

Serotype and Virulence Gene of *E.coli* and Associated with Rota Virus Isolated from Patients with Diarrhea Infection

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Abstract

Diarrheal infection are a sever public health problem and a major causes of mortality of children . *E.coli* is one the most important microbial agent of diarrhea. Rotavirus is the most common causes of sever gastroenteritis and diarrhea in infant and young children in world. Seventy-five diarrheal samples was isolated from children have sign and symptoms as diarrhea, vomiting abdominal pain and dehydration. Samples were isolated from Al-sadder hospital/pediatric department from January to May 2020. In material and methods was been used bacteriological culture media, biochemical test, Gram stain and Vitek-2 to detect *E.coli* in 37C° for 24 hr . For detection of Rota virus use Enzyme linked immunosorbent assay. Cav C gene and Tra t gene are a virulence factors gene in *E.coli* were detected by DNA extraction and Conventional PCR .In the results age groups and sex distribution with diarrheal infection male was 41 (54.7%) and female 34 (45.3%) regarding to sex distribution with *E.coli* and Rota virus male is more effective with *E.coli* 17 (41.5%) and female 20 (58.8%) but Rota virus associated with diarrheal and male was recorded 12 (29.3%) and female (11.82%). *E.coli* was enrolled 37 (49.34%), Rota virus 23 (30.7%) and mix infection 16 (20%) Based on types of feeding bottle feeding was recorded a high rate (40%) which causes diseases. A virulence (gene tra T and cva C) gene in *E.coli* was detected.

Keywords: Serotype of *E.coli* • *E.coli* • Rotavirus • Virulence genes

Description

Diarrheal infection that causes death in age groups in children between (1-5) years old. About 760,000 of children which was dead every year [1]. Some microorganism that causes diarrhea in the world as Escherichia coli Enterhemorrhagic *E.coli* and Rota virus [2]. Escherichia coli is a normal flora of human in large in human and warm blooded animals, some strain are pathogenic and some strain of *E.coli* transfer plasmid DNA responsible for enterotoxin or invasive factors. *E.coli* is responsible about neonatal diarrheal infection, neonate meningitis bacteremia hemolytic uremic syndrome [3]. Escherichia coli is a bacillus or red, gram negative related to Enterobacteriaceae family, facultative anaerobic and spore forming. *E.coli* has capsular Antigen (K-Antigen) or Somatic Antigen (O-Antigen), these causes (40%) of cases septicemia and (80%) of cases meningitis (3). Diarrheal diseases are one of sever health problem and a major causes of morbidity and mortality in infants and younger children [4]. *E.coli* has outer membrane of Lipid Polysaacrile (LPS) this structure referred to O-Ag [5]. The conventional serotypes methods are based on agglutination reaction of O-Ag with antisera that are generated in rabbit against O-antigens 5. Rotavirus infections are a major cause of diarrhea in children and domestic animals, and age of children range between 5 years of life [6]. In the third world and developing countries Rota viral gastrointestinal disease, the development of potential inhibitors of this virus is of great interest [7].

Rotavirus, an icosahedral virus related to family Reoviridae. Bishop was first recognized as a diarrhea infection [8]. Rotavirus particle consists of three protein layers was called triple-layered particle diagnosis by electron-microscopy, it was look like wheels (Latin Rota), and give name Rotavirus for the genus [9]. Rotavirus was the most common cause of severe childhood diarrhea in worldwide which causes diarrheal mortality in developing countries [10]. The World Health Organization (WHO) estimates that (527,000) children under the age (5) years die of rotavirus disease

each year. Currently available rotavirus vaccines protected against severe Rotavirus gastroenteritis and were well tolerated the implementation of immunization programs would be expected to reduce disease burden. Most local previous studies detect the most common cause diarrhea in children as virus infection or bacterial infection in separate studies [11]. A virulence determinant acquired, virulence mechanisms, host colonization and clinical manifestation these factors used for classified pathotypes in to Enteropathogenic *E.coli* (EPEC), Enterohemorrhagic *E.coli* (EHEC), Enteraggregation *E.coli* (EAEC), Enterotoxigenic *E.coli* and Enteroinvasive *E.coli* [12].

Identification and isolation

A total of (75) of diarrheal stool was taken from children with age groups range from (1-24) months were culture on Macconcy, Blood, and Eosin methylene blue agar. *E.coli* colonies were diagnosis by Gram stain, biochemical tests and Vitek-2 [13].

DNA extraction and protocol of amplification of virulence genes

For amplification condition of virulence, genes depend on Yamamoto et al., [14] Table 1.

Name	5' → 3'	Table
Tra T	F-GGTGTGGTGCATGAGCACAG R-CACGGTTTCAGCCATCCCTGAG	290 bp [15]
Cva C	F- CACACACAAACGGGAGCTGTT R'- CTTCCCGCAGCATAGTCCAT	680 bp [15]

Table 1. Oligonucleotide primers for virulence factors genes. Identification of serotypes of *E.coli*.

All strain of *E.coli* were serotyped depend on O-Antigen and H-Antigen

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of agglutination methods was used according to [16].

Enzyme Linked Immunosorbent Assay (ELISA)

All diarrheal samples were detected for *Rota virus* by ELISA kite (RIDASREEN® Germany kite) by monoclonal antibodies are used in a sandwich type method in micro well plate [17]. In accurate results was been showed *E.coli* is more effective is more effective from Rotavirus to causes diarrheal in children, which have sign and symptoms as diarrhea, abdominal pain, vomiting and dehydration .According to age groups of children which was sever from diarrheal infection was range from (1-24) month. All children who has clinical manifestation as diarrheal, high fever, abdominal pain, vomiting and dehydration. In Table 2 age groups associated with sex distribution.

Age groups (months)	Frequency %	Male	Female	Total	P-value
1-4	Frequency	5 (31.3)	3 (37.5)	8 (100)	0.35
5-9	Male	9 (45)	11 (55)	20 (100)	
10-14	Female	1 3 (61.9)	8 (38.1)	21 (100)	
15-19	Total	1 1 (68.9)	5 (31.3)	16 (100)	
20-24	P-value	3 (33.3)	6 (66.7)	9 (100)	
Total	78 (100)	4 1 (54.7)	3 4 (45.3)	75 (100)	

Table 2. Age groups and sex distribution of diarrheal children.

The high percentage was enrolled in age groups (5-9) month was in male is lower 9(45%) than female 11(55%) also age group (10-14) month as in male was a high percentage 13 (61.9%) than female 8 (38.1%) then related to natural of breast feeding natural or bottle feeding, genetic and nutritional factors and other Socioeconomic factors such as mothers education, residences and sample size for study. Some local study in Iraq were enrolled as the following studies in Basra (24%) in 2003, Erbile (37%) in 2006 also in 2011 study was recorded (30%) of diarrheal infection and finally in Ramadi and Hella were (39.26%) and (52.54%) respectively FR [18-20] *E.coli* was recorded 37(100%) in both sex and high rate in female 20(58.8%) and *Rota virus* was reported a lower rate 23(111.7%) and more rate in male 12(29.3%) as in Table 3. Abdollah , et al., was recorded a high rate in female 25(0.30%) these disagrees with this study was (54.67%) in male a high rate and low rate in female (45.33%) [21] (Tables 4-6) (Figures 1-4).

Sex distribution	<i>E.coli</i>	<i>Rota virus</i>	Mix infection
Male	17 (41.5)	12 (29.3)	12 (29.3)
Female	20 (58.8)	11 (82.4)	3 (8.8)
Total	37 (100)	23 (111.7)	15 (38.1)

Table 3.Sex distribution associated with *E.coli* and *Rota virus*.

Age group (month)	<i>E.coli</i>	<i>Rota virus</i>	Mix infection	Total
1-4	5 (62.5)	1 (12.5)	2 (25)	8 (100)
5-9	12 (60)	4 (19)	5 (23.8)	21 (100)
10-14	8 (38.1)	10 (47.6)	3 (14.3)	21 (100)
15-19	8 (38.1)	5 (31.3)	3 (18.8)	15 (100)
20-24	14 (44.4)	3 (33.3)	2 (22.2)	9 (100)
Total	37 (49.3)	23 (30.7)	16 (20)	75 (100)

Table 4. Age groups associated with *E.coli* and *rota virus*.

<i>E.coli</i> serotypes	Positive cases	%
O194:H23	4	26.66
O128:H2	3	20
O142:H6	3	20
O128:H7	2	13.33
O148:H28	2	13.33
O7:H152	1	6.66
Total	15	(100%)

Table 5. Serotype of *E.coli* associated with diarrhea.

Type of feeding	<i>E.coli</i> %	<i>Rota virus</i> %	Mix infection %	P-value
Breast feeding	10 (27)	0	8 (53.3)	0.001*
Bottle feeding	19 (51.4)	14 (60.9)	7 (46.7)	
Mix infection	8 (21.6)	9 (39.1)	0	
Total	37 (100%)	23 (100%)	15 (100%)	

Table 6. Type of feeding associated with diarrheal agent (P ≥ 0.05).

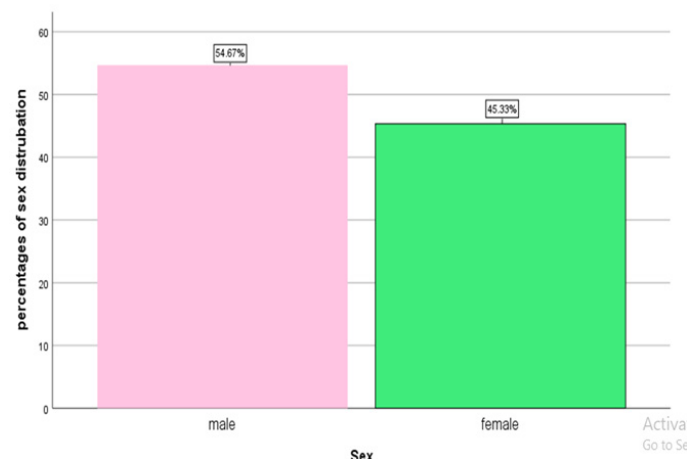


Figure 1. Sex distribution of diarrheal children.

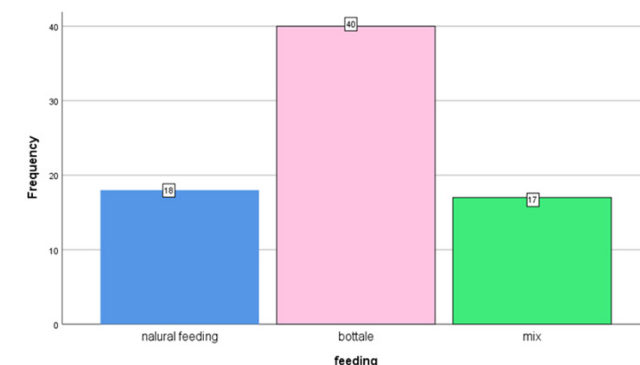


Figure 2. A percentage type of feeding.

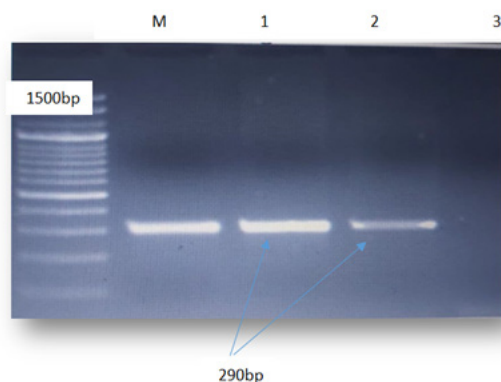


Figure 3. Agarose gel electrophoresis for tra T gene as a virulence genes of *E.coli* ,lane 1,2,3 tra T gene with size gene(290 bp) M lane is (Ladder DNA with 1500 bp) with 1.5 agarose, 70 vol during 60 min).

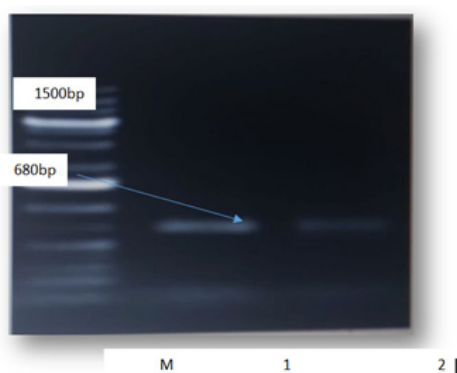


Figure 4. Agarose gel electrophoresis for Cvac C gene as a virulence genes of *E.coli* ,lane 1,2 cva c gene with size gene(680 bp) M lane is (Ladder DNA with 1500 bp) with 1.5 agarose, 70 vol during 60 min).

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