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In the name of God

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**Isolation of Enteropathogenic Escherichia coli from Children with Diarrhoea Attending the National Hospital in Abuja, Nigeria.**

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**Abstract:**

**Background:** Diarrhoeal diseases remain one of the leading causes of childhood morbidity and mortality in most developing countries, with enteropathogenic Escherichia coli (EPEC) being one of the most important aetiological agents of infantile diarrhoea in many of these countries.

**Objective:** To determine the prevalence of EPEC in children (0-24 months) with diarrhoea.

**Methods:** One hundred stool samples from children with diarrhoea attending the National Hospital in Abuja, Nigeria were analysed for the presence of EPEC using standard bacteriological methods. Isolates were also subjected to antimicrobial testing using the disc diffusion method.

**Results:** Fifteen (15.00%) samples were positive for EPEC. The highest number of isolates was recovered from the 6-12 months age group with 10 (20.41%) in total. No isolates were recovered from the 0-5 months age group or from exclusively breast fed children, however, nine (19.15%) children receiving mixed feeding and six (33.33%) children who were not being breast-fed tested positive for EPEC, and the difference was found to be statistically significant ( $P < 0.05$ ). The highest number of isolates, eight (53.33%), belonged to those in the poly 1 sero-group with serotypes O1, O26, O86a, O111, O119, O127 and O128. Fourteen (93.33%) of the isolates were sensitive to norfloxacin, 13 (86.67%) each to ofloxacin and colistin, 11 (73.33%) to nalidixic acid, 10 (66.67%) to cefuroxime, two (13.33%) to cotrimoxazole, and one (6.67%) to tetracycline. None of the isolates were found to be sensitive to ampicillin or chloramphenicol.

**Conclusion:** This study has shown that multidrug resistant EPEC is associated with infantile diarrhoea in Abuja, Nigeria. To address this issue, antibiotic therapy should take into consideration the susceptibility pattern of the pathogen. In addition, the incidence of EPEC in children can be traced primarily to faulty weaning practices and/or poor personal hygiene.

**Keywords:** Enteropathogenic Escherichia coli (EPEC), diarrhoea, children, serotype, Abuja, Nigeria.

## **Introduction:**

Acute diarrhoea has been recognized as a prominent killer of infants and young children in most developing countries.<sup>(1)</sup> Reports in the literature indicate that over five million deaths per year occur in children under the age of five year as a result of diarrhoea in these countries.<sup>(2)</sup> The association between enteropathogenic *Escherichia coli* (EPEC) and diarrhoea has been reported in many countries.<sup>(3, 4, 5)</sup> Studies have also shown that EPEC is an important causative agent in sporadic infantile diarrhoea in many developing countries.<sup>(4, 6)</sup>

In previous studies, it has been demonstrated that the incidence of community acquired EPEC infection is highest in the six month period following childbirth,<sup>(5, 7)</sup> and that the infection is more severe in younger children.<sup>(5)</sup> Infants are more likely to develop diarrhoea during the first episode of colonization with EPEC than during subsequent exposures.<sup>(8)</sup> Illiteracy in parents (especially mothers), faulty weaning practices, and the use of bottle feeding are possible factors that could be behind the high prevalence of this infection in most developing countries.<sup>(9)</sup>

In Nigeria, cases of gastroenteritis due to EPEC have been reported; however, common serotypes associated with the infection have not yet been well established among children from different parts of the country. Hence, this study was undertaken to investigate the prevalence of EPEC sero-groups in children

presenting with diarrhoea at the National Hospital in Abuja, Nigeria.

## **Material and Methods:**

### **Study Population**

The study population included infants and young children between 0 – 24 months attending the Emergency Paediatric Unit (EPU) and Out Patient Department (OPD) of the National Hospital in Abuja, Nigeria. A total of 100 diarrhoea stool samples were collected.

Informed consent was obtained from patients' mothers, hospital authorities, and clinicians involved in the management of the patients examined.

### **Sample Collection**

Stool samples were collected from the patients in clear, transparent, wide mouthed bottles. Information was also obtained from each patient regarding age, sex, feeding patterns, and maternal education.

### **Processing of Specimens**

The specimens were processed according to the guidelines provided by Cheesbrough<sup>(10)</sup> for the laboratory diagnosis of enteric pathogens. These included: macroscopy, microscopy, Gram's stain, motility testing, culture, biochemical testing, and antimicrobial sensitivity testing. Specimens were inoculated onto MacConkey agar plates and incubated aerobically at 37°C for 24 hours. Resultant colonies were identified using standard biochemical tests.

### Biochemical Testing

All lactose fermenting colonies on MacConkey agar plates were subjected to the following tests: Gram staining, urease test, citrate utilization test, indole test, methyl red test, Kligler iron agar test, and a motility test. All tests were done using the methods described by Cheesbrough.<sup>(10)</sup>

### Serotyping

Isolates confirmed to be *E. coli* by Gram staining and biochemical tests were subjected to a slide agglutination test with *E. coli* somatic polyvalent antisera (Biotech Ltd., UK). The following typing sera were used:

*E. coli* Poly 1 (O1, O26, O86a, O111, O119, O127, O128)

*E. coli* Poly 2 (O44, O55, O125, O126, O146, O166)

*E. coli* Poly 3 (O18, O114, O151, O157, O158)

Monovalent anti-sera were not used due to a lack of availability. The testing procedure given by the manufacturer was strictly followed.

### Antimicrobial Susceptibility Testing

Sensitivity of isolates to antimicrobial agents was determined on Mueller-Hinton agar plates using the disc diffusion method,<sup>(11)</sup> and interpreted through zone size analysis.<sup>(12)</sup> All isolates were tested for sensitivity to the following antibiotics: norfloxacin (25 mcg), ofloxacin (25 mcg) colistin (25 mcg), nalidixic acid (30 mcg), cefuroxime (25 mcg), cotrimoxazole (25 mcg) tetracycline (30 mcg), ampicillin (10 mcg), and chloramphenicol (30 mcg). All antibiotics were obtained from Antec Diagnostics, UK.

### Statistical Analysis

The data obtained was analyzed using a chi-squared test and a probability of  $p = 0.05$  as the level of significance.

### Results:

A total of 100 diarrheal stool samples were examined for the presence of enteropathogenic *Escherichia coli* in children between the ages of 0 – 24 months. Of the total number of specimens examined, 15 (15.00%) were positive for EPEC. The highest number of isolates, 10 (20.41%), was recovered from the 6 – 12 month age group, and was followed by the 13 – 18 month age group which produced three (13.04%) isolates, and the 19 – 24 month age group that produced two (10.53%). No isolates were recovered from the 0 – 5 month age group. The difference between these groups was not found to be statistically significant ( $P > 0.05$ ) (table 1).

Table 2 shows the distribution of EPEC serotypes isolated according to age. Eight (53.33%) of the 15 EPECs isolated belonged to serotypes O1, O26, O86a O111, O127, O128, four (26.67%) to serotypes O44, O55, O125, O126, O146, O166, and three (20.00%) to serotypes O18, O114, O142, O151, O157, O158. Serotypes O18, O114, O142, O151, O157, and O158 were isolated from the 6 – 12 month age group only.

Macroscopic examination of the stool specimens showed that the watery samples yielded the highest number of isolates, with a total of five (23.80%). Others had the following results: four (17.39%) were found to be loose and

muroid, three (20.00%) were found to be watery and muroid, two (5.71%) were loose, and one (16.67%) was soft formed (table 3).

Table 4 shows the correlation between feeding patterns and the number of EPECs isolated. Children who had mixed feeding yielded the highest number, with a total of nine (19.15% positive) isolates, while those who were not breast-fed had (33.33% positive). No isolates were recovered from exclusively breast fed children, and the difference was found to be statistically significant ( $P < 0.05$ ).

The relationship between maternal education level and number of EPECs isolated is presented in table 5. Six (33.33%) infants with mothers who had only received primary school education

tested positive. Four (14.51%) infants with mothers who had obtained secondary school education tested positive, and another five (9.09%) infants with mothers who had a postsecondary education tested positive. The results of this analysis were not statistically significant.

In table 6, the in vitro antibiotic susceptibility pattern of EPEC isolates is shown. Fourteen (93.33%) isolates were found to be sensitive to norfloxacin, 13 (86.67%) were sensitive to ofloxacin and colistin, 11 (73.33%) to nalidixic acid, 10 (66.67%) to cefuroxime, two (13.33%) to cotrimoxazole, and one (6.67%) to tetracycline. All isolates were resistant to ampicillin and chloramphenicol.

Table 1: Prevalence of enteropathogenic Escherichia coli in relation to age and sex of children tested.

Age group (Months)	No. samples tested	No. Females Examined	No. (%) Females Positive	No. Males Examined	No. (%) Males positive	Total No. (%) positive
0 - 5	9	4	0(0.00)	5	0(0.00)	0(0.00)
6 - 12	49	21	4(19.04)	28	6(21.42)	10(20.41)
13 - 18	23	9	2(22.22)	14	1(7.14)	3(13.04)
19 - 24	19	8	1(12.50)	11	1(9.09)	2(10.53)
Total	100	42	7(16.67)	58	8(13.79)	15(15.00)

$P > 0.05$

Table 2: Distribution of enteropathogenic Escherichia coli serotypes isolated in relation to age of children (n = 15).

Age Group (Months)	Serotypes of EPEC isolated No. (%) Positive		
	Poly 1	Poly 2	Poly 3
	O1, O26, O86a, O111, O119, O127, O128	O44, O55, O125, O126, O146, O166	O18, O114, O142, O151, O157, O158
0 - 5	0(0.00)	0(0.00)	0(0.000)
6 - 12	5(33.33)	2(13.33)	3(20.00)
13 - 18	2(13.33)	1(6.67)	0(0.000)
19 - 24	1(6.67)	1(6.67)	0(0.00)
Total	8(53.33)	4(26.67)	3(20.00)

$P > 0.05$

Table 3: The number of enteropathogenic E coli isolated from the various types of stool samples examined.

Forms of stool samples	No. Tested	No. (%) positive for EPEC
Watery	21	5(23.80)
Watery and mucoid	15	3(20.00)
Loosed and mucoid	23	4(17.39)
Loosed	35	2(5.71)
Soft formed	6	1(16.67)
Total	100	15(15.00)
P > 0.05		

Table 4: Correlation of feeding pattern of infants with number of enteropathogenic Escherichia coli Isolated.

Type of feeding	No. Examined	No. (%) positive
Exclusive breast feeding	35	0(0.00)
Mixed feeding	47	9(19.15)
No breast feeding	18	6(33.33)
Total	100	15(15.00%)
P < 0.05		

Table 5: Correlation of mothers' level of education to number of enteropathogenic Escherichia coli isolated from their children.

Level of Education	No. Examined	No (%) Positive
Above secondary school level	55	5(9.09)
Up to secondary school	27	4(14.51)
Primary school and below	18	6(33.33)
Total	100	15(15.00%)
P > 0.05		

Table 6: Invitro antibiotic susceptibility pattern of enteropathogenic Escherichia coli isolated n = 15.

Antibiotic	Conc.(Mcg)	No. (%) sensitive
Norfloxacin	25	14(93.33)
Ofloxacin	25	13(86.67)
Colistin	25	13(86.67)
Nalidixic acid	30	11(73.33)
Cefuroxime	25	10(66.67)
Cotrimoxazole	25	2(13.33)
Tetracycline	30	1(6.67)
Ampicillin	10	0(0.00)
Chloramphenicol	30	0(0.00)

**Discussion:**

A total of 100 stool specimens were analysed in this study, with fifteen percent of samples testing positive for enteropatho-

genic *Escherichia coli*. This is lower than the 26% prevalence rate documented by Olanipekun<sup>(13)</sup> for children with diarrhoea attending the Jos University Teaching Hospital in Jos, Nigeria. One probable reason for this difference may be due to the number of children being exclusively breast fed in the two studies. Thirty-five percent of the mothers in this study exclusively breast fed their children compared to 6 percent in the Jos study. Breast milk (colostrum) from mothers living in endemic areas has been reported to contain high levels of immunoglobulin A (IgA) antibodies against the EPEC virulence factors.<sup>(14, 15, 16)</sup>

Enteropathogenic *E. coli* was not isolated from infants younger than 6 months old. In contrast, the highest isolation rates were found in children between the ages of 6 – 12 months. This finding is similar to reports published by several authors<sup>(13, 17, 18)</sup> where they observed that the highest incidence of gastroenteritis in children was found within the age range of 7 – 12 months, when weaning prac-

tices begin in many parts of the world (Nigeria inclusive).

Data obtained in this study indicate that all children below six months old were exclusively breast fed, whereas those between 7 – 12 months had their breast feeding interrupted with mixed feeding, if they had not stopped completely. These findings therefore corroborate findings from previous studies regarding the protective role of breast milk against bacterial gastroenteritis.<sup>(2, 13, 18)</sup>

Faulty weaning practices and poor hygiene during food preparation may also contribute to increased gastroenteritis around the age of 7 – 12 months. The low isolation rate of EPEC in children older than 12 months may be associated with the development of immunity or the loss of receptors for some specific adhesion molecules.<sup>(19)</sup>

The isolation rate of EPEC was found to be slightly higher in females (16.67%) than males (13.79%), however, the difference was not statistically significant.

The highest number of EPECs isolated belonged to the poly 1 sero-group with following serotypes O1, O26, O86a, O111, O119, O127 and O128. Twelve<sup>(12)</sup> EPEC sero-groups have been recognised by the World Health Organization (WHO). These include: O26, O65, O86, O111, O114, O119, O125, O126, O127, O128, O142 and O158.<sup>(20, 21)</sup> The majority of these strains were included in the poly 1 typing sera, a probable reason why the highest number of isolates was found in this group. However, due to the unavailability of the monovalent typing sera, further specific typing was not carried out to show which serotype occurs more frequently in infantile diarrhoea within the study's geographic area.

Macroscopic examination of the stool samples showed that a higher number of EPEC was isolated from watery and watery/ mucoid stools than from other forms of stools. This agrees with literature reports which state that acute watery diarrhoea is a common clinical feature in diarrhoea due to EPEC. The differ-

ence, however, was not found to be statistically significant.

Illiteracy of mothers has been reported to be a predisposing factor that contributes to infants and young children acquiring the infection.<sup>(9)</sup> However, our findings suggest that there is no significant difference in the number of EPECs isolated in relation to the mother's level of education.

The antimicrobial susceptibility profile of the isolates showed that most were sensitive to the fluoroquinolones (norfloxacin and ofloxacin). High sensitivity rates were also observed for colistin, nalidixic acid, and cefuroxime. These antimicrobial agents are expensive and may have adverse side effects.<sup>(22)</sup> Fortunately, EPEC diarrhoea is usually self-limited and rehydration is the most effective treatment. The use of antibiotics in general is of minor importance and has been criticised on the grounds of drug toxicity and the risk of increased of wide-spread antimicrobial resistance.<sup>(10, 12)</sup>

High rates of resistance were recorded for ampicillin (100%), chloramphenicol (100%), tetracycline (93.33%) and cotrimoxazole (86.67%). Several reports have indicated that these drugs are also less effective against other bacterial agents isolated in Nigeria,<sup>(23, 24)</sup> largely because they are inexpensive and can be obtained easily without a doctor's prescription. Resistance is probably due to indiscriminate antibiotic usage (drug abuse) which could result in plasmid-mediated antibiotic resistance found to be common in *Escherichia coli*.<sup>(10)</sup>

### Conclusion:

This study has shown that multi drug-resistant enteropathogenic *Escherichia coli* is associated with infantile diarrhoea in Abuja, Nigeria, and that norfloxacin and ofloxacin should be used in the treatment of EPEC infections in this region. In addition, our findings indicate that the incidence of EPEC in children can be traced primarily to faulty weaning practices and/or poor personal hygiene.

### Recommendation

Replacement of fluids and electrolytes should be the standard treatment in this environment. Antibiotic therapy must take into consideration the susceptibility pattern of the pathogen and the side effects of the drugs. Also, there should be effective legislation by the government to control the indiscriminate purchase of antibiotics to prevent its abuse in Nigeria.

Mothers should be encouraged to practice good personal hygiene and to breast-feed infants for at least 12 months because of the protective role that breast milk plays against bacterial gastroenteritis.

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