AMERICAN JOURNAL OF BIOLOGY AND NATURAL SCIENCES
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Article

Isolation and Identification of Antibiotic Resistance Genes in Multi-Drug Resistance Escherichia coli Isolates from With Urinary Tract Infections

Marwah Ali Oudah[1], Nidda F. Aziz[2], Bushra Hamad Obaid[3]
[1 2 3] AL-Furat AL-Awsat Technical University, Al-Qadisiyah Polytechnic College, Iraq,
*Corresponding author E-mail: Dw.mrw23@atu.edu.iq [1], nedaa.azeezb.idi14@atu.edu.iq [2], bushraa.obaid@atu.edu.iq [3]

Citation: Oudah, M. A, Aziz, N. F & Obaid, B. H. Isolation and Identification of Antibiotic Resistance Genes in Multi-Drug Resistance Escherichia coli Isolates from With Urinary Tract Infections. American Journal of Biology and Natural Sciences 2026, 3(3), 74-85

Received: 10th Des 2025
Revised: 11th Jan 2026
Accepted: 20th Feb 2026
Published: 05th Mar 2026

Abstract: Escherichia coli is an intestinal bacterium that frequently causes UTIs and nosocomial infections. Resistance of E. coli to various antibiotics has been widely reported, making treatment difficult for UTI patients. The purpose of this study is to determine the sensitivity of E. coli in the urine of UTI patients to ciprofloxacin antibiotics. Bacterial samples were obtained from the isolation of patients suffering from UTI using Endo's agar medium selective media, the growing E. coli was then subjected to a sensitivity test at a temperature of 37oC for 24 hours to observe and measure the inhibition zone formed. This research was a descriptive laboratory study with a completely randomized design, data were analysed using the Kruskal-Walli's test and continued with post hoc. The study results showed that the incidence of UTI was highest in women (60%), while in men it was 40%. Most of the isolates had fluoroquinolone resistance determining genes located on the chromosome gyr A and parC, while resistance genes located on the plasmid were found in 5 isolates, qnr (n=1), oqxA (n=2) and oqxB (n=2). Although the frequency of PMQR genes, including qnr, oqxA, and oqxB, was low in 21MDR ciprofloxacin-resistant E. coli isolates, their existence may allow for horizontal gene transfer among other Enterobacteriaceae bacteria and aid in the selection of resistant mutants following ciprofloxacin exposure. It is highly likely that the presence of a ciprofloxacin resistance gene, either on the plasmid or chromosome, is what causes the MDR ciprofloxacin resistance pattern in E. coli isolates.

Keywords: urinary tract infection, Escherichia coli, antibiotics, ciprofloxacin, MDR, Genes

Introduction

Infectious diseases are a problem in the world of health, where almost every country experiences problems with infectious diseases. Infectious diseases are diseases caused by pathogenic microbes and are very dynamic. Urinary tract infections (UTIs) are the most prevalent infectious disease [1]. Up to 8.3 million cases of UTI are reported annually, making UTIs the second most common infection after respiratory tract infections. One important factor contributing to morbidity and death is UTI [2]. Age and gender have a significant impact on the prevalence of UTIs, with women experiencing the infection more frequently than men because of anatomical differences. Microorganisms in the urinary tract, which includes the kidneys, ureters, bladder, and urethra, are typically the cause of UTIs.

Most UTIs are caused by bacteria, although sometimes fungi and viruses can be the etiological agents of UTIs. The bacteria that often cause UTIs are Escherichia coli (7-95%) and other types of Enterobacteriaceae.[3] Escherichia coli is a normal intestinal flora bacterium that is gramme-negative and rod-shaped. Certain strains of E. coli can cause intestinal or extra-intestinal infections in humans or certain animals. E. coli can also cause urinary tract infections, which is the main cause of nosocomial infections in hospitals in Iraq [4]. Providing scientific insight for health workers into the rational use of fluoroquinolone antibiotics, especially ciprofloxacin, in treating infectious patients, especially UTIs, to avoid the risk of developing MDR E. coli strains [5].

Currently, a molecular-based identification method has been developed because it can be detected quickly with a high level of sensitivity and specificity, namely using the 16S rRNA (16S ribosomal RNA) gene sequence for identification of microorganisms and phylogenetic construction because it has a low rate of evolution [6]. The high phenomenon of antibiotic resistance against bacteria that cause urinary tract infections in different places and times has different resistance patterns. Thus, it is necessary to research biomolecular identification using the 16S rRNA gene to identify bacterial species that are resistant to the antibiotic ciprofloxacin, which is the most frequently used antibiotic [7].

Ciprofloxacin is a member of the fluoroquinolone antibiotic class that is most frequently used to treat infections brought on by E. coli [8]. Therefore, ciprofloxacin is widely used for various types of infections, such as those in the bloodstream, intestines or respiratory tract. Overdose and incorrect use can lead to the emergence of ciprofloxacin resistance. Gyr A and par C gene chromosomal mutations are typically the cause of E. coli resistance to fluoroquinolone antibiotics. However, recent studies have shown that plasmids can also mediate low-level resistance through the acquisition of the qnr gene via the plasmid pMG252 [9]. Plasmids are circular, double-stranded DNA molecules that are separate from chromosomes (extra-chromosomal). They can replicate independently and play a significant role in the dissemination of resistance genes, such as extended-spectrum beta-lactamase (ESBL) and the PMQR gene [9]. The finding of resistance in Enterobacteriaceae has been reported all over the world. This is a cause for concern because the ESBL gene makes bacteria resistant to third-generation cephalosporins, while the PMQR gene makes bacteria resistant to low levels of fluoroquinolones [10]. The dissemination of genes that confer resistance to antibiotics in Enterobacteriaceae, particularly in E. coli, constitutes a risk to public health. Based on what was said above, this study's goal is to find ciprofloxacin resistance genes in MDR E. coli isolates. Specifically, it wants to find the PMQR gene, which stands for "plasmid-mediated quinolone resistance." isolated from UTI women patients attending Azadi Teaching Hospital, Duhok, Duhok province, Iraq.

The Aim and Objective of the study

The purpose of this study is to determine the sensitivity of E. coli in the urine of UTI patients to ciprofloxacin antibiotics. Bacterial samples were obtained from the isolation of patients suffering from UTI using Endo's agar medium selective media, the growing E. coli was then subjected to a sensitivity test at a temperature of 37oC for 24 hours to observe and measure the inhibition zone formed. This research was a descriptive laboratory study with a completely randomized design, data were analysed using the Kruskal-Walli's test and continued with post hoc.

Materials and Methods

The research was conducted from August 2023 to December 2023 at the Azadi Teaching Hospital, Duhok, Duhok province, Iraq. The research tools used in this research were micropipettes, 1 set of white, yellow and blue microtips, shaker , incubator, 1.5 ml and 2.0 ml Eppendorf tubes, an Eppendorf tube rack, 1 set of 0.2 ml PCR tubes, a PCR tube rack, a vortex shaker, autoclave, incubator, 1.5 ml and 2.0 ml Eppendorf tubes; Eppendorf tube rack, 1 set of 0.2 ml PCR tubes, PCR tube rack, vortex shaker, microcentrifuge, analytical balance, freezer, refrigerator, water bath, Presto™ Mini Plasmid Kit, 100 preps/kit, Wizard® Genomic DNA Purification Kit, 100 Isolations, microwave, Gentier 96E Real-Time PCR System, BR Biochem MP-8001 model Semi-Automatic electrophoresis and Elico Model SL 159, UV-Visible Spectrophotometer. Before being used for research, equipment and materials/media that will be used for research must be sterilized first by placing them in an autoclave at a temperature of 121^{o}C at a pressure of 1.5 Atm for 2 hours, then the sterilized equipment is stored

in an oven at a temperature of 150°C for 2 hours. For media containing sterile agar, leave it at a temperature of 50°C. After that, pour it into a sterile petri dish aseptically and wait until it solidifies.

Samples of 21 isolates of E. coli MDR ciprofloxacin from women patients with age of 46 to 55 years attended at the outpatient department at Azadi Teaching Hospital, Duhok, Duhok province, Iraq between August to December 2023.

Urine samples were taken from the middle portion of urine (midstream urine) of 21 participants (Women only) who were willing and had indications of Urinary Tract Infection (UTI) who had or were currently using the antibiotic ciprofloxacin and were hospitalised. Isolation of E. coli bacteria from Urine of UTI patients. Isolation of E. coli bacteria was carried out by preparing urine samples from different UTI patients, and then 1 mL of each urine sample was taken and inoculated.

Preparation of the E. coli Bacterial Inoculum: After identifying and finding positive E. coli bacteria on the EMBA plate media, it will be metallic green in colour, the next step is to prepare the inoculum by transferring the E. coli bacteria to the EMBA medium so that it is 2 times into the media. NB, then incubated in an incubator for 24 hours at 37 0C.

Bacterial Sensitivity Test: Against Antibiotics 10, 100µL of E. coli bacterial inoculum in NB media that had been aged overnight was taken, then spread onto MHA plate media and spread evenly using a Drigalski spreader until the MHA surface looked rough.

Resistance Test: The test bacteria are collected using a sterile needle and then placed in a tube with 5 mL of a 0.9% NaCl solution until they reach the same level of cloudiness as the 0.5 MacFarland standard solution. 200 µL of the test bacterial suspension was pipetted and poured onto the entire surface of the LB agar medium [11]. The suspension was levelled using L-Glass and left for 5 minutes. The ciprofloxacin (5µg) disc was placed on the surface of the LB agar medium, pressed with forceps to ensure perfect adhesion, and then incubated at 37°C for 18 to 24 hours. Following incubation, the appearance of a transparent area surrounding the antibiotic disc is noted. The measured diameter is subsequently compared to the standards of CLSI [12].

Primer selection of gene identification [13]: A pair of qnr, oqx A, oqxB, parC and gyr A gene-specific primers (Twist Bioscience, India) for DNA amplification and sequencing as given below:

qnr gene-specific primers	Primer QP1:	5'- GAA AGA ATG TTT CTT CAGA GA G - 3'
	Primer QP2:	5'- AGT TTG GAG ATC ACC CAA GA - 3'.
oqx A gene specific primers	Primer oqx A-F:	5'- CCG CCG GTT ACT ATT GCA AT - 3'
	Primer oqx A-R:	5'- GTA GAG TGG TAT GGG TTA AGG TG - 3'.
oqxB gene specific primers	Primer oqxB-F:	5'- GCG TTT AGG CTG CGA TTT - 3'
	Primer oqxB-R:	5'- TAC CGG ACT ACC GAT ATC C - 3'.
gyrA gene specific primer	Primer gyrA – F:	5'- AGA CTA GGC ATG CGT ACA TG - 3'
	Primer gyrA – R:	5'- AGG ATC GCC AAG CGA TAG TA - 3'
parC gene specific primers	Primary parC – F:	5'- TATTCGTATGCGTGTGAACG - 3'
	Primary parC – R:	5'- CCA GCT CGG AAT TCA AAT GA - 3'

Plasmid Isolation of E. coli Isolate: In this study, plasmid isolation was carried out using the Genomic DNA Mini Kit (Geneaid). In principle, plasmid isolation is carried out to separate DNA from protein and RNA and separate plasmid DNA from chromosomal DNA. The working stages of the Plasmid Kit use the principles of the alkaline lysis solution method [21]. A schematic representation of plasmid DNA isolation from bacteria can be seen in Figure 1.

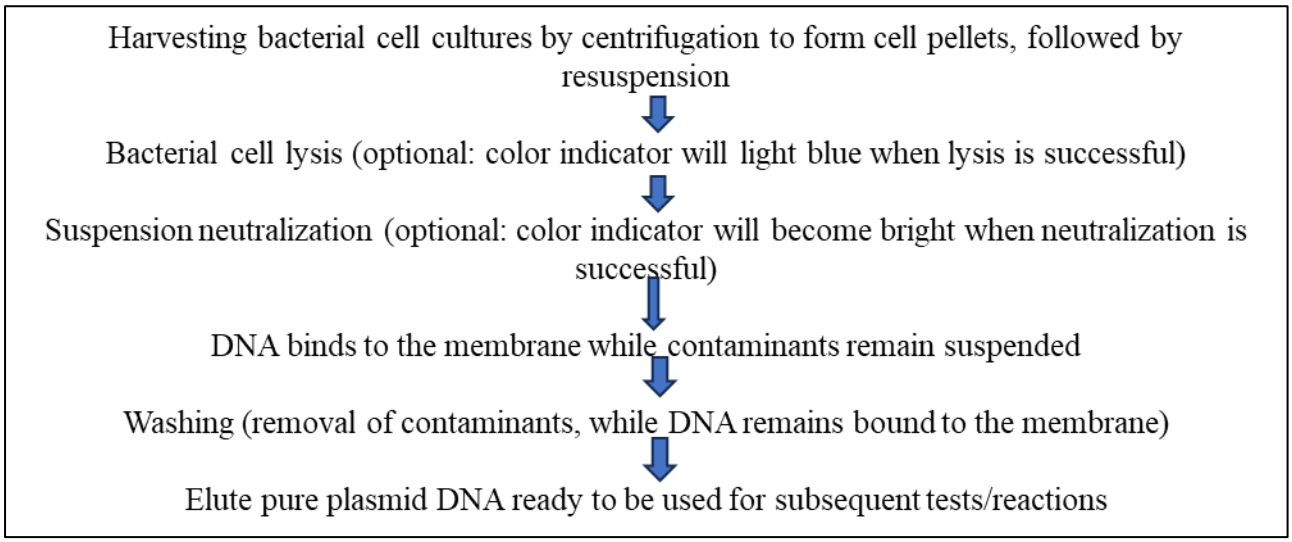

Figure 1. Plasmid DNA isolation scheme.

Electrophoresis and Visualization: PCR products were electrophoresed using a 1% agarose gel in 1X TBE (Tris-Boric EDTA) buffer. The electrophoresis process was carried out using an electric voltage of 130 Volts for 20 minutes, then visualized via a UV Transilluminator and documented. Observe the DNA fragment bands formed from each amplicon sample and determine their position or location, whether they are parallel to the base pair band lines of the DNA ladder ribbon marker. DNA band visualization results from each amplicon sample, save on the computer.

Amplification of the 16S rRNA gene using PCR techniques: The primers used for the PCR process are a new primer pair developed by Dan Sune et al (2020) [14], namely BK-X (forward) 5'- GCA YGT TYT AAC AGC AAC G -3' and BK-XR (reverse) 5'- TTG ATC CAC TCG TCC CAC CTC -3'. Amplification using the PCR technique is carried out with variations in reagent composition and optimized PCR reaction conditions. The composition of the PCR mixture is 15 µL milliQ water, 2 µL DNA template, 20 µL 2x Master Mix, and 1.5 µL each primer. The temperature setting for the PCR machine begins with initial denaturation at 95^{o}C for 3 minutes which is then followed by a denaturation stage at 95^{o}C for 20 seconds, attachment of the primer to the DNA at 55^{o}C for 20 seconds, and elongation of the DNA strand at 72^{o}C for 1 minute which is repeated in 35 cycles. The final DNA strand elongation was carried out at 72^{o}C for 5 minutes and determine bacterial species that are resistant to ciprofloxacin

Biomolecular Identification of Resistant Bacteria: Ciprofloxacin 1. Isolation of Genomic DNA. Isolation of Genomic DNA from bacterial cultures was carried out using the spincolumn method [15] using the Genomic DNA Mini Kit (Geneaid).

Chromosomal DNA Isolation of E. coli Isolates: Isolate chromosomal DNA from E. coli bacteria using the Wizard® Genomic DNA Purification Kit. A schematic of the isolation of genomic DNA from bacteria can be seen in Figure 2. Finally overall research work given in the flow figure 3

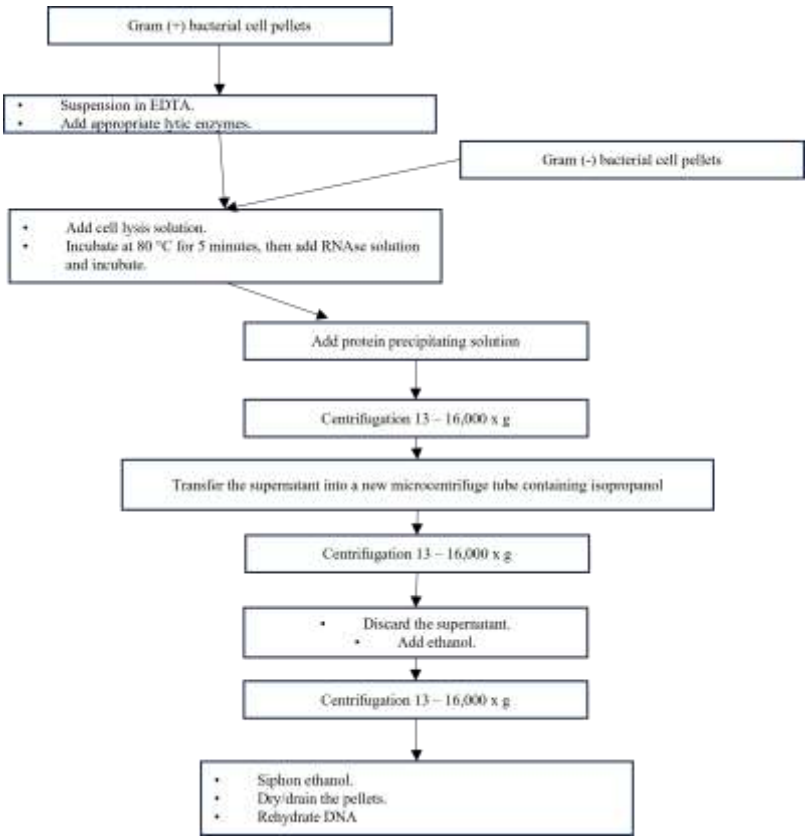

Figure 2. Scheme of chromosomal DNA isolation

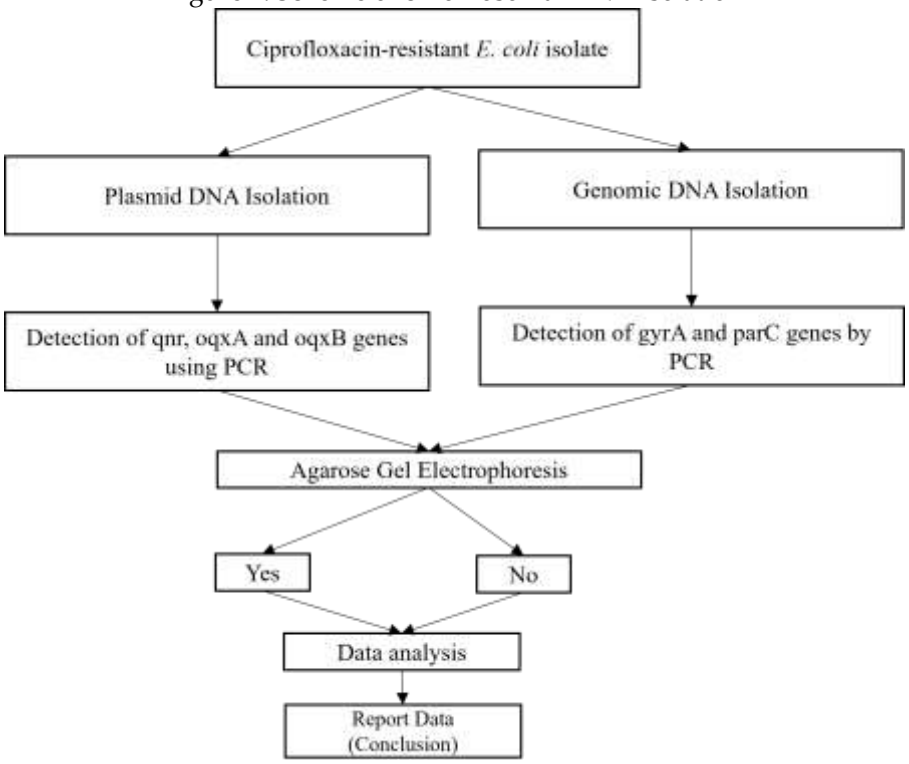

Figure 3. Research flow diagram.

Result and Discussions

Results

E. coli bacteria identification: Based on the findings of bacterial isolation from urinary tract infection patients' urine, 10 isolates of E. coli bacteria were obtained which were grown and verified on EMBA plate media and colonies were obtained that were metallic green in color which is an interpretation if E. coli bacteria were growing on the EMBA media. seen in Figure 4.

Figure 4. E. coli bacteria isolated from UTI patients on metallic green EMBA plate media.

Frequency Distribution: Based on Gender From the results of research on the frequency distribution of UTI sufferers based on gender at study location, the results showed that women had a greater risk (60%) while men had 40%.

Testing Bacterial Resistance to Antibiotics: Bacterial resistance testing is carried out using the diffusion method (Kirby-Bauer disk diffusion method). A comparison of the diameters of the inhibition zones shows that of the 21 isolates tested, 9 of them showed a high level of resistance, while the other 12 were still classified as sensitive and none were intermediate.

In collecting secondary data through interviews, 2 out of 5 participants admitted to having a history of UTI, in other words, they were experiencing recurrent UTI (recurrence) while being treated during the research period. This is in line with the results of the resistance test, namely the discovery of isolates from the participant's urine which showed quite high resistance.

Results of Biomolecular Identification Using the 16S rRNA Gene: Isolates that were continued to the biomolecular identification stage using the 16S rRNA Gene were bacteria with a high level of resistance, namely bacteria with the code CD41 [16]. Based on a search on the network database in GenBank, it shows that the sequence obtained from the antibiotic-resistant bacterial isolate (CD41) is Klebsiella pneumoniae with 99% identity. This is in line with the results of Gram staining which showed that this isolate was a bacillus and was Gram negative. The 16S rRNA gene may have similar threshold sequences to be used as an indicator, and not as a definitive tool for classification of bacterial strains [17].

A) PMQR Gene:

1) qnr gene. Of the 21 clinical isolates of E. coli MDR ciprofloxacin obtained from UTI patients, 1 sample isolate (no. 2E) was found to have the qnr gene, based on a DNA band measuring 593 bp (figure 5).

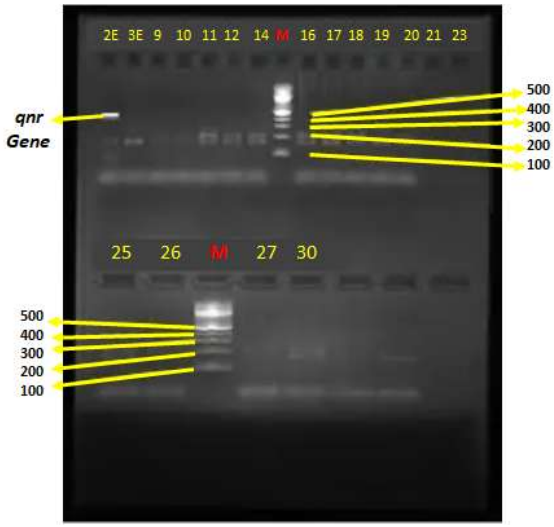

Figure 5. Visualization of the qnr gene amplicon in the DNA band at 593 bp. Only 1 isolate was positive (sample no. 2E).

2) The oqxAB gene (oqxA and oqxB): PCR was used to screen 21 clinical isolates of ciprofloxacin-resistant E. coli with a 5.5% prevalence of the oqxA gene. These isolates were obtained from patients at the Azadi teaching hospital in Duhok who had UTIs.

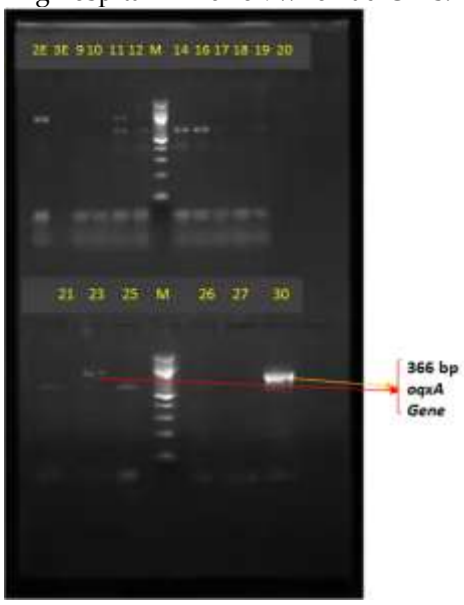

Figure 6. Visualization of the oqxA gene amplicon using DNA bands at 366 bp (sample no. 23 and 30)

The results showed that 2 isolates were positive for the oqxA gene, namely samples no. 23 and 30, based on DNA bands measuring 866 bp (figure 6). Meanwhile, the isolates that were positive for the oqxA gene were also tested for the oqxB gene. The results of PCR screening on oqxB gene on the 21 clinical isolates found 1 positive isolate for oqxB gene, namely sample number 30, based on DNA bands measuring 781 bp (figure 7), it can be said that the prevalence of the oqxB gene was 5.5% of the 21 clinical isolates.

B) QRDRs gene (Quinolone Resistance Determining Regions): GyrA gene Based on the DNA band size at 264bp, from 21 clinical isolates of E. coli

Figure 7. Visualization of the oqxB gene amplicon in the DNA band at 781 bp (sample no. 30).

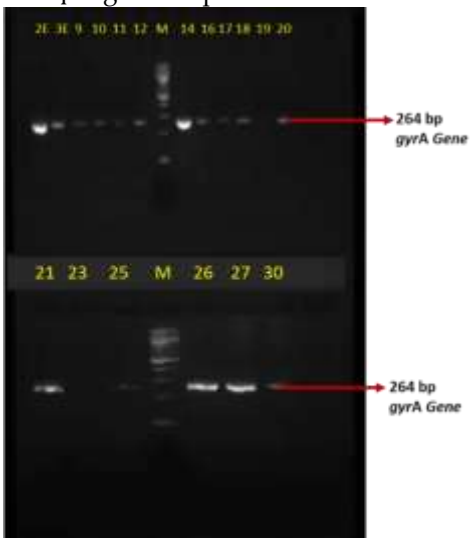

Figure 8. Visualization of the gyrA gene amplicon in the DNA band at 264 bp (all positive samples)

Based on the DNA band size of 191 bp, all of the 21 clinical isolates of E. coli MDR ciprofloxacin were found to be parC gene positive (figure 9).

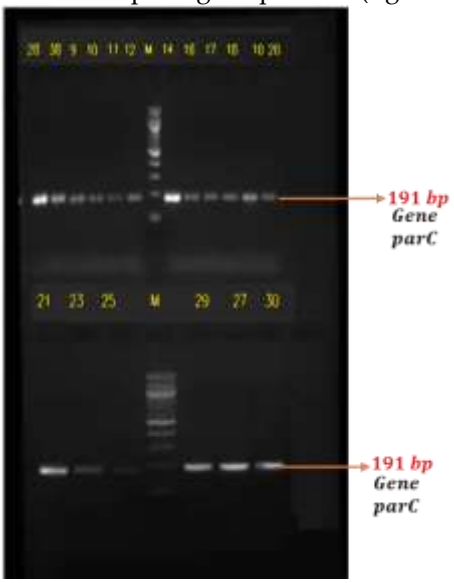

Figure 9. Visualization of amplification of gene parC on the 191 bp band, all samples were positive.

Discussion

The results of the identification research were obtained from all urine samples with 100% isolates of E. coli bacteria in patients suffering from UTI at the Azadi teaching hospital, Duhok, Duhok province, Iraq. Isolates that were positive for E. coli grew on EMBA media with the appearance of metallic green colonies. This is because E. coli is able to ferment lactose and produce acid, thereby precipitating metallic green pigment on the EMBA media [18].

The frequency distribution by gender revealed that women (60%) were the majority of UTI sufferers, with men accounting for 40%. This is consistent with research indicating that women have more UTIs than men, as reported by Czajkowski et al. (2021) [19]. Predisposing factors include the anatomy of the female urinary tract, previous pregnancy history, menopause, and sexual behaviour. The female urethra's concise length and close proximity to the anus facilitate the ingress of bacteria into the urinary tract. Pregnancy is linked to a higher likelihood of developing pyelonephritis (0.5-2%) and asymptomatic bacteriuria (4.7%). Following menopause, there is a substantial decrease in the secretion of oestrogen, which serves as a protective factor against the colonisation of the vagina by Enterobacteriaceae. Sexual activity has been associated with the reappearance of UTIs in premenopausal women.

In another study conducted by Biswas (2014) [20] at Dhaka Square hospital, Bangladesh, 40% of the uropathogens tested were sensitive and 60% were resistant to ciprofloxacin. According to a study by Ali et al (2010) [22], patients receiving inpatient treatment at hospitals had a higher presentation of ciprofloxacin resistance in their bacteria (46.9%) compared to outpatients (30.8%). When treating urinary tract infections, a common issue is making the diagnosis without conducting the necessary testing, which results in treating asymptomatic bacteria with antibiotics. Antibiotics can kill beneficial bacteria in our bodies without knowing which specific bacteria to target, which can impair our body's defences against pathogenic microorganisms. Antibiotic-resistant target bacteria can alter their cell surface and develop resistance to the drugs, rendering them incapable of killing bacterial cells [23].

Genetic Study

1 sample isolate (sample no.2E) was found that had the qnr gene, based on a DNA band measuring 593 bp (figure 5). These results are in accordance with Pereira, et al (2007) [24], Martín et al (2017) [25] and Wang, et al (2012) [26]. While the presence of the qnr gene is not commonly observed in ciprofloxacin-resistant Escherichia coli isolates, its detection could suggest the presence of a mechanism that confers resistance to fluoroquinolones. The qnr gene only provides low-level resistance to fluoroquinolones; Nevertheless, this gene has the ability to horizontally transfer to other E. coli bacteria and promote the emergence of resistant mutants following exposure to Ciprofloxacin. PMQR genes confer minimal resistance to quinolones and facilitate the emergence of highly resistant strains through chromosomal mutations.

The mechanism of the qnr gene in causing resistance to ciprofloxacin can be explained as follows: the qnr protein (coded for the qnr gene) plays a role in protecting the target enzyme DNA gyrase (coded for the gyrA gene) and topoisomerase IV (encoded by the parC gene) in E. coli from ciprofloxacin inhibition. This causes ciprofloxacin to be unable to bind the DNA gyrase and topoisomerase IV enzymes, so that DNA replication continues.

Two positive isolates for oqxA gene were found, namely samples no. 23 and 30, based on DNA bands measuring 866 bp (figure 6). Meanwhile, isolates that were positive for the oqxA gene were also tested for the oqxB gene. The results of PCR screening on the oqxB gene in the 21 clinical isolates found 1 positive isolate for oqxB gene, namely sample number 30, based on DNA bands measuring 781 bp (figure 7), it can be said that the prevalence of the oqxB gene was 5.5% of the 21 clinical isolates.

The prevalence of the oqxAB gene in this study is almost the same as the results of research of Kim (2009), Ciesielczuk (2013 and Yuan (2012) [29] who said that the prevalence of gene oqxA and oqxB was 6.6% (9) of 136 clinical isolates of E. coli resistant to ciprofloxacin.

The presence of PMQR genes (qnr, oqxA, oqxB) located in the plasmid in E. coli bacteria is rare and only provides low level resistance, but they can transmit or spread this resistance trait horizontally among other E. coli bacteria and facilitate the selection of resistant mutants after exposure to ciprofloxacin. This is due to the nature of plasmids, which are circular, double-stranded DNA

molecules that exist outside of bacterial chromosomes (extra-chromosomal) and can replicate autonomously. According to Barreto et al. (2012) [30], plasmids play an important role in the transmission of resistance genes from Enterobacteriaceae spp. bacteria, such as ESBL and PMQR genes.

The resistance mechanism caused by these PMQR genes (qnr, oqxA and oqxB) is:

The qnr gene uses a target change mechanism. Namely by protecting the DNA gyrase and topoisomerase IV enzymes from fluoroquinolone inhibition.

The oqxAB gene via pump efflux activation. Namely, by increasing the efflux produced by the qepAB and oqxAB pumps, the result is a decrease in the accumulation of ciprofloxacin because it is pumped out of the cell.

The study's findings demonstrated that the gyrA and parC genes, which are located on chromosomes in the quinolone resistance determining region, were present in all E. coli MDR ciprofloxacin isolates. Amino acid substitution can lessen the binding of quinolones and fluoroquinolones on the surface of the DNA binding sites [30]. Enterobacteriaceae species typically need multiple mutations to develop clinically significant resistance; one or more QRDR mutations are nearly invariably present in germs that are resistant to quinolone [31].

Conclusion

All isolates (21 samples) had chromosomal genes (QRDR), namely gyr A and parC, while 3 isolates were positive for containing the plasmid gene (PMQR), namely isolates no.2E (positive for the qnr gene), no.23 (positive for oqxA) and no.30 (positive oqxA and oqxB). PMQR genes such as qnr, oqxA and oqxB in 21 E. coli MDR ciprofloxacin isolates were rarely found, but their presence could transmit or spread this resistance trait horizontally among other Enterobacteriaceae bacteria and enable the choice of resistant mutants following ciprofloxacin treatment. Normal genes seen in clinical isolates of E. coli MDR ciprofloxacin are QRDR genes such gyrA and parC.

Recommendations

Further research needs to be carried out to detect other PMQR genes in the MDR ciprofloxacin E. coli isolates (for example the qepA and aac[6']-lb-cr) which plays a role in the spread of E. coli resistance to ciprofloxacin. To detect mutations in the chromosomal DNA, it is essential to sequence the gyrA and parC genes in isolates that test positive for the qnr,oqxA,and oqxB genes. Close monitoring is essential when administering the antibiotic ciprofloxacin, which is prescribed for the treatment of infectious patients, particularly those with urinary tract infections, at the Azadi teaching hospital in Duhok, Duhok province, Iraq.

REFERENCES

[1] P. Pardeshi, "Prevalence of urinary tract infections and current scenario of antibiotic susceptibility pattern of bacteria causing UTI," Indian Journal of Microbiology Research, vol. 5, no. 3, pp. 334-338, Jul. - Sep. 2018.

[2] M. Shahzad, E. M. Khan, O. Q. Khan, U. B. Tariq, and A. Sabahat, "Urinary Tract Infections: Epidemiology, Mechanisms of Infection, and Treatment," Pakistan Journal of Medical & Health Sciences, vol. 16, no. 10, pp. 810, 2022.

[3] C. C. Y. Chan and I. A. Lewis, "Role of metabolism in uropathogenic Escherichia coli," Trends in Microbiology, vol. 30, no. 12, pp. 1174-1204, 2022.

[4] M. A. Alhadlaq, M. I. Mujallad, and S. M. I. Alajel, "Detection of Escherichia coli O157:H7 in imported meat products from Saudi Arabian ports in 2017," Sci Rep, vol. 13, pp. 4222, 2023.

[5] S. E. Stapleton, F. M. E. Wagenlehner, A. Mulgirigama, and M. Twynholm, "Escherichia coli Resistance to Fluoroquinolones in Community-Acquired Uncomplicated Urinary Tract Infection in Women: A Systematic Review," Antimicrob Agents Chemother., vol. 64, no. 10, p. e00862-20, Sep. 2020.

[6] R. Rosselli, O. Romoli, N. Vitulo, et al., "Direct 16S rRNA-seq from bacterial communities: a PCR-independent approach to simultaneously assess microbial diversity and functional activity potential of each taxon," Sci Rep, vol. 6, p. 32165, 2016.

[7] J. S. Johnson, D. J. Spakowicz, B. Y. Hong, et al., "Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis," Nat Commun, vol. 10, p. 5029, 2019.
[8] V. Tchesnokova, L. Larson, I. Basova, et al., "Increase in the community circulation of ciprofloxacin-resistant Escherichia coli despite reduction in antibiotic prescriptions," Commun Med, vol. 3, p. 110, 2023.
[9] Z. Neyestani, F. Khademi, R. Teimourpour, M. Amani, and M. Arzanlou, "Prevalence and mechanisms of ciprofloxacin resistance in Escherichia coli isolated from hospitalized patients, healthy carriers, and wastewaters in Iran," BMC Microbiol., vol. 23, no. 1, p. 191, Jul. 17, 2023.
[10] M. B. Amin, S. R. Saha, M. R. Islam, et al., "High prevalence of plasmid-mediated quinolone resistance (PMQR) among E. coli from aquatic environments in Bangladesh," PLoS One, vol. 16, no. 12, p. e0261970, Dec. 29, 2021.
[11] M. Balouiri, M. Sadiki, and S. K. Ibnsouda, "Methods for in vitro evaluating antimicrobial activity: A review," J Pharm Anal., vol. 6, no. 2, pp. 71-79, Apr. 2016.
[12] Clinical and Laboratory Standards Institute (CLSI), Performance Standards for Antimicrobial Susceptibility Testing, CLSI Approved Standard M100-S15, 2018.
[13] Lopez-Rincon, A. Tonda, L. Mendoza-Maldonado, et al., "Classification and specific primer design for accurate detection of SARS-CoV-2 using deep learning," Sci Rep, vol. 11, p. 947, 2011.
[14] D. Sune, H. Rydberg, Å. Nilsdotter Augustinsson, et al., "Optimization of 16S rRNA gene analysis for use in the diagnostic clinical microbiology service," J. Microbiol. Methods, vol. 170, p. 105854, 2020.
[15] R. Shi, R. S. Lewis, and D. R. Panthee, "Filter paper-based spin column method for cost-efficient DNA or RNA purification," PLoS One, vol. 13, no. 12, p. e0203011, Dec. 7, 2018.
[16] R. Srinivasan, U. Karaoz, M. Volegova, et al., "Use of 16S rRNA Gene for Identification of a Broad Range of Clinically Relevant Bacterial Pathogens," PLoS One, vol. 10, no. 2, p. e0117617, 2015.
[17] D. L. Church, L. Cerutti, A. Gürtler, et al., "Performance and Application of 16S rRNA Gene Cycle Sequencing for Routine Identification of Bacteria in the Clinical Microbiology Laboratory," Clin Microbiol Rev., vol. 33, no. 4, p. e00053-19, Sep. 9, 2020.
[18] D. J. Leininger, J. R. Roberson, and F. Elvinger, "Use of Eosin Methylene Blue Agar to Differentiate Escherichia Coli from Other Gram-Negative Mastitis Pathogens," J. Vet. Diagn. Invest., vol. 13, no. 3, pp. 273-275, 2001.
[19] K. Czajkowski, M. Broś-Konopielko, and J. Teliga-Czajkowska, "Urinary tract infection in women," Prz Menopauzalny, vol. 20, no. 1, pp. 40-47, Apr. 2021.
[20] R. Biswas, R. Rabbani, H. Ahmed, et al., "Antibiotic sensitivity pattern of urinary tract infection at a tertiary care hospital," Bangladesh Crit. Care J., vol. 2, no. 1, pp. 21-24, 2014.
[21] S. Carson, H. B. Miller, D. S. Witherow, et al., Molecular Biology Techniques, 4th ed., Academic Press, 2019, pp. 13-22, ISBN 9780128180242.
[22] S. Q. Ali, Z. A. Zehra, B. S. Naqvi, et al., "Resistance pattern of Ciprofloxacin against different pathogens," OMJ, vol. 25, no. 4, pp. 294-298, 2010.
[23] L. Schulz, R. Hoffman, J. Pothof, et al., "Top Ten Myths Regarding the Diagnosis and Treatment of Urinary Tract Infections," J. Emerg. Med., vol. 51, no. 1, pp. 25-30, 2016.
[24] S. A. Pereira, S. S. Andrade, J. Monteiro, et al., "Evaluation of the Susceptibility profiles, genetic similarity and presence of qnr gene in Escherichia coli resistant to ciprofloxacin isolated in Brazilian hospitals," Braz J Infect Dis, vol. 11, no. 1, pp. 40-43, 2007.
[25] G. M. Martín-Gutiérrez, J. M. Rodríguez-Martínez, Á. Pascual, et al., "Plasmidic qnr Genes Confer Clinical Resistance to Ciprofloxacin under Urinary Tract Physiological Conditions," Antimicrob Agents Chemother., vol. 61, no. 4, p. e02615-16, Mar. 24, 2017.
[26] C. H. Wang, C. C. Fang, S. S. Chen, et al., "Cranberry-containing products for prevention of urinary tract infections in susceptible populations," Arch. Intern. Med., vol. 172, no. 13, pp. 988-996, 2012.
[27] H. B. Kim, M. Wang, C. H. Park, et al., "oqxAB Encoding a Multidrug Efflux Pump in Human Clinical Isolates of Enterobacteriaceae," Antimicrob Agents Chemother., vol. 53, no. 8, pp. 3582-3584, 2009.

[28] H. Ciesielczuk, M. Hornsey, V. Choi, et al., "Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants," J. Med. Microbiol., vol. 62, pp. 1823-1827, 2013.
[29] J. Yuan, X. Xu, Q. Guo, et al., "Prevalence of the oqxAB gene complex in Klebsiella pneumonia and Escherichia coli clinical isolates," J. Antimicrob Chemother., vol. 67, pp. 1655-1659, 2012.
[30] S. C. Barreto, M. Uppalapati, and A. Ray, "Small Circular DNAs in Human Pathology," Malays J Med Sci., vol. 21, no. 3, pp. 4-18, May 2014.
[31] Shaheen, A. Tariq, M. Iqbal, et al., "Mutational Diversity in the Quinolone Resistance-Determining Regions of Type-II Topoisomerases of Salmonella Serovars," Antibiotics (Basel), vol. 10, no. 12, p. 1455, Nov. 26, 2021.