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Detection of Some Antibiotic-Resistance Genes (*qnrB, qnrS, parE*, and *armA*) for *Escherichia coli* Isolated from Tigris River, Baghdad, Iraq

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Abstract

Safe water is a crucial resource for the survival and sustenance of life, and its scarcity is a major cause of water-related ailments, particularly in underdeveloped nations. The aim of this study was to determine the antibiotic sensitivity pattern of *Escherichia coli* isolated from the surface waters of the Tigris River and the detection of resistance genes (*qnrB, qnrS, parE,* and *armA*) for *E. coli*. A total of 32 bacterial isolates of *E coli* were collected for analysis from four distinct sites: S1 Al-Muthana Bridge, S2 Al-Sarrafia Bridge, S3 Al-Senak Bridge, and S4 Al-Jadriyah Bridge. Water samples were collected from November 2023 until July 2024. The isolation of *E. coli* is performed using membrane filtration techniques and biochemical analysis followed by the VITEK2. The antimicrobial susceptibility of *E. coli* isolates was assessed using the Kirby-Bauer disk diffusion method, employing a selection of 10 specific antibiotics for evaluation. The resistance genes for *E. coli* isolates were detected using the PCR technique. The results showed that both nitrofurantoin and ampicillin showed significantly higher resistance (100%), significant proportions of resistance to some β-lactam antibiotics (ceftazidime, cefoxitin, and ampicillin were 94%,94%,100% respectively) , another significant resistance for aminoglycosides antibiotics (gentamycin and amikacin, 81-84%), cephalosporins antibiotics group showed significant differences (ceftriaxone and cefuroxime , 59-93.8%), carbapenems antibiotics (imipenem,72%), quinolones group presented low resistance proportion (ciprofloxacin, 43.8%). All identified genes are complete sets with two, three, or four genes. Fourteen isolates showed resistance to fluoroquinolones (ciprofloxacin), and 26 showed resistance to aminoglycosides (gentamycin). Nine isolates (28.1%) showed the presence of *qnrB,* and 7 isolates (21.9%) showed the presence of *qnrS* genes. Of the 26 resistant isolates to gentamycin, 20 isolates (62.5%) showed the presence of the *armA* gene, and the *parE* gene was found among all selected isolates for fluoroquinolones and aminoglycosides (100%). The double association *qnrB* and *qnrS* was found in 12 isolates (37.5%), This research highlights the necessity of implementing regular surveillance of urban rivers to control the spread of antimicrobial resistance (ARM) genes in aquatic environments.

Keywords: Antibiotic resistance, *E. coli, qnrB, qnrS, parE*, *armA* genes, Tigris River

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الكشف عن بعض الجينات المقاومة للمضادات الحيوية (*qnrB, qnrS, parE, armA*) لبكتيريا الأشريكية القولونية المعزولة من نهر دجلة، بغداد، العراق

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الخلاصة

المياه النظيفة هي مورد أساسي لوجود واستمرار الحياة وعدم توفر المياه النظيفة هو سبب مهم للأمراض المرتبطة بالمياه، وخاصة في البلدان النامية. كان الهدف من هذه الدراسة هو تحديد نمط حساسية المضادات الحيوية لبكتريا الأشريكية القولونية المعزولة من المياه السطحية لنهر دجلة في مدينة بغداد والكشف عن جينات المقاومة (*qnrB، qnrS، parE، armA*) للأشريكية القولونية. جمعت 32 عزلة بكتيرية من الأشريكية القولونية لتحليلها من أربعة مواقع مختلفة: جسر المثنىS1 ، جسر الصرافيةS2 ، جسر السنكS3 وجسر الجادرية S4. جمعت عينات المياه من شهر تشرين الثاني 2023 حتى شهر تموز 2024. تم تحديد عزلات الأشريكية القولونية من خلال تقنية الترشيح الغشائي والتحليل الكيميائي الحيوي متبوعًا بـ VITEK2. تم تحديد حساسية المضادات الحيوية من خلال طريقة انتشار القرص Kirby–Bauer. تم اختبار عزلات الإشريكية القولونية ضد 10 مضادات حيوية انتقائية. تم الكشف عن جينات المقاومة (*qnrB، qnrS، parE، armA*) لهذه العزلات من الإشريكية القولونية باستخدام تقنية تفاعل البلمرة المتسلسل. وأظهرت النتائج أن كل من النتروفورانتوين والأمبيسلين أظهرا مقاومة أعلى بشكل ملحوظ (100%)، ونسب مقاومة ملحوظة في بعض المضادات الحيوية بيتا لاكتام (سيفتازيديم، سيفوكسيتين، أمبيسلين كانت 94%,94%, 100% على التوالي)، ومقاومة ملحوظة أخرى للمضادات الحيوية الأمينوغليكوزيدات (جنتاميسين، أميكاسين، 81–84%)، وأظهرت مجموعة المضادات الحيوية السيفالوسبورينات فروقاً ملحوظة (سيفترياكسون، سيفوروكسيم، 59–93.8%)، والمضادات الحيوية الكاربابينيمات (ايميبينيم، 72 %)، أما مجموعة الكينولونات فقد أظهرت نسبة مقاومة منخفضة (سيبروفلوكساسين، 43.8%). جميع الجينات التي تم تحديدها هي مجموعات كاملة تضم اثنين أو ثلاثة أو أربع جينات معاً. (14) عزلة أظهرت مقاومة للفلوروكينولونات (سيبروفلوكساسين) و (26) عزلة أظهرت مقاومة للأمينوغليكوزيدات (جنتاميسين). (9) عزلات 28.1% أظهرت وجود جين *qnrB* و (7) عزلات 21.9% أظهرت وجود جينات *qnrS*. من بين 26 عزلة مقاومة للجنتاميسين، أظهرت (20) 62.5% من العزلات وجود جين *armA*، وتم العثور على جين *parE* 100% بين جميع العزلات المختارة لجينات الفلوروكينولون والأمينوغليكوزيدات. تم العثور على الارتباط المزدوج *qnrB* و*qnrS* في اثنتي عشرة (12) عزلة (37.5٪). يُسلّط هذا البحث الضوء على ضرورة إجراء مراقبة دورية منتظمة للأنهار الحضرية للحد من انتشار جينات مقاومة مضادات الميكروبات في البيئات المائية.

1. Introduction

Antibiotic resistance is a worldwide public health concern [1]. Aquatic settings, in particular, are suspected of serving as both a reservoir and a conduit for the propagation of antibiotic-resistant bacteria (ARBs) and antibiotic-resistance genes (ARGs)[2]. Aquatic ecosystems receive a large diversity of anthropogenic inputs, including wastewater, recycled water, and stormwater, positioning them as crucial players in the genesis and dissemination of ARBs [1]. ARBs propagate both horizontally and vertically, meaning that resistance genes are transferred between bacterial species and passed on to new generations [3]. ARGs can be transferred to humans through food and drinking water [4]. The proliferation of ARBs and the prevalence of ARGs in wastewater are well-established factors contributing to the reduced potency of antibiotics used in healthcare worldwide [5]. Most recently, microbial resistance to

antibiotics has been studied as another facet of water microbiology [6]. ARBs were formerly thought to be mostly acquired in hospitals and clinics, but additional studies revealed that they can also be found in food, soil, and water [7].

E. coli is a multifaceted bacterial species that is released into the environment by wastewater treatment facilities and human waste. Some strains of this bacteria can be harmful or commensal [8]. *E. coli* is remarkably capable of both giving and receiving genes that cause antimicrobial resistance (AMR). For this reason, it is considered a key source of AMR genes that can spread to other bacteria through horizontal gene transfer. Therefore, AMR in *E. coli* is seen as one of the main issues facing both people and animals globally. In addition to β-lactams, the main antibiotics used to treat *E. coli* infections are quinolones and aminoglycosides [9]. Quinolones are a class of synthetic antimicrobials that have a wide range of action [10]. They are widely used, primarily in the treatment of infections caused by Gram-negative bacteria, and are today a key therapeutic alternative in human and animal medicine [11]. Thus, considering the widespread use of this class of antibiotics, it is logical to conclude that the aquatic environment and the animals that live there play a major role in the persistence and spread of quinolone-resistance genes, posing a threat to the effectiveness of antimicrobial treatments in both human and animal health [10]. Aminoglycosides are a family of antibiotics used to treat infections caused by Gram-negative bacteria, including *E. coli*. Bacterial resistance to aminoglycosides can result from both chromosomal mutations and the acquisition of mobile genetic elements (plasmids, integrons, and transposons) [12].

Due to current conditions contributing to the increase in resistant bacterial species and their resistance patterns, the purpose of this investigation was to detect the existence of resistance genes (*qnrB*, *qnrS*, *parE*, and *armA*) and the spread of antibiotic-resistant *E. coli* in the Tigris River at Baghdad city.

2. Materials and Methods

2.1. Study area

The study region comprised four sites on the Tigris River within Baghdad city, which were selected for use in the current study approach. Site1 was the Al-Muthana Bridge, site 2 was the Al-Sarrafia Bridge, site 3 was the Al-Senak Bridge, and site 4 was the Al-Jadriyah Bridge, as shown in Figure1 . Locations coordinate points have been determined, which summarized in Table 1.

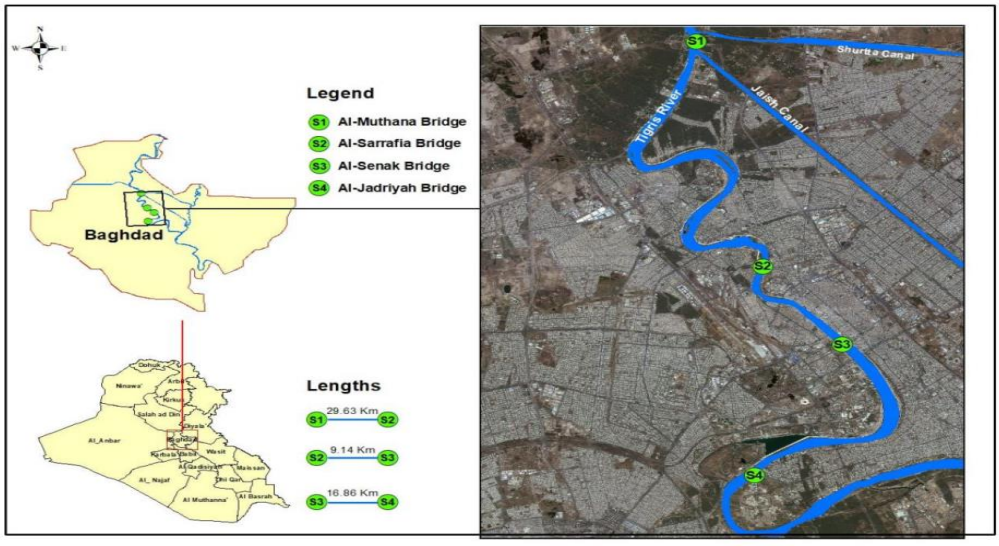

Figure 1: Satellite image of selected sampling sites along the Tigris River, Baghdad (S1-S4) representing the study sites codes.

Table 1: Geographical locations of the Tigris River section sample sites in Baghdad city

Site no.	Site name	Coordinates	
		Longitude (E)	Latitude (N)
1	Al-Muthana Bridge	44.346125°E	33.429255° N
2	Al-Sarrafia Bridge	44.372625°E	33.353972° N
3	Al-Senak Bridge	44.399351°E	33.328607° N
4	Al-Jadriyah Bridge	44.375470°E	33.282625° N

2.2 Water Samples Collection

Water samples were collected on the same day from November 2023 until July 2024 to cover the different seasons; the sample time collection on average was between 07: 00 AM and 12:00 PM. Each water samples were taken from the subsurface about 20-30 cm below water surface using sterile and well-labelled polyethylene bottles. The volume of each water sample was 500 mL. Sampling in each site was taken from different river points and then mixed to get the best representation of the site's environmental reality. The samples were preserved in an icebox for transportation. They were promptly conveyed to the microbiological laboratory. Preliminary microbiological testing was conducted early to prevent unforeseen fluctuations (ideally within 1-2 hours of arrival).

2.3 Detection and Isolation of E. coli

2.3.1 Membrane filter technique

In this study, the membrane filtering approach methodology was employed to determine the presence of contamination by *E. coli*. Sterile membrane filters with 0.45 µm holes and a 47 mm diameter were used to filter a defined volume (100 mL) of water samples taken from the Tigris River in Baghdad [13] using a sterile filtration unit. The membrane filters were placed on the surfaces of Eosin methylene blue agar plates to isolate enteric bacteria and on MacConkey agar plates to isolate lactose and non-lactose-fermenting bacteria. The plates were then incubated at 37 °C for 24 h. *E. coli* isolates were confirmed on Hichrome agar medium, where the presumptive colonies of *E. coli* were distinguished by green.

2.4 Identification of E. coli

Presumptive bacterial colonies were selected and sub-cultured to facilitate additional bacterial identification. *E. coli* was identified by colony morphology and Gram staining. Under compound microscopy, *E. coli* micelles are frequently Gram-negative and have a short, rod-shaped morphology. Traditional biochemical assays include (indole, methyl red, Simon citrate, and urea) [14]. The identities of the isolates were further confirmed using the VITEK 2 compact system (BioMérieux, Marcy, l'E'toile, France), a standardized colorimetric identification system is a method that combines conventional tests with carbohydrate utilisation assays [15].

2.5 Antimicrobial Susceptibility Testing

Using the Kirby Bauer disc diffusion method, antibiotic susceptibility testing (AST) was conducted on Muller-Hinton (MH) agar and interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) standard 2023 [16]. After making bacterial suspensions and adjusting their opacity to match 0.5 red using a McFarland standard densitometer, the inoculum was put onto Mueller Hinton agar plates that had been thoroughly dried . Mueller-Hinton agar plates were streaked all over with sterile, nontoxic cotton swabs dipped in the standardized inoculum. Gram-negative bacteria were tested against 10 different antibiotics:

ciprofloxacin (5 µg), amikacin (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), gentamicin (10 µg), imipenem (10 µg), nitrofurantoin (100 µg), ampicillin (10 µg), ceftriaxone (25 µg), and cefuroxime (30 µg) [17], and according to the Clinical and Laboratory Standards Institute's (CLSI) 2023 criteria, these antibiotic concentrations were chosen. Subsequently, the plates were incubated at 37 °C for 24 hours. The results were classified as sensitive, intermediate sensitive, or resistant according to the 2023 CLSI standards after the inhibitory zone diameter was measured with a ruler [16].

2.6 Isolation of DNA and PCR amplification of genes from Escherichia coli isolates

The bacteria's whole genome DNA was retrieved utilising FavoPrep™ genomic DNA extraction Mini kit, according to the manufacturer procedure (FAVOR GEN biotech corp) (Taiwan) to detect the antibiotic-resistance genes (*qnrB*, *qnrS*, *parE*, and *armA*). Primers for genes were chosen according to previous studies [18,19,12]. The Master Mix (Promega, USA) was used for the PCR mixture, as the negative control distilled water was used. The list of primers, amplicon size, amplification conditions, and reaction components is mentioned in Table 2. PCR was performed using Mj Mini Gradient Thermal Cycler (Bio-Rad, USA). The amplified PCR products were analysed utilizing electrophoresis in a 1.5% agarose gel for 30 minutes at 100V. (Cleaver Scientific, UK), and finally visualized with gel documentation (Bio-Rad, USA) [20].

Table2: The sequence of primer programs, composition of PCR mixtures and PCR product size used in the study

Gene	Sequence 5'-3'	PCR conditions	Composition of PCR mixtures	Amplicon size,
qnrB	F 5'-GATCGTGAAAGCCAGAAAGG-3' R 5'- ACGATGCCTGGTAGTTGTCC-3'	5 minutes at 95°C, 30 cycles of 95 °C for 30s, 53 °C for 30s, 72°C for 30s and final extension 72°C for 7minute.	for 25 µl: 12.5 µl master mix, 2 µl of DNA sample, 1 µl forward ,1µ reverse primer, 8.5 µl water	516bp
qnrS	F 5'-ACGACATTCGTCAACTGCAA-3' R 5'-TAAATTGGCACCCTGTAGGC-3'			469bp
parE	F 5'-TACCGAGCTGTTCCTTGTGG-3' R 5'-GGCAATGTGCAGACCATCAG-3'	5 minutes at 95°C, 30 cycles of 95 °C for 30s, 56 °C for 30s, 72°C for 30s and final extension 72°C for 7minute		265bp
armA	F 5'-ATTCTGCCTATCCTAATTGG-3' R 5'-ACCTATACTTTATCGTCGTC-3'	5 minutes at 95°C, 30 cycles of 95 °C for 30s, 60 °C for 30s, 72°C for 30s and final extension 72°C for 7minute		315bp

3. Results

3.1 Identification and isolation of E.coli from a River

Our isolation methodology consisted of selection and culturing in differentiation media. Firstly, it was performed with MacConkey and Eosin methylene blue agar and secondly with HiCrome *E. coli* agar. This method yielded 32 *E. coli* isolates. Traditional biochemical assays, which include (indole, methyl red, Simon citrate, and urea) showed identical positive and negative results for *E. coli* (Figure 2). as described by Shoaib *et al.,*[14],

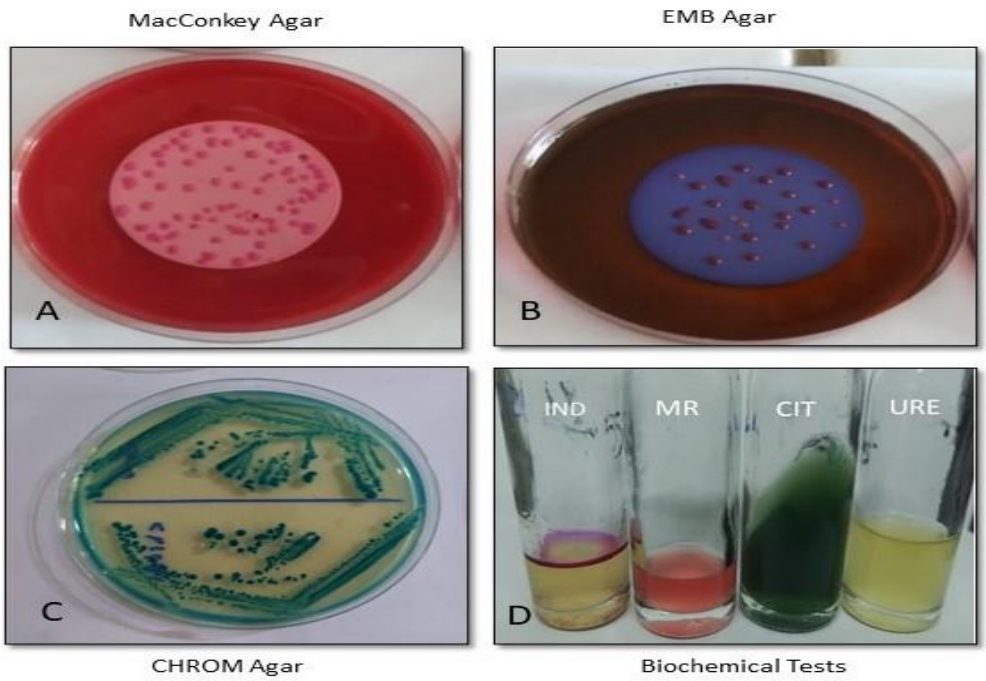

Figure 2: (A) The growth of *Escherichia coli* on membrane filter on MacConkey agar. **(B)** The growth of *Escherichia coli* on membrane filter on EMB agar. **(C)** *E. coli* on HiCrom E. coli agar show colonies that are green in colour. **(D)** Biochemical (IMVIC) tests were used for the characterization of *E. coli* isolates, and the results were as follows: from left (+ + - -) indol (IND), methyl red (MR), citrate utilization (CIT) and Urea (URE).

3.2 Classification of Escherichia coli isolates into resistant and multi-resistant

E. coli was classified as resistant or multi-resistant based on drug resistance. Isolates that were identified as resistant demonstrate phenotypic resistance to one or two antibiotic classes and multi-resistant to three or more (Table 3). Overall, all tested *E. coli* isolates showed phenotypic resistance to the antibiotics under test. The incidence of antibiotic resistance among the *E. coli* isolates varied from 43.8-100 %. All the tested isolates exhibited multiple resistance as, presented in Figure 3.

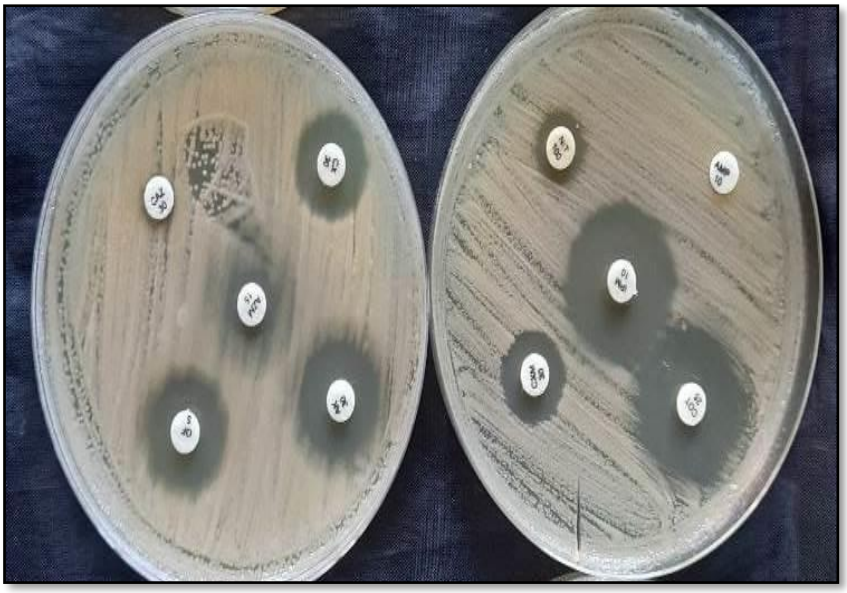

Figure.3**:** The effect of antibiotics on bacterial growth and multiple antibiotics resistance profile of *E. coli* isolates

The 32 *E. coli* isolates studied were subjected to sensitivity evaluation to 10 molecules belonging to 5 different antimicrobial classes. Two antibiotics, namely nitrofurantoin and ampicillin, showed significantly higher resistance (100%, n=32), significantly resistance proportions in some β-lactam antibiotics (ampicillin, ceftazidime, cefoxitin 93.8-100%, n=32) , another significant resistance for aminoglycosides antibiotics (gentamycin and amikacin, 81.3-84.4%, n=32), cephalosporins antibiotics group showed significantly differences (ceftriaxone and cefuroxime , 59.4-93.8%, n=32), carbapenems antibiotics (imipenem,71.9% n=32), quinolones group presented low resistance proportion (ciprofloxacin, 43.8%, n-32) (Table 3).

Table 3: Isolates with ten or more resistance to antibiotics. R indicates resistance to antibiotics; S susceptibility and I intermediate

Bacterial isolate no=32	Names of antibiotics										Resistance gene			
	CAZ	CXM	CX	CTR	AMP	GEN	AK	IPM	CIP	NIT	*qnrB*	*qnrS*	*parE*	*armA*
E1IS	R	I	R	I	R	R	I	I	I	R	+	+	+	-
E2IS	R	R	R	I	R	I	I	R	I	R	+	-	+	+
E3IS	S	R	R	I	R	R	R	S	I	R	+	+	+	+
E4IS	R	I	R	I	R	I	I	I	R	R	-	-	+	+
E5IS	R	R	R	I	R	R	R	R	R	R	+	-	+	+
E6IS	R	R	R	R	R	R	R	R	R	R	-	+	+	+
E7IS	R	R	R	S	R	R	R	I	S	R	-	+	+	+
E8IS	R	R	R	I	R	R	R	R	S	R	+	-	+	+
E9IS	R	R	R	I	R	R	R	R	I	R	+	-	+	-
E10IS	R	R	I	R	R	S	R	I	S	R	+	-	+	+
E11IS	R	R	S	R	R	S	S	S	I	R	+	-	+	-
E12IS	R	R	R	R	R	I	R	S	R	R	+	+	+	-
E13IS	R	R	R	R	R	R	R	R	I	R	-	+	+	+
EI4IS R	R	R	R	R	R	R	S	R	R	R	+	+	+	+
E15IS	R	R	R	R	R	R	R	R	R	R	-	-	+	+
E16IS	I	R	R	R	R	R	R	I	I	R	+	-	+	+
E17IS	R	R	R	R	R	R	R	R	R	R	-	-	+	-
E18IS	R	R	R	R	R	R	R	R	S	R	+	-	+	+
E19IS	R	R	R	R	R	R	R	R	I	R	+	-	+	+
E20IS	R	R	R	I	R	R	R	R	R	R	+	-	+	+
E21IS	R	R	R	R	R	R	R	R	I	R	+	-	+	+
E22IS	R	R	R	R	R	R	R	R	R	R	+	+	+	-
E23IS	R	R	R	R	R	R	R	R	R	R	+	+	+	+
E24IS	R	R	R	R	R	R	R	R	S	R	+	+	+	+
E25IS	R	R	R	S	R	R	R	I	R	R	+	-	+	+
E26IS	R	R	R	I	R	R	R	R	S	R	+	+	+	+
E27IS	R	R	R	R	R	R	R	R	R	R	+	+	+	+
E28IS	R	R	R	R	R	R	R	R	R	R	+	+	+	-
E29IS	R	R	R	R	R	I	R	R	I	R	-	-	+	+
E30IS	R	R	R	I	R	R	R	R	R	R	-	-	+	+
E31IS	R	R	R	R	R	R	R	R	I	R	-	-	+	+
E32IS	R	R	R	I	R	R	R	R	I	R	+	-	+	-
Total %	93.8	93.8	93.8	59.4	100	81.3	84.4	71.9	43.8	100	28.1	21.9	100	62.5

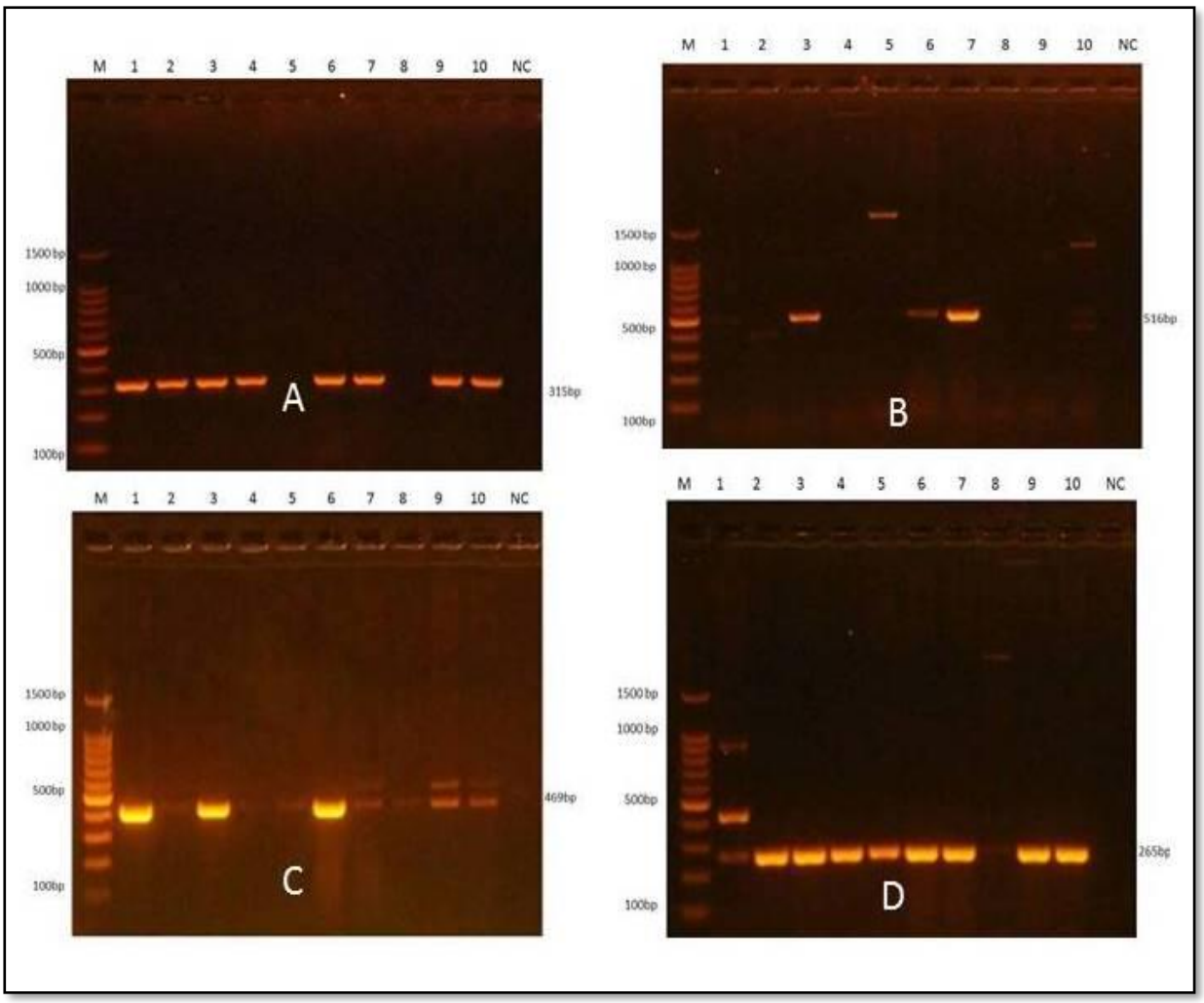

Figure. 4 PCR bands of mediated antibiotics resistance genes, (A) *armA* 315 bp, (B) *qnrB* 516 bp, (C) *qnrS* 469 , and (D) *parE* 265bp.

3.3 Gene analysis of E. coli isolates

3.3.1 Gene evaluation, both phenotypic and genotypic

PCR was used to detect three quinolone genes, two plasmid-mediated quinolone resistance (PMQRs) (*qnrB* and *qnrS*), *parE*, and an aminoglycoside (*armA*). All identified genes are complete sets with two, three, or four genes. Fourteen isolates that showed resistance to fluoroquinolones (ciprofloxacin) and 26 that showed resistance to aminoglycosides (gentamycin) from the Kirby Bauer disc diffusions test were selected for PCR (Figure 4). Nine isolates showed the presence of *qnrB* (28.1%), and 7 isolates showed the presence of *qnrS* genes (21.9%). Of the 26 resistant isolates to gentamycin, 20 isolates showed the presence of the *armA* gene (62.5%), and *parE* gene was found among all selected isolates for fluoroquinolones and aminoglycosides genes (100%), as shown in Table 3 and Figure 5.

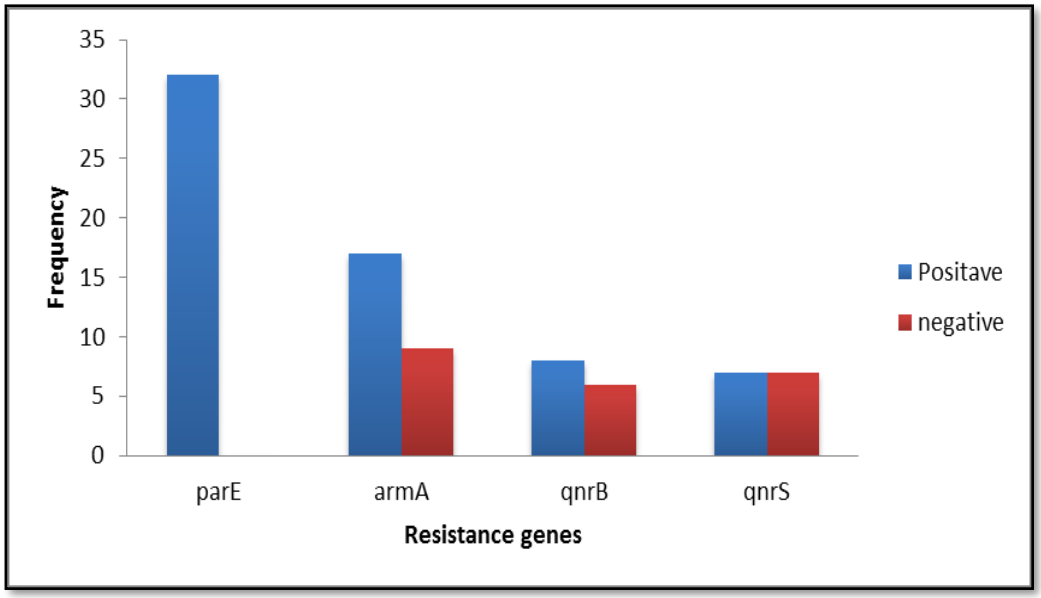

Figure 5: Prevalence of some antibiotic's-resistant genes among *E. coli* isolates

However, *qnr* genes were detected in 7 resistant isolates. The concomitant presence of two *qnr* genes was detected. The double association *qnrB* and *qnrS* were found in 12 isolates (37.5%), regardless of whether the bacterial isolate is resistant, susceptible or intermediate, as indicated in Table 3.

Discussion

The resistance pattern to β-lactam antimicrobial drugs appeared to be higher compared to some similar studies previously conducted on *E. coli* isolates from surface water [21]. The antibiotic that showed low sensitivity in *E. coli* isolates was ciprofloxacin (Table 3). It has been shown that Enterobacteriaceae develop resistance to quinolones [22]. It is indicated that one of the fluoroquinolone resistance genes belonging to the *qnr* family encode proteins that bind bacterial DNA gyrase and thereby prevent the antibiotic from interacting with its target. In general, the acquired genes do not confer a high level of resistance to fluoroquinolones but represent a selective variant of fluoroquinolone resistance [23]. This study showed moderate levels of resistance (50 and 59 %) compared to previous studies reporting resistance to third-generation cephalosporins in Enterobacteriaceae isolated from different aquatic environments [24]. According to Sophie [25], the reduced susceptibility is due to reduced permeability and enzyme inactivation, which may explain the observed low susceptibility to quinolones. Therefore, it has been hypothesized that the regulation of some bacterial genes may be regulated in a complex manner involving interactions between certain abiotic properties of water [26]. Very few studies have been reviewed in Iraq to detect resistance genes for *E. coli* in aquatic environments. Among these studies, Mahdi *et al.,* study [27] found that in the Tigris River, *E. coli* isolates exhibited minimal resistance to quinolones, sulfa drugs, and aminoglycosides. Additionally, a small number of isolates had resistance genes (*gyrA, drf1,* and *drf17*), which could be explained by the horizontal transfer of plasmids carrying genes and their distribution within the Enterobacteriaceae family. These results are consistent with the results of the current study. Another study by Mahdi *et al*., [28] aimed to detect the presence of carbapenems genes and the prevalence of antibiotic-resistant *E. coli* in the Tigris River. According to a European study [29], the overall *E. coli* resistance to cephalosporins, and aminoglycosides was less than 2% and disagreed with the current results, as shown in Table 3.

Plasmid-mediated resistance to quinolones may facilitate the spread and increase the frequency of quinolone-resistant isolates. So far, the *qnr* genes have been widely detected in different parts of the world. This is the first study to report the frequency and diversity of *qnr* genes among *E. coli* isolates from the Tigris River. This study indicated a percentage (50.0-57.0%) of PMQR genes in quinolone-resistant *E. coli* and a predominance of the *parE* gene, which is comparable to earlier findings [30]. Despite the presence of many PMQR genes, our study discovered no statistically significant link between the identified *qnr* genes and the related quinolone/fluoroquinolone resistance phenotype. A considerable fraction of *E. coli* isolates lacking the *qnr* gene show phenotypic resistance to quinolone/fluoroquinolone antimicrobials (Table 3). Other PMQR genes, such as *qnrC, qnrD, qnrE*, and *qnrVC*, which were not investigated in this work, could explain the discrepancy in the genotype-phenotype association [31]. The isolates in our investigation that lacked the PMQR genes were resistant to the quinolones that were examined. These findings add to the evidence that chromosomal alterations QRDR in *gyrA*-encoding regions are essential for quinolone production, as shown by Moon *et al*.,[32]. All isolates that revealed at least one or more resistance to aminoglycosides were treated with an *armA* gene PCR method. To the best of our knowledge, this is the first time this gene(*armA)* has been identified in the Tigris River in Iraq. According to the results of the current study, 62.5% (20/32) of bacterial isolates tested by PCR harboured *armA*. This result disagrees with Djagbare *et al*., [12] study in Burkina Faso, also with Khalaf *et al*., [33] study in Iraq, which was negative for *armA*. These results support the notion that *armA* is disseminated through conjugation and transposition, making its further spread likely, although the origin of the *armA* gene remains unknown.

The cooccurrence of fluoroquinolone and aminoglycoside resistance genes was observed in 7 *E. coli* isolates (Table 3). Very few studies have reported the distribution of the aminoglycoside's genes in aquatic strains of *E. coli* [34]. Singh and his colleague emphasised in their studies that the methylase gene (*armA*) in the plasmid was absent in the *E. coli* strains from the Yamuna River [9]. On the contrary, another study reported that *E. coli* strains isolated from Indian coastal waters were highly resistant to gentamycin [35]. In particular, unlike quinolones, phenotypic resistance to a particular aminoglycoside antibiotic is precisely correlated with the presence of the corresponding resistance gene. It has been reported that genes encoding enzymes that modify aminoglycosides have spread globally and these genes are often found in transposons that may play an important role in the spread of aminoglycoside resistance across interspecific and intraspecific boundaries.

5. Conclusions

E. coli isolated from Tigris River water in Baghdad were resistant to β-lactams, aminoglycosides and minor quinolones. Aquatic environments are significant reservoirs for *qnr* genes, as evidenced by the growing number of studies revealing their presence in aquatic bacteria. However, since *qnr* genes are nearly exclusively studied in quinolone-resistant bacteria, it is also likely that these environments are underappreciated. Therefore, bacteria with modest degrees of antibiotic resistance are typically excluded from research on antibiotic resistance in aquatic environments, which typically only look at bacterial isolates with high levels of antimicrobial resistance. Our study thus emphasises the significance of regular microbial population monitoring in urban water bodies to check for the widespread occurrence of antibiotic resistance determinants. This study showed that these isolates had low sensitivity in all species to ciprofloxacin and were more resistant to gentamycin. The distribution of *qnrB* (28.1%), *qnrS* (21.9 %), and *parE* 100% are primarily responsible for fluoroquinolones (ciprofloxacin) resistance in *E. coli*. All isolates that revealed at least one or more resistance to aminoglycosides were subjected to PCR for the *armA* gene. According to

findings, 62.5% (20/32) of the bacterial isolates tested by PCR, harboured *armA* allowed us to strengthen the monitoring and control of resistant strains. The heavy use of antibiotics in hospitals and probably in human society may lead to bacteria acquiring multidrug resistance. This is a health risk to humans and the water environment. Antibiotic use and water treatment in hospitals and homes must be controlled.

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Competing interest

The authors declare that they have no conflicts of interest

Ethics approval and consent to participate

Authors attest to the approval of ethical considerations. The study received ethical clearance from the local ethical council at the University of Baghdad, and the study protocol was approved by the Scientific Research Commission, Research & Technology Centre of Environment, Water & Renewable Energy / Ministry of Higher Education & Scientific Research Disclosure and conflict of interest.

References

[1] E.G.Milligan, J.Calarco, B.C.Davis, I.M.Keenum, K.Liguori, A.Pruden and V.J.Harwood, " A systematic review of culture-based methods for monitoring antibiotic resistant *Acinobacte*, *Aeromonas*, and *Pseudomonas* as environmentally relevant pathogens in wastewater and surface water," *Current Environmental Health Reports* , vol. 10, pp. 154-171, 2023. https://doi.org/10.1007/s40572-023-00393-9.

[2] S. Hernando-Amado, T.M.Coque, F.Baquero, and J.L.Martinez, " Defining and combating antibiotic resistance from one health and global health perspectives," *Nature Microbiology* , vol. 4, pp. 1432-1442, 2019 https://doi.org/ 10.1038/s41564-019-0503-9

[3] Z. Jian, L.Zeng, T.Xu, S. Sun,S.Yan,L.Yang,Y.Huang, J.Jia, and T.Dou, " Antibiotic resistance genes in bacteria: occurrence, spread, and control," *Journal of Basic Microbiology*, vol. 61, pp. 1049-1070, 2021. https://doi.org/10.2002/jobm.202100201.

[4] M. Kumar, B.Ram, H.Sewwandi,Sulfikar,R.Honda, and T.Chaminda, " Treatment enhances the prevalence of antibiotic resistant bacteria and antibiotic resistance genes in the wastewater of Sri Lanka, and India," *Environmental Research*, vol. 183, pp. 109179, 2020. https://doi.org/10.1016/j.envers.2020.109179.

[5] A.Obayiuwana, and A.M.Ibekwe, " Antibiotic resistance genes occurrence in wastewaters from selected pharmaceutical facilities in Nigeria," *Water*, vol. 12, no.1897 pp. 1-11, 2020. http://doi:10.3390/w12071897.

[6] K.M.S. Ana, J.Madriaga, and M.P.Espino, " Beta- lactam antibiotic and antibiotic resistance in Asian lakes and rivers: an overview of contamination,source and detection methods,"

Environmental Pollution , vol. 275, no.116624, pp. 1-13, 2021. https://doi:10.1016/j.envpol.2021.116624. z.

[7] N. Skandalis, M. Maeusli,D.Papafotis,S.Miller,B.S.Lee, I.Theologidis, and B. Luna, " Environmental spread of antibiotics resistance," *Antibiotics Basel* , vol. 10, no. 6, pp. 1-14, 2021. https://doi: 10.3390/antibiotics10060640.

[8] P. Saini, V. Bandsode, A.Singh, S.K.Mendem,T.Semmler, M.Alam, and N. Ahmed, " Genomic insights into virulence,antimicrobial resistance,and adaptation acumen of Escherichia coli isolated from an urban environment," *Environmental Microbiology/Research Article* , vol. 15, no.3, pp. 1-18, 2024. https:// doi:10.1128/mbio.03545.23.

[9] N.S. Singh, N. Singhal, M.Kumar, and J.S. Virdi, " Public health implications of plasmid mediated quinolone and aminoglycoside resistance genes in *Escherichia coli* inhabiting a major anthropogenic river of India ," *Epidemiology and Infection* , vol. 150, e78, pp. 1-8, 2022. https://doi.org/10.1017/S095026882200053x.

[10] C.D. Miranda, C. Concha, F.A.Godoy, and M.R. Lee, " Aquatic environments as hotspots of transferable low-level quinolone resistance and their potential contribution to high level quinolone resistance," *Antibiotics* , vol. 11, no.1487, pp. 1-31, 2022. https://doi.org/10.3390/antibiotics11111487.

[11] G.A. Richards, A.J. Brink, and C.Feldman " Rational use of the fluoroquinolones," *South African Medical Journal* , vol. 109, no.6, pp.378-381, 2019. doi:10.7196/SAMJ. 2019.v109i6.14002

[12] P.Djagbare,C.Nadembega, T.Sagna,A.K.Ouattara, E.Sampo, T.Zohoncon, M.Ouedrago, M.Belemgnegre, D.Obiri-Yeboah, and J. Simpore, " Detection of *aac(3)IIc,aac(6)Ib, armA* genes coding for *Escherichia coli* resistance to aminoglycosides in Burkina Faso ," *Advances in Infectious Diseases* , vol. 13, pp. 574-585, 2023. https://doi.org/10.4236/aid.2023.134047.

[13] D. Dhengesu, H.Lemma, L.Asefa, and D. Tilahun, " Antimicrobial resistance profile of enterobacteriaceae and drinking water quality among households in Bule Hora Town,South Ethiopia," *Risk Management and Healthcare Policy* , vol. 15, pp. 1569-1580, 2022. https://doi.org/10.2147/RMHP.S370149.

[14] M. Shoaib, I.Muzammil, M.Hammad,Z.A.Bhutta, and I. Yaseen, " A mini review on commonly used biochemical tests for identification of bacteria," *International Journal of Research Publications* , vol. 54,no.1, pp. 1-9, 2020. https://doi.org/10.47119/IJRP100541620201224.

[15] L.B.R.O. dela Pena, M.A.G..Nacario, N.R. Bolo, and W.L. Rivera. " Multiple antibiotic resistance in Esherichia coli isolates from fecal and water sources in Laguns lake,Philippines," *Water* , vol. 14,no.1517, pp. 1-12, 2022. https://doi.org/10.3390/w14091517.

[16] Clinical and laboratory standards institute (CLSI). Performance standards for antimicrobial susceptibility testing. 33rd edition. CLSI supplement M100, ISBN 978-1-68440-170-3 (Print), ISBN 978-1-68440-171-0 (Electronic). Clinical and laboratory standards institute, USA: 2023. vol ,43, no 3. E:customersservice@clis.org/ www.clsi.org.

[17] A.S. Ripanda, M.J.Rwiza, E.C.Nyanza, H.Miraji, N.L.Bith, A.Mzula, E.Mwega, K.N.Njau, S.A.H.Vuai and R.L.Machunda, " Antibiotic resistant microbial populations in urban receiving waters and wastewaters from Tanzania," *Environmental Chemistry and Ecotoxicology*, vol. 5, pp. 1-8, 2023. http://dx.doi.org/10.1016/j.enceco.2022.10.003.

[18] A.Robicsek, J. Strahilevitz, D.F.Sahm, G.A.Jacoby, and D.C. Hooper, " *qnr* Prevalence in Ceftazidime resistant enterobacteriaceae isolates from the United State," *Antimicrobial Agents and Chemotheraby* , vol. 5, no.8, pp. 2872-2874, 2006. https://doi.org/10.1128/AAC.01647-05.

[19] M.Iranzad, and M. Hakemi-Vala, " The role of *parC,parE*, and *qnrB* genes in ciprofloxacin resistant *Escherichia coli* isolates from urinary tract infections," *Archives of Pediatric Infectious Diseases* , vol. 5, no.3, pp. 1-7, 2017. https://doi.org/10.5812/pedinfect.41504 .

[20] T. Bogiel, A.Mikucka and P. Kanarek. " Agarose gel electrophoresis based RAPD-PCR-an optimization of the conditions to rapidly detect similarity of the alert pathogens for the purpose of epidemiological studies ," *Gels*, vol. 8, no.12:760, pp. 1-8, 2022.https://doi: 10.3390/gels8120760.

[21] H. Blaak, G. Lynch, R.Italiaander, R.A.Hamidjaja, F.M.Schets, and A.M.de Roda Husman , "Multidrug resistant and extended spectrum Beta-lactamase-producing *Escherichia coli* in Dutch surface water and wastewater," *PLOS One* , vol. 10, no. 6, pp. 1-16, 2015. https://doi.org 10.1371/journal.pone.0127752.

[22] L. Dhara, and A. Tripathi, " Contribution of genetic factors towards cefotaxime and ciprofloxacin resistance development among extended spectrum beta lactamase producing quinolone resistant pathogenic enterobacteriaceae," *Gene*, vol. 893, pp. 147921, 2024. https://doi.org/10.1016/j.gene.2023.147921.

[23] Shafiurrahman, S.M. Hasan,K.Singh,A.Kumar,J.Bano,M.O.Shahanawaz,S.Ahmad, and A. S.P.Kushwaha, " Revolutionizing quinolone development for DNA gyrase targeting;discovering the promising approach to fighting microbial infections," *Anti Infective Agents* , vol. 23, no. 5, Article ID:e22113525318200, 2024. https://doi:10.2174/0122113525318200240902062055.

[24] L. Carisen, M.Grottker,M.Heim,B.Knobling,S.Schlaub,K.Wellbrock,and J,K,DuarteKnobloch," High genetic diversity in third generation cephalosporin resistant Escherichia coli in wastewater systems of schleswig holstein ," *Pathogens* , vol. 13, no. 1, pp. 90, 2024. https://doi.org/ 10.3390/pathogens 13010090.

[25] Z. Sophie, " Bacterial resistance to antibiotics: occurrence and control strategies," Doctorate in Pharmacy , France:University of Limoges, 2014.

[26] J.S. Eheth, C.L. Djimeli, P.A.Nana, A.T.Arfao,O.V.N.Ewoti, L.M.Moungang, G.Bricheux, T.Sime-Ngando and M. Nola, " Less effect of wells physicochemical properties on the antimicrobial susceptibility *Pseudomonas aeruginosa* isolated in equatorial region of Central Africa," *Applied Water Science* , vol. 9, no. 30 pp. 1-9, 2019. https://doi.org/10.1007/s13201-019-0909-9.

[27] Z.M. Mahdi, S.H. Mahmood and N.N. Bager, " Detection of resistance genes (gyrA,qepA,drf1,drf17) for *E.coli* in Iraqi aquatic environment," *Baghdad Science Journal* , vol. 21, no. 2, pp. 0328-0337, 2024. https://doi.org/10.21123/bsj.2023.7782.

[28] Z.M. Mahdi, Z.H. Hatif, M.H.Younis, and R.A. Abed Allah, " Detection of resistance genes (*blaNDM* and *blaVIM*) of *Escherichia coli* in aquatic environment," *Iraqi Journal of Science* , vol. 64, no. 12, pp. 6193-6203, 2023. https://doi.org/10.24996/ijs.2023.64.12.9.

[29] R.Zamudio,P.Boerlin,R.Beyrouthy,J.Y.Madec,S.Schwarz, M.R.Mulvey, G.G.Zhanel, A.Cormier,G.Chalmers,R.Bonnet,M.Haenni,I.Eichhorn,H.Kaspar,R.Garcia-Fierro, J.L .N .Wood , and A.E. Mather, " Dynamics of extended spectrum cephalosporin resistance genes in *Escherichia coli* from europe and north America," *Nature Communication* , vol. 13, no.7490, 2021. https://doi.org/10.1038/s41467-022-34970-7

[30] M.B. Amin, S.R. Saha, M.R.Islam, S.M.A.Haider, M.I.Hossain, A.S.M.H.K.Chowdhury, E.K.Rousham, and M.A. Islam, " High prevalence pf plasmid mediated quinolone resistance (PMQR) among *E.coli* from aquatic environments in Bangladesh," *PLOS ONE* , vol. 16,no.12, pp. 1-15, 2021. https://doi.org/10.1371/journal pone.0261970.

[31] K. Juraschek, C.Deneke, S.Schmoger, M.Grobbel,B.Malorny, A.Kasbohrer, S.Schwarz, D.Meemken, and J.A.Hammerl, " Phenotypic and genotypic properties of fluoroquinolone resistant ,qnr-carrying Escherichia coli isolated from the german food chain in 2017," *Microorganisms* , vol. 9, no. 1308, pp. 1-16, 2021. https://doi: 10.3390/microorganisms9061308.

[32] D.C. Moon, S.Y. Seol, M.Gurung, J.S.Jin, C.H.Choi,J.Kimm,Y.C.Lee,D.T.Cho, and J.C. Lee, "

Emergence of a new mutation and its accumulation in the topisomerase IV gene confers high levels of resistance to fluoroquinolones in *Escherichia coli* isolates," *International Journal of Antimicrobial Agents* , vol. 35, pp. 76-79, 2010. https://doi.org/10.1016/j.ijantimicag.2009.08.003.

[33] S.A.Khalaf,N.N.Mahmood,and M.A.D.Saleh, " Detection of tetM,armA,blaPER-1and blaIMP genes in E.coli isolates among the gram negative bacteria that cause urinary tract infections ,"*Journal of Physics:Conference Science* ,vol,1999, pp,2-12, 2021. https://doi.10.1088/1742-6596/1999/1/012021.

[34] L. Pradier, and S.Bedhomme , "Ecology, more than antibiotics consummption, is the major predictor for the global distribution of aminoglycoside modifying enzymes" *Ecology ,Epidemiology and Global Health* , vol. 12, no. e77015, pp. 1-30, 2023. https://doi.org 10.7554/eLife.77015.

[35] A. Maloo, A.B. Fulke, N.Mulani, S.Sukumaran, and A. Ram, " Pathogenic multiple antimicrobial resistant *Escherichia coli* serotypes in recreational waters of Mumbai,India: a potential public health risk," *Environmental Science and Pollution Research International*, vol. 24, no. 12, pp. 11504-11517, 2017. https://doi:10.1007/s11356-017-8760-8.