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Phylogeny of Antibiotic Resistance Genes of Escherichia coli B2 Isolated from Urinary Tract Infection Patients

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ORIGINAL STUDY

Phylogeny of Antibiotic Resistance Genes of *Escherichia coli* B2 Isolated from Urinary Tract Infection Patients

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Abstract

Background: Urinary tract infections (UTIs) are the most prevalent bacterial illnesses. Antibiotic resistance is a common problem in *E. coli* bacteria, which can develop or acquire a wide range of resistance mechanisms. *E. coli* strains B2 carry more virulence factors that cause antibiotic resistance.

Objectives: The study aims to detect the phylogeny of antibiotic resistance genes of *Escherichia coli* B2 and identify the presence of the *yjaA*, *chuA* genes. It also aims to measure the susceptibility of antibiotic samples obtained from individuals diagnosed with UTI in Babylon, Iraq.

Materials and Methods: The antibiotic susceptibility was measured using the disk diffusion method. These isolates were previously identified using the VITEK-2 System. The presence of the *yjaA* and *chuA* genes was then detected using specific primer pairs by polymerase chain reaction to phylogenetic *E. coli* B2.

Results: The total number of samples was 315, 210 (67%) positive growth and 105 (33%) showing no growth. Out of 210, 46 (22%) were *E. coli*. Out of 46 *E. coli* isolates, the percentage of B2 was 24 (52%). *E. coli* infection rate was 39 (85%) in females and 7 (15%) in males. Ampicillin and cephalothin showed the highest resistance at 94%, while the antibiotics Colistin and Nitrofurantoin exhibited the lowest resistance at 0% and 6%.

Conclusion: The study demonstrated that *E. coli* phylogenetics indicated that the most prevalent strain was B2. The antibiotics colistin, Imipenem, and Nitrofurantoin showed the lowest resistance among other antibiotics, Beta-lactam antibiotics, such as ampicillin and cephalothin exhibited the highest resistance among other antibiotics.

Keywords: Antibiotic resistance, *Escherichia coli* B2, *yjaA*, *chuA*, UTI, Phylogenetics

1. Introduction

Escherichia coli is a Gram-negative bacterium that belongs to the family Enterobacteriaceae. It is a facultative anaerobic bacterium that does not produce spores [1]. A, B1, B2, and D were classified into categories into which the *E. coli* isolates were initially classified by Clermont's typing system. To distinguish between these categories, genetic markers such as *chuA* and *yjaA* might be employed [2]. There are more genes for virulence factors in Group B2 bacteria compared to the other groups [3]. Most isolates of extraintestinal pathogenic *E. coli* (ExPEC)

and Uropathogenic *E. coli* (UPEC) are members of the B2 phylogenetic group [4]. A urinary tract infection, also referred to as a UTI, is an illness that affects the urinary system and can affect either the upper or lower urinary tracts or both [5]. Urinary tract infections are a relatively prevalent bacterial infection, especially in women [6]. The main agents of UTI are Gram-negative bacilli of the family Enterobacteriaceae. Among them, uropathogenic *Escherichia coli* (UPEC) is the most frequent [7].

The widespread and indiscriminate use of antimicrobial medications to treat infectious illnesses has led to an increase in antibiotic-resistant bacteria, which

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is now an important global issue [8]. Antimicrobial resistance is referred to as the capacity of a bacteria to resist the effects of an antibiotic to which it was previously susceptible. The common antimicrobial resistance to bacterial infections occurs through the random use of antibiotics and the development of microbial characteristics [9]. The World Health Organization has identified *E. coli* as a significant and accurate indication of the extent and severity of the worldwide antimicrobial resistance (AMR) problem [10]. Bacteria can develop defensive mechanisms against antibiotics through genetic encoding in their DNA, which determines their resistance to the medications. Bacteria possess genes for many forms of resistance. When some highly resistant bacteria have the appropriate combination of resistance mechanisms, it makes all antibiotics ineffective, leading to diseases that cannot be treated [11]. *ChuA* is an outer membrane protein that functions as a heme receptor and is synthesized in response to environments with low iron levels. Heme uptake through *chuA* is critical for the invasion of the bladder and kidneys by UPEC (Uropathogenic *E. coli*). Additionally, *chuA* is included in the phylogenetic typing schemes of *E. coli*. *ChuA*, which is located on the chromosome, is used for the purpose of categorizing *E. coli* based on their phylogenetic groups [12]. The study aims to detect and Phylogeny of antibiotic resistance genes of *Escherichia coli* B2 and identify the presence of the *yjaA*, *chuA* genes and measure the susceptibility of antibiotic samples obtained from individuals diagnosed with UTI in Babylon, Iraq.

2. Materials and methods

A total of 315 urine samples were collected from UTI patients aged 5 to 70 years of both genders. These samples were obtained between October 2023 and February 2024 from Aliskandariyah Hospital and Marjan Hospital in the Babylon province. The bacterial isolates were detected with the VITEK 2 system (Biomérieux, France). In addition, antibiotic sensitivity was determined on Mueller Hinton agar via disk diffusion with a 0.5 McFarland standard. They were placed in an incubator at a temperature of 35°C for 16–18 hours [13], following the guidelines set by CLSL, 2023. The antibiotic disks include Gentamicin (10 µg), Amikacin (10 µg), Ciprofloxacin (10 µg), Tetracycline (10 µg), Colistin (10 µg), Nalidixic acid (30 µg), and Nitrofurantoin (100 µg).

2.1. DNA extraction and polymerase chain reaction protocol

The Plasmid Extraction Mini Kit is manufactured by FAVORGEN Company in Taiwan. It was used to

Table 1. The primer pair sequences and amplicon sizes.

Gene	5'–3' sequence	Size (bp)	References
<i>chuA</i>	F-GACGAACCAACGGTCAGGAT R-TGCCGCCAGTACCAAAGACA	279	[12]
<i>yjaA</i>	F-TGAAGTGTCTCAGGAGACGCTG R-ATGGAGAATGCGTTCCTCAAC	211	[12]

extract and prepare the plasmid DNA templates of the isolates following the guidelines set by the manufacturer. The PCR was used to detect *chuA* and *yjaA*. Specific primers were used shown in Table 1. A 25-µL reaction mixture was prepared for PCR tests. It consisted of 12.5 µL of Taq DNA Polymerase Master Mix green (Promega, UK), primers (2 µL each at a concentration of 10 pmol/µL), 5 µL of extracted DNA (at a concentration of 50 ng), and sterile deionised water to reach a final volume of 25 µL. The amplifications were conducted using a thermocycler (Biometra tad-advanced, Germany), following the settings specified in Table 2.

2.2. Ethical considerations

All subjects consented and agreed to participate in the scientific and ethically sound Babylon's Public Health Laboratory and hospitals in Hillah City. According to document number 103 dated December 05, 2023, the study protocols, subject information, and agreement form were examined and approved by a local ethics committee of the Al-Furat Al-Awsat Technical University/College of Health and Medical Techniques/ and Babil Health Directorate.

2.3. Statistical analysis

The statistical study used Microsoft Office Excel 2021 and the findings were evaluated by the Statistical Packages for the Social Science (SPSS version 20). The study used percentages to compare data from different groups.

3. Results

Out of 315 samples, 210 (67%) exhibited positive bacterial growth, while 105 (33%) indicated negative bacterial growth. Methods such as morphological identification, microscopic examinations, and diagnosis using the biochemical tests and Vitek2® system were conducted. *Escherichia coli* was the predominant bacteria in the UTI, accounting for 46 out of 210 isolates, or 22%. The percentage of females with *E. Coli* was 39 (85%). In males, it was 7 (15%). The age group 21–30 exhibited the highest prevalence of *Escherichia coli* infection, with a rate of 26%, and the age group

Table 2. PCR conditions for heat cycling.

Gene name	Temperature (C°)/Time					Number of cycles
	Initial denaturation	Cycling conditions			Final extension	
		Denaturation	Annealing	Extension		
<i>chuA</i>	94 C°/5 min	94 C°/30 sec	55 C°/30 sec	72 C°/30 sec	72 C°/7 min	30
<i>yjaA</i>	94 C°/5 min	94 C°/30 sec	55 C°/30 sec	72 C°/30 sec	72 C°/7 min	30

Table 3. Prevalence of *Escherichia coli* according to the age groups.

Age group (years)	Numbers of samples	(%)
5 to 10	7	15%
11 to 20	4	9%
21 to 30	12	26%
31 to 40	7	15%
41 to 50	6	13%
51 to 60	7	15%
61 to 70	3	7%
Total	46	100%

61–70 demonstrated the lowest infection rate, reaching 7%. The others aged 5 to 10 year 7 (15%), 11 to 20 years were 4 (9%), 31 to 40 years were 7 (15%), 41 to 50 years were 6 (13%), 51 to 60 years were 7 (15%) are shown in Table 3. The study also revealed that Gentamicin resistance was 23%, Amikacin 57%, Ciprofloxacin 51%, Tetracycline 40%, Colistin 0%, Nalidixic acid 69%, Nitrofurantoin 6%, as shown in Fig. 1. The study also found that 80% were multidrug-resistant (MDR), 11% (not categorized as MDR), 9% were classified as Extensively drug-resistant (XDR), and 0% were classified as potentially Pandrug-resistant (PDR), as shown in Fig. 3. Monoplex PCR was used to explore genes for 46 isolates, and the percentage of the *yjaA* gene was 29 (63%) (p-value = 1.0). Indicating no statistically significant difference between the two proportions, and the rate of the *chuA* gene was 29 (63%) (p-value = 1.0). Indicating no statistically significant difference between the two proportions, as shown in Fig. 2. *E. coli* phylogenetics indicated that the prevalent strain was B2, 24 (52%), as shown in Fig. 4.

4. Discussion

The current study revealed that *E. coli* was the most prevalent bacterium, accounting for 46 out of 210 isolates (22%). A previous study in Babylon province revealed that *E. coli* was the most prevalent bacterium at 34% [14]. Another study in Babylon province revealed *E. coli* was the most prevalent bacterium at 45.83% [15].

The current study revealed that the percentage of females with *E. Coli* was higher than males. A previous study in Babylon province demonstrated the propor-

tion of *E. coli* in females, 83% and 17% in males [16]. Another study conducted in the Baghdad province reported that the percentage of *E. coli* in females was 62.2% and 37.8% in males [17]. Women are much more susceptible to UTIs than men, mostly due to the female lower urinary tract anatomy and its proximity to the reproductive system. The female urethra is relatively short, decreasing the distance for bacterial entry [18]. The current study revealed that the prevalence of penicillin resistance was 94%.

The current study revealed that the age group 21–30 had the highest prevalence of *E. coli* infection, with a rate of 12 (26%), and the age group 61–70 demonstrated the lowest infection rate, reaching 3 (7%). The other age groups were 5 to 10 years 7 (15%), 11 to 20 years 4 (9%), 31 to 40 years 7 (15%), 41 to 50 years 6 (13%), and 51 to 60 years 7 (15%). A previous study in Katurdistan province [19] reported that The age group from 20 to 39 years was the highest prevalent at 46%. An additional study in Baghdad province [20] reported the highest percentage of infection in the age group 21 to 30 years, 11%, while those from 70-74 years 1%. A different study in Babylon province [21] reported that the highest percentage of the age group is 30–39 years, 53.33%, and 60–69 years, 50%. Another study in Babylon province [22] demonstrated that the highest percentage of infection was 34.63% for the age group 15 to 28 years, while the lowest percentage was 7.69% of infection for the age group between 1 to 14 years.

The current study revealed that the prevalence of Gentamicin resistance was 23%. A previous study in the Babylon Province [23] reported that the rate of Gentamicin resistance was 25%. Additional study in the Salahadin Province [24] revealed that the rate of Gentamicin resistance was 20%. another study in the Najaf Province [25] reported that the rate of Gentamicin resistance was 100%. A different study in the Najaf Province [26] reported that the rate of Gentamicin resistance was 15%. The low resistance rate is because it contains weak virulence factors, such as a change in the permeability of the outer membrane, and it does not contain efflux pumps. This is due to the bacteria's weak ability to resist the antibiotic [27].

The current study revealed that the prevalence of Amikacin resistance was 57%. A previous study in the

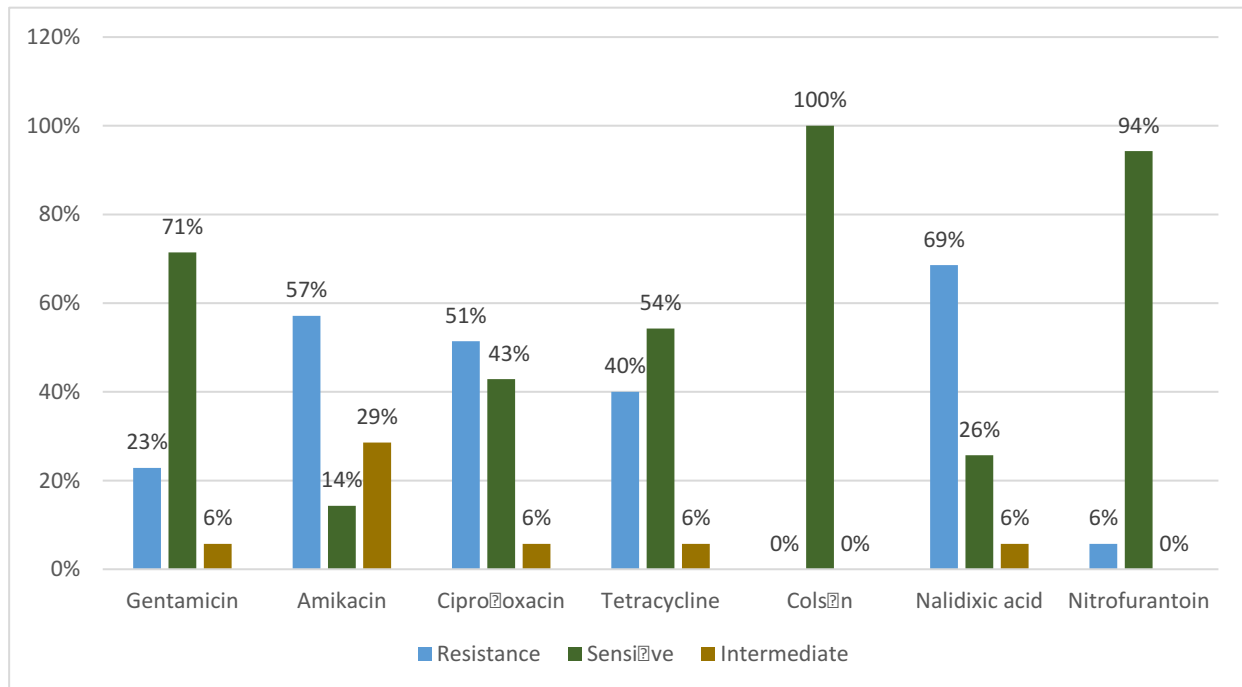


Fig. 1. Percentage of antibiotics susceptibility to *Escherichia coli*.

Najaf Province [28] reported that the rate of Amikacin resistance was 17%. An additional study in the Babylon Province [29] revealed that the rate of Amikacin resistance was 21.42%. Another study [30] demonstrated that the rate of Amikacin resistance was 97.9%. A different study in the Babylon Province [31] reported that the rate of Amikacin resistance was 10.7%. Aminoglycosides are an important class of antibiotics to treat serious infections caused by Gram-negative bacteria, including *E. coli*. One of the most common mechanisms of resistance to aminoglycosides in *E. coli* is the production of aminoglycoside-modifying enzymes (AMEs) [32].

The current study revealed that the prevalence of Ciprofloxacin resistance was 51%. A previous study in the Babylon Province [33] reported that the rate of Ciprofloxacin resistance was 53%. Additional study in the Babylon Province [34] revealed that the rate of Ciprofloxacin resistance was 52.9%. Another study in the Misan Province [5] reported that the rate of Ciprofloxacin resistance was 25.9%. A different study in the Duhok Province [35] illustrated that the rate of Ciprofloxacin resistance was 38.7%. In mutations involving genes encoding DNA gyrase and topoisomerase IV, Ciprofloxacin targets bacterial enzymes that aid in DNA replication. Resistance is frequently caused by mutations in the genes that encode these enzymes [36]. Efflux pumps actively remove the an-

tibiotic ciprofloxacin from the bacterial cell, hence decreasing its effectiveness.

The current study revealed that the prevalence of Tetracycline resistance was 40%. A previous study in the Babylon Province [33] reported that the rate of Tetracycline resistance was 60%. An additional study in the Kurdistan Region [37] revealed that the rate of Tetracycline resistance was 48.51%. Another study in the Karbala province [38] demonstrated that the rate of Tetracycline resistance was 80%. A different study in the Sulaimani province [39] reported that the rate of Tetracycline resistance was 100%. Tetracycline resistance is mediated by about forty acquired tetracycline-resistant genes that encode efflux pumps, enzymatic inactivation, or ribosome protection genes [40].

The current study revealed that the prevalence of colistin resistance was 0%. The previous study in the Karbala Province [41] reported that the rate of Colistin resistance was 2.8%. An additional study in the Babylon Province [34] revealed that the rate of Colistin resistance was 100%. Antibiotics like colistin are the final choice to deal with infections caused by carbapenem-resistant Enterobacteriaceae (CREB) [42] prevalence of colistin resistance has been very little reported in the literature [43] some strains of *E. coli* have developed resistance to most other antibiotics, making colistin an effective option because it targets

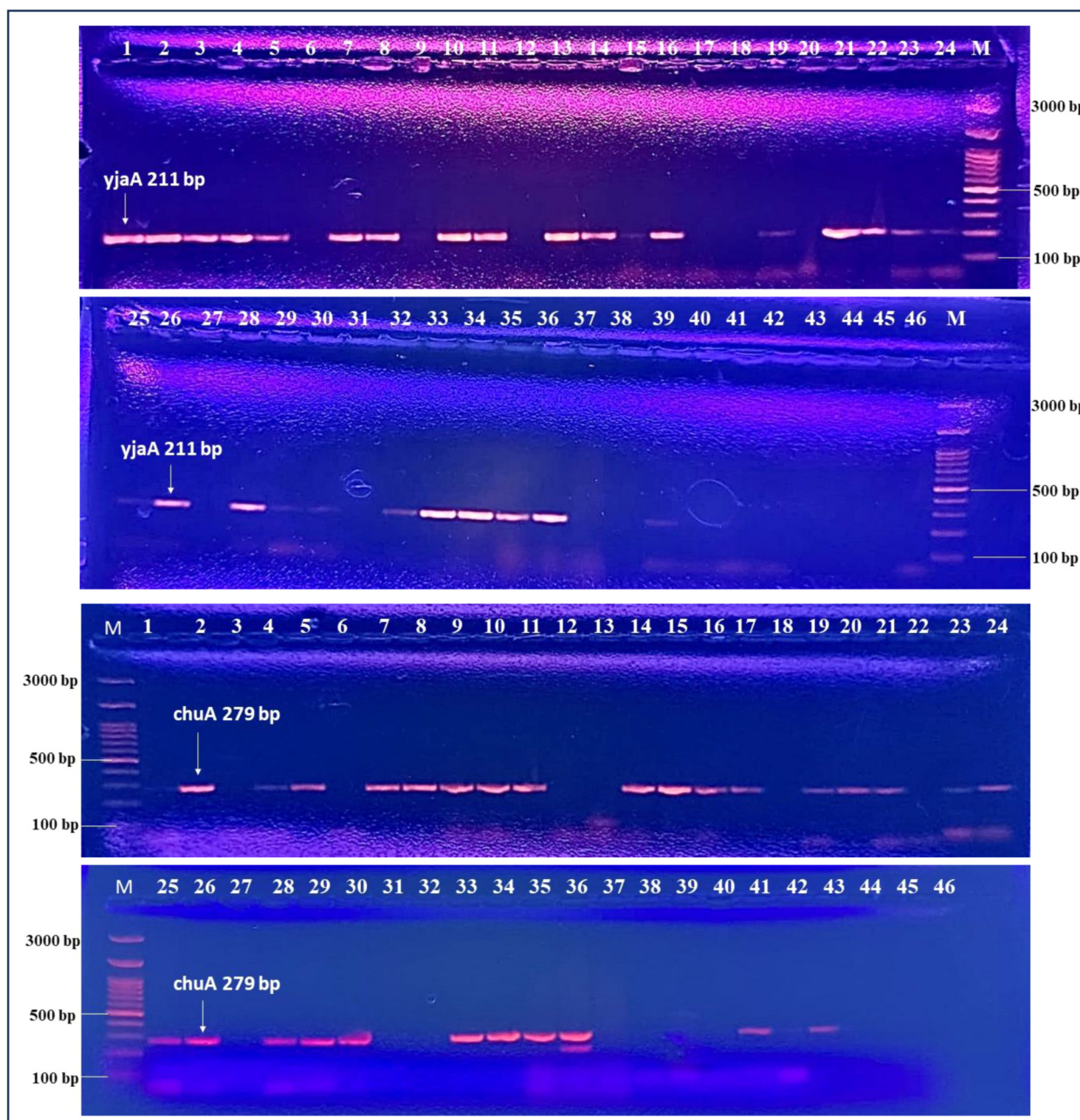


Fig. 2. Gel electrophoresis of amplified *yjaA* and *chuA* genes by Monoplex PCR.

Ethidium bromide-stained agarose gel electrophoresis of Monoplex PCR amplified products from extracted total DNA of *E. coli*. Lane (46 isolates) amplified with diagnostic genes *yjaA* and *chuA* show positive results at 211 bp and 279 bp. Respectively. The electrophoresis was performed at 80 volts for 95 minutes. (M): DNA molecular size markers (3000 ladders).

a portion of the bacterium that is less susceptible to resistance development. Moreover, the resistance rate remains lower than numerous other antibiotics because of its limited use.

The current study revealed that the prevalence of nalidixic acid resistance was 69%. A previous study in the Babylon Province [29] reported that the rate of Nalidixic acid resistance was 64.28%. Additional

study in the Babylon Province [33] revealed that the rate of Nalidixic acid resistance was 75%. Another study in the Mosul province [44] illustrated that the rate of Nalidixic acid resistance was 72.6%. A different study in the Misan province [5] demonstrated that the rate of Nalidixic acid resistance was 23.4%. Resistance can be acquired through changes in specific genes, making antibiotics less effective.

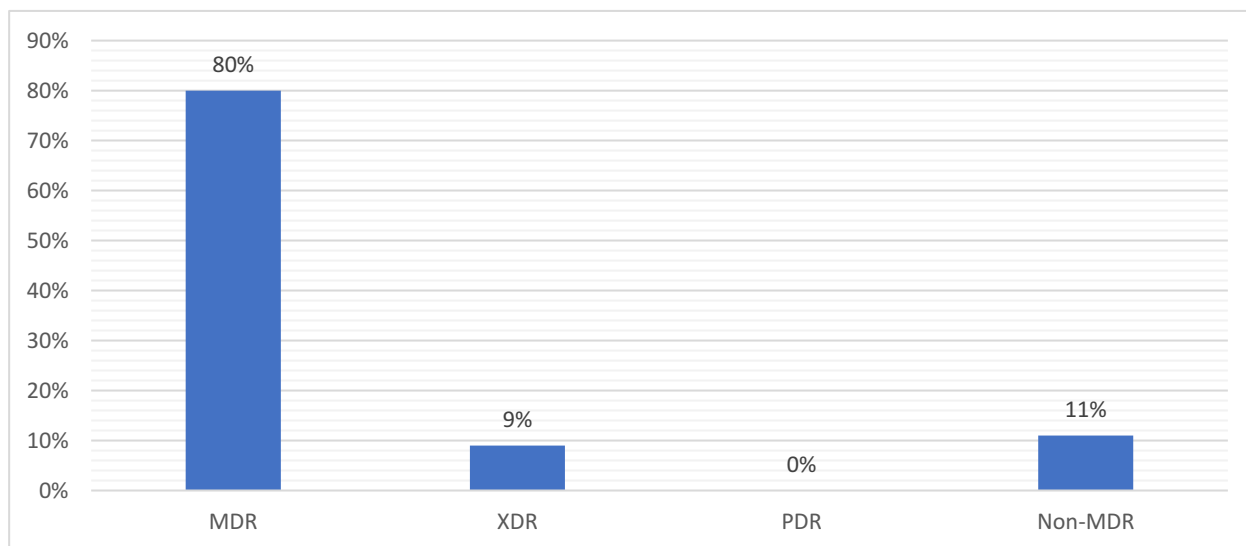


Fig. 3. Percentage of the MDR, XDR, and PDR *Escherichia coli*.

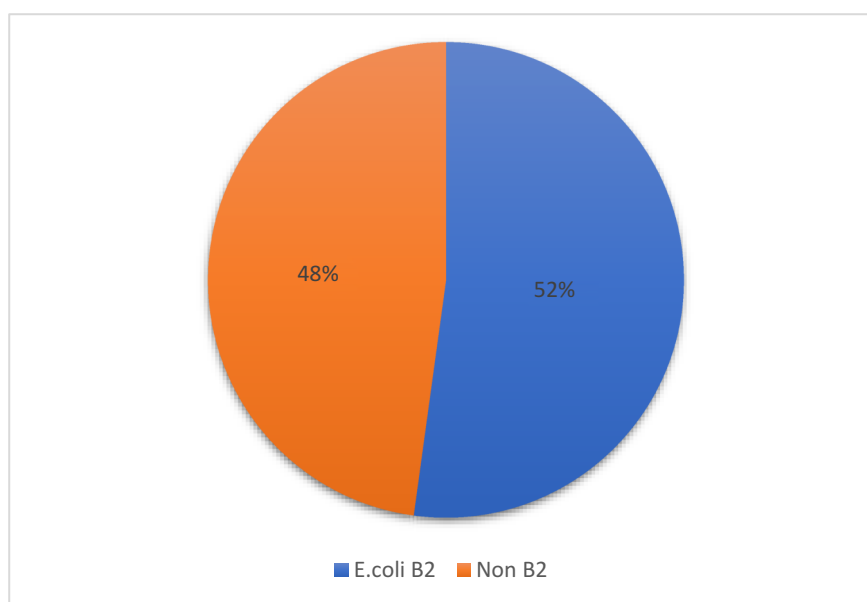


Fig. 4. Percentage of *Escherichia coli* B2 phylogeny and Non B2.

The current study revealed that the prevalence of nitrofurantoin resistance was 6%. A previous study in the Babylon Province [45] reported that Nitrofurantoin resistance was 9.09%. Another study in the Erbil Province [46] reported that the rate of Nitrofurantoin resistance was 2.1%. Additional study in the Karbala province [38] revealed that the Nitrofurantoin resistance was 36%. A different study in the Zakho province [47] illustrated that the Nitrofurantoin resistance was 82.4%. Nitrofurantoin is effective against several common uropathogenic bacterial species, including *E. coli* [48]. Nitrofurantoin has high effectiveness because its unique chemical struc-

ture and mode of action reduce cross-resistance with other antibiotic classes. This feature reduces the likelihood of bacteria developing multidrug resistance pathways, and also due to its rare use.

The antimicrobial susceptibility test of the current study exhibited that out of 46 *E. coli* isolates, 80% were classified as Multidrug-resistant (MDR). While 11% were susceptible to almost all antimicrobial agents (not classified as MDR), 9% were classified as Extensively drug-resistant (XDR), and 0% were classified as possibly Pandrug-resistant (PDR). The previous study in Babylon Province demonstrated that the MDR ratio was 80%, and the XDR ratio was 11% [29].

Another study conducted in the Baghdad Province [49] demonstrated that the MDR ratio was 98.23%, the XDR ratio was 21.24%, and the PDR ratio was 1.77%.

5. Conclusion

Escherichia coli found in different clinical samples had varying patterns of antibiotic susceptibility, and they were highly resistant to common antibiotics. Colistin, Nitrofurantoin, and Imipenem were the antibiotics with the lowest resistance against *E. coli* samples, with a rate of 0%, 6%, and 6%. Beta-lactam antibiotics, such as ampicillin and cephalothin, have the highest resistance among other antibiotics, with a rate of 94% and 94%. *Escherichia coli* phylogenetic analysis indicated that the most prevalent strain was B2, with a rate of 52%.

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