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Detection of Rotavirus A and *Escherichia coli* from Diarrhea Cases in Children and Coliphage Characterization

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A B S T R A C T

Acute gastroenteritis is a common disorder in young children. The purpose of this study was to comprehensive determination of main diarrheagenic pathotypes in children with acute gastroenteritis in the pediatric population in Basra city/Iraq, and characterization of *E.coli* phag. This study determined *Rotavirus* A and bacterial pathogens in 300 stool samples of children by using different techniques. In our study among children with gastroenteritis was 93/300 (31%) *Rotavirus* positive cases by Immunochromatographic (IC) test as monoinfection, coinfection, and mixing infections. Out of 50 IC positives fecal samples were tested using EM, 50(100%) were found positive. A total of 80 stools were examined for *Rotavirus* using polyacrylamide gel electrophoresis. The overall agreement was 68/80(85%). Out of 277/300 (92.33%) bacterial pathogens isolated, 163 (54.33%) children had infections with EPEC *Escherichia coli*, 39/300 (13%) cases with *Salmonella* spp., While, *Shigella* spp. was reported in 12/300 (4.01%) samples. Also parasitic causes were found in 6/300 (2%) samples**.** Coinfection with another pathogen was observed in 109/300 (36.34%) cases, coinfection with *Rotavirus* and EPEC *Escherichia coli* were the most common and occurred in 75/300 (25%). The phage ϕEC-MH1 was isolation successfully from sewage. The phage titer was determined by serial dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}) of the sample by counting the number of plaque forming units (p.f.u.) for each dilution. Our results revealed that dilution factor 10^{-2} was the best countable number of plaques. Effects of chloroform on phage titer during different times was completely inactivated, while sensitivity to saline environments was $3.0*10^{-4}$, $4.2*10^{-4}$, $4.2*10^{-4}$, $5.6*10^{-4}$, $6.0*10^{-4,}$ $6.7*10^{-4}$, $8.2*10^{-4}$, $8.0*10^{-4}$, and $8.4*10^{-4}$ during 5,10, 15, 20, 25, 30, 35 and 40 minutes. The statistical analysis was significantly decrease P≤ 0.05 in phage titer at the temperature 50C° and 65C° comparing with phage titer at the temperature 37C°. We concluded that *Rotavirus* A could be diagnosed in stool samples of children with gastroenteritis by IC test as a rapid technique. Rotavirus and EPEC *Escherichia coli* were the most common coinfectious agents responsible for gastroenteritis.

Keywords

Acute gastroenteritis, *Rotavirus, Escherichia coli, Coliphage.*

Article Info

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Introduction

Fecal pollution of water resources is a problem of increasing worldwide concern (Sauer, 2000). Acute gastroenteritis is a common disorder in young children, and the associated dehydration is a leading cause of admission to hospital in industrialized countries and a major source of mortality in developing countries (Parkin *et al*., 2009). Acute nonbacterial gastroenteritis is one of the most important infectious diseases that severely affects infants and young children (Liu *et al*., 2010). Group A rotavirus is a major pathogen of severe gastroenteritis in infants and young children worldwide (Parashar *et al*., 2006). The virus is transmitted through the fecal-oral route. There are seven species of this virus referred to as A,B,C.D,E,F and G with Rotavirus A being the most common species also identified as a major cause of dehydrating gastroenteritis in infants and young children (Armah *et al*., 2003). Rotaviruses belong to the family Reoviridae. The genome of rotavirus consists of 11 double helix molecules of RNA containing 18,555 base pairs. Each helix is a gene, numbered 1 to 11 by decreasing size. Each gene codes for one protein, except genes 9 and 11 codes for two. The RNA is surrounded by a three-layered icosahedral protein capsid. Viral particles are up to 76.5 nm in diameter and are not enveloped (Surendran, 2008). Each year, worldwide, rotavirus causes approximately 111 million episodes of gastroenteritis, 25 million that result in clinic visits and 2 million hospitalizations. Children in the poorest countries accounting for 82% of rotavirus deaths (Parashar *et al*., 2003). Traditionally, electron microscopy has been used to screen stool samples taken from suspected viral gastroenteritis patients (Atmar and Estes, 2001). Enteropathogenic *Escherichia coli* (EPEC) are a major cause of diarrhea amongst infants in developing countries (Gomes *et al.,* 1991; Mangia *et al.,* 1993). *Escherichia coli* were first isolated by Theodor Escherich in 1885 as *Bacterium coli commune*, which was isolated from the feces of healthy newborns (Berg, 2004). Bacteriophages are the most abundant entities on earth. These bacterial viruses have genetic material in the form of either DNA or RNA, encapsidated by a protein coat (Clark and March, 2006). Phages infect bacteria and can propagate in two possible ways; lytic life cycle and lysogenic life cycle. When phages multiply vegetatively they kill their hosts and the life cycle is referred to as lytic life cycle. On the other hand some phages known as temperate phages can grow vegetatively and can integrate their genome into host chromosome replicating with the host for many generations (Inal, 2003). For this global issue on public health, we undertook this study in order to find out the distribution of main diarrheagenic pathotypes; *Rotavirus* A and *E.coli* among hospitalized children with diarrhea in Basra city/Iraq, and determination of coinfections between these pathotypes. Also the first aim was to develop easily performable detection method for *Rotavirus* A infections, furthermore, characterization of *E.coli* phag.

Materials and Methods

Stool samples were collected between 15/11/2014 and 1/4/2015 from children 0 to 59 months of age who were hospitalized in Basra hospital for women and children, Basra/Iraq. A total of 300 children with acute gastroenteritis were enrolled, including 199 males and 101 females. The stool samples were collected in sterilized plastic container, transported under ice and stored at - 20 C till further processing. Approximately 10% (Wt/vol) suspension of stool specimens was prepared with distilled sterile water or phosphate - buffered saline

(PBS) and clarified by centrifugation at 2000g for 10 minutes twice (Kageyama *et al.,* 2003). Data on the clinical manifestations, such as age, gender and monthly distribution were analyzed.

General Stool Examination (GSE)

Stool specimens of the children were subjected to direct examination for *Entamoeba histolytica*, *Giardia lamblia* and other cysts or ova of parasites.

Detection of *Rotavirus* A

The samples were checked for group A *Rotavirus* by Immunochromatographic (IC) test, direct electron microscopy (EM) and polyacrylamide gel electrophoresis (PAGE).

Detection of Rotavirus by IC Test (one Step Rotavirus Test Device)

The one step rotavirus test device (Acon, Germany) is a rapid chromatographic immunoassay for the qualitative detection of rotavirus in human feces specimens to aid in the diagnosis of rotavirus infection. This test was performed according to the manufacturer's instructions.

Direct Electron Microscope (EM)

A 50 rotavirus-positive samples by IC test were confirmed by electron microscope (Zeiss supra 55vp, Germany) according to Bishop, *et al.,* 1974 with some modifications. About 10% of stool count was suspended PBS. Fecal suspension was clarified at 2000g for 10 min twice.For negative staining, after staining by 3% phosphotungistic acid, a drop of about 10 µl of the virus suspension to be studied was applied to surface of a Petri dish, after drying, the grid was immediately coated with it.

Polyacrylamide Gel Electrophoresis (PAGE)

PAGE was carried out on the faecal suspensions for 80 Rotaviruses positive by IC using a standard method which includes extraction of RNA genome according to the Exprep™Plus Viral DNA/RNA Kits (Bioneer, Korea) by using automated extraction (Automated Nucleic Acid Extraction System, Bioneer, Korea) according to the manufacturer's instructions. The RNA was subsequently electrophoresed in 10% acrylamide gels for 6 - 8 h. at 100 V at room temperature and segments were visualized by Ethidium bromide staining according to the method of Herring *et al.,* 1982 with some modifications.

Isolation and Identification of Bacterial Species

Standard microbiology laboratory techniques were used to isolate and identify *Escherichia coli*, *Shigella* sp. and *Salmonella* sp. from stool samples in MacConkey agar (Salucea, The Netherland) and X.L.D agar (LabM Limited, UK) as previously described (Forbes *et al*., 1998; Collee *et al*., 1996; Gupta, 1995). The identification of bacterial pathogens was later confirmed by routine bacteriological, biochemical assays and api 20 Enterobacteraceae system. *Escherichia coli* were tested for antimicrobial susceptibility onto Muller-Hinton agar (Salucea,The Netherland) according Baur *et al*. (1966) by the standard disk diffusion method, using commercially prepared antibiotic disks containing Impenien (IMP), Trimethoprim (TMP), Ampicillin (AM), Ceftriaxone (CRO) and Doxycyclin (DO). Accordingly, the size of inhibition zone determines whether isolated bacteria were resistant, intermediate, or sensitive.

Bacteriophage Isolation and Purification

A 200 ml sewage samples for phage isolation were obtained from Al-Sadar hospital in Basra/Iraq according to Sambrook *et al.* 2001. The host range of phages was determined by the spot test. The phage titer was determined by serial dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}) of the sample and then plated 0.1 ml each of the dilution and the *E.coli* culture onto NA plates. The plates were incubated overnight at 37 ºC and examined for the presence of plaques. The phage titer was determined by counting the number of plaque forming units (p.f.u.) for each dilution. At this stage choosing dilution factor in which gave the best countable number of plaques for using in further experiments including temperature, chloroform and saline sensitivity.

Statistical Analysis

Analysis of the data obtained was made by using SSPS software. Data of the present study was analyzed by chi square test, P values ≤ 0.05 were considered statistically significant. Calculation of mean values and standard deviation (SD) were made for some clinical manifestations.

Results and Discussion

Characterization of Study Population

The characteristics of the children whose stool samples showed positive results for *Rotavirus* and bacterial pathogens were summarized in Table 1 and 2. Age of the 300 recruited children ranged from 0 to 59 month (mean ± standard deviation: 11.02±12.3 months) and their male/female ratio was 199(66.33%):101(33.67%). So present study showed the males were more susceptible to the infection with significantly elevated value (P< 0.001) than females. Boys are twice as likely as girls to be admitted to hospital (Rheingans *et al.*, 2006). Males are more frequently affected than females (Broor and Singh, 1984). The age period between (0-5) and (6 -11) month was significantly prone (34% and 36%, respectively) to the infection (P< 0.001). In contrast, the age period (24-59) month was significantly decreased (7.34%) to the infection (Table, 1). Almost every child has been infected with rotavirus by age five (Parashar *et al.*, 2006). Because at this age feeding starts and child put things in his mouth. Rotavirus affects 95% of all children by the age of 5 years. Infection rates for rotavirus are highest in the under 5-year old age group and decrease progressively towards adulthood as immunity acquired in childhood protects most adults(Parashar *et al.*1998). Regarding clinical manifestations, all diarrheagenic pathotypes (Table, 2) were complained with diarrhea 300/300(100%), Vomiting 129/300(43%), dehydration 8/300(2.67%) and fever 247/300 (82.34%) with highly significant differences (P< 0.001). Symptoms include a profuse watery diarrhea, vomiting, abdominal pain and possibly fever and severe cases may lead to death, mainly through acute dehydration (Diggle, 2007). Within study population, during 15/11/2014 and 1/4/2015, present study shows diarrheal children cases in all months and become increased in December 96/300 (32%), January 60/300 (20%), March 56/300 (18.67%), February 50/300 (16.66%) and November 38/300 (12.67%) with significant differences (P≤ 0.05). Rotavirus infections rates vary seasonally with the majority of cases in temperate climates occurring in the winter months between November and February (Gleizes *et al.*, 2006). In tropical and developing countries this seasonality is less marked, and infection occur year-round (Parashar *et al.*, 1998). Overall, diarrheagenic pathotype

cases become increased from December to January.

Rotavirus Detection

IC Test

With an increasing number of reports on *Rotavirus* and an estimated increase in the number of patients of *Rotavirus* infection, the demand for rapid diagnosis of this infectious disease is dramatically expanding. IC has been developed as a rapid diagnostic test, ELISA although it still takes more than 4 hours to obtain the results. In this study, a simple, easy-to-read, and rapid detection test for *Rotavirus* using an IC membrane strip was developed. This method took a shorter time; approximately 30 minutes to complete the assay with limited equipment needed such as centrifuge machines and micropipettes. Based on the results in the current study by IC test, rotaviruses were detected in 93/300 (31%) samples (Figure, 1; Table, 1).

Immunochromatographic test is one of the representative methods in rapid diagnosis, and it is widely used to detect various infectious diseases, such as influenza virus, rotavirus, and adenovirus (Fujimoto *et al*., 2004; Hara, 2002, Tsutsumi *et al*., 1999, Bon *et al*., 2007, Hara *et al*., 2006). The IC can theoretically detect 1/100 to 1/10 of the viral load found in clinical samples, which is almost equivalent to the detection power of electron microscopy (Atmar and Estes, 2001). Therefore it may be justified to use IC for screening the stool samples.

PAGE

Electron microscopy and polyacrylamide gel electrophoresis are also used to determine the virus (Beards, 1988). Basic evaluation was performed by comparison of the results of IC with those obtained by EM and on the results of PAGE.A photograph of electrophoresis for samples shown in Figure 2. A total of 80 stools were examined for *Rotavirus* using polyacrylamide gel electrophoresis. The overall agreement was 68/80(85%) with no significant differences. The occurrence of rotavirus-positive samples that yielded negative results by PAGE was possibly due to an insufficient RNA concentration. The rotavirus RNA segments which are different in size are separated by polyacrylamide gel electrophoresis and are observed as RNA pattern after staining of the RNA in gel. The RNA patterns are distinct among different rotavirus species and also different strains (Kobayashi *et al*., 2007). Studies on the electrophoretic migration patterns of viral genomic dsRNA segments (electropherotyping) have allowed the classification of rotaviruses into two major groups, the long (L) and the short (S) electropherotypes (Kapikian *et al*., 2001).

Direct EM

IC test for group A rotavirus could be used as an alternative rapid detection method, then were confirmed by electron microscopy. A total of 50 IC positives fecal samples were tested using EM. A 50(100%) were found positive and showed the characteristic morphology of rotavirus of wheel- like appearance of rotavirus particles (Figure, 3). Rotavirus is shed in high concentration in the stool (~10^{12} viruses/g) of children with gastroenteritis (Surendran, 2008), and thus can be easily identified on electron microscopy of stool samples which is one of the most specific tests for diagnosis. The method is also useful in evaluating the sensitivity and specificity of commercial virus detection kits (Curry *et al*., 2006).

Table.1 The Age Distribution of Study Population

Pathotypes /300	Age/ month				
	0-5	**6-11**	**12-17**	**18-23**	**24-59**
Monoinfections					
Rotavirus	4	1	0	0	0
Escherichia coli	61	55	28	9	10
Salmonella spp.	4	0	0	2	0
Shigella spp.	2	0	0	0	0
Entamoeba histolytica	0	0	1	0	2
Giardia lamblia	0	1	0	2	0
Total	71	57	29	13	12
Coinfections					
Rotavirus + *E. coli*	27	30	6	8	4
Rotavirus + *Salmonella* spp.	0	2	0	0	0
Rotavirus + *Shigella* spp.	0	1	1	0	0
E. coli i + *Salmonella* spp.	0	11	4	3	5
E. coli + *Shigella* spp.	0	3	2	1	1
Total	27	47	13	12	10
Mixing infections					
Rotavirus + *E. coli* + *Salmonella* spp.	4	3	1	0	0
Rotavirus + *E. coli* + *Shigella* spp.	0	1	0	0	0
Total	4	4	1	0	0
Final total (%)	102 (34%)	108 (36%)	43 (14.33%)	25 (8.33%)	22 (7.34%)
P ≤0.05					

Table.2 Clinical Information of Positive Cases

Pathotypes /300	Sign and symptom			
	Diarrhea	**Vomiting**	**Dehydration**	**Fever**
Monoinfections				
Rotavirus	5	5	0	1
Escherichia coli	163	27	2	151
Salmonella spp.	6	1	0	6
Shigella spp.	2	1	0	2
Entamoeba histolytica	3	3	0	3
Giardia lamblia	3	3	0	3
Total	182	40	2	166
Coinfections				
Rotavirus + *E. coli*	75	68	0	47
Rotavirus + *Salmonella* spp.	2	1	0	1
Rotavirus + *Shigella* spp.	2	1	0	0
E. coli i + *Salmonella* spp.	23	5	1	19
E. coli + *Shigella* spp.	7	7	4	7
Total	109	82	5	74
Mixing infections				
Rotavirus + *E. coli* + *Salmonella* spp.	8	7	1	6
Rotavirus + *E. coli* + *Shigella* spp.	1	0	0	1
Total	9	7	1	7
Final total (%)	300 (100%)	129 (43%)	8 (2.67%)	247 (82.34%)
P ≤0.05				

Table.3 Monoinfections, Coinfections and Mixing Infections of Positive Cases

Pathotypes /300	Monoinfections	Coinfections	Mixing infections
Rotavirus	**5(1.66%)**	***Rotavirus* + *E. coli*** **75(25%)** ***Rotavirus* + *Salmonella* spp.** **2(0.67%)** ***Rotavirus* + *Shigella* spp.** **2(0.67%)** ***E. coli i* + *Salmonella* spp.** **23(7.67%)** ***E. coli* + *Shigella* spp.** **7(2.33%)**	***Rotavirus* + *E. coli* + *Salmonella* spp.** **8(2.66%)** ***Rotavirus* + *E. coli* + *Shigella* spp.** **1(0.34%)**
Escherichia coli	**163(54.33%)**		
***Salmonella* spp.**	**6(2%)**		
***Shigella* spp.**	**2(0.67%)**		
Entamoeba histolytica	**3(1%)**		
Giardia lamblia	**3(1%)**		
Total	**182(60.66%)**	**109(36.34%)**	**9(3%)**
P ≤0.05			

Table.4 Antibiotic Susceptibility Test

Antibiotic	R %	S%
Impenien (IMP)	**0**	**100**
Trimethoprim (TMP)	**100**	**0**
Ampicillin (AM)	**100**	**0**
Ceftriaxone (CRO)	**76.66**	**23.34**
Doxycyclin (DO)	**100**	**0**

Table.5 Determination of Phage Titer

Plate	Volume of Phage Plated (ml)	Dilution	Dilution factor (DF)	Plague Per Plate	Titer =Plague * DF\ Volume of Phage Plated(ml)	
1	0.1	10^{-1}	10^{-1}	80	$80*10^{1}/0.1$	$800*10^{1}$
2	0.1	10^{-1}	10^{-2}	85	$85*10^{2}/0.1$	$850*10^{2}$
3	0.1	10^{-1}	10^{-3}	76	$76*10^{3}/0.1$	$760*10^{3}$
4	0.1	10^{-1}	10^{-4}	56	$56*10^{4}/0.1$	$560*10^{4}$
5	0.1	10^{-1}	10^{-5}	32	$32*10^{5}/0.1$	$320*10^{5}$
6	0.1	10^{-1}	10^{-6}	27	$27*10^{6}/0.1$	$270*10^{6}$
7	0.1	10^{-1}	10^{-7}	4	$4*10^{7}/0.1$	$40*10^{7}$
8	0.1	10^{-1}	10^{-8}	2	$2*10^{8}/0.1$	$20*10^{8}$
9	0.1	10^{-1}	10^{-9}	0	$0*10^{9}/0.1$	$0*10^{9}$
P ≤0.05						

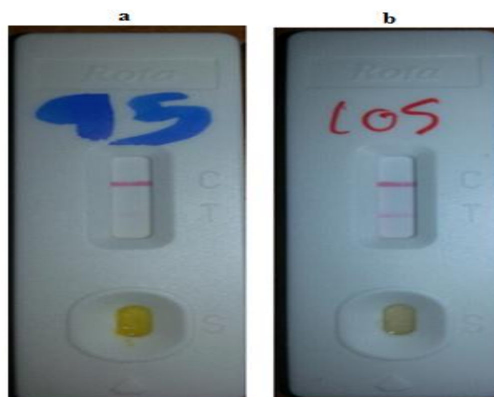
Table.6 Chloroform and Saline Sensitivity of Phage

Time (min.)	Volume of Phage Plated (ml)	Dilution factor (DF)	Titer : Plaque forming unit (PFU)	
			Chloroform	Saline
5	0.1	10^2	0	$3.0*10^{-4}$
10	0.1	10^2	0	$4.2*10^{-4}$
15	0.1	10^2	0	$5.6*10^{-4}$
20	0.1	10^2	0	$6.0*10^{-4}$
25	0.1	10^2	0	$6.7*10^{-4}$
30	0.1	10^2	0	$8.2*10^{-4}$
35	0.1	10^2	0	$8.0*10^{-4}$
40	0.1	10^2	0	$8.4*10^{-4}$
$P \leq 0.05$				

Table.7 Phage Titer in Relation to Temperature (37 C°, 50 C° and 65 C°)

Time (min.)	Volume of phage plated (ml)	DF	Titer : PFU		
			37 C°	50 C°	65 C°
10	0.1	10^2	$2.3*10^{-4}$	$5.4*10^{-4}$	$3.2*10^{-4}$
20	0.1	10^2	$3.0*10^{-4}$	$3.2*10^{-4}$	$2.0*10^{-4}$
30	0.1	10^2	$5.6*10^{-4}$	$2.0*10^{-4}$	0
40	0.1	10^2	$5.4*10^{-4}$	$1.3*10^{-4}$	0
50	0.1	10^2	$7.3*10^{-4}$	$1.2*10^{-4}$	0
60	0.1	10^2	$8.9*10^{-4}$	$1.0*10^{-4}$	0
Means			$5.41*10^{-4}$	$2.35*10^{-4}$	$0.86*10^{-4}$
Std.Deviation			$1.39*10^{-4}$	$1.69*10^{-4}$	$1.06*10^{-4}$
Minimum			2.3*10-4	$1.0*10^{-4}$	0
Maximum			8.9*10-4	$5.4*10^{-4}$	$3.2*10^{-4}$
$P \leq 0.05$					

Figure.1 Photograph of the IC for *Rotavirus*



(a) Negative sample; (b) positive sample

Figure.2 Polyacrylamide Gel Electrophoresis of dsRNA *Rotavirus*

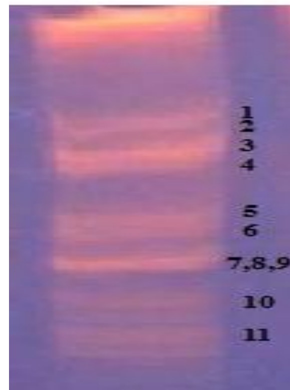
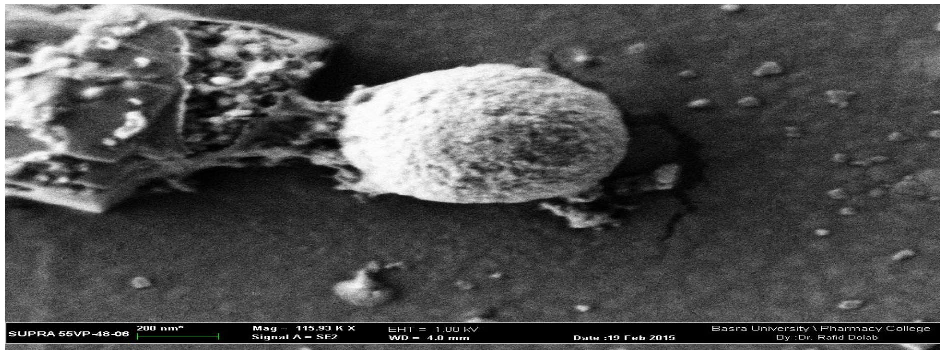
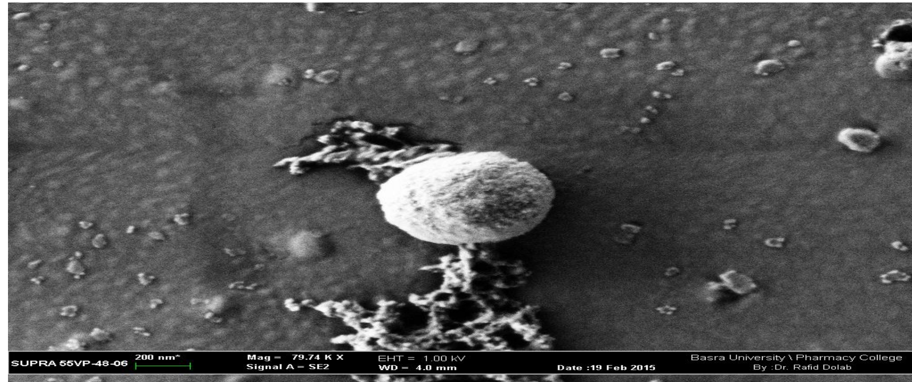


Figure.3 Rotavirus Particles Stained with Negative Staining under EM





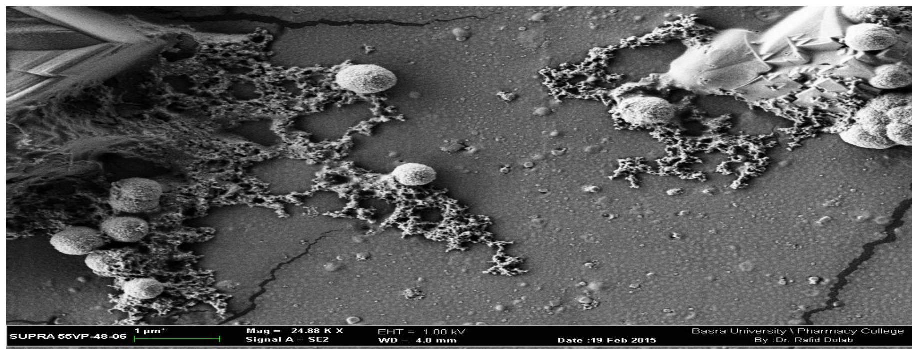


Figure.4 Api 20 Enterobacteraceae System for *E. coli*

Figure.5 Spot Test of Phage

Figure.6 Plaques caused by Bacteriophage ϕEC-MH1

Detection rates of Infectious Agents

As shown in Table 3, in monoinfection 182(60.66%) cases, viral agents (rotaviruses) were detected in 5/300 (1.66%) samples, while the bacterial pathogens were found in 171/300 (57%) samples, also parasitic causes were found in 6/300 (2%) samples, in addition, coinfections and mixing infections were detected in 118/300(39.34%) samples, with highly significant differences (P< 0.001). Out of 93/300 (31%) children had infections with *Rotavirus*, 5 (1.66%) cases had monoinfection, 79(26.34%) coinfection, and 9(3%) mixing infections. Regarding bacterial pathogens, 277/300 (92.33%) children had infections with EPEC *Escherichia coli*: 163 (54.33%), 105(35%) and 9(3%) children had monoinfections, coinfection and mixing infections, respectively. Furthermore, among 39/300 (13%) cases with *Salmonella* spp., 6(2%) cases had monoinfection, 25(8.34%) cases had coinfection and 8(2.66%) cases had mixing infection. While, *Shigella* spp. was reported in 12/300 (4.01%) samples, 2(0.67%), 9(3%) and 1(0.34%) children had monoinfections, coinfection and mixing infections, respectively. Furthermore, in parasitic pathogens, *Entamoeba histolytica* and *Giardia lamblia* were found in 3/300 (1%) samples as monoinfection cases (Table, 3).

Morbidity and mortality rates caused by diarrhea in developing countries remain high despite efforts to improve sanitary conditions, water quality, and the healthcare infrastructure (Sánchez-Fauquier *et al.*, 2006). Rotavirus is ubiquitously distributed to humans and animals. Rotavirus has been recognized as a cause of infantile diarrhea since 1970s, and is now established as the most common cause of gastroenteritis in infants and young children (Kobayashi *et al.*, 2007). After entrance orally into gastrointestinal tract, propagation of rotavirus occurs in epithelial cells of villi of small intestine. Cell lysis occurs finally by the viral propagation, causing curtailment of the villi. Diarrhea due to rotavirus infection is considered to be caused by some different mechanisms (Ramig, 2004). Transmitted by the fecal-oral route, rotavirus infects cells that line the small intestine producing an enterotoxin (NSP4) that induces gastroenteritis (Diggle, 2007). Although good hygiene measures can help prevent spread of the disease, the robustness of rotavirus and the low infectious dose (10–100 virus particles), makes standard sanitary measures to halt transmission of the virus relatively ineffective (Gray, 2011). The extended programme on immunization was initiated in Iraq in 2012. Rotavirus vaccine was introduced as a result of the increasing mortality and morbidity associated with acute gastroenteritis. All 3 doses of vaccine

are required for maximum protection (Vesikari *et al*., 2006). In addition, although there has been a downward trend in the number of cases of gastroenteritis caused by bacteria and parasites in young children over the last ten years, the proportion of gastroenteritis cases due to viruses, and to rotavirus in particular, has remained stable (Iturriza-Gomara *et al*., 2008). In our study, coinfection with another pathogen was observed in 109/300 (36.34%) cases (Table, 3), coinfection with *Rotavirus* and EPEC *Escherichia coli* were the most common and occurred in 75/300 (25%). Overall, 93/300(31%) showed positive results for rotavirus. These results were lower than previous findings on rotavirus prevalence (51.98%) in Najaf governorate (Al-Kelaby, 2008). Al-Ameen *et al*., 2012 study showed the positive cases of rotavirus to total samples of diarrhea were 278 (39.66 %) in Basra Province/Iraq from 2008-2011. Also Thwiny, 2013 showed that group A rotavirus, sapovirus, norovirus, astrovirus and adenovirus were detected in 40.5%, 21.5%, 8%, 2.5% and 2.5% of the study population in Basra Province/Iraq, respectively.

Furthermore, this was lower than the prevalence of rotavirus attained in Syria (61%) (Teleb, 2008), Oman (50%) (Al-Awaidy *et al*., 2009) and Kuwait (44%) (Marmash *et al*., 2007). Otherwise, Lee *et al*., 2007 studied the etiologic agents in 962 Korean children hospitalized with gastroenteritis that rotavirus, norovirus, adenovirus and astrovirus were detected in 25.7%, 13.7%, 3.0%, and 1.1% of the study population, respectively. These different detection rates may be explained by different conditions of the studies, such as the season of sampling and the sampling methods, also because rotaviral infection rates can vary both over time and geographically within the same country.

Bacterial Pathogens Isolation and Antimicrobial Susceptibility Test

The bacterial pathogens were isolated from 289(96.34%) children with acute diarrhea including: EPEC *Escherichia coli*, *Salmonella* spp. and *Shigella* spp. according to routine bacteriological and biochemical assays that were later confirmed by api 20 Enterobacteraceae system (Figure, 4). Antibiotic susceptibility testing was performed on Muller Hinton agar against five different antibiotics to 30 isolates of EPEC *Escherichia coli*. The antibiotic resistance/susceptibility profile of EPEC *E.coli* isolates revealed that most of the isolates were resistant to three tested antibiotics (Table, 4).

Coliphage Characterization

The phage was isolation successfully from sewage. The host range of phage was determined against four isolates of EPEC *E. coli*; the phage was showed lytic activity against all isolates (Figure, 5). The phage was named ϕEC-MH1, and then it was selected for further experiments. Number of p.f.u. for each dilution (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}), was $800*10^{1}$, $850*10^{2}$, $760*10^{3}$, $560*10^{4}$, $320*10^{5}$, $270*10^{6}$, $40*10^{7}$, $20*10^{8}$, $0*10^{9}$, respectively(Table, 5; Figure, 6). This result revealed that dilution factor 10^{-2} was the best countable number of plaques. Table (6) showed Chloroform and saline sensitivity of phage. Chloroform sensitivity on number of plaques and phage titer during different time was 0, which revealed to complete inactivated of phage. Sensitivity of phage titer to saline environments (Min. - Max.; mean ± standard deviation: $3.0*10^{-4}$ - $8.4*10^{-4}$; $6.26*10^{-4}\pm1.96*10^{-4}$) was $3.0*10^{-4}$, $4.2*10^{-4}$, $4.2*10^{-4}$, $5.6*10^{-4}$, $6.0*10^{-4,}$ $6.7*10^{-4}$, $8.2*10^{-4}$, $8.0*10^{-4}$, and $8.4*10^{-4}$ during 5,10, 15, 20, 25, 30, 35 and 40

minutes respectively. Also by using different time 10, 20,30,40,50, and 60 minutes and different temperature 37 C° (as control group), 50 C° and 65 C°, temperature sensitivity effect on phage titer was $2.3*10^{-4}$, $3.0*10^{-4}$, $5.6*10^{-4}$, $5.4*10^{-4}$, $7.3*10^{-4}$, $8.9*10^{-4}$ and $5.4*10^{-4}$, $3.2*10^{-4}$, $2.0*10^{-4}$, $1.3*10^{-4}$, $1.2*10^{-4}$, $1.0*10^{-4}$ and $3.2*10^{-4}$, $2.0*10^{-4}$, 0, 0, 0, 0, respectively (Table, 7).

Bacteriophages have very effective bactericidal activity and several advantages over other antimicrobial agents. Most notably, phages replicate at the expense of infectious bacteria, are available in abundance where they are most required, and so far, no serious or irreversible side effects of phage therapy have been described (Sulakvelidze and Kutter, 2005). If bacteria become resistant to phages then phages do evolve naturally to infect the aforementioned resistant bacteria, hence minimizing the chances of bacterial escape, which scores another advantage of phage over antibiotics (Hausler, 2007). At the moment it seems a bit far that phage therapy will replace antibiotics exclusively, but there is the hope that it will be used complementary to antibiotics especially for antibiotic resistant strains (Clark and March, 2006).

In conclusion, this study added several data to the knowledge on the epidemiology of main diarrheagenic pathotypes infections in one of the country's regions. In our study the use of different tests would allow monitoring of the diversity of circulating rotavirus strains. We found that rotaviruses easy detected because the viruses shedding in large quantities and has a circular shape size of 65-70 nm ranges. Rotavirus A could be diagnosed in stool samples of children with gastroenteritis by IC test as a rapid technique. Negative staining EM was a valuable technique to monitor the presence rotaviruses infection. *Rotavirus* and EPEC *Escherichia coli* were the most common coinfectious agents responsible for gastroenteritis. Children between an age of 0 and 11 months were at greatest risk for developing severe disease from diarrheagenic pathotypes infection. The phage was showed lytic activity against all EPEC *Escherichia coli* isolates.

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