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DETECTION OF DIARRHEAGENIC *E. COLI* AMONG CHILDREN UNDER 5 YEAR'S AGE IN TIKRIT CITY OF IRAQ BY USING SINGLE MULTIPLEX PCR TECHNIQUE

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ABSTRACT

A variety of diarrheagenic *E coli* (DEC) are responsible for causing diarrhea in children under five years age especially in developing courtiers**.** The aim of this study was to isolate and bacteriological characterizing of DEC from diarrheic children's stool and using molecular identification of DEC pathotypes for better discrimination and investigate their antibiotic resistance patterns. Total of 200 fresh stools specimens were collected from children with diarrhea in Salah Aldin Hospital in Tikrit city, Iraq. The samples were cultured on selective media such as (MacConkey and EMB). Colonies were identified through biochemical reaction and api 20E system and then antibiotic susceptibility profiles were determined. *E. coli* were isolated and characterized from 200 diarrheagenic stool positive samples, collected from hospitalized children less than 5 years old. A total of 75(40.5%) samples were yielded positive for growth of *E. coli* Out of these, 46 genotypically-identified DEC were then subjected to multiplex PCR assay targeting certain virulence factors (SHIG, bfpA, eae, LT, ST, EA, vt1, vt2, daaE, uidA) for discrimination of pathotyes**.** 21/46(45.7%**)** EPEC with 18 atypical and **3** typical, 11/46(24%) ETEC, 3/46 /(6.5%) EAEC, 2/46 (4.3%) for EIEC was detected, 2/46(4.3%) EHEC was also detected, while no DAEC was detected, Also mix pathotypes were detected, more than one pathotype was observed in a number of samples 7/46(15.2%), 5 (aEPEC + ETEC), 2 (aEPEC + EAEC). Moreover, all pathotypes expressed high resistant to Cefotaxime, ampicillin, Piperacillin, Azitromycin, Amikacin. while little resistance to Imipenem, Meropenem, Ciprofloxacin, Norfloxacin was observed. The study concludes EPEC is the dominant pathotype between DEC pathotypes in our society that causes diarrhea in children, and emphasizes the importance of using mPCR assay for best discrimination.
Keywords: Diarrheagenic, E. coli, Multiplex PCR technique

Introduction

Diarrheal disease is still a global problem around the world especially in children under five years in developing countries. In the most recent Global Burden of Disease (GBD) study, Diarrhea was the fourth-leading cause of death among children under 5 years old, responsible for 499,000 deaths (95% UI: 447,000–58,000), representing 8.6% of all deaths in this age group (GBD 2015 Risk Factors Collaborators 2016). For those who survive these illnesses and suffer from repeated infections by enteric pathogens during the critical early years of life, Diarrhea can lead to serious, lifelong health consequences such as environmental enteric dysfunction (EED), growth faltering, impaired cognitive development, and reduced immune response to infection and vaccinations (Guerrant *et al*., 2013). Diarrhea pathogen etiologic contribution may vary depending on the study's geographic location, duration, or the population sampled (Lindsay *et al.* 2015). These infections are believed to be different in the developing world compared to the developed world with regard to a number of features, including earlier age of onset, multiple repeated exposures, greater diversity of pathogens, nutritional status of the host, and a number of others, such as co-infection, diet, and genetics (Heidt *et al*.2014; GBD 2015 Eastern Mediterranean Region Diarrhea Collaborators, 2018). The microbial causes of diarrhea are variety of bacterial, viral and parasite (Navaneethan and Giannella, 2008; Kotloff *et al*., 2013). Among these pathogens, diarrheagenic *E. coli* (DEC) play a major role in causing diarrhea in children under 5 years (Thakur *et al*., 2018; Saka *et al*., 2019; Ramakrishnan *et al*., 2018).

When the microbial agent is bacteria, *E. coli* consider one of the major causes, specially to infantile diarrhea (Ramakrishnan *et al*., 2019; Spano *et al.,* 2017). Depending on specific virulence gene, clinical features, and serotypes DEC into 6 stains: Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* and Diffusely Adherence *E. coli* (DAEC) (Croxen *et al*., 2013). 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, multiplex PCR provide detection to many DEC strains virulence genes with high sensitivity and specificity (Ghoshal and Tejan, 2018).

The aim of this study was to detect the distribution of DEC pathotypes among children with diarrhea in Tikrit city, Salah Aldin province, Iraq by multiplex PCR, and assessing

the antimicrobial susceptibility profile of DEC, 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 July 2018 to March 2019, a total of 200 stool samples were collected from children (males and females) with an ages under five years presented with diarrhea whom had been admitted to hospital or attended at private clinics in Tikrit city, Iraq. The stool samples cultured on MacConkey agar, XLD, EMB, Blood agar and incubated aerobically at 37 °C for 24 hours. the isolated bacteria was identified according to morphological, biochemical tests (Mahon, 2007) and the identification was confirmed by using analytical profile index (API 20 E) system.

Antibiotic susceptibility test:

Performed by Kirby-Bauer procedure on Muller Hinton agar, and results interpreted according to Clinical and Laboratory Standards Institute (Bauer *et al*., 1966; CLSI, 2019).

DNA extraction:

Genomic DNA was extracted from bacterial cultures for 75 isolates of *E. coli* by using of manual extraction methods according to Chen and Kuo (1993).

Multiplex PCR technique:

mPCR reactions were performed under sterile conditions for 75 isolates of *E.coli* bacteria using specific primers (*LT, ST, bfpA, EA, daaE, eae, vt1, vt2, SHIG, uidA*) encoded for the virulence factors, for the purpose of diagnosing pathotypes of DEC. these virulence factors divided into two groups to ensure that no interference occurred between the results of a reaction, because the expected size of the product of some primers is very close and the difficulty of distinguishing the difference between the results on the agarose, where the first group included primeres (*LT, ST, bfpA, EA, daaE*) while the second group included primeres (*eae, vt1, vt2, SHIG, uidA*). The mixture reaction was performed in a total volume 20 µl of PCR PreMix (DNA Template 3 µl, 0.8 µl for each forward primer and reverse primer, except vtI, which added 1 µl of forward primer and the same of reverse primer, The remaining volume was supplemented free water ddH_2O). PCR cycling program parameters used in this reaction for detection of (*LT,ST, bfpA, EA, daaE, eae, vt1, vt2, SHIG, uidA)* genes as shown in table (1), Samples were amplified for 35 cycles, Initial denaturation 96°C for 5 min. 1cycle), (Denaturation 94°C for 20 sec 35 cycle), (Annealing 55°C for 20 sec. 35 cycle), (Extension 72°C for 20 sec. 35cycle), (Final extension 72° C for 7min. 1 cycle) (Holding 4° C 1 cycle).PCR products were visualized following electrophoresis through 1.5% agarose gels stained with red safe, and the amplicons were identified based only on the size of the amplified product.

Table 1 : Primers used for multiplex PCR reaction.

Primers	Sequence (5′ – 3′)	Product size (bp)	References
LT	**-3**-TCTCTATGTGCATACGGAGC**5** 5-CCATACTGATTGCCGCAAT-3	322	Nguyen *et al*., 2005
ST	5-CTAAACCAGTAGAGGTCTTCAAAA-3 5-CCCGGTACAGAGCAGGATTACAACA-3	147	
bfpA	5-TTCTTGGTGCTTGCGTGTCTTTT-3 5-TTTTGTTTGTTGTATCTTTGTAA-3	367	
EA	5-CTGGCGAAAGACTGTATCAT-3 5-CAATGTATAGAAATCCGCTGTT-3	630	
daaE	5-AACGTTGGTTAATGTGGGGTAA-3 R-TATTCACCGGTCGGTTATCAGT	542	Vidal *et al*., 2005
Vt1	5-GAAGAGTCCGTGGGATTACG-3 5-AGCGATGCAGCTATTAATAA-3	130	Nguyen *et al*., 2005
Vt2	5-ACCGTTTTTCAGATTTTGACACATA-3 5-TACACAGGAGCAGTTTCAGACAGT-3	298	
eae	5-CACACGAATAAACTGACTAAAATG-3 5-AAAAACGCTGACCCGCACCTAAAT-3	376	
SHIG	5-CTGGTAGGTATGGTGAGG-3 5-CCAGGCCAACAATTATTTCC-3	320	
uidA	5-CCAAAAGCCAGACAGAGT-3 5-GCACAGCACATCAAAGAG-3	623	Moyo *et al*., 2007

Results

E. coli were isolated in 75 (40.5 %) of 200 collected stool samples from children under five age with diarrhea. The results of primary diagnosis to these *E. coli* isolates by selective and differential culture media were consistent with the microscopic and biochemical tests results. Multiplex PCR applied on theses 75, the results showed that DEC in were detected in 46/ 75 (61.3 %) among diarrheal children. The distribution of 46 DEC pathotype isolates were: EPEC was found in 21 (45.7%), ETEC in 11 (24%), EAEC in 3(6.5%), EIEC in 2 (4.3%), EHEC in 2 (4.3%) and 0 (0%) in DAEC as shown in figure (1). From 21 isolates detected as EPEC which was watery diarrhea 18 (85.7%) isolates of them are aEPEC showed *eaeA* gene found without *bfpB* gene, and 3 (14.3 %) were tEPEC which showed *eaeA* gene together with *bfpB* gene. in addition just one isolate of aEPEC don't produce uidA, but the rest of the aEPEC isolates and tEPEC isolates were all produced uidA gene. ETEC 11 (24%) isolates came second after EPEC as

causative agent of diarrhea among Diarrheagenic *E. coli* pathotypes in our study, *st* gene was appeared in all ETEC isolates detected in our study. EAEC account 3 isolates (6.5%), EIEC and EHEC was detected in 2 isolates (4.3%) that suggested these pathotype maybe play a lessimportant role in childhood diarrhoea in developing countries.

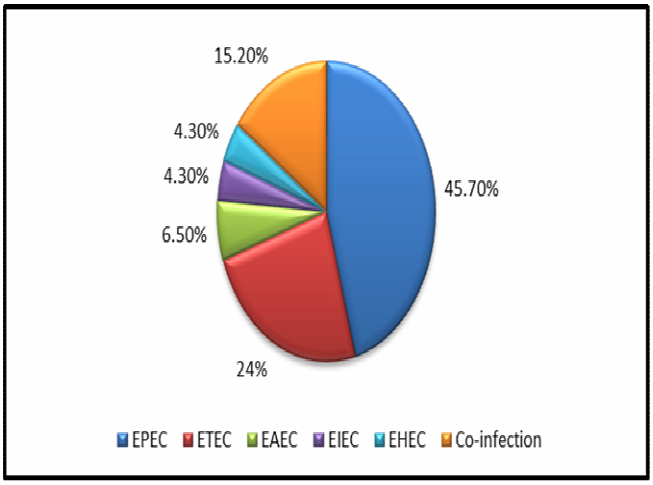

Fig. 1 : Distribution ratios of different DEC pathotypes

High incidence of EPEC recorded in first and second age group flowed by fourth and fifth age group, and then third age group. The most notable feature of the epidemiology of the disease due to EPEC is the striking age distribution of the patients. EPEC infection is primarily a disease of infants younger than 2 years of age (Nguyen *et al*., 2005).The prevalence of EPEC and ETEC infections with their distribution to most age groups, but the highest were in first group, also all EAEC infections were detected under 2 years (groups 1,2), while EIEC and EHEC infections were all under 1 year (group1) as shown in figure (2).

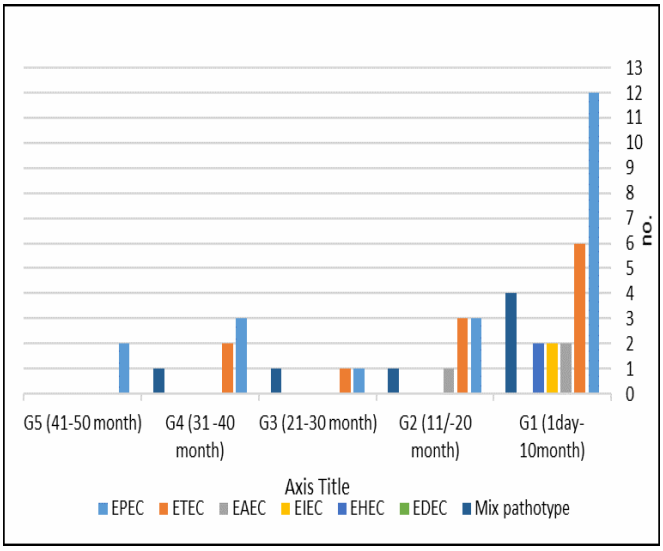

Fig. 2 : Prevalence diarrheagenic *E. coli* with age groups.

DEC pathotypes, in our study were identified and isolated successfully by using mPCR. PCR products visualized to measured product size results from amplification the primers in compared with (100 bp) ladder as shown in figures (3,4,5,6,7,8,9,10,11).

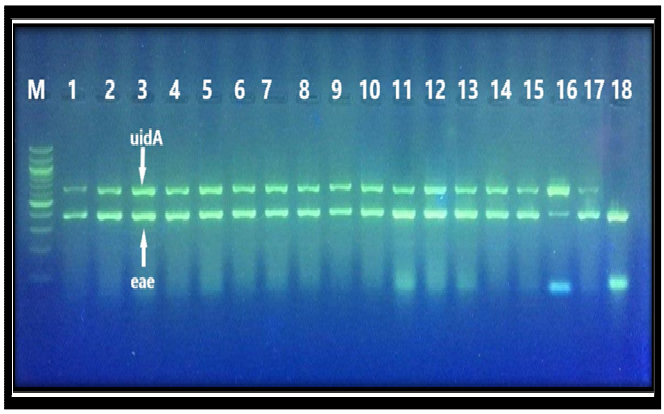

Fig. 3 : Gel electrophoresis of amplified (*eae ,uidA*) genes, the product size (376 , 623 bp) respectively, of aEPEC using conventional PCR . Agarose 1.5%, and SB (1X) at (75 V/cm for 90 min., stained with Red dye and visualized on a UV transilluminator. Lane(M): DNA ladder (100-1500 bp), Lanes: (1-18) samples.

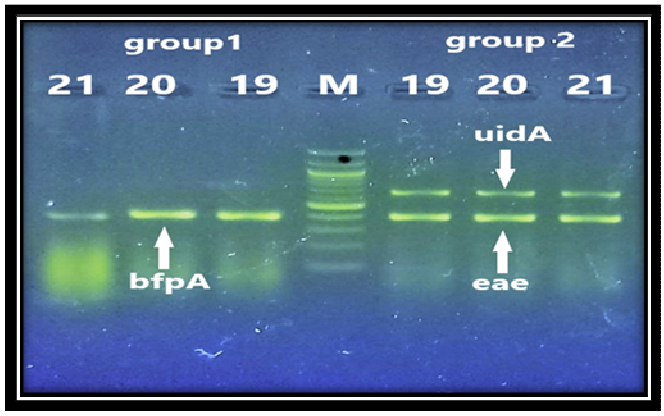

Fig. 4 : Gel electrophoresis of amplified (bfpA , *eae ,uidA*) genes, the product size (367,376 , 623 bp) respectively, of tEPEC using conventional PCR. Agarose 1.5%, and SB (1X) at (75 V/cm for 90 min., stained with Red dye and visualized on a UV transilluminator. Lane(M): DNA ladder (100-1500 bp), Lanes: (19-21) samples.

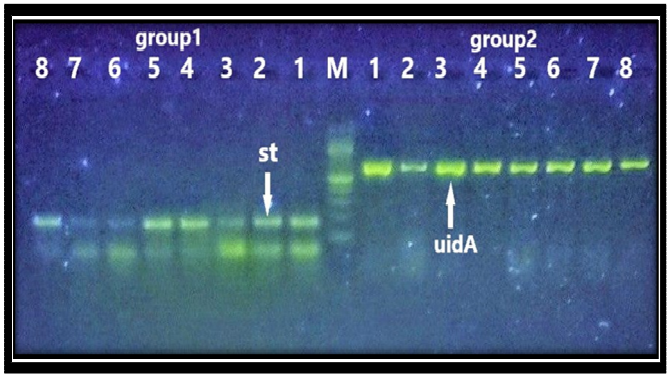

Fig. 5 : Gel electrophoresis of amplified (*st,uidA*) genes, the product size (147, 623 bp) respectively, of ETEC using conventional PCR. Agarose 1.5%, and SB (1X) at (75 V/cm for 90 min., stained with Red dye and visualized on a UV transilluminator. Lane (M): DNA ladder (100-1500 bp), Lanes: (1-8) samples.

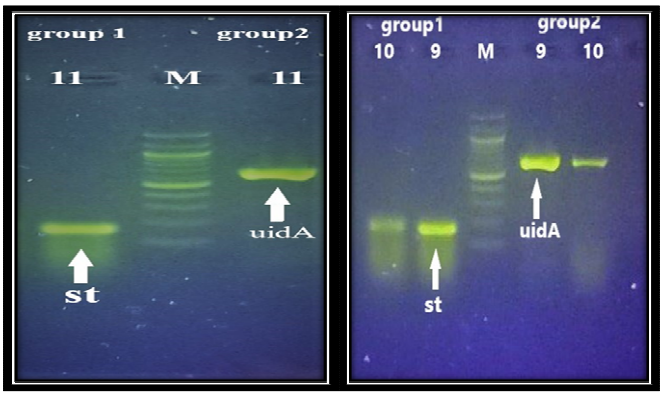

Fig. 6 : Gel electrophoresis of PCR reaction product of ETEC isolates samples (9-11) is positive for presence of *st* gene (147 bp) (from first group primers) and for *uidA* gene (623 bp) (from the second group). Agarose 1.5%, and SB (1X) at (75 V/cm for 90 min., stained with Red dye and visualized on a UV transilluminator Lane (M): DNA ladder (100-1500 bp), Lanes: (9-11) samples.

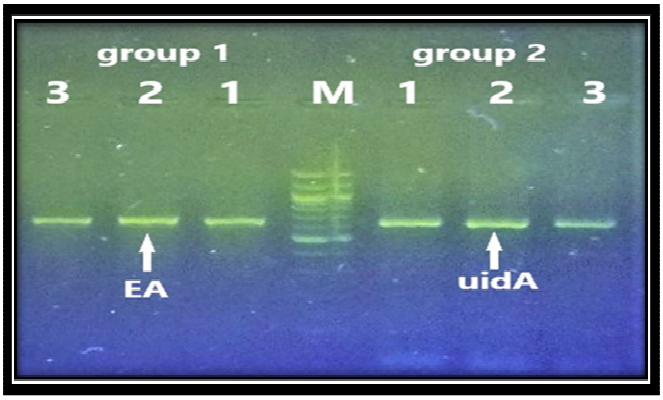

Fig. 7 : Gel electrophoresis of PCR reaction product of EAEC isolates samples (1-3) is positive for presence of *EA* gene (630 bp) (from first group primers) and for *uidA* gene (623 bp) (from the second group). Agarose 1.5%, and SB (1X) at (75 V/cm for 90 min., stained with Red dye and visualized on a UV transilluminator Lane (M): DNA ladder (100-1500 bp), Lanes: (1-3) samples.

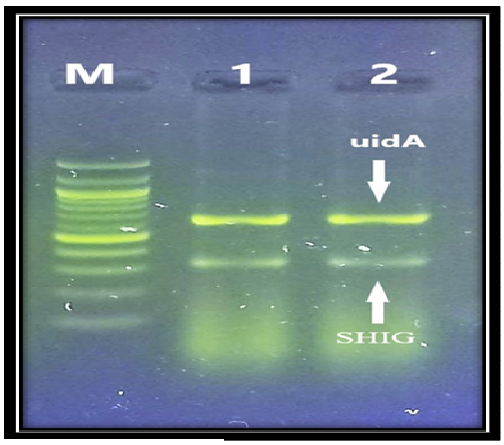

Fig. 8 : Gel electrophoresis of PCR reaction product of EIEC isolates samples (1,2) is positive for presence of *SHIG* gene (320 bp) and for *uidA* gene (623 bp) (from the second group). Agarose 1.5%, and SB (1X) at (75 V/cm for 90 min., stained with Red dye and visualized on a UV transilluminator Lane (M): DNA ladder (100-1500 bp), Lanes: (1-2) samples.

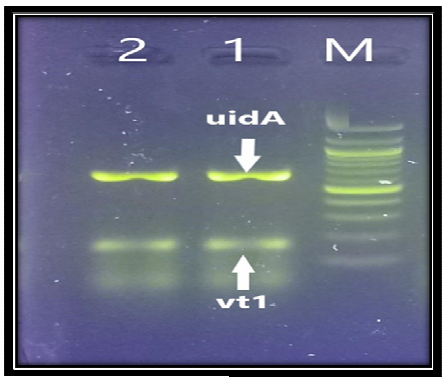

Fig. 9 : Gel electrophoresis of PCR reaction product of EHEC isolates samples (1,2) is positive for presence of vt1 gene (320 bp) and for *uidA* gene (623 bp) (both from the second group). Agarose 1.5%, and SB (1X) at (75 V/cm for 90 min., stained with Red dye and visualized on a UV transilluminator Lane (M): DNA ladder (100-1500 bp), Lanes: (1-2) samples.

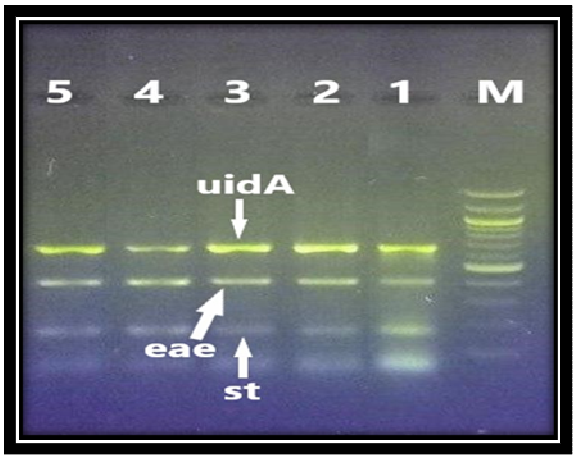

Fig. 10 : Gel electrophoresis of PCR reaction product of mix pathotypes isolates (aEPEC+ ETEC) samples (1-5) is positive for presence of *eae* gene (376 bp), *st* gene (147 bp) and for *uidA* gene (623 bp). Agarose 1.5%, and SB (1X) at (75 V/cm for 90 min., stained with Red dye and visualized on a UV transilluminator Lane (M): DNA ladder (100-1500 bp), Lanes: (1-5) samples.

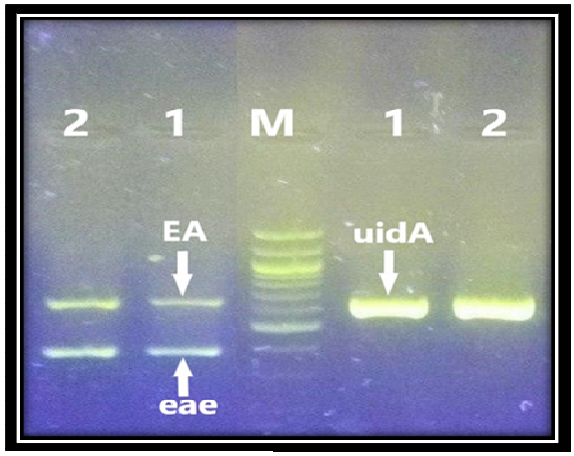

Fig. 11: Gel electrophoresis of PCR reaction product of mix pathotype (aEPAC+ EAEC) isolates samples (1,2) is positive for presence of *EA* gene (630 bp), eae (376 bp) and for *uidA* gene (623 bp). Agarose 1.5%, and SB (1X) at (75 V/cm for 90 min., stained with Red dye and visualized on a UV transilluminator Lane (M): DNA ladder (100-1500 bp), Lanes: (1-2) samples.

Antibiotic susceptibility test results were showed in table (3). All isolates were multiresistance. The highest level of resistance were to Cefotaxime (100%), followed by Ampicillin (97.8%), Piperacillin (97.8%), Azitromycin (95.7%), Amikacin (87%). Lower resistance rates were observed for Norfloxacin, Ciprofloxacin, Meropenem and Imipenem with rates of 2.2%, 6.5%, 28.3%, and 32.6% respectively

Table 3 : Antibiotic resistance rates for each DEC pathotype isolated from children with diarrhea.

Antibiotics	DEC	EPCE	ETCE	EACE	EICE	EHCE	Mix pathotypes
Meropenem	6.50%	9.50%	4.80%	0%	0%	0%	0%
Imipenem	2.20%	0%	4.80%	0%	0%	0%	0%
Ciprofloxacin	28.30%	28.60%	18.20%	66.70%	50%	50%	14.30%
Norfloxacin	32.60%	42.90%	18.20%	33.30%	100%	50%	14.30%
Amikacin	87%	90.50%	81.80%	66.70%	100%	100%	85.70%
Ampicillin	97.80%	95.20%	91%	100%	100%	100%	100%
Piperacillin	97.80%	100%	100%	100%	100%	50%	100%
Azitromycin	95.80%	95.20%	100%	100%	100%	100%	85.70%
Cefotaxime	100%	100%	100%	100%	100%	100%	100%

Discussion

The prevalence of DEC in the present study was 46(61.3%) among 75 *E. coli* isolates. Among all the DEC pathotypes, EPEC were found to be the most common pathotypes for children with (45.7%), our result compatible with local study by Abdul-hussein *et al.* (2018) who showed EPEC as most than other pathotypes (45.3%), and in contrast with Khalil (2015) and Al-Dulaimi (2015) where they show it came second after EAEC. Our finding was, however, similar to globally studies with Verma *et al*. (2019), Zhou *et al*. (2018), Thakur *et al*. (2018) that also reported a high frequency of EPEC pathotypes associated with diarrhea. Whereas in Saka. *et al*. (2019) study, in Nigeria EPEC isolation rate was 6% (13/215) lower than the rest of the pathotypes present in children with diarrhea because of DEC. while EPEC has not been isolated in the studies of Hasan *et al*. (2020) and Omran et al. (2014). EPEC is also a very important pathogen in children with diarrhea. Numerous case–control studies in many countries have found that EPEC are isolated more frequently from children with diarrhea than from healthy controls (Nguyen *et al*., 2005). EPEC are sub-grouped into typical (tEPEC, eae+ bfpA+) and atypical (aEPEC, eae+ bfpA**-**) strains that differ in several respects Naji and Nasser (2015) **.**From 21 isolates detected as EPEC, 18 (85.7%) isolates of them are aEPEC showed *eaeA* gene found without *bfpA* gene, and 3 (14.3 %) were tEPEC which showed *eaeA* gene together with *bfpA* gene. Global reports by Darbandi *et al*. (2016) in Iran, and Nataraja *et al*., (2018) in India, that showed the distribution of atypical EPEC was higher than typical EPEC. Ochoa and Contreras (2011) report that aEPEC are more prevalent than tEPEC in both developed and developing countries, and that aEPEC are important in both pediatric endemic diarrhea and diarrhea outbreaks.

In this study, ETEC account 11 (24%) isolates came second after EPEC among DEC pathotypes, the detection of ETEC is in consonance with previous local findings by Al-Marzoqi *et al*. (2019) and concur with global study by Abbasi *et al*. (2020) in Iran. Our study differ from other reports by Verma *et al*. (2019), Thakur *et al.* (2018) in India which suggested low prevalence of ETEC 6%, 5.7%. While in Huber *et al.* (2019), ETEC was recognized ETEC as major pathotype among children less than 5 years, and in the study by Hasan *et al.* (2020) in Dohuk /Iraq, the isolation rate of ETEC was the highest. Low-grade hygienic state of family and artificial feeding may account for this high rate of ETEC.

EAEC 3(6.5%) isolates came after ETEC causative agent of diarrhea in our study**,** that agree with reports by Omran *et al.,* (2014) in Libya, also Globally with Thakur *et al*., (2018) and by Enrique *et al.,* (2019) in Brazil. But EAEC considered the major cause of diarrhea between DEC pathotypes in local studies by Al-Marzoqi *et al*. (2019), Khalil (2015) and Al-Dulami (2015) also Globally, Taborda *et al*., (2018) in Brazil, Ali *et al*. (2014) in Egypt.

ETEC and EAEC pathotypes are important etiological agents causative of diarrhea in children younger than 5 years of age in Mexico and in developing countries, where they cause numerous deaths. Both have been associated with delayed growth in children and are the main causative agents of traveler's diarrhea (Ríos-Muñiz *et al.,* 2019).

EIEC was detected in 2 isolates (4.3%) of DEC. Our study is nearly close with local study by Abdul-hussein *et al*. (2018) (7.1%) and globally with (9.3%) by Abbasi *et al*. (2020) and (3.7%) by Zhou *et al*. (2018). While none of EIEC was detected by Al-Marzoqi *et al*. (2019) and Enrique *et al.,* (2019).Vieira *et al*. (2007) suggested that this pathotype may be play a less important role in childhood diarrhea in developing countries**.**

EHEC was detected in 2 (4.3%) were detected this result is similar to local reports of Al-Marzoqi *et al*. (2019) (5.27%) and Al-Dulaimi (2015) (7.8%). Globally, Abbasi *et al*. (2020) 9.3%. Whereas, by Abdul-hussein *et al.* (2018), Enrique *et al*. (2016), Verma *et al*. (2019) and Ali *et al.,*(2014) were not detected any EHEC pathotype in their study. observed in developing countries where in EHEC is much less frequently isolated than other DEC strains, such as ETEC or EPEC, The much lower incidence of EHEC in developing countries than in developed countries (Nataro and Kaper,1998).

In the current study, none of DAEC pathotype was detected when we used a specific primer for *daaE* gene. Unlike the results of study conducted in Tikrit/Iraq by Jameel (2014) was 4% and in Brazil by Enrique *et al*. (2019) was 16.98%. Patzi-Vargas *et al*. (2015) found that DAEC was the top DEC group Among children with DEC infections (35%) in Mexico.

Our results confirm the possibility that mixed infections by two pathotypes of DEC can be detected in a sample, as

mentioned in other studies (Huber *et al*., 2019; Odetoyin *et al.,* 2016). This PCR was highly specific with the primers chosen for the detection of six categories of DEC. The most common case of mixed infection observed by Natarajan *et al.* (2018) study with the participation of aEPEC was more frequent pathotype in mixed infections than other DEC types. In Mexico, a 10% incidence of mixed infections was observed by Patzi-Vargas *et al*. (2015).

These difference between our results and other studies may be attributed to The possibility of using more than one primer may yield positive amplification, rout of infection, virulence factors, pathogen strains, difference in population selection, time of collection, size of samples in addition geographical differences may also effect on the prevalence of DEC pathotypes.

All DEC isolates (100%) were multiresistant to antibiotics, showing resistance to four classes of antibiotics used. High rates of resistance were recorded against antibiotics Ampicillin, Piperacillin, Cefotaxime, Amikacin, Neomycin, and Azitromycin these antibiotics are the most widely used and widely used in developing countries to treat diarrhea due to their low cost and availability, which leads to increased resistance to these antibiotics. Lower resistance rates were observed for Norfloxacin, Ciprofloxacin, Meropenem, Imipenem in this study.

The isolated DEC pathotypes showed high resistance rates to Ampicillin agreement with another study had been reported high resistance in studies done by Li *et al*., (2019), Abbasi *et al*., (2020) and Hussein (2016). while there was no resistance for Piperacillin. Konaté *et al*., (2017) observed that (64.5%) of diarrheal cases were resistance to Piperacillin. High rate of resistance were recorded for penicillin group Several reports have indicated that this drug was less effective against *E.coli* and other bacterial isolate as reported by many studies (Abdulkareem, 2016; Abdul-hussein *et al*., 2018) because these groups of antibiotic are inexpensive and can be obtained easily without a medical prescription, so resistance is probably due to indiscriminate antibiotic usage (drug abuse) which could result in plasmid-mediated antibiotic resistance found to be common in *E. coli*. According to this result, the penicillin group should not be used for treating diarrhea and other disease caused by *E.coli*. therefore, local information about antibiotic resistance should be used in clinical management and treatment guideline should be updated routinely.

High rate of resistance were recorded for amikacin was agreement with studies of Abdulkareem (2016) and Jameel (2014) was 92%, 70%, respectively, while in Li *et al* (2019), Abdul-hussein *et al* (2018) and Hussein (2016) low resistance were 8.25%, 7.1% and 3.3% respectively.

There was a high resistance of DEC to Azitromycin and this disagree with the result of Abbasi *et al.* (2020) that found low resistance to Azitromycin (25%), and this is nearly similar to the study found the prevalence of resistance to Verma *et al*., (2019) reach to 74.32%.

Our study for Cefotaxime was agreement with previous local studies by Jameel (2014) 93%, while disagreement Hussein (2016) 10%.

Ciprofloxacin and Norfloxacin showed low resistant similar with local studies by Abdulkareem (2016) and Abdul-hussein *et al*. (2018) Globally by Yadav *et al*., (2019) and Konaté *et al*., (2017). Ciprofloxacin was one of the most active antimicrobial agent which currently recommended to treated diarrhea in children (Ayatollahi *et al*., 2013).

Low resistant for Imipenem and Meropenem, Impenim was the most effective antibiotic against DEC followed by Meropenem, Ciprofloxacin and Norfloxacin. Impinem has been highly effective against gram negative bacteria, They should be used in life threatening multidrug resistance infections where there is no other alternative (Ventola, 2015).

The antimicrobial susceptibility testing results for the DEC strains are shown in Table 3. In our study, the frequency of antimicrobial resistance to various antibiotics was high in DEC strains. The high incidence of antibiotic-resistant isolates of DEC may be due to the widespread use of antibiotics. The transfer of resistance genes that may occur between species could lead to the construction of diverse resistance to usual antibiotics (Aslani *et al.,* 2011), and worldwide prevalence of high resistance in DEC could be attributed to the inappropriate and wide use of different antibiotics to treat infection in children of a young age.

In conclusion, EPEC continues to be an important agent associated with diarrhea in children from Tikrit/Iraq. This study highlights the using of multiplex PCR in identifying and successful isolation of DEC 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 diarhea in children and decreasing the problem of increasing antibiotic resistance. The results of antibiotic sensitivity test revealed that the most active compound against DEC isolates was Imipenem followed by Meropenem, Ciprofloxacin and Norfloxacin.

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