

## HAEMAGGLUTINATION PATTERNS OF ENTEROPATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM DIARRHEIC CHILDREN OF DIFFERENT BLOOD GROUPS

<sup>1</sup>\*Mohemid Maddallah Al-Jebouri, <sup>2</sup>Ibraheem Mohammed Al-Rahaley

<sup>1</sup>\*Department of Medical Laboratory Technology, Health and Medical College of  
Technology, Al-Qalam University, Kirkuk, Iraq.

<sup>2</sup>Department of Biology, College of Science, University of Mosul, Mosul, Iraq.

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### \*Corresponding Author

Mohemid Maddallah Al-Jebouri

Department of Medical Laboratory  
Technology, Health and Medical  
College of Technology, Al-Qalam  
University, Kirkuk, Iraq.



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### ABSTRACT

**Background:** Because of bacterial pili, the agglutination of erythrocytes with bacteria has been demonstrated to be facilitated. The usage of sugars such as mannose for inhibition of hemagglutination with certain bacterial strains giving differential patterns of haemagglutination for different types of bacteria depending on types of pili including enteropathogenic *Escherichia coli* (EPEC). **Materials and Methods:** A total of 614 children feces samples (363 diarrhetic from children hospital and 251 healthy children from Mosul nurseries). All children were under three years of age. Fecal samples were transported to research laboratory using nutrient broth as transport medium for rectal swabs. EPEC identification, serotyping and haemagglutination were carried out. **Results:** The data indicated that blood group O exhibited the highest overall frequency of enteropathogenic *E. coli* infection (41.4%), followed by blood group B (31.7%), A (24.4%), and

AB (2.4%). Cases from the hospital were predominantly associated with blood groups O and B, whereas blood group A was observed exclusively among nursery children. The present study revealed that serotypes O126 and O142 were the most common (7 isolates each). But Serotype O126 was strongly associated with blood group O (5 isolates) and serotype O142 was concentrated in blood groups A and B. Blood group AB contained only one isolate. Among the serotypes examined, O86, O142, and the mixed serogroup OI+II+III exhibited the

broadest spectrum of positive reactions across multiple erythrocyte types, indicating stronger and more versatile adherence capabilities. Conversely, several isolates of O111, O125, O126, and O127 showed predominantly negative reactions, suggesting lower hemagglutinating potential. **Conclusions:** The data indicated that blood group O exhibited the highest overall frequency of enteropathogenic *E. coli* infection (41.4%), followed by blood group B (31.7%), A (24.4%), and AB (2.4%). Cases from the hospital were predominantly associated with blood groups O and B, whereas blood group A was observed exclusively among nursery children. Analysis of 600 pediatric subjects demonstrated an overall diarrhea prevalence of 6.8%. Blood group B exhibited the highest prevalence of *Escherichia coli* diarrhea (8.55%), followed by blood groups O (7.26%) and A (5.35%). Serotypes O126 and O142 were the dominant serotypes, accounting for the highest frequencies observed. Blood group A exhibited the highest frequency of positive hemagglutination reactions under both mannose-treated and untreated conditions, suggesting that A-group erythrocytes possess receptors that are highly favorable for EPEC attachment.

**KEYWORDS:** children, diarrhea, EPEC, serotypes, haemagglutination.

## INTRODUCTION

Nine identified pathovars of *Escherichia coli* strains isolated from humans can cause diarrheagenic and extraintestinal diseases.<sup>[1,2,3,4]</sup> Seven of these pathotypes are enteric pathogenic *E. coli*, including Enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), and a recently discovered pathotype, adherent-Invasive *E. coli* (AIEC).<sup>[5,6,7,8]</sup> These particular pathotypes are mainly responsible for triggering diarrhea and various intestinal disorders. For instance, enterohemorrhagic *E. coli* (EHEC) pathotypes pose significant public health concerns as they are known foodborne pathogens and have been linked to fatal outbreaks in both developed and developing countries.<sup>[9,10,11,12]</sup> These pathotypes cause diseases by expressing genes that encode virulence factors, and recent studies have emphasized their potential impact on a range of disorders.<sup>[13,14,15,16,17,18]</sup> It was found that Gram-negative bacteria particularly *E. coli* are associated with many infectious diseases attacking cancer patients like bacteraemia<sup>[19]</sup> who concluded that 13.8% of patients with cancer of bladder were suffered from urinary tract infection due to incidence of *E. coli*. It was postulated by many investigators that there is a relationship between natural selection and blood group types. They found a relation between

ABO and duodenal ulcer, carcinoma of stomach and diabetes mellitus.<sup>[20,21,22,23,24]</sup> The haemagglutination has been used for detection of many infectious diseases as a cheap tool for identification compared to other sophisticated techniques such as serology, biochemical and phage typing.<sup>[25, 26,27,28,29,30]</sup> Because of bacterial pili, the agglutination of erythrocytes with bacteria has been demonstrated to be facilitated. The usage of sugars such as mannose for inhibition of haemagglutination with certain bacterial strains giving different patterns of haemagglutination for different types of bacteria depending on types of pili. It was shown that type-I was very common causing mannose-sensitive haemagglutination (MSHA) as discovered by Duguid *et al.*<sup>[31,32]</sup> Generally, there are two main types of direct haemagglutination which are mannose-sensitive which utilized for identification of type-I-fimbriae(MSHA) and mannose-resistant haemagglutination(MRHA) which was commonly used for epidemiological studies. This is because that most of the virulent pathogens possess fimbriae of MRHA.<sup>[33]</sup> It was found a relation between O,K and H bacterial antigens and fimbrial antigen of isolates causing urinary tract infection<sup>[34,35,36]</sup> of *Escherichia coli* causing this disease. It was also demonstrated that there is a difference between haemagglutination patterns occurred with human RBCs and that of animals like cattle, chicken, guinea pig and rabbit due to different fimbriae.<sup>[8,37]</sup>

## MATERIALS AND METHODS

### *Patients and sampling*

A total of 614 children feces samples (363 diarrhetic from children hospital and 251 healthy children from Mosul nurseries). All children were under three years of age. Fecal samples were transported to laboratory using nutrient broth as transport medium for rectal swabs.<sup>[16,38]</sup>

### *Isolation*

All samples were inoculated on MacConkey agar (Oxoid). Inoculated plates were incubated at 37 °C for 24 hours.<sup>[38,39]</sup>

### *Identification of *Escherichia coli**

The purified isolates of suspected *E.coli* were conventionally identified following methods of workers.<sup>[40,41]</sup>

### *Haemagglutination typing*

Type A human blood was drawn from volunteers and placed into a tube containing 1.0 ml of 3.8% citric acid in distilled water per 9.0 ml of blood. Blood was diluted 1:4 with phosphate-

buffered saline to test for HA and 1:4 with 1% mannose in phosphate buffered saline to test for MRHA. The same procedure was used for blood freshly drawn from guinea pigs and for bovine, adult chicken, and African Green monkey erythrocytes obtained from Flow Laboratories, Inc., McLean, Va. HA tests were performed by slide agglutination as follows. Bacterial cells from CFA agar cultures (see above) were picked up with a sterile wooden toothpick and mixed with a drop of the appropriate species of blood (approximately 20 g/l) on a glass slide at room temperature. After observation for HA for about 1 min, the slides showing less than maximum HA were placed on the surface of ice and observed for a least 2 min, with intermittent mixing by rotation of the slide. Results were recorded at 4+ when the HA reaction was instantaneous and complete, involving all the erythrocytes. Lesser degrees of HA were recorded as 3+, 2+, 1+, or negative (11). HA was denoted as resistant (MRHA) if the same degree of HA occurred with and without mannose and as sensitive (MSHA) if HA was prevented or grossly reduced by the presence of mannose. For consistency each test strain was tested first with erythrocytes in phosphate-buffered saline in the following order: human, bovine, chicken, monkey, guinea pig. If positive for HA, the test was repeated with the appropriate species of erythrocytes in phosphate-buffered saline plus mannose. There was only one circumstance, which occurred infrequently, in which an HA pattern could not be obtained; a few rough (untypeable) isolates of *E. coli* were found to produce MRHA with every species of erythrocyte tested, but this HA was not typical and therefore, was easily recognized.<sup>[42]</sup>

### ***Statistical analyses***

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics such as means, standard deviations, and frequency distributions were computed to summarize the data. A paired sample t test correlation coefficient (R) and coefficient of determination (R<sup>2</sup>) were calculated.<sup>[28,43,44]</sup>

## **RESULTS**

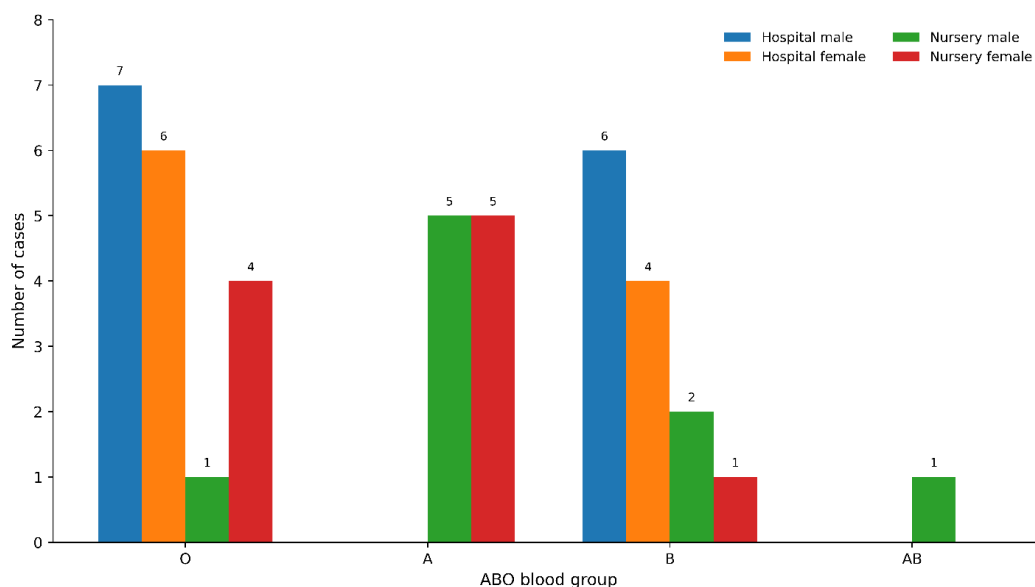
### ***Diarrhea and blood groups of children***

The data indicated that blood group O exhibited the highest overall frequency of enteropathogenic *E. coli* infection (41.4%), followed by blood group B (31.7%), A (24.4%), and AB (2.4%). Cases from the hospital were predominantly associated with blood groups O and B, whereas blood group A was observed exclusively among nursery children (Table 1). The marked differences in distribution suggest a potential association between ABO blood

group and susceptibility to enteropathogenic *E. coli* infection, which should be formally evaluated using Pearson's Chi-square test and Cramer's V effect size. The predominance of blood group O among hospitalized children may reflect increased susceptibility of intestinal epithelial receptors to enteropathogenic *E. coli* adhesion. Several studies have reported enhanced bacterial attachment and toxin-mediated effects among individuals with blood group O. Conversely, blood group A was predominantly associated with nursery cases, suggesting that environmental or age-related exposure factors may interact with host blood-group antigens. The very low frequency of blood group AB indicates either a protective effect or simply reflects its naturally lower prevalence within the population. The distribution of enteropathogenic *E. coli* diarrhea varied markedly among ABO blood groups. Blood group O predominated among hospitalized children, whereas blood group A was concentrated in nursery cases. These findings suggest a potential association between ABO blood-group antigens and susceptibility patterns to enteropathogenic *E. coli* infection in pediatric populations (Figure 1).

**Table 1: Distribution of enteropathogenic *Escherichia coli* causing diarrhea among Children of different blood groups in pediatric hospital and nursery.**

Blood group	Gender	Children with diarrhea at:				Total	
		Hospital		Nursery			
		No.	%	No.	%	No.	%
O	Male	7	17.1	1	7.4	8	11.5
	Female	6	14.6	4	7.3	10	21.1
	Total	13	31.7	4	9.7	17	41.4
A	Male	0	0	5	12.2	5	12.2
	Female	0	0	5	12.2	5	12.2
	Total	0	0	10	24.4	10	24.4
B	Male	6	14.6	2	4.1	8	19.5
	Female	4	9.8	1	2.4	5	12.2
	Total	10	24.4	3	7.3	13	31.7
AB	Male	0	0	1	2.4	1	2.4
	Female	0	0	0	0	0	0
	Total	0	0	1	2.4	1	2.4
Overall	Overall	23	56.1	18	47.8	41	100



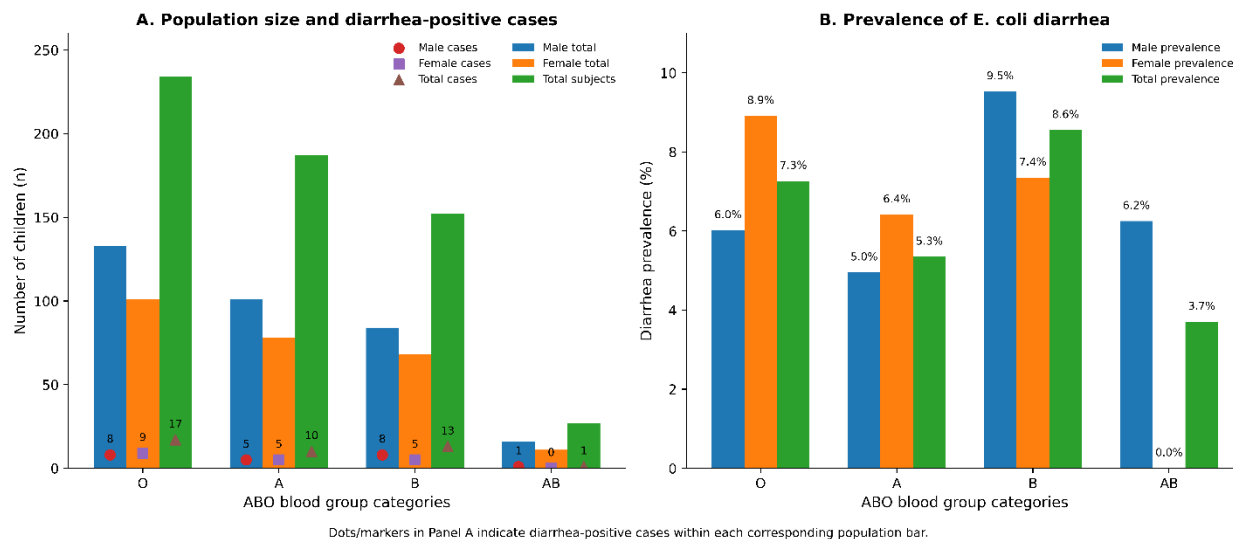
**Figure 1: Distribution of enteropathogenic *Escherichia coli* diarrhea cases according to ABO blood group, gender, and source of admission among pediatric patients.**

#### ***Children blood group and diarrhoea***

Analysis of 600 pediatric subjects demonstrated an overall diarrhea prevalence of 6.8%. Blood group B exhibited the highest prevalence of *Escherichia coli* diarrhea (8.55%), followed by blood groups O (7.26%) and A (5.35%), whereas blood group AB showed the lowest prevalence (3.70%). Female children displayed a greater prevalence than males, suggesting that both ABO blood-group antigens and gender may influence susceptibility to diarrheagenic *E. coli* infection. Pearson's Chi-square test and odds-ratio analysis are recommended to quantify the strength and significance of these associations (Table 2, Figure 2).

**Table 2. Distribution of diarrhea of *Escherichia coli* among children of different blood groups.**

Blood group	Gender:				Total	
	Male:		Female:			
	Number	No.(%) with diarrhea	Number	No.(%) with diarrhea	Number	NO.(%) with diarrhea
O	133	8(6)	101	9(8.9)	234	17(7.3)
A	101	5(4.6)	78	5(6.4)	187	10(5.3)
B	84	8(9.5)	68	5(7.4)	152	13(8.6)
AB	16	1(6.3)	11	0	27	1(3.7)
Overall	334	14(2.3)	258	19(3.7)	600	41(6.8)



**Figure 2. Gender-specific distribution of *Escherichia coli* diarrhea among pediatric ABO blood groups.**

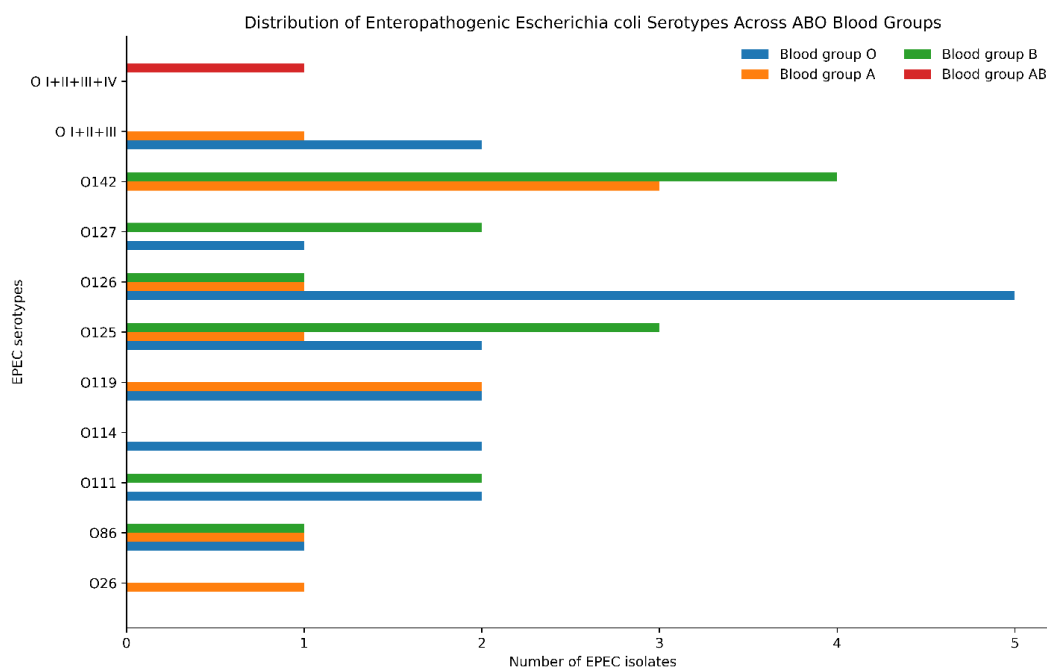
***Escherichia coli* serotypes causing diarrhoea**

Blood group O showed the highest overall frequency (17 isolates; 41.5%) whereas the blood group B was second (13 isolates; 31.7%). The present study revealed that serotypes O126 and O142 were the most common (7 isolates each). But Serotype O126 was strongly associated with blood group O (5 isolates) and serotype O142 was concentrated in blood groups A and B. Blood group AB contained only one isolate, limiting statistical power for that category (Table 3). The distribution of enteropathogenic *Escherichia coli* serotypes varied among ABO blood groups, with blood group O harboring the greatest number of isolates. Serotypes O126 and O142 were the dominant serotypes, accounting for the highest frequencies observed. The concentration of specific serotypes within particular blood groups suggests a possible host–pathogen interaction that can be formally evaluated using Pearson's Chi-square test and correspondence analysis (Figure 3).

**Table 3. Distribution of serotypes of enteropathogenic *Escherichia coli* causing diarrhea to children of different blood groups.**

Serotype	Number of serotypes associated with following blood groups				Total
	O	A	B	AB	
O26	0	1	0	0	1
O86	1	1	1	0	3
O111	2	0	2	0	4
O114	2	0	0	0	2
O119	2	2	0	0	4

O125	2	1	3	0	6
O126	5	1	1	0	7
O127	1	0	2	0	3
O142	0	3	4	0	7
O I+II+III	2	1	0	0	3
OI+II+III+IV	0	0	0	1	1
Total	17	10	13	1	41



**Figure 3: Frequency of enteropathogenic *Escherichia coli* serotypes according to ABO blood group.**

### ***Haemagglutination***

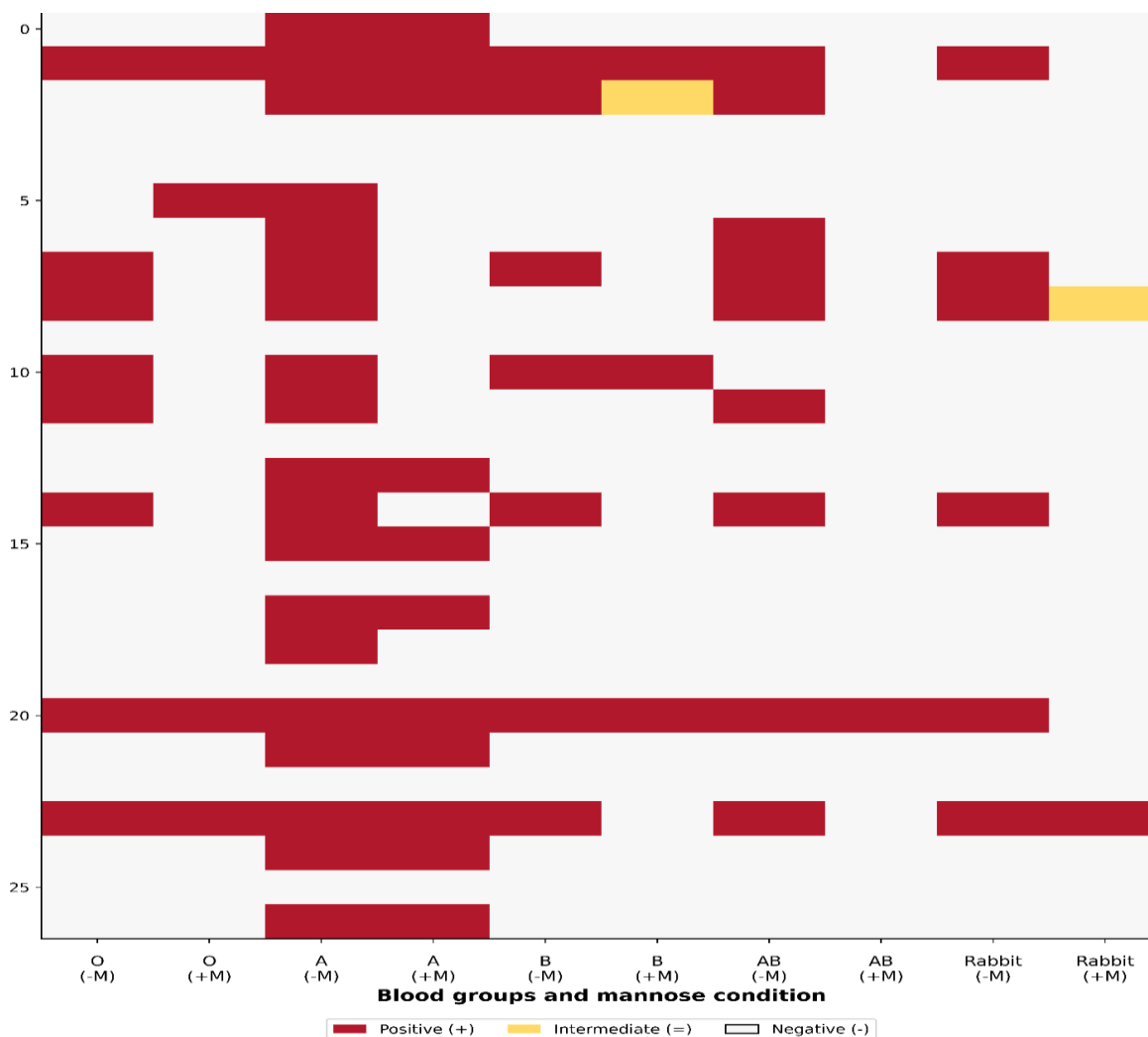
Blood group A exhibited the highest frequency of positive hemagglutination reactions under both mannose-treated and untreated conditions, suggesting that A-group erythrocytes possess receptors that are highly favorable for EPEC attachment. In contrast, blood groups O, B, and AB showed lower and more variable levels of reactivity, while rabbit erythrocytes displayed limited susceptibility to bacterial agglutination. The presence of mannose reduced hemagglutination activity in several isolates, indicating that a proportion of the adhesive interactions were mediated by mannose-sensitive fimbrial adhesins. However, persistence of positive reactions in certain serotypes despite mannose treatment suggests the existence of mannose-resistant adhesion mechanisms, reflecting heterogeneity in virulence-associated surface structures among EPEC isolates. Among the serotypes examined, O86, O142, and the mixed serogroup OI+II+III exhibited the broadest spectrum of positive reactions across multiple erythrocyte types, indicating stronger and more versatile adherence capabilities.

Conversely, several isolates of O111, O125, O126, and O127 showed predominantly negative reactions, suggesting lower hemagglutinating potential (Table 4). The occurrence of only a few intermediate reactions demonstrates that most isolates could be clearly categorized as either hemagglutination-positive or hemagglutination-negative, supporting the robustness of the observed adhesion patterns. Overall, the heatmap reveals marked serotype-dependent differences in hemagglutination behavior and demonstrates that blood group A erythrocytes are the most receptive targets for EPEC adhesion. The reduction of reactions following mannose treatment further confirms the involvement of mannose-sensitive adhesins in many isolates, while residual positive reactions indicate the contribution of alternative mannose-resistant attachment mechanisms. These findings highlight the diversity of colonization strategies employed by EPEC and emphasize the importance of host-cell receptor specificity in bacterial adherence and pathogenicity (Figure 4).

**Table 4: Patterns of Haemagglutination of different blood groups of children with diarrhea with reference to enteropathogenic *Escherichia coli* in presence and absence of mannose sugar.**

Serotype	Isolates No.	Hemagglutination of blood groups with(+ve) and without (-ve) mannose for 44 isolates of EPEC <i>Escherichia coli</i> ::								Rabbit blood	
		O		A		B		AB			
		- ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
026	2	-		+	+	-		-		-	
086	1	+	+	+	+	+	+	+	-	+	-
086	1	-		+	+	+	-	+	-	-	
086	2	-		-		-		-		-	
0111	1	-		-		-		-		-	
0111	2	-	+	+		-		-		-	
0114	2	-		+	-	-		+	-	-	
0119	1	+	-	+	-	+	-	+	-	+	-
0119	1	+	-	+	-	-		+	-	+	-
0119	2	-		-		-		-		-	
0125	1	+	-	+	-	+	+	-		-	
0125	1	+	-	+	-	-		+	-	-	
0125	2	-		-		-		-		-	
0125	3	-		+	+	-		-		-	
0126	1	+	-	+	-	+	-	+	-	+	-
0126	2	-		+	+	-		-		-	
0126	4	-		-		-		-		-	
0127	1	-		+	+	-		-		-	
0127	1	-		+	-	-		-		-	
0127	1	-		-		-		-		-	
0142	1	+	+	+	+	+	+	+	+	+	-
0142	2	-		+	+	-		-		-	

0142	2	-		-		-		-		-	
O I+II+III	1	+	+	+	+	+	-	+	-	+	+
O I+II+III	1	-		+	+	-		-		-	
O I+II+III	1	-		-		-		-		-	
O I+II+III+	1	-		+	+	-		-		-	



**Figure 4: Heatmap illustrating hemagglutination reactions of enteropathogenic *Escherichia coli* (EPEC) serotypes against human blood groups O, A, B, AB and rabbit erythrocytes in the presence (+M) and absence (-M) of mannose. Red cells indicate positive hemagglutination (+), yellow cells indicate intermediate reactions (=), and white cells indicate negative reactions (-). The figure highlights differences in erythrocyte-binding affinity and the inhibitory effect of mannose on bacterial adhesion patterns.**

**DISCUSSION**

The data indicated that blood group O exhibited the highest overall frequency of enteropathogenic *E. coli* infection (41.4%), followed by blood group B (31.7%), A (24.4%), and AB (2.4%). Cases from the hospital were predominantly associated with blood groups O

and B, whereas blood group A was observed exclusively among nursery children. The very low frequency of blood group AB indicates either a protective effect or simply reflects its naturally lower prevalence within the population. The distribution of enteropathogenic *E. coli* diarrhea varied markedly among ABO blood groups. Blood group O predominated among hospitalized children, whereas blood group A was concentrated in nursery cases. These findings suggest a potential association between ABO blood-group antigens and susceptibility patterns to enteropathogenic *E. coli* infection in pediatric populations. It was recently studied diarrheagenic *E. coli* prevalence in Peru using 8,000 *E. coli* strains previously isolated from 8 different studies in children mainly younger than 36 months of age and primarily from cohort studies in peri-urban Lima.<sup>[45]</sup> Diarrheagenic *E. coli* were detected by a multiplex real-time PCR using specific primers for each of the six diarrheagenic *E. coli*.<sup>[46,47,48,49,50]</sup> The same methodology was used in all samples. Overall, the average EPEC prevalence in diarrhea samples (n=4,243) was 8.5% (95% CI: 7.6–9.3), second only to enteroaggregative *E. coli* (EAEC, 9.9%). EPEC prevalence increased with age. EPEC was found in 3% of diarrheal samples in children <6mo, in 11% of children 6–12mo, and in 16% of children 13–24mo. However, in this setting exclusive breastfeeding is very prevalent (>80% for infants younger than 6mo), and therefore, small infants may be protected from symptomatic EPEC infection. Among control samples collected from asymptomatic children from the same study sites (n=3,760), EPEC was the most prevalent pathotype with an average prevalence of 10.9% (95% CI: 9.4–11.4), followed by EAEC (10.4).<sup>[51,52,53]</sup>

The present study showed that the analysis of 600 pediatric subjects demonstrated an overall diarrhea prevalence of 6.8%. Blood group B exhibited the highest prevalence of *Escherichia coli* diarrhea (8.55%), followed by blood groups O (7.26%) and A (5.35%), whereas blood group AB showed the lowest prevalence (3.70%). Female children displayed a greater prevalence than males, suggesting that both ABO blood-group antigens and gender may influence susceptibility to diarrheagenic *E. coli* infection. Pearson's Chi-square test and odds-ratio analysis are recommended to quantify the strength and significance of these associations. However, it is likely that some of the A blood group–positive individuals did not express the A antigen on their gastrointestinal epithelia. Theoretically, this might account for some individuals in the A blood group who failed to become ill following challenge. Conversely, while the A blood group is a risk factor in this model, the development of severe diarrheal illness among non-A volunteers demonstrates that expression of A glycans is not an absolute requirement for disease. *V. cholerae* infections provide an important analogous

paradigm for severity of illness related to blood group. While severe cholera is significantly associated with the O blood group, it is not exclusively observed in blood group O individuals.<sup>[54,55,56,57]</sup> We should also note that the ETEC-challenge studies were done with a relatively high inoculum of bacteria ( $\sim 10^7$ ) designed to assure a reasonable attack rate.<sup>[58,59,60]</sup> a feature that may not be reflected in naturally occurring infections in young children where the A blood group also appears to be a risk for development of symptomatic infections.<sup>[61,62,63,64]</sup> It is also worth noting that volunteers in these studies were treated with antibiotics to clear the infection once they met the definition of severe diarrheal illness, a feature that might also have affected the ultimate outcome.<sup>[65]</sup> Moreover, the present study showed Blood group O showed the highest overall frequency (17 isolates; 41.5%) whereas the blood group B was second (13 isolates; 31.7%). The present study revealed that serotypes O126 and O142 were the most common (7 isolates each). But Serotype O126 was strongly associated with blood group O (5 isolates) and serotype O142 was concentrated in blood groups A and B. Blood group AB contained only one isolate, limiting statistical power for that category (Table 3). The distribution of enteropathogenic *E. coli* serotypes varied among ABO blood groups, with blood group O harboring the greatest number of isolates. Serotypes O126 and O142 were the dominant serotypes, accounting for the highest frequencies observed. The concentration of specific serotypes within particular blood groups suggests a possible host-pathogen interaction that can be formally evaluated using Pearson's Chi-square test and correspondence analysis.

However, It was reported by the present study that Blood group A exhibited the highest frequency of positive hemagglutination reactions under both mannose-treated and untreated conditions, suggesting that A-group erythrocytes possess receptors that are highly favorable for EPEC attachment. In contrast, blood groups O, B, and AB showed lower and more variable levels of reactivity, while rabbit erythrocytes displayed limited susceptibility to bacterial agglutination. The presence of mannose reduced hemagglutination activity in several isolates, indicating that a proportion of the adhesive interactions were mediated by mannose-sensitive fimbrial adhesins. However, persistence of positive reactions in certain serotypes despite mannose treatment suggests the existence of mannose-resistant adhesion mechanisms, reflecting heterogeneity in virulence-associated surface structures among EPEC isolates. Among the serotypes examined, O86, O142, and the mixed serogroup OI+II+III exhibited the broadest spectrum of positive reactions across multiple erythrocyte types, indicating stronger and more versatile adherence capabilities. Conversely, several isolates of

O111, O125, O126, and O127 showed predominantly negative reactions, suggesting lower hemagglutinating potential (Table 4). Moreover, The other type which was mannose-resistant haemagglutination (HRHA) which was considered by Evans *et. al.*,<sup>[42,47,48,49]</sup> has no significant value because the serotypes specific for this patten are virulence carrier causing various infections. Moreover, colonization factor antigens (CFA) were also considered as able to haemagglutinate in presence of mannose sugar (MRHA). It was also demonstrated that CFA were specifically reacted with host membrane receptors.<sup>[42,50]</sup> However, there are two types of CFA, CFA/I which agglutinates with erythrocytes of human, cattle and poultry and the other type CFA/II was able to agglutinate with erythrocytes of cattle and poultry only<sup>[8,52]</sup> who found two types of haemagglutination which were mannose-sensitive and mannose-resistant. In addition, Al-Jebouri and Al-Rahaley concluded a unique pattern of negative haemagglutination for all blood group types with 16 frequency among 44 serotypes of EPEC *E.coli* whereas the second was seen with blood group A only with 17 frequency.<sup>[8]</sup> Furthermore, the cross-reactions were postulated by Orskov and Orskov<sup>[33,53,54,55]</sup> who noticed a strong cross-reaction occurred between serotype 086 of EPEC *E.coli* and blood group B which almost to the present findings (Table 2). It was suggested by Evans *et al.*,<sup>[42,56,57,58]</sup> that haemagglutination should be carried out at the same time with EPEC serotyping to identify the most distributed variant causing diarrhoea for children. However, the purpose of the present utilization of CFA/I and CFA/II was to assess of application of these patterns for preparation of vaccines specific for *E.coli* strains causing gastrointestinal infections.

Moreover, It was found recently by other workers that diarrheagenic *E. coli* prevalence in Peru using 8,000 *E. coli* strains previously isolated from 8 different studies in children mainly younger than 36 months of age and primarily from cohort studies in peri-urban Lima.<sup>[51,66,67]</sup> Diarrheagenic *E. coli* were detected by a multiplex real-time PCR using specific primers for each of the six diarrheagenic *E. coli*.<sup>[68,69,70,71]</sup> However, in this setting exclusive breast feeding is very prevalent (>80% for infants younger than 6mo), and therefore, small infants may be protected from symptomatic EPEC infection. Among control samples collected from asymptomatic children from the same study sites (n=3,760), EPEC was the most prevalent pathotype with an average prevalence of 10.9% (95% CI: 9.4–11.4), followed by EAEC (10.4%).<sup>[51,72,73]</sup> In a study carried out elsewhere it was concluded that only EPEC, ETEC, and EAEC were detected in stool samples, with only EAEC being significantly found in diarrheic children (30.7%) compared with controls (9.3%) ( $p < 0.01$ ). Compared

with our findings, lower prevalence rates of EAEC from diarrheic children have been reported from Iraq (8%), Kuwait (2.6%), Libya (4.1%), and Tunisia (11.3%).<sup>[74,75,76,77,78,79,80,81]</sup>

## CONCLUSIONS

The data indicated that blood group O exhibited the highest overall frequency of enteropathogenic *E. coli* infection (41.4%), followed by blood group B (31.7%), A (24.4%), and AB (2.4%). Cases from the hospital were predominantly associated with blood groups O and B, whereas blood group A was observed exclusively among nursery children. Analysis of 600 pediatric subjects demonstrated an overall diarrhea prevalence of 6.8%. Blood group B exhibited the highest prevalence of *E. coli* diarrhea (8.55%), followed by blood groups O (7.26%) and A (5.35%). Serotypes O126 and O142 were the dominant serotypes, accounting for the highest frequencies observed. Blood group A exhibited the highest frequency of positive hemagglutination reactions under both mannose-treated and untreated conditions, suggesting that A-group erythrocytes possess receptors that are highly favorable for EPEC attachment.

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## Statement of Ethics

All the procedures involving human participation were conducted in strict accordance with ethical standards of Institutional Research Committee, Department of Scientific Research, Mosul University as well as the 1964 Helsinki Declaration and its subsequent amendments or equivalent ethical norms.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Conflict of Interest Statement

The author declares that he has no conflicts of interest, financial or otherwise.

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