

THE OCCURRENCE OF DIARRHOEAGENIC ESCHERICHIA COLI AMONG CHILDREN UNDER FIVE YEARS OF AGE IN BASRAH, SOUTHERN IRAQ

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Objective: Examine the occurrence of diarrhoeagenic Escherichia coli (E. coli) among children under five years of age and the distribution of enteropathogenic (EPEC) serotypes in relation to some hygienic factors.

Design: A structural questionnaire form administered principally to mothers for obtaining the relevant informations.

Setting: Basrah General Hospital and Basrah University Teaching Hospital, Iraq.

Subjects: A total of 1124 stool specimens or rectal swabs collected from children with acute diarrhoea under the age of 5 years.

Methods: Detection of EPEC serotypes through serotyping and the enterotoxin of both LT and ST production as well as the estimation of E. coli invasiveness.

Results: Diarrhoeagenic E. coli was found in 148 (13.2 %) of examined specimens. Among these 77 (52 %) were enteropathogenic (EPEC), 69 (46.6 %) were enterotoxigenic (ETEC) and 2 (1.4 %) were enteroinvasive Escherichia coli (EIEC). EPEC serotypes 0119K69, 0114K90 and 0111K58 were the more frequent among twelve other serotypes. Diarrhoeagenic Escherichia coli showed high rates of resistance to antibiotics especially ampicillin (98 %), tetracycline (93 %) and chloramphenicol (86 %).

Conclusions: The diarrhoeagenic Escherichia coli were common and they represent critical medical problem in our area and antibiotic resistance is a part of this problem that deserve a major commitment of attention and resources. Bahrain Med Bull 1996;18(2):

Diarrhoeal diseases are important causes of mortality and morbidity in various age groups especially infants in developing countries¹. Research into the role of Escherichia coli (E. coli) as a cause of gastroenteritis established the

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following points: (1) only few strains causes gastroenteritis, (2) the illness occur most often in infants, (3) infections as a distinct form of illness is very common and (4) pathogenic strains vary over short period in their ability to cause disease².

In hospital based studies of acute diarrhoea, the rate of enteropathogenic E. coli (ETEC) ranged between 10 % to 50 % with an average of about 20 % in children under five years of age^{1,4,5}. EPEC is also an important cause of sporadic infant diarrhoea in Brazil and South African infants accounting for 30 % of cases⁶. Pathogenic E. coli was recovered from 4.8 % of patients with gastroenteritis in Nigeria⁷. Frequencies of EPEC isolation rates ranging from 7.9 % to 30 % were reported throughout the world in infants^{2,4,5,7,8}. EPEC serotypes 0111K58, 0119K69, 055K59 and 0126K71 were reported to be the most frequent isolates among infants^{4,7-9}. Enteroinvasive E. coli (EIEC) strains were

detected in 9 % of acute diarrhoea in infants of Columbia⁸ and Nigeria⁷. An isolation rate of EIEC less than 2 % was also reported in other studies¹⁰.

Feeding practice plays a role in spreading the organisms and breast feeding is an important factor in the prevention of infantile enteritis². Seasonal peak of *E. coli* diarrhoea is often observed during warmer season^{1,12,13}.

Diarrhoeagenic *E. coli* have been reported to develop resistant to antibiotics^{2,13-15}. In South East Asia 72 % of diarrhoeagenic *E. coli* isolates were found to be resistant to at least four drugs^{6,16}.

The objective of this study are: (1) examine the impact of diarrhoeagenic *E. coli* in diarrhoeal diseases in Basrah, Southern Iraq, (2) identify the various EPEC serotypes implicated in infant enteritis in relation to some hygienic and sanitary measures on feeding practice, (3) examine the antibiotic susceptibility of these diarrhoeagenic isolates.

METHODS

Stool and rectal swabs were collected from 1124 children (66 hospital admitted patients and 1058 outpatients) under five years of age with acute diarrhoea seen at Basrah General Hospital and Basrah University Teaching Hospital, Iraq over the period from November 1989 to April 1993. Information about each child was obtained by interviewing mostly the mothers using specially designed questionnaire form to include age, sex, feeding practice, water sources and other relevant hygienic factors.

Specimens were cultured on MacConkey's agar plates and incubated at 37°C for 24 hours. Ten lactose fermenter colonies with typical appearance of *E. coli* were selected from the plates, stored on nutrient agar slabs and tested within two weeks of isolation for heat-labile (LT) enterotoxin production by Biken test³ using suitable media and reagents. They were also tested for heat-stable (ST) toxin production by infant mice assay as described by the WHO guidelines for enteric isolates³. Anti-LT antisera and standard LT were obtained from the WHO collaboration Centre for Reference and Research on Escherichia, Copenhagen, Denmark. The EPEC serotypes were identified according to standard method using bioMerieux diagnostic antisera: polyvalent and monovalent antisera for each serotype (bioMerieux Laboratories, France). Sereny test was carried out to detect the invasiveness of *E. coli* isolates also in accordance with the WHO guidelines³. Control stains were donated by WHO collaboration Centre for Reference and Research on Escherichia, Copenhagen, Denmark.

E. coli isolates were tested by agar diffusion test for antibiotic susceptibility using the commercially available antibiotic discs recommended by the WHO guideline³. The antibiotics used were ampicillin (10 ug), kanamycin (30 ug), chloramphenicol (30 ug), tetracycline (30 ug), sulfamethoxazole-trimethoprim (25 ug), gentamycin (10 ug) and cephalothin (30 ug).

X² test was done to show the significant values.

RESULTS

Of the 1124 children in this study there were 614 (54.5 %) boys and 510 (45.4 %) girls with age range from one day to 56 months.

A total of 148 pathogenic *E. coli* were isolated from 1124 patients with acute diarrhoea showing an isolation rate of 13.2 %. Among them 77 (52 %) were EPEC, 69 (46.6 %) enterotoxigenic *E. coli* (ETEC), and 2 (1.4 %) EIEC.

Table 1. Distribution of pathogenic E. coli according to age groups

Age groups (months)	No tested	EPEC		ETEC*		EIEC		Total	
		No	%	No	%	No	%	No	%
0-5	186	23	12.3	4	2.2	0	0.0	27	14.5
6-12	184	39	21.2	6	3.3	0	0.0	45	24.4
13-24	182	9	4.9	10	5.5	1	0.5	20	11.0
25-36	184	0	0.0	13	7.0	0	0.0	13	7.0
37-48	191	4	2.1	17	8.9	0	0.0	21	11.0
49- above	197	2	1.0	19	9.6	1	0.5	22	11.2
Total	1124	77	52.0	69	46.6	2	1.4	148	13.2

* ETEC produced LT-type enterotoxin accounted for 60.9 %, ST-type 29 %, and both types 10.1 %.

Table 1 shows the proportional distribution of E. coli isolates according to age of patients. There was no differences in the rates of recovery of various E. coli isolates in different age groups but there were a higher rates of EPEC serotypes recovery among age groups 0-5 months (12.3 %) and 6-12 months (21.2 %), $P < 0.01$. Most of the 77 EPEC isolates were detected in children below one year while the recovery of ETEC isolates increased as the age increased. EIEC strains were detected on two occasions only which showed a positive Sereny test. None of the EPEC serotypes were enterotoxigenic or enteroinvasive. The majority of ETEC isolates were producer of LT-type toxin (60.9 %) followed by ST-type (29 %) while ETEC isolates producing both types accounted for 10 % (Table 1).

Twelve different serotypes of EPEC were encountered; serotype 0119K69 (22 %) was the most frequent isolate, although other serotypes were also recovered in varying percentages; 0114K90 and 0111K58 in 14.3 % and 13 % respectively. When the data analyzed to detect the prevalence and frequency of EPEC in infants in their first year of life (0-12 months), the recovery rate was increased. There were 62 (80.5 %) EPEC isolates recovered from this group with an isolation rate of 32.9 %.

Table 2. Proportional distribution of EPEC serotypes in relation to season of isolation

EPEC serotypes (n)	Seasons			
	Spring (312)*	Summer (353)	Autumn (288)	Winter (171)
1. 055K59 (3)	1	1	0	1
0111K58 (10)	1	7	2	0
026K60 (2)	0	2	0	0
2. 086K61 (6)	1	0	4	1
0119K69 (17)	0	14	2	1
0127K63 (7)	1	5	0	1
3. 0125K70 (4)	0	2	2	0
0126K71 (8)	2	4	0	2
0128K67 (2)	0	2	0	0
4. 0114K90 (11)	2	5	4	0

0124K72(1)	0	0	1	0
0142K86(6)	2	0	4	0
-----Total and (%) (77)				
10(13)	42(54.5)	19(24.7)	6(7.8)	

* Number tested

Table 2 shows the proportional distribution of the 12 EPEC serotypes which were detected along the different seasons of the year. The highest proportion was recovered during summer (54.5 %) and autumn (24.7 %). Serotypes 0119K69, 0114K90, 0111K58 and 0126K71 were the predominant isolate during summer and autumn seasons.

Table 3. The distribution and frequency of EPEC serotypes in relation to types of feeding practice

Type of feeding	No. tested	No. of EPEC (%)	Frequent serotypes
Breast-fed	341	9 (11.6)	055K59, 026K60, 0111K58, 0128K67, 0124K72
Bottle-fed	358	41 (53.3)	0111K58, 0119K69, 0126K71, 086K61, 0114K90, 0142K86
Combined feeding	425	27 (35.0)	0114K90, 0111K58, 0127K63, 0125K70, 0119K69, 055K59
Total	1124	77 (6.9)	

The frequency of EPEC serotypes in relation to types of feeding practice is presented in Table 3. EPEC recovery rates accounted for 6.9 %, among these 9 (11.9 %) were from breast fed babies. On the other hand, 41 (53.3 %) and 27 (35 %) of EPEC isolates were recovered from bottle-fed and combined-fed babies respectively which showed a significant differences (P <0.01). Serotypes 0119K69, and 0114K90 were the more frequent among artificially fed babies but not in breast-fed babies. However, serotypes 026K60 and 055K59 were frequent in breast fed babies only.

Table 4. Antibigram of pathogenic E. coli isolates:

Antibiotics	Numbers of resistant isolates (%)			Total (148)*
	EPEC (77)*	EPEC (69)*	EIEC (2)*	
Ampicillin	77(100)	67(97)	2(100)	146(98)
Tetracycline	72(93)	64(93)	1(50)	137(93)
Chloramphenicol	68(88)	59(81)	1(50)	128(86)
Gentamicin	54(70)	33(48)	0	87(59)
Sulfamethoxazole-trimethoprim	42(54)	49(71)	1(50)	92(62)
Kanamycin	77(100)	36(52)	2(100)	115(78)
Cephalothin	49(63)	41(59)	1(50)	91(61)

* Number examined of each isolate

Antibiogram of diarrhoeagenic *E. coli* are shown in Table 4. These isolates showed high rates of resistance to all antibiotics used especially ampicillin (98 %) and tetracycline (93 %).

DISCUSSION

Enteropathogenic, enterotoxigenic and enteroinvasive *E. coli* are all important causes of diarrhoeal disease in tropical and semitropical countries^{1,13,16}. However, the recovery rates reported in this study were higher than those reported elsewhere⁷. This may be due to the use of better methods for the isolation and identification or that pathogenic *E. coli* are an important aetiological agent in patients with acute diarrhoeal diseases from whom a recognised pathogen cannot be isolated^{1,2,7-9}. This was supported by another study carried out in Basrah².

The majority of EPEC serotypes in this study were detected during infancy (0-12 months) and this is in agreement with other studies from the third world countries^{1,2,4,5,7-9}. However, some EPEC serotypes were detected in older age groups and this may raise the possibility that EPEC shedding may continue even after infantile period and act as a potential enteric pathogen or may form a carriage status after primary infection. Further study is needed to clarify this point. EPEC serotypes 0111K58, 055K59, and 012K71 were reported to be the most frequent isolates of EPEC strains^{4,7-9}. In this study EPEC serotypes 0119K69, 0114K90 and 0111K58 were found to be the more frequent isolates while the other serotypes appeared in less frequencies in our area. There were differences of isolation rates and distribution of these EPEC serotypes in different age groups and the frequency of the serotypes in various seasons of the year which is not been reported previously.

The high rates of EPEC recovery may be influenced by the fact that in this study ETEC was investigated in the symptomatic cases only because the isolates can still be recovered in asymptomatic cases^{1,2,17}. LT-type enterotoxin was predominantly produced by ETEC isolates which was in agreement with other reported results^{1,13,18}. However, other studies showed that ETEC enteritis has been attributed to ST-type only¹⁸, whereas ST-type in this study form only about one-third of ETEC isolates. ETEC was recovered from only few cases and the detection of these strains may need a more sensitive assay for their detection.

Poor hygienic standard and poor sanitation, unwashed hands and utilisation of unboiled water in preparation of feeding bottles and drinking play a significant role in transmitting the organisms^{1,2,11}. Feeding practice also influence the prevalence of EPEC serotypes as breast feeding has been reported to be associated with lower rates of pathogenic *E. coli* detection^{1,8,19}. This trend was also found in this study.

The isolation rates and frequencies of pathogenic *E. coli* decreased as the age increased. These observations were also reported by other^{1,2}. The peak of diarrhoeagenic *E. coli* recovery was seen during the warmer seasons which is also in consistence with other reported data from developing countries^{1,12}.

Diarrhoeal diseases and other infections due to enteric bacterial pathogens represent critical public health problem in large areas of the world and antibiotic resistance is a part of this problem which deserve a major commitment of attention and resources. Diarrhoeagenic *E. coli* have been reported to be resistant to multiple antibiotics^{2,13,10,20}. These results were in agreement with the findings obtained from this study.

CONCLUSION

Diarrhoeagenic *E. coli* is common among patients with acute gastroenteritis. EPEC serotypes were a major cause of infantile diarrhoea, their frequencies and distribution were much influenced by age, season of isolation and feeding practices. The frequency of their shedding may give an explanation of different pattern of epidemiology. The diarrhoeagenic *E. coli* isolates were multiple resistant to antibiotics. The patterns of high rates of resistance to antibiotics imply an urgency to control the extent of use of various antimicrobial agents.

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