Archives of Razi Institute, Vol. 76, No. 3 (2021) 561-573

DOI: 10.22092/ari.2021.355392.1679

Original Article

Plasmid-Mediated Mechanism of Quinolone Resistance on *E. coli* Isolates from Different Clinical Samples

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Received 24 July 2021; Accepted 9 August 2021
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Abstract

Quinolone antimicrobials are widely used in clinical medicine due to their wide spectrum with high tissue penetration and ease of use; but increasing resistance with clinical use appears to be common in some bacterial pathogens, including Escherichia coli *(E.coli*). The aim of this study was to investigate plasmid-mediated quinolone resistance determinants (PMQR) including, *qnr*A, *qnr*B, and *qnr*S as the emerging mechanisms of quinolone resistance of E.coli isolates from different clinical sites in Karbala province, Iraq. A total of 200 clinical samples were collected from patients suffering from infections such as UTI, gastro enteritis (diarrhea), vaginitis, and wound infections; 30 samples were diagnosed as E.coli clinical strain from both sexes and different ages after identification by biochemical test, VITEK-2 compact system, and by molecular method using 16Sr DNA marker. Antimicrobial susceptibility and minimal inhibition concentration (MIC) testing for nalidixic acid, norfloxacin, ciprofloxacin, levofloxacin, and gatifloxacin was performed using the broth microdilution method. All strains were screened for PMQR genes *qnr*A, *qnr*B, and *qnr*S by the PCR method after DNA extraction from tested clinical isolates of E.coli. The results showed that *E. coli* is largely isolated from vaginal (40%) and urine (32%) samples, followed by wound infections (24%) and stools (21%).The high occurrence rate of *E. coli*(33.33%) isolates was observed in participants aged 31-45 years, while a lower occurrence (10%)was recorded in a group of > 60-year-old female participants. Females have a notably increased frequency of *E.coli* compared to males, with the female to male ratio being 87%:13%. Molecular investigation showed the total percentage of *E.coli* isolates harboring *qnr* genes to be 21/30 (70%); this figure is composed of 14/30 isolates harboring *qnr* in combined or mixed form (46.66%) and 7/30 (23.33%) isolates harboring *qnr* in single form (3 isolates harboring *qnr*A alone, 1 isolate harboring *qnr*B alone, 3 isolates harboring *qnr*S alone).The prevalence rates of *qnr*A, *qnr*B, and *qnr*S were 40%, 43.33%, and 53.33%, respectively. The results also showed that among *E.coli* isolates encoding *qnr* genes A, B, and S, 24%, 12%, and 36% were resistant to nalidixic acid, respectively. Among those isolates carrying *qnrA*, *qnr*B, and *qnr*S genes, 15.8%, 5.3%, and 26.3%, respectively, were resistant to ciprofloxacin. Moreover, Norfloxacin resistance was seen in 20.0%, 5.0%, and 30.0% of *E.coli* isolates harboring *qnr* A, B, and S genes, respectively. Levofloxacin resistance was seen in 37.5%, 75.0%, and 37.5% of the isolates carrying the *qnrA*, *qnr*B, and *qnr*S genes, respectively. The lowest resistance rates of *qnr*A, B, and S-positive *E.coli* strains were against gatifloxacin (0,0, and 25%, respectively).A high prevalence of *qnr* genes enhances the increasing resistance rate of *E.coli* against the quinolone antibiotic under study.

Keywords: *qnr*, quinolone antibiotics, *E.coli*, plasmid mediated resistance

Mécanisme a Médiation Plasmidique de la Résistance aux Quinolones sur les Isolats d'*E. coli* Provenant de Différents Echantillons Cliniques

Résumé: Les quinolones antimicrobiennes sont largement utilisées en médecine clinique en raison de leur large spectre, de leur pénétration tissulaire élevée et de leur facilité d'utilisation; mais l'augmentation de la résistance

avec l'utilisation clinique semble être courante chez certains agents pathogènes bactériens, y compris Escherichia coli (*E. coli*). Le but de cette étude était d'étudier les déterminants de la résistance aux quinolones à médiation plasmidique (RQMP), y compris *qnr*A, *qnr*B et *qnr*S en tant que mécanismes émergents de la résistance aux quinolones des isolats d'*E. coli* provenant de différents sites cliniques dans la province de Karbala, en Irak. Au total, 200 échantillons cliniques ont été collectés auprès de patients souffrant d'infections telles que les infections urinaires, la gastro-entérite (diarrhée), la vaginite et les infections des plaies; 30 échantillons ont été diagnostiqués comme étant une souche clinique d'*E. coli* des deux sexes et d'âges différents après identification par test biochimique, système compact VITEK-2 et par méthode moléculaire utilisant le marqueur ADN 16Sr. Les tests de sensibilité aux antimicrobiens et de concentration minimale d'inhibition (CMI) pour l'acide nalidixique, la norfloxacine, la ciprofloxacine, la lévofloxacine et la gatifloxacine ont été effectués à l'aide de la méthode de microdilution en bouillon. Toutes les souches ont été criblées pour les gènes PMQR *qnr*A, *qnr*B et *qnr*S par la méthode RCP après extraction de l'ADN à partir d'isolats cliniques testés d'*E. coli.* Les résultats ont montré qu'*E. coli* est largement isolé des échantillons vaginaux (40%) et d'urine (32%), suivi des infections des plaies (24%) et des selles (21%). Le taux d'occurrence élevé des isolats d'*E. coli* (33.33%) a été observé chez les participants âgés de 31 à 45 ans, tandis qu'un taux d'occurrence plus faible (10%) a été enregistré dans un groupe de participantes > 60 ans. Les femelles ont une fréquence considérablement plus élevée d'*E. coli* par rapport aux mâles, le ratio femelle/mâle étant de 87%: 13%. L'enquête moléculaire a montré que le pourcentage total d'isolats d'*E. coli* contenant des gènes *qnr* était de 21/30 (70%); ce chiffre est composé de 14/30 isolats hébergeant *qnr* sous forme combinée ou mixte (46.66%) et 7/30 (23.33%) isolats hébergeant *qnr* sous forme unique (3 isolats hébergeant *qnr*A seul, 1 isolat hébergeant *qnr*B seul, 3 isolats hébergeant *qnr*S seul). Les taux de prévalence de *qnr*A, *qnr*B et *qnr*S étaient de 40%, 43.33% et 53.33%, respectivement. Les résultats ont également montré que parmi les isolats d'*E. coli* codant pour les gènes *qnr* A, B et S, 24%, 12% et 36% étaient respectivement résistants à l'acide nalidixique. Parmi les isolats portant les gènes *qnr*A, *qnr*B et *qnr*S, 15.8%, 5.3% et 26.3%, respectivement, étaient résistants à la ciprofloxacine. De plus, une résistance à la norfloxacine a été observée dans 20.0%, 5.0% et 30.0% des isolats d'*E. coli* hébergeant les gènes *qnr* A, B et S, respectivement. Une résistance à la lévofloxacine a été observée dans 37.5%, 75.0% et 37.5% des isolats portant les gènes *qnr*A, *qnr*B et *qnr*S, respectivement. Les taux de résistance les plus faibles des souches d'*E. coli qnr*A, B et S-positives étaient contre la gatifloxacine (0, 0, et 25%, respectivement). Une forte prévalence des gènes *qnr* augmente le taux de résistance croissant d'*E. coli* contre l'antibiotique quinolone à l'étude.

Mots-clés: *qnr*, antibiotiques quinolones, *E. coli*, résistance à médiation plasmidique

1. Introduction

Escherichia coli (E. coli) is a bacterium in which antimicrobial resistance is dramatically increased due to the increased use of antibiotics which has been recognized as a major factor driving E. coli resistance over recent decades (1). Fluoroquinolones (FQs) are broad spectrum antibiotics widely used in the treatment of infections caused by E. coli that have high tissue penetration and are easy to use (2). Quinolone resistance most often develops in bacteria as a result of spontaneous chromosomal mutations in the quinolone resistance determining region (QRDR) of the genes encoding DNA gyrase or topoisomerase IV (3, 4) or by point mutations in the DNA gyrase and by plasmid-mediated transfer through the acquisition of plasmid-mediated quinolone resistance (PMQR) determinants, including *qnr*S, *qnr*A, *qnr*B (3, 5-7). Additionally, PMQR determinants have been shown to coexist with resistance genes, causing resistance toward other antimicrobials, and thus enabling co-selection (8, 9). Until now, *qnr* genes have been widely detected in different parts of the world. Data on these genes, however, is not available for Karbala province, Iraq. In this study, three *qnr* groups were detected and are described.

2. Material and Methods

2.1. Specimens

For this study, 200 samples were collected from patients of both genders and different age groups

(ranging from 5 to 66 years) who were suffering from urinary tract infections, vaginitis, diarrhea, and wounds, who referred to two main hospitals in the Karbala governorate, Iraq, at Al-Husseini General Teaching Hospital for Maternity and Pediatrics during the period from February to June 2020. Strains were identified by biochemical test, VITEK-2 compact system, and the molecular method using 16Sr DNA markers. Each strain exhibits either susceptible, reduced susceptible (intermediate), or resistant fluoroquinolone phenotypes based on the routine diagnostic procedure carried out by disk diffusion method. The Kirby-Bauer method is a standardized system for this test that takes all variables into consideration (10). The quinolone antibiotics used in the current study are nalidixic acid 30 mg/µl, ciprofloxacin 5mg/µl, norfloxacin 10 mg/µl, levofloxacin 5 mg/µl, and gatifloxacin 5 mg/µl.

2.2. Determination of MIC and Statistical Analysis

The E-test was used to determine the MICs of antibiotics. A nonporous plastic strip was covered with the preformed exponential gradient of an antimicrobial along 60 mm of length.The gradient of the agent covered concentration ranges of 0.002 to 32 mg/L, 0.016 to 256 mg/L, or 0.064 to 1024 mg/L, depending on the agent. This range corresponds to 15 two-fold dilutions in (11) a conventional MIC method. The results were analyzed according to the Clinical and Laboratory Standards Institute (12).

2.3. PMQR Gene Detection

DNA was extracted from clinical isolates. One colony of each isolate was cultured and inoculated into 5 ml of BHI (Brain Heart Infusion) and grown overnight at 37 ºC. From these isolate cultures, DNA was purified from bacterial cells using a genomic DNA kit supplied by the manufacturing company (Geneaid, UK). The extracted DNA was used as templates for all PCR experiments. The DNA concentration was measured by a nanodrop machine at 260/280nm; it gave 1.8 purified DNA (13). The upstream and downstream primers were prepared according to the manufacturing company instructions (Bioneer, Korea) and stored at-20 ºC. Polymerase Chain Reaction was performed in a final volume of 25 µl according to the manufacturer's instructions (Bioneer, Korea) (Table 1). Successful PCR amplification was confirmed by agarose gel electrophoresis (14).

Table 1. Primers sequences and PCR condition

Primer name	Primer sequence (5´-3´)	Size product	Condition	Reference
16SrDNA	F:5'-AGAGTTTGATYMTGGCTCAG R: 5' CTACGGCTACCTTGTTACG	1500	94 ºC 5min 1x 94 ºC 1 min 54 ºC 1min 35x 72 ºC 7 min	(15)
qnrA	F: 5-ATTTCTCACGCCAGGATTTG-3 R:5-GATCGGCAAAGGTTAGGTCA-3	516	95ºC 3min 1x 95ºC 30sec 51.2ºC 1min 35x 72ºC 2min 72ºC 5min 1x	(16)
qnrB	F: 5-GATCGTGAAAGCCAGAAAGG-3 R: 5-ACGATGCCTGGTAGTTGTCC-3	469bp	94ºC 5min 1x 94ºC 45sec 53ºC 45 sec 32x 72ºC 1min 72ºC 5min 1x	
qnrS	F: 5-ACGACATTCGTCAACTGCAA-3 R: 5-TAAATTGGCACCCTGTAGGC-3	417bp	94ºC 5min 1x	

3. Results

In the present study, a total of 200 clinical samples were tested; 30 isolates were recovered as *E. coli*. The internally transcribed 16S–23Sr DNA spacer (ITS1) regions of *E.coli* were analyzed after amplifying by PCR, and the product was subjected to gradient analysis, which allowed the identification of the bacteria. The results revealed that all 30 samples of *E. coli* isolated from different sources gave positive results (100%) containing 16 Sr DNA gene sequences, as shown in Figure 1.

From the 30 isolates recovered, 13 (32%) were obtained from urine samples, 4 (21%) from stool samples, 8 (40%) from vaginal swabs, and 5 (24%) from wound swabs, as shown in Table 2.

3.1. E.coli Resistance to the Studied Quinolone Antibiotics and its Relationship with *qnr* Genes

Eleven isolates were positive for the *qnrA* gene (40%) (Figure 2), 13 isolates were positive for the *qnrB* gene (43.33%) (Figure 3), and 16 bacterial isolates were *qnr*S positive (53.33%) (Figure 4). The antibiotic resistance of different source of *E. Coli* is shown in Table 3. The prevalencese of *E. Coli* in different age groups are presented in Table 4. The distribution of *qnr* genes prevalence in Clinical *E.coli* isolates are shown in Figure 5.

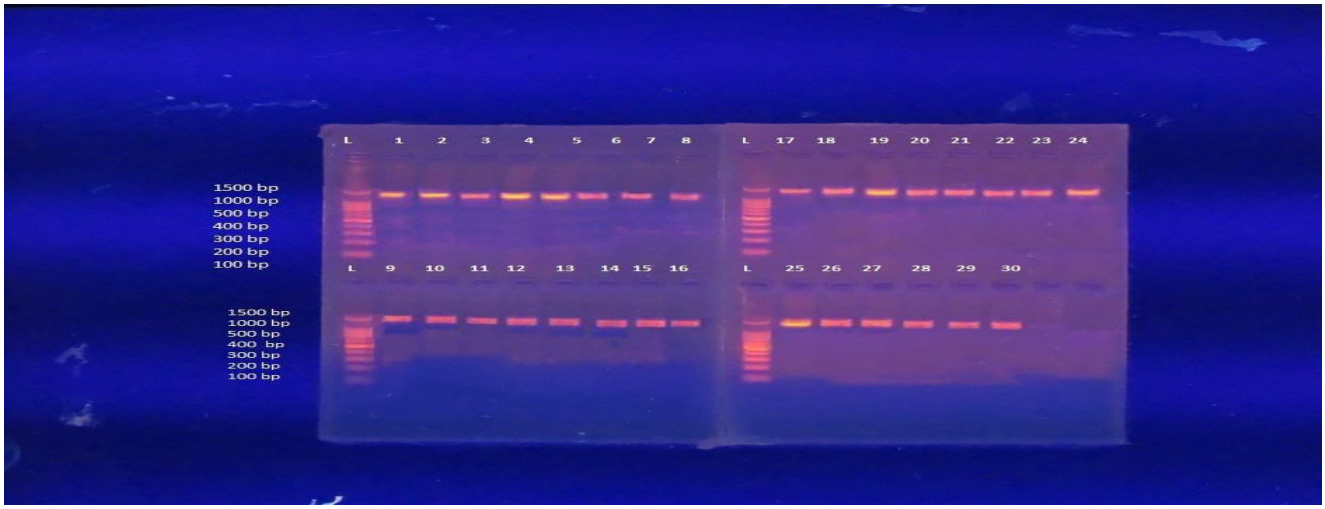

Figure 1. Gel electrophoresis of PCR product (1500bp), for Escherichia coli 2% agarose gel at 5 volt /cm for 2 hours. Lanes 1-30: PCR product positive for 16S rDNA genes

Table 2. Numbers and Percentages of *E.coli* Isolates from Different Sources

Source of the sample	Total no. of samples	No. of E.coli isolates	Percentage (%)
Urine	81	13	**32%**
Stool	38	4	**21%**
Vaginal swabs	40	8	**40%**
Wound swabs	41	5	**24%**
Total	200	30	**15%**

Table 3. Antibiotic resistance of different sources of *E.coli* isolates

Antibiotics	Rate of Resistance (%)		Rate of intermediate		Rate of sensitive	
Norfloxacin	19/30	63.33%	5	16.66%	6	20%
Gatifloxacin	4/30	13.33%	6	20.00 %	20	66.66%
Moxifloxacin	0	0.0%	0	0.0%	0	0.0%
Ciprofloxacin	20/30	66.66%	2	6.66%	8	26.66%
Nalidixic acid	25/30	86.36%	0	0.00%	5	16.66%
Levofloxacin	8/30	27.27%	10	33.33 %	12	40%

Table 4. Frequency of *E.coli* isolates in different age groups

Age groups (Years)		No. of isolates		Patients	
		Female	Male	No.	%
1.	1-15	3 (10.00 %)	0(0.00%)	4	13.33
2.	16-30	6(20.00%)	2(6.67%)	8	26.66
3.	31-45	8(26.67%)	2(6.67%)	10	33.33
4.	46-60	4(13.13%)	1(3.33%)	5	16.66
5.	> 60	2(6.67%)	1(3.33%)	3	10
Total		25 (83.33%)	5 (16.67)	30	100%

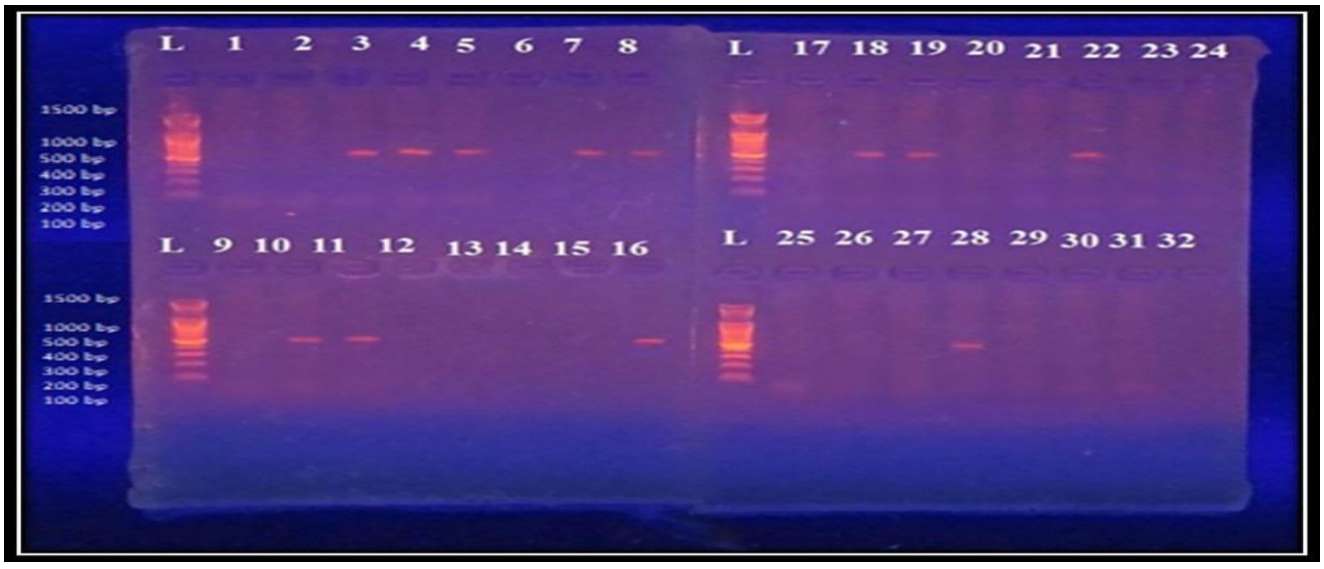

Figure 2. 1% L1: 15000 bp DNA marker; lanes (3, 5, 7, 8, 10, 11, 16, 18, 19, 22, 28) were positive for *qnr* A. The size of the product is 516 bp.

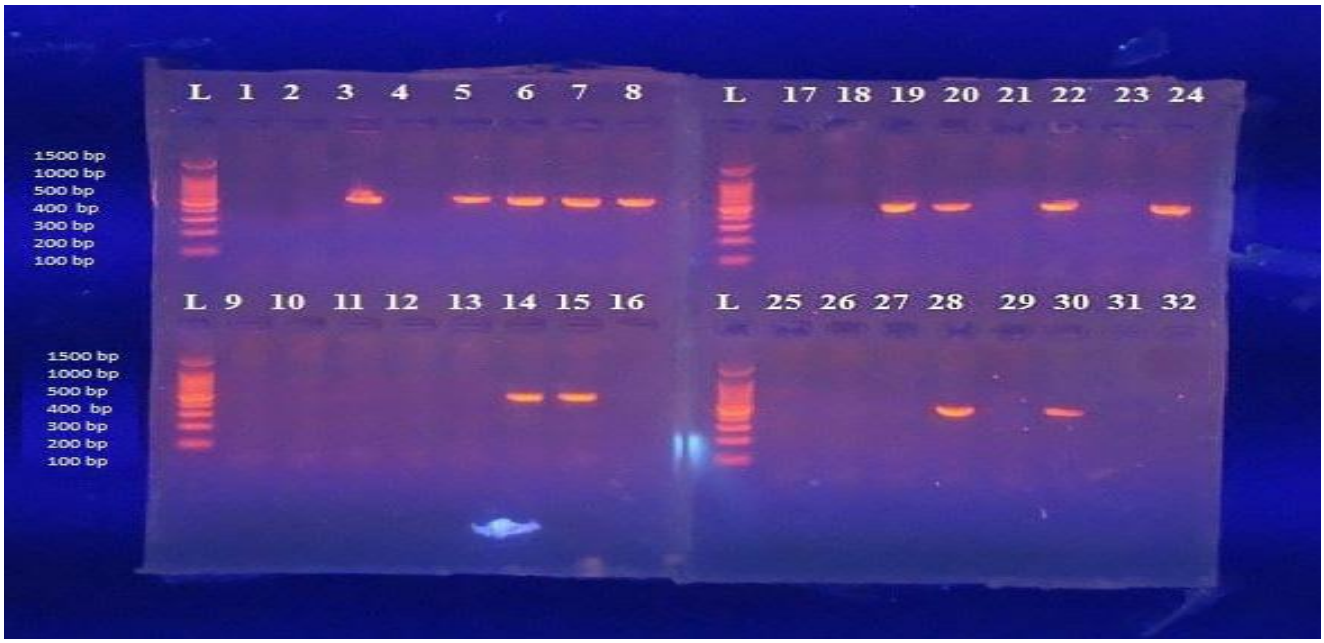

Figure 3. *Qnr*B: lanes (3,5,6,7,8,14,15,19,20,22,24,28,30) were positive for *qnrB*. The size of the product is 469 bp**.**

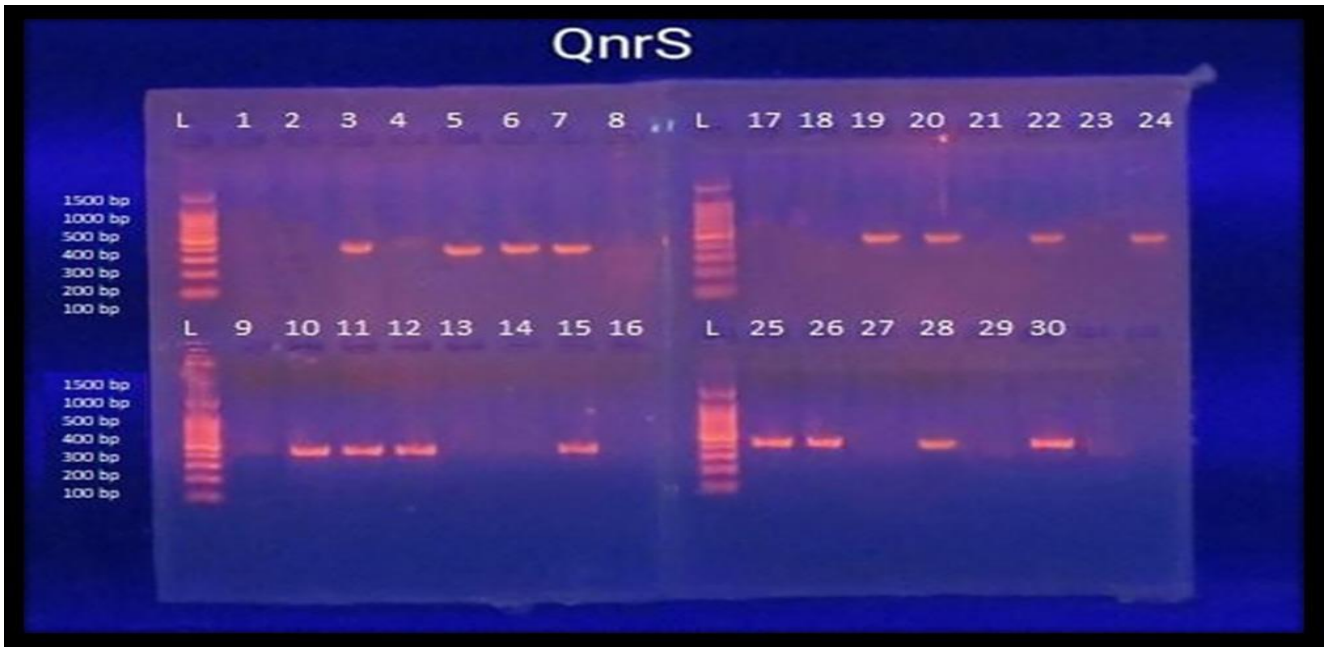

Figure 4. Lanes (3,5,6, 7, 10,11, 12, 19, 20, 22, 24,25,26,28,30) were positive for *qnrS*. The size of the product is 417 bp.

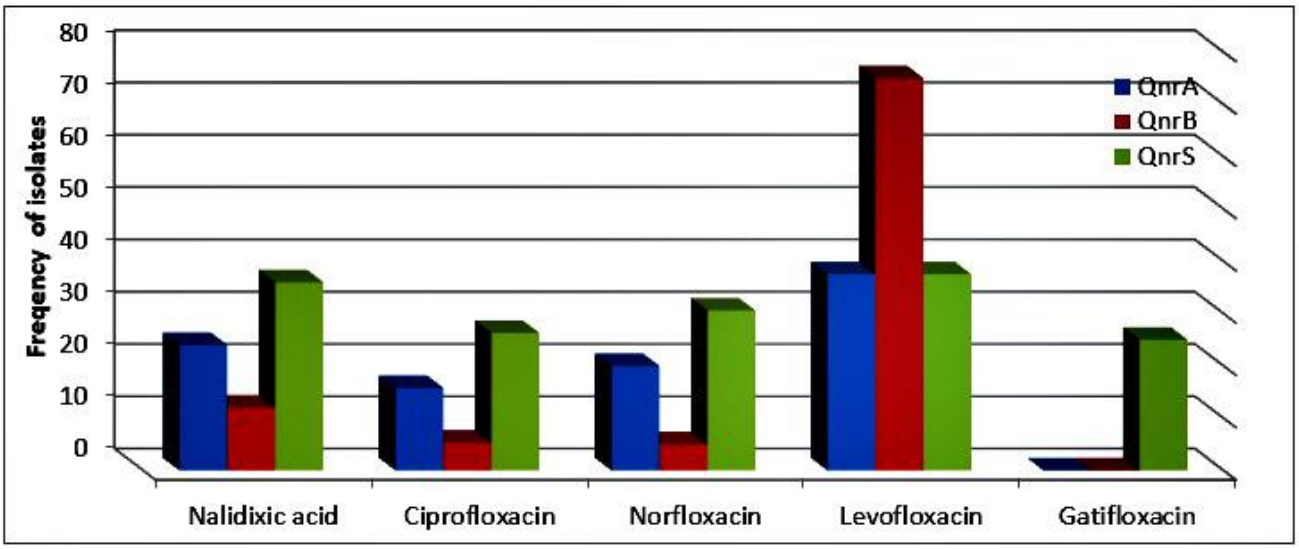

Figure 5. Distribution of *qnr* gene prevalence in clinical *E.coli* isolates.

4. Discussion

From 30 isolates, 13 (32%) were recovered from urine samples, 4 (21%) from stool samples, 8 (40%) from vaginal swabs, and 5 (24%) from wound swabs, as shown in Table 2. The results are close to those obtained by AL-saadi (17) who found that the prevalence of *E.coli* was approximately (46%) in the vagina, (33%) in urine, and (30%) in wounds. Nevertheless, the data recorded in the present study showed a decrease compared to that mentioned by Russo and Johnson (18), who found *E. coli* to be present in individuals at rates of (11%), (8%), (5%), and (4%) in urine, stool, vagina, and wound, respectively. In the present study, 32% of *E.coli* were isolated from urine. When compared to other studies, this result may correlate with those of Hamza (19), who found the prevalence of *E.coli* to be 36.84% in urine samples and the prevalence of *E.coli* to be 38% in urine samples. However, the rate is lower than those obtained in some local studies. For example, Brusch (20) found that 7.5% of UTIs were caused by *E. coli.* Parvin, Rahman (21) found that UTIs due to *E.coli* are quite common in patients who have undergone instrumentation or catheterization of the urinary tract, and 80% of subjects had *E. coli* among Gram-negative pathogens as a cause of catheter-associated UTIs. The current study revealed that the percentage of *E. coli* isolated from stool samples (21%) was lower from that found by Otaiwi, Tarrad (22). *E. coli* isolated from patients with diarrheal stool represented about 12/46(26.1%), a lower percentage than that obtained by Aljanaby and Alfaham (23) who reported the prevalence *E.coli* to be (36%) in children with heavy diarrheal stool in Iraq. This difference may be due to different criteria for the collection of samples. The results concerning *E. coli* isolated from vaginal swab samples (40%) are in agreement with those obtained by Hassan, Tantawy (24), who reported that *E.coli* prevalence in *Kirkuk* province as a causative agent of vaginitis was 50% and by Al-Mayahie (25) in Wasit province, who indicated that *E.coli* caused (45%) of vaginitis cases in women. Finally *E. coli* isolated from wound swab samples (24%) were in agreement with a study done by Ali (26) in Iraq, who revealed that *E.coli* was found in 30% of collected clinical samples. The

results are also compatible with those of Hassan Abdulqader and Towfeeq Saadi (27), who reported a prevalence rate of 29%. Adhikari, Basnyat (28) reported the prevalence rate to be about 24.2%. The isolation rate of *E.coli* from urine, vaginal swabs, and wounds depends on several factors, such as the virulence of isolates, health status of patients, and effects of environmental conditions. Moreover, Gessese, Damessa (29) reported that UTIs caused by *E. coli* affect both sexes and all ages groups across the life span, but women are more susceptible than men due to their shorter urethra, pregnancy, easy contamination of the urinary tract with fecal flora, and anatomical malformations of the urinary tract. The studied isolates revealed a high level of resistance of *E. coli* clinical isolates to most antibiotics (Table 2). The bacterial isolates showed resistance to first generation (nalidixic acid, 86.36%), second generation (ciprofloxacin, 66.66%; norfloxacin, 63.33%), third generation (levofloxacin, 27.27%), and fourth generation (gatifloxacin, 13.33%) antibiotics.

4.1. Antibiotic Resistance of *E.coli* Isolates from Different Sources

The current results were also in agreement with AL-saadi (17), (30) in Iraq, who showed that resistance to ciprofloxacin and levofloxacin ranged from 50% to 70% and was>70% to nalidixic acid. However in Pakistan, Ali, Rafaque (31) reported resistance rates against ciprofloxacin, levofloxacin, and norfloxacin of 60%, 58%, and 57%, respectively. Resistance rates in the present study were higher than those in Nigeria recorded by Ekwealor, Ugwu (32),who reported a resistance rate against ciprofloxacin of27%. The increased resistance rate among isolates was mostly due to overuse, disuse of medical prescription with the empirical therapy that increased antibiotic pressure and increased probability of resistance transfer, such as plasmid-mediated antibiotic resistance that was found to be common in *E. coli* isolates.

E. coli are highly isolated from vaginal (40%) and urine (32%) samples, followed by wound infection (24%) and stool (21%) samples.

4.2. Frequency of *E.coli* Isolates in Different Age Groups

Table 4 shows that the highest occurrence rate of *E. coli* isolates was seen in patients aged 31-45 years (33.33%), while the lowest occurrence rate was seen in patients aged > 60 years (10%), in females. These results agreed with those of Tabasi, Asadi Karam (33), who found that the incidence of *E. coli* in female patients in the age group of 31-40 years (45.2%) was highest, while the age group 46-60 years had the lowest incidence of *E.coli* (16.66%). Moreover, the highest incidence of *E. coli* in male patients was seen in the age group 31-45 years (6.67%), while the lowest incidence was seen in the age group of 1-15(0%). The high occurrence of *E.coli* in both males and females in the age range of 31-40 years may be attributable to sexual activity in both genders at this age, pregnancy in females as well as a prior UTI, vaginal infection, diabetes, obesity, and genetic susceptibility (34). These results differ from the results of Tabasi, Asadi Karam (33), who reported that the highest prevalence of *E.coli* was observed in the age group of 51-60 years (46.9%), and the lowest was seen in the age group of 31-40 years (12.5%). Moreover, the results of Dawood (35) revealed that the highest prevalence of *E.coli* was observed in the age group of >70 years (46%), and the lowest was seen in the age group of 14-40 years (8%) among male patients. Females had a notably increased frequency of *E. coli* versus males, which could be explained by several clinical factors, including anatomic differences, hormonal effects, and behavioral patterns (33). This result is in agreement with those of Prakapaite, Saab (36) who reported the female-to-male ratio as being 87%:13%.

4.3. *E.coli* Resistance to the Studied Quinolone Antibiotics and Its Relationship with *qnr* Genes

The total percentage of *E. coli* isolates harboring *qnr* genes was 70% (21/30); this was composed of 14/30 isolates harboring *qnr* in combined or mixed form (46.66%) and 7/30 (23.33%) isolates harboring *qnr* in single form (3 isolates harboring *qnr*A alone, 1 isolate harboring *qnr*B alone, 3 isolates harboring *qnr*S alone).

There seems to be a high frequency of transconjugation or transformation mechanisms of the *qnr* genes. Another study revealed that 59.88% of the *E.coli* isolates from clinical samples harbored the *qnr* genes (37); however, Nsofor, Tattfeng (38) reported that 93.3% of *E.coli* isolates from different sources harbored at least one *qnr* genes. Mokhtari-Farsani, Doosti (39) reported the percentage as 80.34%.This difference may be due to the selected *E. coli* population and changes in environmental conditions and geographical areas.

qnrS was the most prevalent, followed by *qnrB* and finally *qnrA*. The present findings were in agreement with a previous European survey that reported *qnrS* to be more frequently detected than other *qnr* genes in clinical *Enterobacteriaceae* isolates (40). Although the results of this study were found to be lower to those obtained by Mokhtari-Farsani, Doosti (39) who reported 19.15% of isolates were carrying *qnr*A, whereas *qnr*B, *qnr*S were detected in 88.30% and 78.72% of isolates, respectively. Data obtained in the present study revealed a higher percentage than those of Al-Hasnawy, Jodi (41) in Iraq, who reported the percentage of *qnr* genes to be 31.8%, 56.5%, and 28.9% for *qnr*A, *qnr*B, and *qnr*S, respectively. This difference may be due to the selected *E. coli* population and changes in environmental conditions and geographical areas. *E. coli* tested from different clinical samples were found to be harboring at least one of the *qnr* genes; 47.74% harbored *qnrB*, 47.10% harbored *qnrS*, and 12.58% harbored *qnrA* (42).

The prevalence of PMQR determinant has been investigated in many countries. Tarchouna, Ferjani (43) found that 32% of *E. coli* strains isolated from different clinical samples in several Arab countries, such as Tunisia, were positive for the presence of *qnr* genes, yet only 12.5% of them carried the *qnrB* gene.

4.4. Distribution of *qnr* Gene Prevalence in Clinical *E.coli* Isolates According to Resistance Pattern of Quinolone Antibiotics Under Study

Among nalidixic acid-resistant *E.coli* isolates, 25 clinical isolates encoded the studied *qnr* genes (A,B, and S).In the current study, 24%,12%, and 36%,respectively, were resistant to nalidixic acid. These results are compatible with those obtained by Taha, Omar (44) in Iraq, who reported that the frequency of *qnr*A in NA-resistant *E.coli* was 22.5%; to the results of Al-Hasnawy, Jodi (41), who found the frequency of *qnr*B in NA-resistant *E.coli* to be 14.0%; and to the results of Malekzadegan, Rastegar (45) who found the frequency of *qnr*S in NA-resistant *E.coli* to be 34.7%. Ciprofloxacin- resistant isolates that carried the *qnrA*, *qnr*B, and *qnr*S genes were 15.8%, 5.3%, and 26.3%, respectively. These isolates exhibited a high level of ciprofloxacin resistance (average MIC>128 µg/mL). Doma, Popescu (46) reported that the *qnrA* gene was observed only in 12.0% of isolates of CFX–resistance-*E.coli* obtained from human subjects. Sedighi, Arabestani (47) reported the prevalence of CFX-resistant isolates that carried *qnrB* was 6.7%, which is also compatible with the present results, while Firoozeh, Zibaei (48) reported that 14% of CFX–resistant isolates harbored *qnr*B. The prevalence rate of the *qnr*S gene was higher than the other tested *qnr* types (*qnr*A, *qnr*B). This result is compatible with FarajzadehSheikh, Veisi (37), (49), who reported that *qnr* genes were harbored by 32.5% of ciprofloxacin-resistant *E.coli* isolates from blood and stool samples. This means that the *qnr*S gene seems to play a more significant role than *qnr*A or *qnr*B with regard to quinolone resistance, as 62.5% (10/16) of the isolates harboring the *qnr*S gene were resistant to all quinolones.

The combination of *qnr*S and *qnr*B detected in five strains increased the level of resistance to quinolone-containing antibiotics to nearly 100% (4 of 5), suggesting that the synergistic effects resulting from this combination in a strain may increase their defensive positions. However, some other factors such as the over-expression of the efflux pump or reduced intracellular concentration and mutations (DNA gyrase

enzymes) could also be equally effective in this regard. The percentage of norfloxacin–resistant *E.coli* isolates harboring *qnr*A, *qnr*B, and *qnr*S in the present study were 20.0%, 5.0%, and 30.0%, respectively. All isolates showed higher MIC values than ciprofloxacin (MIC>64 μg/mL). Other studies have reported that resistance to norfloxacin and ciprofloxacin in clinical *E.coli* is interrupted by the presence of *qnr* genes, as reported by Alm'amoori and Hadi ZJand Almohana (40), (50) in Iraq as well as Hooper and Jacoby (4), who mentioned that *qnr* genes not only partially protect*E. Coli* gyrase against ciprofloxacin and norfloxacin inhibition, but also inhibit the ATP-dependent super coiling activity of gyrase. However horizontal transferable resistance genes are often compensated by the regulation of transcription factors encoded by other genes harboring onto the same plasmid (51).

Levofloxacin-resistant isolates that carried *qnrA*, *qnr*B,and *qnr*S genes were 37.5%,75.0%,and 37.5%, respectively. The results of the current study indicate that levofloxacin presents the highest frequencies of *qnr* A, B, and S genes, i.e. greater than the other tested FQ antibiotics. This result is compatible with those of Malekzadegan, Rastegar (45), who mentioned that levofloxacin presented the highest frequencies of *qnr* in their study of quinolone-resistant pathogenic *Escherichia coli* in tertiary care hospitals in Iran. The current results are also compatible with those of several other studies concerning MIC values (52, 53) that reported *qnr* A,B, and S positive clinical strains of determinant raised MICs of levofloxacin to 32-fold compared to wild type, increasing MIC values up to 8 mg/L. Finally, the lowest prevalence of *qnr*A,B, and S-positive *E.coli* strains was against the antibiotic gatifloxacin (0%,0%, and 25%, respectively).This may be because gatifloxacin is the newest and latest reported generation of flouroquinolone antibiotics. It is necessary to monitor the spread of PMQR genes among clinical isolates and to prescribe antibiotics with caution in hospital settings.

Authors' Contribution

Study concept and design: I. F. A.
Acquisition of data: M. S. A.
Analysis and interpretation of data: I. F. A.
Drafting of the manuscript: M. S. A.
Critical revision of the manuscript for important intellectual content: I. F. A.
Statistical analysis: M. S. A.
Administrative, technical, and material support: I. F. A.

Ethics

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Al-Qasim Green University, Babylon Province, Iraq under the project number of 2021-7897896-2

Conflict of Interest

The authors declare that they have no conflict of interest.

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