



Detection of Antibiotic Resistance of the Phylogenetic Group E among *E. coli* Isolated from Diarrheal Cases in Children Under Five Years

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Abstract

From 50 stool samples collected from children with diarrhea of both sexes who visited various hospitals in Baghdad, 26 isolates of *E. coli* were found to belong to the phylogenetic group E. The findings revealed that the percentage of *E. coli* for the phylogenetic group E is (52%), making it the dominant group among the other phylogenetic groups. The findings demonstrated that 100% of the *E. coli* isolates from phylogenetic group E are resistant to penicillin, and only 15% are resistant to imipenem. Multi-drug resistance (MDR) was found to be 15%, while XDR reached 85%. The results of the phylogenetic group for the remaining species of isolates in this study were group A (2/50 and by 4%), group B2 (1/50 and by 2%), group C (12/50 and by 24%), group D (6/50 and by 12%), group F (3/50 and by 6%), group B1 by 0%, and group Clade 1 by (0%).

Keywords: Diarrhea, phylogenetic group E, *E. coli*.

1. Introduction

Escherichia coli is one of the important bacteria belonging to the Enterobacteriaceae family; it is part of the normal flora in the colon of humans and other animals. The characteristics of *E. coli* is negative, facultative anaerobic, widespread in nature, positive indole and methyl red, negative oxidase test, positive catalase test, negative Voges-Proskauer, H₂S, and gelatin [1].

E. coli is described as containing a large number of virulent factors, which are cytotoxic necrotizing factor, cilia that help attach to the surface of the host, flagella, capsula, lipopolysaccharides (LPS), haemolysin, toxins, siderophores, and also flagellar antigen H, somatic antigen O and capsular antigen K capsules, enzymes and Type III secretion systems, and alkaline protease. *E. coli* cause infection and disease in humans; the most important of these are intestinal diseases represented by watery and bloody diarrhea, which is called diarrheagenic *E. coli* (DEC), urinary tract infections (UTIs) which are called uropathogenic *E. coli* (UPEC), sepsis, and meningitis [3].



Diarrhea is a general health problem and a common disease that affects all age groups; it is the largest and most influential percentage, especially in children under five years. Determine which groups of *E. coli* include Shiga-toxin. *E. coli* (STEC) or Enterohemorrhagic *E. coli* were classified into two main groups: O157, which causes hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUC). The second group that includes the most common serotypes of EHEC/STEC related to human infection is O24, O26, O45, O103, O111, O121, O55, and O145, which are the most dangerous and cause diseases caused by food and are transmitted through food-producing animals such as cows and chickens, which are the main reservoirs of many foodborne pathogens that are represented by strains of *E. coli* producing Shiga-toxins that cause millions of sporadic disease conditions such as diarrhea and chronic complications. Studies have shown that 1.5 billion cases of diarrhea annually in children under 5 years of age are caused by intestinal pathogenic bacteria, which lead to more than 3 million deaths annually [1, 2]. Epidemiological studies have shown a rise in the rate of STEC infection in humans, leading to a broad challenge to public health services in many countries with high incidence and mortality rates worldwide [3].

The resistance of *E. coli* to antibiotics is one of the biggest health problems in the world, which prompted many researchers to generate new antibiotics to defeat the resistant strains. *E. coli* has several mechanisms of resistance to antibiotics, such as Amoxicillin, Ampicillin, Cefepime, Ceftriaxone, Cefotaxime, Imipenem, Gentamicin, Amikacin, Ciprofloxacin, Norfloxacin, Chloramphenicol, Amoxicillin, /Clavulanic acid, Aztreonam, Piperacillin, and Tazobactam. especially beta-lactams through their effect on the inhibition of transpeptidase and carboxypeptidase enzymes, which affect the side connections in the cell wall that surrounds the bacteria, leading to its weakness and thus cell decomposition. Additional mechanisms that *E. coli* possess for resistance to beta-lactams include the production of beta-lactamase enzymes, a change in cell membrane permeability, an efflux pump, and target site modification [4].

The study aimed to determine the relationship between antibiotic resistance patterns and phylogenetic group E in *E. coli* that cause diarrhea in children under five years of age.

2. Materials and Methods

2.1. Collection of samples from patients

In three hospitals in Baghdad (the Children's Protection Teaching Hospital/City of Medicine, the Central Children's Teaching Hospital, and Al-Alwiya Teaching Hospital), from January 2022 to the end of April 2022, 120 fecal samples were collected from children with diarrhea who were under five years old, both males and females.

2.2. Isolation and diagnosis of *E. coli*

Oxidase, catalase, and IMViC, which included Indole methyl red, voges proskauer, and citrate utilization, were used to identify *E. coli* samples in addition to cultural media, MacConkey agar, Blood agar, and Eosin methylene blue agar [5]. with the vitek-2 compact system, identify the final diagnosis of isolates (France, Biomerieux).

2.3. Resistance of *E. coli* isolates to antibiotics

The sensitivity test to bacterial isolates under study was carried out using the Kirby-Bauer method according to CLSI 2021 [6] for 15 antibiotics, which include: Amoxicillin (30 µg), Ampicillin (10 µg), Cefepime (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Imipenem (10 µg), Gentamicin

(10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), NORfloxacin (10 µg) Chloramphenicol (30 µg), Amoxicillin/ Clavulanic acid (30µg), Aztreonam (30 µg) Piperacilli/Tazobactam (100/10 µg) , Trimethprim-Sulfamethozal (1.25/23.75 µg).

2.4. DNA isolation using a special kit to extract genomic DNA(HiPurA®Bacterial Genomic DNA Purification Kit ®): the isolation of bacteria is carried out in accordance with the manufacturer's recommendations.

2.5. Measuring the purity of the DNA output in the Nano drop device

The purity of DNA was measured using Nano-drop, by applying 1µl of the extracted DNA to the sampling port of the device.

Detection of phylogenetic groups of genes in *E.coli* using a multiplex PCR device as shown in **Table 1**, including the sequence of specific primers.

Table 1. Sequence of Primers used in this study.

Gene name		Primer sequence (5-3)	Product size (base pair)	Reference
<i>ChuA</i>	F	ATGGTACCGGACGAACCAAC	288	[7]
	R	TGCCGCCAGTACCAAAGACA		[8]
<i>YjaA</i>	F	CAAACGTGAAGTGTCAGGAG	211	[7]
	R	AATGCGTTCCCTCAACCTGTG		
<i>TspE4C2</i>	F	CACTATTCGTAAGGTCATCC	152	[7]
	R	AGTTTATCGCTGCGGGTTCGC		
<i>AceK</i>	F	AACGCTATTCGCCAGCTTGC	400	[9]
	R	TCTCCCCATACCGTACGCTA		
<i>ArpAgpE</i>	F	GATTCATCTTGTCAAAATATGCC	301	[10]
	R	GAAAAGAAAAAGAATTCCCAAGAG		
<i>trpAgpC</i>	F	AGTTTTATGCCAGTGCGAG	219	[10]
	R	TCTGCGCCCGGTCACGCC		
<i>trpBA</i>	F	CGGCGATAAAGACATCTTCAC	489	[11]
	R	GCAACGCGGCTGGCGGAAG		

2.6. Investigation of phylo-groups genes in *E.coli*

Prepare a PCR mixture of 12.5 µl of Master Mix equipped by Promega (USA), 1 µl per F-Primer and R-Primer primer, 3 µl of DNA tamplate and 2.5 µl of Deionized Sterile Distilled Water and Equipped by Promega (USA) to obtain the final size of 25 µl then the contents of the PCR tubes were thoroughly mixed using the vortex and then placed in a PCR column. The optimal conditions for detecting the Phylo-group genes included (AceK, ArpAgpE, ChuA, trpBA, trpAgpC, TspE4C2, YjaA) for *E. coli* using Multiplex PCR are only one cycle for 4 minutes at 94°C for the first denaturation, 30 cycles including 5 seconds at 94 °C for the denaturation of the DNA template, 20 seconds at 57 °C for the primer to bind with the DNA template, 1 minute at 72°C to elongate the associated primer, and then transfer 5 µl of the duplication gene product for electrophoresis on the prepared agarose gel at 2% with a voltage difference of (100) volts and for 60 minutes, and use the volumetric guide (100-1500 base pairs).

3. Results and Discussion

After conducting the tests to diagnose the bacteria, 26 isolates were obtained, and 52% belong to *E. coli* and the phylogenetic group E, which were collected from cases of diarrhea in children

under five years of age. Out of the 50 *E. coli* that belong to the phylogenetic group E showed a clear variation in resistance to the antibiotics used in this study, as these isolates showed resistance to Ampicillin and Amoxicillin 100%, resistance to Chloramphenicol and Ceftriaxone 69%, and resistance to Aztreonam 77%, Ciprofloxacin 62%, Sulfamethoxazole-Trimethoprim 59%, Cefotaxime 54%, Amoxicillin-clavulanic acid 53%, Cefepime 46%, Gentamicin 38%, Amikacin 35%, resistance to Piperacillin-tazobactam 31%, Norfloxacin 23%, and resistance to Imipenem 15% [11] (CLSI, 2022) as shown in **Figure 1**.

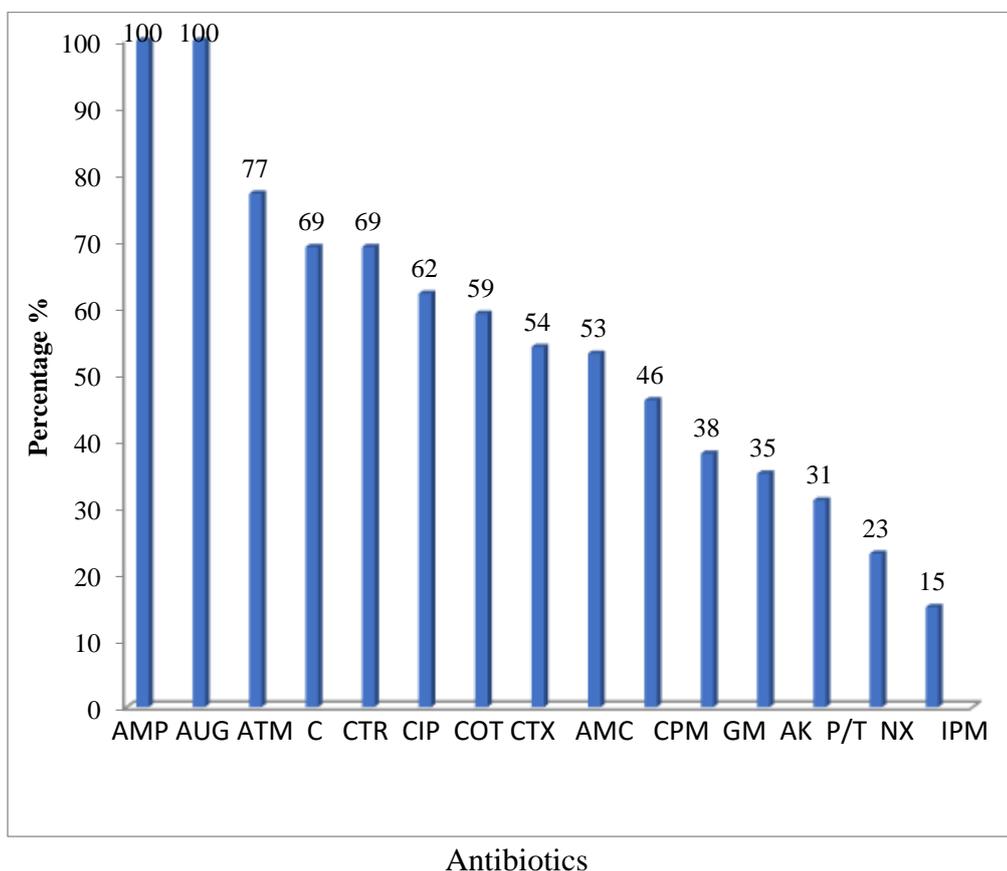


Figure 1. Percentage of resistance to *E. coli* isolates from phylogenetic group E of antibiotics; Amoxicillin (AUG), Ampicillin (AMP), Cefepime (CPM), Ceftriaxone (CTR), Cefotaxime (CTX), Imipenem (IMP), Gentamicin (GM), Amikacin (AK), Ciprofloxacin (CIP), NORfloxacin (NX), Chloramphenicol (C), Amoxicillin /Clavulanic acid (AMC), Aztreonam ATM, Piperacillin / Tazobactam P/T.

The presence of genes (TrpBA, arpA, ArBAGpE, ChuA, TspE4c2, yjaA) was detected, and the results showed that isolates E7-E25 – E39– E40 possess genes (ArpA-TrpBA – ChuA) and they are resistant to three groups of antibiotics by 15%. The isolates E3-E6-E8-E12-E32-E33-E35-E37 that possess genes (ArpAgp.E-arpA-TrpBA-ChuA) were resistant to six groups of antibiotics by 31%, while the isolates E28-E16-E10-E34-E38-E36-E48 that possess genes (arpA-TrpBA-ChuA-TspE4C2) resistant to seven groups of antibiotics by 30%, whereas the isolates E1-E9-E15-E24-E41-E44-E50 that possess genes (ArpAgp.E-arpA-TrpE4C2) were resistant to eight groups of antibiotics by 27%, as shown in **Figures (2) and (3)**, as shown in **Figures (2) and (3). Table (1)**. The results of [13] in a study on 33 isolates of *E. coli* conducted in Iraq (Baghdad) and the results of [14] conducted in the city of Shiraz in southern Iran on 113 isolates and isolated cases of diarrhea in children determined the percentage of resistance to Ampicillin and Amoxicillin by 100%. The resistance of bacteria to the antibodies of the Cephalosporin group (Cefepime, Ceftriaxone, and

Cefotaxime) is indicated by the results of [15], which were conducted in India and isolated from 200 children, where the resistance rate to Cefotaxime was 67%, as well as [16], which showed that the resistance to Ceftriaxone 88%. Whereas, the results of the [17] study determined the resistance percentage of Cefepime to be 44%. While the findings of [15] determined a resistance rate of 15% to Monobactams, which include Aztreonam and Carbapenem, which include Imipenem, the results of [15] showed the resistance percentage was determined at 15%. One of the most important reasons for resistance to beta-lactam by *E.coli* is due to the production of beta-lactamase enzymes (Cephalosporinase, Penicillinase), as well as their production of broad-spectrum beta-lactamase enzymes (ESBLs), their possession of efflux pumps, and a change in the permeability of the outer membrane due to the loss of outer membrane openings [18].

The results of the current study showed the resistance of *E.coli* to the aminoglycoside group, which includes the antibiotics Gentamicin and Amikacin. The results of [15] indicate that the percentages of resistance to the Gentamicin and Amikacin were 31% and 28%, respectively. Local isolates have shown resistance to the Aminoglycoside group, and the reason for this resistance is due to the production of the modified enzymes Phosphotransferase and N-acetyl transferase, which have the ability to modify the antibiotic molecule to an ineffective form [19].

E.coli isolates in the current study showed resistance to Quinolone antibiotics that include both Ciprofloxacin and Norfloxacin; the results of [17] determined the resistance percentage of Ciprofloxacin at 49%, and the study of [15] indicated the resistance percentage of Norfloxacin (25%). *E.coli* resistance to quinolone antibiotics is caused by stopping the action of the DNA gyrase enzyme or by a mutation in the DNA gyrase enzyme [20]. *E.coli* isolates have shown resistance to Chloramphenicol this agrees with the [14] study on 113 patients conducted in southern Iran, in which they mentioned the resistance percentage of this antibiotic as 36%.

While [16] showed a resistance to Chloramphenicol by 87%, the resistance to chloramphenicol is caused by the disruption of the activity of the enzyme chloramphenicol acetyl transferase (CAT) by an enzyme that enters new acetylcysteine groups in antibiotics [21]. In the current study, the isolates of *E.coli* bacteria have shown resistance to Trimethoprim-Sulfamethoxazole, and the results of the [17] study determined the resistance percentage of this antibiotic to be 66%. This indicates that the mechanism of action of this group is evidenced by mixing these two antibiotics to become more powerful than the action of each antibiotic separately and thus work to inhibit the metabolic pathway of making folic acid, which plays an important role in the synthesis of bacterial cells nucleic acid [22]. *E.coli* isolates have shown resistance to Piperacillin and Tazobactam, as indicated by the results of [15], which indicate that the resistance percentage to this antibiotic is 21%. As for Amoxicillin and Clavulanic Acid, the results of the [17] study that determined the resistance percentage of this antibiotic to 23% indicate that these inhibitors work to form a stable covalent bond between the inhibitor and the active site of enzymes of beta-lactamase, which in turn inhibits these enzymes and at the same time works to protect the antibiotic [23].

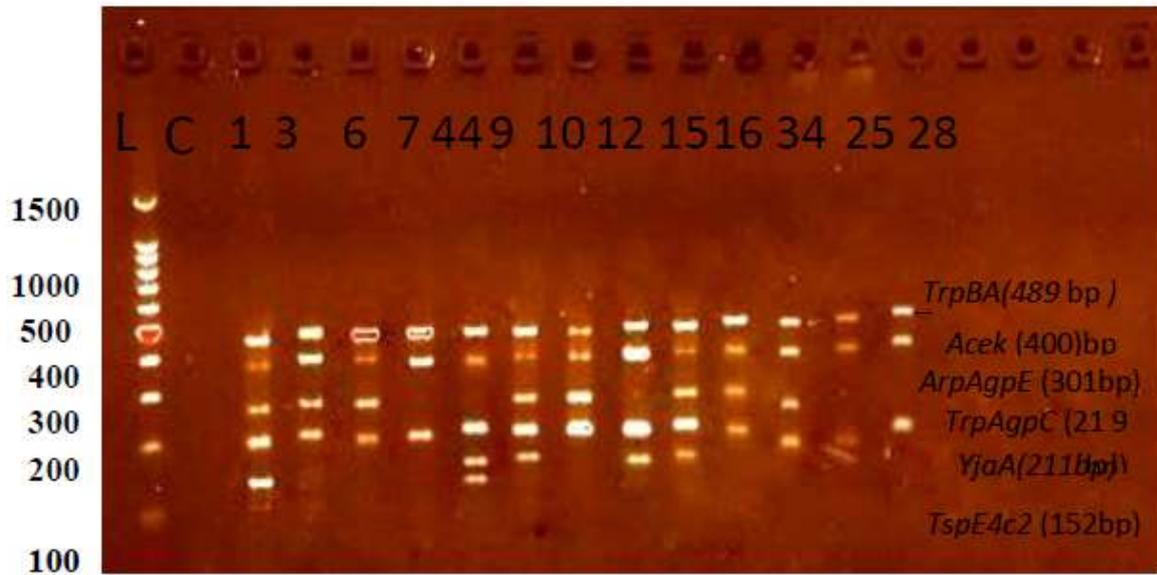


Figure 2. Electrophoresis of the polymerase chain reaction product of *trpBA* gene (489bp), *Acek* gene (400bp), *ArBAgpE* gene (301bp), *ChuA* gene (288bp), *TrpAgpc* gene (219bp), *YjaA* gene (211bp) and *TSPE4C2* gene (152bp) for the isolates of *E.coli* on the agarose gel at a concentration of 2% and a voltage d 100 V for 60 minutes. L : ladder marker (100-1500 bp).

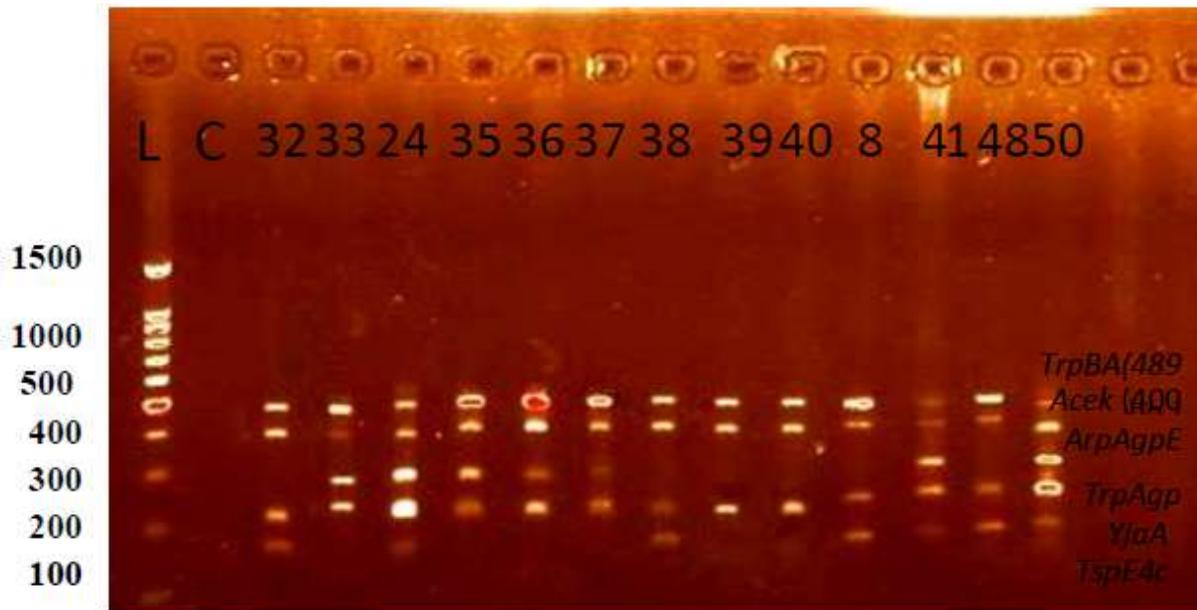


Figure 3. Electrophoresis of the polymerase chain reaction product of *trpBA* gene (489bp), *Acek* gene (400bp), *ArBAgpE* gene (301bp), *ChuA* gene (288bp), *TrpAgpc* gene (219bp), *YjaA* gene (211bp) and *TSPE4C2* gene (152bp) for the isolates of *E coli* on the agarose gel at a concentration of 2% and a voltage 100 V for 60 minutes. L:ladder marker (100-1500 bp).

Table 1. The percentages of the phylogenetic group E resistant to different groups of antibiotics.

Isoletes	Possession of genes	Antibiotics	Percentage of resistance%
E7– E259-E3-E40	<i>ArpA -TrpBA -ChuA</i>	3	15
E3-E6-E8-E12- E32-E33-E35- E37	<i>YjaA- TrpBA – arpA- TspE4C2- ArpAgp.E</i>	6	31
E10-E16-E28- E34-E36 -E38- E48	<i>arpA -TrpBA -ChuA- TspE4C2</i>	7	30
E1-E9-E15-E24- E41– E44-E50	<i>-ArpAgp.E - arpATrpBA - TspE4C2 -ChuA</i>	8	27

Resistance to antibiotic groups can be gained through mutations or gene transmission by conjugation, transduction, transformation, or the possession of plasmids, transposons, or integrons that are carriers of antibiotic-resistant genes. Several studies have indicated a lack of association between sensitivity to antibiotics and the phylogenetic group assigned to the isolates of *E.coli* [24]. The information obtained emphasizes the importance of regular monitoring of the distribution of the phylogenetic group and antibiotic resistance to commensal and pathogenic strains of *E. coli* in each geographical region, as such studies can be useful in developing appropriate guidelines for the administration of antibiotics among the child population in the region.

4. Conclusions

The phylogenetic group was divided into 8 groups, including A, B1, B2, C, D, F, Clade 1, and the E group, which was the common group. The E group had TrpBA, ArpA, ArBAgpE, ChuA, TspE4c2, and yjaAgenes.

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