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Research Article

Quinolone resistance (*qnrA*) gene in isolates of *Escherichia coli* collected from the Al-Hillah River in Babylon Province, Iraq

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Abstract

Aquatic environment contamination remains a foremost global public health hazards, and symbolizes a significant reservoir of releasing antibiotic resistant bacteria. The survival of *Escherichia coli* in aquatic environments serves as a potential reservoir of antibiotic resistance, encompassing but not restricted to a plasmid-mediated quinolone resistance (PMQR) mechanism. The current study aimed to detect the presence of the PMQR-*qnrA* gene in quinolone-resistant *E. coli* isolates. Sixty-one waterborne *E. coli* with known phylogroups/subgroups isolated from the Al-Hillah River in Babylon Province, Iraq, were screened for the phenotypic resistance to third-generation quinolones (levofloxacin and ofloxacin) and were further analysed for the presence of the *qnrA* gene using polymerase chain reaction (PCR). Fifty-seven (93.4%) of 61 *E. coli* isolates were levofloxacin-resistant, and 55 (90.2%) were ofloxacin-resistant. Among the 57 quinolone-resistant *E. coli*, 40 (65.57%) isolates were found to carry the PMQR-*qnrA* gene. Among the 40 *qnrA*-positive *E. coli*, 22 (36.1%) isolates were in phylogroup B2, followed by 8 (13.1%) isolates in phylogroup D, 6 (9.8%) isolates in phylogroup B1, and 4 (6.6%) isolates in phylogroup A. The presence of the PMQR-*qnrA* gene in *E. coli* belonging to phylogroup B2 and D reflects the need for routine monitoring of antibiotic resistance genes (ARGs) in the Al-Hillah River.

Keywords

Aquatic environment, *Escherichia coli*, *qnrA* gene, Quinolone resistance

Introduction

The emergence of antibiotic-resistant bacteria (ARB) jeopardizes the efficacy of the antibiotics that have changed medicine and saved millions of lives (Ventola 2015; Suzuki et al. 2017). While much more attention continues to be devoted to clinical bacteria, there is a need for more information regarding the role of environmental bacteria in the rise of antibiotic resistance to slow its spread and increase the lifespan of the antibiotic arsenal (Wright 2010). In particular, the aquatic environment can serve not only as a channel for the dispersal of ARB but also as a reservoir of clinical resistance genes (Young 1993; Michael et al. 2013).

Surface water is one of the crucial bacterial habitats on Earth, and elevates the dissemination of human pathogens along with the spread of antibiotic resistant bacteria (ARB) that confer resistance to other bacteria (Young 1993; Austin et al. 1999). Several of these bacteria carry antibiotic resistance genes (ARGs) that are ultimately introduced into genetic mobile elements (plasmids, transposons, integrons) and can be disseminated among water bacterial species (Alonso et al. 2001). A potential reservoir of antibiotic-resistant Gram-negative bacteria is the commensal

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microbiota in the gut of humans and warm-blooded animals, especially after the excessive use of antibiotics (Khan et al. 2019). In particular, *Escherichia coli* (non-spore forming rods, facultative anaerobic coliform bacterium), a gut microbiota, can enter aquatic environments through direct discharge, particularly from agriculture, animal farmlands, and domestic life (Guentzel 1991; Alves et al. 2014; Ghaderpour et al. 2015). Recently, *E. coli* has been deemed to be a significant reservoir of genes coding for antibiotic resistance and is extremely adept at horizontal gene transfer (HGT), which is considered a vehicle for antibiotic resistance spread (Hammerum and Heuer 2009; Odonkor et al. 2018; Chuppava et al. 2019). Altogether, the rivers used for human activities can be effective vectors that spread ARB and ARGs (Pereira et al. 2013; Suzuki et al. 2017).

Quinolones are a class of synthetic and broad-spectrum antibacterial agents that interfere with bacterial DNA gyrase (bacterial topoisomerase II) and topoisomerase IV, preventing the supercoiling of DNA, and ultimately promoting DNA strand breakage (Sánchez and Martínez 2012; Rezazadeh et al. 2016). In addition to their use in human medicine, quinolones are extensively utilized as therapeutics and prophylactic additives to prevent the occurrence of disease in livestock and fish farms (Aly and Albutti 2014; Guidi et al. 2018). Resistance to quinolone in *E. coli* can occur by either chromosomal mutations in DNA gyrase genes or acquisition of transferable plasmid-mediated quinolone resistance (PMQR) genes (Salah et al. 2019). PMQR is mediated by *qnr* genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*) encoding proteins that belong to the pentapeptide repeat family (Andres et al. 2013). The *qnrA* gene encoding a 218-amino acid QnrA protein of the pentapeptide repeat family protects topoisomerase II and topoisomerase IV against quinolone inhibition (Poirel et al. 2005; Wang et al. 2008). Generally, plasmid-mediated resistance is a rising concern and can be transferred among various bacterial species and stimulate their transfer into other pathogenic species through horizontal gene transfer (HGT) (Davies and Davies 2010).

The Al-Hillah River in Babylon Province, Iraq, usually serves the residents in this city as the main source for human activities, such as irrigation, recreational activities, fishing and bathing. Unfortunately, the Al-Hillah River has been significantly impacted by direct raw sewage and wastewater discharge from agriculture areas, livestock and fish farms, and hospitals. Previous study by our research group characterized the emergence of multiple-drug resistance in phylogroups/subgroups of *E. coli* isolated from the Al-Hillah River (Alwash and Al-Rafyai 2019). Therefore, we decided to detect the presence of the *qnrA* gene in quinolone-resistant *E. coli* isolated from the Al-Hillah River.

Materials and methods

Site description

The Al-Hillah River in the city of Babylon Province, Iraq, is a tributary stream of the Euphrates River, which serves as a water source for irrigation and domestic activities according to the intermittent provision of water supply throughout the country (Alwash and Al-Rafyai 2019). The sampling was performed in mid-December 2017. Briefly, 75 water samples were collected from each site along the Al-Hillah River, as described previously in Alwash et al. (2019). All three sampling sites (Figure 1) are impacted by different sources of human activities. The first site (S1) is situated in an intense agriculture land, the second site (S2) is situated close to the Marjan Internal Medicine and Cardiology Hospital, and the third site (S3) corresponds to the main urban areas.

E. coli isolates

A collection of 61 *E. coli* isolates with known phylogenetic groups were retrieved in HiCrome *E. coli* (HiMedia Laboratories, Mumbai, India) from S1 site (n = 21), S2 site (n = 19) and S3 site (n = 21) (Alwash and Al-Rafyai 2019). The phylogenetic diversity of the *E. coli* isolates was inspected by a polymerase chain reaction-based assay (PCR) with primers and conditions previously described by Clermont et al. (Clermont et al. 2000).

Detection of quinolone resistance among *E. coli* isolates

Antimicrobial susceptibility tests were performed by the Kirby-Bauer disk diffusion method using Mueller–Hinton agar (HiMedia Laboratories, Mumbai, India) according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2015). After incubating the inoculated plates at 37 °C for 18–24 h, the susceptibility of the *E. coli* isolates to the third-generation quinolones levofloxacin (LEV, 5 µg) and ofloxacin (OFL, 5 µg) (Biomaxima, Poland) was determined, and the results were interpreted in accordance with criteria provided by the Clinical Laboratory Standards Institute (CLSI, CLSI 2015). The bacterial isolates were designated as resistant, intermediate and susceptible as recommended by the CLSI (CLSI 2015). Resistant and intermediate isolates of *E. coli* were regarded as non-susceptible, while sensitive isolates were regarded as susceptible.

Extraction of genomic DNA

Genomic DNA was extracted from *E. coli* isolates after 24 h of incubation. The DNA extraction was carried out using the G-spin Genomic DNA Extraction Kit (iNtRON Biotechnology, Korea). The DNA quality and quantity were assessed using a NanoDrop spectrophotometer (Implen, Germany). Genomic DNA was extracted in duplicate from each independent sample. The DNA samples were stored at -20 °C until further analysis.

Screening for the *qnrA* gene in *E. coli* isolates

The presence of the *qnrA* gene in quinolone-resistant *E. coli* isolates was detected by PCR amplification as pre-

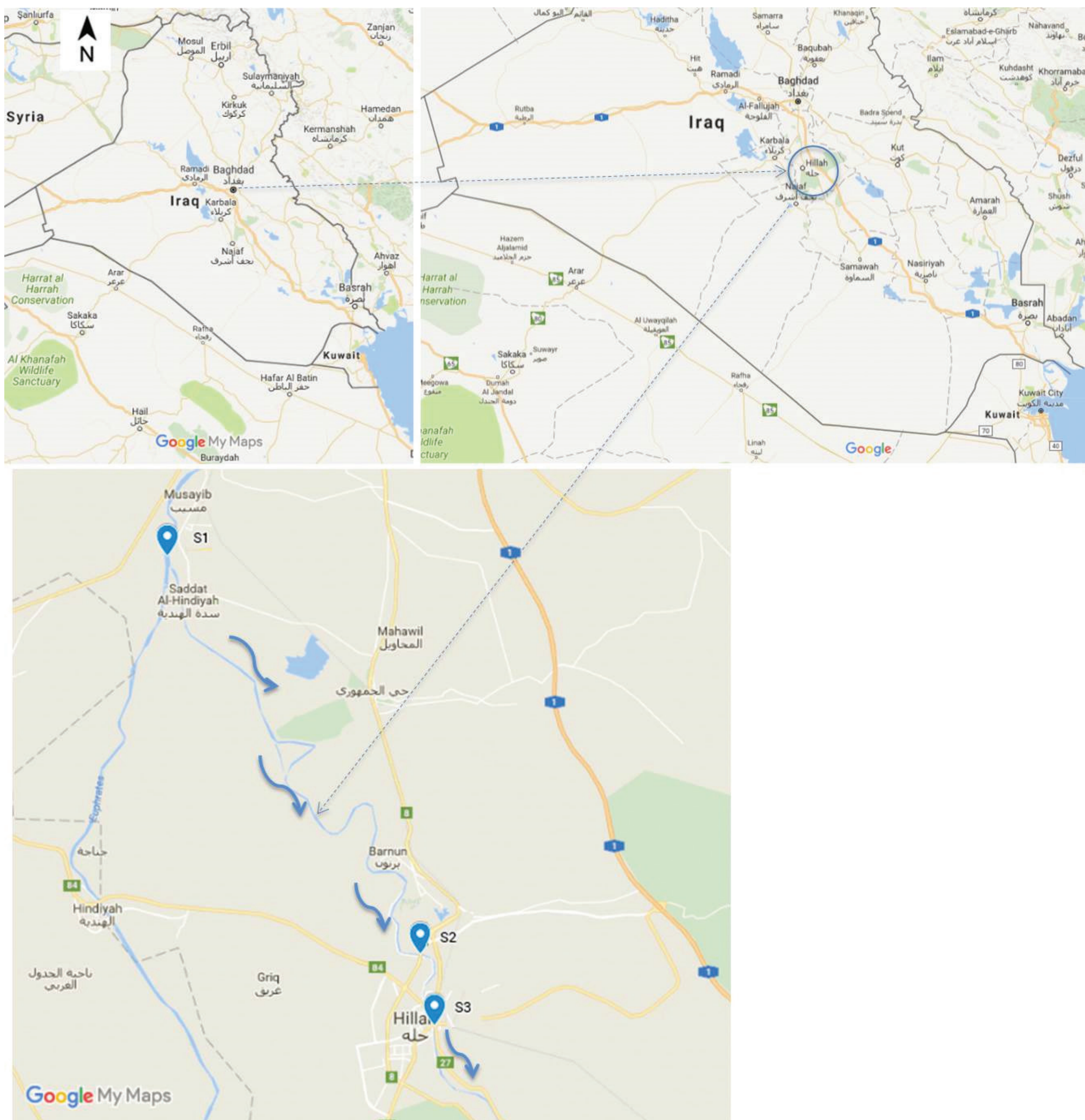

Figure 1. Geographic locations of the three sampling sites (S1–S3) along the Al Hillah River.

viously described by Wang et al. (2008). The primer set used for *qnrA* was as follows: '5-ATTTCTCACGCCAGGATTTG-3' and 5'-GATCGGCAAAGGTTAGGTCA-3' for a 516-bp product (Macrogen, Korea). The PCR program was performed using the following conditions: 1 minute (min) at 94 °C for denaturation, 1 min at 55 °C for annealing, and 1 min at 72 °C for extension, with a cycle number of 30, and without DNA template as a negative control in each run. The amplification products were separated by electrophoresis in 1.5% agarose gel containing ethidium bromide. After electrophoresis, amplification products were visualized and photographed under a ultra-violet trans-illuminator light (Mishra et al. 2010).

Results

Sixty-one *E. coli* isolates were previously recovered from 75 water samples along the Al-Hillah River in mid-December 2017. Out of the 61 *E. coli* isolates, 21 of the isolates were from S1 site, 19 isolates were from S2 site, and 21 isolates were from S3 site. Regardless of the sampling sites, a high prevalence of *E. coli* isolates resistant to levofloxacin and ofloxacin was detected. Among the 61 *E. coli* isolates examined, 57 (93.4%) and 55 (90.2%) were resistant to levofloxacin and ofloxacin, respectively. Fifty-seven *E. coli* isolates were screened for the PMQR-*qnrA* gene, which confers resistance to quinolone. Only 40 (70.2%) out of the 57 isolates were *qnrA*-positive (Figure 2). Among the 40 *qnrA*-positive iso-

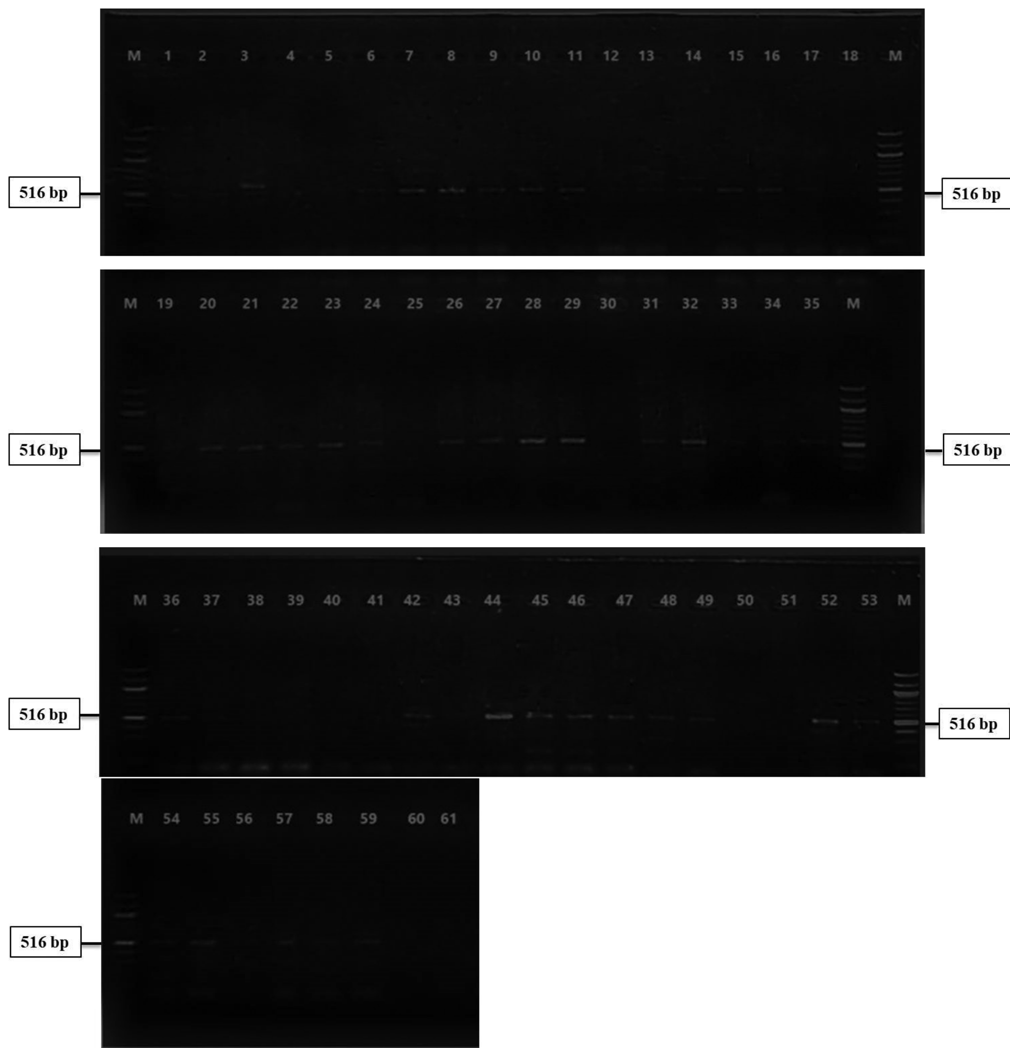

Figure 2. Agarose gel electrophoresis of PCR products derived from amplifying the *qnrA* gene of 61 *E. coli* isolates (516-bp). Lane M is a 1-kb DNA Ladder, and lanes 1 to 61 are *E. coli* isolates. Samples were amplified on 1.5% agarose gel, and isolates are labelled at the top of the figure.

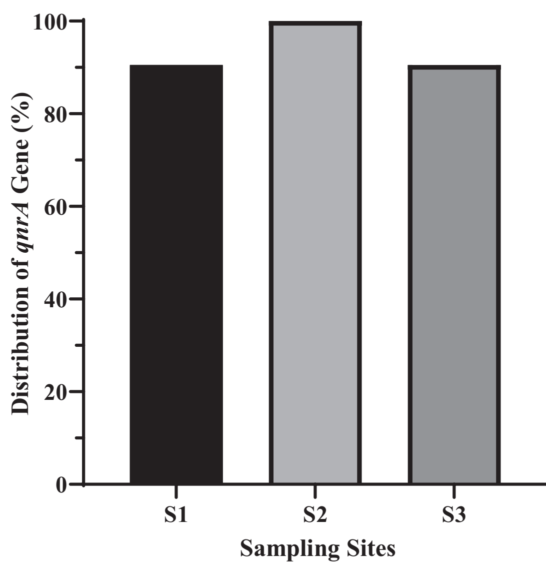

Figure 3. Distribution of the *qnrA* gene among quinolone-resistant *E. coli* isolates in the three sampling sites along the Al-Hillah River.

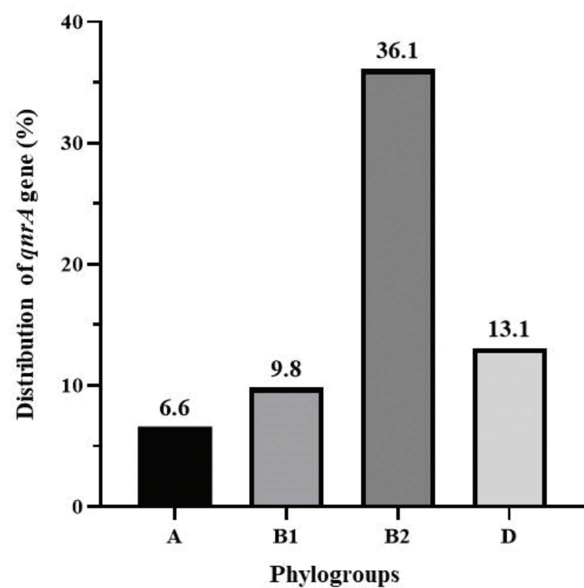

Figure 4. Distribution of the *qnrA* gene among phylogenetic groups of quinolone-resistant *E. coli* isolates recovered from the three sampling sites.

lates, 11 (27.5%) were from S1 site, 18 (45%) were from S2 site, and 11 (27.5%) were from S3 site (Figure 3). The *qnrA* gene was not found in 17 isolates (29.8%).

The distribution of *qnrA*-positive *E. coli* isolates among phylogroups is summarized in Figure 4. Among the 40 *qnrA*-positive *E. coli*, 22 (36.1%) isolates belonged to phylogroup B2, followed by 8 (13.1%) isolates in phylogroup D, 6 (9.8%) isolates in phylogroup B1, and 4 (6.6%) isolates in phylogroup A (Figure 4). The subgroups of each phylogroup (A_0/A_1, $B2_2/B2_3$, and D_1/D_2) were not distributed equally among *qnrA*-positive *E. coli* isolates. The highest prevalent subgroup among *qnrA*-positive *E. coli* isolates was $B2_3$ (16 isolates, 26.2%), followed by $B2_2$ (6 isolates, 9.8%), D_2 (5 isolates 8.2%), A_0 (4 isolates, 6.6%), and D_1 (3 isolates, 4.9%) (Figure 5).

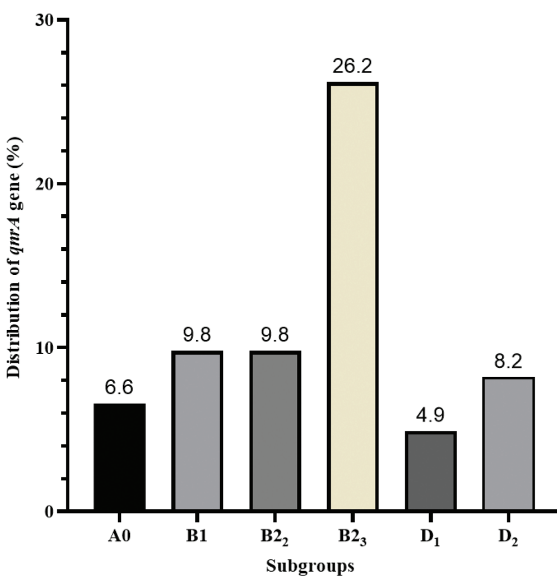

Figure 5. Distribution of the *qnrA* gene among phylogenetic subgroups of quinolone-resistant *E. coli* isolates recovered from the three sampling sites.

Discussion

The inappropriate use of antibiotics has promoted the worldwide dispersal of ARB and ARGs (Khan et al. 2019). Over last decade, antibiotic resistance has been extensively reported in clinical settings; however, the focus has been switched to studies on the emergence of resistant bacteria and ARGs in aquatic environments influenced by anthropogenic activities according to their persistent detection in aquaculture and, thus, their threat to human and animal health (Suzuki and Hoa 2012; Xiong et al. 2014; Devarajan et al. 2016; Liu et al. 2018; Kumar et al. 2019; Felis et al. 2020). In a previous study by our research group, 61 *E. coli* isolates distributed in different phylogroups/subgroups were collected from three sampling sites along the Al-Hillah River (Alwash and Al-Rafyai 2019). The majority of *E. coli* isolates (80.3%) exhibited the multiple-drug resistance phenotype (Alwash and Al-Rafyai 2019).

In the present study, high percentage rates of resistance to levofloxacin (93.4%, 57/61 isolates) and ofloxacin (90.2%, 55/61 isolates) were detected at the three sites. In addition to the high consumption of these drugs in human and veterinary medicine, quinolones are widely used in aquaculture and are mobile in the water system due to their hydrophilic properties (Wang et al. 2008; Hanna et al. 2018; Felis et al. 2020). Altogether, the Al-Hillah River is mostly vulnerable to the evolution of quinolone-resistant *E. coli* according to the heavy use of this drug in human and veterinary medicines, accompanied by the lack of sufficient sewage and wastewater treatment systems.

The percentage rate of isolates carrying the *qnrA* gene among quinolone-resistant *E. coli* bacteria was 70.2% (40/57) at the three sampling sites. The *qnrA* gene is usually PMQR and can easily disseminate among the *Enterobacteriaceae* family by HGT (Strahilevitz et al. 2009; Jacoby et al. 2014). A previous study by Jeong et al. (2005) noted that the presence of a *qnrA* gene enhances resistance to quinolones four- to eight-fold. However, no PMQR-*qnrA* genes were found in 17 (29.8%) quinolone-resistant *E. coli* isolates, which might be linked to the presence of another mechanism of resistance to quinolones, such as mutations in the topoisomerase II and topoisomerase IV genes (Jacoby 2005; Redgrave et al. 2014; Salah et al. 2019).

The highest percentage rate of *qnrA*-positive isolates observed in *E. coli* isolates was from the S2 site (94.7%, 18/19 isolates, Figure 3). This finding is likely due to its nearness to the Marjan Internal Medicine and Cardiology Hospital, where there is a direct discharge of raw sewage and wastewater from the hospital into the S2 site; in addition, levofloxacin and ofloxacin are common antibiotics used in fish cages at the S2 site. The PMQR-*qnrA* gene could be acquired through HGT and can also be further spread by river flow from the S2 site to the S3 site, as indicated by the percentage rate of *qnrA*-positive *E. coli* isolates at the S3 site (52.4%, 11/21 isolates). Detection of *qnrA*-positive *E. coli* isolates at the S1 site (52.4%, 11/21 isolates) is perhaps associated with the extensive use of quinolones in modern agricultural practices and/or veterinary medicine at this site. Altogether, there is a high possibility that the Al-Hillah River was heavily polluted by the wastewater from the Marjan hospital and from the livestock and fish cages that are adjacent to the Al-Hillah River.

From a genetic background, *E. coli* isolates fall into four main phylogroups (A, B1, B2, and D). Commensal *E. coli* isolates are commonly associated with phylogroups A and B1, while virulent extra-intestinal pathogenic *E. coli* (ExPEC) isolates belong mainly to phylogroups B2 and, to a lesser extent D (Clermont et al. 2000; Johnson et al. 2001). Generally, a relationship between isolates of *E. coli* carrying the *qnrA* gene and phylogroups was found at the three sites impacted by different anthropogenic pressures along the Al-Hillah River (Figure 4). The 40/57 *qnrA*-positive *E. coli* isolates investigated belonged mainly to phylogroups B2 (22/40 isolates, 36.1%) and D (8/40, 13.1% isolates), followed by group B1 (6/40 isolates, 9.8%) and group A (4/40 isolates, 6.6%) (Figure 4). Similarly, phylogroup $B2_3$ (16/40 isolates, 26.2%) was the most common among the isolates carrying the *qnrA* gene in this stu-

dy (Figure 5). Altogether, the majority of *qnrA*-positive *E. coli* isolates (30/40 isolates, 75%) are associated with the ExPEC isolates (groups B2 and D). The high percentage of ExPEC isolates (75%) carrying the quinolone resistance *qnrA* gene at sampling sites might reflect the impacts of hospital raw wastewater discharges and livestock and fish farms as possible sources of quinolone-resistant ExPEC isolates. The ExPEC strains reside as commensals in the gastrointestinal tracts of humans and animals, where they might acquire resistance genes through horizontal transfer (Hamelin et al. 2006). Furthermore, ExPEC isolates are associated with human and warm-blooded extraintestinal infections (Cherifi et al. 1991).

Conclusions

The current study provides the first report on the presence of the PMQR-qnrA gene in *E. coli* isolates recovered from the Al-Hillah River in Babylon Province, Iraq. Recent significant results regarding antibiotic resistance are as follows: (1) the Al-Hillah River is principally vulnerable to the development of ARB due to contamination with antibiotics in the absence of sufficient wastewater treatment systems, (2) a high percentage rate of isolates carrying the qnrA gene in quinolone-resistant *E. coli* (70.2%) was found at the three sampling sites, indicating that quinolone contamination correlates strongly with the prevalence of qnrA-positive *E. coli* isolates in the Al-Hillah River, and (3) the majority of qnrA-positive *E. coli* isolates (30/40, 75%) are associated with the ExPEC (groups B2 and D), reflecting the impacts of raw wastewater discharges as possible sources of quinolone-resistant ExPEC isolates. Although this study underlines the presence of the PMQR-qnrA gene, which contributed to the development and spread of quinolone resistance among *E. coli* isolates in the Al-Hillah River, further studies will focus on the presence of qnrA and its variants in a broader range of bacterial strains known to be common in aquatic environments. This information could be valuable in detecting the origin of the qnrA gene and its variants in isolates from the Al-Hillah River. Taken together, the current study has generated scientific evidence that aquatic environments are a potential source for the dispersal of ARB and ARGs. Furthermore, the presence of qnrA-positive *E. coli* isolates belonging to phylogroups B2 and D highlights the necessity for routine surveillance of ARGs in the Al-Hillah River.

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