Prevalence of the fluoroquinolone resistance genes in the *Escherichia coli* sequence type 131 clone isolated from hospitalized patients with urinary tract infection

Running Title: Fluoroquinolone resistance genes in the *Escherichia coli* sequence type 131 clone

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Abstract

Background: The emergence of fluoroquinolones (FQ) resistance in the *Escherichia coli* (*E. coli*) sequence type 131 (ST131) has become a major challenge in the management of urinary tract infections (UTI). Chromosomal mutations and plasmid-mediated quinolone resistance (PMQR) determinants play an important role in the FQ resistance.

Methods: This cross-sectional study was conducted in 2020 on 300 urine samples. Aimed to investigate the prevalence of the chromosomal mutations, the PMQR genes including *qnr*, *aac* (*6′*)-*Ib-cr*, and efflux pumps among the FQ-resistant ST131 and non-ST131 *E. coli* causing UTI. Initially, the ST131 clone was detected using allele-specific PCR and confirmed by multilocus sequence typing.

Results: Among 95 FQ-resistant *E. coli* isolates, 29 (30%) as belonging to the ST131 clone. The most frequently detected PMQR genes in FQ-resistant isolates were *aac (6)'-lb-cr* and *qnr*S. However, statistical analysis revealed a stronger association between *aac (6')-Ib-cr* and the ST131 clone (62%; p<0.03). The *oqx*A gene was the most prevalent efflux pump gene observed in both ST131 (n=11; 38%) and non-ST131 (n=16; 24%) isolates. Analysis of the *gyr*A and *par*C genes revealed significantly higher in ST131 compared to non-ST131. Double mutations, S80I+E84V, were significantly more prevalent in both *gyr*A (76% ST131 vs. 43% non-ST131; p=0.004) and *par*C (55% ST131 vs. 26% non-ST131; p=0.002). Notably, the substitution E84G was exclusive to non-ST131 isolates (n=4). High-level resistance (MIC ≥32 μg/mL) was observed in 96.6% (28/29) of ST131 isolates compared to 65% (43/66) of non-ST131 isolates.

Conclusion: The double mutations confer a high level resistance to FQs in the ST131 clone. These findings on resistance mechanisms can guide infection control strategies.

Keywords: Mutation, Plasmid, Drug resistance, Quinolones, Fluoroquinolones

Introduction

Fluoroquinolones (FQs) like ciprofloxacin (CIP) and levofloxacin (LEV) are broad-spectrum antimicrobial to treat a wide range of hospital and community-acquired infections such as urinary tract infections (UTIs) (1, 2). The extensive use of antibiotics, particularly FQs, has inadvertently driven the selection and proliferation of multidrug-resistant (MDR) strains like *Escherichia coli* (*E. coli*) sequence type 131 (ST131). The ST131, a pandemic clone linked to the spread of extended-spectrum beta-lactamases (ESBLs), has emerged as a global threat to public health (3). The ST131 exhibits multi-resistance to a broad spectrum of antibiotics including aminoglycosides, cephalosporins, and FQs. The ST131 is the most common MDR high-risk clone in UTIs worldwide. The emergence and spread of the MDR *E. coli* ST131 clone, particularly resistant to FQs, is a major concern worldwide (4, 5). Chromosomal mutations in the quinolone resistance-determining regions (QRDRs) of DNA gyrase and topoisomerase IV- necessary enzymes for bacterial DNA reproduction and repair- are the key mechanisms of quinolone resistance including nalidixic acid and reduced susceptibility to FQs. These mutations can allow bacteria to produce enzymes that destroy FQs or prevent them from entering bacterial cells (6, 7). The plasmid-mediated quinolone resistance (PMQR) is another common mechanism contributing to quinolones and FQs resistance among gram-negative bacilli. The PMQR based on their mechanism categorized into three classes including various qnr allels such as *qnr*A, *qnr*B, and *qnr*S, efflux pump genes QepA and OqxAB, and avarient of aminoglycoside amino transferase as aac-(6′)-Ib-cr (8-10). FQ-resistant bacteria can use efflux pumps to transport FQ out of their cells. The aim of this study was the finding of the ST131 clone in the FQ-resistant *E. coli* isolates from patients with UTI. Additionally, it investigated mutations in the DNA gyrase and topoisomerase IV genes, as well as the presence of the PMQR and efflux pumps genes, using polymerase chain reaction (PCR) in the FQ-resistant *E. coli* isolates.

Methods

Bacterial isolates

A total of 250 *E. coli* isolates were obtained from the urine of hospitalized patients with UTI in hospital wards in Tehran, Iran, in 2020. Standard bacteriological methods were employed for the identification of these *E. coli* isolates (11). Before participating in the research, each patient or their parent/guardian providing written informed consent and gave an explanation regarding the study's purpose.

Susceptibility of the *E. coli* isolates against FQs

The Kirby-Bauer disk diffusion technique was employed for the determination of the susceptibility of the *E. coli* isolates against antibiotic disks (MAST, England) including nalidixic acid (NAL; 30 µg), ciprofloxacin (CIP; 5 µg) according to the CLSI guidelines (12). Then, the minimum inhibitory concentration (MIC) of the CIP was determined by the E-test. The CLSI breakpoints for CIP were susceptible to ≤ 1 µg/mL; and resistant to≥ 4 µg/mL). The *E. coli* ATCC 25922 and *K. pneumonia* were used as quality control strains.

DNA extraction

The genomic DNA of *E. coli* isolates from pure culture was extracted by boiling method and stored at -20°C until use. The quality of extracted DNA was proved by NanoDrop (Thermo Scientific, Roskilde, Denmark). The absorbance ratio at 260/280 nm above 1.7 has an acceptable purity (13).

Screening of the ST131 clone

After DNA extraction of *E. coli* isolates, the ST131 clone was screened through the ST131-specific sequence polymorphisms in the *mdh* and *gyrB* (14). The O25b-ST131 variant was detected using allele-specific PCR targeting the *pabB* gene (4). The *trpA* gene was amplified as an internal control of the PCR reactions (15). The Multilocus Sequence Typing (MLST) system was employed for the confirmation of the ST131 clone using seven housekeeping genes including *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*

(http://enterobase.warwick.ac.uk/ species/ecoli/allele_st_search). Table 1 lists the sequences of the primers used in this study.

Detection of chromosomal mutations in the QRDRs

The mutations within the *gyrA* and *parC* genes of the FQ-resistant isolates were identified through amplification and sequencing of the QRDRs (16). First, the *gyrA* and *parC* genes were amplified by PCR using primers in the Table 1. Eventfully, sequencing of the PCR products was done with an ABI 3730XL DNA analyzer (Macrogen Inc., Korea). Nucleotide sequences were compared with the reference sequences using BLAST tools of the National Center for Biotechnology Information GenBank database; (NCBI; http://www.ncbi.nlm.nih.gov/blast).

Detection of the PMQR genes

Detection of the PMQR genes *qnrA, qnrB, qnrS, qnrC, qnrD,* and *aac (6')-Ib-cr* was accomplished via previously described PCR methods (17-20).

Detection of the efflux pump genes

The efflux pump-encoding genes *oqx*A, *oqx*B, and *qep*A were detected by PCR using specific primers as described previously (20, 21).

In PCR process, isolates with target gene confirmed by sequencing used as positive control and the isolates without the target gene used as negative control.

Statistical analysis

The statistical analysis of the data was carried out by the R software version 3.3.3 and interpreted based on the prevalence distribution and percentage. Data with a p-value less than or equal to 0.05 were regarded as statistically significant.

Ethical statement

The Islamic Azad University, Jahrom Branch, Iran ethically approved this study.

Results

Distribution of the FQ resistance in the *E. coli* isolates

In this study, 200 *E. coli* isolated from the patients with ages range from 15 to 85 years. Female patients accounted for 57.8% (n=115/200), compared to 42% (n=85/200) in the males. Of the 200 *E. coli* isolates, 47.5% (n=95/200) were resistant to both NA and CIP, categorizing them as FQ-resistant. Approximately 75% (n=71/95) of the *E. coli* isolates had CIP MIC ≥32 µg/mL, with 25% of isolates (n=24/95) having MIC 4-6 µg/mL. Among the 95 FQ-resistant *E. coli* isolates, 30% (n=29/95) were screened as the ST131 clone using PCR assay. All the screened ST131 isolates belonged to the O25 serotype, also known as the O25-ST131 clone. High-level CIP resistance (MIC ≥ 32 mg/L) was found in 96.5% (n=28/29) of the ST131 and 65% (n=43/66) of the non-ST131 isolates (p=0.001). The remaining 24 FQ- resistant isolates exhibited low-level CIP resistance (MIC 4-16 µg/mL).

Mutations in the *gyr*A and the *par*C

DNA sequence analysis of the QRDR of *gyr*A and *par*C shown in table 2 revealed the FQ-resistant *E. coli* isolates had two mutation types in the *gyr*A and three in the *par*C. The point mutations in the *gyr*A occurred at positions 83 (Serine →Leucine) and 87 (Aspartic *acid* → Asparagine). In the *par*C, the mutations were found at positions 80 (Serine→ Isoleucine), 84 (Glutamic acid →Valine), and 84 (Glutamic acid → glycine). Single mutations S83L and D87N in the *gyr*A were found in 20% (n=19), and 11.5% (n=11) of the FQ-resistant isolates, respectively. Single mutations identified in the *par*C were S80I, E84V, and E84G, occurring in 10.5% (n=10), 23% (n=22), and 4% (n=4) of the FQ-resistant isolates, respectively. Mutations in the *gyr*A (single or double) within QRDRs were the most frequently observed in 96% (n=28/29) of the ST131 and 82% (n=54/66) of the non-ST131 isolates (p=0.05). Double amino acid substitutions in the *gyr*A and *par*C were identified in 89% (n=85/95) of FQ-resistant isolates, so these substitutions were observed in 53.6% (n=51/95) at the *gyr*A gene compared to 35.6% (n=34/95) at the *par*C gene (p= 0.01). Comparative analysis revealed a significant difference in the presence of

double mutations, S80I+E84V in the *gyr*A (76% ST131 vs. 43% non-ST131; p=0.004), and S80I+ E84V in the *par*C, (55% ST131 vs. 26% non-ST131; p=0.002). Isolates with the double mutation exhibited significantly higher levels of CIP resistance (p≤0.05). Among the FQ-resistant isolates, six non-ST131 isolates had a wild-type QRDR, while the remaining isolates (n=89/95, 93.6%) had amino exchanges in one or both the *gyr*A or the *par*C genes.

Prevalence of the resistance PMQR genes and *aac(6)'-lb-cr* variant

Table 2 shows the prevalence of resistance genes to FQ in FQ-resistant *E. coli* isolates, 74.7% (n=71/95) isolates possessed at least one PMQR gene. The more common PMQR gene was the *qnr*S, being found in 42% (n=40/95) *E. coli* isolates, followed by *aac(6')-Ib-cr* (n=35/95; 36%), *gnr*B (n=15/95; 16%), *qnr*C (n=5/95; 5.2%), and *qnr*D (n=1/95; 1%). *The qnr*A gene was not found in any of the FQ-resistant *E. coli* isolates. The *aac(6)'-lb-cr* and *qnr*S were the most frequently observed PMQR genes in the ST131 (n=16/29, 55%) and non-ST131 isolates (n=28/66, 42%). Statistical analysis indicated a significant relationship between the presence of the *aac(6')-Ib-cr* with the ST131 clone compared with non-ST131 (p<0.03).

Twelve ST131 isolates carried more than one PMQR gene in the following combinations: *qnr*S+*qnr*B (n=2), *qnr*S+*qnr*D (n=1), *qnr*S+*aac(6)'-lb-cr* (n=5), *qnr*S+*qnr*B+*aac(6)'-lb-cr* (n=2), and *qnr*S+*qnr*C+ *aac(6)'-lb-cr* (n=2). Whereas, the combination of the PMQR genes in 18 non-ST131 was as follows: *qnr*S+*qnr*B (n=7), *qnrB+aac(6)'-lb-cr*(n=2), *qnr*S+*aac(6)'-lb-cr* (n=4), *qnr*B+*aac(6)'-lb-cr* (n=3), *qnr*S+*qnr*B+*aac(6)'-lb-cr* (n=1), *qnr*S+*qnr*C+*aac(6)'-lb-cr* (n=1).

Prevalence of the efflux pump genes

In FQ-resistant *E. coli* isolates, 37% (n=35/95) were positive for at least one of three efflux pump genes: *qep*A (n=11/95, 11.5%), *oqx*A (n=27/95, 28%), and *oqx*B (n=13/95, 13.5%) genes. Notably, the *oqx*A had the highest prevalence in both ST131 (n=11/29; 38%) and non-ST131 (n=16/66; 24%) isolates.

Discussion

The FQs are a class of antibiotics commonly used to treat UTI. The rise and distribution of FQ-resistant E. coli strains-especially the ST131 clone-represent a major public health concern due to their strong capacity for widespread, international dissemination (22, 23). This study screened the prevalence of the *E. coli* ST131 and FQ resistance genes in patients with UTIs.

Identifying how common these resistance determinants are and how they are distributed is essential for selecting suitable antibiotics and strengthening infection-control strategies. In the current study, the FQ resistance rate in the *E. coli* isolates exceeded 47.5%. Damavandi et al observed 48% FQ resistance in the *E. coli* isolates obtained from the inpatients which is very close to the present research (24). The prevalence of FQ resistance varies across different countries, with rates reported as, 20-30% in Turkey, 62.25% in China, 51.8% in Pakistan, 55.6% in Iran (25-28). Variations in reported FQ resistance rates among E. coli isolates can be attributed to factors such as local antibiotic-prescribing habits, infection-control quality, and circulation of high-risk lineages like ST131.(29). Our study found that the ST131 clone accounted for 30% (n=29 / 95) of FQ-resistant isolates, regardless of whether the isolates produced extended-spectrum beta-lactamases (ESBLs). Raoulinasab et al. reported that among FQ-resistant isolates producing ESBLs, 55% (n=30/60) belonged to the ST131 clone (30).

The FQ antibiotics exert their bactericidal effect by inhibiting DNA gyrase and topoisomerase IV, enzymes essential for bacterial DNA replication. However, the primary target for these antibiotics can vary depending on the bacterial species and the type of FQs used (31, 32). This study demonstrated a significantly higher prevalence of mutations in the *gyrA* gene compared to the *parC* gene. Moreover, the double mutations within *gyrA* (53.6%) were also significantly more frequent than double mutations in the *parC* (35.6%; p=0.01). Research evaluating E. coli isolates carrying mutations in both enzymes indicates that DNA gyrase is typically the main target of fluoroquinolones and is more readily inhibited by these drugs. Topoisomerase IV appears to play a secondary role. Consequently, mutations in the *gyrA*

gene, which codes for the gyrase A subunit, often confer initial resistance to FQs. In contrast, mutations in the *parC*, encoding topoisomerase IV subunits, typically arise later during the development of multidrug resistance (33). There are few reports about evaluating of the FQ-resistance genes in the ST131 clone (30, 34, 35). The current study showed a significantly higher frequency of double mutation in the *gyrA* (S83L and D87N) and *parC* (S80I and E84V) among the ST131 isolates compared to the non-ST131 isolates. In a study conducted in Iran, a significant frequency (80%) of double mutations in the *parC* was observed in the ST131 isolates (30). The emergence of double mutations in the ST131 clone highlights the urgent need for comprehensive strategies to prevent the spread of these extensively drug-resistant strains, including rigorous surveillance, infection control protocols, and antibiotic stewardship programs. The substitution E84G in the *parC* was not observed in any ST131 isolates, consistent with the findings of previous studies (30, 34). Chromosomal mutations lead to alterations in the topoisomerase enzymes, rendering them less susceptible to the inhibitory effects of ciprofloxacin. In the present study, the E-test MIC experiments revealed high-level ciprofloxacin resistance (MIC ≥32 µg/mL) in 96.6% (28/29) of the ST131 compared to 65% (43/66) of the non-ST131 isolate which this difference was statistically significant (p=0.001). The higher frequency of double mutations in the *gyr*A and *parC* genes within ST131 isolates may explain this disparity. Consistent with our findings, a previous study from Iran reported that all ST131 isolates exhibited MIC ≥32 µg/mL to ciprofloxacin (30). Interestingly, six non-ST131 bacteria with wild-type QRDR had low levels of ciprofloxacin resistance in our study.

The PMQR genes are another FQ resistance mechanism. Among the FQ-resistant *E. coli* isolates, 74.7% harbored at least one PMQR gene *qnr* and *aac(6)'-lb-cr* . The *qnr*S gene emerged as the most prevalent in 63% of FQ-resistant isolates. The *aac(6)'-lb-cr* and *qnr*S was the most frequently occurring PMQR gene in the ST131 (n=16, 55%) and non-ST131 isolates (n=28, 42%). Statistical analysis indicated a significant relationship between the presence of the *aac(6')-Ib-cr* with the ST131 clone (62%; p<0.03). In a study in Mexico similar to our study, the O25-ST131 linage 100% (n=14/14) isolates had *aac(6')-lb-cr*; and 7% (n=1/14) had *qnr*A1 (35). The significant association (p<0.03) of the *aac(6')-Ib-cr* gene with the ST131 lineage in this study suggests a potential role for this gene in FQ resistance. Further research is needed to elucidate the specific contribution of the *aac(6')-Ib-cr* to fluoroquinolone resistance in the ST131. The aac(6′)-Ib-cr enzyme as a bifunctional acetyltransferase can modify aminoglycosides and quinolones simultaneously, conferring resistance to clinically relevant aminoglycosides (36, 37).

In these multidrug-resistant (MDR) bacteria, efflux pumps work overtime to push antibiotics out of the cell, lowering the concentration of the drug inside and making the bacteria less susceptible (38). The spread of efflux pump genes like *oqx*AB among bacteria raises serious concerns for public health (39). Our results showed that 37% of FQ-resistant isolates were positive for at least one *oqx*A, *oqx*A, and *oqx*B gene, with *oqx*A being the most prevalent efflux pump gene (28%). The ST131 isolates exhibited a higher prevalence of *oqx*A and *oqxB* genes compared to non-ST131 isolates, however, this difference was not statistically significant (p>0.05). In another study in Iran, the most common FQ resistance efflux pumps genes were *oqxB* (34%), followed by *oqxA* (25%), and *qnrB* (18%) (40). The results alert for the continuous monitoring of quinolone resistance determinants in order to minimize the emergence and selection of high risk *E. coli* clones showing reduced susceptibility or resistance to quinolones.

The limitation of our study come with more surveys which include different resistance genes or and more isolates by different laboratory methods.

Conclusions

This study revealed a worrying prevalence of FQ-resistant *E. coli*, particularly within the ST131 clone, highlighting the need for continuous monitoring to inform infection control strategies and minimize the spread of these resistant strains. The high frequency of the mutations in the *gyr*A and the presence of PMQR genes like *aac(6')-Ib-cr* were alarming for FQ resistance. In addition, the significant association

between the double mutations and the *aac(6')-Ib-cr* gene with the ST131 lineage warrants further investigation in resistance development.

Conflict of interest

The authors declare they have no conflicts of interest.

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Author contribution

Mehdi Bozorgi Mazandarani, Mohammad Kargar, Farshid Kafilzadeh have supervised the study, collected samples, performed the work, and written and edited the manuscript.

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Ethical statement

This study was approved by the Islamic Azad University, Jahrom Branch, Iran (NO. p-162376730).

Data availability statement

No new data were created or analyzed in this study. Data sharing does not apply to this article.

Table 1: Primers used to detect the ST131 clone and fluoroquinolone resistance genes

Target gene	Primer	Sequence (5´- 3´)	Amplicon size (bp)	Annealing temp (ºC)	Reference
*pab*B	O25pab-Bspe.F	TCCAGCAGGTGCTGGATCGT	347	63	(4)
	O25pab-Bspe.R	GCGAAATTTTTCGCCGTACTGT			
*trp*A	TrpA1.F	GCTACGAATCTCTGTTTGCC	427	63	(15)
	TrpA1.R	GCAACGCGGCCTGGCGGAAG			
rfbO25b	gndbis.F	ATACCGACGACGCCGATCTG	300	60	(41)
	rfbO25b.R	TGCTATTCATTATGCGCAGC			
*qnr*A	QnrA.F	AGAGGATTTCTCACGCCAGG	619	57	(17)
	QnrA.R	GCAGCACTATKACTCCCAAGG			
*qnr*B	QnrB.F	GGMATHGAAATTCGCCACTG	264	57	(17)
	QnrB.R	TTTGCYGYYCGCCAGTCGAA			
*qnr*S	QnrS.F	GCAAGTTCATTGAACAGGCT	428	57	(17)
	QnrS.R	TCTAAACCGTCGAGTTCGGCG			
*qnr*C	QnrC.F	GGGTTGTACATTTATTGAATC	447	58	(20)
	QnrC.R	TCCACTTTACGAGGTTCT			
*qnr*D	QnrD.F	CGAGATCAATTTACGGGGAATA	582	58	(18)
	QnrD.R	AACAAGCTGAAGCGCCTG			
*oqx*A	OqxA.F	GACAGCGTCGCACAGAATG	339	61	(21)
	OqxA.R	GGAGACGAGGTTGGTATGGA			
*oqx*B	OqxA.F	CGAAGAAAGACCTCCCTACCC	240	61	(21)
	OqxA.R	CGCCGCCAATGAGATACA			
*qep*A	QepA.F	GCAGGTCCAGCAGCGGGTAG	218	60	(20)
	QepA.R	CTTCCTGCCCGAGTATCGTG			
aac(6')Ib-cr	AAC.F	ATGACTGAGCATGACCTTGC	519	55	(19)
	AAC.R	TTAGGCATCACTGCGTGTTC			
*par*C	ParC.F	TGCGTTGCCGTTTATTGG	470	56	(16)
	ParC.R	GCAGGTTATGCGGTGGAAT			
*gyr*A	GyrA.F	GCGATGTCGGTCATTGTT	490	56	(16)
	GyrA.R	ACTTCCGTCAGGTTGTGC			

bp, Base Pair; F, Forward; R, Reverse.

Table 2. Prevalence of the FQ resistance genes in the FQ-resistant *E. coli* isolates

n (%) of the FQ-R genes in the *E. coli* isolates			
Resistance genes	**ST131 clone; n=29**	**Non-ST131 clone; n=66**	**p-value***
Mutations in the QRDR			
Mutation in *gyr*A			
S83L: Ser83Leu	4 (10)	16 (24)	0.19
D87N: Asp87Asn	2 (6.8)	9 (13)	0.28
S83L+ D87N	22 (75)	29 (43)	0.004
Mutation in *par*C			
S80I: Ser80Ile	2 (7)	8 (14)	0.35
E84V: Glu84Valin	4 (3)	18 (27)	0.11
E84G: Glu84Gly	0	4 (6)	0.51
S80I+ E84V	16 (55)	15 (26)	0.004
S80I+ E84G	0	3 (4.5)	0.48
PMQR genes			
Quinolone-encoding *qnr* and *aac(6')-Ib-cr* genes			
*qnr*S	12 (41)	28 (42)	0.55
*qnr*B	4 (13.7)	11 (16.6)	0.47
*qnr*C	3 (10%)	2 (3)	0.16
*qnr*D	1 (%)	0	0.52
aac(6')-Ib-cr	16 (55)	19 (28)	0.02
Efflux pumps-encoding genes			
*qep*A	3 (10%)	8 (12%)	0.55
*oqx*A	11 (38%)	16 (24%)	0.13
*oqx*B	6 (20%)	7 (10%)	0.16

n: Number; FQ-R: Fluoroquinolone resistance; QRDR: quinolone resistance-determining regions; PMQR: Plasmid-Mediated Quinolone Resistance.
*A p-value less than or equal to 0.05 is typically considered to be statistically significant.

References

1. Kim Y, Kim B, Wie SH, Kim J, Ki M, Cho YK, et al. Fluoroquinolone can be an effective treatment option for acute pyelonephritis when the minimum inhibitory concentration of levofloxacin for the causative *Escherichia col*i is ≤ 16 mg/L. Antibiotics. 2021;10(1):37.
2. Wagenlehner FM, Wagenlehner C, Redman R, Weidner W, Naber KG. Urinary bactericidal activity of doripenem versus that of levofloxacin in patients with complicated urinary tract infections or pyelonephritis. Antimicrobial agents and chemotherapy. 2009;53(4):1567-73.
3. Nicolas-Chanoine M-H, Blanco J, Leflon-Guibout V, Demarty R, Alonso MP, Caniça MM, et al. Intercontinental emergence of *Escherichia coli* clone O25: H4-ST131 producing CTX-M-15. Journal of Antimicrobial Chemotherapy. 2008;61(2):273-81.
4. Clermont O, Dhanji H, Upton M, Gibreel T, Fox A, Boyd D, et al. Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. Journal of Antimicrobial Chemotherapy. 2009;64(2):274-7.
5. Ismail MD, Ali I, Hatt S, Salzman EA, Cronenwett AW, Marrs CF, et al. Association of *Escherichia col*i ST131 lineage with risk of urinary tract infection recurrence among young women. Journal of global antimicrobial resistance. 2018;13:81-4.
6. Minarini LA, Darini ALC. Mutations in the quinolone resistance-determining regions of gyrA and parC in Enterobacteriaceae isolates from Brazil. Brazilian Journal of Microbiology. 2012;43:1309-14.
7. Ruiz J. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. Journal of Antimicrobial Chemotherapy. 2003;51(5):1109-17.
8. Hooper DC, Jacoby GA. Mechanisms of drug resistance: quinolone resistance. Annals of the New York academy of sciences. 2015;1354(1):12-31.
9. Azargun R, Gholizadeh P, Sadeghi V, Hosainzadegan H, Tarhriz V, Memar MY, et al. Molecular mechanisms associated with quinolone resistance in Enterobacteriaceae: review and update. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2020;114(10):770-81.
10. Yamane K, Wachino J-i, Suzuki S, Arakawa Y. Plasmid-mediated qepA gene among *Escherichia coli* clinical isolates from Japan. Antimicrobial agents and chemotherapy. 2008;52(4):1564-6.
11. Zhou J, Zhou J, Chen M, Lü P, Jiang C. Prevalence of β-lactam antibiotic resistance of *Escherichia coli* isolated from a neonatal intensive care unit. BMC pediatrics. 2025;25(1):95.
12. Wayne P. Clinical and Laboratory Standards Institute: Annapolis Junction. MD, USA. 2025.
13. Hammond DS, Schooneveldt JM, Nimmo GR, Huygens F, Giffard PM. bla SHV genes in Klebsiella pneumoniae: different allele distributions are associated with different promoters within individual isolates. Antimicrobial agents and chemotherapy. 2005;49(1):256-63.
14. Johnson JR, Clermont O, Johnston B, Clabots C, Tchesnokova V, Sokurenko E, et al. Rapid and specific detection, molecular epidemiology, and experimental virulence of the O16 subgroup within *Escherichia col*i sequence type 131. Journal of Clinical Microbiology. 2014;52(5):1358-65.
15. Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, et al. The CTX-M-15-producing Escherichia coli diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. Journal of Antimicrobial Chemotherapy. 2008;61(5):1024-8.
16. Zhao L, Zhang J, Zheng B, Wei Z, Shen P, Li S, et al. Molecular epidemiology and genetic diversity of fluoroquinolone-resistant *Escherichia coli* isolates from patients with community-onset infections in 30 Chinese county hospitals. Journal of Clinical Microbiology. 2015;53(3):766-70.
17. Cattoir V, Poirel L, Rotimi V, Soussy C-J, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. Journal of antimicrobial chemotherapy. 2007;60(2):394-7.

18. Cavaco LM, Hasman H, Xia S, Aarestrup FM. qnrD, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. Antimicrobial agents and chemotherapy. 2009;53(2):603-8.
19. Platell JL, Cobbold RN, Johnson JR, Heisig A, Heisig P, Clabots C, et al. Commonality among fluoroquinolone-resistant sequence type ST131 extraintestinal *Escherichia coli* isolates from humans and companion animals in Australia. Antimicrobial agents and chemotherapy. 2011;55(8):3782-7.
20. Wang M, Guo Q, Xu X, Wang X, Ye X, Wu S, et al. New plasmid-mediated quinolone resistance gene, qnrC, found in a clinical isolate of Proteus mirabilis. Antimicrobial agents and chemotherapy. 2009;53(5):1892-7.
21. Chen X, Zhang W, Pan W, Yin J, Pan Z, Gao S, et al. Prevalence of qnr, aac (6′)-Ib-cr, qepA, and oqxAB in *Escherichia coli* isolates from humans, animals, and the environment. Antimicrobial agents and chemotherapy. 2012;56(6):3423-7.
22. Dalhoff A. Global fluoroquinolone resistance epidemiology and implictions for clinical use. Interdisciplinary perspectives on infectious diseases. 2012;2012.
23. Nicolas-Chanoine M-H, Bertrand X, Madec J-Y. Escherichia coli ST131, an intriguing clonal group. Clinical microbiology reviews. 2014;27(3):543-74.
24. Damavandi M-S, Gholipour A, Pour ML. Prevalence of class D carbapenemases among extended-spectrum β-lactamases producing Escherichia coli isolates from educational hospitals in Shahrekord. Journal of clinical and diagnostic research: JCDR. 2016;10(5):DC01.
25. Afsharikhah S, Ghanbarpour R, Mohseni P, Adib N, Bagheri M, Jajarmi M. High prevalence of β-lactam and fluoroquinolone resistance in various phylotypes of Escherichia coli isolates from urinary tract infections in Jiroft city, Iran. BMC microbiology. 2023;23(1):114.
26. Akgoz M, Akman I, Ates AB, Celik C, Keskin B, Ozmen Capin BB, et al. Plasmidic fluoroquinolone resistance genes in fluoroquinolone-resistant and/or extended spectrum Beta-Lactamase-Producing Escherichia coli Strains Isolated from Pediatric and adult patients diagnosed with urinary tract infection. Microbial Drug Resistance. 2020;26(11):1334-41.
27. Bullens M, de Cerqueira Melo A, Raziq S, Lee J, Khalid G, Khan S, et al. Antibiotic resistance in patients with urinary tract infections in Pakistan. Public Health Action. 2022;12(1):48-52.
28. Zeng Q, Xiao S, Gu F, He W, Xie Q, Yu F, et al. Antimicrobial resistance and molecular epidemiology of uropathogenic *Escherichia coli* isolated from female patients in Shanghai, China. Frontiers in cellular and infection microbiology. 2021;11:653983.
29. McDermott E. The threat of antibiotic resistance on global health. 2019.
30. Rasoulinasab M, Shahcheraghi F, Feizabadi MM, Nikmanesh B, Hajihasani A, Aslani MM. Distribution of ciprofloxacin-resistance genes among ST131 and non-ST131 clones of *Escherichia col*i isolates with ESBL phenotypes isolated from women with urinary tract infection. Iranian Journal of Microbiology. 2021;13(3):294.
31. Hiasa H. DNA topoisomerases as targets for antibacterial agents. DNA topoisomerases: Methods and protocols. 2018:47-62.
32. Spencer AC, Panda SS. DNA Gyrase as a Target for Quinolones. Biomedicines. 2023;11(2):371.
33. Heisig P. Genetic evidence for a role of parC mutations in development of high-level fluoroquinolone resistance in *Escherichia coli.* Antimicrobial agents and chemotherapy. 1996;40(4):879-85.
34. Hajihasani A, Ebrahimi-Rad M, Rasoulinasab M, Aslani MM, Shahcheraghi F. The Molecular Characterization and Risk Factors of ST131 and Non-ST131 *Escherichia coli* in Healthy Fecal Carriers in Tehran, Iran. Jundishapur Journal of Microbiology. 2022;15(5).
35. Reyna-Flores F, Barrios H, Garza-Ramos U, Sánchez-Pérez A, Rojas-Moreno T, Uribe-Salas FJ, et al. Molecular epidemiology of *Escherichia coli* O25b-ST131 isolates causing community-acquired UTIs in Mexico. Diagnostic Microbiology and Infectious Disease. 2013;76(3):396-8.

36. Bush NG, Diez-Santos I, Sankara Krishna P, Clavijo B, Maxwell A. Insights into antibiotic resistance promoted by quinolone exposure. Antimicrobial Agents and Chemotherapy. 2025;69(1):e00997-24.
37. Serwacki P, Hareza D, Gajda M, Świątek-Kwapniewska W, Adamowska M, Serwacka K, et al. Fluoroquinolone consumption and resistance after an Antibiotic Stewardship Team intervention—an interventional study in a single hospital in southern Poland from 2018–2023. American Journal of Infection Control. 2025.
38. Nikaido H, Pagès J-M. Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. FEMS microbiology reviews. 2012;36(2):340-63.
39. Li J, Zhang H, Ning J, Sajid A, Cheng G, Yuan Z, et al. The nature and epidemiology of OqxAB, a multidrug efflux pump. Antimicrobial Resistance & Infection Control. 2019;8:1-13.
40. Ghotaslou R, Baghbani S, Ghotaslou P, Mirmahdavi S, Leylabadlo HE. Molecular epidemiology of antibiotic-resistant *Escherichia coli* among clinical samples isolated in Azerbaijan, Iran. Iranian Journal of Microbiology. 2023;15(3):383.
41. Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. Antimicrobial agents and chemotherapy. 2009;53(7):2733-9.