

Investigation serotype of Enter pathogenic Escherichia coli

Rawaa Abdulkhaleq Hussein, Walaa Najm Abood, Tamara Amer Taha

**Investigation serotype of Enter pathogenic Escherichia coli
prevalence among the causative agent of acute diarrhea among
children of Baghdad province**

**Rawaa Abdulkhaleq Hussein, Walaa Najm Abood, Tamara Amer
Taha**

**College of medicine/Diyala University/college of medicine/Diyala University/college of
basic education/ Diyala University**

Receiving Date: 12-04-2010 - Accept Date: 09-06-2010

Abstract

In this study two hundred seventeen children with acute diarrhea between Januarys to the end of July 2008 were involved. One hundred thirty were male (59.9%) and eighty seven female (40.1%) with (mean age $\pm$ SD)(35.15 $\pm$ 24.26)months. This study appear the high frequency of the rota virus (36.9%) Followed by Entamoeba histolytica was (28.6%), then diarrheagenic Enteropathogenic Escherichia coli (EPEC) (17.1%) and Salmonella was (11.5%) and the lowest frequency to Giardia lamblia was (5.9%). The important result in this study ,that limitation the serotypes of EPEC that cause acute diarrhea , 46% was EPEC serotype(O:86,O:119,O:127),27%was EPEC serotype (O:125,O:126,O:128) while EPEC serotype(O:114,O:124,O:142)was 27%,The highest occurrence of children diarrhea in age group between (14-42) months.

Key word: Rota virus, Entamoeba histolytica, EPEC, Salmonella, Giardia lamblia

1. Introduction

Diarrhea has many definition but in epidemiological studies it is defined as passage of three or more loose , that takes the shape of the container , watery stool in 24 hours period [1] .It is also can be defined as increased total daily stool output ,it is usually associated with increased stool water content . For infants and children this would result in stool output greater than 10g /kg/24h^[2].

Diarrhea is a leading cause of illness and death in young children would wide. More than 50% of the deaths of children under two year of age are a result of dehydration due to diarrhea. diarrhea and dehydration have been considered as the single biggest threat to child survival in developing countries today^[3]. Diarrhea leads to malnutrition , the malnutrition leads to reduced immunity leading to further episodes of diarrhea . It was found that those at particular risk of diarrhea are infants who are artificially feed and those who are at the weaning age (due to inadequate feeding and contaminated foods)^[1].

The incidence of persistent diarrhea is highest in the youngest age group especially in the first year of life and among low birth weight infants and those of low weigh for age. Persistent diarrhea carries a substantial diarrhea related morbidity and accounts for (36-54)% of diarrhea related death .^[4] More recent reviews indicate that although global mortality of children under five years has decreased but the incidence of diarrhea remains unchanged at more 3.2 episodes per child per year , indicating a continuing need to focus on prevention and management of diarrhea disease in children especially in developing countries.^[5] In Iraq ,diarrhea is considered to be an important cause for infant morbidity and mortality , bacterial diarrhea contributes to about one third of the total cases of acute diarrhea in infancy and early childhood^[6]. Finally the WHO estimated that the annual incidence of diarrhea in Iraq was six episodes per child per year^[7].

The most causative agent rota virus, *Giardia lamblia*, *Entamoeba histolytica*, *Salmonella*, *Shigella* and Enterpathgenic *Escherichia coli*. The species *E.coli* is serologically divided in serogroupes and serotype on the basis of its antigenic composition(somatic or O antigens for serogroups and flagelar or H antigens for serogroups). Many strains express a third class of antigens (capsular or k antigens) that although important in pathogenesis only occasionally are used in serotyping^[8].

Investigation serotype of Enter pathogenic *Escherichia coli*

The species comprise intestinal and extraintestinal pathogens. The intestinal pathogens are also known as diarrheagenic *E.coli* (DEC) of which six categories have been characterized :enteropathogenic *E.coli* (EPEC),enterohaemorrhagic *E.coli* (EHEC), enterotoxigenic *E.coli* (ETEC),enteroinvasive *E.coli* (EIEC), enteroaggregative *E.coli*(EAEC),and diffusely adhering *E.coli*(DAEC) ^[8].According to the World Health Organization *E.coli* O serogroups should be considered EPEC O serogroups : O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158(WHO1987). All strains were studied in regard to adhesion patterns, virulence genes implicated in the definition of the DEC category, and serotypes.

This study was carried out to determine the serotypes of EPEC that cause child diarrhea.

2. Material and methods:

3. Selection of patients:

This study was conducted among patients complaining of diarrhea who attended out patients clinics of Protecting Hospital for Pediatrics and Baghdad Teaching Hospital during the period from January to the end of July 2008. 217 patients from both sexes and with age range of 1-140 months from various socioeconomic statuses had been examined for detecting the causative agents of diarrhea in their stool by using direct examination, culture, biochemical and serological test. In addition to use API 20 E system ^[9].

The diagnosis of the cases was based on the presences of blood ,pus, and mucous , in addition to determine the color ,stool consistency and enteropathogens identification for each sample a detailed questionnaire was filled with all necessary information for each patient. A fresh stool specimens were collected from diarrhea patients and examined for intestinal parasites by saline and lugol' s iodine preparations Then, for bacteriological examination. Stool specimens were cultured on the differential and selective media MacConkey agar and Xylose Lysine Deoxycolate (XLD) media .These inoculated media were incubated for 24 hours at 37c. after that sub culture were made on the same media ,any non lactose fermented colonies from the first and second isolates were subjected to different biochemical tests.

In addition E system (API 20E) from bio Merieux-france was used for biochemical characterization of *E.coli* according to manufacturer instructions. *E.coli* was typed by direct slide agglutination technique in to four types (*E.coli* type1, *E.coli* type2, *E.coli* type3, *E.coli* type4) using specific trivalent antisera from Bio-RAD-france, contain (antibodies specific for

Investigation serotype of Enter pathogenic Escherichia coli

O111,O55,O26 antigens for *E.coli* type1 ; antibodies specific for O86,O119,O127 antigens for *E.coli* type2; antibodies specific for O125,O126,O128 antigens for *E.coli* type3; antibodies specific for O114,O124,O142 antigens for *E.coli* type4).

For virological examination, stool sample were analysed for group A rota virus using the rota kit for ELISA(DAKO Ltd,UK) ^[10]. This test is a qualitative enzyme immunoassay for the detection of group A rota virus in human fecal samples. This test utilizes a polyclonal antibody in a solid- phase sandwich enzyme immunoassay to detect group specific antigen present in group A rota virus .Fecal suspension is added to the micro well and incubated simultaneously with a rota virus specific polyclonal antibodies conjugated between antibodies on the solid phase and the enzyme-conjugated antibodies the wells were read by spectrophotometer at the wavelength of 450 nm.

4. Statistical analysis:

Data analysis was performed using SPSS for window TM version 11,and micro soft excel for windows 2007. The correlations between the groups done by Spearman's rho the significant at the 0.05 level.

5. Results:

In this study two hundred seventeen children with acute diarrhea were involved . one hundred thirty were male (59.9%) and eighty seven female (40.1%) with (mean age $\pm$ SD) (35.15 $\pm$ 24.26) months as shown in table (1).The distribution diarrheal causative agent in male more than in female as shown in table (2).

Table (1): general description of gender for patients that enrolled in the present study

Gender	Number (%)
Male	130 (59.9%)
Female	87 (40.1%)
total	217 (100%)

Investigation serotype of Enter pathogenic Escherichia coli

Table (2): distribution diarrheal causative agent with the gender

Gender	<i>Salmonella</i>	EPEC	<i>E.histolytica</i>	<i>G.lamblia</i>	Rota virus	Total
Male	16	23	33	9	49	130
Female	9	14	29	4	31	87
Total	25	37	62	13	80	217

In this study investigated the causative agent of acute diarrhea among children between January to July 2008, and the result appear the frequency of the causative agent was the rota virus get a high percentage (36.9%), then *E. histolytica* (28.6%), then Enteropathogenic *E.coli* (EPEC) (17.1%), the *Salmonella* was (11.5%), while the less percentage was to *G.lamblia* (5.9%) as shown in table (3).

Table (3): frequency distribution of the diarrhea causative agent

Diarrhea causative agent	Number (%) of cases
Rota virus	80 (36.9 %)
<i>E.histolytica</i>	62 (28.6 %)
EPEC	37 (17.1%)
<i>Salmonella</i>	25 (11.5%)
<i>G.lamblia</i>	13 (5.9 %)
total	217 (100%)

Table (4) shows the frequency distribution of the causative agent according to the age group , the results appear that the high infection with rota virus among age group between (1-14) months, while the frequency of infection to the other causative agent that participate in this study was a high among the age group between (15-56) months.

Investigation serotype of Enter pathogenic Escherichia coli

Table (4): distribution of the causative agent according to the age group/ month

Age group/m	salmonella	EPEC	<i>E.histolytica</i>	<i>G.lambliia</i>	Rota virus
1-14	1	11	16	2	57
15-28	3	13	14	1	12
29-42	6	1	15	5	4
43-56	6	0	11	1	2
57-70	2	4	1	2	2
71-84	3	5	3	2	1
85-98	1	0	1	0	0
99-112	1	0	0	0	0
113-126	2	2	0	0	0
127-140	0	1	1	0	2
Total	25	37	62	13	80

Table (5) show the distribution of serotype of EPEC type 1 was 0% , EPEC type 2was 46% , EPEC type 3 was 27% , EPEC type 4was27% .

Table (5): the distribution of serotype of EPEC

serotype	Number	Percentage
Type1	0	0%
Type2	17	46%
Type3	10	27%
Type4	10	27%
total	37	100%

This study shows that the higher distribution of the types of the EPCE with age group between(1-22) months table (6).

Investigation serotype of Enter pathogenic Escherichia coli

Table (6): show the distribution each type of EPCE with age /month

Age group/month	EPCE Type2	EPCE Type3	EPCE Type4	Total
1-22	9	5	5	19
23-44	2	2	2	6
45-66	2	1	1	4
67-88	2	1	2	5
89-110	2	0	0	2
111-132	0	1	0	1
Total	17	10	10	37

In table (7) show the correlation between the serotype of EPCE ,gender and age from the result that appear there was no significant correlation between the serotype of EPCE and each gender and age.

Table (7): show the correlation between the type of the EPCE ,gender and age

Correlation			TYPE OF EPCE	Gender	Age
Sperman's rho	TYPE OF EPCE	correlation coefficient	1.000	.105	.035
		Sig.(2-tailed)		.274	.717
		N	37	37	37
	Gender	correlation coefficient	.105	1.000	.079
		Sig.(2-tailed)	.274		.411
		N	37	37	37
	Age	correlation coefficient	.035	.079	1.000
		Sig.(2-tailed)	.717	.411	
		N	37	37	37

Correlation is significant at the 0.05 level(2-tailed)

6. Discussion:

The problem of diarrhea remains one of the most common illnesses of children and one of the major causes of infant and childhood mortality in developing countries in which approximately one billion episodes of illness and 3-5 billion deaths annually have been registered^[11]. This study revealed that from 217 children admitted to the hospital were suffering from diarrhea. In this study was found the majority of children diarrhea cases were male (59.9%) this finding is in agreement with the finding that had been studied in Baghdad that made constituted (79.8%)^[12]. This result might be attributed to the deeply rooted preference of many families for male children which motivates them for quick consultation for male ill children. However other studies showed that no sex difference in the occurrence of diarrhea was found as the risk factors associated with diarrhea are environmental and sociodemographic rather than biological factors^[13,14,15].

This study reveals that most frequent enter pathogen isolated was rotavirus constituting (36.9%) of the cases followed by *E. histolytica* (28.6%) then EPEC (17.1%) and *salmonella* (11.5%) while *G. lamblia* take lowest frequent (5.9%). These results to rotavirus are approximately higher than the percentage was observed in Iraq (23%) by Findukly^[16]. But this result is consistent with study which was done in Iraq (37%)^[17]. But, a higher percentage of rotavirus diarrhea cases were reported in Iraq (40%) by Abbass *et al.* 1988^[18]. This study showed that highest occurrence of children diarrhea in age group (14-42) months, and the higher frequency distribution of infection with rotavirus was (71.25%) in age group between (1-14) months. Rotavirus tends to spread in children younger than two years old, as older children may have sufficient immunity, these data were in keeping with those obtained from local studies^[12,17,19]. This result might be attributed to the time of introduction of solid foods and due to start of infant crawling with increased ingestion of contaminated materials especially in an unhygienic environment.

The percentage of other diarrheal pathogens that investigated in this study was higher than that recorded by Nabeel, (1986). EPEC was (6.67%) and *Salmonella* (3%)^[20]. While, in Arbil the prevalence rate of *G. lamblia* was (35%) and *E. histolytica* was (27%)^[21]. In Kerbala the prevalence rate of *G. lamblia* (9.9%) followed by *E. histolytica* (3.5%)^[22]. In Al-Najaf province, *G. lamblia* was the most common intestinal parasite detected with a rate of (17.5%) followed by *E. histolytica* (9.6%)^[23]. In Samarra province the prevalence rate

Investigation serotype of Enter pathogenic Escherichia coli

was found (36.7%) for *E. histolytica* and (20%) for *G. lamblia* ^[24]. In Baghdad in the year 2003 the prevalence rate of *E. histolytica* was (48.9%) , *G. lamblia* was (13.3%) and *Salmonella* was (1.5%) ^[25]. These results were approach to that found in the neighboring countries. In Al-Riyadh city the prevalence are of *G. lamblia* was (60.7%) and (7.4%) for *E. histolytica* ^[26] . But, in Turkey the prevalence rate of *G.lamblia* was (17.7%) and (2.6%) for *E. histolytica* ^[27]. Previous studies confirm the high prevalence of intestinal parasites with low socioeconomic conditions ,inferior sanitary and environmental conditions, and poor personal and community hygiene . all the above reasons ,become popular problem in 2006,2007and 2008 and that reason to spread the diarrheal diseases in Baghdad city .In addition to contamination of municipal water supplies with raw sewage or from inadequate water treatment system.

The importance of classical O-serogroup of Enteropathogenic *E.coli* (EPEC) was ignored through previous studies that focused on diarrial disease .The result of this study comes in agreement with that recorded by Rodrigues *etal*,(2002) ^[28].They stated that classical O-serogroup of EPEC(O:55,O:126,O:127,O:142,O:125) represent (11.86%) out of all diarreogenic *E.coli* (DEC) isolated from acute cases of diarrhea . Torres *etal*, (2001) ^[29] mentioned that *E.coli* pathgenic virotype especially EPEC were the microorganism most frequently associated with diarrhea of infants from low- income families admitted to the public pediatric hospital in Montevideo.In this study the most serogroup of EPEC(O:86,O:119,O:125,O:126,O:127,O:128) and PEC/EIEC(O:114,O:129,O:142) while there is no infected with serotype (O:111,O:55,O:26).In the previous studies they illustrated that the serotypes O:86,O:119,O:125,O:126,O:127,O:128 as EPEC and depended on the effecting the microorganism on infected cells. EPEC express characteristic adhesion patterns(localized, aggregative,and diffuse respictively) to epithelial cells and are thus referred to as enteroadherent *E.coli* ;EIEC is distinguished by the capacity to invade epithelial cells and the inability to produce toxins ^[30].EPEC was the first pathotype of *E.coli* to be described. Large outbreaks of infant diarrhea in United Kingdom Bray,in1945, to describe agroup of serologically distinct *E.coli* strains that were isolated from children with diarrhea but not from healthy children ^[31]. The hallmark of infections dueto EPEC is the attaching and effacing histopathology , which can be observed in intestinal biopsy specimens from patients or infected animals ^[32]. The bacteria intimately attach to intestinal epithelial cells and cause striking cytoskeletal changes, including the accumulation of polymerized actin directiy

Investigation serotype of Enter pathogenic Escherichia coli

beneath the adherent bacteria. The microvilli of the intestine are effaced and pedestal-like structures on which the bacteria perch frequently rise up from the epithelial cell^[28].

This ability is encoded by genes on a 35-kb called the locus of enterocyte effacement (LEE). The LEEs have a gene *eaeA* encoding a protein called intimin, which mediates the intimin attachment of EPEC to epithelial cell^[33,34]. EPEC also has other virulence factors such as a protein named lymphostatin, which inhibits lymphocyte activation^[35]. The most special feature of the epidemiology of disease due to EPEC infection is primarily a disease of infants younger than 2 years^[32,36]. In addition to watery diarrhea, vomiting and low grade fever are common symptoms of EPEC infection. As compared to the developing countries since it is the major cause of diarrhea. EPEC also cause diarrheal disease in many settings: nosocomial outbreaks, out patient clinics, patients admitted to hospitals, urban areas^[37,38].

In conclusion, our results show that: some serogroups such as EPEC (O:86, O:119, O:125, O:126, O:127, O:128) and EPEC/EIEC (O:114, O:129, O:142) are common in cause the diarrhea in children younger than 2 years and it is recommended to investigate the type of *E. coli* serotype to know the dangerous of the evolution the ill's case.

7. Reference

- [1] WHO. Reading on diarrhea :Student's manual ,WHO,Geneva,1992.3-11.
- [2] Ghishan, F.Chronic diarrhea . Beherman RE;KliegmanRM;JensenHB.Nelson text book of pediatrics . 2004.chapter 322.philadelphia.17th edition :p-1276.
- [3] Forsberg,BC;PetzoldMG; Tomson G; and Allebeck, P.Diarrhea case management in low and middle income countries . Bulletine of the WHO. 2007. 85: 42-48.
- [4] International working group on persistent diarrhea . Evaluation of an algorithm for the treatment of persistent diarrhea. A multicentre study . Bull WHO.1996. 74: 479-489.
- [5] Kosek M;Bern G;and Guerrant, R.L;The global burden of diarrhea disease as estimated from studies published between 1990 and 2000. Bull who .2003.81:197 – 204 .
- [6] Makkia M;Al-Tikriti S;and Al-Shahwani, Y. Diarrheal disease in infancy and early childhood . Iraqi Med J. 1978.26:27.
- [7] WHO. Thired inter country work shop on child healthy policy initiative , Tunisia. 2006. 10-13Dec.

Investigation serotype of Enter pathogenic *Escherichia coli*

- [8] Trabulsi LR, Keller R, and Gomes, T.A.T. Typical and atypical enteropathogenic *E.coli*. *emerg infec dis*. 2002. 8:508-513.
- [9] Finegold, S.M. and Martin, W.J. *Diagnostic microbiology*, 6th ed, C.V. Mosby Co. 1982.
- [10] Altindi, M.; Yavru, S; Simsek, A; and Rawitz, E. Rota virus infection in children with acute diarrhea as detected by latex agglutination, ELISA and polyacrylamide gel electrophoresis. *Indian pediatrics*. 2004. 41(17):590-594.
- [11] Qadri, SM; *Infectious diarrhea. Managing a misery that is still world wide*. *Postgraduate Med J*. 1990. 88(5): 169-75.
- [12] Al-Ani, Nafi. *Clinic-epidemiological study of diarrhea cases among infants Admitted to the children welfare teaching hospital during 15th Oct 2007 to 15th May 2008*. 2008. Thesis.
- [13] Manunabo, M.N. Influence of demographic, socioeconomic and environmental variables on childhood diarrhea in a rural area of Zaire. *J Trop Med and hygiene*. 1994. 97:31-8.
- [14] El-Zanaty, F.H; *Childhood illness and treatment, in Egypt demographic and health survey*. Egypt National population council and macro international inc. 2002. 141-7.
- [15] El-Gilany, A. H and Hammad S. Epidemiology of diarrheal disease among children under 5 years in Dakahlia, Egypt. *Eastern Mediterranean health J*. 2005. 11(4) : 765.
- [16] Findukly F.M; Al-Janabi, N.Y; and Al-Barazanji HR. Role of the human rotavirus in childhood diarrhea of hospitalized young children in Baghdad. *Iraq Medical J*. 1989. 38.
- [17] Arif, H and Hamody K. Chronic diarrhea in under 2 years aged children, a ten years retrospective analysis. *J faculty medicine Baghdad*. 2007. 49(2) : 205-210.
- [18] Abbas, N.I; Al-Hadithi TS; A.L-Attar A; Omer A.R; and Al-Obaidi S. Incidence of rotavirus gastroenteritis among infants and young children in Baghdad. *J Comm Med*. 1988. 1(1) : 39-45.
- [19] Tawfeek, H.T; Najim N.H and Al-Mashikhis. Studies on diarrhea illness among hospitalizes under 5 years of age in Baghdad during 1990- 1997. *Eastern Mediterranean health J*. 2002. 8(1) : 181-188.
- [20] Abbas, Nabeel Ibraheem. *Rota virus and acute infectious gastroenteritis in children under five years of age*. 1926. Thesis.

Investigation serotype of Enter pathogenic Escherichia coli

- [21] Mlan , Abdul L. and Farag , Abbas M. Prevalence of intestinal parasites in school children of Arbil , northen , Iraq. Saudi Medical Journal. 1989. 10(2): 107-110.
- [22] AL-Dujaili AA ; Naizi , AD and AL-Mosawi , AM. Prevalence of intestinal parasitic infection among primary school children in Kerbla 1993.Iraqi Medical Journal. 1997. 46 : 33.
- [23] AL-Kubaisy , W.A ; AL-Rubiae , MGand Salih , SS. Prevalence of intestinal parasitic infection among primary school children in AL-Najaf province. Iraqi medical J.2000. 49(4) : 70-74
- [24] Mohammed A.K; Nashwan N.A ;and Hatim M.A. A study on protozoal diarrhea in Samarra district .J Fac. Med. Baghdad. 2000. 42(4).
- [25] Saleem , Rand M. A ssociation between *Giardia lamblia* some gram negative bacterial diarrhea-2003. Thesis.
- [26] Qadri Hussain S.M and khalil S.H. Intestinal parasites : incidence and etiology in over 1,000 patients at king faisal apecialist hospital in Riyadh . Annal sof Saudi Medicine .1987. 7(7) : 207-211.
- [27] Celiksoz A ; Guler N ; Guler G ; Yasemin A.O and Serpil D. Prevalence of intestinal parasite in three socioeconomically-different regions of Sivas , Turkey. J Health popul. Nutr. 2005. 23(2) : 184-191.
- [28] Alvarez, F., C. E. Hormaeche, R. Demarco, C. Alí'a, and F. Schelotto. Clinical and bacteriological observations on acute diarrhea of hospitalized infants. Arch. Pediatr. Urug. 1974; 45:210–221.
- [29] Rivas M,Cacace L,Ayala A,Baschkier Eand Alvars O.,Cases of gastroenteritis associated with non O1vibrio cholerae in Oran ,salta.rev.argent.microbiol. 1996.28:163-169.
- [30] Rodrigues J, Thomazini CM, Morelli A, and Georgea CM. Reduced etiological role for entropathogenic *E.coli* in cases of diarrhea in Brazilian infants. J Clin microbiol.2004.42(1):398-400.
- [31] Kaper JB, JP Nataro and HL Mobley. Pathgenic *E.coli*. Nat Rev microbiol.2004.2:123-140.
- [32] Nataro JP, and JB Kaper. Diarrheagenic *E.coli*.Clin microbiol Rev.1998.11:142-201.
- [33] Jerse AE, YuJ, Tall BD,and Kaper JB. Genetic locus of enteropathogenic *E.coli* necessary for the production of attaching and effacing lesion on tissue culture cells .Proc Natal Acad Sci USA.1990.87:7839-7843.

Investigation serotype of Enter pathogenic Escherichia coli

- [34] Mcdaniel TK, Jarvis KG, Donnenberg MS and Kaper. A Genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proc Natl Acad Sci USA.1995.92:1664-1668.
- [35] Klapproth JM, Scaletsky IC, Mcnamara BP, Lai LC, and knight a. A large toxin from pathogenic *E.coli* strains that inhibits lymphocyte activation. Infec immun.2000.68:2148-2155.
- [36] Scaletsky IC, Pedroso MZ, and Fangundes U. Attaching and effacing enteropathogenic *E.coli*O18ab invades epithial cells and causes persistent diarrhea. Infec immun.1996.64:4876-4881.
- [37] Robin –browne RM, Bordun A, Tauschek M, Rosner H. *E.coli* and community acquired gasroentritis , Melbourne, Australia. Emerging infection Dis.2004.10(10):1797-1805.
- [38] Branth PV, Steinsland H, Fischer TK, Perch M, bore,k.. Cohort study of guinean children : incidence ,pathogenicity , conferred protection , and attributable risk for enteropathogens during the first 2 years of life. J of Clin Micr.2003.41(9):4238-4254.

