Letter to the Editor
Clinical Microbiology

Ann Lab Med 2018;38:384-386
https://doi.org/10.3343/alm.2018.38.4.384
ISSN 2234-3806 • eISSN 2234-3814

ANNALS OF LABORATORY MEDICINE

Prevalence of Plasmid-Mediated Quinolone Resistance Genes Among *Escherichia coli* in the Gut of Healthy People in Fuzhou, China

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Dear Editor,

The intestinal tract may be an important reservoir for antibiotic-resistant genes [1]. Determining the prevalence of quinolone resistance in intestinal bacteria within the community is important for both healthy subjects and hospital patients as quinolone is one of the most commonly used antibiotics in China. The prevalence of plasmid-mediated quinolone resistance (PMQR) gene-harboring *Enterobacteriaceae* in the gut flora among healthy humans was previously unknown, so we assessed the prevalence of PMQR genes among commensal *Escherichia coli* in healthy persons from one region in China. This study was approved by the Ethics Committee of the Fujian Medical University Union Hospital (No. 2012KY085). Written informed consent was obtained from all participants.

From July to October 2013, fecal samples were collected at a teaching hospital in Fuzhou (China) from healthy subjects at their annual physical examination. None of the subjects had been exposed to antibiotics or a hospital environment in the three months prior to sample collection. A total of 429 fecal samples, one per person, were examined. Samples were screened using MacConkey agar plates supplemented with levofloxacin (0.125 μg/mL). Among the 429 participants, 220 (51.3%) were males and 209 (48.7%) were females. The age range was 6–84 years (median age: 48 years). Four participants were under 18 years old, and the rest were over 18 years.

In this study, 89.0% (382/429) of the samples were colonized with *E. coli* with reduced quinolone susceptibility. Previous research on community carriage of quinolone-resistant *Enterobacteriaceae* described carriage rates that were highly different from our findings [2, 3], probably because of factors such as regional differences in climate, eating patterns, and sanitary conditions.

PMQR determinants were screened by PCR in 382 levofloxacin-resistant *E. coli* fecal isolates as previously described [4]. The results showed that 31.4% (120/382) of fecal *E. coli* harbored PMQR determinants. Among 120 PMQR-positive isolates, *qnr* genes were the most common determinants (41.7%), followed by *aac (6')-Ib-cr* (36.7%) and *oqxAB* (35.8%) (Table 1). Among 50 *qnr*-positive *E. coli*, *qnrS1* was the most common PMQR gene (94.0%), as reported by previous studies [3, 4]. *qnrB1* was detected in four isolates (Table 1); *qnrA, qnrC,* and *qnrD* were not found. Usually, *aac (6')-Ib-cr* is more common than *qnr* genes [5]. A previous study showed that strain selection criteria may underestimate the presence of bacteria harboring *aac (6')-Ib-cr* [6], which probably explains the low prevalence of this PMQR determinant in our study.

Received: August 4, 2017
Revision received: September 18, 2017
Accepted: March 12, 2018

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Table 1. Distribution of PMQR genes in *E. coli* phylogenetic groups and the corresponding fluoroquinolone minimal inhibitory concentration (MIC) ranges

PMQR genes	N	Phylogenetic group (N, %)				Ciprofloxacin (μg/mL)			Levofloxacin (μg/mL)		
		A	B_1	B_2	D	MIC range	MIC_{50}	MIC_{90}	MIC range	MIC_{50}	MIC_{90}
qnrS1	38	25 (65.8)	9 (23.7)	4 (10.5)	0	1–64	4	32	1–128	4	32
aac(6´)Ib-cr	33	16 (48.5)	6 (18.2)	3 (9.1)	8 (24.2)	1–256	32	128	2–64	8	32
oqxA+oqxB	32	18 (56.3)	10 (31.3)	0	4 (12.5)	1–64	8	32	1–32	8	16
qnrS1+oqxA+oqxB	6	3 (50)	3 (50)	0	0	1–8	2	8	1–32	4	32
aac(6´)Ib-cr+oqxA+oqxB	4	2 (50)	2 (50)	0	0	8–32	16	32	16–32	16	32
qnrS1+ aac(6´)Ib-cr	2	2 (100)	0	0	0	-	-	-	-	-	-
qnrB1+aac(6´)Ib-cr	2	2 (100)	0	0	0	-	-	-	-	-	-
oqxA	1	0	0	0	1	-	-	-	-	-	-
qnrS1+qnrB1+aac(6´)Ib-cr	1	1	0	0	0	-	-	-	-	-	-
qnrB1+aac(6´)Ib-cr+oqxA+oqxB	1	1	0	0	0	-	-	-	-	-	-
Total	120	70 (58.3)	30 (25.0)	7 (5.8)	13 (16.7)						

Abbreviations: MIC_{50}, minimum inhibitory concentration required to kill 50% of the microbial population; MIC_{90}, minimum inhibitory concentration required to kill 90% of the microbial population.

Table 2. Association between antimicrobial susceptibility and β-lactamase genes in PMQR-positive *E. coli* isolates

Antimicrobial agents	MIC (μg/mL)			Resistance rate (%)						*P**
				Total (N=120)		β-lactamase gene-positive strains (N=99)		β-lactamase gene-negative strains (N=21)		
	MIC range	MIC_{50}	MIC_{90}	S	R	S	R	S	R	
Nalidixic acid	16–512	512	512	0	100	0	100	0	100	-
Ciprofloxacin	0.5–512	8	64	9.2	78.3	8.1	81.8	14.3	57.1	0.407
Levofloxacin	0.5–256	8	32	10.8	66.7	10.1	71.7	14.3	42.9	0.463
Cefotaxime	0.125–512	0.125	128	56.7	43.3	51.5	48.5	85.7	14.3	0.004
Gentamicin	0.125–512	2	128	52.5	45.8	49.5	48.5	71.4	28.6	0.081
Cefepime	0.125–512	0.125	8	92.5	4.2	92.9	3.0	95.2	4.8	0.555
Amikacin	0.125–512	2	2	97.5	2.5	97.0	3.0	100	0	1.000
Imipenem	0.125–4	0.25	0.5	100	0	100	0	100	0	-

*Statistical significance (*P*) was calculated using the Pearson Chi-square test in terms of the number of resistant strains and susceptible strains in the β-lactamase gene-positive and β-lactamase gene-negative groups. Intermediate isolates were considered non-susceptible isolates.
Abbreviations: S, susceptible; R, resistant; MIC, minimum inhibitory concentration; MIC_{50}, minimum inhibitory concentration required to kill 50% of the microbial population; MIC_{90}, minimum inhibitory concentration required to kill 90% of the microbial population.

PMQR determinants are frequently associated with extended spectrum beta-lactamase (ESBL) in *Enterobacteriaceae* [5]. In this study, β-lactamase genes (*bla*TEM, *bla*SHV, and *bla*CTX-M) were detected as described previously [7]. Ninety-nine (82.5%) strains co-harbored β-lactamase genes. bla_{TEM-1}, $bla_{CTX-M-1}$ group, and $bla_{CTX-M-9}$ group were found in 72 (72.3%), 24 (24.2%), and 21 (21.2%) isolates, respectively. Overall, $bla_{CTX-M-15}$ was the predominant ESBL genotype (20.2%, 20/99), followed by $bla_{CTX-M-14}$ (13.1%, 13/99), $bla_{CTX-M-65}$ (5.1%, 5/99), $bla_{CTX-M-79}$ (4.0%, 4/99), $bla_{CTX-M-27}$ (2.0%, 2/99), and $bla_{CTX-M-98}$ (1.0%, 1/99). bla_{SHV}, $bla_{CTX-M-2}$ group, and $bla_{CTX-M-8}$ group were not found. Different PMQR genes tend to co-exist with specific cephalosporin resistance genes. For example, *qnrA* and *qnrB* are usually found with *bla*CTX-M and *bla*SHV, and *qnrS* is always located on plasmids carrying *bla*TEM-1 [8]. These might explain why in our study, most PMQR-positive isolates produced *bla*TEM-1 and less than half of the strains co-harbored *bla*CTX-M.

PMQR-harboring fecal *E. coli* were tested for susceptibility toward nine different antimicrobials according to the CLSI [9]. These isolates showed high resistance against nalidixic acid (100%,

120/120), ciprofloxacin (78.3%, 94/120), and levofloxacin (66.7%, 80/120). Resistance rates to gentamicin, cefotaxime, cefepime, and amikacin were less than 50% (Table 2). All strains were susceptible to imipenem. Antibiotic susceptibilities of β-lactamase gene-positive and β-lactamase gene-negative PMQR-positive *E. coli* are shown in Table 2. A higher percentage of resistance to cefotaxime was observed in β-lactamase gene-positive isolates than in negative isolates (Table 2).

Molecular characteristics of PMQR-positive *E. coli* fecal isolates were determined, including phylogenetic grouping and random amplified polymorphic DNA analysis as previously described [2, 7]. The distribution of PMQR genes in *E. coli* phylogenetic groups is shown in Table 1. PMQR-positive *E. coli* fecal strains (120) were categorized into 71 genotypes; three strains were non-typeable, suggesting a non-clonal origin.

In conclusion, our study demonstrated a high prevalence of quinolone-resistant *E. coli* colonization in the gut flora of healthy subjects in one region of China. PMQR determinants were widely disseminated among those strains. Rational antibiotic use is imperative, and intervention programs and policies are urgently needed to control the colonization and dissemination of quinolone-resistant *Enterobacteriaceae* to promote public health in China.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgment

This study was supported by the National Natural Science Foundation of China (grant no. 81201328), and the Medical Elite Cultivation Program of Fujian, China (grant no. 2015-ZQN-ZD-15).

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https://doi.org/10.3343/alm.2018.38.4.384