



Prevalence of Extended-Spectrum B-Lactamase (ESBL) and Quinolone Resistance (*qnr*) Genes among Cytotoxic Necrotizing Factor-1-Producing Uropathogenic *Escherichia coli* in Babylon, Iraq

ARTICLE INFO

Article Type
Original Article

Authors

Haider Qassim Raheem, *PhD*^{1*}
Lamiaa Al-Maliki, *PhD*²
Mohammed Alaa Abdulzahra, *PhD*¹
Thaer Shafi Hussein, *MSc*³

¹ DNA Research center, University of Babylon, Al-Karamah Street - 51001, Babylon state, Iraq

² Department of Molecular and Medical biotechnology, College of Biotechnology Al-Nahrain University.

³ Department of Pharmacy, Al-Amal College For Medical Specialized sciences, Karbala-56001, Iraq

* Correspondence

DNA Research center, University of Babylon, Al-Karamah Street - 51001, Babylon state, Iraq.
E-mail:haider.qassim@uobabylon.edu.iq

How to cite this article

Raheem HQ., Al-Maliki L., Abdulzahra MA., Hussein TS. Prevalence of Extended-Spectrum B-Lactamase (ESBL) and Quinolone Resistance (*qnr*) Genes among Cytotoxic Necrotizing Factor-1-Producing Uropathogenic *Escherichia coli* in Babylon, Iraq. Infection Epidemiology and Microbiology. 2023;9(3): 209-218.

Article History

Received: June 19, 2023

Accepted: October 04, 2023

Published: October 18, 2023

ABSTRACT

Background: Pathogenic *Escherichia coli* (*E. coli*) is usually known as the principal agent of hospital-acquired infections, particularly urinary tract infections (UTIs). This study aimed to determine ESBL (extended-spectrum B-lactamase) production and quinolone resistance (*qnr*) genes in cytotoxic necrotizing factor 1 (CNF-1)-producing *E. coli* isolates from UTIs in Iraq. **Materials & Methods:** A total of 996 *E. coli* isolates were obtained from UTIs in two general hospitals in Hillah, Babylon, Iraq (during 2014-2022), and 100 uropathogenic *E. coli* (UPEC) were *cnf-1* carriers. ESBL production was evaluated using the double-disk synergy test. *qnr* genes were detected using polymerase chain reaction (PCR). **Findings:** Nalidixic acid and chloramphenicol resistance was 70 and 30%, respectively. ESBL production was observed among 46% of *cnf-1* carriers. *qnrA*, *qnrB*, and *qnrS* genes were detected in 18, 21, and 11% of the isolates, respectively. ESBL-producing isolates mainly carried the *qnrB* gene and showed the highest resistance levels to quinolones. Major risk factors of pathogenic *E. coli* isolation included older age (68%, $p=.031$), previous hospitalization (76%, $p=.021$), and urinary catheter (83%, $p=.018$). **Conclusion:** Although the prevalence of *cnf-1* gene was not high among UPEC isolates, its prevalence was high among quinolone-resistant and ESBL-producing isolates. Continuous investigation of virulence and resistance genes is essential for monitoring and controlling infections. It is necessary to determine virulence factors and resistance genes among UPEC in Iraq and take timely measures to hinder the spread of resistance genes to other nosocomial isolates.

Keywords: UTIs, Virulence, Antibiotics, Quinolone resistance, *Escherichia coli*, ESBL

CITATION LINKS

[1] McDanel J, et al. Incidence of... [2] Jiang X, Detection of... [3] Rodríguez-Martínez JM, Plasmid-mediated... [4] Ezzaroug Ezzraimi A, Platelets and... [5] Ramuta T, The antibacterial... [6] Shah C, Baral R, Virulence factors... [7] Kaur H, Computational... [8] Holmbom M, et al. Risk factors... [9] Gatyia Al-Mayahie SM, Prevalence of... [10] Tseng CH, Extended-spectrum... [11] Karlowsky JA, et al. Prevalence of... [12] Palmeira JD, Ferreira HMN. Extended... [13] Ortiz-Díez G, et al. Prevalence, incidence... [14] Lima LS, High genetic diversity... [15] Briaies A, et al. Prevalence of... [16] Kariuki K, et al. Plasmid-mediated... [17] Jacoby GA, Plasmid... [18] Akshay SD, Differential... [19] Rodríguez-Martínez JM, Plasmid-mediated... [20] Aldred KJ, Mechanism of... [21] Doma AO, Comparative, ... [22] Amador P, Prevalence of... [23] Aworh MK, Quinolone-resistant ... [24] Ruiz E, et al. *qnr*, *aac(6')*... [25] Mohamed Ali I, et al. Microbiology and ... [26] Sora VM, Extraintestinal... [27] Raimondi S, Antibiotic... [28] Wang MH, Cytotoxic necrotizing... [29] Li XZ. Quinolone resistance... [30] Herrera-Vázquez A, Detection of... [31] Alhadidi HA, Prevalence and... [32] Chat H, Cytotoxic... [33] Harwalkar A, Lower prevalenc... [34] Da Silva GJ, Association between... [35] Marin J, et al. The population... [36] Biggel M, Convergence of... [37] Jia Y, Study on... [38] Guiral E, Prevalence of... [39] Sfeir MM. Adoption of... [40] Quesada MD, et al. Performance of... [41] Cattoir V, Multiplex PCR... [42] Azam MW, Updates on... [43] Akiyama T, Molecular... [44] Chong Y, Current epidemiology, ... [45] Onishi R, et al. Impact on... [46] Rahn DD. Urinary tract... [47] Rippere-Lampe KE, Mutation of ... [48] Morgan RN, Prevalence and... [49] Dolejska M, Plasmid-mediated... [50] Alawi M, Plasmid-mediated... [51] Meng M, Plasmid-mediated... [52] Azargun R, The prevalence ... [53] Liang H, Fluoroquinolone... [54] Allou N, Impact of... [55] Firoozeh F, Detection of... [56] Ghasemian A, The association of... [57] Al Hamdan AS, et al. Evaluating the... [58] Yalaw GT, et al. Prevalence of... [59] San Millan A. Evolution of plasmid... [60] DelaFuente J, et al. Within-patient...

Introduction

A considerable range of nosocomial infections is caused by *Escherichia coli* (*E. coli*) [1]. In spite of being as a member of the normal flora of humans and wildlife, [2] *E. coli* isolates cause urinary and gastrointestinal tract infections (UTI and GIT, respectively), skin and respiratory infections, sepsis, and neonatal meningitis [3-5]. Infections could range from severe or lethal to insignificant depending on the level of antibiotic resistance and encoding of bacterial virulence factors. A considerable part of nosocomial UTIs and the majority of community-acquired UTIs are caused by this bacterium, which has a significant impact on morbidity and medical costs globally. UTIs and invasive infections caused by multidrug-resistant (MDR) *E. coli* strains have recently increased globally. MDR strains have extremely low therapeutic success owing to few ongoing treatment options available [3, 6]. Furthermore, the population biology of uropathogenic *E. coli* (UPEC) has not been well understood, making it difficult to prevent and control the spread of related infections [7-9]. Extended-spectrum β -lactamases (ESBLs) in Gram-negative bacteria include plasmid-mediated enzymes hydrolyzing cephalosporins mainly of third generations and aztreonam [10]. These enzymes are widely distributed worldwide [11]. The highly diverse nature of ESBL-producing bacterial strains and numerous mutations in resistance encoding genes have resulted in high morbidity and resistance rates with huge clinical impact [12, 13]. Quinolones are a class of antibiotics effective against *Enterobacteriaceae* infections. Common antibiotic classes used in humans and animals include beta-lactams and quinolones [14]. Unfortunately, fluoroquinolone resistance is rising due to chromosomal alterations (for example mutations in topoisomerase IV and DNA gyrase enzymes) or acquisition of

plasmids [15,16]. In recent years, the frequency of plasmid-mediated quinolone resistance (PMQR) has progressively increased, resulting in a decrease in effective elimination of infections. The primary mechanisms of quinolone resistance are plasmid-mediated [17, 18]. Major alterations in quinolone targets occur in *qnr* proteins through quinolone resistance genes. *qnr* genes, which are of PMQR determining factors, were initially found in *Klebsiella pneumoniae*. These genes, for example, *qnrA*, *B*, and *S*, are three kinds of *qnr* determining factors found in numerous *Enterobacteriaceae* members [19, 20]. Other gene classes such as *qnrC* and *D* have also been found [21, 22]. Penta-peptide repeat proteins encoded by *qnr* genes blockade the action of quinolones and cause low-level resistance [17, 23]. Various *qnr* genes differ in sequence by 35% [23]. Following antibiotic pressure, structural changes in arrangement might occur, resulting in non-susceptibility to trimethoprim, cefepime, ceftriaxone, and co-amoxiclav among *E. coli* isolates [24, 25]. *qnr* genes are found worldwide, particularly among hospitalized patients. As an opportunistic pathogen, *E. coli* is usually known as a major cause of hospital-acquired infections and various diseases, particularly UTIs. *E. coli* carries various virulence factors such as adhesins, secretory systems, and toxic proteins [26, 27]. The chromosomally encoded cyclomodulin, called cytotoxic necrotizing factor 1 (CNF-1) and produced by UPEC and other pathogenic strains, is associated with bacterial colonization, virulence, invasion, survival, and distribution in the body, it is a leading cause of host cell cycle changes such as colorectal tumorigenesis, apoptosis, inflammation, and tissue damage [28-32]. The association of *cnf-1* gene carriage and quinolone resistance or ESBL production has not been demonstrated previously. A study exhibited a low prevalence of *cnf-1* gene among quinolone-resistant and ESBL-

bearing isolates [33]. As virulence factors play a substantial role in the distribution of isolates, the inefficiency of common antibiotics results in the failure to hinder the distribution of pathogens in the body [34-36].

Objectives: The purpose of this study was to determine quinolone resistance and the presence of *qnr* genes in ESBL-producing and non-producing *E. coli* isolates collected from UTIs.

Materials and Methods

Bacterial isolation: During April 2014 to November 2022, a total of 996 *E. coli* isolates were collected from UTIs in two general hospitals in Hillah, Babylon, Iraq. Bacteriological culture techniques such as culture on MacConkey agar (Merk, Germany) and blood agar (Merk, Germany) media and biochemical tests (IMVIC test and Vitek-2 system) were applied to identify bacterial isolates. Positive results included methyl red (M-R), indole, mannitol and lactose (MAL), glucose (GLU), and maltose [37]. Antibiotic susceptibility pattern was determined by Viteck 2 compact system [11]. For the separation of *cnf-1* carrying isolates, polymerase chain reaction (PCR) was performed to amplify the *cnf-1* gene using forward (5-AAG ATG GAG TTT CCT ATG CAG GAG-3) and reverse (5-CAT TCA GAG TCC TGC CCT CAT TAT T-3) primers [38]. Inclusion criteria included frequency and urgency of urination, dysuria, suprapubic pain, and positive culture in the hospital. Patients receiving antibiotics during

two weeks prior to sampling were excluded. **Antibacterial susceptibility test:** Vitek 2 system (Biomerieux, USA) was used for identification (Gram negative card) and susceptibility analysis (AST-N256 card). Accordingly, bacterial cultures on nutrient agar and suspensions equal to 0.5 McFarland were prepared. After culturing the isolates on each card for a specific antibiotic, the minimum inhibitory concentration (MIC) was measured. The antibiotics used included ciprofloxacin (CIP, 5 µg), ofloxacin (OF, 5 µg), chloramphenicol (CL, 30 µg), cefotaxime (CF, 30 µg), ceftazidime (CAZ, 30 µg), nalidixic acid (NA, 30 µg), and aztreonam (AZT, 30 µg). The resistance profile was determined using the Viteck-2 system method as per the manufacturer's guidelines [39, 40].

ESBLs screening test: The isolates were evaluated for ESBL production using the double-disk synergy test (DDST) in compliance with Clinical and Laboratory Standards Institute (CLSI) recommendations. The disks included ceftazidime, cefotaxime, and clavulanic acid, where the appearance of an inhibition zone around the disks indicated ESBL production.

Extraction of plasmid DNA and amplification: Plasmid DNA content was extracted utilizing the Plasmid Miniprep kit (Favorgen, Taiwan) and kept at -20 °C and served as a template for the amplification of *qnr* genes using the primer sets depicted in Table 1.

Table 1)Primer sequences of quinolone resistance genes in this study

Genes	Sequence: 5'-3'	Amplicon (bp)	Reference
<i>qnrA</i>	F- AGAGGATTTCTCACGCCAGG R- TGCCAGGCACAGATCTTGAC	580	
<i>qnrB</i>	F -GGMATHGAAATTCGCCACTG R-TTTGCGYGYCGCCAGTCGAA	264	[41]
<i>qnrS</i>	F-GCAAGTTCATTGAACAGGGT R-TCTAAACCGTCGAGTTCGGC	428	

Ethical statement: The protocols of this study were carried out in accordance with the ethical permissions for scientific research, designed (BRC/HO-14314) by the Ethics Committee of the Iraqi Ministry of Health and Ministry of Higher Education and Scientific Research.

Statistical analysis: Sigma Plot software Version 12.5 and Microsoft office Excel 2019 were both used to process and analyze data at a significance level of 0.5. The analysis of variance (ANOVA) and multivariate linear regression model were used to evaluate differences or relations.

Findings

Patients' demographic data: Out of 996 UPEC isolates, 100 (10.04%) isolates carried the *cnf-1* gene. Among 100 patients infected with *cnf-1* carrying UPEC, 46% were male, and 54% were female. Underlying diseases including diabetes mellitus (4%, n=4), kidney disorder (11%, n=11), ICU residence (16%, n=16), alcohol consumption (1%, n=1), smoking (19%, n=19), immunomodulatory treatment (2%, n=2), and previous antibiotic usage (21%, n=21) were not significantly associated with the isolation of *cnf-1*-carrying *E. coli*. However, older age (68%, n=68, $p = .031$), previous hospitalization (76%, n=76, $p = .021$), and urinary catheter (83%, n=83, $p = .018$) were significant associated factors in this regard.

Antibacterial sensitivity, MIC levels, and ESBL production: The antibiotic sensitivity testing of *cnf-1*-carrying *E. coli* isolates revealed that resistance to the tested quinolones was high among the isolates. Resistance to ciprofloxacin, levofloxacin, ofloxacin, and norfloxacin was found in 54% (n=54), 52% (n=52), 56% (n=56), and 50% (n=50) of the isolates, respectively. Additionally, resistance to nalidixic acid, aztreonam, cefotaxime, ceftazidime, and chloramphenicol was 70% (n=70), 68% (n=68), 50% (n=50), 62% (n=62), and 30% (n=30), respectively.

The minimum inhibitory concentration (MIC) levels of ceftazidime, cefotaxime, ciprofloxacin, and ofloxacin are shown in Table 2. The highest resistance rate to beta-lactams was related to ceftazidime (62%, n=62) with MIC ≥ 4 $\mu\text{g/mL}$. Moreover, the highest resistance to quinolones was related to ofloxacin (56%, n=56) with MIC ≥ 16 $\mu\text{g/mL}$. Moreover, ESBL production was observed among 46% (n=46) of *cnf-1*-carrying UPEC isolates during 2014-2022. **Amplification of *qnr* genes:** Amplification of *qnr* genes using PCR is shown in Figures 1, 2, and 3. Accordingly, 50% (n=50) of ESBL-bearing *E. coli* isolates carried *qnr* genes including *qnrA* (18%, n=18), *qnrB* (21%, n=21), and *qnrS* (11%, n=11).

Table 2) MIC levels of ceftazidime, cefotaxime, ciprofloxacin, and ofloxacin among uropathogenic *E. coli* in this study

Antibiotics/MIC	%(N) in Susceptibility Range	%(N) in Resistance Range
Ceftazidime	38 (n=38) (MIC:0.5-2 $\mu\text{g/mL}$)	62 (n=62) (MIC ≥ 4 $\mu\text{g/mL}$)
Cefotaxime	50 (n=50) (MIC:0.5-2 $\mu\text{g/mL}$)	50 (n=50) (MIC ≥ 4 $\mu\text{g/mL}$)
Ciprofloxacin	54 (n=54) (MIC:0.5-4 $\mu\text{g/mL}$)	46 (n=46) (MIC ≥ 16 $\mu\text{g/mL}$)
Ofloxacin	56 (n=56) (MIC:0.5-4 $\mu\text{g/mL}$)	44 (n=44) (MIC ≥ 8 $\mu\text{g/mL}$)

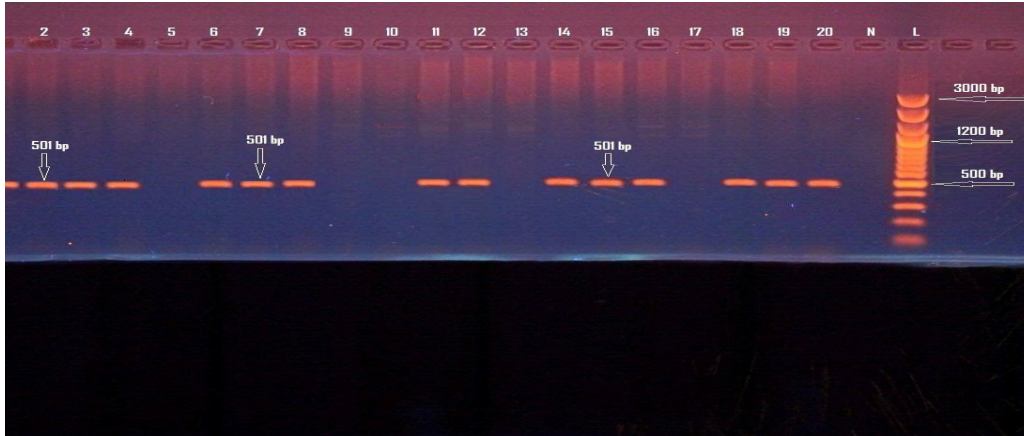


Figure 1) Gel electrophoresis products of the *qnrA* gene of *E. coli* with a 501 bp size; L: +DNA marker, wells 1-3, 6-8, 11, 12, and 18-20: positive samples, and N: negative control in this study



Figure 2) Gel electrophoresis products of the *qnrB* gene of *E. coli* with a 300 bp size; L: DNA marker, wells 8, 11, 14, 17, 18, and 19: positive samples, and N: negative control in this study

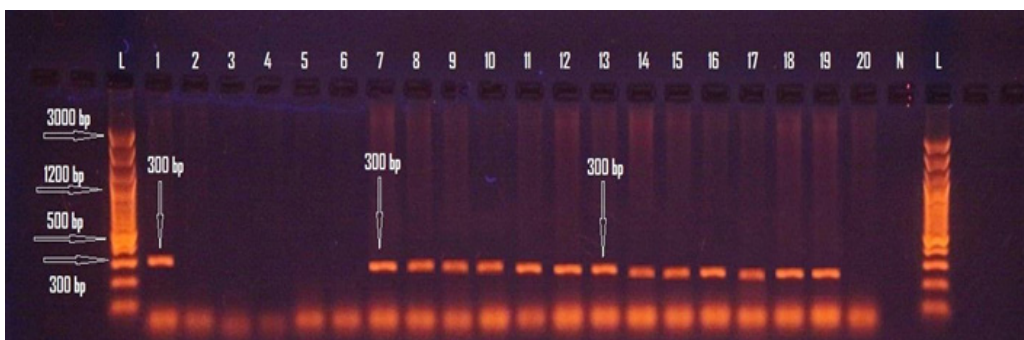


Figure 3) Gel electrophoresis products of the *qnrS* gene of *E. coli* with a 457 bp size; wells L: DNA marker, 1: positive control, N: negative control, and 7-19: positive samples in this study

Discussion

UTIs are among the major human infections worldwide, which are mainly (90%) caused by UPEC. In addition, UTIs affect 50% of all women [42]. Drug-resistant UPEC carrying virulence factors has been among the major causes of nosocomial infections and related deaths around the sphere in recent decades. Moreover, plasmid-encoded genes involved in resistance are responsible for the rapid spread of antibiotic resistance [43]. Plasmid-encoded *qnr* and ESBL genes remain as the furthestmost common agents of resistance to quinolones and β -lactams, respectively; however, resistance to other antibiotics has also recently increased dramatically [44, 45]. The majority of UTI-diagnosed patients in this study (68%, n=68) were female. This could be due to the structure and site of the urinary tract in women, which is near the anus channel. Also, most infections occur in individuals in the age range of 20 to 42 years, who have the most sexual activity. As a result, according to Rahn (2008) [46] regarding the prevalence of UTIs, age and sex are significant variables. The results showed that approximately 10.04% of UPEC isolates carried the *cnf-1* gene. However, more than half of them were ESBL producers and resistant to quinolones. A previous study inferred that 12.3% of ciprofloxacin-resistant UPEC carried the *cnf-1* gene, which is lower than the present study result [33]. The *cnf-1* gene encodes CNF-1, which is almost always associated with hemolysin and provokes actin stress fibers formation and cytoskeleton rearrangement. Additionally, it causes effacement of intestinal microvilli, increased permeability in polarized intestinal cell monolayers, apoptosis, and inflammation in murine model of UTI [33, 47, 48]. The isolates in the current investigation showed the highest resistance to nalidixic acid (70%, n=70) and the lowest resistance to chloramphenicol (30%, n=30). However,

because chloramphenicol is not commonly used in clinical practice, aztreonam may be a better option for UTIs. Moreover, the low chloramphenicol resistance in this study could be due to its minimal use in routine UTI treatments. Surprisingly, half of the UPEC isolates were quinolone-resistant, possibly due to uncontrolled consumption of antibiotics or previous hospitalization [49-51]. Relevantly, significant related risk factors in this study included older age (68%, n=68, p= .031), previous hospitalization (76%, n=76, p= .021), and urinary catheter (83%, n=83, p= .018). According to the findings, ESBL production was also high among UPEC isolates. However, the prevalence of ESBL-encoding genes was not investigated. ESBL and quinolone resistance gene carriage by *E. coli* isolates has created considerable health care costs due to the importance of target antibiotics [52]. Fluoroquinolone resistance by mutations in *gyr* genes such as *gyrA* is an important phenomenon which was not assessed in this study [53]. According to the results, the antibiotic sensitivity pattern of these pathogenic bacteria was significantly variable, and this may be due to the antibiotic consumption without a valid prescription or laboratory supervision, misuse, indiscriminate usage, differences in geographical location and bacterial strains, and the acquisition of resistance mechanisms [54-56]. We observed that older age (68%, n=68, p= .031), previous hospitalization (76%, n=76, p= .021), and urinary catheter (83%, n=83, p= .018) were significantly associated with isolation of *cnf-1*-producing UPEC. There are several studies regarding the main risk factors of isolation of drug-resistant bacterial isolates, such as older age, previous hospitalization, and consumption of antibiotics [57, 58]. Therefore, prior exposure to antibiotics is a substantial factor needing to be controlled. It was observed that the highest resistance rate to beta-lactams was related to ceftazidime (62%) with MIC \geq 4 μ g/mL. Additionally, the highest resistance rate to quinolones was related to ofloxacin (56%) with MIC \geq 16 μ g/mL. Moreover, ESBL production

was observed among 46% of *cnf-1*-carrying UPEC isolates during 2014-2022. In addition, the findings revealed that 50 out of 100 (50%, n=50) ESBL-bearing *E. coli* isolates carried *qnr* genes including *qnrA* (18%, n=18), *qnrB* (21%, n=21), and *qnrS* (11%, n=11). The rapid spread of plasmid-mediated resistance genes (such as those encoding *qnr* and mediated resistance genes (such as those encoding *qnr* and ESBLs) is a concern in the era of extensive drug resistance rates [59, 60]. Limitations of this study mainly included low sample size, limited study area, and lack of assessment of DNA mutations responsible to quinolone resistance, expression analysis of genes, and genetic typing.

Conclusions

Although the prevalence of *cnf-1* gene was not high among UPEC isolates, its prevalence was high among quinolone-resistant and ESBL-producing isolates. The quinolone-resistant, ESBL-producing, *cnf-1*-carrying UPEC isolates, mainly carrying the *qnrB* gene, had the highest rate of resistance to quinolones. The co-carriage of virulence determinants and plasmid-encoded resistance genes is a crisis considering the failure in infection eradication. We observed that more than half of the isolates were resistant to quinolones and third generation cephalosporins. Determining resistance mechanisms to common antibiotic classes facilitate the control of infections in any epidemiological area. Additionally, targeting enzyme proteins (using novel synthetic or natural drugs) is a promising approach after screening for prevalent drug-destroying bacterial enzymes. Considering the various possible mechanisms participating in this kind of resistance, more investigation is required to determine the rate and level of quinolone resistance in Iraq and to take rapid measures to prevent the spread of resistance genes to other nosocomial isolates.

Acknowledgements

The authors would like to express their gratitude to the University of Babylon DNA Research Center. Additionally, we would like to express our deepest gratitude to the hospital staff in Hillah, Babylon, Iraq for their contributions to this work.

Ethical permission: This study was performed after obtaining the approval of the Ethics Committee of the University of Babylon. There was no human or animal study.

Conflict of interest: None declared by authors.

Authors' contributions: H.Q.R. and L.A. conceptualized the study. M.A.A. and T.S.H. performed the work and wrote the draft.

Funding: None declared by authors.

Consent to participate: Consent was obtained from the ministry of health, Iraq, committee.

References

1. McDanel J, Schweizer M, Crabb V, Nelson R, Samore M, Khader K, et al. Incidence of extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* infections in the United States: A systematic literature review. *Infect Control Hosp Epidemiol.* 2017;38(10):1209-15.
2. Jiang X, Yu T, Wu N, Meng H, Shi L. Detection of *qnr*, *aac(6')-Ib-cr*, and *qepA* genes in *Escherichia coli* isolated from cooked meat products in Henan, China. *Int J Food Microbiol.* 2014;187:22-5.
3. Rodríguez-Martínez JM, Cano ME, Velasco C, Martínez-Martínez L, Pascual A. Plasmid-mediated quinolone resistance: An update. *J Infect Chemother.* 2011;17(2):149-82.
4. Ezzaroug Ezzraimi A, Hannachi N, Mariotti A, Rolain JM, Camoin-Jau L. Platelets and *Escherichia coli*: A complex interaction. *Biomedicines.* 2022;10(7)1636:.
5. Ramuta T, Tratnjek L, Janev A, Seme K, Starčić Erjavec M, Kreft ME. The antibacterial activity of human amniotic membrane against multidrug-resistant bacteria associated with urinary tract infections: New insights from normal and cancerous urothelial models. *Biomedicines.* 2021;9(2)218:.
6. Shah C, Baral R, Bartaula B, Shrestha LB. Virulence factors of uropathogenic *Escherichia coli* (UPEC) and correlation with antimicrobial resistance.

- BMC Microbiol. 2019;19(1):6-1.
7. Kaur H, Modgil V, Chaudhary N, Mohan B, Taneja N. Computational guided drug targets identification against extended-spectrum beta-lactamase-producing multi-drug resistant uropathogenic *Escherichia coli*. *Biomedicines*. 2023;11(7):2028.
 8. Holmbom M, Möller V, Kristinsdottir L, Nilsson M, Rashid MU, Fredrikson M, et al. Risk factors and outcome due to extended-spectrum β -lactamase-producing uropathogenic *Escherichia coli* in community-onset bloodstream infections: A ten-year cohort study in Sweden. *PLoS One*. 2022;17(11):e0277054.
 9. Gatyia Al-Mayahie SM, Al-Guranie DRT, Hussein AA, Bachai ZA. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic *Escherichia coli* phylogroup B2 isolates from outpatients in Wasit province, Iraq. *PLoS One*. 2022;17(1):e0262984.
 10. Tseng CH, Liu CW, Liu PY. Extended-spectrum β -lactamases (ESBL) producing bacteria in animals. *Antibiotics*. 2023;12(4):661.
 11. Karlowsky JA, Lob SH, DeRyke CA, Siddiqui F, Young K, Motyl MR, et al. Prevalence of ESBL non-CRE *Escherichia coli* and *Klebsiella pneumoniae* among clinical isolates collected by the SMART global surveillance programme from 2015 to 2019. *Int J Antimicrob Agents*. 2022;59(3):106535.
 12. Palmeira JD, Ferreira HMN. Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* in cattle production—a threat around the world. *Heliyon*. 2020;6(1):e03206.
 13. Ortiz-Díez G, Mengíbar RL, Turrientes MC, Artigao MR, Gallifa RL, Tello AM, et al. Prevalence, incidence, and risk factors for acquisition and colonization of extended-spectrum beta-lactamase- and carbapenemase-producing *Enterobacteriaceae* from dogs attended at a veterinary hospital in Spain. *Comp Immunol Microbiol Infect Dis*. 2023;92:101922.
 14. Lima LS, Proietti-Junior AA, Rodrigues YC, da Silva Vieira MC, Lima L, de Oliveira Souza C, et al. High genetic diversity and antimicrobial resistance in *Escherichia coli* highlight *Arapaima gigas* (Pisces: *Arapaimidae*) as a reservoir of quinolone-resistant strains in Brazilian Amazon rivers. *Microorganisms*. 2022;10(4):808.
 15. Briales A, Rodríguez-Martínez JM, Velasco C, de Alba PD, Rodríguez-Baño J, Martínez-Martínez L, et al. Prevalence of plasmid-mediated quinolone resistance determinants *qnr* and *aac(6')-Ib-cr* in *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β -lactamases in Spain. *Int J Antimicrob Agents*. 2012;39(5):431-4.
 16. Kariuki K, Diakhate MM, Musembi S, Tornberg-Belanger SN, Rwigy D, Mutuma T, et al. Plasmid-mediated quinolone resistance genes detected in ciprofloxacin non-susceptible *Escherichia coli* and *Klebsiella* isolated from children under five years at hospital discharge, Kenya. *BMC Microbiol*. 2023;23(1):129.
 17. Jacoby GA, Strahilevitz J, Hooper DC. Plasmid-mediated quinolone resistance. *Microbiol Spectr*. 2014;2(5):10.
 18. Akshay SD, Nayak S, Deekshit VK, Rohit A, Maiti B. Differential expression of outer membrane proteins and quinolone resistance determining region mutations can lead to ciprofloxacin resistance in *Salmonella Typhi*. *Arch Microbiol*. 2023;205(4):136.
 19. Rodríguez-Martínez JM, Machuca J, Cano ME, Calvo J, Martínez-Martínez L, Pascual A. Plasmid-mediated quinolone resistance: Two decades on. *Drug Resist Updat*. 2016;29:13-29.
 20. Aldred KJ, Kerns RJ, Osheroff N. Mechanism of quinolone action and resistance. *Biochemistry*. 2014;53(10):1565-74.
 21. Doma AO, Popescu R, Mitulețu M, Muntean D, Dégi J, Boldea MV, et al. Comparative evaluation of *qnrA*, *qnrB*, and *qnrS* genes in *Enterobacteriaceae* ciprofloxacin-resistant cases, in swine units and a hospital from western Romania. *Antibiotics*. 2020;9(10):698.
 22. Amador P, Fernandes R, Prudêncio C, Duarte I. Prevalence of antibiotic resistance genes in multidrug-resistant *Enterobacteriaceae* on Portuguese livestock manure. *Antibiotics (Basel)*. 2019;8(1):23.
 23. Aworh MK, Kwaga JK, Hendriksen RS, Okolocha EC, Harrell E, Thakur S. Quinolone-resistant *Escherichia coli* at the interface between humans, poultry, and their shared environment—a potential public health risk. *One Health Outlook*. 2023;5(1):1-16.
 24. Ruiz E, Sáenz Y, Zarazaga M, Rocha-Gracia R, Martínez-Martínez L, Arlet G, et al. *qnr*, *aac(6')-Ib-cr*, and *qepA* genes in *Escherichia coli* and *Klebsiella* spp.: Genetic environments and plasmid and chromosomal location. *J Antimicrob Chemother*. 2012;67(4):886-97.
 25. Mohamed Ali I, Duman C, Bozdağ İ, Artan Abdi A, Nor Abdi M, Karakurt SE, et al. Microbiology and drug susceptibility pattern of bacterial isolates from patients with chronic suppurative otitis media at a tertiary care hospital in Somalia. *Infect Drug Resist*. 2022;15:7733-9.
 26. Sora VM, Meroni G, Martino PA, Soggiu A, Bonizzi L, Zecconi A. Extraintestinal pathogenic *Escherichia coli*: Virulence factors and antibiotic resistance. *Pathogens*. 2021;10(11):1355.
 27. Raimondi S, Righini L, Candelieri F, Musmeci

- E, Bonvicini F, Gentilomi G, et al. Antibiotic resistance, virulence factors, phenotyping, and genotyping of *E. coli* isolated from the feces of healthy subjects. *Microorganisms*. 2019;7(8):251.
28. Wang MH, Kim KS. Cytotoxic necrotizing factor 1 contributes to *Escherichia coli* meningitis. *Toxins (Basel)*. 2013;5(11):2270-80.
 29. Li XZ. Quinolone resistance in bacteria: Emphasis on plasmid-mediated mechanisms. *Int J Antimicrob Agents*. 2005;25(6):453-63.
 30. Herrera-Vázquez A, Arellano-Aranda R, Hernández-Cueto D, Rodríguez-Miranda E, López-Briones S, Hernández-Luna MA. Detection of cyclomodulin CNF-1 toxin-producing strains of *Escherichia coli* in pig kidneys at a slaughterhouse. *Microorganisms*. 2023;11(8):2065.
 31. Alhadidi HA, Al-Qaysi SA, Al-Halbosiy MM. Prevalence and cytotoxic effects of some colibactin and *cnf* genes among *Escherichia coli* isolated from urinary tract infections. 2022;50(2):283-92.
 32. Chat H, Dalmasso G, Godfraind C, Bonnin V, Beyrouthy R, Bonnet M, et al. Cytotoxic necrotizing factor 1 hinders colon tumorigenesis induced by colibactin-producing *Escherichia coli* in *ApcMin/+* mice. *Gut Microbes*. 2023;15(1):2229569.
 33. Harwalkar A, Gupta S, Rao A, Srinivasa H. Lower prevalence of *hlyD*, *papC*, and *cnf-1* genes in ciprofloxacin-resistant uropathogenic *Escherichia coli* than their susceptible counterparts isolated from southern India. *J Infect Public Health*. 2014;7(5):413-9.
 34. Da Silva GJ, Mendonça N. Association between antimicrobial resistance and virulence in *Escherichia coli*. *Virulence*. 2012;3(1):18-28.
 35. Marin J, Clermont O, Royer G, Mercier-Darty M, Decousser JW, Tenaillon O, et al. The population genomics of increased virulence and antibiotic resistance in human commensal *Escherichia coli* over 30 years in France. *Appl Environ Microbiol*. 2022;88(15):e00664-22.
 36. Biggel M, Moons P, Nguyen MN, Goossens H, Van Puyvelde S. Convergence of virulence and antimicrobial resistance in increasingly prevalent *Escherichia coli* ST131 *papGII+* sublineages. *Commun Biol*. 2022;5(1):752.
 37. Jia Y, Mao W, Liu B, Zhang S, Cao J, Xu X. Study on the drug resistance and pathogenicity of *Escherichia coli* isolated from calf diarrhea and the distribution of virulence genes and antimicrobial resistance genes. *Front Microbiol*. 2022;13:992111.
 38. Guiral E, Bosch J, Vila J, Soto SM. Prevalence of *Escherichia coli* among samples collected from the genital tract in pregnant and nonpregnant women: Relationship with virulence. *FEMS Microbiol Lett*. 2011;314(2):170-3.
 39. Sfeir MM. Adoption of the updated CLSI fluoroquinolone breakpoints for Gram-negative bacteria in microbiology laboratories. *Clin Microbiol Infect*. 2021;27(2):308-10.
 40. Quesada MD, Giménez M, Molinos S, Fernández G, Sánchez MD, Ravelo R, et al. Performance of VITEK-2 compact and overnight microscan panels for direct identification and susceptibility testing of Gram-negative bacilli from positive FAN BacT/ALERT blood culture bottles. *Clin Microbiol Infect*. 2010;16(2):137-40.
 41. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother*. 2007;60(2):394-7.
 42. Azam MW, Zarrilli R, Khan AU. Updates on the virulence factors produced by multidrug-resistant Enterobacterales and strategies to control their infections. *Microorganisms*. 2023;11(8):1901.
 43. Akiyama T, Khan AA. Molecular characterization of strains of fluoroquinolone-resistant *Salmonella enterica* serovar Schwarzengrund carrying multidrug resistance isolated from imported foods. *J Antimicrob Chemother*. 2012;67(1):101-10.
 44. Chong Y, Shimoda S, Shimono N. Current epidemiology, genetic evolution, and clinical impact of extended-spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*. *Infect Genet Evol*. 2018;61:185-8.
 45. Onishi R, Shigemura K, Osawa K, Yang YM, Maeda K, Tanimoto H, et al. Impact on quinolone resistance of plasmid-mediated quinolone resistance gene and mutations in quinolone resistance-determining regions in extended spectrum beta lactamase-producing *Klebsiella pneumoniae* isolated from urinary tract infection patients. *Pathog Dis*. 2022;80(1):ftac030.
 46. Rahn DD. Urinary tract infections: Contemporary management. *Urol Nurs*. 2008;28(5):333-41.
 47. Rippere-Lampe KE, O'Brien AD, Conran R, Lockman HA. Mutation of the gene encoding cytotoxic necrotizing factor type 1 (*cnf 1*) attenuates the virulence of uropathogenic *Escherichia coli*. *Infect Immun*. 2001;69(6):3954-64.
 48. Morgan RN, Saleh SE, Farrag HA, Aboulwafa MM. Prevalence and pathologic effects of colibactin and cytotoxic necrotizing factor-1 (*Cnf 1*) in *Escherichia coli*: Experimental and bioinformatics analyses. *Gut Pathog*. 2019;11:1-

- 18.
49. Dolejska M, Papagiannitsis CC. Plasmid-mediated resistance is going wild. *Plasmid*. 2018;99:99-111.
 50. Alawi M, Torrijos TV, Walsh F. Plasmid-mediated antimicrobial resistance in drinking water. *Environ Adv*. 2022;8:100191.
 51. Meng M, Li Y, Yao H. Plasmid-mediated transfer of antibiotic resistance genes in soil. *Antibiotics*. 2022;11(4):525.
 52. Azargun R, Sadeghi MR, Soroush Barhaghi MH, Samadi Kafil H, Yeganeh F, Ahangar Oskouee M, et al. The prevalence of plasmid-mediated quinolone resistance and ESBL-production in Enterobacteriaceae isolated from urinary tract infections. *Infect Drug Resist*. 2018;11:1007-14.
 53. Liang H, Zhang J, Hu J, Li X, Li B. Fluoroquinolone residues in the environment rapidly induce heritable fluoroquinolone resistance in *Escherichia coli*. *Environ Sci Technol*. 2023;57(12):4784-95.
 54. Allou N, Cambau E, Massias L, Chau F, Fantin B. Impact of low-level resistance to fluoroquinolones due to *qnrA1* and *qnrS1* genes or a *gyrA* mutation on ciprofloxacin bactericidal activity in a murine model of *Escherichia coli* urinary tract infection. *Antimicrob Agents Chemother*. 2009;53(10):4292-7.
 55. Firoozeh F, Zibaei M, Soleimani-Asl Y. Detection of plasmid-mediated *qnr* genes among the quinolone-resistant *Escherichia coli* isolates in Iran. *J Infect Dev Ctries*. 2014;8(07):818-22.
 56. Ghasemian A, Mobarez AM, Peerayeh SN, Abadi AB. The association of surface adhesin genes and the biofilm formation among *Klebsiella oxytoca* clinical isolates. *New Microbes New Infect*. 2019;27:36-9.
 57. Al Hamdan AS, Alghamdi AA, Alyousif GF, Hamza FA, Shafey MM, AlAmri AM, et al. Evaluating the prevalence and the risk factors of Gram-negative multi-drug resistant bacteria in eastern Saudi Arabia. *Infect Drug Resist*. 2022;15:475-90.
 58. Yalew GT, Muthupandian S, Hagos K, Negash L, Venkatraman G, Hagos YM, et al. Prevalence of bacterial vaginosis and aerobic vaginitis and their associated risk factors among pregnant women from northern Ethiopia: A cross-sectional study. *PLoS One*. 2022;17(2):e0262692.
 59. San Millan A. Evolution of plasmid-mediated antibiotic resistance in the clinical context. *Trends Microbiol*. 2018;26(12):978-85.
 60. DelaFuente J, Toribio-Celestino L, Santos-Lopez A, León-Sampedro R, Alonso-Del Valle A, Costas C, et al. Within-patient evolution of plasmid-mediated antimicrobial resistance. *Nat Ecol Evol*. 2022;6(12):1980-91.