York: John Wiley & Sons; 1999.
14. Bukar AM, Isa MA, Bello HS, Isa A, Baba, FA. Prevalence of enteropathogenic *E. coli* among hospitalized diarrhoea children in state specialist hospital maiduguri, Borno, Nigeria. International Journal of Research (IJR). 2014;1(11).
[ISSN 2348-6848]
15. Cheesbrough M. District laboratory practice in tropical countries Part 2. Cambridge University Press, Cambridge. 2006;99–105.
16. Tsukamoto T. *Escherichia coli,* Rinsho-to-biseibutu. 1989;15:69.
17. Sakazaki R. Serotyping of diarrhoeagenic *E. coli.* Media Circle. 1992;34:117.
18. Adegunloye DV. Carrier rate of enteric bacteria associated with diarrhoea in children and pupils in Akure, Ondo State, Nigeria. African Journal of Biotechnology. 2005;5(2):162-164.
19. Sethi S, Sehgal R, Malla N, Dubey ML, Mahajan RC. Changing trends of intestinal parasitic infections in Chandigarh (Northern India): Hospital based study. Indian Journal of Medical Microbiology. 2000;18:106-9.
20. Ifeanyi CI, Isu RN, Akpa AC, Ikeneche NF. Enteric bacteria pathogens associated with diarrhoea of children in the federal capital territory abuja, Nigeria. New York Science Journal. 2010;3(1).
21. Patwari AK, Manorama D, Ridie D. Clinical and laboratory prediators of invasive diarrhoea in children less than five years old. Journal of Diarrhoea Disease Research. 1993;11(4):211–216.
22. Oni GA, Schumann DA, Oke EA. Diarrhoeal disease morbidity, risk factors and treatment in a low socioeconomic area of Ilorin, Kwara State, Nigeria. Journal of Diarrhoeal Diseases Research. 1991;9:50-7.
23. Nweze EI. Virulence properties of diarrhoeagenic *E. coli* and etiology of diarrhoea in infants, young children and other age groups in Southeast, Nigeria. American-Eurasian Journal of Scientific Research. 2009;4(3):173-179.
24. Abdullahi M, Olonitola SO, Inabo IH. Isolation of bacteria associated with diarrhoea among children attending some hospitals in Kano Metropolis, Kano State, Nigeria. Bayero Journal of Pure and Applied Sciences. 2010;3(1):10–15.
25. Sule EI, Aliyu AM, Abdula ziz BM. Isolation of diarrhegenic bacteria in children attending some selected hospitals within kaduna metropolis, Kaduna state, Nigeria. Continental Journal of Applied Sciences. 2011;6(1):1-6.
26. Ako-Nai AK, Lamikanra A, Ola O, Fadero FF. A study of the incidence of enterotoxigenic *Escherichia coli* (ETEC) secreting heat-labile toxin in two communities in south-western Nigeria. American Journal of Tropical Medicine and Hygiene. 1990;93:116–8.
27. Al-Jurayyan NA, al Rashed AM, al-Nasser MN, al-Mugeiren MM, al Mazyad AS. Childhood bacterial diarrhoea in a regional hospital in Saudi Arabia: Clinico-etiological features. American Journal of Tropical Medicine and Hygiene. 1994;97:87–90.
28. Abdullahi M, Olonitola SO, Inabo IH. Isolation of bacteria associated with diarrhoea among children attending some hospitals in Kano Metropolis, Kano State, Nigeria. Bayero Journal of Pure and Applied Sciences. 2010;3(1):10–15.
29. Okeke IN, Lamikanra A, Steinruck H, Kaper JB. Characterization of *Escherichia coli* strains from cases of childhood diarrhoea in provincial southwestern Nigeria. Journal of Clinical Microbiology. 2000;38:7–12.
30. Huge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand. Journal of Clinical Infectious Diseases. 1998;26:341-345.
31. Enayat K, Fariborz S, Heiman S, Mohammad MSD. Frequency, antimicrobial susceptibility and plasmid profiles of *E. coli* pathotypes obtained from children with acute diarrhea. Jundishapur Journal of Microbiology. 2011;4(1):23-28.
32. Barletta F, Contreas C, Mercado E. New insight into the epidemiology of enteropathogenic *Escherichia coli* infection. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2008;102:852–856.

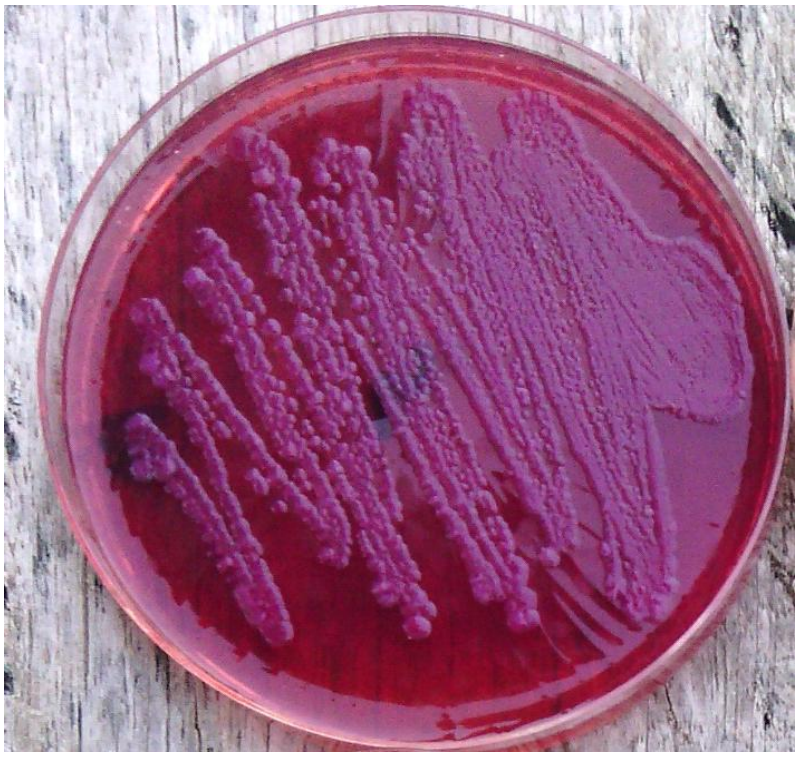
33. Okeke IN. Diarrhoeagenic *Escherichia coli* in sub-Saharan Africa: Status, uncertainties and necessities. Journal of Infection in Developing Countries. 2009;3:817–842.
34. Ochoa TJ, Contreas C. Enteropathogenic *Escherichia coli* infection in children. Current Opinion in Infectious Diseases. 2011;24:478–483.
35. Bukar AM, Isa MA, Bello HS, Isa A, Baba FA. Prevalence of enteropathogenic *E. coli* among hospitalized diarrhoea children in state specialist hospital Maiduguri, Borno, Nigeria. International Journal of Research (IJR). 2014;1(11). [ISSN 2348-6848]
36. Adebola O, Oluwatoyin I, Adebayo L. A study of the prevalence of diarrhoeagenic *Escherichia coli* in children from Gwagwalada, Federal Capital Territory, Nigeria. Pan African Medical Journal. 2014;17:146.
37. Ome KA, Nonye CM. Association of Enteropathogenic *Escherichia coli*with or Without Diarrhoea Among Children in Aba, Nigeria. International Journal of Microbiology and Application. 2015;2(2):38-44.
38. Iman KB, Emad AA, Entsar AA, Rania SL. Enteropathogenic *Escherichia coli* associated with diarrhoea in children in Cairo, Egypt. Scientific World Journal. 2011;11:2613–2619.
39. Addy PAK, Antepim G, Frimpong EH. Prevalence of pathogenic *Escherichia coli* and parasites in infants with diarrhoea in Kumasi, Ghana. East African Medical Journal. 2004;81:7.
40. Alikhani MY, Mirsalehian A, Aslani MM. Detection of typical and atypical enteropathogenic *Escherichia coli* (EPEC) in Iranian children with and without diarrhoea. Journal of Medical Microbiology. 2006;55:1159-1163.
41. Scaletsky IC, Fabbricotti SH, Silva SO, Morais MB, Fagundes-Neto U. HEp-2-adherent *Escherichia coli* strains associated with acute infantile diarrhea, Sao Paulo, Brazil. Emerging Infectious Diseases. 2002;8:855–8.
42. Araujo JM, Tabarelli GF, Aranda KRS, Fabbricotti SH, Fagundes-Neto U, Mendes CMF, et al. Typical enteroaggregative and atypical enteropathogenic types of *Escherichia coli* are the most prevalent diarrhea-associated pathotypes among brazilian children. Journal of Clinical Microbiology. 2007;45:3396–9.
43. Ochoa TJ, Contreas C. Enteropathogenic *Escherichia coli* infection in children. Current Opinion in Infectious Diseases. 2011;24:478–483.
44. Scaletsky ICA, Pedroso MZ, Oliva CAG, Carvalho RLB, Morais MB, Fagundes-Neto UA. Localized adherence-like pattern as a second pattern of adherence of classic enteropathogenic *Escherichia coli* to HEp-2 cells that is associated with infantile diarrhoea. Infection and Immunity. 1999; 67:3410–5.
45. Jenkins C, Lawson AJ, Cheasty T, Willshaw GA, Wright P, Dougan G, et al. Subtyping intimin genes from enteropathogenic *Escherichia coli* associated with outbreaks and sporadic cases in the United Kingdom and Eire. Molecular and Cellular Probes. 2003;17(4):149-56.
46. Ochoa TJ, Barletta F, Contreras C, Mercado E. New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. Transactions of The Royal Society of Tropical Medicine and Hygiene. 2008;102(9):852-856.
47. Nair GB, Ramamurthy T, Bhattacharya MK, Krishnan T, Ganguly S, Saha DR, Rajendran K, Manna B, Ghosh M, Okamoto K, Takeda Y. Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrhoeal patients in Kolkata, India. Gut Pathogens. 2005;2(1):4.
48. Hernandes RT, Elias WP, Vieira MAM, Gomes TAT. An overview of atypical enteropathogenic *Escherichia coli*. FEMS Microbiology Letter. 2009;292:137–149.
49. Levine MM, Edelman R. Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhoea: Epidemiology and pathogenesis. Epidemiology Review. 1984;6:31-51.
50. Nataro JP, Kaper JB. Diarrhoeagenic *Escherichia coli*. Clinical Microbiology Review. 1998;11:142-201.
51. Levine MM, Edelman R. Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhoea: Epidemiology and pathogenesis. Epidemiology Review. 1984;6:31-51.
52. Scotland SM, Willshaw GA, Smith HR, Said B, Stokes N, Rowe B. Virulence properties of *Escherichia coli* strains belonging to serogroups O26, O55, O111 and O128 isolated in the United Kingdom in 1991 from patients with diarrhoea.

Epidemiology Infection. 1993;111:429–438.

53. Viljanen MK, Peltola T, Junnila SY, Olkkonen L, Jarvinen H, Kuistila M, et al. Outbreak of diarrhoea due to *Escherichia coli* O111:B4 in schoolchildren and adults: Association of Vi antigen-like reactivity. Lancet. 1990;336:831–4.
54. Echeverria P, Orskov F, Orskov I, Knutton S, Scheutz F, Brown JE, Lexomboon U. Attaching and effacing enteropathogenic *Escherichia coli* as a cause of infantile diarrhoea in Bangkok. Journal of Infectious Diseases. 1991;164:550-554.
55. Gomes TT, Beatric ECG, Sonia RTS, Aloysio MF. Phenotypic and genotypic characteristics of shiga toxin-producing *E. coli* strains isolated from children i9n Sao Paulo. Memorias do instituto Oswaldo Cruz. 2002;97(8):1085-1089.
56. Dulguer MV, Fabbricotti SH, Bando SY, Moreira-Filho CA, Fagundes-Neto, Scaletsky IC. Atypical enteropathogenic *Escherichia coli* strains: Phenotypic and genetic profiling reveals a strong association between enteroaggregative *E. coli* heat-stable enterotoxin and diarrhoea. Journal of Infectious Diseases. 2003; 188:1685-1694.
57. Bokete TN, Whittam TS, Wilson RA, Clausen CR, O'Callahan CM, Moseley SL, Fritsche TR, Tarr PI. Genetic and phenotypic analysis of *Escherichia coli* with enteropathogenic characteristics isolated from Seattle children. Journal of Infectious Diseases. 1997;175:1382-1389.
58. Vieira MA, Andrade JR, Trabulsi LR, Rosa AC, Dias AM, Ramos SR. et al. Phenotypic and genotypic characteristics of *Escherichia coli* strains of non-enteropathogenic *E. coli* (EPEC) serogroups that carry EAE and lack the EPEC adherence factor and Shiga toxin DNA probe sequences. Journal of Infectious Diseases. 2001;183:762–72.
59. Vieira MA, Andrade JR, Trabulsi LR, Rosa AC, Dias AM, Ramos SR. et al. Phenotypic and genotypic characteristics of *Escherichia coli* strains of non-enteropathogenic *E. coli* (EPEC) serogroups that carry EAE and lack the EPEC adherence factor and Shiga toxin DNA probe sequences. Journal of Infectious Diseases. 2001;183:762–772.
60. Yatsuyanagi J, Saito S, Sato H, Miyajima Y, Amano K, Enomoto K. Characterization of enteropathogenic and enter oaggrega-tive *Escherichia coli* isolated from diarrheal outbreaks. Journal of Clinical Microbiology. 2002;40:294–297.

Appendix i

Pink Colonies on MacConkey Agar Indicating Lactose Fermentation



Appendix ii

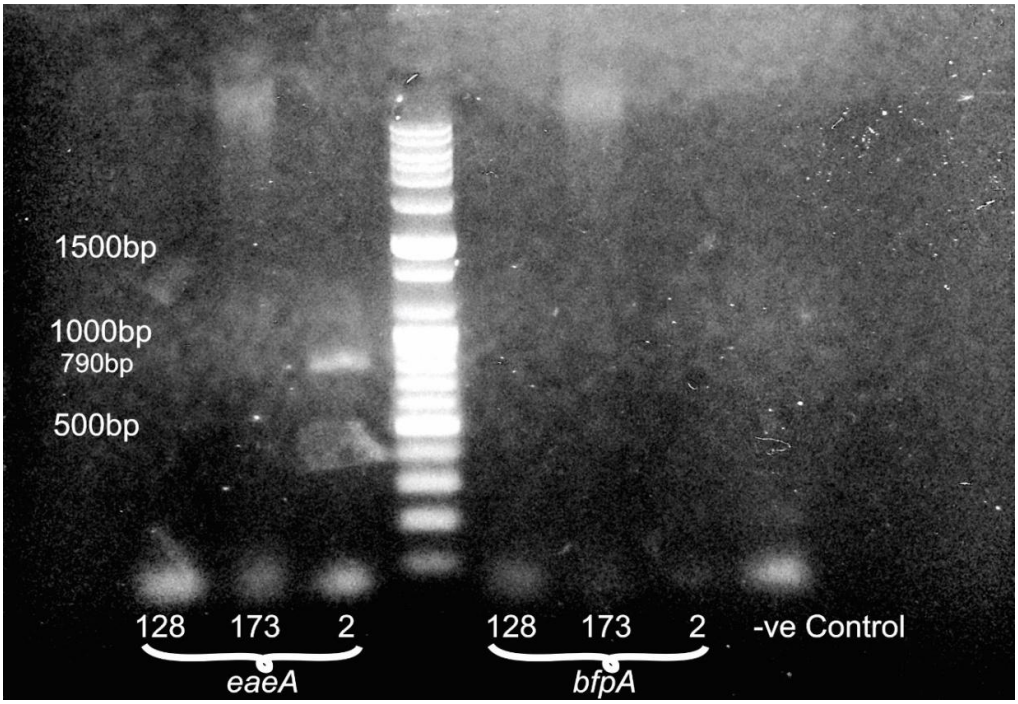


Plate 1. Agarose gelelectrophoresisshowing the band positive for *eaeA* gene at 790bp fragment size

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25792