

Evaluation some virulence factor of Escherichia coli that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

Dr. Thekra A. Hamada , Dr. Ahmed H. Abdelghafoor, Affaf S. Hussein

Abstract

The presented study was carried out from May2015 to September 2015 in the Pediatric General Hospital in Kirkuk. A total of 102 samples from children detect some virulence factor of Escherichia coli. The identification of these bacteria is depended on several biochemical tests including (indole test , methyl red test , vogas proskaur test , citrate utilization test , urease test, oxidase test and motility test) and the identification was confirmed by using Analytical profile index (API 20 E system). Antibiotic Susceptibility test was done for these isolates to detect the ability of this bacteria to drug resistance . Finally the genes that are responsible for diarrhea in E.coli isolates (eae gene for EPEC, ial gene for EIEC, It gene for ETEC, stx gene for EHEC) detected using PCR.

E.coli were the most common pathogen isolated from children (30isolates), and pathogenic Ecoli was 11 isolated ,4 was ETEC, 4EIEC,3EPEC, and absent of EHEC.

Introduction

Infections of the gastrointestinal tract are among the world's leading causes of illness and death among children. Recently such infections were reported to cause more than 3.2 disease episodes per year in children under the age of five in developing countries (1). They have also been estimated to be the third most common cause of death by infectious diseases, only preceded by lower respiratory tract infections and HIV/AIDS (2). Globally 21% of all deaths in children under five years of age are estimated to be due to diarrhoeal infections (1). In recent years studies from several developing countries have shown that diarrhoeal diseases also cause considerable lasting disabilities both in physical growth and

fitness, and in cognitive skills and school performance (3).

Escherichia coli was first described as Bacterium coli commune by the German paediatrician Theodore Escherich in 1885. This name was used until the genus Escherichia with the type species E. coli. E. coli strains associated with diarrhea have been classified into six groups, based on clinical, epidemiological and molecular criteria: enteropathogenic E. coli (EPEC), enterohaemorrhagic E. coli (EHEC), enteroinvasive E. coli (EIEC), enterotoxigenic E. coli (ETEC), enteroaggregative E. coli (EAaggEC) and diffusely adherent E. coli (DAEC)(4).

The identification of DEC cannot be based only on cultural and biochemical criteria, since they are indistinguishable from the non-pathogenic E. coli commonly found in human feces. Moreover, specific serotyping

Evaluation some virulence factor of *Escherichia coli* that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

is not always correlated with pathogenicity. Since several virulence factors and DNA sequences of DEC have been identified, they can be determined by the presence of genes coding for specific virulence factors, which are absent in non-pathogenic strains. Polymerase chain reaction (PCR) is a commonly used method that gives rapid, reliable results, and shows high sensitivity and high specificity. Several PCR methods, with both single and multiple target genes, have been reported for detecting the different DEC pathotypes.

Aim of study

the aim of this study is to decrease the severity of diarrhea caused by *E. coli* by detection of some virulence factors of the bacteria using PCR.

Materials and methods

patients

Patients were children with diarrhea attending Pediatric General Hospital in Kirkuk city between May 2015 to September 2015.

During the study period, 102 stool samples from children less than 5 years old were investigated for DEC. Of them, 82 were with diarrhea and 20 without diarrhea (control group).

The selection criteria for inclusion of children patients with diarrhea was having at least 3 or more soft, semi solid or liquid bloody faeces within 24 hours.

Also, selection was made on basis of a questionnaire, providing information

regarding other gastrointestinal disorder, frequency of diarrhea episodes, type of feeding, source of water, age and sex, place of residence, previous and current antibiotic treatment, and other associated diseases.

Methods

❖ **Specimen Collection:** 102 stool samples were collected by sterile Cary-Blair transport medium, from children under five years of age.

General Stool Examination (GSE): This test involves two steps, macroscopic and microscopic examinations. The macroscopic examination of stool sample is done visually according to [7], for consistency (formed, unformed 'soft', or liquid), color (white, yellow, brown, or black), and presence of any abnormal components (mucus or blood). While the Microscopic examination of stool sample is done to demonstrate RBCs, pus cells, Monilia, bacteria, intestinal protozoa, fatty drops, undigested food, and normally small to moderate epithelial cells. The presence of large number of epithelial cells indicates that the intestinal mucosa is irritated [3].

A Stool Culture: Culture of fresh stool specimens remains the standard for determining an etiologic diagnosis [4]. The sample will be inoculated on differential culture media (MacConkey agar, Salmonella - Shigella agar, nutrient agar), through the use of streaking plate method technique, followed by overnight incubation at 37°C for 24 hours.

Purification of bacterial isolates

Evaluation some virulence factor of *Escherichia coli* that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

The selection of bacterial colonies fermented sugar lactose that growth on MacConky agar and plating on nutrient agar for the purpose of purification.

Identification of bacterial isolates

1- Microscopic examination

The smear was prepared and fixed on clean glass slide and stained with gram stain by added crystal violet for 30 seconds then smear washed with distilled water. Gram's iodine was added for 10 seconds then the smear was washed with water and decolorized with 95% Acetone alcohol and finally safranin a secondary dye was added to the smear for 30 seconds washed with water, air dried and observed under oil immersion objective(100X).

2-Morphological characteristics

Observed colonies according to size, shape and color and ferment or not ferment lactose.

3-Biochemical tests

Analytical Profile Index (API 20 E System) test:

API 20 E (Analytical profile index) is a standardized identification system for *Enterobacteriaceae* and other non-fastidious, Gram negative rods which uses 21 miniaturized biochemical tests and a database. The complete list of those organisms that it is possible to identify with this system is given in the identification.

Preservation and Maintenance of bacterial isolates:

After identification of bacterial isolates and identification if *E. coli*, preserved *E. coli* by

slant method in tube contains nutrient agar (Washington *et al.*, 2006). Which commonly used for G+ve bacteria and some G-ve bacteria, and repeated every month for the purpose of active survival for the duration of the study.

Antimicrobial Susceptibility Testing:

Disk diffusion test method:

The disk diffusion susceptibility method is simple and practical and has been well-standardized. The test is performed by applying a bacterial inoculum of approximately $1-2 \times 10^8$ CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton agar plate. Up to 12 commercially-prepared, fixed concentration, paper antibiotic disks are placed on the inoculated agar surface. Plates are incubated for 16–24 h at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter.

Molecular diagnosis of *Escherichia coli*

Genomic DNA isolation

Wizard Genomic purification DNA kit from (PROMEGA, USA) provide fast and easy method for purification DNA for PCR test.

Agarose gel electrophoresis:

It is a method of [gel electrophoresis](#) used in [biochemistry](#), [molecular biology](#), and clinical chemistry to separate a mixed population of DNA or proteins in a matrix of [agarose](#) (Sambrook *et al.*, 2001).

PCR amplification:

Primer selection The primers were selected to detect several different gene for *E. coli*, It

gene for ETEC, *hly* gene for EIEC, *stx* gene for EHEC, *eae* and *bfp* gene for EPEC (Elsabeth *et al*, 2003), these primers create according to the manufacturer's instructions (Bioneer-Korea)

Specific genes, primers, and expected products for PCR assays for analysis of diarrheagenic *Escherichia coli* shown table (1).

PCR premix kit

It contains DNA polymerase, dNTPs, a tracking dye and reaction buffer in a premixed format, freeze-dried into a pellet. The patented chemical stabilizer of this product enables to maintain the activity of pre mixture for over a month even when stored at room temperature (25°C), over 2 years in the freezer.

PCR procedure:

The PCR consists of a series of 20-40 repeated temperature changes, called cycles, with each cycle commonly consisting of 2-3 discrete temperature steps, usually three. The cycling is often preceded by a single temperature step at a high temperature ($> 90^{\circ}\text{C}$), and followed by one hold at the end for final product extension or brief storage. The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters. These include the enzyme used for DNA synthesis, the concentration of divalent ions and dNTPs in the reaction, and the melting temperature of the primers (Rychlik *et al.*, 1990). These steps are: Initialization step, Denaturation step, Annealing step, Extension/elongation step, Final elongation, Final hold:

Results

Study sample was 102 subject: acute diarrhea 61 (59.8%), chronic diarrhea 21 (20.6%), and non-diarrheal 20 (19.6%).

Distribution of the study groups according to direct stool examination results.

Direct stool examination show that *Entamoeba H* found among 26 (31.7%) of the cases as compared with 3 (15%) of the control group, *Giardia* found among 5 (6.1%) of cases and non of the control group, this relation was statistically not significant, as shown in table 2.

Distribution of the study groups according to stool culture results.

Most common infection among cases was *E. coli* 28 (34.1%), followed by *Klebsiella* 17 (20.7%), while among controls was *Klebsiella* 10 (50%), followed by no growth 5 (2%), this relation was statistically not significant as shown in table 2, 3.

Distribution of the study groups according to *E. coli* infection and age

Among cases about 10 (35.7%) of the infected was from 1-12 months, among cases about 6 (21.4%) of the infected was from the age group 13-24 months versus 9 (16.7%) of the non-infected with *E. coli*, this relation was statistically not significant as shown in table 6. Among controls about 1 (50%) of the infected was from the age group 25-36 months versus 5 (27.8%) of the non-infected with *E. coli*, and 1 (50%) of the infected was from the age group 37-48 months versus 1 (5.6%) of the

non- infected with *E. coli* this relation was statistically not significant as shown in table 4.

Distribution of the study groups according to *E. coli* infection gender

Among cases about 18(64.3%) of the infected was female versus 20(37%) of the non- infected with *E. coli*, this relation was statistically significant as shown in table 7. Among controls about 1(50%) of the infected was female versus 6(33.3%) of the non- infected with *E. coli*, and 1(50%) of the infected male versus 12(66.7%) of the non- infected with *E. coli* this relation was statistically not significant as shown in table 5.

Distribution of the study groups according to *E. coli* infection and feeding

Among cases about 13(46.4%) of the infected was bottle feed versus 15(27.8%) of the non- infected with *E. coli*, this relation was statistically not significant as shown in table 8. Among controls 2 (100%) of the infected was mixed feed versus 7(38.9%) of the non- infected with *E. coli*, this relation was statistically not significant as shown in table 6.

Distribution of the study groups according to PCR results

Among cases ETEC was commonly detected 4(14.3%), followed by EPEC and EIEC 3(10.7%) for each one, while among the controls EIEC was detected 1(50%) and 1(50%) non- pathogenic *E. coli* was detected, this relation was statistically not significant as shown in table 7.

Sensitivity test results of the isolated *E. coli*

Cefotaxime was the most drug that the bacteria sensitive to 20(66.7%), followed by Amikacin 18(60%), while the organism was resistant to Amoxicillin 28(93.3%) and Ampicillin 27(90%), as shown in table 8

Discussion

Diarrheal diseases remain a leading cause of preventable death, especially among children under five years in developing countries.

Distribution of the study groups according to direct stool examination

The direct stool examination show that *Entamoeba histolytica* and *Giardia lamblia* in 28.4% and 4.9% respectively. *E. histolytica* was the most common enteric protozoan found in the present study 28.4% in cases and control groups, this result in close to reports from Erbil^(5, and 7), which was 39.1%, 39.7% and 6.4% respectively

Distribution of the study groups according to stool culture results

In stool culture result the most frequent isolated pathogen was *E. coli* 29.40% and *Klebsiella* 26.5%, this result is agreement with study in Chad⁽⁸⁾ which was the isolation rate for *E. coli* 34.3%, similar result reported in Iran^(9,10), and also in Senegal⁽¹¹⁾.

Distribution of the study groups according to *E. coli* infection and age

There was prevalence of diarrhea in early age group in children aged 0-2 years which was more than 50% as reported in Baghdad^(12,13)

Evaluation some virulence factor of *Escherichia coli* that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

Similar to Palestinian report⁽¹⁴⁾, our study showed that bacterial infection was significantly higher in children less than two years old.

Distribution of the study groups according to *E. coli* infection and gender

In this study a slight higher microbial infection rate were recorded among boys than girls 53.7% and 46.3%, this result agreed with other studies performed in Erbil⁽¹⁵⁾ and Saudi Arabia⁽¹⁶⁾, this slightly increasing may be due to boys more involved in out and indoor activities than girls.

no difference between male and female in infected with *E. coli*, that agreement with other studies done in Bagdad and India^(17,18).

Distribution of the study groups according to *E. coli* infection and feeding

In the present study, out of 30 infected cases and control with *E. coli*, about 15 of these cases and control with mixed feeding, these finding therefore corroborate findings from previous study in^(19,20).

Distribution of the study groups according to pathogenic *E. coli* and PCR results.

Results showed prevalence of pathogenic *E. coli* 11 (36.7 %) of the total 30 infected with *E. coli*, this result is similar with other reported in Oman⁽⁶⁾ and in Iraq⁽¹⁷⁾, India, Chile and Peru^(18,21,22).

Most common type of diarrheagenic *E. coli* incases was the ETEC with *elt* gene 4 (14.3%). This study is approximately similar

to those reported by Qadri⁽²³⁾, in which they found that the rate of ETEC was 18.5 and 18%, respectively.

The prevalence of EPEC in this study with *eae* gene was 3 (10.7%) among all patients. Result agreed with study in Iraq, 12.5% by Al-Kaissi⁽²⁴⁾ and 13 % by Tawfeek⁽²⁵⁾.

The prevalence of EIEC in this study with *ial* gene was 3 (10.7%) in cases and 1 in control, this result agreed with other study in Kenya⁽²⁶⁾.

Sensitivity test results of the isolated *E. coli*.

The treatment with antibiotics can lead to shorter disease period in children with diarrhea, in this study, most antibiotic resistance to *E. coli* was amoxicillin 93.3%, ampicillin 90.0 %, ofloxacin 76.7% and sensitive to amikacin 60.% and cefotaxime 66.7%. this study is similar to study done by Turkey in Anbar⁽²⁷⁾, which found that all isolation strains were sensitive to amikacin and imipenem antibiotics while it was found that all the isolation strains were resistance to ampicillin 83% , amoxicillin 97% .

Similarly to Mitra in Iran⁽²⁸⁾, which found that *E. coli* was resist 100% to ampicillin , 84.3% to cephalothin , and 74.5% to ceftriaxone .

References

1. Kosek, M., C. Bern, and R. L. Guerrant. 2003. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull. World Health Organ 81:197-204.

Evaluation some virulence factor of *Escherichia coli* that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

2. Mathers, C. D., A. D. Lopez, and C. J. L. Murray. 2007. The Burden of Disease and Mortality by Condition: Data, Methods and Results for 2001., p. 45-93. In A. D.Lopez, S. Begg, and E. Bos (eds.), Global Burden of Disease and Risk Factors. Oxford ,University Press, New York.
3. Guerrant, R. L., M. Kosek, A. A. Lima, B. Lomtz, and H. L. Guyatt. 2002. Updating the DALYs for diarrhoeal disease. Trends Parasitol. 18:191-193.
4. NATARO J.P., KAPER J.B. (1998). Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11, 142-201
5. Rabatti A.A., Rasheed N.E. Etiology of Bloody Diarrhea among Children Admitted to Maternity and Childrens Hospital-Erbil AL- Kindy Col Med J 2008; Vol .4(2):19-24
6. Ameen S.M., Darogha S.N., and Shikhani K.A., A study on the causative agents of diarrhoea in patients of maternity and pediatric hospital in Erbil, JDU, 1999; 2:397-400 .
7. Faraj.Ab ,Koyee.Qa. .Evaluation and Efficiency of Grape juice in diagnosing intestinal parasites .Zanco.J.Med.Sci .2007;11(2).
8. Bessimbaye N. , TidjaniA. , Moussa AM., et al .Gastroenteritis with *Escherichia coli* in pediatric hospital in N Djamena-chad. Journal of Applied Biology&Biotechnology2013 ;1(02:013-017).
9. Aslani MM. , Alikhani MY. Serotypes of Enteropathogenic *Escherichia coli* Isolated from Children Under 5 years of Age. Iranian J publ Health 2009;38(3):
10. Diouf S , Sarr M ,Sy H, Abdalahi OC , Fall M. Malnutrition and diarrhea in children at the University Hospital of Dakar.Clinical epidemiological and biological aspects .Med Afr Nre. 1990;3:29-32,
11. Sanou I, Kam KL, Tougouma A, Sangare L , Nikiema JHP. Acute diarrhea in children : epidemiological, clinical and developmental pediatric hospital in Ouagadougou . pediatric Research . 1999; 12http ://scialer.net/abstract ,doi=jms. 2008.371.377
12. Hasanain FG, Jamsiah M ,Zaleha MI, Azmi MT umil , Mohammed AA .Association between drinking water sources anddiarrhea with mulnutrition among kindergartens children in Baghdad city . Iraq. Malaysian Journal of Public Health Medicine 2012; 12(1);45-48.
13. UNESCO .Nutritional status survey under 5 years children in Baghdad .Iraq.2003. United Nations Children Fund. 2003.
14. Abu-Alhoof R.A.T.Diarrheagenic *Escherichia coli* Prevalence and related factors Among Children . Jenin Governorate An -Najah National University ,Palestine ,2013 Msc thesis.
15. Hamad N.R . and Ramzy I.A. Epidemiology of *Entameba histolytica* among children in Erbil.Ku Journal of Research in biology 2012 :1;057-062.
16. Alshammari S,Khoja T .El-Khwasky F. and Gad A. Intestinal parasitic diseases in Riyadh , Saudia Arabia: prevalence

Evaluation some virulence factor of Escherichia coli that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

sociodemographic and environmental associates. *Trop. Med IntHealth* 2001;6(3):184-189.

17. Tawfeek, H.I., Najim, N.H., Al-Mashikhi, S, Studies on diarrhoeal illness among hospitalized children under 5 years of age in Baghdad during 1990-97, *Eastern Mediterranean Health Journal*, 2002 Jan Vol 8 (1):181-8.

18. Bhan, MK, Bhandari, N, Sazawal, S, Clemens, J, Raj, P, Levine, MM, Kaper, JB, Descriptive epidemiology of persistent diarrhea among young children in rural northern India, *Bulletin of World Health Organization*, 1989;67(3):281-8.

19. Kebede D, Ketsela T, Asfaw W, Pattern of breast feeding in Western Ethiopia and their relationship to acute diarrhea in infants, *J. Paediatric* 1990;36:180-183.

20. Kandakai -Olukemi YT, Mawak JD, Ochai U, Olukemi MA, Isolation of *Areomonas* species from children with and without diarrhea in Jos, Nigeria *Afr. J. Exper. Microbiol* 2007;8(1):54-62.

21. Patel, P.K, Mercy, J, Shenoy, J, Ashwini, B, Factors associated with acute diarrhea in children in Dhahira-Oman: a hospital-based study, *Eastern Mediterranean Health Journal*, 2008 May- Jun;14(3):571-8.

22. Levine, MM, Ferreccio, C, Prado, V, Cayazzo, M, Abrego, P, Martine, J, Maggi, L, Baldini, MM, Martin, W, Maneval, D, et al, Epidemiologic studies of *Escherichia coli* diarrheal infections in low socioeconomic

level peri-urban community in Santiago, Chile, *National Library of Medicine*, 1993 Nov 15;138(10):849-69.

23. O. Theresa, E. Lucie, B. Francesca.....etc. Age-related susceptibility to infection with diarrheagenic *E. coli* in infants from peri-urban areas of Lima, Peru. *J Clinical infection disease*.2009 Dec 1;49(11) 1694-1702.

24. Qadri, M.H., M.H. Al-Ghamdi and M. Imadulhaq, Acute diarrheal disease in children under five years of age in the eastern province of Saudi Arabia. *Ann. Saudi Med.*,1990, 10(3): 280-284.

25. Al-Kaissi, E.N., M. M akki and M. Al-Khoja Etiology and epidemiology of severe infantile diarrhoea in Baghdad, Iraq. *Eur. J. Sci. Res*.2006, 14(3):

26. Tawfeek, H.I., N.H. Najim and S. Al-Mashikhi, 2002. Studies on diarrhoeal illness among hospitalized children under 5 years of age in Baghdad during 1990-1997. *Eastern Mediterranean Health J.*, 8(1): 12-19.

27. Turkey A.M, Abdul Rahman I. A, and Abd J.J. Multiple antibiotic resistance of enteropathogenic *Escherichia coli* which caused diarrhea for infant (Msc thesis), college of science, Al-Anbar University.201205(2).

28. Mitra M., Ahmad P., Hosein A., Ahmad K. Multiple drug resistance of enteropathogenic *Escherichia coli* isolated from children with diarrhea in Kashan, Iran. *African Journal of Microbiology Research* 2011;5(20):3305-3309.

Evaluation some virulence factor of *Escherichia coli* that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

Table (1) Specific genes, primers, and expected products for PCR assays for analysis of diarrheagenic *Escherichia coli*

Pathotype	specific gene	primers(5→3)	Product size
EPEC	eaeA	F: GAC CCG GCA CAA GCA TAA GC R: CCA CCT GCA GCA ACA AGA GG	384
ETEC	Li	F: GGC GAC AGA TTA TAC CGT GC R: CGG TCT CTA TAT TCC CTG TT	450
EHEC	Stx	F: CTG GAT TTA ATG TCG CAT AGT G R: AGA ACG CCC ACT GAG ATC ATC	150
EIEC	Ial	F: CTGGATGGTATGGTGAGG R: GGA GGC CAA TTA TTT CC	320

Table 2 Distribution of the study groups according to direct stool examination results.

Direct stool examination		Study groups		Total
		case	control	
	Entamoeba H	26 31.7%	3 15.0%	29 28.4%
	Giardia	5 6.1%	0 0.0%	5 4.9%
	Normal	51 62.2%	17 85.0%	68 66.7%
Total		82 100.0%	20 100.0%	102 100.0%

$\chi^2 = 4.05$, $df = 2$, $P \text{ value} > 0.05$ NS

Evaluation some virulence factor of *Escherichia coli* that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

Table 3 Distribution of the study groups according to stool culture results.

stool culture	Study groups		Total
	case	control	
Klebsila	17 20.70%	10 50.00%	27 26.50%
Proteus	9 11.00%	2 10.00%	11 10.80%
E. coli	28 34.10%	2 10.00%	30 29.40%
No growth	17 20.70%	5 25.00%	22 21.60%
Staph. aureus	3 3.70%	0 0.00%	3 2.90%
Shigella	1 1.20%	0 0.00%	1 1.00%
Pseudomonas	7 8.50%	1 5.00%	8 7.80%
Total	82 100.00%	20 100.00%	102 100.00%

$\chi^2 = 9.77$, $df=6$, $P \text{ value} > 0.05$ NS

Evaluation some virulence factor of *Escherichia coli* that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

Table 4 Distribution of the study groups according to *E. coli* infection and study groups and age.

Study groups			culture code		Total	X ² , P value
			Infected with <i>E. coli</i>	Not infected		
case	age in months	1-12	10 35.7%	21 38.9%	31 37.8%	1.3, > 0.05 NS
		13-24	6 21.4%	9 16.7%	15 18.3%	
		25-36	5 17.9%	13 24.1%	18 22.0%	
		37-48	4 14.3%	8 14.8%	12 14.6%	
		49-60	3 10.7%	3 5.6%	6 7.3%	
		Total	28 100.0%	54 100.0%	82 100.0%	
control	Age	1-12	0 .0%	3 16.7%	3 15.0%	5.2, > 0.05 NS
		13-24	0 .0%	5 27.8%	5 25.0%	
		25-36	1 50.0%	5 27.8%	6 30.0%	
		37-48	1 50.0%	1 5.6%	2 10.0%	
		49-60	0 .0%	4 22.2%	4 20.0%	
		Total	2 100.0%	18 100.0%	20 100.0%	

Evaluation some virulence factor of *Escherichia coli* that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

Table 5 Distribution of the study groups according to *E. coli* infection and study groups and gender

Study groups		culture code		Total	X ² , P value
		Infected with <i>E. coli</i>	Not infected		
case	Male	10 35.7%	34 63.0%	44 53.7%	5.5, < 0.05 S
	Female	18 64.3%	20 37.0%	38 46.3%	
	Total	28 100.0%	54 100.0%	82 100.0%	
control	Male	1 50.0%	12 66.7%	13 65.0%	0.2, > 0.05 NS
	Female	1 50.0%	6 33.3%	7 35.0%	
	Total	2 100.0%	18 100.0%	20 100.0%	

Evaluation some virulence factor of *Escherichia coli* that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

Table 6 Distribution of the study groups according to *E. coli* infection and study groups and feeding

Study groups			culture results		Total	X ² , P value
			Infected with <i>E. coli</i>	Not infected		
case	Feeding	Bf	4 14.3%	17 31.5%	21 25.6%	6.1, > 0.05 NS
		bottle	4 14.3%	14 25.9 %	18 22.0%	
		Mixed	13 46.4%	15 27.8%	28 34.1%	
		Solid	7 25.0%	8 14.8%	15 18.3%	
	Total		28 100.0%	54 100.0%	82 100.0%	
control	Feeding	bottle	0 0.0%	4 22.2%	4 20.0%	2.7, > 0.05 NS*
		Mixed	2 100.0%	7 38.9%	9 45.0%	
		Solid	0 .0%	7 38.9%	7 35.0%	
	Total		2 100.0%	18 100.0%	20 100.0%	

Evaluation some virulence factor of *Escherichia coli* that causes diarrhea in children by using polymerase chain reaction technique in kirkuk city

Table 7 Distribution of the study groups according to PCR results

PCR	Study groups				Total
	case	gene	control	gene	
EPEC	3	eae	0		3
	10.70%		0.00%		10.00%
ETEC	4	elt	0		4
	14.30%		0.00%		13.30%
EIEC	3	ial	1	ial	4
	10.70%		50.00%		13.30%
Negative	18		1		19
	64.30%		50.00%		63.30%
Total	28		2		30
	100.00%		100.00%		100.00%

Likelihood Ratio= 2.36,df=3,P value> 0.05

Table 8 Sensitivity test results of the isolated E coli

Antibiotic	Disk code	disk potency in Mcg	Zone size in mm	Resistant		Intermediate No. (%)		Sensitive	
				No.	(%)	No	(%)	No	(%)
Ciprofloxacin	cip	10	23	3	10.0	10	33.3	17	56.7
Ampicillin	AM	10	0	27	90.0	3	10.0	0	0.0
Ofloxacin	ofx	5	19	23	76.7	6	20.0	1	3.3
Amikacin	AK	10	16	1	3.3	11	36.7	18	60.0
Amoxicillin	AX	25	0	28	93.3	2	6.7	0	0.0
gentamycin	GN	10	14	2	6.7	18	60.0	10	33.3
Tobramycin	Tab	10	15	5	16.7	17	56.7	8	26.7
Cefotaxime	CTX	30	22	3	10.0	7	23.3	20	66.7