RESEARCH ARTICLE

Molecular Diagnosis of Diarrheagenic *E. coli* Infections Among the Pediatric Patients in Wasit Province, Iraq

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Abstract

Diarrheagenic *Escherichia coli* still an important pathogen that cause diarrhea which lead to hospital admissions and death specially in children. In order to identify the common pathotypes of *E. coli* via investigate different virulence genes. A total of 210 stool samples were collected from children under five years presented with diarrhea from different hospitals and private clinics in Wasit province, Iraq, on the other hand, 40 stool samples were collected from healthy children considered as control group. regarding to culture, biochemical tests and API 20E results 100 isolates were supposed to be *E. coli*. The DNA were extracted to that 100 isolates from diarrheal cases and for 40 isolates of control, concentration of DNA samples were between (50-360 mg/µl) and the purity between (1.8-2). All isolates studied for detectionvirulence gene of five Diarrheagenic *Escherichia Coli* strains based on using multiplex Polymerase Chain Reaction technique, by amplified 13 primer (*eaeA, bfpB, aggR, astA, pic, hly, stx1, stx2, invE, ipaH, elt, estIa, estIb*), and showed the distribution of the strains and its susceptibility to antibiotics. The most frequent pathotypes was Enteropathogenic *E.coli* 19/42 (45.3%)) with 9 typical and 10 atypical, followed by Enteroaggregative *E. coli* 17/42 (40.5%), Enterotoxigenic *E. coli* 3/42 (7.1%), Enteroinvasive *E. coli* 3/42 (7.1%), and 0/42 (0%) in Shigatoxin producing *E .coli* and no DEC in all control patients. The highest resistance to antibiotics was (95.2%) to Amoxicillin and Ampicillin, respectively, Sulfa-Trimethoprim 92.9%, followed by 85.7% for Tetracycline and Cephalothin, Ceftriaxone 81% and Cefotaxim "clavulanic acid 71.4%. While the lowest resistance was to Chloramphenicol (19 %), Ciprofloxacin (16.7%), Amikacin (7.1%) and no resistance was detected toward Imipenem. We can conclude in this study, multiplex PCR is a swift, and accurate procedure can be used for Diarrheagenic *E.coli* identification and isolation successfully of strains.

Key words: Diarrheagenic *E.coli* , virulence genes , Multiplex PCR.

INTRODUCTION

Diarrheal disease is still a global problem around the world specially in children under five years in developing countries[1]. According to the World Health Organization (WHO), diarrheal diseases are the second leading cause of death (~760,000 per year) in children [2,3]. The microbial causes of diarrhea are variety of bacterial, viral and parasite [4], Among these pathogens, diarrheagenic *E. coli* play a major role in causing diarrhea in children under 5 years [5,6,7].

When the microbial agent is bacteria, *E. coli* consider one of the major causes, specially to infantile diarrhea [8,9,10]. Depending on specific virulence gene, clinical features, and serotypes diarrheagenic *E.coli* divided into 6 stains: Enteropathogenic *E. coli*, Enteroaggregative *E. coli*, Enteroinvasive *E. coli*, Enterotoxigenic *E. coli*, Shiga toxin-producing *E. coli* and Diffusely Adherence *E. coli* [11]. Culture and biochemical test can't distinguished between commensal or pathogenic strains of *E. coli* in stool, therefor PCR used to detect the virulence genes in pathogenic strains [12], multiplex PCR provide detection to many Diarrheagenic *E.coli* strains virulence genes with high sensitivity, specificity [13].

The aim of thisstudy was detecting the distribution of diarrheagenic *E. coli* pathotypes among children with diarrhea in Wasit province, Iraq by multiplex PCR, and assessing the antimicrobial susceptibility profile of diarrheagenic *E. coli*, in order to contribute to the establishment of a more effective empirical antibiotic therapy for the disease.

MATERIALS AND METHODS
Collection of samples

During period from middle of September 2017 to middle of December 2017 a total of 210 stool samples were collected from children

Table 1. Primers used for multiplex PCR reaction

E.coli strain	Primers	Primers (Sequence (5' – 3'))	Product size (bp)	References
	aggR	aggR-F: ACGCAGAGTTGCCTGATAAAG		
EAEC		aggR-R: AATACAGAATCGTCAGCATCAGC	400	
	astA	astA-F: TGCCATCAACACAGTATATCCG		
		astA-R: ACGGCTTTGTAGTCCTTCCAT	102	16
	pic	pic-F: AGCCGTTTCCGCAGAAGCC		
		pic-R: AAATGTCAGTGAACCGACGATTGG	1,111	
	hly	hly-F: TTCTGGGAAACAGTGACGCACATA	688	
		hly-R: TCACCGATCTTCTCATCCCAATG 0.1		17
STEC	**stx1**	stx1A-F: CGATGTTACGGTTTGTTACTGTGACAGC	244	
		stx1A-R: AATGCCACGCTTCCCAGAATTG		
	stx2	stx2AF: GTTTTGACCATCTTCGTCTGATTATTGAG	324	16
		stx2A-R: AGCGTAAGGCTTCTGCTGTGAC		
	invE	invE-F: CGATAGATGGCGAGAAATTATATCCCG	766	
		invE-R:CGATCAAGAATCCCTAACAGAAGAATCAC		
EIEC	**ipaH**	ipaH-F: GAAAACCCTCCTGGTCCATCAGG	437	18
		ipaH-R: GCCGGTCAGCCACCCTCTGAGAGTAC		
	eaeA	eae-F: TCAATGCAGTTCCGTTATCAGTT	482	
		eae-R: GTAAAGTCCGTTACCCCAACCTG		
EPEC	**bfpB**	bfpB-F: GACACCTCATTGCTGAAGTCG	910	
		bfpB-R: CCAGAACACCTCCGTTATGC		
	elt	elt -F: GAACAGGAGGTTTCTGCGTTAGGTG	655	
		elt -R: CTTTCAATGGCTTTTTTTTGGGAGTC		16
ETEC	**estIa**	estIa-F:CCTCTTTTAGYCAGACARCTGAATCASTTG	157	
		estIa -R: CAGGCAGGATTACAACAAAGTTCACAG		
	estIb	estIb-F: TGTCTTTTTCACCTTTCGCTC	171	
		estIb-R: CGGTACAAGCAGGATTACAACAC		

(males and females) with an ages under five years presented with diarrhea had been admitted at hospitals and attended at private clinics in Wasit province, Iraq. Otherwise, 40 stool samples were collected from healthy children considered as control. The stool samples transported on Carry Blair swabs and cultured on MacConkey agar, XLD, EMB, Blood agar, and CHROMagarSTEC and incubated aerobically at 37 °C for 24 hours, the isolated bacteria was identified according to morphological, biochemical tests and API 20E kit.

Antibiotic susceptibility test: performed by Kirby-Bauer procedure on Muller Hinton agar [14] and results interpreted according to Clinical and Laboratory Standards Institute[15]

DNA extraction: was performed according to the procedure (Geneaid Genomic DNA extraction Kit).

Multiplex PCR technique: was used for amplifying the genes. The mixture reaction was performed in a total volume 50 µl of PCR Mastermix Gold Multiplex 50x (DNA Template 4 µl, Forward primer 1 µl for each primer, Reverse primer 1 µl for each primer, free water ddH2O 20µl). PCR cycling program parameters used in this reaction for detection of (*bfpB* , *eaeA* ,*pic* , *aggR* , *astA* ,*invE* , *ipaH* , *hly*, *stx1*,*stx2*, *elt* ,*estla* ,*estlb*) genes as shown in table (1), the thermal cycling program(Initial denaturation 95°C for 5 min. 1cycle), (Denaturation 94°C for 30 sec 35 cycle), (Annealing 58°C for 30 sec. 35 cycle), (Extension 72°C for 1 min. 35cycle), (Final extension 72° C for 7min. 1 cycle) (Holding 4° C 1 cycle). The amplification products were electrophoresed through a 2 % agarose gel and visualized with UV transilluminator after ethidium bromide staining. A 100 bp DNA ladder was used as a molecular size marker in gel. The statistical analysis of all the evidence was done using the system SPSS IBM version 20 software, Chi-squire test. P- value ≤ 0.05 was considered statistically significant.

RESULTS

E. coli were isolated in 100 (47.6 %) of 210 collected samples followed by 78 (37.2%) of other gram negative bacteria (Salmonella, Klebsiella, Proteus, Pseudomonas) and 32 (15.2 %) samples that were no growth. The results of primary diagnosis to these 100 *E. coli* isolates by selective and differential culture media were consistent with the microscopic and biochemical tests results.

Multiplex applied on theses 100 and 40 control samples and the results showed that DEC in were detected in 42/ 100 (42 %) among diarrheal children compared with 0/100 (0%) among control children. The distribution of 42 DEC pathotype isolates were: EPEC was found in 19 (45.3%), EAEC in 17(40.5%), ETEC in 3 (7.1%), EIEC in 3 (7.1%) and 0 (0%) in STEC and controls.

From 19 isolates detected as EPEC which was watery diarrhea 10 (52.6%) isolates of them are atypical EPEC showed *eaeA* gene found without *bfpB* gene, and 9 (47.4 %) were typical EPEC which showed *eaeA* gene together with *bfpB* gene. All 19 isolates in our study don't produce nether stx1 or stx2, in addition one of aEPEC showed *astA* gene.

Enteroaggregative *E. coli* 17 (40.5%) isolates came second after Enteropathogenic *E.coli* as causative agent of diarrhea among Diarrheagenic *E. coli* pathotypes in our study, aggR

Table 2. Antibiotic susceptibility test

Antibiotics	CEC	AMC	CTR	TE	SXT	AMP	CTL	C	CIP	IPM	AK
Sensitive	8	1	7	4	2	2	3	31	33	42	36
	19%	2.4%	16.7%	9.5%	4.8%	4.8%	7.1%	73.8%	78.6%	100%	85.7%
Intermediate	4	1	1	2	1	0	3	3	2	0	3
	9.5%	2.4%	2.4%	4.8%	2.4%	0%	7.1%	7.1%	4.8%	0%	7.1 %
Resistance	30	40	34	36	39	40	36	8	7	0	3
	71.4%	95.2%	81%	85.7%	92.9%	95.2%	85.7%	19%	16.7%	0%	7.1%
Chi squire	28	72.4	44.14	52	67	34.38	51.8	31.8	39.57	34.38	51.8

AK=Amikacin, AMC=Amoxiclav, AMP=Ampicillin, CTR=Ceftriaxone, CTL=Cephalothin, C= Chloramphenicol, CIP=Ciprofloxacin, CEC=Cefotaxime/Clavulanic, IPM=Imipenem TE=Tetracycline, STX=Sulfa-Trimethoprim

gene was appeared in all EAEC isolates detected in our study that mean all of them were typical EAEC. Enterotoxigenic *E.coli* account 3 isolates (7.1%) and Enteroinvasive *E. coli* was detected in 3 isolates (7.1%) that suggested these pathotype maybe play a lessimportant role in childhood diarrhoea in developingcountries. When age stratification was analysed high incidence of DEC *E. coli* recorded in first and second age group flowed by third and fourth age group, and there were no cases recorded in fifth age group.

The prevalence of Enteropathogenic *E. coli* infection infections was high in first and second years , also all Enteroaggregative *E.coli* infections were detected under 2 years, while Enteroinvasive *E. coli* were high between 2-3 years also cause infection in first age group, in time all Enterotoxigenic *E.coli* infections were all above 1 year as shown in figure (1).

E. coli pathotypes, in our study were identified and isolated successfully by using Multiplex PCR. PCR products visualized to measured product size results from amplification the primers in compared with (100 bp) ladder as shown in figures (2, 3, 4, 5, 6, 7, 8, 9).

Antibiotic susceptibility test results were showed in table (2): The highest level of resistance were to Amoxiclav (95.2%), Ampicillin (95.2%), Sulfa -Trimethoprim (92.9%) followed by Tetracycline (85.7%), Cephalthin (85.7%) Ceftriaxone (81%) Cefotaxime/clavulanicacid 71.4%. The maximum *E. coli* sensitivity was to Imipenem (100%) flowed by Amikacin (85.7%), Ciprofloxacin (78.6%) Chloramphenicol (73.8%).

DISCUSSION

The distribution of DEC in our study was 42 (20%) among 210 diarrheal cases. Our result concur to other study in Iraq reported by Hamada *et al* [12] in Kirkuk (36%) and globally with other studies in Iran Heidary *et al* [19] (28 %), while our result contrast with other studies were showed less prevalence to Diarrheageic *E. coli* Konate*et al* [20] revealed (7.4%) in Burkina Faso, Salmani*et al* [21] in Iran who showed (88%). These differences reflecting the difference in distribution of geographical areas , quality of sanitation .

Among all the Diarrheagenic *E.coli* pathotypes , Enteropathogenic *E.coli* (EPEC) were found to be the most common pathotypes for children with (45.3%), our result compatible with localstudy by Sakhi [22] who showed EPEC as most than other pathotypes (63%), and in contrast with Khalil [11] and Al-Dulaimi[23] where they show it came second after EAEC . Our finding was , however , similar to globally studies with Zhou *et al*[1], Thakur *et al*[7] and Chellapandi*etal*[6] that also reported a high frequency of EPEC pathotypes associated with pediatric diarrhea.

EPEC are sub-grouped into typical (tEPEC, *eae+ bfpA+*) and atypical (aEPEC, *eae+ bfpA-*) strains that differ in several respects Naji and Nasser [24] . From 19 isolates detected as EPEC which was watery diarrhea 10 (52.6%) isolates of them are atypical EPEC showed eaeA gene found without *bfpB* gene, and 9 (47.4 %) were typical EPEC which showed *eaeA* gene together with *bfpB* gene.
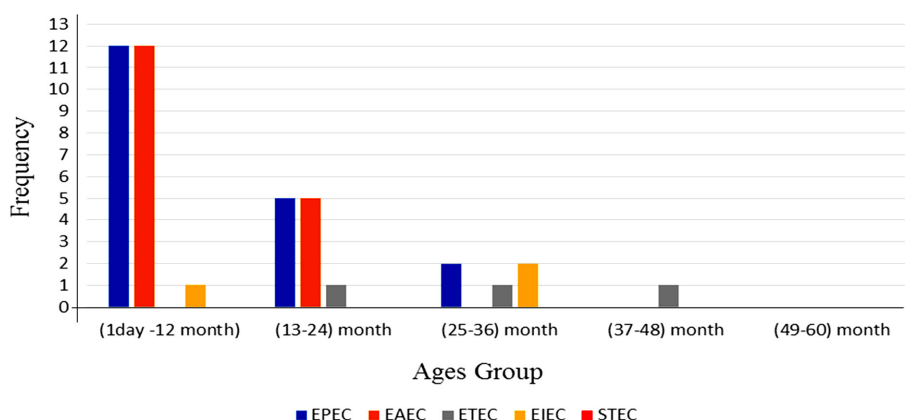

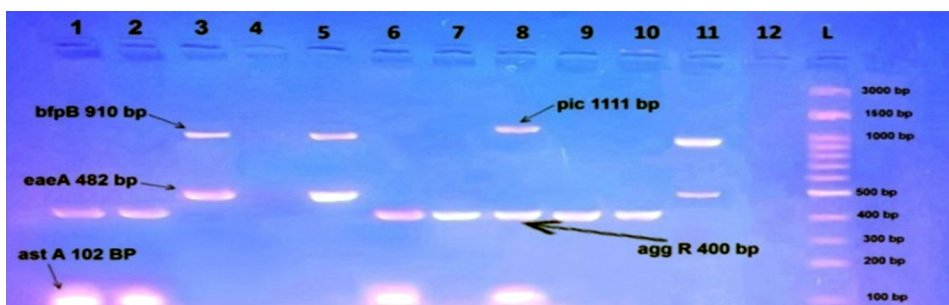**Fig.1.** Prevalence diarrheagenic *E. coli* with age groups.

Fig. 2. Gel electrophoresis of amplified (*eaeA , bfpB, aggR ,astA ,pic ,hly ,stx1 ,stx2 invE , ipaH, elt ,estIa ,estIb*) genes, the product size (482 , 910 , 400 , 102 , 1,111 , 688 , 244 , 324 , 766 , 437 , 655 , 157 , 171 (bp) respectively), of E .coli strains using conventional PCR . Agarose 2%, and TBE (1X) at (75 V/cm for 90 min., stained with Ethydium bromide dye and visualized on a UV transilluminator. Lane(L): DNA ladder (100-3000 bp), Lanes: (1-12) stool samples.
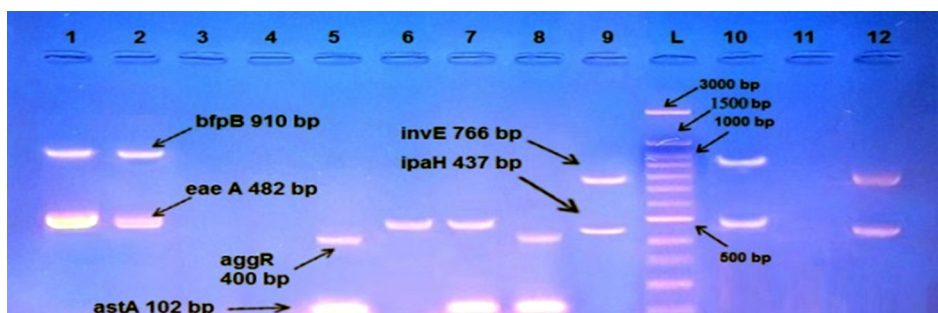

Fig. 3. Gel electrophoresis of amplified (*eaeA, bfpB, aggR, astA, pic, hly, stx1, stx2, invE, ipaH, elt ,estIa, estIb*) genes, the product size (482, 910, 400, 102, 1, 111, 688, 244, 324, 766, 437, 655, 157, 171 (bp) respectively), of *E .coli* strains using conventional PCR. Agarose 2%, and TBE(1X) at (75 V/cm for 90 min., stained with Ethydium bromide dye and visualized on a UV transilluminator. Lane(L): DNA ladder (100-3000bp) Lanes: (1-12) stool samples.

Fig. 4. Gel electrophoresis of amplified (*eaeA , bfpB, aggR, astA, pic, hly, stx1, stx2, invE, ipaH, elt ,estIa, estIb*) genes, the product size (482, 910, 400, 102, 1, 111, 688, 244, 324, 766, 437, 655, 157, 171(bp) respectively), of *E .coli* strains using conventional PCR. Agarose 2%, and TBE (1X) at (75 V/cm for 90 min., stained with Ethidium bromide dye and visualized on a UV transilluminator. Lane (L): DNA ladder (100-3000bp), Lanes: (1-12) stool samples.
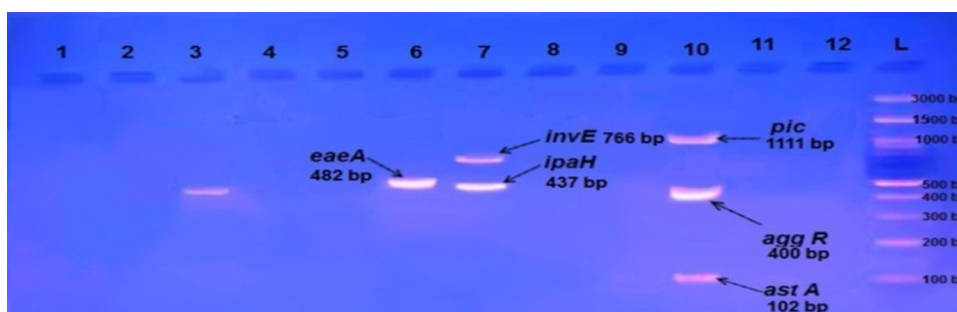
All 19 isolates in our study don't produce nether stx1 or stx2, in addition one of aEPEC showed *astA* gene. Our study is close to study by Arif and Salih[23] in Sulaimani, Iraq, and global reports by Malvi [25] in India, that showed the distribution of atypical EPEC was higher than typical EPEC. Ochoa and Contreras [26] report that atypical EPEC (aEPEC) are more prevalent than typical-EPEC (tEPEC).
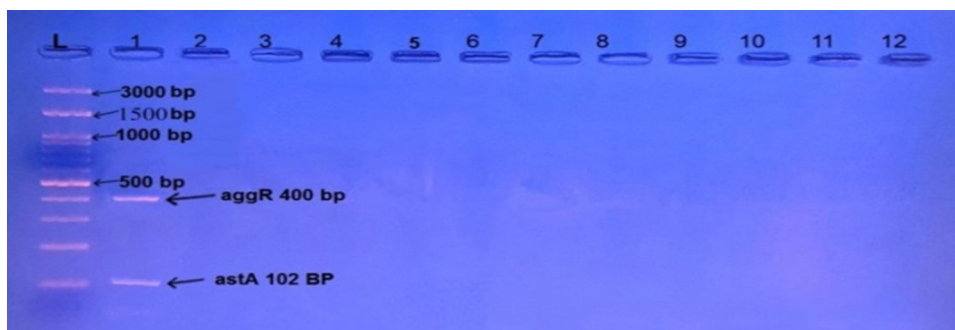
Fig. 5. Gel electrophoresis of amplified *(eaeA, bfpB, aggR, astA, pic, hly, stx1, stx2, invE, ipaH, elt, estIa, estIb)* genes, the product size (482, 910, 400, 102, 1, 111, 688, 244, 324, 766, 437, 655, 157, 171 (bp) respectively), of *E .coli* strains using conventional PCR . Agarose 2%, and TBE(1X) at (75 V/cm for 90 min., stained with Ethidium bromide dye and visualized on a UV transilluminator. Lane (L): DNA ladder (100-3000bp), Lanes: (1-12) stool sample.

Fig. 6. Gel electrophoresis of amplified (*eaeA, bfpB, aggR, astA, pic, hly, stx1, stx2, invE, ipaH, elt, estIa, estIb*) genes, the product size (482, 910, 400, 102, 1, 111, 688, 244, 324, 766, 437, 655, 157, 171 (bp) respectively), of *E .coli* strains using conventional PCR. Agarose 2%, and TBE (1X) at (75 V/cm for 90 min., stained with Ethidium bromide dye and visualized on a UV transilluminator. Lane (L): DNA ladder (100-3000 bp), Lanes: (1-12) stool samples.
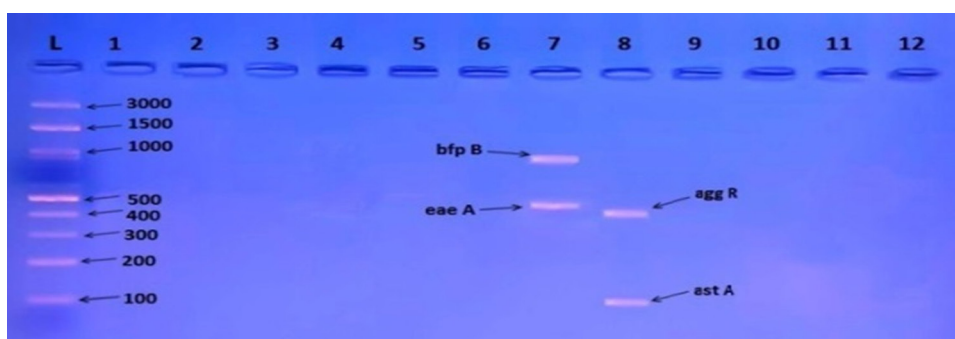

Fig. 7. Gel electrophoresis of amplified (*eaeA, bfpB, aggR, astA, pic, hly, stx1, stx2, invE, ipaH, elt, estIa ,estIb*) genes, the product size (482, 910, 400, 102, 1, 111, 688, 244, 324, 766, 437, 655, 157, 171 (bp) respectively), of *E. coli* strains using conventional PCR . Agarose 2%, and TBE (1X) at (75 V/cm for 90 min., stained with Ethidium bromide dye and visualized on a UV transilluminator. Lane (L): DNA ladder (100-3000bp), Lanes: (1-12) stool samples.
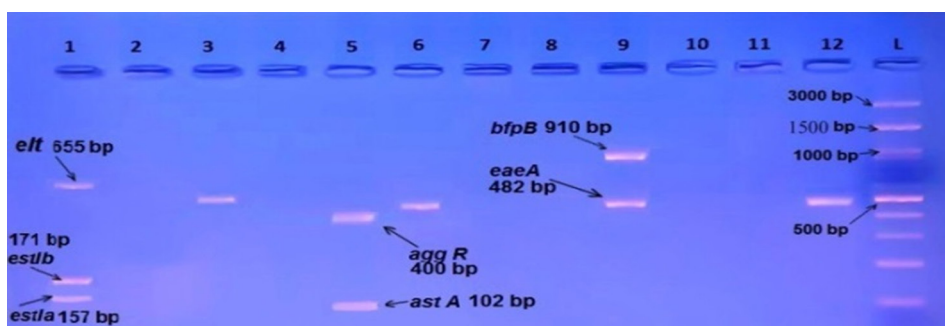
Enteroaggregative *E.coli* 17 (40.5%) isolates came second after Enteropathogenic *E. coli* as causative agent of diarrhea among Diarrheagenic *E. coli* pathotypes in our study, that agree with reports by Sakhi [22] in Dhi-Qar city, also Globally with Thakur *et al* [7] . But EAEC considered the major cause of diarrhea between diarrheagenic *E. coli* pathotypes in local studies by Khalil [11] and Al-Dulami [23] , also Globally, Rajendran*et al* [27] in India, Ali *et al* [28] in Egypt,

Fig. 8. Gel electrophoresis of amplified (eaeA, bfpB, aggR, astA, pic, hly, stx1, stx2, invE, ipaH, elt, estIa ,estIb) genes, the product size (482, 910, 400, 102, 1, 111, 688, 244, 324, 766, 437, 655, 157, 171 (bp) respectively), of *E. coli* strains using conventional PCR. Agarose 2%, and TBE (1X) at (75 V/cm for 90 min., stained with Ethidium bromide dye and visualized on a UV transilluminator. Lane (L): DNA ladder (100-3000bp), Lanes: (1-12) stool samples.

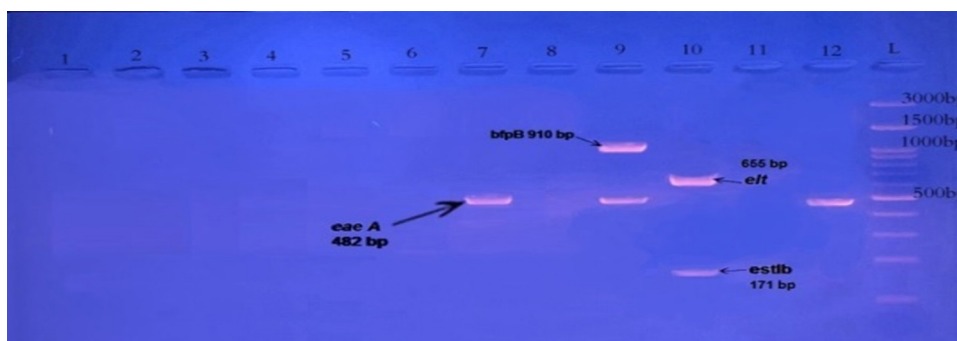**Fig. 9.** Gel electrophoresis of amplified (*eaeA, bfpB, aggR, astA, pic, hly, stx1, stx2, invE, ipaH, elt, estIa, estIb*) genes, the product size (482, 910, 400, 102, 1, 111, 688, 244, 324, 766, 437, 655, 157, 171 (bp) respectively), of *E. coli* strains using conventional PCR. Agarose 2%, and TBE (1X) at (75 V/cm for 90 min., stained with Ethidium bromide dye and visualized on a UV transilluminator. Lane (L): DNA ladder(100-3000 bp), Lanes: (1-12) stool samples.
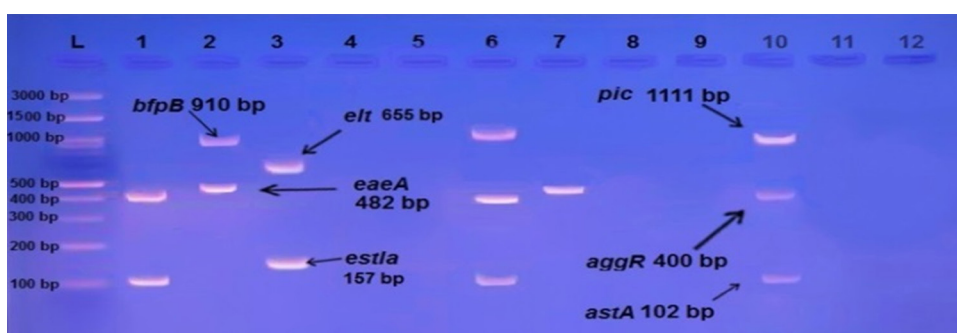
and Konate*et al* [20] in Burkina Faso.

EPEC and EAEC were reported as the most common Diarrheagenic *E. coli* pathotypes Bueris*et al* [29]; Moyo*et al* [30]; Wang *et al* [31].

Enterotoxigenic *E. coli* account 3 isolates (7.1%) of Diarrheagenic cases, the detection of ETEC is in consonance with previous local findings by Hamada *et al* [12], and concur with global study by Raghavan*et al* [32] in India. Our study differ from other reports by Chiyangi*et al* [33] in Zambia which suggested high prevalence of ETEC 40%.

Enteroinvasive *E. coli* was detected in 3 isolates (7.1%) of Diarrheagenic isolates. Our study is nearly close with local study by Hamada *et al* [12] (10%), and globally with (0.5%) by Moshtagian [34], and (12.9%) by Konate*et al* [20], and (3.7%) by Zhou*et al* [1].

Vieira *et al* [35] also showed low rate of prevalence Enteroinvasive *E. coli* and suggested

that this pathotype may be play a less important role in childhood diarrhea in developing countries. In this study, no Shiga toxin-producing *E. coli* were detected this result is similar to local reports of Sakhi [22] and Hamada *et al* [12]. Globally, Ali *et al* [28] and Canzalez-Roman [36], also showed no isolates of STEC were detected in children with diarrhea. STEC appears to be more frequent in adults than children Okeke*et al* [37]. These difference between our results and other studies may be attributed to rout of infection, virulence factors, pathogen strains, difference in population selection, time of collection and size of samples.

Antibiotic susceptibility test

Resistance to Amoxiclave (95.2%) agreement with another study had been reported high resistance in studies done by Al-Hilali [38] 83.4 % in Al-Najaf. Our result; disagreed with previous study in Alkut by Shamkhi[39] who recorded low

resistance to Amoxiclav with 7.1% AL-Shuwaikh *et al* [39] in Baghdad report 33.3%. Increased Amoxiclav resistance coincided with growing Amoxiclav consumption at the community level, similarly, the isolated Diarrheagenic *E. coli* pathotypes showed high resistance rates to Ampicillin and Sulfa-Trimethoprim Konate*et al* [41], as mention in study by Goossens*et al* [42]; Llor and Bjerrum [43] about antibiotic resistant, there is high resistant in the most consumption antibiotic. Sulfa-Trimethoprim is widely used in developing countries to treat diarrhea because of their availability over the countries Nguyen *et al* [44]. Attention should be given while prescribing Amoxiclav, Ceftriaxone, and Ampicillin to avoid increasing resistance pathotype by *E. coli*. Similar study conducted by Konate *et al* [41] in Burkina Faso revealed that 85 % of *E. coli* isolates were resistance to Tetracycline.

Our study for Cefotaxime was agreement with previous local studies by, AL Hilali [38] 68%, Sakhi [22] 85.7%, Ugwa *et al* [45], also reported resistance to Ceftriaxone (91%) by *E. coli* isolates. Rajeshwari *et al* [46] reported similar finding for the high resistance of Ceftriaxone 75% and cefotaxime (77.5%) in Indian children with diarrhea, while disagreement Khalil [11] 4% and Hamada *et al* [12] 10%. Antibiotic susceptibility testing of isolates showed high resistance rate to Cephalothin (85.7%). The emergence of multidrug resistance especially in *E. coli* has become a critical public concern, which was designated as resistance to one agent in three or more antibiotic classes. Kamwati [47]; Alizadi [48]. Many factors responsible for an increase in rates of antimicrobial resistance include misuse/over use of antibiotic by healthcare professionals and general public Magiorakos *et al* [49]; WHO [50]; Konate*et al* [41], and inadequate surveillance systems and independence on reliable microbiological techniques which leads to inappropriate prescription of antibiotics Wellington *et al* [51].

Ciprofloxacin showed low resistant similar with local studies by Hamada *et al* [12] 10 %, Khalil [11] 8%. Globally Kamwati [47] 4%, Canizalez-Roman *et al* [36] 21 %. Ciprofloxacin was one of the most active antimicrobial agent which currently recommended to treated diarrhea in children Ayat ollahi *et al* [52].

Amikacin showed low resistant agreement with 3.3 % reported by Hamada *et al*[12]and

Al-Hilali [38] 0% , and globally with Zhou *et al* [1] 7.4%.

Imipenem with no resistant agreement with 0% resistant from Al-Hilali [38] and Shamkhi [39]. The Impenim was the most effective antibiotic against DEC followed by Amikacin, Ciprofloxacin and Chloramphenicol. Impinem has been highly effective against gram negative bacteria Mohammed *et al* [53], Alam *et al* [54]. They should be used in life threatening multidrug resistance infections where there is no other alternative.

The statistical analysis to susceptibility test results in this study showed high resistance among Diarrheagenic *E. coli* isolates which were collected from hospitalized children than isolates collected from private pediatric clinics (without history of hospital admitted) as shown in table (3).

This result goes with report of Kamwati[47] who showed isolates from children who had been hospitalized were more resistance than those isolated from children not previously hospitalized , and he conclude that recent history of antimicrobial use and hospitalization is a serious predisposing factor to carriage of Multi Drug Resistant strains. Fox-Lewis [55] also mention that hospital-acquired *Escherichia coli* isolates were multidrug resistant than isolates were community-acquired. Multi drug resistance MDR may be acquired from other patients who have received antibiotics. Infections caused by Multi drug resistance gram negative bacteria are difficult to treat and so may cause more prolonged symptoms in the site of infection Hawkey *et al* [56].

CONCLUSION

Enteropathogenic *E. coli* and Enteroaggregative *E. coli* was the most common types of Diarrheagenic *E. coli* among children less than 2 years of age presented with diarrhea in Wasit province. Enterotoxigenic *E. coli* and Enteroinvasive *E. coli* were more common in children more than 2 years of age in Wasit province.

This study highlights the Using of multiplex PCR in identifying and successful isolation of Diarrheagenic *E.coli* from normal flora and can be used as a rapid and accurate method for the isolation of pathogenic strains of *E. coli*, this will greatly help pediatricians to decrease the use of antibiotic in treatment of diarrhea in

children and decreasing the problem of increasing antibiotic resistance. The results of antibiotic sensitivity test revealed that the most active compound against Diarrheagenic *E. coli* isolates was Imipenem followed by Amikacin, Ciprofloxacin and Chloramphenicol .

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