

# Correlation Between Antibiotic Resistance and Phylogenetic Groups of *Escherichia Coli* Isolated from Children with Acute Diarrhea in Baqubah City

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## ABSTRACT

Diarrhea remains a significant public health concern among children in Iraq, leading to high rates of morbidity and mortality. *Escherichia coli* is a common bacterial pathogen associated with acute diarrhea in children, making the investigation of its genetic diversity and antibiotic resistance patterns essential. This study aimed to evaluate the relationship between antibiotic resistance and the phylogenetic groups of *E. coli* isolated from children with acute diarrhea in Baqubah City. A total of 367 stool samples were collected between November 15, 2022, and February 1, 2023, from pediatric patients at Al-Batool Hospital for Obstetrics, Gynecology, and Children, as well as private laboratories. The samples included 207 males and 160 females, aged from less than one year to seven years old. Antibiotic susceptibility testing was performed for 15 antibiotics, with resistance rates observed as follows: Ampicillin (96%), Amoxicillin-clavulanate (94%), Ceftazidime (88%), Cefotaxime (92%), Aztreonam (68%), Tobramycin (26%), Amikacin (56%), Azithromycin (52%), Doxycycline (60%), Tigecycline (0%), Ciprofloxacin (26%), Levofloxacin (28%), Imipenem (14%), Trimethoprim-sulfamethoxazole (74%), and Sulfonamides (68%). Phylogenetic analysis of *E. coli* isolates was conducted based on the presence of seven specific genes, categorizing them into eight phylogenetic groups: A, B1, B2, C, D, E, F, and UP. The highest prevalence was observed in group E (13 isolates, 26%), followed by group C (8 isolates, 16%). Groups A, B2, and UP each accounted for 6 isolates (12%), while group B1 had 5 isolates (10%). The lowest prevalence was seen in groups D and F, with 3 isolates each (6%). This study highlights the high prevalence of antibiotic resistance among *E. coli* isolates and provides valuable insights into the phylogenetic distribution of *E. coli* in children with acute diarrhea in Baqubah City. These findings emphasize the need for continuous surveillance of antimicrobial resistance and phylogenetic diversity to guide effective treatment strategies and public health interventions.

**Keywords:** *Escherichia Coli*, Antibiotic Resistance, Phylogenetic Groups Genes

## INTRODUCTION

Enterobacteriaceae is a family of bacteria that includes *Escherichia coli*. They can be sticky or mobile, with some strains having certain traits while others do not. They are commonly found in agricultural settings and can grow with or without oxygen, and do not produce spores [1]. Most of these bacteria produce CO<sub>2</sub> gas and different types of toxins and virulence agents and have complex antigen compositions [2, 3]. Diarrheagenic *E. coli* (DEC) is the most common bacterial pathogen worldwide and often contributes to intestinal diseases caused by foodborne pathogens [4]. DEC generally causes watery diarrhea, which in some cases can develop into a more severe disease associated with a range of virulence factors. *E. coli* bacteria are the most studied organisms and have been instrumental in developing fundamental concepts in biology [5]. They are well-suited for biochemical and genetic research, and their study is now prominent due to their prevalence, ease of isolation, and the development of molecular biological techniques [6]. A major advantage of studying *E. coli* is understanding how bacteria combat antibiotics. This can provide valuable information about the connection between these bacteria and the development of antibiotic resistance in humans [7]. Additionally, poor hygiene practices, such as transmission of bacteria through oral-fecal routes between humans, environmental sources, and animals, contribute to exacerbating this issue [8]. Diarrheagenic *E. coli* is believed to be the most common infectious agent that causes diseases. Its ability to cause infection is

determined by the virulence and host susceptibility to infection and pathological patterns can be expressed through a range of clinical symptoms [9]. There is growing evidence that many *E. coli* strains are specialized. Biochemical and Phylogenetic group evidence supports the classification of *E. coli* as a distinct species based on high genomic diversity, intra-strain variability, phenotype, altered metabolic pathways, and different areas of injury [10]. It includes *E. coli* bacteria from many groups, the pathogens of which are classified into several types, most of which are linked to diarrhea and are known as Diarrheagenic *E. coli* [11]. Beta-lactam antibiotics are widely used antimicrobials that play a crucial role in healthcare around the world. They are derived from common chemical structures and are modified through the addition of active groups. In some cases, two beta-lactam rings are combined to create a single multifunctional compound [12]. Antibiotic-resistant bacteria pose a significant global threat to human health, particularly those producing  $\beta$ -lactamases, representing a monumental development in antibiotic resistance [13]. Inactivation of antibiotic activity by plasmid-encoded enzymes and transposons is the most common mechanism of resistance to aminoglycosides [14]. Modified enzymes aminoglycosides are classified into three types: acetyltransferases (AAC), adenylyltransferase (ANT), and phosphotransferases (APH) [15]. Chloramphenicol and Florfenicol are phenols that inhibit protein synthesis by blocking binding to peptidyltransferase in the Ribosome subunit S50, inhibiting peptide

transport for peptide chain elongation [16]. Resistance to chloramphenicol in bacteria is usually caused by acquiring plasmids that express chloramphenicol acetyltransferases (CAT), which disrupt treatment enzymatically. Three forms of CAT enzymes were detected in esteemed bacteria. , resistance occurs due to reduced external membrane permeability and the acquisition of extrachromosome flow pumps [17]. Tetracycline functions by inhibiting protein synthesis. Bacteria enter via an energy-dependent mechanism and bind reversely to the bacteria's 30S ribosome subunits. This method prevents aminoacyl-tRNA from reaching the RNA-ribosome complex, thus hindering the production of bacterial polypeptides [18]. Trimethoprim resistance is due to the acquisition of DNA that determines DHFR, which reduces trimethoprim inhibition by enzymes [19]. Fluoroquinolones work by targeting bacterial enzymes DNA gyrase and DNA topoisomerase IV. These medications stabilize the enzyme-DNA complex, causing the DNA to break into strands and leading to cell death. This is an effective way to kill bacteria [20].

### Phylogenetic Grouping of E. coli

Understanding the relationships between species in the phylogenetic group is essential for many biological studies. The phylogenetic tree provides valuable insights into evolutionary changes, such as the development of new metabolic patterns. It is crucial for determining the origin of genes, identifying molecular adaptations, comprehending the morphological evolution of shapes, and reconstructing recent demographic changes. However, accurately constructing a phylogenetic tree presents various challenges, including identifying genes or proteins and aligning multiple sequences [21]. The classification of E. coli isolates by Phylogenetic group is crucial not just for understanding E. coli populations, but also for clarifying the relationship between strains and diseases [22]. Clermont et al. [9] divided the extracellular E.coli bacteria into four classes using a PCR reaction and amplified three genetic markers, TspE4.C2, chuA, and yjaA, in 2000. These isolates were obtained from

various sources[23]. Clermont et. al.[9] added a new arpA gene to the previous three genes in 2013 to create a chain reaction of four more precisely than the previous division. E. coli isolates were classified into eight groups using this method (B1, B2, A, D, F, E, C, (clade). Here, a new PCR-based method was developed that enables the identification of E. coli isolation from the eight Clermont ethnic groups and others, 2013). Some Phylogenetic groups are more resistant to antibiotics than others. Prevalence of resistance in groups B2 and D has also been observed, so specific antibiotic treatment strategies should be developed.

### MATERIALS AND METHODS

Between November 15, 2022, and February 1, 2023, patients hospitalized to Al-Batool Hospital for Obstetrics, Gynecology, and Children and private laboratories provided discharge samples (stool). The samples were incubated at 37°C for 24 hours after being placed straight into the middle of the MacConkey agar. They were then plated in EMB medium, where isolates with a bright metallic green color emerged after a 24-hour incubation period at 37°C. Following a plating process for purification on a nutrient agar medium, all of the isolates were cultured in accordance with Atlas et al. The study made use of standard antibiotic tablets that are mentioned in CLSI (2022).

#### Extraction DNA

Boiling the bacterial culture in a tiny tube with sterile distilled water for ten minutes at 100°C was the method used to extract the bacterial DNA [24]. After centrifuging the mixture, the floating material was utilized right away as a DNA template for PCR procedures [25]. Using the specialized initiator trpA, E. Coli was identified [26].

#### PCR Master Mix

Attended the PCR interaction mix using Go Taq ® G2 Green Master Mix provided by Promega, following the instructions outlined in the table 3.

### Primers Used in the Study

**Table 1:** Primers Used in the Study

| Primers ID                | Gene     | Nucleotide Sequence (5'–3')                            | (bp) | References             |
|---------------------------|----------|--------------------------------------------------------|------|------------------------|
| Phylogenetic groups Genes | chuA     | F: ATGGTACCGGACGAACCAAC<br>R: TGCCGCCAGTACCAAGACA      | 288  | (Clermont et al. 2013) |
|                           | yjaA     | F: CAAACGTGAAGTGCAGGAG<br>R: AATGCGTTCCTCAACCTGTG      | 211  |                        |
|                           | TspE 4C2 | F: CACTATTGTAAGGTCATCC<br>R: AGTTTATCGCTGCGGGTCCG      | 152  |                        |
|                           | arpA     | F: AACGCTATTGCGCAGCTTGC<br>R: TCTCCCATACCGTACGCTA      | 400  |                        |
|                           | arpA     | F: GATTCCATCTTGTCAAATATGCC<br>R: GAAAAGAAAAAGATTCCCAAG | 301  |                        |
|                           | trpA     | F: AGTTTATGCCCAGTGCAGAG<br>R: TCTGCGCGGTCACGCC         | 219  |                        |
|                           | trpA     | F: CGGCGATAAGACATCTTCAC<br>R: GCAACGCGCCTGCGGAAG       | 489  |                        |

### PCR Interaction Steps

**Table 2:** PCR Interaction Steps

| PCR Interaction Steps     |                                | trpA        | chuA | yjaA | TspE 4C2 | trpA | Gp. C arpA | Gp. E arpA | Time  |
|---------------------------|--------------------------------|-------------|------|------|----------|------|------------|------------|-------|
|                           |                                | Temperature |      |      |          |      |            |            |       |
| 40 Cycle                  | One Cycle Initial Denaturation | 95c°        | 95c° | 95c° | 95c°     | 95c° | 95c°       | 95c°       | 95c°  |
|                           | Denaturation                   | 95c°        | 95c° | 95c° | 95c°     | 95c° | 95c°       | 95c°       | 45Sec |
|                           | Annealing                      | 68c°        | 68c° | 68c° | 68c°     | 68c° | 59c°       | 57c°       | 45Sec |
|                           | Extension                      | 72c°        | 72c° | 72c° | 72c°     | 72c° | 72c°       | 72c°       | 1min  |
| One Cycle Final Extension |                                | 72c°        | 72c° | 72c° | 72c°     | 72c° | 72c°       | 72c°       | 10min |

**Table 3:** PCR Master Mix

| PCR Master Mix               | Volume  |
|------------------------------|---------|
| Go Taq ® G2 Green Master Mix | 12.5 µL |
| DNA Template                 | 5 µL    |
| Forward Primer               | 1.5 µL  |
| Reverse Primer               | 1.5 µL  |
| Free Nuclease Water          | 4.5 µL  |
| Total                        | 25 µL   |

**Table 4:** Distinguishes Phylogroups by the Presence or Absence of Genes

| Quadruplex Genotype |             |             |                | Gp. C       | Gp. E       | <i>trpA</i> | Phylo-Groups       |
|---------------------|-------------|-------------|----------------|-------------|-------------|-------------|--------------------|
| <i>arpA</i>         | <i>chuA</i> | <i>yjaA</i> | <i>TspE4C2</i> | <i>trpA</i> | <i>arpA</i> |             |                    |
| +                   | -           | -           | -              | -           | -           | +           | Phylo-Groups A     |
| +                   | -           | +           | -              | -           | -           | +           |                    |
| +                   | -           | -           | +              | -           | -           | +           | Phylo-Groups B1    |
| -                   | -           | -           | +              | -           | -           | +           |                    |
| -                   | +           | +           | +              | -           | -           | +           | Phylo-Groups B2    |
| -                   | +           | +           | -              | -           | -           | +           |                    |
| -                   | -           | +           | +              | -           | -           | +           |                    |
| +                   | -           | +           | -              | +           | -           | +           | Phylo-Groups C     |
| +                   | +           | -           | +              | -           | -           | +           | Phylo-Groups D     |
| +                   | +           | -           | -              | -           | -           | +           |                    |
| +                   | +           | +           | -              | -           | +           | +           | Phylo-Groups E     |
| +                   | +           | -           | -              | -           | -           | +           |                    |
| -                   | +           | -           | -              | -           | -           | +           | Phylo-Groups F     |
| +                   | +           | +           | +              | -           | -           | +           |                    |
| +                   | -           | +           | +              | -           | -           | +           | Phylo-Groups UP    |
| -                   | -           | -           | -              | -           | -           | +           |                    |
| -                   | -           | -           | -              | -           | -           | -           | Not <i>E. coli</i> |

## Results

### Escherichia Coli Diagnosis and Isolation

Between November 15, 2022, and February 1, 2023, 367 stool samples from patients hospitalized in Al-Batoul Hospital for Gynecology, Obstetrics, and Children and private laboratories yielded fifty isolates of *E. coli*, which cause diarrhea. The patient's details were broken down into age groups, including 207 samples of males and 160 samples of females, ages ranging from under a year to seven. of males

and females, with 207 samples of males and 160 samples of females. The presence of bacteria causing diarrhea was investigated in 367 Stool samples from advanced patients, as the number of samples that gave a negative result for bacterial culture was 84, or 22.88%.

In contrast, 283 samples gave a positive result, at a rate of 77.12%, with 207 samples from males, at 79.71%, and 160 from females, at 73.75%. PCR technology was used for molecular detection of the isolates under study using the *trpA* gene. This technique is considered a confirmed diagnosis of *E. coli* [27].

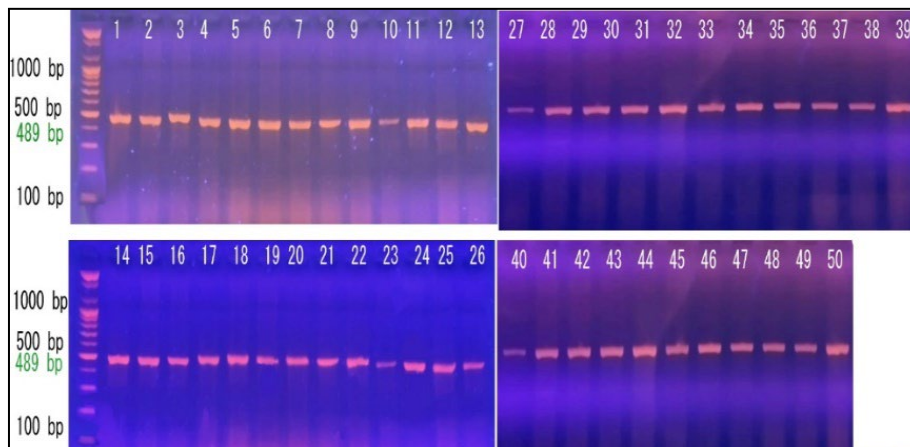


**Figure 1:** The Figure Represents the Electrophoresis of the PCR Reaction Product to Detect the Trap Gene of *E. Coli* Using 1.5% Agarose Gel and Ethidium Bromide Dye at a Voltage of 100 Volts for 60 Minutes. Track M: Standard Size Guide DNA Ladder (100-100 Base Pairs).

This agreed to some extent with the study of Hasan et al. in Dohuk Governorate, as 349 (87.2%) positive samples of *E. coli* were obtained. It also converged with the study of Ali et al. [28] in Baghdad Governorate, which was 66.23%. The results of the current study differed from the study of Otaiwi et al. [29] in Babil Governorate, as it was 30.66%. This difference and variation in outcomes could be related

to age differences, socio-demographic and socio-environmental background, unhealthy dietary behaviors, and lack of awareness and personal hygiene [30]. Patients may have taken antibiotics before taking the samples, which negatively affected the absence of bacterial growth. Age increases the infection rate due to decreased immunity as one gets older and increased exposure to hospital infections.

### Testing the Sensitivity of *E. coli* to Antibiotics

The results shown in Appendix (2) showed an apparent variation in the extent of the isolates' response to the antibiotics used. The highest percentage was for the antibiotic (Ampicillin), which belongs to the group (Penicillins), and the resistance rate was 96% [31]. This result differed from the results of the study by Renzhammer et al. [32], as the resistance rate for this antibiotic was 71.9%. It also differed from the study of Salleh et al. [33], with a resistance rate of 73.5% to this antibiotic. As for the antibiotic (Amoxicillin-clavulanate) from the group (B-lactam combination), the resistance rate was 94%. This result also differed from what was reported in the study of Bonyadian et al., as this study found that the resistance rate was 65.6%. As for the two antibiotics (Ceftazidime and Cefotaxime) from the group of (Cephalosporins), the resistance rate was 88% and 92%, respectively. This result also differed from the study of Jafari et al. [1], where the rates of resistance to the antibiotics above were 16.9% and 35.4%, respectively. The resistance rate to the folate pathway antibiotics (Trimethoprim-sulfamethoxazole and sulfonamides) was 74% and 76%, respectively [34].

The results of resistance to Trimethoprim-sulfamethoxazole matched what was reported in the study of Saka et al. (2019), where the resistance rate was 77.6%. The results of resistance to the anti-sulfonamide also agreed to some extent with the results of Vidal et al. [24], with a rate of 87.3%. As for the anti-Aztreonam from the

Monobactams group, the resistance rate reached 64.4%. This differed from the results of Renzhammer et al. [32], and the rate of resistance to this antibiotic was 2%. Meanwhile, the rate of resistance to the antibiotics (Tobramycin and Amikacin) was 26% and 56%, respectively. The resistance rate to the anti-tobramycin was identical in the study of Khan et al. [35] and was 20.28%, and the same study differed in the resistance to the anti-amikacin, which was 9.42%. The rate of resistance to Azithromycin among the Macrolides group was 52%. This also differed from the study by Guiral et al. [36], as the resistance rate was 23%. The resistance rate to Doxycycline from the Tetracycline group was 60%, which agrees with the study of YANG et al. [31], where the resistance rate to this antibiotic was 56%. Also, the resistance rate to Ciprofloxacin and Levofloxacin from the Fluoroquinolones group was 26% and 28%, respectively [37].

This converged to some extent with the study of Jia et al. [38] if the resistance rates were 48% and 33%, respectively, and the rate of resistance to the antibiotic (Imipenem) from the group (Carbapenems) amounted to 14%. This converged to some extent with the study of Jafari et al. [1], which showed that all isolates under study were sensitive to the antibiotic (Imipenem). As for the antibiotic (Tigecycline), which belongs to the (Tetracycline) group, none of the isolates were resistant. This result was close to what was reported in the study of Li et al. (2021), where the resistance rate was only 7% with seven isolates out of 100

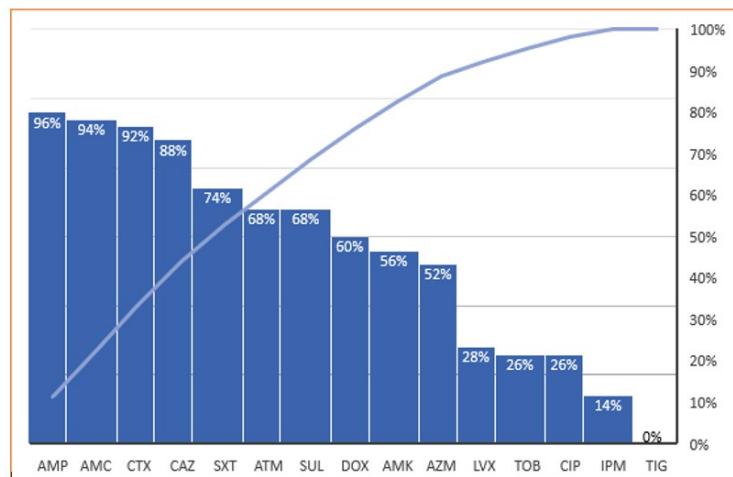


**Figure 2:** Figure Represents Antibiotics and their Percentages

The use of antibiotics to treat Diarrhea *Escherichia coli*, especially in children, is limited by increasing antibiotic resistance [39]. Therefore, routine antimicrobial susceptibility testing should be performed, identifying the effective antibiotic and identifying the causes of antibiotic-resistant bacteria to limit the increase in bacterial resistance to antibiotic [34]. The high rate of antibiotic resistance can be explained by the fact that antibiotics are more easily used as a treatment for infections, and individuals have been purchasing them without a prescription for self-treatment in recent years [36]. This may be a reflection of how these drugs are used and abused in society.

This conclusion is because, outside the hospital setting, people can easily take many antibiotics from any pharmacy without a prescription. Furthermore, random medical rules lead to many free medicines to treat and manage common diseases [40]. Antibiotic resistance in *E. coli* is primarily due to genes located on conjugative plasmids, which are transferred to susceptible recipients via

conjugation, transformation, or transfer processes [26]. Alternatively, resistance genes may be present on the bacterial chromosome and jump to resistance plasmids via transposition. Many transposons carry antibiotic resistance genes, and some contain more than one resistance gene [41]. Antibiotics have played a crucial role in treating and preventing common diseases. However, the rules of evolution and natural selection and their misuse and misuse have led to an alarming increase in antibiotic-resistant use globally [42]

### Multiple Antibiotic Resistance

The results showed that 48 isolates (96%) carried the multiple resistance (MDR) trait. It ranged from 3 to 14 antibiotics, and no isolate showed sensitivity to all of the antibiotics used in the study, which confirms the presence of multiple resistance among the local isolates [43]. As for the isolates with resistance (XDR), there were 10 isolates, at a rate of 20%. The results showed that the majority of isolates showed multiple resistance to the antibiotics tested [44].

**Table 5:** Multiple Antibiotic Resistance

| Isolates | Antibiotics that you Resisted | Resistance Type | %   | Isolate Numbers and Type of Isolate            |
|----------|-------------------------------|-----------------|-----|------------------------------------------------|
| 2        | 2                             | Do not have     | %4  | E.12, E.50                                     |
| 2        | 3                             | MDR             | %4  | E.9, E.19                                      |
| 3        | 4                             | MDR             | %6  | E.1, E.17, E.48                                |
| 5        | 5                             | MDR             | %10 | E.3, E.11, E.15, E.29, E.31                    |
| 4        | 6                             | MDR             | %8  | E.4, E.8, E.16, E.47                           |
| 6        | 7                             | MDR             | %12 | E.2, E.5, E.20, E.21, E.22, E.27               |
| 8        | 8                             | MDR             | %18 | E.13, E.23, E.24, E.28, E.30, E.37, E.40, E.45 |
| 6        | 9                             | MDR             | %12 | E.33, E.35, E.42, E.43, E.46, E.49             |
| 4        | 10                            | MDR             | %8  | E.34, E.28, E.39, E.44                         |
| 5        | 11                            | XDR             | %10 | E.7, E.10, E.14, E.36, E.41                    |
| 3        | 12                            | XDR             | %6  | E.6, E.18, E.32                                |
| 2        | 13                            | XDR             | %4  | E.25, E.26                                     |

Bacteria adapt to the effectiveness of antibiotics through two basic genetic strategies: mutations in a gene, which are usually associated with a mechanism of action. Secondly, horizontal gene transfer to obtain the input DNA coding [45]. The broad use of antibiotics has contributed to the growth of multiple antibiotic-resistant pathogens [29]. Bacteria have demonstrated broad antimicrobial potential by targeting key determinants of pathogenicity, antibiotic resistance, and spread such as cell membrane, efflux pumps, quorum sensing, biofilms, and plasmids. The extensive and excessive use of broad-spectrum antibiotics has also led to the emergence of many *E. coli* isolates resistant to multiple antibiotics [33].

#### Clermont Classification of *E. Coli*

*E. coli* were classified and analyzed into phylogroups based on what was stated by Clermont et al.[9]. The phylogenetic analysis showed that all isolates belong to one of seven main Phylogenetic groups. *E. coli* isolates showed high levels of diversity for groups and thus provided information and evaluation of genetic characteristics and valuable data on the epidemiology of diseases in different geographical regions [46]. The results showed that the bacteria were classified as 8, and the highest group was E, with 13 isolates (26%). followed by group C, which had eight isolates (16%), while groups A, B2, and Up had six isolates (12%) each. As for group B1, there were five isolates, at a rate of (10%), and the lowest group in D and F was three isolates, at a rate of (6%) for each. The results of the current study agreed with the results of Jafari and Bouzari [1], as the E group had a percentage

of (26%), the B2 group had a percentage of (13.9%), the C group had a percentage of (12.3%), the D group had a percentage of (3.7%). The F group had a percentage of (0%). These results differed from the study of Al-Azzawi and Abdullah [47] on children with diarrhea in Baghdad with different proportions of developmental groups [32]. The distribution of phylogenetic groups in our current study may differ from previous studies due to various factors. These factors include differences in geographic regions, the health status of the host, nutritional factors, patterns of antibiotic use, genetic factors, and the overall epidemiological importance of the disease. It's important to note that the distribution of *E. coli* strain populations in humans and animals can be influenced by pediatric pathogens in different geographic regions. *E. coli* is the most common and virulent infecting food-producing animals and is primarily transmitted to humans via the food chain [48]. These populations are documented by variation in populations due to host physical health, nutritional and genomic determinants, environmental and topographic factors, changes in collection location and techniques, Virulence factors, antibiotic resistance, growth rate, carbohydrate fermentation, and genome size may all reflect the variable frequency of Phylogenetic clustering documented in different studies [42]. Many studies have shown the relationship between phylogeny and pathogenicity of *E. coli* [45]. *E. coli* strains are genetically diverse, falling into four major Phylogenetic groups: A, B1, B2, and D. Determining the phylogenetic group allows understanding of the pathogenic potential of bacteria to an organism, including potential complications [43].

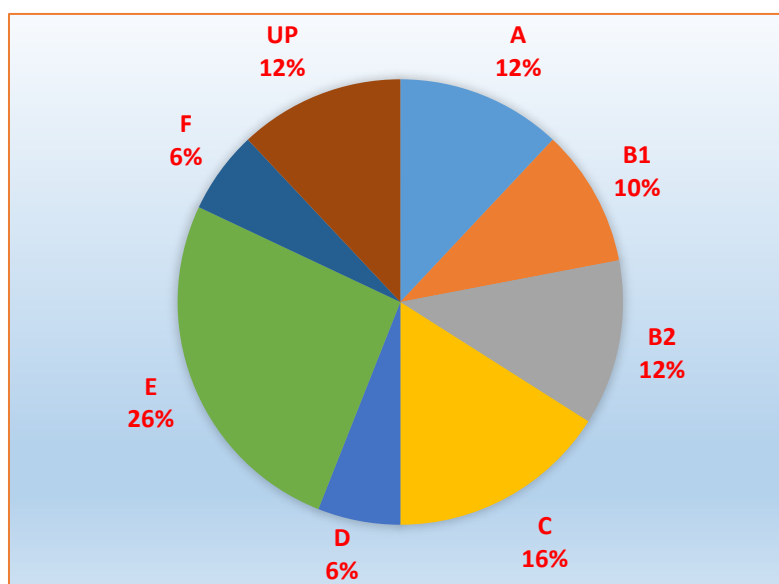
**FIGURE 3:** The Figure Represents Phylogroup Totals and their Percentages.

**Table 6:** The Table Represents the Results of Phylogroups for E. coli

| Group | arpA   | chuA   | yjaA   | TspE4C2 | Gp. C          | Gp. E          | Number         |
|-------|--------|--------|--------|---------|----------------|----------------|----------------|
|       | 301 bp | 288 bp | 211 bp | 152 bp  | TrpA<br>219 bp | arpA<br>400 bp | TrpA<br>489 bp |
| A     | +      | -      | +      | -       | -              | -              | 15.            |
| A     | +      | -      | -      | -       | -              | -              | 18.            |
| A     | +      | -      | -      | -       | -              | -              | 35.            |
| A     | +      | -      | +      | -       | -              | -              | 38.            |
| A     | +      | -      | +      | -       | -              | -              | 40.            |
| A     | +      | -      | -      | -       | -              | -              | 50.            |
| B1    | +      | -      | -      | +       | -              | -              | 1.             |
| B1    | -      | -      | -      | +       | -              | -              | 4.             |
| B1    | +      | -      | -      | +       | +              | -              | 24.            |
| B1    | +      | -      | -      | +       | -              | -              | 44.            |
| B1    | +      | -      | -      | +       | -              | +              | 49.            |
| B2    | -      | +      | +      | +       | -              | -              | 14.            |
| B2    | -      | -      | +      | +       | -              | -              | 27.            |
| B2    | -      | +      | +      | -       | -              | +              | 37.            |
| B2    | -      | +      | +      | -       | +              | -              | 42.            |
| B2    | -      | +      | +      | -       | -              | +              | 43.            |
| B2    | -      | +      | +      | -       | -              | -              | 45.            |
| C     | +      | -      | +      | -       | +              | -              | 5.             |
| C     | +      | -      | +      | -       | +              | -              | 11.            |
| C     | +      | -      | +      | -       | +              | -              | 17.            |
| C     | +      | -      | +      | -       | +              | -              | 26.            |
| C     | +      | -      | +      | -       | +              | -              | 28.            |
| C     | +      | -      | +      | -       | +              | -              | 29.            |
| C     | +      | -      | +      | -       | +              | -              | 34.            |
| C     | +      | -      | +      | -       | +              | -              | 41.            |
| D     | +      | +      | -      | -       | -              | -              | 19.            |
| D     | +      | +      | -      | -       | -              | +              | 23.            |
| D     | +      | +      | -      | -       | -              | -              | 32.            |
| E     | +      | +      | -      | +       | -              | +              | 6.             |
| E     | +      | +      | -      | +       | -              | +              | 8.             |
| E     | +      | +      | +      | -       | -              | +              | 10.            |
| E     | +      | +      | -      | +       | -              | +              | 12.            |
| E     | +      | +      | -      | -       | -              | +              | 16.            |
| E     | +      | -      | +      | +       | -              | +              | 20.            |
| E     | +      | +      | -      | +       | -              | +              | 21.            |
| E     | +      | +      | +      | -       | -              | -              | 22.            |
| E     | +      | +      | +      | -       | -              | +              | 25.            |
| E     | +      | +      | -      | -       | -              | +              | 30.            |
| E     | +      | +      | -      | -       | -              | +              | 33.            |
| E     | +      | +      | -      | +       | -              | +              | 36.            |
| E     | +      | +      | +      | -       | -              | +              | 47.            |
| F     | -      | +      | -      | -       | -              | -              | 9.             |
| F     | -      | +      | -      | -       | -              | +              | 13.            |
| F     | -      | +      | -      | -       | -              | -              | 31.            |
| UP    | +      | -      | +      | -       | -              | -              | 2.             |
| UP    | -      | -      | -      | -       | -              | -              | 3.             |
| UP    | +      | -      | +      | +       | -              | -              | 7.             |
| UP    | +      | -      | +      | +       | -              | -              | 39.            |
| UP    | +      | -      | +      | +       | -              | -              | 46.            |
| UP    | +      | +      | +      | +       | +              | -              | 48.            |

### Distribution of Antibiotic Resistance Among Phylogenetic Groups

This study found that patterns of antibiotic resistance were more widespread. As for the MDR resistance type, it was more prevalent in evolutionary groups (B1, B2, D, F, and UP) with a percentage of (100%), followed by evolutionary groups (E, A, and C) with a

percentage of (92.3%, 83.3%, 16.6), respectively). The XDR resistance type was also more prevalent in developmental group D with a percentage of (33.3%), followed by group E with a percentage of (30.7%), followed by evolutionary groups C, B2, UP, and A with a percentage of (25%, 16.6%, 16.6%, and 16.6%, respectively). Groups B1 and F did not have an XDR pattern.

**Table 7:** A Table Representing the Distribution of Antibiotic Resistance Patterns into Phylogenetic Groups

| Phylogenetic Groups | Patterns of Antibiotic Resistance |           |
|---------------------|-----------------------------------|-----------|
|                     | MDR                               | XDR       |
|                     | N=48 (%)                          | N=10 (%)  |
| Phylogenetic A      | 5 (83.3%)                         | 1 (%16.6) |
| Phylogenetic B1     | 5 (%100)                          | 0         |
| Phylogenetic B2     | 6 (%100)                          | 1 (%16.6) |
| Phylogenetic C      | 8 (16.6%)                         | 2 (%25)   |
| Phylogenetic D      | 3(%100)                           | 1 (33.3)  |
| Phylogenetic E      | 12 (%92.3)                        | 4 (%30.7) |
| Phylogenetic F      | 3 (%100)                          | 0         |
| Phylogenetic UP     | 6 (%100)                          | 1 (%16.6) |

The current study disagreed with several studies, as Pereira et al.'s study found that isolates belonging to phylogenetic groups B2 and B1 showed a reduced prevalence of MDR in Europe. The current study agreed with what the study in Iran indicated that the majority of MDR strains belong to phylogenetic group B2 [48]. It also agreed with the study of Nazir et al. [49] which shows that Pakistani strains have a wide range of MDR and differed at the same time group. B2. The current study agreed with the findings of the study conducted by Mosquito et al. and the study of Ghaderpour et al. [41] that the MDR resistance pattern was distributed among groups A and B1. This is somewhat consistent with the current study. The proportions of the MDR pattern have varying distribution across the evolutionary groups of E. coli. Isolates belonging to the evolutionary groups (B1, B2, D, F, and UP) have a higher prevalence of many antibodies, followed by groups (E, A, and C). This is consistent with what the study of Mohsin et al. [26] indicated that the prevalence of MDR is highest in phylogenetic group F, followed by groups C, B2, E, and D. Differences in results between studies could be related to different geographic regions,

climatic factors, and the origins of E. coli isolates found at each site that play a major role in the global E. coli demographics. Furthermore, Phylogroup E contains the largest number of multi-resistant isolates, which can be attributed to a larger genome that tends to have many more genes and express diverse resistance genes and pathogenicity islands compared to other strain groups, and these resistance genes are the result of horizontal gene transfers.

## Conclusions

Escherichia coli is the most common in children with diarrhoea. The resistance of bacterial isolates to most antibiotics and their possession of the characteristic of multiple resistance to antibiotics observed to be common among the isolates under consideration. The diversity of isolates under study between the evolutionary aggregates in which Escherichia coli divides, the highest of which was the E group, which took the highest percentage of the presence of disguises and the high percentage of antibiotic resistance.

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