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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

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Escherichia coli is the leading etiological agent of community-acquired urinary tract infection (UTI). Fluoroquinolones have long been the choice of empirical treatment for UTIs. Plasmid-mediated fluoroquinolone-resistance (PMFR) is important not only for conferring resistance to fluoroquinolones but also because of the presence of PMFR genes on plasmids carrying genes encoding resistance to other antimicrobials. In this study, we aimed at investigating the frequency of PMFR genes in fluoroquinolone-resistant and/or expanded spectrum beta-lactamase (ESBL)-producing *E. coli* strains isolated from pediatric and adult patients diagnosed with UTI. *E. coli* strains isolated from urine cultures of 141 adult and 117 pediatric outpatients were evaluated. Antimicrobial susceptibilities were interpreted according to the EUCAST criteria. The presence of PMFR genes (*qnrA, qnrB, qnrC, qnrS, qepA, aac(6′)-Ib,* and *aac(6′)*-Ib-cr) was investigated by multiplex PCR analysis. One hundred-three (73.05%) adult and 92 (78.63%) pediatric isolates were fluoroquinolone resistant and/or ESBL producers. One third (92/258) of all isolates carried at least one PMFR gene, the most prevalent one being *qnrS* (67.4%). None of the isolates carried *qnrC* and *qepA* genes. PMFR determinants were found to be widespread among adult and pediatric isolates. Rational antimicrobial use is crucial for prevention of resistance in both adult and pediatric populations.

Keywords: fluoroquinolone resistance, plasmid, *qnr*, *qepA*, *aac(6′)-Ib*

Introduction

Urinary tract infection (UTI) is the most prevalent community acquired (CA) bacterial infection, and *Escherichia coli* is the leading etiological agent. In the era of antimicrobial resistance, UTIs are the main cause of antibiotic consumption. Frequently prescribed agents for the empirical therapy of UTIs are beta-lactams, trimethoprim-sulfamethoxazole, and fluoroquinolones. Fluoroquinolones have the advantage of availability in both oral and parenteral forms, good absorption from the gastrointestinal tract, and high urinary excretion rate.[1] However, due to the frightening increase in fluoroquinolone resistance and low efficacy, recent guidelines recommend the use of nitrofurantoin, trimethoprim-sulfamethoxazole, fosfomycin-trometamol, or pivmecillinam for the empirical therapy of acute uncomplicated cystitis and discourage the use of fluoroquinolones or beta-lactams.[2,3]

The most crucial mechanism conferring fluoroquinolone resistance is the acquisition of sequential chromosomal mutations in the genes encoding the gyrase (*gyrA, gyrB*) and topoisomerase IV (*parC* and *parE*) target enzymes as a result of direct response to drug pressure.[1,3,4] Overexpression of certain efflux pumps and porin alterations are other mechanisms of resistance.[4,5]

Though once believed to be impossible due to the plasmid curing effect of quinolones, recent studies underlined the emergence of plasmid-mediated fluoroquinolone resistance

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(PMFR) mechanisms.[6,7] Since 1998, three PMFR mechanisms have been discovered:

1. Proteins of the pentapeptide repeat family encoded by the *qnr* genes that protect target enzymes from quinolone inhibition.
2. Acetylation of quinolones with appropriate amino-nitrogen targets by a variant of the common aminoglycoside acetyltransferase encoding gene, the *aac(6′)*-Ib-cr.
3. Enhanced efflux due to genes encoding QepAB and OqxAB pumps.

PMFR mechanisms usually confer low-level resistance to fluoroquinolones, but either alone or together with chromosomal target mutations, they widen the mutant selection window by elevating the mutant prevention concentration (MPC).[5,6] They increase the minimum inhibitor concentrations (MICs) of the strains and facilitate selection of higher-level resistance.[5,6] Another important feature of these PMFR genes is their presence on plasmids carrying genes encoding resistance to other antimicrobials such as beta-lactams and aminoglycosides.[5,6] Thus, their presence may be regarded as a marker of multi-drug resistance.[5,6]

This study was aimed at investigating the presence and frequency of PMFR genes in fluoroquinolone-resistant and/or expanded spectrum beta-lactamase (ESBL)-producing *E. coli* strains isolated from pediatric and adult patients diagnosed with UTI.

Materials and Methods

Study strains

E. coli strains were prospectively isolated from midstream clean-catch voided urine cultures of 141 adult and 117 pediatric outpatients clinically diagnosed with UTI between 01.06.2015 and 31.03.2016 in Ibni Sina and Cebeci Hospitals of Ankara University. Only isolates of $>10^5$ CFU/mL pure cultures were included. Replicate samples from the same patient were excluded from the study. Identification of the isolates was performed by MALDI-TOF MS (Maldi Biotyper, Bruker). All the study strains were preserved in brain-heart infusion broths with 20% glycerol at −20°C.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities of the isolates against ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefixime, cefuroxime-axetil, cefotaxime, cefepime, ertapenem, imipenem, meropenem, ciprofloxacin, levofloxacin, gentamycin, amikacin, trimethoprim-sulfamethoxazole, nitrofurantoin, and fosfomycin were routinely determined by the Kirby-Bauer disk diffusion method and interpreted according to the EUCAST criteria.[8] An isolate was regarded as ''fluoroquinolone-resistant'' if it was at least resistant to ciprofloxacin. ESBL production was determined by combination disk test by using cefepime and cefepime-clavulanic acid disks as suggested by EUCAST.[9] For the isolates with fluoroquinolone resistance, MICs for norfloxacin, ofloxacin, ciprofloxacin, and levofloxacin were determined by using gradient strips (BioMérieux, France).

Molecular analyses

DNA was extracted by boiling the harvested colonies in 500 μL sterile distilled water at 95°C for 10 min. After centrifugation at 10,000 *g* for 10 min, the supernatant was used as template.

The presence of PMFR genes (*qnrA, qnrB, qnrC, qnrS, qepA,* and *aac(6′)-Ib*) was investigated by multiplex polymerase chain reaction (PCR) as described in the literature with minor modifications (Table 1). For the determination

Table 1. Primers and Reaction Conditions Used for the Determination of Plasmid-Mediated Fluoroquinolone Resistance Genes

Gene	*Primer*	*Primer sequence*	*Product (bp)*	*Reaction conditions*
qnrA	QnrAm-F	5′-AGAGGATTTCTCACGCCAGG-3′	580	Multiplex touchdown PCR: 5 min initial denaturation at 95°C followed by a total of 34 cycles consisting of 1 min denaturation at 95°C, 1 min annealing at 55°C (2 cycles), 53°C (2 cycles), 50°C (30 cycles), and 1 min extension at 72°C. A final extension was performed for 5 min at 72°C
	QnrAm-R	5′-TGCCAGGCACAGATCTTGAC-3′		
qnrB	QnrBm-F	5′-GGMATHGAAATTCGCCACTG-3′	264	
	QnrBm-R	5′-TTTGCYGYYCGCCAGTCGAA-3′		
qnrS	QnrSm-F	5′-GCAAGTTCATTGAACAGGGT-3′	428	
	QnrSm-R	5′-TCTAAACCGTCGAGTTCGGCG-3′		
qnrC	QnrC-F	5′-GGGTTGTACATTTATTGAATC-3′	447	Multiplex touchdown PCR: 5 min initial denaturation at 95°C followed by a total of 34 cycles consisting of 1 min denaturation at 95°C, 1 min annealing at 55°C (2 cycles), 53°C (2 cycles), 50°C (30 cycles), and 1 min extension at 72°C. A final extension was performed for 5 min at 72°C
	QnrC-R	5′-TCCACTTTACGAGGTTCT-3′		
qepA	QepA-F	5′-GCAGGTCCAGCAGCGGGTAG-3′	199	
	QepA-R	5′-CTTCCTGCCCGAGTATCGTG-3′		
aac(6′)-Ib-cr	aac(6′)-1b-F	5′-TTGCGATGCTCTATGAGTGGCTA-3′	482	
	aac(6′)-1b-R	5′-CTCGAATGCCTGGCGTGTTT-3′		

Table 2. Characteristics of the Isolates Showing Low-Level Resistance to Fluoroquinolones

Strain no.	Phenotype	MICs (μg/mL) NOR	OFL	CIP	LEV	FQ-resistance gene
36P	ESBL (+) + FQ-R	4	1	1.5	0.50	*qnrB*
83P	FQ-R	6	2	1.5	0.75	None
90P	ESBL (+) + FQ-R	4	1.5	0.75	1	None
29A	ESBL (+) + FQ-R	6	3	0.75	1.5	None

A, adult; CIP, ciprofloxacin; ESBL, extended-spectrum beta-lactamase; FQ-R, fluoroquinolone-resistant; LEV, levofloxacin; MIC, minimum inhibitor concentration; NOR, norfloxacin; OFL, ofloxacin; P, pediatric.

of the *aac(6′)*-Ib-cr variant, the amplified region was digested by *BseGI* restriction endonuclease (Thermo Fisher Scientific).[10,11] Positive control strains carrying plasmidic fluoroquinolone-resistance determinants used in this study were kindly provided by Marmara University Faculty of Medicine, Department of Medical Microbiology, and were subjected to the same culture-identification and storage steps.

The data obtained was analyzed by chi-square test by using the SPSS software version 20.0. *p*-values of <0.05 were regarded significant.

Results

Adult patients were 20–90 years old (mean 58.7 years): 31 were male, and 110 were female. Pediatric patients were 8 days to 16 years old (mean 5.2 years): 20 were male, and 97 were female.

Thirty eight adult (26.95%) and 25 (21.37%) pediatric isolates were susceptible to all the antimicrobials tested. Of the adult isolates, 37 (35.92%) were fluoroquinolone-resistant, 30 (29.12%) were ESBL-positive, and 36 (34.95%) were fluoroquinolone-resistant and ESBL-positive. Five (5.43%) of the pediatric isolates were fluoroquinolone-resistant, 68 (73.91%) were ESBL-positive, and 19 (20.65%) were both fluoroquinolone-resistant and ESBL-positive. ESBL production was significantly more prevalent among pediatric isolates ($p < 0.001$), and fluoroquinolone resistance was more prevalent among adult isolates ($p < 0.001$).

All but 4 of the 97 fluoroquinolone-resistant isolates showed high-level resistance to fluoroquinolones (MICs for norfloxacin, ofloxacin, ciprofloxacin, levofloxacin >32 μg/mL). The characteristics of the four low-level fluoroquinolone-resistant isolates are shown in Table 2.

About one third (92/258) of all isolates and half (92/195) of the resistant ones carried at least one PMFR gene (Fig. 1). The most prevalent gene detected was *qnrS* (67.4%), followed by *aac(6′)*-1b-cr variant (42.4%), *qnrB* (7.6%), and *qnrA* (3.2%). None of the isolates carried the *qnrC* and *qepA* genes. In 54.3% of the strains, a single determinant (either *qnrS* or *qnrB*) was detected.

The *qnrS* gene was present in 67.4% (62/92) of the isolates carrying PMFR genes, either alone (48/62, 77.4%) or with other PMFR determining genes (14/62, 22.6%). The *aac(6′)*-1b-cr variant was found in 39 (42.4%) isolates. In 26 (66.7%) of these isolates, it was the only PMFR determinant. The *qnrB* gene was found in seven isolates (7.6% of the isolates with PMFR genes), being the only PMFR determinant in 2 (0.78%) of them (Table 3). When adult and pediatric isolates were compared for the presence of PMFR determining genes, only the *aac(6′)-1b-cr* variant was found to be more prevalent among adult isolates (55.5% vs. 23.7% in pediatric isolates, $p = 0.002$).

Among the *E. coli* strains susceptible to all antimicrobials tested ($n = 63$), seven (11.1%; two adult, five pediatric) isolates were found to carry PMFR genes: Five (two adult, three pediatric) isolates carried the *qnrS* gene, one pediatric isolate carried the *qnrB* gene, and one pediatric isolate carried the *aac(6′)*-1b-cr variant.

Only in the adult population, fluoroquinolone-resistant and/or ESBL-positive strains were more likely to carry PMFR genes when compared with the susceptible isolates ($p < 0.001$ for ESBL-positives, $p < 0.001$ for fluoroquinolone-resistant ones, and $p = 0.001$ for both ESBL-positive and

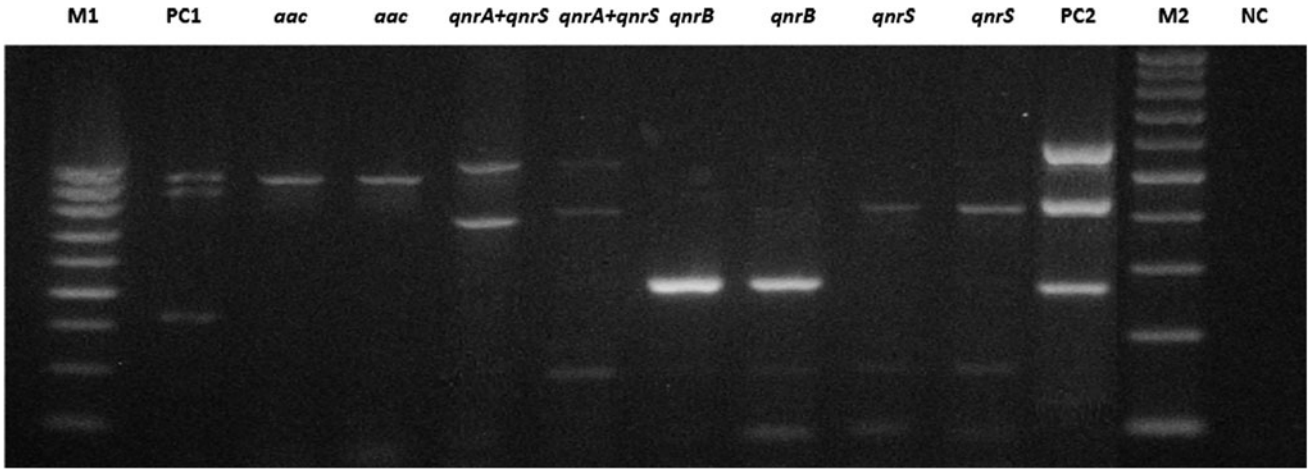


FIG. 1. PCR analyses of plasmid-mediated fluoroquinolone resistance genes. M1 and M2 are molecular markers (M1: 50 bp DNA ladder [Amresco], M2: 100 bp DNA ladder [Thermo Scientific]) PC1 and PC2 are positive control mixes (PC1 contains PCR amplicons of *aac(6′)1b, qnrC*, and *qepA*; PC2 contains PCR amplicons of *qnrA, qnrS*, and *qnrB*). NC is negative control.

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Table 3. Distribution of Plasmidic Fluoroquinolone Resistance Genes with Respect to the Study Populations and Phenotypes of Antimicrobial Susceptibility

Population		*Adult*					*Pediatric*					*Total*				
Phenotype		*ESBL (+)*	*FQ-R*	*ESBL (+) & FQ-R*	*Susceptible*	*Total*	*ESBL (+)*	*FQ-R*	*ESBL (+) & FQ-R*	*Susceptible*	*Total*	*ESBL (+)*	*FQ-R*	*ESBL (+) & FQ-R*	*Susceptible*	*Total*
N		30	37	36	38	141	68	5	19	25	117	98	42	55	63	258
FQ resistance genes [*n* (%)]	*qnrB*	—	—	—	—	—	—	—	1 (5.26)	1 (4)	2 (1.71)	—	—	1 (1.82)	1 (1.59)	2 (0.78)
	qnrS	10 (33.3)	6 (16.21)	5 (13.89)	2 (5.26)	23 (16.31)	18 (26.47)	1 (20)	3 (15.79)	3 (12)	25 (21.37)	28 (28.57)	7 (16.67)	8 (14.55)	5 (7.94)	48 (18.60)
	qnrA & qnrS	—	1 (2.70)	—	—	1 (0.71)	1 (1.47)	—	—	—	1 (0.85)	1 (1.02)	1 (2.38)	—	—	2 (0.78)
	qnrB & qnrS	—	—	—	—	—	1 (1.47)	—	—	—	1 (0.85)	1 (1.02)	—	—	—	1 (0.39)
	aac(6′)1b-cr	—	8 (21.62)	11 (30.56)	—	19 (13.48)	2 (9.94)	1 (20)	3 (15.79)	1 (4)	7 (5.98)	2 (2.04)	9 (21.43)	14 (25.45)	1 (1.59)	26 (10.08)
	aac(6′)1b-cr & qnrB	—	1 (2.70)	—	—	1 (0.71)	—	—	1 (5.26)	—	1 (0.85)	—	1 (2.38)	1 (1.82)	—	2 (0.78)
	aac(6′)1b-cr & qnrS	1 (3.33)	2 (5.41)	5 (13.89)	—	8 (5.67)	—	—	—	—	—	1 (1.02)	2 (4.76)	5 (9.09)	—	8 (3.01)
	aac(6′)1b-cr & qnrA & qnrS	—	—	1 (2.78)	—	1 (0.71)	—	—	—	—	—	—	—	1 (1.82)	—	1 (0.39)
	aac(6′)1b-cr & qnrB & qnrS	—	—	1 (2.78)	—	1 (0.71)	—	—	1 (5.26)	—	1 (0.85)	—	—	2 (3.64)	—	2 (0.78)
	None	19 (63.33)	19 (51.35)	13 (36.11)	36 (94.73)	87 (61.70)	46 (67.65)	3 (60)	10 (52.63)	20 (80)	79 (67.52)	65 (66.32)	22 (52.38)	23 (41.82)	56 (88.89)	166 (64.34)

fluoroquinolone-resistant ones). This difference was not observed in the pediatric group.

When compared with the susceptible isolates, fluoroquinolone-resistant isolates, ESBL-positive isolates, fluoroquinolone-resistant isolates, and ESBL-positive isolates were about 9 (odds ratio [OR] = 9.24, 95% CI = 3.83–22.32), 6 (OR = 5.91, 95% CI = 2.539–13.81), and 8 (OR = 7.65, 95% CI = 2.87–20.37) times more likely to carry the PMFR genes, respectively (Table 4).

Discussion

E. coli strains frequently express plasmidic ESBLs. The frequency of ESBL producing *E. coli* strains is highest in East Europe (28.9%), and lowest (7.5%) in North America.[5,12] In Turkey, 16–29.9% of *E. coli* strains are reported as ESBL producers.[13–15] In our study, 46.80% of the adult and 74.36% of the pediatric isolates were ESBL producers. The high frequency may reflect the increase in ESBL-producing strains over time, but it may more likely be due to the selection bias of the strains, as strains with resistance mechanisms other than fluoroquinolone resistance or ESBL production were not included in the study.

Fluoroquinolone resistance emerged in the mid-1990s due to their extensive use, and resistance rates continue to increase.[3–6] Fluoroquinolone resistance rate is low in the United States (7%), and high in Europe and Asia (28%). Higher resistance rates are reported in the developing countries.[1] Approximately 40% of *E. coli* strains isolated from complicated, and 20% from uncomplicated CA-UTIs

Table 4. Comparison of the Adult and Pediatric Isolates According to Their Phenotypic Resistance Patterns and Presence of Plasmidic Fluoroquinolone Resistance Genes

	Plasmidic FQ-resistance gene (n)			
Study population	*Positive*	*Negative*	p	*OR (95% CI)*
Adult isolates ($n = 141$)				
Resistant ($n = 103$)	52	51		
All susceptible ($n = 38$)	2	36	0.0001	9.59 (4.19–80.25)
ESBL-positive ($n = 66$)	34	32		
ESBL-negative ($n = 75$)	20	55	0.002	2.92 (1.44–5.90)
All susceptible ($n = 38$)	2	36	0.0001	19.13 (4.25–86.14)
FQ-resistant ($n = 73$)	41	32		
FQ-susceptible ($n = 68$)	13	55	<0.0001	5.42 (4.53–11.60)
All susceptible ($n = 38$)	2	36	<0.0001	23.06 (5.16–103.06)
ESBL-positive & FQ-resistant ($n = 36$)	23	13		
Susceptible + only ESBL-positive + only FQ-resistant ($n = 105$)	31	74	0.0004	4.22 (1.89–9.38)
All susceptible ($n = 38$)	2	36	<0.0001	31.85 (6.57–154.30)
Pediatric isolates ($n = 117$)				
Resistant ($n = 92$)	33	59		
All susceptible ($n = 25$)	5	20	0.139	2.24 (0.77–6.5)
ESBL-positive ($n = 87$)	31	56		
ESBL-negative ($n = 30$)	7	23	0.219	1.81 (0.70–4.71)
All susceptible ($n = 25$)	5	20	0.147	2.21 (0.76–6.78)
FQ-resistant ($n = 24$)	11	13		
FQ-susceptible ($n = 93$)	27	66	0.121	2.07 (0.82–5.19)
All susceptible ($n = 25$)	5	20	0.059	3.38 (0.95–12.01)
ESBL-positive & FQ-resistant ($n = 19$)	9	10		
Susceptible + only ESBL-positive + only FQ-resistant ($n = 98$)	29	69	0.135	2.14 (0.79–5.82)
All susceptible ($n = 25$)	5	20	0.059	3.6 (0.95–13.62)
Total ($n = 258$)				
Resistant ($n = 195$)	85	110		
All susceptible ($n = 63$)	7	56	<0.0001	6.18 (3.83–22.32)
ESBL-positive ($n = 153$)	65	88		
ESBL-negative ($n = 105$)	27	78	0.006	2.13 (1.24–3.67)
All susceptible ($n = 63$)	7	56	<0.0001	5.91 (2.53–13.81)
FQ-resistant ($n = 97$)	52	45		
FQ-susceptible ($n = 161$)	40	121	<0.0001	3.49 (2.05–5.97)
All susceptible ($n = 63$)	7	56	<0.0001	9.24 (3.83–22.32)
ESBL-positive & FQ-resistant ($n = 55$)	22	23		
Susceptible + only ESBL-positive + only FQ-resistant ($n = 203$)	60	143	0.014	2.28 (1.18–4.40)
All susceptible ($n = 63$)	7	56	<0.0001	7.65 (2.87–20.37)

OR, odds ratio.

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are fluoroquinolone resistant.[5] Fluoroquinolone resistance rates in CA-UTIs in Turkey was reported as 20–30%.[1] In a meta-analysis conducted in Turkey to evaluate the change in the antimicrobial susceptibility patterns of urinary *E. coli* isolates between 1996 and 2012, ciprofloxacin resistance and ESBL production rates were found to increase significantly in both adult and pediatric populations.[16] In our study, 51.78% of the adult isolates and 20.51% of the pediatric isolates were fluoroquinolone resistant.

Strahilevitz *et al.*[17] have reviewed top publications investigating PMFR determinants and have shown an overall 1.5% prevalence for *qnrA*, 4.6% for *qnrB*, 2.4% for *qnrS*, and, more commonly, the presence of *aac-(6′)*-Ib-cr (10.8%) in *E. coli* strains.

The *qnr* genes are the major PMFR determinants in Enterobacteriaceae.[6,18] Strains carrying these determinants usually show low-level resistance to fluoroquinolones comparable to that provided by the first-step mutations in the target genes, but 16–32 times increased MICs have also been reported. Their presence increases the MPC and facilitates the selection of mutants with additional resistance mechanisms.[3,5,7,19] They are often associated with plasmid-encoded beta-lactamases, ESBLs, and AmpC enzymes.[7,17]

Depending on the selection criteria of the resistant strains, the prevalence of *qnr* determinants ranges between 0.2% and 94%.[19–21] In Iran and Greece, the two neighbors of Turkey, its prevalence is reported as 20% and 10%, respectively.[21,22] In our study, 25.58% of all strains and 29.89% of fluoroquinolone-resistant isolates were *qnr*-positive. The presence of *qnr* genes was correlated with the production of ESBLs, as observed in other studies.

Although *qnrB* was reported as the most frequent PMFR determinant among *E. coli* strains in many studies,[19,23,24] *qnrB* was found in only 7 (2.71%) isolates in our study and *qnrS* was predominant (24.03%).

A few studies have found bacteria harboring more than one *qnr* gene, most frequently *qnrS* with either *qnrB* or *qnrA*. Whether they have an additive effect on MICs or not is unclear. They may as well compete for binding to DNA gyrase, as experiments show that the effect of both genes on ciprofloxacin MIC is the same as that of either one alone.[17] In our study, 6 (2.33%) strains harbored more than one *qnr* determinant (Table 3).

The *aac(6′)*-Ib-cr variant acetylates not only aminoglycosides but also fluoroquinolones with an amino-terminal on the piperazynil ring such as ciprofloxacin and norfloxacin. It reduces their activity by about two- to four-fold and facilitates the survival of target mutants by increasing the MPC.[5–7] This gene may be found with other PMFR genes and on plasmids encoding ESBLs. Thus, strains carrying these plasmids can show resistance to beta-lactams, aminoglycosides, and fluoroquinolones.[5–7,17]

The prevalence of *aac(6′)*-Ib-cr ranges from 0.4% to 77.5% depending on the characteristics of the studied strains.[19,24] In a study evaluating 718 bloodstream *E. coli* isolates collected between 1991–1997 and 1998–2005 periods, ciprofloxacin-resistance rate and the frequency of *aac(6′)*-Ib-cr were found to increase from 11.3% to 46.6% and from 0% to 7.1%, respectively ($p < 0.001$). Of the *aac(6′)*-Ib-cr carriers, 78% were ESBL producers in this study.[25] In our study, *aac(6′)*-Ib-cr was found in 15.12% of the strains, and 66.67% were ESBL producers.

The *qepA* gene encodes a quinolone efflux pump belonging to the major facilitator superfamily of protein-dependent transporters. Its main targets are mainly hydrophilic fluoroquinolones such as norfloxacin and ciprofloxacin.[6,26] The presence of QepA efflux pump is responsible for an increase in the MICs of these fluoroquinolones about 8–64-fold. It is found on a transposable element along with a methyltransferase encoding gene, and it may also select for the co-resistance of fluoroquinolones, aminoglycosides, and beta-lactams.[5,7,17,19] It is the least prevalent (0.2–3.6%) PMFR mechanism.[17,24,27] In our study, none of the strains were *qepA*-positive.

In studies evaluating the presence of PMFR genes in Turkey, *qnr* and *aac(6′)*-Ib-cr genes were found with low frequencies and different distribution patterns. Oktem *et al.*[28] have found *qnrA* genes in 6.4% of 78 ESBL-positive Enterobacteriaceae blood culture isolates. In the studies of Nazik *et al.*,[29,30] the presence of *qnr* determinants was as low as 1% (4% in ESBL-producing isolates). About 15% of the *qnr*-positive isolates were also *aac(6′)*-Ib-cr determinant positive.[30] Aktepe *et al.*[10] reported the presence of *aac(6′)*-1b-cr in 59.8% of 112 quinolone-resistant *E. coli* isolates. Fifty-eight percent of these strains were ESBL producers. In the study of Coban *et al.*,[31] 0.3%, 0.9%, and 0.4% of the isolates were found to be *qnrA, qnrB*, and *qnrS* carriers, respectively. In the study of Nazic *et al.*,[32] *qnr* positivity was 4% among ESBL-producing strains. None of the studies from Turkey have reported the presence of *qepA*. Our results indicate a higher prevalence of *qnr* genes (25.58%, 32.02% among ESBL producers), followed by *aac(6′)*-Ib-cr (15.12%).

The first study reporting the presence of PMFR determinants in susceptible strains have shown the presence of *qnrA* in 10% of susceptible *Klebsiella pneumoniae* and 1% of susceptible *Enterobacter* spp. isolates.[33] In our study, 23% of fluoroquinolone-susceptible and 9.5% of full susceptible isolates were PMFR gene carriers. The presence of PMFR determinants in full susceptible strains with no identified phenotypical resistance mechanisms may not only help to select for full resistance in the future but may also lead to therapeutic failure. Studies have shown that growth under physiological urinary tract conditions confer clinical levels of ciprofloxacin resistance in *qnr*-expressing strains and can lead to therapeutic failure in patients with UTIs. The presence of physiological urinary tract conditions plus effective *qnr* horizontal transfer and co-selection with other resistance genes may play a critical role in the rapid spread of multidrug resistance throughout uropathogenic *E. coli*.[34,35]

The results of this study have shown that PMFR determinants are widespread among clinical isolates of adult and pediatric populations. Their presence in pediatric isolates is worrisome. It has been suggested that fluoroquinolone resistance may, to some extent, be dependent on cross-infection from adults to children,[36] but its emergence due to other antimicrobial resistance mechanisms carried on plasmids may also be possible. Fluoroquinolone resistance in healthy fecal flora is reported as 24% in adults and 16% in children.[5] In our study, 20% of the susceptible pediatric isolates were carrying PMFR determinants. Fluoroquinolone-resistance rate in clinical isolates is more common in countries with frequent non-prescription antimicrobial use and is proven to increase with the increased

prescription rates and decrease when its use is restricted.[37–39] In Turkey, though recently restricted by law, nonprescription antimicrobial use is still a major problem.

To conclude, the high fluoroquinolone resistance rates highlight the significance of local resistance monitoring and investigating underlying mechanisms. We recommend sparing fluoroquinolones for more complicated infections, restricting their use in human and veterinary medicine and livestock, avoiding their prescription in children and in uncomplicated cases, and we highlight the importance of the rational use of antimicrobials.

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