Pro Index | Journal of Science in Medicine and Life
Volume: 3 Issue: 1 Year: 2025
ISSN: 2992-9202 | http://journals.proindex.uz

Evaluation of B-Lactam Resistance among Phylogenetic Groups of Diarrheagenic *Escherichia Coli*

Nawras Najah Mubark
Wasit University, College of Science, Department of Forensic Evidence
nawras@uowasit.edu.iq

Heyam Emad Al. Qurabiy, Nedhal Hameed Sajet
Wasite university College dentist
naldresawy@uowasit.edu.iq, halqurapy@uowasit.edu.iq

Abstract: **Background:** Diarrheagenic *Escherichia coli* (DEC) is one of the common causes of diarrhea in all ages and its treatment is necessary with appropriate antibiotics, so the current study aims to isolate and diagnose DEC according to the phylogenotype to know which group is the causative agent of diarrhea and know its resistance to the most common antibiotics represented by beta-lactams. **Methods:** The current research included 150 stool samples from individuals with diarrhea, and after diagnosing bacteria by culture media and biochemical tests, it was found that only 118 samples had DEC as the causative pathogen of diarrhea. Antibiotic sensitivity test for all DEC isolates preform by 10 commonly used antibiotics belonging to the beta-lactam. After DNA extraction, the genotypes were determined using multiplex PCR technique. **Results:** According to multiplex PCR-based phylogenetic analysis, group B2 represent the majority of the collected DEC isolates (70.3%) followed by D (17%) and group B1 (8.5%) but group A appeared in 5 samples only. Moreover, all phylogenetic groups (100%) and subgroups (100%) were resist to penicillin and amoxicillin. Group D and their subgroups showed complete resistance (100%) to Penicillin, Ampicillin, Cefalexin, Cefepime and Amoxicillin, at the same time they showed high resistance to the Cefotaxim, while they were more sensitive to Ceftriaxone. groups A and B1 showed complete resistance (100%) to both penicillin and amoxicillin also they have high resistance to Cefalexin (80% and 90% respectively) and Cefotaxim (80% for each group), while they were 100% sensitive to ceftriaxone. In addition, group A have 100% sensitivity to Ampicillin. Subgroup D2 isolates were the most resistant among the subgroups, as they were 100% resistant to other antibiotics such as Ampicillin, Cefalexin, Cefixime and Cefepime. Subgroup A0 showed a high sensitivity (100%) to Ampicillin, Cefixime, Ceftriaxone, Imipenem, Meropenem and Cefepime. **In conclusion**: Group B2 and their subgroups were the main cause of diarrhea and they showed high resistance to most antibiotics, while the effective treatment for it was Ceftriaxone and Meropenem.

Key points: DEC, phylogenetic groups, B- lactam, antibiotic resistance, multiplex PCR.

INTRODUCTION

Diarrheal infections are a major cause of morbidity and mortality in developing countries. Various enteric pathogens, including bacteria, viruses, fungi, and parasites, contribute to the incidence of diarrhea . The two most well-known microorganisms that may cause diarrhea are Escherichia coli (DEC) and Shigellaspp[1]. Rise and scattering of antibiotic resistance is very much archived in bacterial segregates around the world, especially in non-industrial countries[2,3]. Antibiotic resistance in E. coli serves as a good indicator of the overall level of resistance in a given population [2,4]. *E. coli* is popular to be productively fit for tolerating and moving hereditary materials and, under

pressure, promptly moves those hereditary materials to intestinal microbes including *Salmonella*, *Yersinia*, *Vibrio*, and *Shigella* species. That's why it's a significant source of antibiotic resistance that may spread to other organisms [2,3,4]. Many different types of enteric pathogens, including bacteria, viruses, fungi, and parasites, may lead to diarrhea[5] . Escherichia coli (DEC) and Shigella spp. are the two most well-known germs that may cause diarrhea[6]. Antibiotic overuse on a global scale may contribute to the evolution of resistance mechanisms in both harmless and harmful E. coli strains [7].

B-lactams are among the most often prescribed classes of antibiotics for both people and animals [4]. The -lactamases responsible for destroying the B-lactam ring in successive generations of B-lactam antibiotics are principally responsible for B-lactam resistance in Enterobacteriaceae. While many different types of -lactamases have been identified, Enterobacteriaceae are notorious for producing TEM, CTX-M, and SHV enzymes[7,8]. The enzyme's action may be broadened to encompass penicillins, extended-spectrum cephalosporins, and aztreonam if the genes producing them are mutated. Extended-spectrum -lactamases (ESBLs) are the term given to these enzymes [8].

"Antibiotic resistance among bacteria poses a serious public health challenge, necessitating ongoing surveillance to inform treatment strategies and resistance management protocols[2]. The development of novel approaches to counteracting this phenomena may also benefit from a deeper understanding of the molecular basis of resistance [9,10]. Effective efforts to reduce the spread of these infectious agents need a thorough understanding of the risk factors for infections caused by ESBL-producing bacteria. Multiple research have attempted to determine risk factors for ESBL-producing bacterial infections, but their findings have been inconsistent. However, the epidemiology of the different epidemics may account for some of these variations [10,11, 12.]

Several methods, including serotyping, phenotyping, and the molecular approach, may identify DEC pathotypes. PCR is an excellent test since it is rapid and accurate [13]. PCR assay can also categorize DEC into different phylogenetic groups[A,B1,B2,D] [14]. These phylogenetic groups differ in their virulence and cause injuries in several places in the human body, such as the urinary system, nervous system, blood, etc. Our current study aims to find out which phylogenetic group is responsible for the occurrence of diarrhea, and at the same time, we determine the appropriate antibiotics for its treatment, which are available in Iraq, including beta-lactam antibiotics.

METHODS

Study Design and Samples Size : The current study is a cross-sectional study that used data acquired from a population at a certain point in time. The current study determines sample size based on the frequency or percent (p) of diarrhea using a specific equation {sample size = z2 * p(1-p) / d2} with an estimated stander normal variation (Z) of 1.96 and precision (d) of 0.05, as per previous studies. According to this equation, the current study's sample size consists of 150 fecal samples[20].

Inclusion Criteria : All participants provided verbal informed permission in accordance with Al-Kut city's ethical standards .Criteria for selecting samples may include :

- ✓ Individual age range from 1 to 80 years.
- ✓ Fecal samples collected from individuals who have diarrhea for at least one hour and
- ✓ Performed a microscopic examination to exclude infectious diarrhea caused by parasites .
- ✓ Diarrhea is not caused by chemical poisoning .

Sample Collection : About 1 gram of feces from each participant is collected in individual , sealed containers and submitted to the lab . .

Transportable Cary-Blair media may be used to preserve samples until they can be delivered to the microbiological lab [20].

Isolation and Identification of *Escherichia coli*: To determine whether bacterial cells, parasites, or other materials are present, all specimens undergo a general stool examination. In a bacteriological incubator, selected specimens are seeded on MacConkey agar and incubated under aerobic

conditions for the entire night at 37˚C. The rose-pink color of the colonies on MacConkey agar plates and their shape helped identify E. coli. This was confirmed by growing a subculture on Eosin Methylene Blue agar and keeping it at 37 °C for 24 hours. The result was then supported by biochemical tests .Following identification, E. coli bacteria were grown on Muller-Hinton agar for antibiotic susceptibility testing and in Nutrient broth for DNA extraction for molecular studies [16].

Antibiotic Susceptibility Test: identified *E. coli* isolates were subjected to susceptibility testing by modified disc-diffusion method. Antibiotic resistance was determined by using 10 antibiotic discs listed in Table (1) according to the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI), corresponding to the drugs considered routine testing and reporting on Enterobacteriaceae [13,16].

DNA Extraction : Genomic DNA Mini Kit (Geneaid) instructions were followed to extract DNA from bacterial broth .After confirming the presence of DNA in each sample through electrophoresis on an agarose gel (0.5% agarose stained with 5L of ethidium bromide), the DNA was kept at -20 degrees Celsius in microcentrifuge tubes and later utilized in polymerase chain reaction (PCR).

Primer Preparation: According to the manufacturer's instructions, specific primers (Table 2) were produced by dissolving the lyophilized primers in deionized distal water to make a stock solution with a concentration of 100 pmol / l. Each primer's final working solution (10 pmol / l) was prepared by diluting the stock solution with deionized water following the formula C1V1 = C2V2.

Table (1): Antibiotic discs and their remarks

Antibiotics	Symbol	Concentration µg/disc	Manufacturers Name
Penicillin	P	10	Ankara/ Turkey
Amoxicillin	AMX	25	
Ampicillin	AMP	10	
Cefalexin	CL	30	
Cefixime	CFM	5	
Cefotaxim	CTX	30	
Ceftriaxone	CRO	30	
Imipenem	IMP	10	
Meropenem	MEM	10	
Cefepime	FEP	10	

Table (2): Triplex PCR primers and their references

Gene	Oligoneucleotide primer sequence (5´-3´)		Reference
chuA	1	GACGAACCAACGGTCAGGAT	Clermont *et al*., 2000 (15)
	2	TGCCGCCAGTACCAAAGACA	
YjaA	1	TGAAGTGTCAGGAGACGCTG	Clermont *et al*., (15,17)
	2	ATGGAGAATGCGTTCCTCAAC	
TspE4C2	1	GAGTAATGTCGGGGCATTCA	Clermont *et al*., 2000 (15)
	2	CGCGCCAACAAAGTATTACG	

PCR Reaction Mixtures : The PCR reactions used in this study were prepared ahead of time in accordance with the manufacturer's instructions for the master mix and the genes of interest.

PCR Reaction Mixtures for genotypic Analysis: genotypic groups were determined by multiplex PCR. Amplification reaction for all primers were conducted in 0.2 ml tube of Accu Power PCR Premix tube according to the bioneer corporation's instruction. After the lyophilized pellet was dissolved and all components were well combined in the PCR tube with vigorous vortexing, the tube was placed in a Professional TR/O Thermocycler. . The thermocycling condition for multiplex

PCR reaction was performed with a Professional TR/O Thermocycler under the following Conditions (Figure 1).

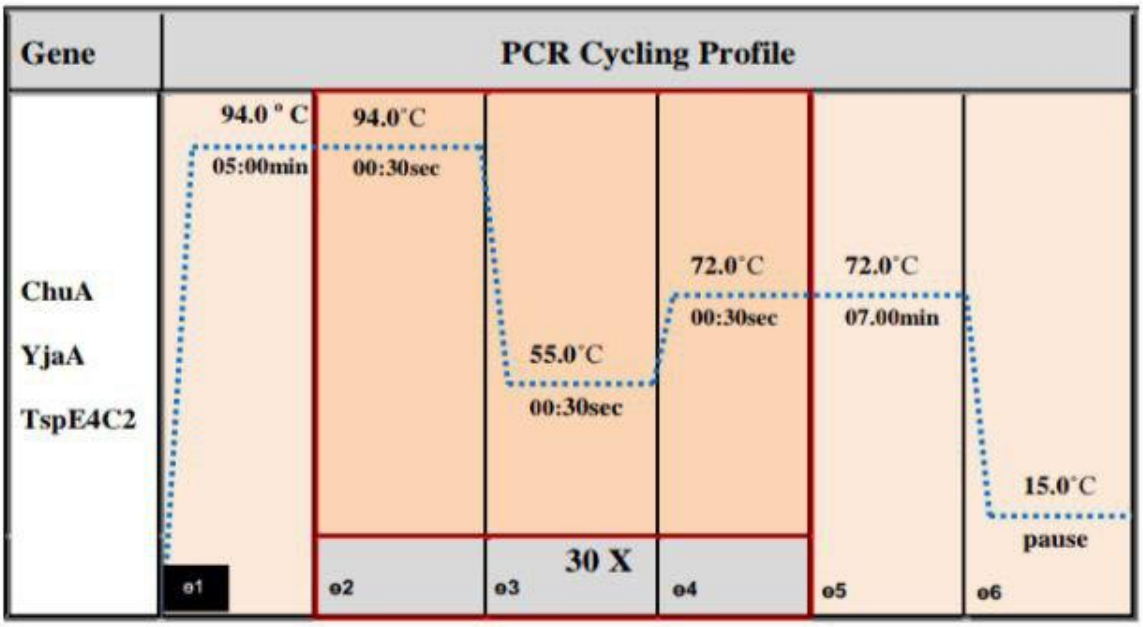

Figure (1): Thermocycling conditions for phylogenetic determine

Phylogenotypic analysis [15] was done on the basis of the presence or absence of the 2 genes and one DNA fragment (*chuA*, *yjaA* and TSPE4.C2) and Phylogenetic groups detected as follows:

Group A: chuA– and TspE4.C2–

- ✓ Subgroup A0:chuA–, yjaA– and TspE4.C2–
- ✓ Subgroup A1: chuA–, yjaA+ and TspE4.C2–

Group B1: chuA–, yjaA– and TspE4.C2+

Group B2: chuA+ and yjaA+

- ✓ Subgroup B22: chuA+, yjaA+ and TspE4.C2–
- ✓ Subgroup $B2_3$: chuA+, yjaA+ and TspE4.C2+

Group D: chuA+ and yjaA–

- ✓ Subgroup D1: chuA+, yjaA– and TspE4.C2–
- ✓ Subgroup D2: chuA+, yjaA– and TspE4.C2+

Statistical analysis

The statistical analysis was conducted using Microsoft Excel 2010 and the Statistical Package for the Social Sciences (SPSS) version 17 for Windows. Frequencies and estimated percentages for age groups, antibiotic susceptibility tests, resistance patterns, and phylogenetic groupings or subgroups were computed in order to perform descriptive analysis. P-values below the 0.05 threshold were deemed statistically significant in all of these tests.

RESULTS

This study's cross-sectional design , 150 stools were collected from people suffering from diarrhea, and after bacteriological diagnosis, it was found that only 118 (79%) samples contained *E. coli*, while the other 32 samples (21%)were either negative or positive for other bacterial isolates, as shown in Figure 2.

The ages of the diarrheagenic *E. coli* infected patients ranged from 1 to 80 years their ages were divided into seven age groups as follows: Group 1 includes 26 patients with an average age of 6.6 ± 5.2 (14 females and 12 males), Group 2 includes 18 patients with a mean age of 22 ± 1.9 (8 females and 10 males), and Group 3 also includes 18 patients with a mean age of 28.6 ± 1.3 (9 females and 9 males), group 4 consisted of 10 patients with a mean age of 37.7 ± 2.3 (4 females and 6 males), group 5 consisted of 14 patients with a mean age of 47.8 ± 3.3 (7 females and 7 males), group 6 included 12 patients with an average age of 59.2 ± 3.5 (7 females and 5 males), and group 7 consisted of 20 elderly patients with an average age of 74 ± 7.3 (9 females and 11 males) as shown in Table (3). However, most children and adolescents in group 1 were more likely to have intestinal diarrhea (22%), followed by the elderly in group 7 (17%), while adults in group 4 were less likely to have diarrhea (9%).

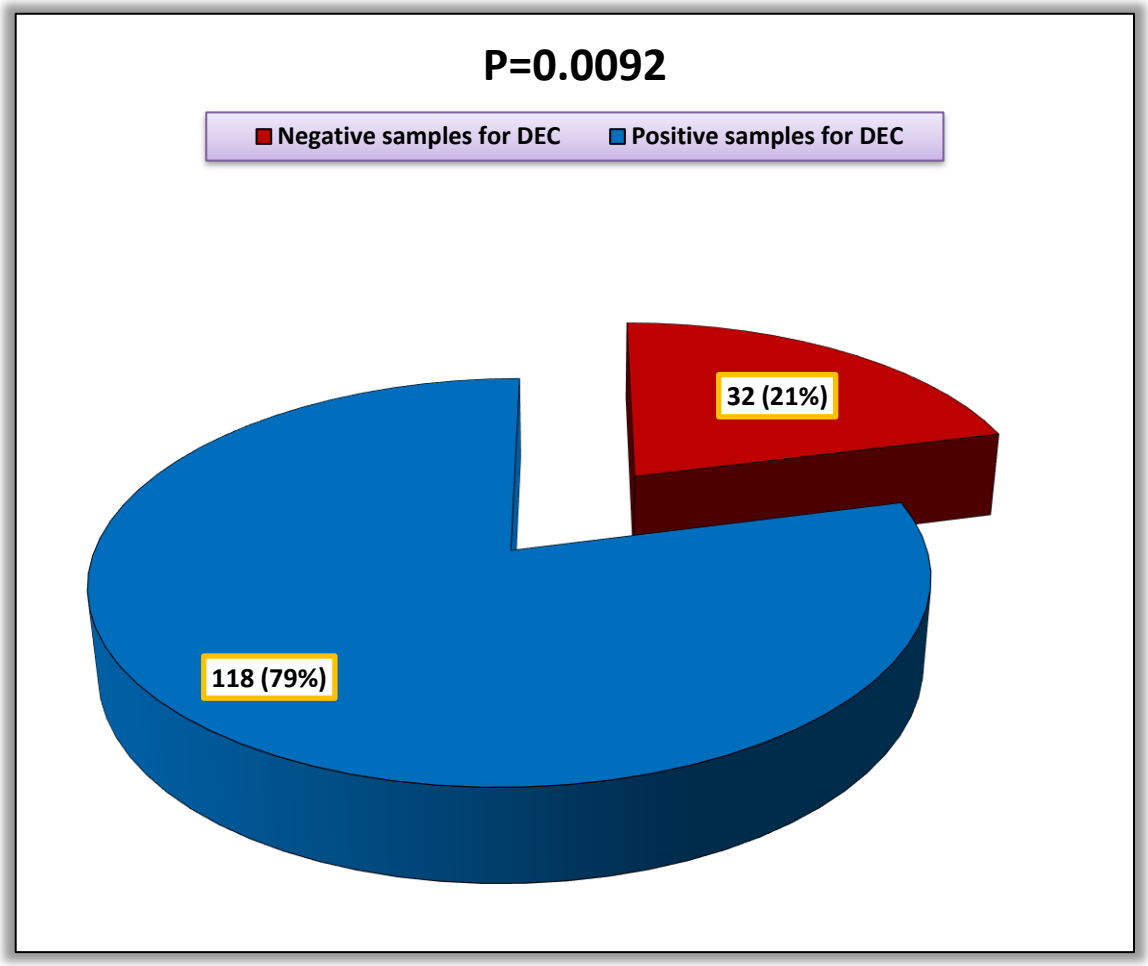

Figure (2): Frequency of DEC among collected diarrhea samples

Table (3): Age group and gender of DEC infected patients

Age range	Age groups	Mean ± SD	Females N (%)	Males N (%)	Total number (%)
1-15	Group 1	6.6± 5.2	14 (12)	12 (10)	26(22)
16-25	Group 2	22 ± 1.9	8 (7)	10 (8)	18 (15)
26-35	Group 3	28.6 ± 1.3	9 (7.5)	9 (7.5)	18 (15)
36-45	Group 4	37.7 ± 2.3	4 (3)	6 (6)	10 (9)
46-55	Group 5	47.8 ± 3.3	7 (6)	7 (6)	14 (12)
56-65	Group 6	59.2 ± 3.5	7 (6)	5 (4)	12 (10)
66-80	Group 7	74 ± 7.3	9 (8)	11 (9)	20 (17)

The results of the antibiotic sensitivity test in tables 4 and 5 showed that all *E. coli* that isolated from different age groups were resistant (100%) to amoxicillin and penicillin, as well as high resistance 98% and 90% to cephalexin and ampicillin respectively, especially in patients in the age group 36 to 80 years (100% resistance). On the contrary, DEC were more sensitive 88% and 86% to ceftriaxone and meropenem respectively where these antibiotics were effective in treating infection, especially in children (group 1 included 17 % resist to Ceftraxine and 25% to Meropenem. Moreover, the more effective antibiotic for treatment DEC infection in elders (group 7) is Meropenem (resistance 30%) whereas Ceftriaxone appeared the best option for treatment DEC infection in age groups 2, 3, 4, 5 and 6 (resistance 17%, 20%, 25%, 24% and 27% respectively).

Table (4): General antibiotic resistance among DEC

B-lactams antibiotics	Antibiotic sensitivity test		P value
	Resistance	Sensitivity	
Antibiotics	**N (%)**	**N (%)**	
Penicillin	118 (100)	0 (0)	>0.0001*
Amoxicillin	118 (100)	0 (0)	>0.0001*
Ampicillin	106 (90)	12 (10)	0.0012*
Cefalexin	116 (98)	2 (2)	0.0005*
Cefixime	105 (89)	13 (11)	0.0018*
Cefotaxim	110 (93)	8 (7)	0.0011*
Ceftriaxone	30 (25)	88 (75)	0.0076*
Imipenem	35 (30)	83 (70)	0.0098*
Meropenem	32 (27)	86 (73)	0.0083*
Cefepime	104 (88)	14 (12)	0.0021*

***significant differences (P<0.05)**

Table (5): Distribution of antibiotic resistance of DEC among age groups

B-lactams antibiotics	Resistance % among age groups							P value
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	
Penicillin	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**1**
Amoxicillin	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**1**
Ampicillin	**60**	**80**	**90**	**100**	**100**	**100**	**100**	**0.733**
Cefalexin	**90**	**98**	**98**	**100**	**100**	**100**	**100**	**0.863**
Cefixime	**80**	**80**	**88**	**77**	**99**	**90**	**99**	**0.052**
Cefotaxim	**93**	**93**	**92**	**90**	**93**	**95**	**95**	**0.948**
Ceftriaxone	**17**	**17**	**20**	**25**	**24**	**27**	**45**	**0.0593**
Imipenem	**30**	**30**	**28**	**33**	**28**	**30**	**31**	**0.894**
Meropenem	**25**	**27**	**23**	**30**	**27**	**27**	**30**	**0.899**
Cefepime	**87**	**88**	**87**	**90**	**90**	**86**	**90**	**0.888**

Multiplex PCR-based phylogenetic analysis (Figure 3) revealed that the majority of the DEC isolates belong to group B2 (70.3%), followed by group D (17%) and group B1 (8.5%). Group A was detected in only 5 samples." (Table 6). Most strains of group A (3 isolates, 2.5%) belonged to the subgroup A1 and the others (2 isolates, 1.7%) were related to subgroup A0. Furthermore, 60 isolates (50.8%) of group B2 belonged to subgroup $B2_3$ and 23 isolates (19.5%) to subgroup $B2_2$. On other hand 9 isolates of group D (8%) fitted in subgroup D1 and others 11 isolates (9%) constituted to the subgroup D2.

Table (6): Distribution of diarrheagenic *E. coli* according to genotypic groups & subgroups

E.coli groups	N (%)	*E.coli* subgroup	N (%)
A	5 (4.2)	A0	2 (1.7)
		A1	3 (2.5)
B1	10 (8.5)		
B2	83 (70.3)	$B2_2$	23 (19.5)
		$B2_3$	60 (50.8)
D	20 (17)	D1	9 (8)
		D2	11 (9)

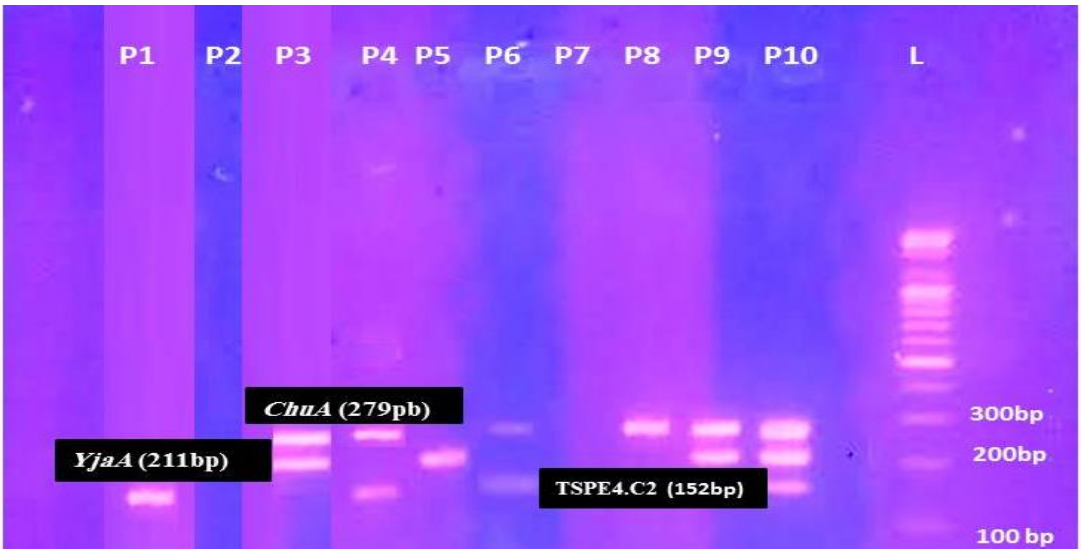

Figure (3): Triplex PCR profiles specific to E. coli phylogenetic groupings. The chuA gene, yjaA gene, and DNA fragment TSPE4.C2 are amplified to detect the phylogenetic groupings and subgroups of E. coli isolates. Group A (P2/A0, P5/A1, P7/A0); group B1 (P1); group B2 (P3/$B2_2$, P9/$B2_2$, P10/$B2_3$); group D (P4/D2, P6/D2, P8/D1)

The most common DEC isolates represented by group B2 were resistant to most antibiotics, especially Penicillin, Amoxicillin and Cephalexin, where the percentage of resistance to these antibiotics was 100%, while 96% of them were resistant to each of Ampicillin and Cefixime and Cefotaxim so 92% of them showed resistance to Cefepime while its resistance to Meropenem was weak (17%).On the other hand, all isolates of group D showed complete resistance (100%) to Penicillin, Ampicillin, Cefalexin, Cefepime and Amoxicillin, at the same time they showed high resistance (90%) to the Cefotaxim, while they were more sensitive (45% resist) to Ceftriaxone as shown in Table 7.

Less common isolates belonging to groups A and B1 showed complete resistance (100%) to both Penicillin and Amoxicillin also they have high resistance toward Cefalexin (80% and 90% respectively) and Cefotaxim (80% for each group), while they were 100% sensitive to Ceftriaxone.In addition, group A have 100% sensitivity to Ampicillin.

On the same line, all the subgroups (A0, A1, $B2_2$, $B2_3$, D1 and D2) are 100% resistant to Penicillin and Amoxicillin. It should also be mentioned that subgroup D2 isolates were the most resistant among the subgroups, as they were 100% resistant to other antibiotics added to what were mentioned such as Ampicillin, Cefalexin, Cefixime and Cefepime.In the second place in the resistance scale was subgroup D1, which showed complete resistance (100%) to Ampicillin, Cefalexin and Cefepime, added to Penicillin and Amoxicillin .The $B2_2$ and $B2_3$ subgroups also had a high resistance to the previously mentioned antibiotics, but they were sensitive to Ceftriaxone (resistance 48% and 17% respectively) and Imipenem (39% and 20 respectively). Moreover, The two isolates in the subgroup A0 showed a high sensitivity (100%) to Ampicillin, Cefixime, Ceftriaxone, Imipenem, Meropenem and Cefepime while the three isolates in the subgroup A1 were

highly resistant to most antibiotics (100%) except Ampicillin (0%), Cefixime (33%), Ceftriaxone (0%), Imipenem (33%) and Meropenem (33%) as shown in Table 8.

Table (7): Frequency antibiotics resistance among DEC phylogenotypic groups

B-lactams antibiotics	*E. coli* phylogenotypic groups				P value
	A	B1	B2	D	
	N (%)	N (%)	N (%)	N (%)	
Penicillin	5 (100)	10 (100)	83 (100)	20 (100)	1
Amoxicillin	5 (100)	10 (100)	83 (100)	20 (100)	1
Ampicillin	0 (0)	6 (60)	80 (96)	20 (100)	0.042*
Cefalexin	4 (80)	9 (90)	83 (100)	20 (100)	0.277
Cefixime	1 (20)	7 (70)	80 (96)	17 (85)	0.035*
Cefotaxim	4 (80)	8 (80)	80 (96)	18 (90)	0.046*
Ceftriaxone	0 (0)	0 (0)	21 (25)	9 (45)	0.039*
Imipenem	1 (20)	3 (30)	21 (25)	10 (50)	0.044*
Meropenem	1 (20)	2 (20)	14 (17)	15 (75)	0.049*
Cefepime	3 (60)	5 (50)	76 (92)	20 (100)	0.010*

Table (8): Frequency antibiotics resistance among *E.coli* phylogenetic subgroups

B-lactams antibiotics	Antibiotics resistance among *E.coli* phylogenetic subgroups N (%)						P value
	A0	A1	$B2_2$	$B2_3$	D1	D2	
Penicillin	2 (100)	3 (100)	23 (100)	60 (100)	9 (100)	11 (100)	1
Amoxicillin	2 (100)	3 (100)	23 (100)	60 (100)	9 (100)	11 (100)	2
Ampicillin	0 (0)	0 (0)	20 (87)	60 (100)	9 (100)	11 (100)	0.043*
Cefalexin	1(50)	3 (100)	23 (100)	60 (100)	9 (100)	11 (100)	0.275
Cefixime	0 (0)	1 (33)	22 (96)	58 (97)	6 (67)	11 (100)	0.033*
Cefotaxim	1 (50)	3 (100)	20 (87)	60 (100)	9 (50)	9 (50)	0.045*
Ceftriaxone	0 (0)	0 (0)	11 (48)	10 (17)	3 (33)	6 (67)	0.038*
Imipenem	0 (0)	1 (33)	9 (39)	12 (20)	7 (78)	3 (27)	0.048*
Meropenem	0 (0)	1 (33)	7 (50)	7 (50)	7 (78)	8 (73)	0.046*
Cefepime	0 (0)	3 (100)	20 (87)	56 (93)	9 (100)	11 (100)	0.011*

DISCUSSION

Disease caused by diarrheagenic E. coli strains is a global health concern, particularly in underdeveloped regions. Furthermore, it has significantly added to death, illness, and healthcare expenses [36].

Using data from three genes and a DNA fragment (the chuA and yjaA genes and TspE4.C2), Escherichia coli clinical strains have been assigned to one of many phylogenetic groupings (A, B1, B2, C, and D) [17]. The relationship between strains and illness and the frequency with which they occur in the environment may be learned by phylogenetic characterisation of E. coli clinical strains. However, few research have revealed phylogenetic groupings of DEC compared to Uropathogenic E. coli [18] . In the current study, DEC was isolated from 118 out of 150 people with diarrhea and it was found that the main cause of diarrhea was phylogenetic groups and subgroups of B2 (83/118), followed by D (20/118), while only 10/118 isolates belonged to group B1 and 5/118 to group A so

present finding dissimilar to pervious studies that indicated DEC strains belong to groups A, B1 and D [19, 20] in the same line study in southeast of Iran showed that DEC isolates mainly related into phylogenetic groups A and D (21) while our study agrees with the study of Clermont who mentioned that invasive *E. coli* strains caused diarrhea and symbiotic strains mostly belonged to groups A and B1, while those that caused intestinal infections primarily belonged to groups B2 and D . Ahumada-Santos et al. also found that the most common phylogroups among children with DEC were A and B2 [28]. Variations in study settings such as nutrition, habitat, ambient conditions, lifestyle, immune system, and cleanliness may account for observed discrepancies in the distribution of phylogenetic groupings [29].

A critical public health issue that may cause treatment challenges for patients is the growth of multidrug resistance among clinical isolates, which is mostly attributable to the overuse and abuse of antibiotics . The findings of our study show high levels of resistance to Penicillin, Ampicillin, Amoxicillin especially among phylogenetic group and subgroups of B2 and D , confirming results from other countries including Japan, China, South Africa, Iran, Mexico, and Michigan[22,23,24,25,26,27].The predominant mechanism of B- lactam antibiotics resistance among phylogenetic groups B2 and D is the hydrolysis of the antibiotic by beta-lactamases including ESBL and Amp-C B-lactamases that frequently acquired through large plasmids holding many resistance genes[40]. Clinical samples with ESBL-producing strains are cause for concern worldwide .The rapid spread of ESBL-positive isolates severely hinders the efficacy of broad-spectrum antibiotics, posing significant challenges to treatmen [30] . Horizontal gene transfer adds to the spread of resistance determinants, and the fact that ESBL-encoding plasmids often carry genes for resistance to other antimicrobial drugs compounds the problem. Therefore, knowledge of gene variations in bacteria that produce -lactamases is crucial for the proper and efficient treatment of patients [30]

DEC pathotypes showed varying degrees of resistance to Ampicillin, Chloramphenicol, and Trimethoprim-Sulfamethoxazole, ranging from 19.1% to 86.4%, according to a separate investigation by Nguyen *et al*. [31] in Honoi, Vietnam. Among DEC isolates in Egypt, the frequency of resistance to Ampicillin-Sulbactam, Ampicillin, and Trimethoprim-Sulfamethoxazole varied from 24.2% to 68.2% [32]. Similar results were seen in a study of DEC strains isolated from Tehran, Iran, by Bouzari *et al.* [33], who also found widespread resistance to the antibiotics trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol. The widespread prevalence of multidrug-resistant DEC pathotypes has been shown in a number of studies; this phenomenon may be attributable to the acquisition of resistance genes from the environment, the transfer of infections among people of all ages, or zoonosis [34], sometimes because to the careless use of many antibiotics for the treatment of infectious diarrhoea [35]. Another previous study determined a high resistance by bacteria to Cefixime, Penicillin and Ampicillin, and this is consistent with the current study, but our results showed a higher resistance [37]. The high resistance to most antibiotics in current study among DEC is probably due to continuous abuse of it for many years in our population [39]. As the current investigation confirms, Imipenem is a Carbapenem antibiotic that is very active against ESBL-producing Enterobactereaceae, as was shown before by Santo *et al*. This medication has a unique and desirable quality: its resistance to beta-lactamase. For gram-negative bacteria, this has a "post antibiotic" impact [38].

The results of our research showed isolation of DEC from different ages, and its resistance to beta-lactam antibiotics appeared in different age groups, especially the elderly, and this indicates the misuse of antibiotics for a long time by these people. The elderly and children also appeared more susceptible to diarrhea, this may be due to the lack of hygiene in children and the weak immune system of the elderly [38,41]. Furthermore, Canizalez-Roman recall that international and regional mobility raises the possibility of DEC transmission. Health policy makers, thus, need detailed information regarding the spread of these illnesses across different geographic areas [40]. However, few studies have looked into the role of DEC in acute diarrhea in people of all ages (children, adults, and the elderly).

CONCLUSION

Diarrheagenic *Escherichia coli* are common in our society. Molecular analysis showed that phylogenetic groups B2 and D are the main cause of diarrhea due to their virulence factors, in addition to their high resistance to most beta-lactams, especially Penicillin, Amoxicillin, and Ampicillin, which should be discontinued as treatment in hospitals and sold in pharmacies in Iraq.

RECOMMENDATIONS

The high rate of DEC resistance to commonly used antibiotics (beta-lactam class) in the treatment of diarrheal diseases has become a source of concern and a great danger to human health. Therefore, this must be carefully and continuously monitored to determine the extent of its spread to take the appropriate strategy to reduce its risk. At the same time, there must be later studies about phylogenetic groups using quadruple PCR technique to determine the prevalence and relationship of new groups phylogenetic (C, E, and F) with intestinal infection and their sensitivity to antibiotics.

REFERENCES

1. Hien BTT, Scheutz F, Cam PD, *et al*. Diarrheagenic *Escherichia coli* and Shigella strains isolated from children in a hospital case-control study in Hanoi, Vietnam. J. Clin. Microbiol. . 2008;46:996–1004.
2. Okeke IN, Fayinka ST, Lamikanra A. Antibiotic resistance in *Escherichia coli* from Nigerian students, 1986-1998. Emerg. Infec. Dis. . 2000;6:393–396.
3. Aibinu IE, Peters RF, Amisu KO, *et al.* Multidrug resistance in *E. coli* 0157 strains and the public health implication. J. Am. Sci. 2007;3:22–33.
4. Brinas L, Zarazaga M, Sa´enz Y, *et al.* β-Lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. Antimicrob. Agents Chemother. 2002;46:3156–3163.
5. Lietzau S, Raum E, von Baum H, *et al.* Clustering of antibiotic resistance of *E. coli* in couples: suggestion for a major role of conjugal transmission. BMC Infect. Dis. . 2006;6:119.
6. Miles TD, McLaughlin W , Brown PD. Antimicrobial resistance of *Escherichia coli* isolates from broiler chickens and humans. BMC Vet. Res. . 2006;2.
7. Oteo J, Navarro C, Cercenado E, *et al.* Spread of *Escherichia coli* strains with high-level cefotaxime and ceftazidime resistance between the community, long-term care facilities, and hospital institutions. J. Clin. Microbiol. . 2006;44:2359–2366.
8. Steward CD, Rasheed JK, Hubert SK, *et al.* Characterization of clinical isolates of *Klebsiella pneumoniae* from. 2001;39:2864–2872.
9. Ahmed MO, Clegg PD, Williams NJ, *et al.* Antimicrobial resistance in equine faecal *Escherichia coli* isolates from North West England. Ann. Clin. Microbiol. Antimicrob. . 2010;9:12.
10. Lautenbach E, Patel JB, Bilker WB, *et al*. Extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. Clin. Infect. Dis. . 2001;32:1162–1171.
11. Aslani MM, Salmanzadeh-Ahrabi S, Alikhani YM, *et al.* Molecular detection and antimicrobial resistance of diarrheagenic *Escherichia coli* strains isolated from diarrheal cases. Saudi Med. J. . 2008;29:388–392.
12. Aworh MK, Kwaga J, Okolocha E, Mba N, and Thakur S. Prevalence and risk factors for multi-drug resistant Escherichia coli among poultry workers in the Federal Capital Terri tory, Abuja, Nigeria. PLoS One. 2019;14(11):e0225379.
13. Clinical Performance standards for antimicrobial susceptibility testing; Twenty-first informational supplement. CLSI document M100-S21. The Institute, Wayne. 2011.

14. Khairy R.M.M., FathybZ.A., Mahrous D.M. *et al*. Prevalence, phylogeny, and antimicrobial resistance of *Escherichia coli* pathotypes isolated from children less than 5 years old with community acquired- diarrhea in Upper Egypt. BMC Infect Dis. 2020; 20: 908.

15. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol. 2000;66:4555–8.

16. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests. M012 -A12. Wayne: Clinical and Laboratory Standards Institute; 2015.

17. Clermont O, Christenson JK, Denamur E, Gordon DM. The Clermont Escherichia coli phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep. 2013;5(1):58-65.

18. Mosquito S, Pons MJ, Riveros M, *et al*. Diarrheagenic *Escherichia coli* phylogroups are associated with antibiotic resistance and duration of diarrheal episode. ScientificWorldJournal. 2015;2015:610403.

19. Brisse S, Diancourt L, Laouénan C, *et al.* Phylogenetic distribution of CTX-M- and non-extended- spectrum- β-lactamase- producing *Escherichia coli* isolates: group B2 isolates, except clone ST131, rarely produce CTX-M enzymes. J Clin Microbiol. 2012;50:2974–81.

20. Khadhim MM, Kazaal MA. Antibiotics Resistant and Integrin Class 1 among Commonsal *Escherichia coli.* Al-Qadisiyah Medical Journal. 2017; 113 (24):30-38.

21. Alizade H, Ghanbarpour R, Aflatoonian MR. Virulence genotyping of *Escherichia coli* isolates from diarrheic and urinary tract infections in relation to phylogeny in southeast of Iran. Trop Biomed. 2014;31:174–82.

22. Kubomura A, Sekizuka T, Onozuka D, *et al.* Truncated class 1 integron gene cassette arrays contribute to antimicrobial resistance of diarrheagenic *Escherichia coli.* Biomed Res Int. 2020;2:1-9.

23. Zhang SX, Zhou YM, Tian LG, *et al*. Antibiotic resistance and molecular characterization of diarrheagenic *Escherichia coli* and non-typhoidal Salmonella strains isolated from infections in Southwest China. Infect Dis Poverty. 2018 ; 7:7(1):53.

24. Omolajaiye S, Afolabi K, Iweriebor B. Pathotyping and antibiotic resistance profiling of *Escherichia coli* isolates from children with acute diarrhea in amatole district municipality of Eastern Cape, South Africa. BioMed Res Int. 2020;4:1-10.

25. Abbasi E, Mondanizadeh M, van Belkum A, Ghaznavi-Rad E. Multi-drug-resistant diarrheagenic *Escherichia coli* pathotypes in pediatric patients with gastroenteritis from central Iran. Infect Drug Resist. 2020; 13; 1387—1396.

26. Amézquita-López BA, Quiñones B, Soto-Beltrán M, *et al*. Antimicrobial resistance profiles of Shiga toxin-producing *Escherichia coli* O157 and Non-O157 recovered from domestic farm animals in rural communities in Northwestern Mexico. Antimicrob Resist Infect Control. 2016; 5(5):1.

27. Mukherjee S, Mosci RE, Anderson CM, *et al.* Antimicrobial drug–resistant Shiga toxin–producing *Escherichia coli* infections, Michigan, USA. Emerg Infect Dis. 2017; 23(9):1609-1611.

28. Ahumada-Santos YP, Báez-Flores ME, Díaz-Camacho SP, *et al.* Association of phylogenetic distribution and presence of integrons with multidrug resistance in *Escherichia coli* clinical isolates from children with diarrhoea. J Infect Public Health. 2020;13(5):767-772.

29. Jafari E, Oloomi M, Bouzari S. Characterization of antimicrobial susceptibility, extended-spectrum β-lactamase genes and phylogenetic groups of Shigatoxin producing *Escherichia coli* isolated from patients with diarrhea in Iran. Ann Clin Microbiol Antimicrob. 2021; 15:20(1):24.

30. Halawani EM. Beta-lactam antibiotic resistance in *Escherichia coli* commensal fecal flora of healthy population in Taif, Saudi Arabia. African Journal of Microbiology Research. 2010; 5:73–78.

31. Nguyen TV, Le PV, Le CH, Weintraub A. Antibiotic resistance in diarrheagenic *Escherichia coli* and Shigella strains isolated from children in Hanoi, Vietnam. Antimicrob Agents Chemother. 2005;49(2):816-9.

32. Putnam SD, Riddle MS, Wierzba TF, *et al*. Antimicrobial susceptibility trends among *Escherichia coli* and Shigella spp. isolated from rural Egyptian paediatric populations with diarrhoea between 1995 and 2000. Clin Microbiol Infect. 2004;10(9):804-10.

33. Bouzari S, Jafari A, and Zarepoor M. Distribution of genes Encoding toxins and antibiotic resistance patterns in diarrhea-Genic *Escherichia coli* isolates in Tehran. The Eastern Medi-Terranean Health Journa. 2007; 13(2):287-93

34. Karama M, Mainga AO, Cenci-Goga BT, *et al.* Molecular profiling and antimicrobial resistance of Shiga toxin-producing *Escherichia coli* O26, O45, O103, O121, O145 and O157 isolates from cattle on cow-calf operations in South Africa. Sci Rep. 2019;9(1):11930.

35. Usein CR, Tatu-Chitoiu D, Ciontea S, *et al. Escherichia coli* pathotypes associated with diarrhea in Romanian children younger than 5 years of age. Jpn J Infect Dis. 2009;62(4):289-93.

36. Akinjogunla OJ, Eghafona NO, Ekoi OH. Diarrheagenic *Escherichia coli* (DEC): prevalence among in and ambulatory patients and susceptibility to antimicrobial chemotherapeutic agents. J. Biotech. Res. 2009;1:34–38 .

37. Ghorbani-Dalinia S, Kargar M, Doostic A, *et al*. Molecular Epidemiology of ESBLs Genes and Multi-Drug Resistance in Diarrheagenic *Escherichia coli* Strains Isolated from Adults in Iran. Iranian Journal of Pharmaceutical Research. 2015;14 (4): 1257-1262.

38. Santo E, Salvador MM and Marin JM. Multidrug-Resistant urinary tract isolates of *Escherichia coli* from Ribeirão Preto, São Paulo, Brazil. Braz. J. Infect. Dis.2007; 11: 575-578.

39. Hassan SA, Jamal SA and Kamal M. Occurrence of Multidrug resistant and ESBL producing *E. coli* causing Urinary tract infections. J. Basic Appl. Sci. 2011;7: 39-43.

40. Falagas ME, Karageorgopoulos DE. Extended-spectrum beta-lactamase-producing organisms. J Hosp Infect. 2009;73(4): 345–54.

41. Canizalez-Roman A, Flores-Villasenor HM, Gonzalez-Nunez E, Velazquez-Roman J, Vidal JE, *et al.* Surveillance of Diarrheagenic *Escherichia coli* strains isolated from diarrhea cases from children, adults and elderly at northwest of Mexico. Front Microbiol. 2016;7:1924.

42. Suzhaeva LV, Makarova MA, Kaftyreva LA. Phylogenetic groups and virulence genes of Escherichia coli strains isolated from the children gut microbiota. Klin Lab Diagn. 2020;65(4):251-257.