Research Article   **Open Access**

Detection of Some Virulence Genes in Diarrheagenic *Escherichia Coli* Pathotypes in Children Under Five Years in Aleppo City

Ahmad Homsi[1*], Walid Al-Said[1], Zaher Tahan[1]

[1]Department of Plant Biology, Faculty of Science, Aleppo University, Syria.

*Corresponding contact: ahmadhomsi1436@gmail.com

ABSTRACT

Diarrheagenic *Escherichia coli* (DEC) strains can be classified into six main pathotypes based on their specific virulence properties. These pathotypes are associated with certain serotypes and exhibit distinct epidemiological and clinical features. The identification of DEC cannot be based solely on cultural and biochemical criteria, as they are indistinguishable from the non-pathogenic *E. coli*. However, the presence of virulence genes and DNA sequences specific to DEC can be used to determine its identity. The aim of this study is to investigate the prevalence of Diarrheagenic *Escherichia coli* (DEC) pathotypes among infected children under 5 years living in the city of Aleppo Syria. From June 2020 to September 2021, one hundred samples of diarrheal were collected from children under five years suffering from diarrhea admitted at Aleppo University Hospital and from some private laboratories in Aleppo city. *Escherichia coli* pathotypes were detected by using multiplex polymerase chain reaction and Phylogenetic genes were detected by Triplex PCR. The frequency of diarrheagenic *E. coli* was (48%), the most frequently isolated pathotypes were atypical enteropathogenic *E. coli.* (42%), followed by enterotoxigenic *E. coli* (4%), and enteroinvasive *E. coli* (2%). Enterohemorrhagic *E. coli*, enteroaggregative *E. coli* and Diffusely Adherent *E. coli* were not detected in any sample (0%). The most prevalent phylogenetic group of *E. coli* was "Phylogenetic group B2" which represented (62%), followed by "Phylogenetic group D" that comprised 33%, followed by "Phylogenetic group A" strains (3%). Strains of the "Phylogenetic group B1" were rare (2%). This study revealed that DEC strains contribute to cause diarrheal diseases in children, EPEC is the most commonly identified DEC strain. B2 and D groups are the most prevalent phylogenetic groups. In addition, they are virulent because these groups are associated with the presence of several virulence factors.

KEYWORDS: Virulence factor genes, Phylogenetic groups, diarrhea, *Escherichia coli*.

الخلاصة

يمكن تصنيف سلالات الإشريكية القولونية المسببة للإسهال (DEC) إلى ستة أنماط رئيسة ممرضة بناءً على عوامل الفوعة الخاصة بها، والمرتبطة بالأنماط المصلية والخصائص الوبائية والسريرية المختلفة. لا يمكن تحديد الإشريكية القولونية المسببة للإسهال (DEC) فقط من خلال الزراعة الجرثومية والإختبارات الحيوية الكيميائية، حيث لا يمكن التمييز بينها وبين الإشريكية القولونية المعوية غير المسببة للأمراض. نظرًا لأنه تم تحديد العديد من عوامل الفوعة وتسلسل الحمض النووي الخاص بسلالات الإشريكية القولونية الممرضة للأمعاء، فيمكن التحري عنها من خلال الكشف عن مورثات الفوعة المرضية. الهدف من الدراسة هو التحري عن انتشار أنماط الأشريكية القولونية بين الأطفال المصابين بالاسهال الذين يعيشون في مدينة حلب- سوريا. تم جمع مائة عينة من شهر حزيران عام ٢٠٢٠ حتى شهر ايلول ٢٠٢١ من إسهال الأطفال دون سن الخامسة الذين يعانون من الإسهال الذين تم إدخالهم في مستشفى جامعة حلب ومن بعض المخابر الخاصة في مدينة حلب. تم الكشف عن الأنماط المرضية للإشريكية القولونية باستخدام تفاعل البلمرة المتسلسل المتعدد PCR وتم الكشف عن مورثات المجموعات التطورية بواسطة تفاعل ثلاثي البوليميراز المتسلسل Triplex PCR. كانت نسبة عزل الإشريكية القولونية المسببة للإسهال (٤٨٪)، وكانت أكثر الأنماط المعزولة تكرارا هي النمط EPEC (٤٢٪)، تليها الأنماط ETEC (٤٪)، و EIEC (٢٪). في حين لم يتم الكشف عن الانماط EHEC و EAEC و DAEC في أي عينة (٠٪). كانت المجموعة التطورية الأكثر انتشارًا للإشريكية القولونية هي المجموعة التطورية "B2" والتي تمثل (٦٢٪)، تليها المجموعة التطورية "D" والتي تضمنت (٣٣٪)، ثم المجموعة التطورية "A" بنسبة (٣٪). وكانت المجموعة التطورية "B1" الأقل عزلاً بنسبة (٢٪). الاستنتاجات كشفت هذه الدراسة أن سلالات الإشريكية القولونية الممرضة للأمعاء DEC كانت المسبب الرئيسي في حدوث الإسهال عند الأطفال، وكان النمط EPEC هو أكثر سلالات (DEC) شيوعًا. كما أظهرت الدراسة أن المجموعات التطورية B2 و D هي أكثر المجموعات التطورية انتشارًا. بالإضافة إلى ذلك، فهي أكثر المجموعات الحاوية على مورثات الفوعة.

INTRODUCTION

Diarrheal disease is a major public health problem throughout the world, with over 8.8 million children still dying every year before reaching their 5th birthday [1]. *Escherichia coli* strains which cause diarrhea known as diarrheagenic *E. coli* (DEC) especially those with low immunity [1]. "Public health problem" refers to a significant issue that affects the health of a population. One such problem is the high rate of morbidity and mortality in infants and young children particularly in developing countries. This issue is of great concern and requires urgent attention [2]. The DEC can be divided into six categories of pathotypes based on their distinct virulence determinants: enteropathogenic *E. coli* (EPEC), enterohemorrhagic (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC) [3]. Enteropathogenic *Escherichia coli* (EPEC) is a common cause of watery diarrhea in children in developing countries. This type has been classified under two subtypes (tEPEC) in the presence of the adherence factor (EAF) plasmid and atypical Enteropathogenic (aEPEC) in absence of plasmid [4][5]. Enterohemorrgice *Escherichia coli* (EHEC) is one of the most pathogenic intestinal bacteria in the world. EHEC is one of the most serious zoonoses borne by food, associated with the spread of diseases that are a major concern for public health in the world. When the infection occurs by *E. coli* O157:H7, it produces and releases shiga toxin, which is encoded by the stx1 and stx2 genes, and is one of the most important pathogens in EHEC, stx associated with globotriaosylceramide (Gb3). The feature of stx is expressed in the intestinal epithelium as well as on the surface of endothelial cells. The protein synthesis process stops after Stx binds to Gb3 and this leads to induced cell apoptosis [6][7]. Enteroaggregative *Escherichia coli* (EAEC) is one of the most common microorganisms that cause diarrhea in travelers. It is found in the feces of children particularly in developing countries, and its symptoms include recurrent diarrhea and weight loss [8][9]. Enteroinvasive *Escherichia coli* (EIEC) is a type of bacteria that causes dysentery such as shigella, which can cause gastrointestinal disease when the invasion of the medullary mucosa occurs. Its symptoms include abdominal cramps, nausea, fever, and bloody and mucous diarrhea [10][11]. Diffusely adherent *E. coli* causes an inflammation to the mucous membrane of the intestines especially in people with low immunity due to having virulent genes, which are also responsible for the diarrhea for travelers [12]. Enterotoxigenic *Escherichia coli* (ETEC), is a leading cause of diarrhea in children in developing countries, which use adhesives and enteric toxins to induce diarrhea in the host due to having virulence factors such as heat-labile (LT) and heat-stable (ST) enterotoxins along with the colonization factor antigen I (CFA / I) adhesin. These intestinal toxins are responsible for the secretion of water in the intestinal lumen, leading to excessive diarrheas [13][14]. Genotyping analysis has shown that *E. coli* strains are composed of four main phylogenetic groups (A, B1, B2, and D). Virulent extra-intestinal strains are fundamentally related to group B2, which is less prevalent compared to group D. Meanwhile, the most commensal strains belong to groups A and B1. Genotypic grouping was performed using the clermont triplex PCR method, which utilizes primers targeting three genetic markers: *chuA*, *yjaA* and *TspE4.C2*. [15][16]. Multiplex PCR allows fast and accurate detection of more than one gene that is responsible for the virulence factors in a single PCR reaction. For the same strain simultaneously [17][18]. The aim of this study was to identify the different pathotypes of diarrheagenic *Escherichia coli* (DEC) isolated from 100 Syrian children under 5 years old who were experiencing acute diarrhea. Due to the lack of research on DEC in Aleppo, Syria, we focused our efforts on describing the prevalence of DEC in Aleppo city.

MATERIALS AND METHODOLOGIES

The samples were collected using sterile containers with a spoon inside to collect diarrheal stool that contain buffer phosphate PH=8 directly transported to the lab. Then the samples were cultured onto enrichment culture media Selenite broth (A+B) and inoculated at 37°C for 24h. After that Mac-Conkey agar plates were inoculated and incubated at 37°C for 24h. The pink colored (lactose fermentation) colonies from MacConkey agar platea were subcultured on Eosin Methylene Blue (EMB) agar and Hi-Chrome agar (Hi-media) at the same conditions as mentioned above. Colonies producing greenish metallic sheen on EMB agar and blue color were on Hi-Chrome agar considered

as *E. coli*. Various biochemical tests such as Indole production methyl red Voges-Proskauer, and citrate utilization were done for the confirmation of *E. coli* [19][20].

Molecular Typing of Isolates

Genomic DNA Extraction
Cultures grown for 18 hours in Luria Bertani broth (LB) were utilized for DNA extraction.
This procedure was done by using commercially available DUAL Genomic DNA isolation kit (Blood/cultured cell/Fungus) (Gene-Direx). The procedure was explained in detail in the user's manual DNA amplification by PCR.

Primers used for PCR
The primers were prepared according to the manufacturer's instructions (Eurofins Genomics) by dissolving the lyophilized product in sterile deionized water after centrifugation. Briefly, a working primer tube was prepared by diluting it with deionized. The final number of picomoles depended on the specific primer procedure used.

PCR and Thermocycling Conditions
E. coli DNA templates were subjected to PCR using three sets of primers (F and R) to detect virulence factors and determine *E. coli* phylogenetic groups (refer to Table 1). The PCR was performed in a final reaction volume of 25 µL. Multiplex PCR was performed to detect virulence factors in order to differentiate the four pathotypes of EPEC, ETEC, EHEC, and EIEC. Each reaction tube was prepared by adding 10 µl of primer mixture, 2.5 µl of DNA template, and 12.5 µl of Master Mix (GeneDirex). For EAEC and DAEC, a duplex PCR was performed as follows: 1 µl of DNA template was added to 19 µl of the Master Mix and 1 µl of each primer, resulting in a final volume of 20 µl. The *E. coli* isolates were classified into phylogenetic groups using both the original and revised Clermont phylogenetic typing schemes. This was achieved through a triplex PCR reaction, where the amplification mixture was adjusted to a final volume of 25 µl. The mixture contained 2.5 µl of DNA template, 12.5 µl of Master Mix (Genedirex), and 1 µl of each diluted primer. The required volume was completed with sterile distilled water (15-17 µl). The size of the variable regions of virulence factors and *E. coli* phylogenetic groups was determined by PCR assay. The primer sequences, sizes of PCR products, and PCR conditions are presented in Tables 1 and 2. Electrophoresis was performed on 1.5% agarose gels to visualize the amplified targets, followed by staining with ethidium bromide.

PCR was used for Phylogenetic Grouping
The *E. coli* isolates were classified into phylogenetic groups using both the original and revised Clermont phylogenetic typing schemes. This was achieved by performing a triplex PCR reaction that targeted two genes, *ChuA* and *YjaA*, as well as an anonymous DNA fragment *TspE4*.C2 15-21-22, to identify phylogenetic groups A, B1, B2, and D[15-21-22], (See Figure 1).

Table 1. Specific Genes, PCR Conditions, primers, and expected products for PCR Assays for analysis of Diarrheagenic Pathogens.

Athotype Or Assay	Specific Gene	Primers (5′→3′)		Product size (bp)	Primer concentration (pmol) in mixture	References
EPEC	*bfpA*	F:	AAT GGT GCT TGC GCT TGC TGC	324	6.0	17
		R:	GCC GCT TTA TCC AAC CTG GTA		6.0	
EPEC	*eaeA*	F:	GAC CCG GCA CAA GCA TAA GC	384	6.3	17
		R:	CCA CCT GCA GCA ACA AGA GG		6.3	
ETEC	*st*	F:	ATT TTT CTT TCT GTA TTG TCT T	190	6.3	17
		R:	CAC CCG GTA CAA GCA GGA TT		6.3	
ETEC	*lt*	F:	GGC GAC AGA TTA TAC CGT GC	450	6.3	17
		R:	CGG TCT CTA TAT TCC CTG TT		6.3	
EHEC	*stx* 1	F:	CTG GAT TTA ATG TCG CAT AGT G	150	6.0	17
		R:	AGA ACG CCC ACT GAG ATC ATC		6.0	
EHEC	*stx* 2	F:	GGC ACT GTC TGA AAC TGC TCC	255	6.0	17

|		R: TCG CCA GTT ATC TGA CAT TCTG		6.0	
EIEC	*ial*	F: GGT ATG ATG ATG ATG AGT CCA	650	6.3	17
		R: GGA GGC CAA CAA TTA TTT CC		6.3	
EAEC	*aggR*	F: GTA TAC ACA AAA GAA GGA AGC	254	5.0	17
		R: ACA GAA TCG TCA GCA TCA GC		5.0	
DAEC	*daa* E	F: GAA CGT TGG TTA ATG TGG GGTAA	542	5.0	17
		R: TAT TCA CCG GTC GGT TAT CAG		5.0	
Phylogenetic assay	ChuA.1	F: GAC GAA CCA ACG GTC AGG AT	279	10.0	15
	ChuA.2	R: TGC CGC CAG TAC CAA AGA CA		10.0	
Phylogenetic assay	YjaA.1	F: TGA AGT GTC AGG AGA CGC TG	211	10.0	15
	YjaA.2	R: ATG GAG AAT GCG TTC CTC AAC		10.0	
Phylogenetic assay	TspE4C2.1	F: GAG TAA TGT CGG GGC ATT CA	152	10.0	15
	TspE4C2.2	R: CGC GCC AAC AAA GTA TTA CG		10.0	
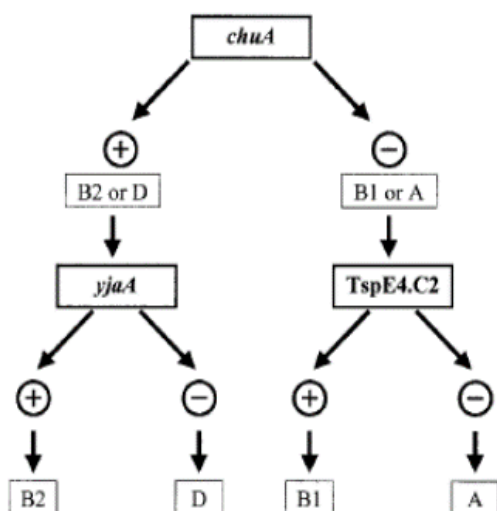
Figure 1 shows a dichotomous decision tree that can be used to determine the phylogenetic group of an *E. coli* strain based on the results of PCR amplification of the *chuA* and *yjaA* genes and DNA fragment TSPE4.C2.

RESULTS AND DISCUSSION

Detection of intestinal *E. coli* pathotypes: Multiplex and duplex PCR were performed to detect the six main categories of *E. coli*. A total of 100 children with diarrhea were included in the study. Diarrheagenic *E. coli* was isolated from 48 out of 100 (48%) of the children. These isolates tested positive for virulence factor genes associated with diarrheagenic *E. coli*. The frequency of each pathotype of DEC is shown in Table 2. According to Figure 2, the most frequently isolated pathotypes were atypical enteropathogenic *Escherichia coli* (EPEC) (42%). Among the bacterial strains, EPEC was the most prevalent followed by ETEC (4%) and EIEC (2%). EHEC, EAEC, and DAEC were not detected in any of the samples (0%). Among all clinical isolates, the most frequent pathotypes were EPEC. Interestingly, most of the strains that tested positive for the *eaeA* gene and they negative for *bfpA*.
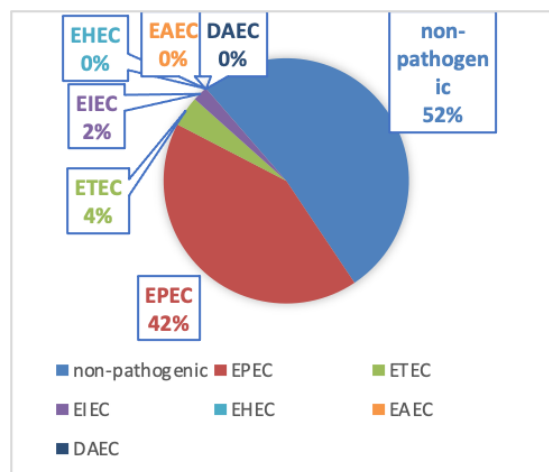
Figure 2. Frequency of *Escherichia coli* pathotypes and none-pathotypes in children with diarrhea.

Table 2. Specific genes, PCR conditions, primers, for PCR assays for analysis of diarrheagenic.

Pathotype or assay	Specific gene	PCR conditions	references
EPEC	*bfpA*		17
EPEC	*eaeA*		17
ETEC	*st*	3 minutes at 94°C; 30 cycles. 1 minute at 94°C, followed by 1 minute at 56°C. 1 minute at 72°C; followed by 5 minutes at 72°C.	17
ETEC	*lt*		17
EHEC	*stx* 1		17
EHEC	*stx* 2		17

|			
EIEC	*ial*		17
EAEC	*aggR*	2 minutes at 94℃; followed by 8 cycles. 15 seconds at 94℃, followed by a 30-second at a temperature range of 66℃ to 59℃.	17
DAEC	*daa* E	30 seconds at 72℃ for 32 cycles. 15 seconds at 94℃, followed by 30 seconds at 59℃. 30 seconds at 72℃; 5 minutes at 72℃.	17
Phylogenetic assay	ChuA.1 ChuA.2	5 minutes at 94℃, followed by 30 cycles of 30 s at 94℃ and 30 seconds at 55℃. and 7 minutes at 72℃. 72℃	15
Phylogenetic assay	YjaA.1 YjaA.2		15
Phylogenetic assay	TspE4C2.1 TspE4C2.2		15

Phylogenetic grouping: The triplex PCR described by Clermont et al, 2000. was used for phylogenetic grouping analysis. The method enabled the detection of the four main phylogenetic groups of *E. coli*, namely A, B1, B2, and D**.** by targeting two marker genes (*chuA* and *yjaA*) and a DNA fragment *TSPE4.C2*.

(*chuA-, yjaA+l-, TSPE4.C2-*) ☐ group A
(*chuA-, yjaA+l-, TSPE4.C2+*)☐ group B1
(*chuA+, yjaA+, TSPE4.C2+l-*)☐ group B2
(*chuA+, yjaA-, TSPE4.C2-l+*)☐ group D

Out of 100 *E. coli* isolates, the most distributed phylogenetic group of *E. coli* was "Phylogenetic group B2" which comprised 62/100 (62%), followed by "Phylogenetic group D" that comprised 33/100 (33%), followed by "Phylogenetic group A" strains 3/100 (3%). Strains of the "Phylogenetic group B1" were rare 2/100 (2%), According to the Figure 3.

Phylogenetic and virulence: by studying the relationship between the Phylogenetic and virulence the results showed that, there were 48/100 (48%) among strains containing virulence genes. The prevalence of all virulence genes was significantly higher in strains belonging to "phylogenetic group B2" (31/48 or 64.5%) compared to the other phylogenetic groups. The next highest prevalence was found in "phylogenetic

group D" (16/48 or 33.34%), followed by "phylogenetic group A" (1/48 or 2.16%).
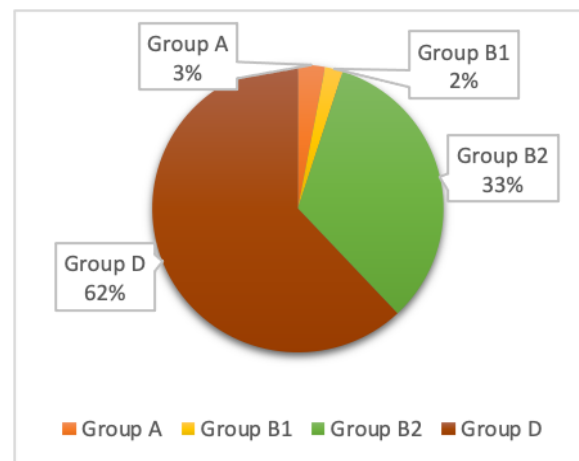

Figure 3. Phylogenetic distributions of *Escherichia coli* isolates.

However, virulence genes were absent for both strains belong to "phylogenetic group B1"(0/48) 0%, and non-pathogenic strains. A total of 52/100 (52%) non-pathogenic strains were belonged mainly to phylogenetic group of B2" which comprised 31/52 (59.7%), followed by "Phylogenetic group D" that comprised 17/52 (32.7%), "Phylogenetic group A and B1" which were rare 2/52 (3.8%) for each one. As shown in (Table 3) and Figures 4, 5 and 6.

Table 3. Distribution of clinical *E. coli* pathotypes among phylogenetic groups.

Characteristics	Group A		Group B1		Group B2		Group D		Total	
	NO	%	NO	%	NO	%	NO	%	NO	%
Pathogenic	1/48	2.08	0/48	0	31/48	64.58	16/48	33.34	48	100
EPEC	1/42	2.4	0/42	0	27/42	64.2	14/42	33.3	42	100
ETEC	0/4	0	0/4	0	2/4	50	2/4	50	4	100
EIEC	0/4	0	0/4	0	2/4	100	0/4	0	2	100

EHEC	0/0	0	0/0	0	0/0	0	0/0	0	0	0
EAEC	0/0	0	0/0	0	0/0	0	0/0	0	0	0
DAEC	0/0	0	0/0	0	0/0	0	0/0	0	0	0
Non-pathogenic	2/52	3.8	2/52	3.8	31/52	59.7	17/52	32.7	52	100
Total.	3/100		2/100		62/100		33/100			100
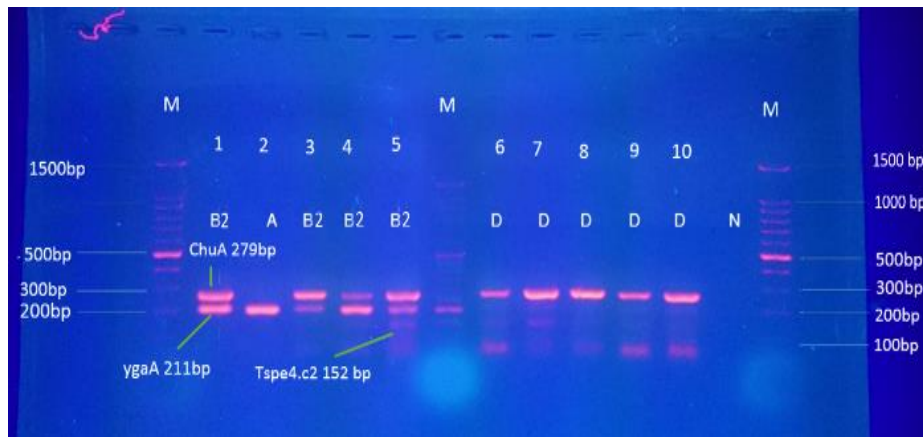
Figure 4. Multiplex PCR results of some clinical samples. The sizes of each of the following genes are shown *ChuA* 279bp, *YgaA* 211bp, Tspe4.c2 152bp Multiplex PCR results of some clinical samples M=DNA marker, N=Negative samples, A, B1, B2, D= the phylogenetic grouping of *E. coli* isolates was made on the basis of the presence of specific PCR-amplified fragments.
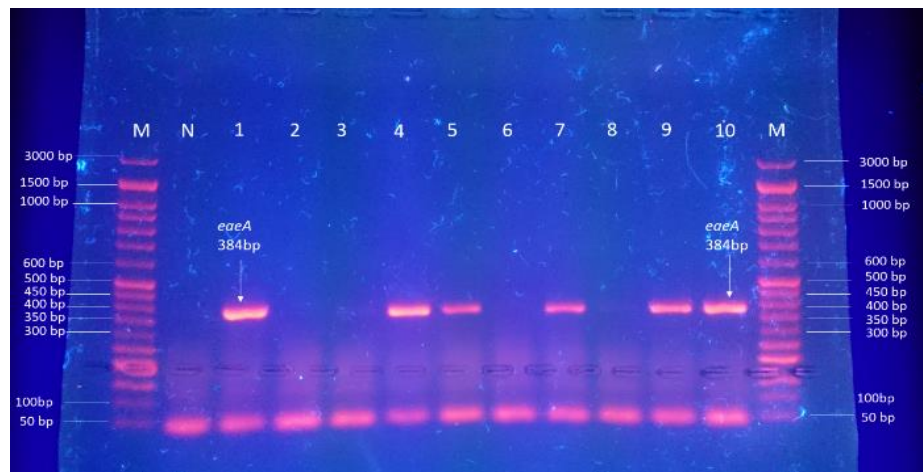
Figure 5. Multiplex PCR results of some clinical samples which show the size of the gene *eaeA* 384bpM=DNA marker, N=Negative samples, 1-10 represents isolates number.
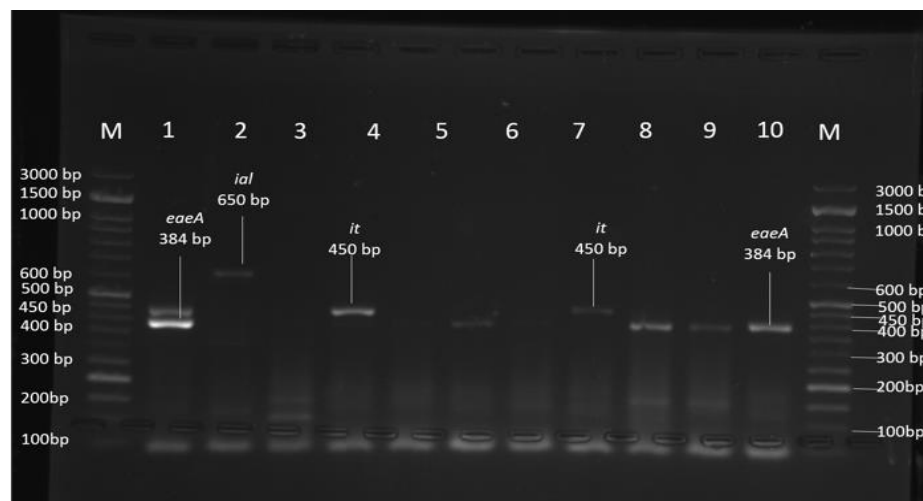
Figure 6. Multiplex PCR results of some clinical samples, the size of each of the following genes are shown *eaeA* 384bp, *ial* 650bp, it 450bp. M=DNA marker, 1---10 represents an isolated number.

E. coli is considered an important cause of diarrhea in children under five years old in all developing countries. The incidence varies from country to country according to different studies. The prevalence of DEC in the current study was significantly higher in children with diarrhea (48/100) 48% in comparison with some global studies as shown in (Table 3), the results were similar to ours. Also, the Iranian study came close to the results of the current study and similar results have also been reported in Sweden. This High rate of *E. coli* isolation can be explained due to the low health care conditions in Syria such as an exposure to stressful conditions such as heat, which leads to sweating and drinking water.

Table 3. comparison with related works.

DEC	Country	references
48%	Syria	current study
38.6%	Iraq	[24]
50.29%	Iran	[25]
53.8%	Nicaragua	[26]

In our study, EPEC was the most common DEC pathotypes spreading in children (42/48) 87.5%, making it the most frequent type among pathogenic *E. coli*. EPEC is known to be one of the major causes of diarrhea in children, particularly those who are under two years old [28]. This result is close to Zhou *et al*, study in China 50% [29] and with study in Brazil 52.6% [30]. However, our results are far a little bit from study in Iraq 42.75% [3], whereas, with other studies, like [17] it was 21.15%. The incompatibility in percentages is due to the difference in environmental conditions and geographical location from which the samples were collected. In addition, it is because of the age variation, ages of children, health status and the type of food and body immunity. On the other hand, the current war situation in the country of study, Syria, has played a great role in spread of many bacterial species in Syrian society, such as *E. coli*, and permitted them to access to many sources that are in direct contact with children, such as water supply, food and even playing areas. in addition to the role of some physiological factors can be added. ETEC was the second most pathotypes spreading in children (4/48) 8.33%, this result is with a study in Cost Rica 7.69% [17]. Also, Brazilian and Iranian study, 6,3%,7.2% [30-32] Respectively. ETEC is more prevalent in developing countries

and is a major cause of travelers' diarrhea [33]. This is consistent with our results. EIEC was found to be less pathogenic in children, with only 2 out of 84 cases (4.16%). Our results are consistent with a study conducted in Brazil, which reported a prevalence of 4.4% [30]. Similarly, Iranian studies have reported prevalence rates of 3.1% and 1.8%, respectively [25-32]. whereas it is far a little bit with a study in Colombia, South America 0.29% [34] whereas with the results of CostRica study 19.23%. EHEC was not detected in any sample (0%), similar results have also been reported in Taiwan [35]. EAEC and DAEC were not detected in any sample (0%) these did not agree with almost all studies. This result can be explained by the non-spread of this pattern in the city of Aleppo Our study showed the *E. coli* isolated from children under 5 years old were belonging to four phylogenetic groups (A, B1, B2, and D), the most prevalent group was phylogroup B2(62%), followed by phylogroup D (33%). Numerous studies conducted worldwide have shown that *E. coli* strains containing extraintestinal virulence factors are mostly found in groups B2 and D36. Phylogroups A and B1 were detected at lower rates of 3% and 2%, respectively. Whereas most commensal *E. coli* belong to group A and B1 [16]. The results showed that Enteropathogenic *E. coli* (EPEC) is most commonly belongs to group B2 (64.2%) and group D with a rate (33.3%). Several studies have shown that groups B2 and D are more virulent due to their association with the presence of virulence factors [37].

CONCLUSIONS
The results of the study suggest that various pathotypes of *Escherichia coli* play an important role in causing diarrhea in children under the age of five. The presence of these pathogenic patterns EPEC, ETEC and EIEC was first recorded in Syria and the ability of this pattern to cause diarrhea in children has been confirmed in contrast to what is believed to be *Escherichia coli* coexisting in the intestines. EPEC was the most dominant group.

Ethical approval

All authors granted approval for the study. Prior to participation, written informed consent was acquired from the interviewees/participants' parents or guardians after a comprehensive explanation regarding the study's content and objectives.

Disclosure and Conflict of Interest: The authors declare that they have no conflicts of interest.

REFERENCES

[1] C.L. Fischer Walker, , M. J. Aryee, C. Boschi-Pinto, and R. E. Black. "Estimating diarrhea mortality among young children in low and middle income countries." PloS one 7, no. 1 (2012): e29151.
https://doi.org/10.1371/journal.pone.0029151

[2] T.A. Gomes, W.P.Elias, I.C.Scaletsky, B.E.Guth, J.F. Rodrigues,R.M. Piazza,L. Ferreira, and M.B. Martinez. Diarrheagenic Escherichia coli. brazilian journal of microbiologyvol 47, pp.3-30, 2016.
https://doi.org/10.1016/j.bjm.2016.10.015

[3] R.C. Dias, B.C. dos Santos, L.F. dos Santos, M.A.Vieira, R.S. Yamatogi, A.L.Mondelli and R.T. Hernandes. Diarrheagenic Escherichia coli pathotypes investigation revealed atypical enteropathogenic E. coli as putative emerging diarrheal agents in children living in Botucatu, São Paulo State, Brazil. Apmis vol 124, no 4), pp. 299-308., 2016.
https://doi.org/10.1111/apm.12501

[4] M.E. Alvarez-Suarez,A. Otero, M.L. Garcia-Lopez, G. Dahbi, M. B., Azucena Mora, J. Blanco, and J. A. Santos. "Genetic characterization of Shiga toxin-producing Escherichia coli (STEC) and atypical enteropathogenic Escherichia coli (EPEC) isolates from goat's milk and goat farm environment." International journal of food microbiology vol 236, pp.148-154, 2016.
https://doi.org/10.1016/j.ijfoodmicro.2016.07.035

[5] L.R. Trabulsi, , R. Keller, and T. A. Tardelli Gomes. "10.321/eid0805. Typical and Atypical Enteropathogenic Escherichia coli." Emerging infectious diseases vol 8, no. 5, pp. 508.2002.
https://doi.org/10.3201/eid0805.010385

[6] J. Liu, R. Zhou, L. Li, B. M. Peters, B. Li, C. Lin, T. Chuang et al. "Viable but non-culturable state and toxin gene expression of enterohemorrhagic Escherichia coli O157 under cryopreservation." Research in Microbiology, Vol. 168, no. 3 pp: 188-19, 2017.
https://doi.org/10.1016/j.resmic.2016.11.002

[7] K. Saito, R. Suzuki, Y. Koyanagi, H. Isogai, H. Yoneyama, and E. Isogai. "Inhibition of enterohemorrhagic Escherichia coli O157: H7 infection in a gnotobiotic mouse model with pre colonization by Bacteroides strains." Biomedical reports vol.10, no.3 pp: 175-182, 2019.
https://doi.org/10.3892/br.2019.1193

[8] B. Hebbelstrup Jensen, A. Poulsen, S. Hebbelstrup Rye Rasmussen, C. Struve, J.H. Engberg,A. Friis-Møller, N. Boisen,R. Jønsson, R.F. Petersen, A.M Petersen andK.A. Krogfelt, K.A. Genetic virulence profile of enteroaggregative Escherichia coli strains isolated from Danish children with either acute or persistent diarrhea. Frontiers in cellular and infection microbiology, vol. 7, pp. 230,2017.
https://doi.org/10.3389/fcimb.2017.00230

[9] E.Mendez Arancibia, C. Pitart, J. Ruiz, F.Marco, J. Gascon, and J Vila, Evolution of antimicrobial resistance in enteroaggregative Escherichia coli and enterotoxigenic Escherichia coli causing traveller's diarrhoea. Journal of antimicrobial chemotherapy, vol 64, no 2 2pp.343-347,2009.
https://doi.org/10.1093/jac/dkp178

[10] E. A. Pettengill, J.B. Pettengill, and R Binet, Phylogenetic analyses of Shigella and enteroinvasive Escherichia coli for the identification of molecular epidemiological markers: whole-genome comparative analysis does not support distinct genera designation. Frontiers in microbiology, vol. 6, pp.1573,2016.
https://doi.org/10.3389/fmicb.2015.01573

[11] V.Michelacci, G., Prosseda, A. Maugliani, R. Tozzoli, S., Sanchez, S., Herrera-León and S., Morabito, Characterization of an emergent clone of enteroinvasive Escherichia coli circulating in Europe. Clinical Microbiology and Infection, 2016,vol. no. 3, 287-e11,2016.
https://doi.org/10.1016/j.cmi.2015.10.025

[12] C.R. da Silva, M.S, Sanches, K.H., Macedo, A.ML., Dambrozio, S.P.D, A., Navarro and J.S. Pelayo, J. S.-Molecular and phenotypic characterization of diarrheagenic Escherichia coli isolated from groundwater in rural areas in southern Brazil. Journal of water and health, vol 17, no 4, pp. 597-608,2019.
https://doi.org/10.2166/wh.2019.142

[13] A. A. Crofts, S.M. Giovanetti, E.J., Rubin, F. M. Poly, R. L., Gutiérrez, K.R., Talaat and M. Maciel, Enterotoxigenic E. coli virulence gene regulation in human infections. Proceedings of the National Academy of Sciences, vol 115, no 38, pp. E8968-E8976,2018.
https://doi.org/10.1073/pnas.1808982115

[14] J. Liu, S. Silapong, P. Jeanwattanalert, P. Lertsehtakarn, L. Bodhidatta, B. Swierczewski,C. Mason, A. L. McVeigh, S. J. Savarino,R. Nshama,E. Mduma,A. Maro,J. Zhang,J. Gratz,E. R. Houpt. Multiplex real time PCR panels to identify fourteen colonization factors of

enterotoxigenic Escherichia coli (ETEC). PloS one, vol 12, no 5 2 e0176882,2017.
https://doi.org/10.1371/journal.pone.0176882

[15] O. Clermont, S., Bonacorsi and E. Bingen. Rapid and simple determination of the Escherichia coli phylogenetic group. Applied and environmental microbiology., vol 66, no 10, pp. 4555-4558,2000.
https://doi.org/10.1128/AEM.66.10.4555-4558.2000

[16] S. Bashir, A. Haque, Y.Sarwar, A. Ali, and M.I. Anwar. Virulence profile of different phylogenetic groups of locally isolated community acquired uropathogenic E. coli from Faisalabad region of Pakistan. Annals of clinical microbiology and antimicrobials, vol 11, no1, pp. 23,2012.
https://doi.org/10.1186/1476-0711-11-23

[17] C. Pérez, O.G. Gómez-Duarte and M.L. Arias. Diarrheagenic Escherichia coli in children from Costa Rica. The American journal of tropical medicine and hygiene, vol 83, no 2, pp. 292-297, 2010.
https://doi.org/10.4269/ajtmh.2010.09-0412

[18] M. Vidal, E. Kruger, C. Durán, R. Lagos, M. Levine, V. Prado, C. Toro, and R. Vidal."Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic Escherichia coli associated with enteric infections." Journal of clinical microbiology vol 43, no. 10. pp. 5362-5365, 2005.
https://doi.org/10.1128/JCM.43.10.5362-5365.2005

[19] P. De Vos, G. Garrity, D. Jones, N.R. Kri, W. Ludwig, F.A. Rainey et al. (Eds.). - Bergey's Manual of Systematic Bacteriology: Volume 3: The Firmicutes (Vol. 3). Sp ringer Science & Business Media.2009 .

[20] G. M. Garrity, J.A. Bell, and T.G. Lilburn. Taxonomic outline of the prokaryotes. Bergey's manual of systematic bacteriology. Springer, New York, Berlin, Heidelberg.2004 .

[21] O. Clermont, J.K. Christenson, E., Denamur and D.M. Gordon. The Clermont E Escherichia coli phylotyping method revisited: improvement of specificity and detection of new phylo-groups. Environmental microbiology reports, vol 5, no 1, pp. 58-65,2013.
https://doi.org/10.1111/1758-2229.12019

[22] C.M Logue, Y. Wannemuehler, B.A. Nicholson, C. Doetkott, N. L. Barbieri, L.K and Nolan. Comparative analysis of phylogenetic assignment of human and avian ExPEC and fecal commensal Escherichia coli using the (previous and revised) Clermont phylogenetic typing methods and its impact on avian pathogenic Escherichia coli (APEC) classification. Frontiers in microbiology, vol 8, pp. 283, 2017.
https://doi.org/10.3389/fmicb.2017.00283

[23] P. Duriez, O. Clermont, S. Bonacorsi, E. Bingen, A. Chaventré, J. Elion and E. Denamur.Commensal Escherichia coli isolates are phylogenetically distributed among geographically distinct human populations. Microbiology, 2001,vol 147, no 6, pp.1671-1676,2001.
https://doi.org/10.1099/00221287-147-6-1671

[24] Z.K Khalil.Isolation and identification of different diarrheagenic (DEC) Escherichia coli pathotypes from children under five years old in Baghdad. Iraqi Journal of Community MedicinE,vol 28, no 3, pp.126-132, 2015.

[25] S. Eybpoosh, S. Mostaan, M.M Gouya, H. Masoumi-Asl, P. Owlia, B. Eshrati and S.Bouzari. Frequency of five Escherichia coli pathotypes in Iranian adults and children with acute diarrhea. BioRxiv, 725952, 2019.
https://doi.org/10.1101/725952

[26] S. Vilchez, D. Reyes, M. Paniagua, F., Bucardo, R. Möllby, and A. Weintraub. Prevalence of diarrhoeagenic Escherichia coli in children from Leon, Nicaragua. Journal of medical microbiology, vol 58, no 5, pp. 630-637, 2009.
https://doi.org/10.1099/jmm.0.007369-0

[27] D. S. NewburG. Oligosaccharides in Human Milk and Bacterial Colonization. Journal of pediatric gastroenterology and nutrition, vol. 30, pp. S8-S17, 2000.
https://doi.org/10.1097/00005176-200000002-00003

[28] T. J. Ochoa, F.Barletta, C. Contreras and E Mercado. New insights into the epidemiology of enteropathogenic Escherichia coli infection. Transactions of the Royal Society of Tropical Medicine and Hygiene, vol 102, no 9, 852-856, 2008.
https://doi.org/10.1016/j.trstmh.2008.03.017

[29] Y. Zhou, X. Zhu, H. Hou, Y. Lu, J. Yu, L. Mao and Z. Sun. Characteristics of diarrheagenic Escherichia coli among children under 5 years of age with acute diarrhea: a hospital-based study. BMC infectious diseases, vol 18, no 1, pp. 63, 2018.
https://doi.org/10.1186/s12879-017-2936-1

[30] E. L. Ori, E.H. Takagi, T.S. Andrade, B.T. Miguel, M.C. Cergole-Novella, B.E.C. Guth, E. C. Romero. Diarrhoeagenic Escherichia coli and Escherichia albertii in Brazil: pathotypes and serotypes over a 6-year period of surveillance. Epidemiology & Infection, 147, e 10 2019.
https://doi.org/10.1017/S0950268818002595

[31] A. M. Turkey, I.A.Abdul-Rahman and J.J. Abed. Multiple antibiotic resistance of enteropathogenic Escherichia coli which caused diarrhoea for infants. Al-Anbar Journal of Veterinary Sciences, vol 5, no2, pp. 211-220, 2012.

[32] Z. Nazari, K. Amini, A. Mokhtari. Detection of diarrhoeagenic Escherichia coli virulence genes by

multiplex-PCR method and their antibiotic susceptibility profile., Scientific Journal of Microbiology, vol 4, no 7, pp. 40-48, 2015.

[33] M. A. Croxen, R.J. Law,R. Scholz, K.M Keeney, M. Wlodarska, and B. Finlay. - Recent advances in understanding enteric pathogenic Escherichia coli. Clinical microbiology reviews, vol 26, no 4, pp. 822-880, 2015.
https://doi.org/10.1128/CMR.00022-13

[34] O. G. Gomez-Duarte, Y.C. Romero-Herazo, C.Z.Paez-Canro, J.H. Eslava-Schmalbach and O. Arzuza.- Enterotoxigenic Escherichia coli associated with childhood diarrhoea in Colombia, South America. The Journal of Infection in Developing Countries, vol 7, no 5, 372-381, 2013.
https://doi.org/10.3855/jidc.2667

[35] L. J. Teng, P. R. Hsueh, S. J. Liaw, S. W. Ho and J. C. Tsai. Genetic detection of diarrheagenic Escherichia coli isolated from children with sporadic diarrhea. Journal of Microbiology, Immunology and Infection, vol 37, no 6, pp. 334, 2004.

[36] J. K. Bailey, J. L. Pinyon, S. Anantham, S., & R.M. Hall. Distribution of human commensal Escherichia coli phylogenetic groups. Journal of clinical microbiology, vol 48, no 9, 3455-3456, 2010.
https://doi.org/10.1128/JCM.00760-10

[37] S. M. Soto, J. Bosch, M. J. De Anta and J. Vila.Comparative study of virulence traits of Escherichia coli clinical isolates causing early and late neonatal sepsis. Journal of clinical microbiology, vol 46 no, pp. 1123-1125, 2008.
https://doi.org/10.1128/JCM.01682-07

How to Cite