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PREVALENCE OF PLASMID MEDIATED QNRA, QNRB AND QNRS AMONG CLINICAL ESCHERICHIA COLI ISOLATED FROM URINARY TRACT INFECTIONS IN DUHOK, KURDISTAN REGION OF IRAQ

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ABSTRACT:

Excessive use of antibiotics (such as fluoroquinolone) and inadequate infection control practices have turned antibiotics resistance (AMR) into a global, public health peril. The current study emphasizes on the prevalence of qnrA, qnrB, and qnrS plasmid in fluroquinolone (FQ) resistant Escherichia coli (E. coli) isolates from urinary tract samples and their correlation with the burden of resistance in these isolates. In this research, several E. coli strains were isolated from outpatients having urinary tract infections (UTIs) from Duhok province, Kurdistan Region of Iraq. Biochemical and Molecular confirmations were performed to confirm the isolates. Antibiotics susceptibility test was performed to detect the isolates antibiogram. Furthermore, polymerase chain reaction (PCR) amplification was used to detect the qnrA, B and S genes. Bioinformatics tools were used to perform the analysis of the results and data visualization. Forty isolates (out of 55) have been confirmed as E. coli. Antibiograms revealed that 70%, 65% and 63% of the isolates were resistant to Ciprofloxacin, Enrofloxacin and Levofloxacin, respectively. While there was variation in resistance to other antibiotics tested. The FQ resistant genes were detected in 17 (42.5%) isolates, with some isolates carrying more than one gene. The variant qnrS was the most predominant, as it has been detected in 14 isolates. The increasing rates of multidrug resistance E. coli isolates from UTIs, including FQ antibiotics, emphasizes the importance of carefully monitoring their use for UTI treatment.

KEYWORDS: E. coli, qnrA, B and S, Fluroquinolone, UTIs, Duhok.

1. INTRODUCTION

Urinary tract infections (UTI) is one of the most common infections which are considered as a leading public health problem (Naqid et al., 2020 a). UTI refers to bacterial colonization of the urinary tract by different kind of pathogens, it affect wide range of people (Foxman, 2014; O'brien et al., 2017; Tamadonfar et al., 2020). In 2017, there were an estimate of 274 million new cases of UTIs globally (including upper and lower cases), combining both genders and all ages (James et al., 2018). Although both male and female can be infected, UTIs are traditionally thought of as a disease of females, as approximately 50% of women affected at least one time across their lifespan (Foxman, 2014).

Clinically, UTIs can be categorized into complicated and uncomplicated, based on the extent and severity of the infection. Even though many uncomplicated UTIs can resolve spontaneously and without antibiotic treatment, more complicated forms of UTI need antibiotics treatment. This is very crucial to prevent more serious consequences, and substantially to affect the life quality of affected individuals (Kostakioti et al., 2012; McLellan et al., 2016). There are numerous microorganisms that can cause UTIs including different kinds of bacteria, and most commonly by *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis, Proteus mirabilis*, and *Staphylococcus saprophyticus* (Foxman, 2014; Assafi et al., 2015; Flores-Mireles et al., 2015; Naqid et al., 2020 b; Hasan et al., 2022).

Although not all strains of *E. coli* can cause UTIs, however it represents approximately 80% for both uncomplicated and complicated infections (WHO, 2022). Uropathogenic *Escherichia coli* (UPEC) is one of the specific subset of extraintestinal pathogenic bacteria; that is the main cause of UTIs (Mobley et al., 2009; Nielubowicz et al., 2010). The World Health Organization report (WHO) indicated that *E. coli* is a very important indicator for antimicrobial resistance (AMR) (WHO, 2022).

Several studies emphasized that there are no optimal therapeutic antibiotic agents that can be used in UTIs treatment due to the prevalence of Multi-drug resistance (MDR) uropathogenes (Gupta et al., 2001; Foxman, 2014). This can lead to a more complicated problem with recurrent UTIs which might be life threatening in both gender (Al-Badr et al., 2013). Antibiotics such as Amoxicillin+ clavulanic acid, Nitrofurantoin, trimethoprim sulfamethoxazole, Ciprofloxacin and third generation cephalosporin such ceftriaxone or cefotaxime combined with gentamicin or amikacin are also recommended as therapeutic agents for different categories of UTIs by the WHO (WHO, 2022).

As to the treatment guidelines for UTIs, Ciprofloxacin is the choice of treatment for mild upper UTIs by the WHO (Zanichelli et al., 2023). This antibiotic is one of the fluoroquinolone groups which include many other therapeutic agents such as Levofloxacin, Ofloxacin and Enrofloxacin (Baggio et al., 2021). Mutations in the quinolone resistance-determining regions (QRDB) of the topoisomerase genes, the overexpression of efflux pumps, and plasmid-mediated resistance can all result in resistance to FQ antibiotics (Bush et al., 2020). Even though QRDR mutations are the primary source of FQ resistance, it is important to highlight that plasmid-mediated quinolone resistance (PMQR) has considerably grown recently. Additionally, earlier research has already demonstrated that PMQR may help to promote QRDR mutations (Piekarska et al., 2015). In 1998s, the quinolone resistance gene *qnr* was initially

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recognized. Then after, other *qnr* determinants have been discovered on plasmids or the bacterial chromosome (Martínez-Martínez et al., 1998).

Even though, the *qnr* genes offer minimal resistance to quinolones in *E. coli*, however, the multi-resistance attribute is important. Recently, studies on *E. coli* resistance to fluoroquinolones and the detection and expression of specific *qnrA*, *qnrB*, and *qnrS* genes have been investigated in many countries (Spellberg et al., 2015; Kuo et al., 2022). Because the current study was concerned with FQ resistance in human UTIs, the current investigation attempted to detect fluroquinolone resistant cases and monitored the *qnrA*, *qnrB*, and *qnrS* genes in *E. coli* using a molecular method. The aim of the current study was to know how prevalent these fluroquinolone resistance genes are and how they relate to other antibiotic resistance. To the best of my knowledge, this sort of study on plasmid mediated FQ resistance genes recovered from *E. coli* UTIs in Duhok, Kurdistan Region of Iraq, were not performed or published before.

2. MATERIALS AND METHODS

2.1 Collection and Storage of Isolates

The presumptive *E. coli* isolates were collected from some of the private laboratories in Duhok city. The source of the isolates was from outpatients which were not hospitalized due to UTIs. The patients were referred to the clinical lab due to having the symptoms of UTIs. The isolates were provided on MacConkey agar medium. The isolates were collected from September 2021 to March 2022.

The provided isolates were immediately sub-cultured on double strength nutrient broth (Neogen, USA) and incubated overnight at 37 °C, then used for subsequent storage. Hence, 400 µL of the overnight culture were added to 400 µL of 50% glycerol in a 2 mL screw top tube and gently mixed and stored in deepfreeze (Warner et al., 2018).

2.2 Isolates Characterization

The isolates were re-grown from frozen stocks for characterization. The collected isolates were characterized originally based on the colonies appearance on MacConkey agar and gram stain results. The characterization then confirmed using phenotypic and molecular methods. The phenotypic confirmation performed by oxidase and IMViC (Indole, Methyl red, Voges Proskauer and Citrate) tests (Johan et al., 2003). Based on the IMViC test results, further confirmation performed using *uidA* as *E. coli* specific gene (Bej et al., 1991).

2.3 Antimicrobial Susceptibility Test

Eleven antimicrobials from various antimicrobial classes were used in the test (Table 1). The Clinical & Laboratory Standards Institute (CLSI) disc diffusion method was used to conduct an antimicrobial susceptibility test (CLSI, 2013). In order to create the inoculum for the test, 2-4 colonies were taken from a Nutrient Agar (NA) (Neogen, USA) plate that had been cultured for 18 hours at 37°C and suspended in 3 mL of Mueller-Hinton broth (Neogen, USA). After that, the bacterial suspension was incubated for approximately 3-4 hours at 37°C. To achieve approximately 1 to 2 _ 10^8 CFU ml^{-1} of bacterial concentration which represent a 0.5 McFarland standard, the suspension was adjusted by adding a sterile distilled water.

Following dilution, 100 µL of the suspension was distributed with a sterile swab over the surface of petri dish containing approximately 25 mL of Mueller-Hinton agar (Oxoid, UK). The plates were allotted to dry at room temperature before locating the antimicrobial discs (Bioanalyse, Turkey) on the agar surface with sterile forceps. For each isolate, three Mueller-Hinton agar plates were used. Then, the Mueller-Hinton agar plates were incubated for 18 to 20 hours at 37 °C, and the findings recorded by measuring the diameter of the inhibition zone throughout the disc. The zone of inhibition was then identified using the usual CLSI measures (CLSI, 2021).

2.4 Molecular Characterization and Fluroquinolone Resistance Genes Detection

2.4.1 DNA extraction

DNA for each single isolate was extracted by separating one colony from an overnight culture on NA agar into 100 µL of sterile 1 × TE buffer (10 mM Tris-Cl, 1 mM EDTA buffer, pH 7.8) (Promega, USA). Boiling method for DNA extraction were used (Ghaffar et al., 2023). The suspension was heated in water bath (Stuart, UK) to 95◦C for 30 min to rupture bacterial cells and then centrifuged (Sigma, Germany) at 11 000 × *g* for 10 min. The crude DNA in the supernatants was transferred into sterile microcentrifuge tubes and stored at –20°C until use.

Table 1: Discs used in antibiogram assays, their symbols, the concentration of antibiotics present on each disc and antibiotics categories.

Antibiotics disc (abbreviation)	Content	Antibiotics class
Ciprofloxacin (CIP)	5 µg	Fluoroquinolone
Enrofloxacin (ENR)	5 µg	Fluoroquinolone
Levofloxacin (LEV)	5 µg	Fluoroquinolone
Cloxacillin (CX)	5 µg	Penicillin
Meropenem (MEM)	10 µg	Penems
Streptomycin (STR)	10 µg	Aminoglycosides
Gentamycin (CN)	30 µg	Aminoglycosides
Nitrofurantoin (F)	300 µg	Nitrofuran
Trimethoprim / Sulfamethoxazole (SXT)	1.25/ 23.75 µg	Folate pathway inhibitors
Tetracycline (TE)	30 µg	Tetracyclines
Chloramphenicol (C)	30 µg	Phenicols

2.4.2 Molecular confirmation of the *E. coli* isolates

All phenotypically confirmed *E. coli* isolates were screened for *uidA* gene for molecular confirmation as described in the study of Bej et al., (1991). Then, 10 µL of Ruby star PCR master mix (2X) (Jenna Bioscience, Germany), 6 µL of nuclease-free water, and 1 µL (10 µM) of each primer were added to a 20 µL reaction mixture along with 2 µL of DNA (50 ng/µL). The primers and

the expected PCR product size for the *uidA* gene are listed in Table 2.

The reactions were conducted using a programmable PCR-Thermal cycler (Applied Biosystem, Singapore). The reaction started with initial denaturation of the DNA at 94°C for 5 minutes, then followed by 35 cycles, each lasting 1 minute at 94°C, 1 minute at 58°C, and 1 minute at 72°C, with a final elongation cycle lasting 5 minutes at 72°C. The amplified PCR product for the gene was run at 80 V for 45 min, and each gel contained a 100 bp DNA size marker from Jena Bioscience in Germany. The agarose gel (2%) (Schuarlau-Spain) prepared with 1x TAE solution and stained with safe DNA coloring dye (GeNetBio, Korea).

2.4.3 PCR determination of the *qnr* genes:

PCR was used to screen all isolates that indicated resistance to one of the FQ antibiotics for *qnrA, qnrB,* and *qnrS* genes. In a 20 µL reaction mixture containing 10 µL of Ruby star PCR master mix (2X), 6 µL of DNase free water, and 1 µL of each primer, 2 µL of total DNA (50ng/L), the PCR reaction was employed. Table 2 shows the primers and predicted PCR product sizes. As a negative control, a PCR reaction mixture devoid of DNA was employed. The reactions were carried out using a programmed PCR-Thermal cycler under the parameters given in Table 3. The amplicon (6 µL) was electrophoresed at 85 V for 1 hour in a 1% w/v agarose gel containing a safe dye solution (0.4 µg /mL) in 1 xTAE. In each gel, a 100 bp DNA size ladder was applied.

Table 2: PCR primers used for molecular characterization of *E. coli* and detection of plasmid mediated fluoroquinolone resistance (*qnrA, qnrB and qnrS*).

Reference	Product size	Primer 5'-3'	Gene
Bej et al., 1991	147 bp	AAAACGGCAAGAAAAAGCAG	*uidA-F-**
		ACGCGTGGTTACAGTCTTGCG	*uidA-R-*
Badamchi et al., 2019	516 bp	ATTTCTCACGCCAGGATTTG	*qnrA-F-*
		GATCGGCAAAGGTTAGGTCA	*qnrA-R-*
Doma et al., 2020	526 bp	GTTGGCGAAAAAATTGACAGAA	*qnrB-F-*
		ACGACATTCGTCAACTGCAA	*qnrB-R-*
Doma et al., 2020	415 bp	ACGACATTCGTCAACTGCAA	*qnrS-F-*
		AATTGGCACCCTGTAGGC	*qnrS-R-*

* All oligonucleotides were synthesized by Macrogen, south Korea.

2.5 Bioinformatics and Statistical Analysis:

Various Bioinformatics programs and software packages were used for antibiogram and molecular data visualization, such as UPGMA clustering (http://genomes.urv. cat/ UPGMA), FigTree (http://tree.bio.ed.ac.uk/ software/ figtree/) and Microsoft Excel. GraphPad Prism 9.4.1 was used to analyze the data. Spearman's method was used for nonparametric correlation between the antibiotic's resistance number of each isolate, and plasmid FQ resistance genes. Statistical significance was established when $p < 0.05$.

2.6 Ethics Statement

There were no data collected regarding the gender, age or any risk factors related to the patients and none will be present in this research paper. The scientific committee of the Biology Department, College of Science, Duhok University, Kurdistan Region of Iraq, agreed to the research design and methodology. This has been further approved by the Collage council meeting (session no. 10, report no. 14).

Table 3: The parameters of PCR amplification need for *qnrA, qnrB,* and *qnrS* detection.

Genes	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	References
qnrA	95 °C 3 min One cycle	94°C 1 min	54 1min	72 1 min	72 5 min 1 cycle	Badamchi et al., 2019
		35 cycles				
qnrB	95 °C 3 min One cycle	94°C 1 min	56 30 sec	72 1 min	72 5 min 1 cycle	Doma et al., 2020
		30 cycles				
qnrS	95 °C 3 min One cycle	94°C 1 min	59 1min	72 1 min	72 5 min 1 cycle	Doma et al., 2020
		35 cycles				

3. RESULTS

Strains Confirmation Using Phenotypic and Molecular Methods:

Out of 55 presumptive *E. coli* isolates, 40 (72.7%) isolates confirmed using phenotypic and molecular methods.

Based on the phenotype confirmation, all the isolates that showed typical colony morphology on MacConkey agar, gram stain negative, oxidase negative, positive for indole and MR test, negative to VP and citrate production test, have been considered

as *E. coli* and further tested using molecular confirmation. Species specific gene (*uidA*) was detected in all the 40 isolates which phenotypically confirmed as *E. coli* (Figure 3).

3.1 Antibiogram Pattern of the Isolates:

All isolates were screened for sensitivity against 11 antibiotics by the disc diffusion method. Resistance to fluoroquinolones antibiotics was exhibited by 70%, 65% and 63% of the isolates to Ciprofloxacin, Enrofloxacin and Levofloxacin, respectively.

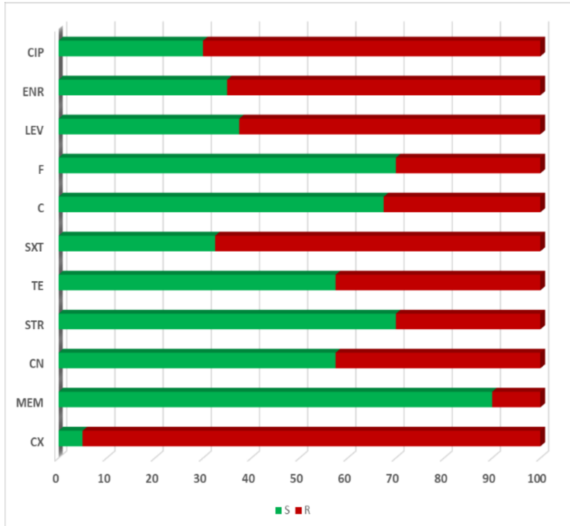

Figure 1. Percentages of resistances to 11 antibiotics for 40 UTIs *E. coli* isolates.
Green indicates sensitivity and Crimsion indicates resistance percentages. The percentage of isolates is depicted on the X-axis. Antibiotics abbreviation: CIP (Ciprofloxacin), ENR (Enrofloxacin), Lev (Levofloxacin), CX (Cloxacillin), MEM (Meropenem), STR (Streptomycin), CN (Gentamycin), F (Nitrofuranton), SXT (Trimethoprim/Sulfamethoxazole), TE (Tetracycline), C (Chloramphencol).

3.2 Isolates Clustering Using Tree Representation:

Unweighted pair group method with arithmetic mean (UPGMA) tree construction was very helpful in representing the isolates clustering based on their resistance/sensitive pattern

The less effective antibiotic was Cloxacillin to which 95% of isolates appeared to be resistant. Furthermore, resistance to sulfamethoxazole + trimethoprim was observed in 68% of the isolates. Resistance to Gentamycin and Streptomycin, representing the aminoglycoside class of antibiotics were 30% and 43 %, respectively. Resistance to Tetracycline and Nitrofurantoin was 30%. About 10% of the isolates were resistant to Meropenem; this percentage of resistance was the lowest among all tested classes of antibiotics (Figure 1)
(Figure 2). The tree represents 5 different clusters, C1 and C3 represent the strains that have moderate resistance toward the tested antibiotics. C2 (highlighted in red) was the largest and the most alarming cluster, representing isolates mostly resistance to 8 antibiotics such ECU11 and ECU32 (Table 4). There were 4 isolates showed resistance to Meropenem (the branches colored in red); ECU2, ECU24 located at the beginning of C2 (resistance to 6 and 9 antibiotics respectively) and ECU15 and ECU17 located at the end of C2. ECU15 and ECU17 represent the most resistance isolates as they showed to be resistant to 10 and 11 (all) antibiotics, respectively (Table 4). C4 and C5 (highlighted in blue) represent the most sensitive isolates (Figure 2).

3.3 The Prevalence of *qnr* Genes:

Molecular identification for the *qnrA, B and S* genes (Figure 3) revealed that 42.5% (17/40) of the isolates carrying at least one of the *qnr* genes. The least prevalence gene was *qnrA* as only one isolate (2.5%) was carrying this gene (Figure 4 and Table 4). Furthermore, *qnrB* detected in 10% (4 isolates) of the isolates, while the most prevalence type was *qnrS* as it was detected in 35% (14) isolates. Some isolates appeared to carry more than one of the *qnr* genes, such as ECU12 which was carrying both *qnrA* and *qnrS* genes, while another isolate (ECU2) was carrying combinations of *qnrB* and *qnrS* (Table 4). The correlation between the presence/absence of plasmid FQ resistance genes with the number of the antibiotics resistance in each isolate showed that the number of resistances increased by the presence of the plasmid FQ resistance genes (Figure 5). This might indicate that the co-carriages of plasmid FQ resistance genes with other resistance genes such as β-lactamase and Aminoglycosides resistance on the same plasmid.

Table 4: Antibiogram profile for the strains that show the carriages of the *qnrA, B and S* genes

Sample ID	Antibiotics resistance profile	*Qnr genes* profile
ECU2	CX, MEM, CN, TE, SXT, CIP	*qnrB, qnrS*
ECU3	CX, STR, TE, SXT, LEV, ENR, CIP	*qnrS*
ECU4	CX, CN, SXT, C, LEV, ENR, CIP	*qnrS*
ECU5	CX, CN, SXT, F, LEV, ENR, CIP	*qnrS*
ECU7	CX, CN, STR, TE, SXT, LEV, ENR, CIP	*qnrS*
ECU8	CX, CN, STR, SXT, C, LEV, ENR, CIP	*qnrB*
ECU9	CX, CN, TE, SXT, F, LEV, ENR, CIP	*qnrS*
ECU11	CX, STR, TE, SXT, C, LEV, ENR, CIP	*qnrS*
ECU12	CX, TE, SXT, C, LEV, ENR, CIP	*qnrA, qnrS*
ECU15	CX, MEM, CN, STR, TE, SXT, C, LEV, ENR, CIP	*qnrB*
ECU19	CX, CN, STR, S, C, LEV, ENR, CIP	*qnrS*
ECU21	CX, CN, STR, SXT, LEV, ENR, CIP	*qnrB*
ECU23	CX, CN, STR, S, SXT, LEV, ENR, CIP	*qnrS*
ECU24	CX, MEM, CN, STR, TE, SXT, LEV, ENR, CIP	*qnrS*
ECU28	CX, CN, S, SXT, LEV, ENR, CIP	*qnrS*
ECU37	CX, CN, TE, SXT, F, LEV, ENR, CIP	*qnrS*
ECU39	CX, STR, TE, SXT, C, LEV, ENR, CIP	*qnrS*

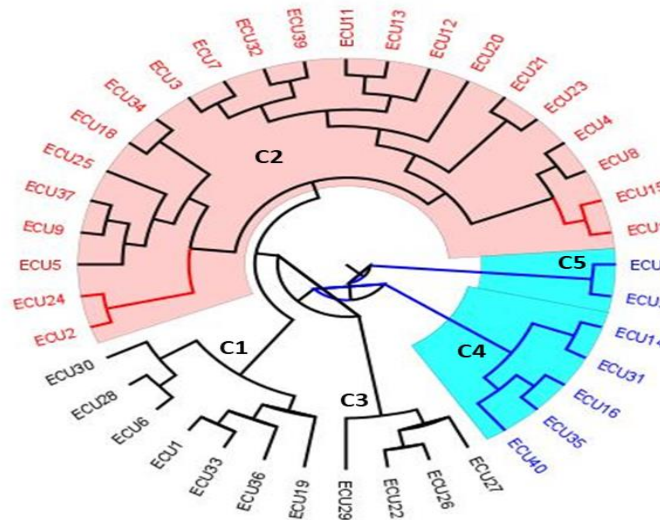

Figure 2: A tree displaying the relationships between several strains based on the phenotypes of their antibiotic sensitivity.

Tree tips (Isolate ID) are color-coded according to the range of resistance against different numbers of antibiotics. Red tips, represent high resistance isolates (at least resistance to 7-9 antibiotics), blue tips represent highly sensitive isolates. The isolates with red branches represent the Meropenem resistance isolates including the isolate EU17 (resistance to all the antibiotics).

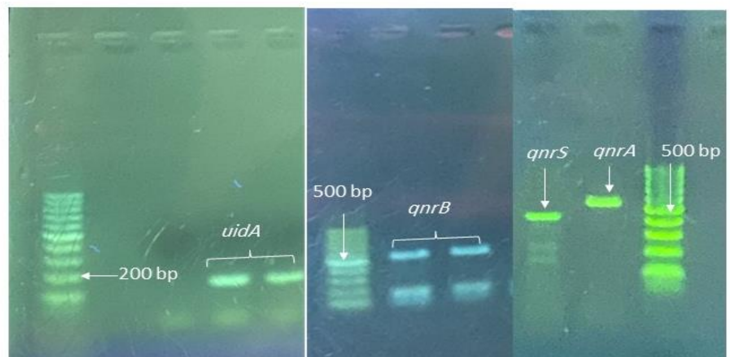

Figure 3: Representative of PCR product for *uidA, qnrA, qnrB* and *qnrS* for some of *E. coli* isolates.
The product size for *uidA* was 147 bp, for *qnrA* was 516, for *qnrB* was 526 bp and for *qnrS* was 415 bp.

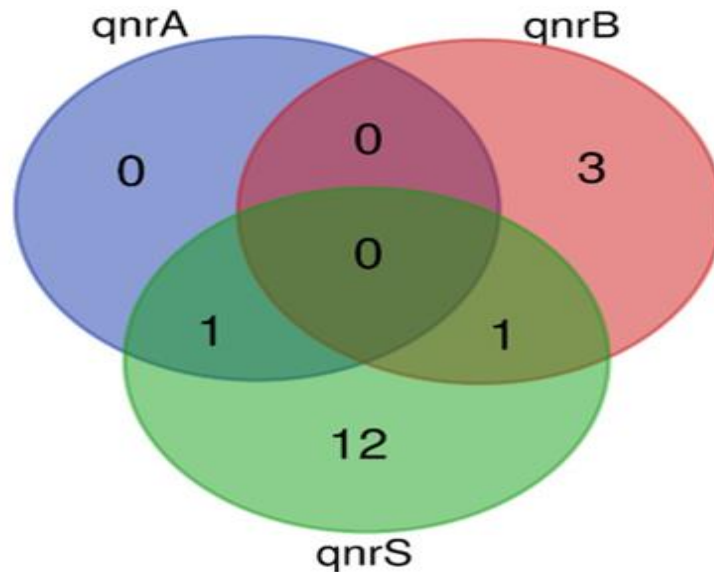

Figure 4: Illustration for the range of FQ resistance genes distribution in 40 UTIs *E. coli* isolates.
Venn diagram was produced online using (http: //bioinformatics.psb. ugent.be/webtools/Venn/)
(Accessed on 06/06/2023).

DISCUSSION

Widespread usage of fluoroquinolone antibiotics in human medicine is linked to an increase in the establishment of fluoroquinolone resistant bacteria (Correia et al., 2017). Here, a group of UTI isolates from some private labs in Duhok city were investigated for FQ resistance and their carriages of plasmid *qnr* genes. The inclusion of fluoroquinolone antibiotics such as Ciprofloxacin in treatment regimens throughout the 1980s was a significant advancement in medicine at that time. Unfortunately, resistance started to show up after just ten years of use, though considerably less frequently than it does now (Jacoby et al., 2008; Strahilevitz et al., 2009; Hooper et al., 2015). This phenomenon's growth over time may be related to the widespread detection of *qnr* genes.

In the current study, 70%, 65%, and 63% of the isolates displayed resistance to Ciprofloxacin, Enrofloxacin, and Levofloxacin, respectively. This was higher than the expected value especially for some studies conducted within the same region. One of these studies showed that the resistance to Ciprofloxacin, Levofloxacin and Norfloxacin within *E. coli* community isolated from UTIs in Azadi hospital in Duhok city were 55.7%, 52.4% and 59.1% respectively (Abdulrahman, 2018). Similarly , another study depicted that approximately 53% of *E. coli* isolates from UTIs in Duhok city were resistant to Ciprofloxacin and levofloxacin (Hussein et al., 2017). Furthermore, many other studies conducted in Kurdistan Region showed variation in the percentages of resistance against members of fluoroquinolones antibiotics from both gram positive and negative bacteria isolated from UTIs (Hasan et al., 2022; Hami et al., 2023). This increase in the trend of resistance for the FQ antibiotics might be due to the overuse and abuse of these antibiotics. It has been shown that there was a significant increase in Ciprofloxacin resistance between 2008 and 2014, going from 25% to more than 40% in Asia, according to a comprehensive review of *E. coli* resistance to FQ in uncomplicated UTIs in women (Stapleton et al., 2020).

The molecular identification for the *qnrA, qnrB and qnrS* genes revealed that 42.5% (19/40) of the isolates carrying at least one of the *qnr* genes. Here, we can observe that some of the isolates that were phenotypically resistance to the fluoroquinolone's antibiotics did not reveal the carriages of the FQ genes. This was expected as there are many others mechanisms for the FQ resistance, such as mutation in *gyrA* gene (Karczmarczyk et al., 2011). Furthermore, *qnr* genes produce roughly the same resistance to some of the FQ antibiotics due to single *gyrA* mutations.

The molecular identification of *qnr genes* revealed that *qnrS* (35% of the isolates) was more prevalent than the other gene types (*qnrA* and *qnrB*). This was consistent with the findings of several other investigations (FarajzadehSheikh et al., 2019; Doma et al., 2020; Fadhil Abdul-Husin et al., 2021) which indicated that *qnrS* was the most prevalent gene among the other plasmids expressing FQ resistance. However, in another study, such as the one conducted by Badamchi and his colleagues on *E. coli* isolates from Iranian UTIs (Badamchi et al., 2019) and the similar finding in Nigerian patients (Nsofor et al., 2021), *qnrB* was found to be the most prevalent variant. Furthermore, Düzgün and his co-workers found that *qnrA* was the most common type within *E. coli* community from UTIs in Turkish patients (Düzgün et al., 2019). This variation might be due to the differences in the empirical therapeutic regimes followed by each region, and it might be due to the variation in the strains subtype and clone.

monitoring and which is of great significance to human health.

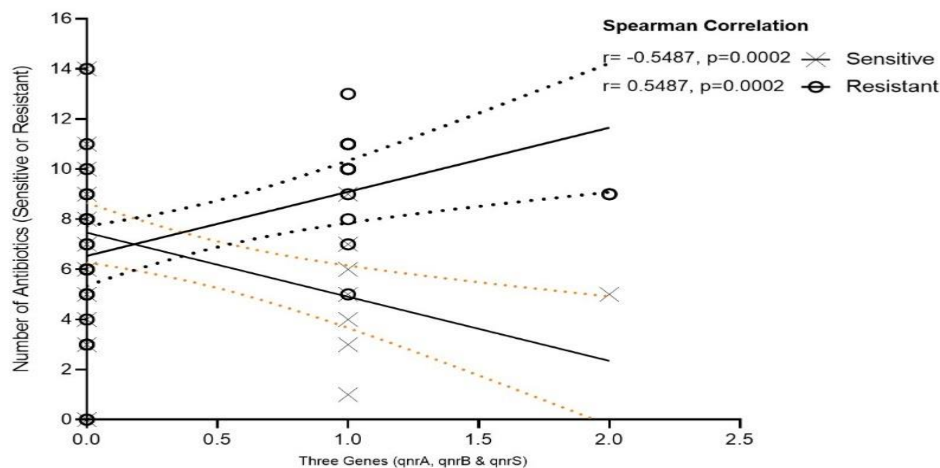

Figure 5: Correlation analysis between the number of antibiotics resistance for each *E. coli* isolates from UTIs and the presence of FQ resistance genes.

According to the correlation analysis, the existence of FQ resistance genes increases the number of antibiotics resistance. GraphPad Prism was used to perform Spearman's correlation, and significance was determined when p 0.05.

With regard to the trend of resistance towards other antibiotics, the isolates of the current study were highly resistant to the B-lactam class member as the percentages of the resistance to Cloxacillin were 95%. Furthermore, we can observe that most of the isolates that were resistant to Ciprofloxacin were also resistant to Cloxacillin, which might indicate the existence of the resistance genes for both antibiotics on one plasmid or any other mobile genetics elements and it might indicate the co-selection of those antibiotics. The correlation between FQ resistance and the β-lactam resistance in *E. coli* has been observed before in many studies, including strains from clinical samples (Sedighi et al., 2015; Salah et al., 2019; Ibrahim et al., 2023) and environmental samples (Literak et al., 2010; Cornejova et al., 2015; Bajaj et al., 2016) .

The most effective antibiotic against UTIs *E. coli* isolates in the current study was Meropenem, as only 4 isolates (10%) found to be resistant to this antibiotic. According to the WHO classification (AWaRe), this antibiotics is a within the Reserve group and should not be used unless the isolate is resistance to all the others antibiotics within access and watch class (Zanichelli et al., 2023). Although Nitrofurantoin and sulfamethoxazole + trimethoprim were within the empirical treatment choice suggested by the WHO for lower UTIs, 30% and 68% of the isolates found to be resistant to those antibiotics, respectively.

Using the tree representation has the advantage of showing major clusters and uncommon patterns like the resistance to Meropenem, which is present in only 4 isolates and whose clustering appeared clearly on the tree representation (Figure 2).

4. CONCLUSION

In this study, a high incidence of MDR *E. coli* isolates was found which about the half were carrying at least one of the FQ resistance genes. The most prevalent FQ resistance variance amongst the detected genes was *qnrS* followed by *qnrB* and *qnrA*. Antibiotic therapy is vital in UTIs treatment; however, it has become more difficult in recent years due to UTIs resistance to commonly used antibiotics. UTIs place a significant burden on healthcare resources, so research is required to improve patient care and advance treatment choices. *E. coli* as one of the most common etiologies of UTIs; that contribute to colonization, pathogenesis, and morbidity in UTIs patients, needs continuous monitoring and which is of great significance to human health. The knowledge of *E. coli* resistance rates in each specific area, as well as established standards for best initial antibiotic treatment, should be vital in the practical treatment of UTIs, remarkably for the regulated usage of FQ antibiotics.

5. ACKNOWLEDGEMENTS

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