

Ciprofloxacin Resistance in *Escherichia coli* Strains Isolated from Urinary Tract Infections

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Abstract

Background: The purpose of this investigation was to show how resistant *E. Coli* is towards the fluoroquinolone treatment Ciprofloxacin. There were 140 specimens in total. It is widely recognized that infections of the urinary tract are a prevalent condition in women and men and different ages. The bacteria that cause *E. Coli* becomes more resistant as a result of increased consumption of antibiotics, which means this symptom is dangerous on a worldwide scale.

Methods: Within Al-Hindya Teaching Hospital, specimens of urine involving individuals of different age have been employed to isolate the *E. Coli* germs. The required separation procedures have been conducted, comprising genetic sequencing of the bacterial strain carbon monoxide gene, microscopical examination, plus biochemical examinations. The resulting isolates were subjected to the procedure called polymerase chain reaction (PCR), and their resistance to antibiotics was assessed by putting discs containing antibiotics on the Mueller_ Hinton agar.

Results: Compared to males, girls had a greater rate of *E. Coli* disease. The bacteria that underwent responsiveness testing employed ciprofloxacin, and that microbes rate of resistance to this antibiotic was somewhat higher than its level of sensitivity. Investigating the growing resistance of Ur pathogenic *E. Coli* bacterium to the antibiotic ciprofloxacin is the goal objective this investigation.

Keywords: *Escherichia coli*, urinary tract infection, ciprofloxacin, antibiotic

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Introduction

A leading source of mortality and morbidity, urinary tract infections constitute among the most prevalent pathogenic bacteria in healthcare facilities and communities [1]. The Ur pathogenic *Escherichia coli* pathotype, which possesses numerous pathogenic characteristics such as adhesives and related products capsules, siderophores which and toxic agents, is responsible for almost Seventy-five of urinary tract infections. [1,2] Ur pathogenic *E. Coli* (a bacterial infection) produces a variety of virulence-associated variables such as P fimbriae, aerobactin, hemolysin, as well as cytotoxicity necrotizing factor type 1 (CNF1) [3]. Additionally, this has demonstrated growing resistance to certain therapies and is acknowledged as an especially prevalent pathogen amongst the microbes in the gut that cause UTIs [4]. Antibiotic-resistant resistant strains of *E. Coli* along with additional urinary tract infections (UTI) microorganisms have become more prevalent, which reduces the efficacy of therapies [5]. Furthermore, the continued use of medicines for the management of UTIs has made this resistance an issue for the general population. It also raises the difficulty of diagnosing and avoiding urinary tract infections [4]. Traditional therapies are limited by the broad incidence of *E. Coli* strains and resistance to antibiotics. In the year 2007, resistance rates have been steadily rising [6,7]. Ciprofloxacin, the main member within the fluoroquinolone group, has become the mainstay of therapy for UTIs since its debut in the 1980s [8,9]. Significant amounts of resistance to influenza viruses are prevented by changes within the genes responsible for producing target enzymes, which also reduce the fluoroquinolone's ability to interact with the DNA enzyme complex. These Mutations like these lower the concentration inside cells of antibiotics by increasing the production protein flowing exchangers and decreasing cellular production of protein porins [10].

The appearance of resistance to multiple medications in bacteria has been largely attributed to the movement of genes that confer resistance to antibiotics. The process of conjugation transduction, and transformation are the three ways that the genes are transferred in *E. Coli* [11]. The management of urinary tract infections could grow more difficult with fewer therapeutic options if *E. Coli* resistance to medications rises [12].

Materials and Methods:

Between July and August, more than 200 spacemen were taken from Al-Hindiya Teaching Hospital in the city of Karbala Province who had urinary tract infections and placed in a sterilized container. Within two hours of being collected, the samples were brought to the laboratory and analyzed.

The bacterial isolation:

Bergey's handbook [13] has been employed to identify the bacterial isolates. based on techniques in [14] and [15]. Regarding the purification of gram-negative (-ve) microorganisms' bacterial samples were cultivated on enriching medium and specific medium such as blood agar and MacConkey agar, correspondingly.

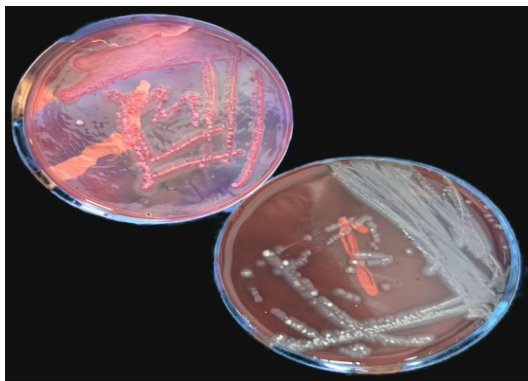


Figure1: *E. Coli* culture in blood agar media

The morphological analysis and gram stain were then used to do microscopic investigation. The biochemical examinations include lactate as well as a substance called fermenting, cytochrome oxidase synthesis, coagulation enzyme enzyme, indole, methylene red, and citrate.

PCR method: Localization of the *E. Coli* CNF1 gene by DNA sequencing the remainder of table 1 lists the primers that were used to extract the polymerase chain reaction (PCR) Genes from the *Escherichia coli* diagnostic products produced by Academic Investigator Co. Ltd./Iraq in accordance with [16].

Table 1. primers sequence and product size used in the present study

Primer name	primer sequences 3' – 5'	Reference
cnf1-F	5'-ATCGTAGTCCCG GAGGTATCTG-3'	17
cnf1-R	5'-GCTTTACAATATTG ACATGCTGC-3	

The steps involved in polymer polymerization employed the E. The bacteria Coli CNF1 gene. The primer in the solutions has been created with a 10 picomol/ μ L level. The subsequent procedures were used to program PCR experiment equipment: The initial step the initial mutation of templates DNA, a 5-minute cycle at 95 °C is used. The second phase of thirty cycles involved denaturing the template DNA for 1 minute at 96° C. These primers attach to the starting point DNA for 30 seconds at 52°C, while the corresponding primers elongate for 1 minute at 72°C. Lastly The last lengthening within a duplicated strand of DNA occurs during a 10-minute cycle at 72 °C.

Antibacterial susceptibility Test:

Mueller-Hinton agar was subjected to resistance to antimicrobial tests using the Kirby Bauer diffusion disk technique in accordance with accepted practices [18]. Ciprofloxacin (5 µg) was the primary antimicrobial agent that was evaluated. The National Committee for Laboratory Standards (NCCLS) was followed in the interpretation of the rejection information.

Results and Discussion

Infected urinary tract individuals provided approximately two hundred biopsy specimens. Utilizing differentiated circumstances for cultivation and a microscope to identify colonies, preliminary tests were conducted to isolate the *Escherichia coli* bacterium. There are just forty hundred *E. Coli* specimens. A Gram stain was subsequently applied, and the results showed that the samples were Gram-negative.

1. Biochemical investigations were conducted, with the findings displayed in table 2 below.

Table 2: The morphological and biochemical tests results for *E. Coli*

No.	Biochemical test	<i>E. Coli</i>
1-	Indole	+
2-	Methyl red	+
3-	Citrate	-
4-	Lysine decarboxylase	+
5-	Lactose Fermentation test	+
6-	Mannitol test	+
7-	Yellow pigment	-
8-	Gram stain	-
9-	oxidase	+
10-	Motility test	+

2- Using the method of polymerase chain reaction (PCR), isolates of bacteria can be quickly diagnosed. Following a biochemical diagnosis of microbes, this response is employed as a confirmatory test. Since it may be used to detect bacteria along with their genomes, this type of response is at present crucial to medical study of these organisms [19,20]. The existence within the CNF 1 gene in *E. Coli* was found using PCR. The bacterial identification as a bacterium that is Gram-negative was confirmed by the extraction of its genetic material [21].

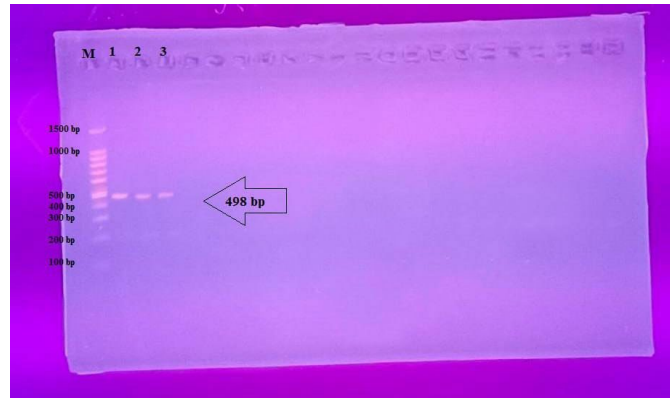


Figure 2. Agarose gel electrophoresis of PCR product using specific primers to detect CNF gene in *E. Coli*

3- The prevalence of the bacteria *E. Coli* infection leading to an infection of the bladder was 21.42% among those aged 1–20 years, 29.28% among those aged 21–30 years, and 17.14% among those aged 31–40 years, according to age categorization. The average percentage for those aged forty-one to fifty were roughly 17.85%. Lastly, table 3 shows that the average incidence frequency for those aged 51 to 60 was 14.28%. It is noteworthy that overall occurrence rate was significantly greater for both kids and adults aged 30 and older than for those aged 31 to 40. The number was lower among the over-50 population.

Compared with the older people, children and young adults had more instances of *E. Coli* in the urinary system, as well. They also had a greater prevalence of getting resistant to the fluoroquinolone group antibiotic levofloxacin. This corresponds to exactly Wang et al. [22] said in a study they did at a Chinese clinic.

Table3: The sample of *E. Coli* from urine

Age group	1-20	21-30	31-40	41-50	51-60
No. of infection	30	41	24	25	20
Infection rate	(21.42) %	(29.28) %	(17.14) %	(17.85) %	(14.28) %

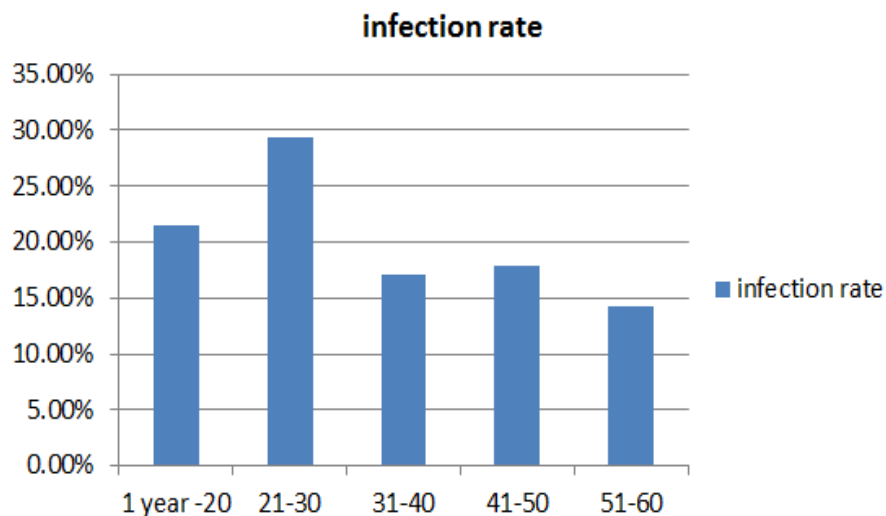


Figure 2: Show the infection rate of *E. Coli* in different age

The efficacy of Ciprofloxacin versus microorganisms is displayed in table 4 following. For these microorganisms, the sensitization ratio was 47.14%. On the other hand, 52.58% of these germs were resistant. As illustrated in image 3 below. The sensitivity rate and the resistance rate are related. This indicates that the bacteria *E. Coli* has become resistant to the antibiotic ciprofloxacin. The amount of resistance rate in comparable research by Gobernado et al. [23] was quite high, hitting 22.8%. Additionally, in a different investigation conducted by Nakhjavani et al. [24] Ciprofloxacin resistant in These bacteria was 42.1% greater than antimicrobial sensitivity by 4.9%, suggesting that the rate of susceptibility was rising quickly. However, it differed from a prior study by Moniri et al. [25], in which the detection percentage was 85.9% and the antibody resistance rate to ciprofloxacin was 14.1%.

Table 4: The antimicrobial drug resistance of *Escherichia coli* from UTI

Susceptible test	No. of isolated	Sensitive (%)	Resist (%)
<i>E. Coli</i>	140	66(.4714)%	74(52.85)%
male	58	16(27.58)%	42 (72.41)%
Female	82	50(60.97)%	32(39.02)%

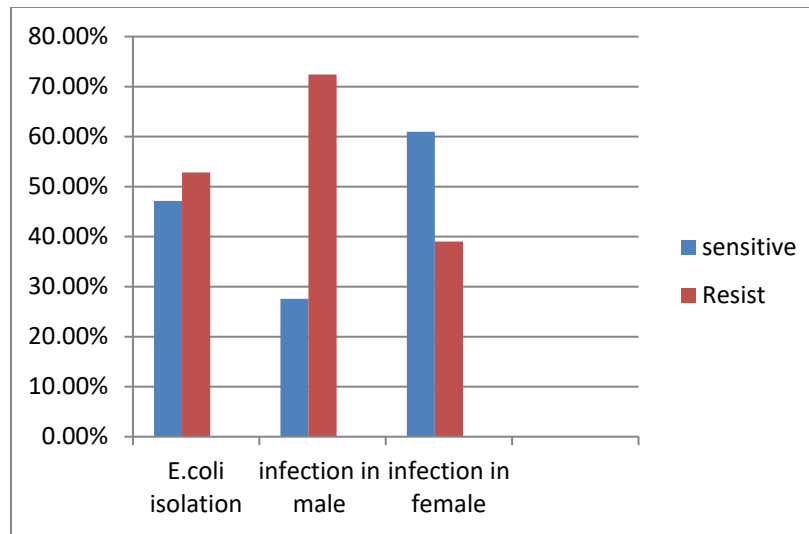


Figure 3: Sensitive and Resist rate for Ciprofloxacin.

Female less urinary system anatomy, intimate closeness with the genital area, and shorter private parts minimize the distance required for bacterial invasion, which is why women experience higher urinary system infection than men [26, 27]. The rate of infection rates for males were lower than those for females, reaching about 41.42% for male with 58.57% with females, as shown by table 5 which has been connected. The rates of infections were nearly the same as those found in an earlier investigation by Bashir et al., [28], in which the incidence of infection in females was greater than that in males. According to the results of our most recent investigation, the *Escherichia coli* prevalence reached the ratio in question was nearly the same as one found in the research conducted by McGregor et al. [29].

Table 5: the infection rates for males and female

Gender	No. of infection	Infection rate
Male	58	(41.42) %
Female	82	(58.57) %

The majority of naturally occurring human urinary tracts have been demonstrated to have immunity impermeable to disease even though contamination alongside organisms that are gram-negative, indicating that bacterial inability to respond is the single most significant variable in averting elementary infections in the urinary tract. Therefore, an alteration within the mucosa inside the urinary is required for bacteria to carry organisms that produce clinical urinary track inflammation [30].

Conclusions:

The data collected through this study's findings demonstrated that *E. Coli* bacteria have a growing propensity to develop resistance to the medication of ciprofloxacin as time passes. The

purpose of this investigation was to evaluate the degree of resistance of *Escherichia coli* to the fluoroquinolone drug ciprofloxacin.

References

- 1- Mwakyoma AA, Kidenya BR, Minja CA, Mushi MF, Sandeman A, Sabiti W, Holden MT, Mshana SE. Allele distribution and phenotypic resistance to ciprofloxacin and gentamicin among extended-spectrum β -lactamase-producing *Escherichia coli* isolated from the urine, stool, animals, and environments of patients with presumptive urinary tract infection in Tanzania. *Frontiers in Antibiotics*. 2023 Jun 5; 2:1164016.
- 2- Afsharikhah S, Ghanbarpour R, Mohseni P, Adib N, Bagheri M, Jajarmi M. High prevalence of β -lactam and fluoroquinolone resistance in various phylotypes of *Escherichia coli* isolates from urinary tract infections in Jiroft city, Iran. *BMC microbiology*. 2023 Apr 22;23(1):114.
- 3- Rippere-Lampe KE, O'Brien AD, Conran R, Lockman HA. Mutation of the gene encoding cytotoxic necrotizing factor type 1 (cnf 1) attenuates the virulence of uropathogenic *Escherichia coli*. *Infection and immunity*. 2001 Jun 1;69(6):3954-64.
- 4- Moniri R, Khorshidi A, Akbari H. Emergence of multidrug resistant strains of *Escherichia coli* isolated from urinary tract infections. *Iranian Journal of Public Health*. 2003;32(4):42-6.
- 5- Arslan H, Azap ÖK, Ergönül Ö, Timurkaynak F. Risk factors for ciprofloxacin resistance among *Escherichia coli* strains isolated from community-acquired urinary tract infections in Turkey. *Journal of Antimicrobial Chemotherapy*. 2005 Nov 1;56(5):914-8.
- 6- Abbott IJ, van Gorp E, Cottingham H, Macesic N, Wallis SC, Roberts JA, Meletiadiis J, Peleg AY. Oral ciprofloxacin activity against ceftriaxone-resistant *Escherichia coli* in an in vitro bladder infection model. *Journal of Antimicrobial Chemotherapy*. 2023 Feb 1;78(2):397-410.
- 7- Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Annals of clinical microbiology and antimicrobials*. 2007 Jan; 6:1-7.
- 8- Jakobsen L, Lundberg CV, Frimodt-Møller N. Ciprofloxacin pharmacokinetics/pharmacodynamics against susceptible and low-level resistant *Escherichia coli* isolates in an experimental ascending urinary tract infection model in mice. *Antimicrobial Agents and Chemotherapy*. 2020 Dec 16;65(1):10-128.
- 9- Nakhjavani FA, Mirsalehian A, Hamidian M, Kazemi B, Mirafshar M, Jabalameli F. Antimicrobial susceptibility testing for *Escherichia coli* strains to fluoroquinolones, in urinary tract infections. *Iranian journal of public health*. 2007;36(1):89-92.
- 10- Neyestani Z, Khademi F, Teimourpour R, Amani M, Arzanlou M. Prevalence and mechanisms of ciprofloxacin resistance in *Escherichia coli* isolated from hospitalized patients, healthy carriers, and wastewaters in Iran. *BMC microbiology*. 2023 Jul 17;23(1):191.

- 11- Ayobola ED, Oscar WO, Ejovwokoghene EF. Plasmid-mediated quinolone resistance genes transfer among enteric bacteria isolated from human and animal sources. AIMS microbiology. 2021 Jun 9;7(2):200.
- 12- Mandal J, Acharya NS, Buddhapriya D, Parija SC. Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant *Escherichia coli*. Indian Journal of Medical Research. 2012 Nov 1;136(5):842-9.
- 13- Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST. Bergey's Manual of Determinative bacteriology, 9th edn. Williams and Wilkins, Baltimore, USA. 1994:749-55.
- 14- JF M. Biochemical tests for identification of medical bacteria. Lippincott, Williams & Williams, Baltimore. 2000.
- 15- Mackie TJ. Practical medical microbiology. Churchill livingstone; 1996.
- 16- Al-Ghanimi AA, Al-Zughaibi AS. Molecular Identification of *Acinetobacter baumannii* and *Staphylococcus aureus* isolated from wound infections in Karbala governorate Iraq. In AIP Conference Proceedings 2022 Dec 2 (Vol. 2547, No. 1). AIP Publishing.
- 17- Mehdipour S, Doosti A, Ghasemi Dehkordi P. Detection of cytolethal distending toxin (cdt) and cytotoxic necrotizing factor (cnf) genes among *Escherichia coli* isolates from Iranian sheep carcasses. Comparative clinical pathology. 2012 Dec; 21:1683-8.
- 18- Perez-Roth E, Claverie-Martin F, Villar J, Mendez-Alvarez S. Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. Journal of clinical microbiology. 2001 Nov 1;39(11):4037-41.
- 19- Jonas D, Speck M, Daschner FD, Grundmann H. Rapid PCR-based identification of methicillin-resistant *Staphylococcus aureus* from screening swabs. Journal of Clinical Microbiology. 2002 May;40(5):1821-3.
- 20- Bauer AW, Kirby M, Sheris JD, Turch M. Antibiotic resistance testing by standard single disc method. Am J Clin Pathol. 1966;45(4):493-6.
- 21- Thong KL, Lai MY, Teh CS, Chua KH. Simultaneous detection of methicillin-resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by multiplex PCR.
- 22- Wang S, Zhao S, Zhou Y, Jin S, Ye T, Pan X. Antibiotic resistance spectrum of *E. Coli* strains from different samples and age-grouped patients: a 10-year retrospective study. BMJ open. 2023 Apr 1;13(4): e067490.
- 23- Gobernado M, Valdés L, Alós JI, Garcia-Rey C, Dal-Ré R, García-de-Lomas J, Spanish Surveillance Group for *E. Coli* Urinary Pathogens. Quinolone resistance in female outpatient urinary tract isolates of *Escherichia coli*: age-related differences. Rev Esp Quimioter. 2007 Jun 1;20(2):206-10.
- 24- Nakhjavani FA, Mirsalehian A, Hamidian M, Kazemi B, Mirafshar M, Jabalameli F. Antimicrobial susceptibility testing for *Escherichia coli* strains to fluoroquinolones, in urinary tract infections. Iranian journal of public health. 2007;36(1):89-92.

- 25- Moniri R, Khorshidi A, Akbari H. Emergence of multidrug resistant strains of *Escherichia coli* isolated from urinary tract infections. Iranian Journal of Public Health. 2003;32(4):42-6.
- 26- Czajkowski K, Broś-Konopielko M, Teliga-Czajkowska J. Urinary tract infection in women. Menopause Review/Przegląd Menopauzalny. 2021 Apr 21;20(1):40-7.
- 27- Griebing TL. Urinary tract infection in women. Urologic diseases in America. 2007; 7:587-619.
- 28- Bashir MF, Qazi JI, Ahmad N, Riaz S. Diversity of urinary tract pathogens and drug-resistant isolates of *Escherichia coli* in different age and gender groups of Pakistanis. Tropical journal of pharmaceutical research. 2008 Sep 11;7(3):1025-31.
- 29- McGregor JC, Elman MR, Bearden DT, Smith DH. Sex-and age-specific trends in antibiotic resistance patterns of *Escherichia coli* urinary isolates from outpatients. BMC family practice. 2013 Dec; 14:1-5.
- 30- Bennett CJ, Young MN, Darrington H. Differences in urinary tract infections in male and female spinal cord injury patients on intermittent catheterization. Spinal Cord. 1995 Feb;33(2):69-72.