Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2018;43(2):295-300
DOI: 10.17826/cumj.341637

ARAŞTIRMA / RESEARCH

Plasmid-mediated fluoroquinolone resistance in clinical isolates of Escherichia coli in Konya, Turkey

Konya, Türkiye'de Escherichia coli klinik izolatlarında plazmid aracılı fluorokinolon direnci

Dhay Ali Azeez[1], Duygu Fındık[1], Hatice Türk Dağı[1], Uğur Arslan[1]

[1]Selcuk University Faculty of Medicine, Department of Medical Microbiology, Konya, Turkey

Cukurova Medical Journal 2018;43(2):295-300

Abstract

Purpose: Resistance to quinolones usually results from mutations in the topoisomerase genes encoded chromosomally and also the expression of efflux pumps, loss of porines and the transfer of plasmid-mediated genes. The aim of this study was to investigate the presence of plasmid-mediated quinolones resistance genes (qnrA, qnrB, qnrC, qnrS, qepA, and aac(6')-1b-cr) in clinical isolates of Esherichia coli from Selcuk University, Konya, Turkey.

Materials and Methods: In this study 115 quinolone-resistant isolates were screened for qnrA, qnrB, qnrC, qnrS, qepA, and aac(6')-1b-cr genes by polymerase chain reaction (PCR). All aac(6')-1b positive amplicons were analyzed by digestion with BseGI restriction enzyme to identify aac(6')-1b-cr variant.

Results: Of the 115 quinolone-resistant E.coli strains, three (2.6%) carried qnrB, nine (7.8%) carried qnrS and 50 (43.5%) carried aac(6')-1b-cr genes. None of them harboured qnrA, qnrC and qepA genes.

Conclusion: We determined that aac(6')-1b-cr gene was responsible for most of the quinolone-resistant E. coli strains from Konya, Turkey. The prevalence of qnrB and qnrS genes was low and qnrA, qnrC and qepA genes were not detected. The surveillance of quinolone resistance genes is important, especially plasmid mediated ones are rapidly spreading all over the world.

Key words: Escherichia coli, plasmid-mediated fluoroquinolone resistance; qnr; qepA, aac(6')-1b-cr

Öz

Amaç: Kinolonlara karşı direnç gelişimi, genellikle kromozomlarla kodlanan topoizomeraz ve efflux pump genlerindeki mutasyonlara, porin kaybına ve plasmid aracılı genlerin transferine bağlı olarak gelişmektedir. Bu çalışmanın amacı, Selçuk Üniversitesi, Konya, Türkiye'de Escherichia coli klinik izolatlarında plazmid aracılı kinolon direnç genlerinin (qnrA, qnrB, qnrC, qnrS, qepA, and aac(6')-1b-cr) varlığını araştırmaktır.

Gereç ve Yöntem: Bu çalışmada, qnrA, qnB, qnrC, qnrS, qepA, and aac (6')-1b-cr genleri kinolon dirençli 115 E. coli suşunda polimeraz zincir reaksiyonu (PZR) ile araştırıldı. Tüm aac (6')-1b-cr varyantını tanımlamak için aac (6')-1b pozitif amplikonlar BseGI restriksiyon enzimiyle kesilerek araştırıldı.

Bulgular: 115 kinolon dirençli E.coli suşundan üçünde (% 2.6) qnrB, dokuzunda (% 7.8) qnrS ve ellisinde (% 43.5) aac (6')-1b-cr genleri pozitif bulundu. Suşların hiçbirinde qnrA, qnrC ve qepA genleri tespit edilmedi.

Sonuç: Türkiye Konya'da E. coli klinik suşlarında kinolon direncinden, aac (6 ')-1b-cr geninin sorumlu olduğu belirlendi. qnrB ve qnrS gen sıklığı düşük olarak tespit edildi ve qnrA, qnrC ve qepA genleri tespit edilmedi. Kinolon direnç genlerinin sürveyansı önemlidir, özellikle plazmid aracılı olanlar tüm dünyaya hızla yayılmaktadır.

Anahtar kelimeler: Escherichia coli; plazmid aracılı florokinolon direnci; qnr; qepA; aac (6')-1b-cr

INTRODUCTION

Although most *Escherichia coli* strains do not cause disease and is a member intestinal microbiota, virulent strains can cause serious infections like gastroenteritis, urinary tract infections, neonatal meningitis and septicemia. It can also be characterized by severe abdominal cramps, diarrhea that typically turns bloody within 24 hours, and

Yazışma Adresi/Address for Correspondence: Hatice Türk Dağı, Selcuk University Faculty of Medicine, Department of Medical Microbiology, Konya, Turkey E-mail: haticeturkdagi@yahoo.com
Geliş tarihi/Received: 27.02.2017 Kabul tarihi/Accepted: 18.09.2017

sometimes fever. More rare virulent strains are also responsible for bowel necrosis and perforation, peritonitis, mastitis, septicemia, and Gram-negative pneumonia[1,2].

Quinolones are widely used antimicrobials against *E.coli* infections and resistance to quinolones has increased markedly since their introduction. Quinolones affect bacterial cells by disarranging DNA synthesis. They inhibit DNA-gyrase and topoisomerase IV; the inhibition leads to cell death. They affect by inhibiting these enzymes and accelerating DNA separation in DNA-enzyme complexes[3,4].

The acquired resistance in susceptible bacteria against quinolones generally consists of single-stage spontaneous chromosome mutations. Chromosome mutations generally reveals in two forms;first is a modification in subunits of DNA gyrase and topoisomerase IV which are target enzymes of quinolones and the second is degradation of membrane permeability[3,4].

Hypothesis of plasmid-mediated quinolone resistance (PMQR) was first suggested in a *Shigella dysenteriae* strain which is resistant to nalidxic acid in 1987; however this hypothesis couldn't be proven[5]. Existance of low level quinolone resistance which was transferable by a plasmid was first shown in *Klebsiella pneumoniae* strain resistant to ciprofloxacin which was isolated in the urine sample of a patient in 1994[6].

The resistance gene site comes up as a result of plasmid cloning called as "*qnr*". This gene encodes a protein (QnrA) of a repetitive pentapeptide family consisting of 218 aminoacides and in further studies similar proteins (QnrS, QnrB, QnrC and QnrD) were discovered which causes PMQR[7].

In 2006, a different plasmid-mediated resistance gene, *aac (6')-Ib-cr* was discovered. *aac (6')-Ib* gene encodes an aminoglycoside acetyl transferase which causes resistance to kanamycin, tobramycin and amikacin. A variant of this gene (*aac (6')-Ib-cr*) causes enzymatic inactivation of some quinolones such as norfloxacin and ciprofloxacin and reduces susceptibility[8]. *qepA (*quinolon efflux pump) another PMQR gene was shown in *E.coli* strains in Japan and Belgium in 2007. *qepA* encodes a protein including 511 aminoacides associated with major facilitator family belonging to 14 trans membrane efflux pumps and causes to pump hydrophylic quinolones out of the cell and increases minimum inhibitory concentrations of these antibiotics[9].

Worldwide PMQR gene analyses in clinical isolates of *E.coli* have been demonstrated. The aim of this study was to investigate the presence of PMQR genes in clinical isolates of *E. coli* at a Medical Center in Selcuk University, Konya/Turkey.

MATERIALS AND METHODS

According to our records, 2663 *E.coli* strains were isolated, January 2013 to December 2013. The samples sent from different clinics were inoculated in 5% sheep blood agar and EosineMethylen Blue (EMB) agar plates and were incubated at 35°C for 24-48 hours. The identification and the antibiotic susceptibility tests were performed by VITEK 2 system (bioMerieux, France) according to the manufacturer's instructions. Some of them were stored at -20°C in beaded tubes. One hundred and fifteen ciprofloxacin and/or levofloxacin resistant strains which could be revitalized were included in the study.

After revitalization on blood and EMB agar, for the 115 isolates broth microdilution method was performed to detect susceptibility of *E.coli* strains against ciprofloxacin (32-0.025µg/ml) and levofloxacin (32-0.025µg/ml) for confirmation and extended-spectrum beta-lactamase (ESBL) production was determined by double-disc synergy test according to Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as control isolates.

After DNA isolation by a commercial QIAamp DNA Mini Kit (Qiagen, Germany) plasmid mediated quinolone resistance genes, *qnrA, qnrB, qnrS, qnrC, qepA*and *aac (6')1b* were investigated by polymerase chain reaction (PCR) with spesific primers (Table 1). The amplification was carried out in Sensoquest thermal cycle (Labcycler, Germany). PCR amplification steps for *qnrA, qnrB, qnrS* genes were at follows: an initial denaturation at 94°C for three minutes and following 32 cycles at 94°C for 45 sec, annealing at 53°C for 45 seconds, elongation at 72°C for one minute, final elongation at 72°C for five minutes. The following amplification steps was applied for*qnrC*, *qepA* and *aac(6')1b* genes; pre-denaturation at 94°C for 3 minutes, following 30 cycles at 94°C for 30 seconds, annealing at 53°C for 45 seconds, elongation at 72°C for one minute, final

elongation at 72°C for five minutes. The PCR products were analyzed on 1.5% agarose gel and visualized with ultraviolet light transilluminator staining with 0.5μg/mL ethidium bromide. Presence of bands at 646, 562,447,416,199 and 482 bp was considered positive for the *qnrA, qnrB, qnrC, qnrS*, *qepA* and *aac(6')1b* genes respectively. After *aac(6')-1b* determinant was amplified by PCR, all *aac(6')-1b* positive amplicons were analyzed by digestion with *BseGI* restriction enzyme (Fermentas, USA) to identify *aac(6')-1b-cr* variant [11].

This cross sectional study was approved by Ethical Committee of Faculty of Medicine, Selcuk University (Number of decision: 335, November 2013).

Table 1.Primer sequences and expected band size used in PCR.

Genes	Primer	Sequence (5'-3')	Base pair
qnrA	QnrA-F QnrA-R	TCAGCAAGAGGATTTCTCA GGCAGCACTATTACTCCCA	626 bp
qnrB	QnrB-F QnrB-R	ATGACGCCATTACTGTATAA GATCGCAATGTGTGAAGTTT	562 bp
qnrC	QnrC-F QnrC-R	GGGTTGTACATTTATTGAATC TCCACTTTACGAGGTTCT	447 bp
qnrS	QnrS-F QnrS-R	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	416 bp
qepA	QepA-F QepA-R	GCAGGTCCAGCAGCGGGTAG CTTCCTGCCCGAGTATCGTG	199 bp
aac(6')1b	Aac(6)-1b-F Aac(6)-1b-R	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	482 bp

Statistical analysis

Descriptive statistics were given as percentage and frequency when statistical evaluation was performed.

RESULTS

In the year 2013, 2663 *E.coli* starins were isolated from different clinical specimens at our hospital microbiology laboratory. Of the 2663 isolates, 36.6% was resistant to ciprofloxacin and 26.6% was resistant to levofloxacin. One hundred and fifteen quinolone-resistant *E.coli* strains were isolated from 50 male and 65 female patients. 77.4% of strains were isolated from urine, 11.3% from wound, 6.0% from blood, 2.6% from drainage fluid, 0.9% from BAL, 0.9% from vaginal discharge and 0.9% from abscess samples.

Of the 115 quinolone-resistant *E.coli* strains 76.5% was found to be ESBL positive. The resistance rates of the isolates to antibiotics were presented in table 2.

Table 2. The resistance rates of *E. coli* isolates to antibiotics

Antibiotics	Number (n:115)	Percent
Ertapenem	1	0.86
İmipenem	1	0.86
Meropenem	1	0.86
Nitrofurantoin	12	10.43
Amikacin	24	20.86
Piperacilline-tazobactam	46	40.00
Gentamicin	56	48.69
Ceftazidime	83	72.17
Ceftriaxone	85	73.91
Cefuroxime	90	78.26
Amoxicillin-clavulanic acid	93	80.86
Ampicillin	109	94.78

Plasmid-mediated resistance genes were detected in 62 strains. *qnrB*, *qnrS* and *aac (6')-1b-cr* genes were found positive in three (2.6%), nine (7.8%) and 50 isolates (43.5%), respectively. *aac (6')-1b-cr* gene was determined positive in two of three *qnrB* positive strains and in five of the nine *qnrS* positive strains. *qnrA*, *qnrC* and *qepA* genes were detected in none of quinolone-resistant isolates by PCR (Figure 1).

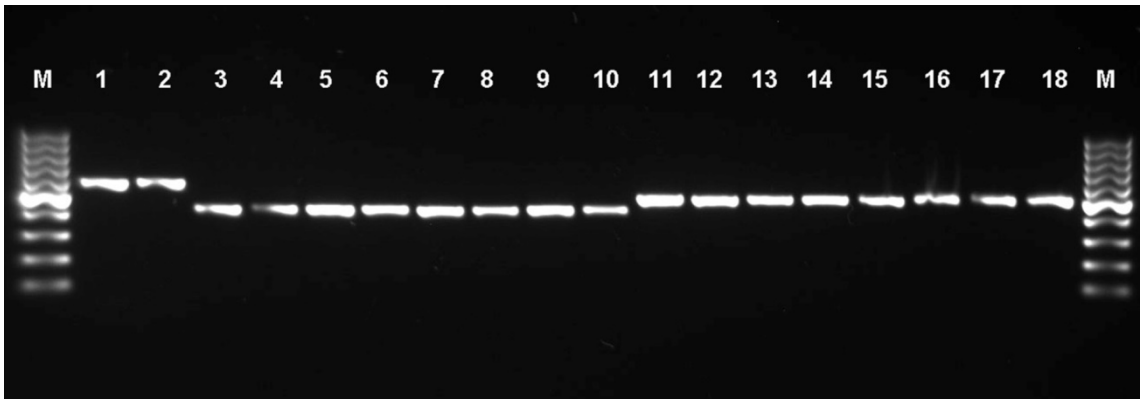

Figure 1: Agarose gel image of PCR products in *qnrS, qnrB* and *aac(6')-1b-cr* genes of *E.coli* strains [M: Marker, 1-2: *qnrB* positive isolates (562bp), 3-10: *qnrS* positive isolates (416bp), 11-18:*aac(6')-1b-cr* positive isolates (482bp)].

DISCUSSION

E. coli is a frequent cause of life-threatening bloodstream infections and other common infections, such as urinary tract infections and is one of the major agents of community and hospital-acquired infections. Rates of resistance exhibit variation in foreign studies on quinolone resistance of *E.coli.* In a study conducted in Greek, the ciprofloxacin resistance of *E.coli* strain was determined to be 21% [12]. In a study conducted in China, ciprofloxacin resistance of 202 *E.coli* strains was found to be 74% whereas their levofloxacin resistance was found to be 71%, both of which were very high rates. The ciprofloxacin resistance of uropathogen *E.coli* in some parts of China reached very high rates such as 41-80% [13]. The rates of ciprofloxacillin resistant *E.coli* strains in Turkey vary between 42 % and 45% [14,15]. In our laboratory in 2013 of the 2663 isolates, 36.6% was resistant to ciprofloxacin and 26.6% was resistant to levofloxacin.

Investigation of a qnrA plasmid from Shanghai that provided more than the expected level of ciprofloxacin resistance led to the discovery in 2006 of a second mechanism for PMQR: modification of certain quinolones by a particular aminoglycoside acetyltransferase, *aac(6')-Ib-cr*. A third mechanism for PMQR was added in 2007 with the discovery of plasmid-mediated quinolone efflux pumps *QepA* and *OqxAB*. In the past decade these genes have been found in bacterial isolates from around the world. They reduce the susceptibility of bacteria to quinolones, usually not to the level of non-susceptibility, but facilitating the selection of more quinolone resistant mutants and treatment failure [6].

The first PMQR in Turkey was reported by Naziket al.,[16] in 2005. Presence of *qnrA* gene was investigated on 49 ESBL-positive strains (36 *E.coli,* 7 *K.pneumoniae*, 4 *Enterobacter* spp. and 2 *Citrobacter* spp.) in Istanbul, and it was found in one *Enterobacter cloacae* and one *C.freundii* strain (4%). In a study conducted in Turkey, *qnrA*, *qnrB*, and *qnrS* genes were investigated in 460 *Enterobacteriaceae* and other gram-negative bacteria isolated from intensive care patients, and *qnrB13* gene was identified in one (0.65 %) and *qnrS1* gene was found in two of the three *E.cloacae* isolates [17]. In an another study from Turkey in which prevalence of *qnrA*, *qnrB*, *qnrS*, and *aac (6')-Ib-cr*genes was investigated, in 248 ESBL-producing *E.coli* and *K.pneumoniae* isolates, *qnrB1* and *aac (6')-Ib-cr* genes were identified in one *K.pneumoniae* isolate, moreover, it was indicated that *aac (6')-Ib-cr* gene was present in 78 %(n: 50) of the ESBL-positive isolates [18]. In 112 quinolone-resistant *E.coli* strains isolated from various clinical samples of which 78 (69.6 %) were ESBL-positive at Afyon Kocatepe University,

none of the *qnrA*, *qnrB*, *qnrS*, *qnrC* and *qepA*-type genes were identified in and *aac (6')-1b-cr* gene was identified at a rate of 59.8 % (67/112) [11].

In our study, *qnrA*, *qnrB*, *qnrS, qnrC, qepA* and *aac (6')-1b-cr* plasmid-based quinolone resistance genes were investigated in a total of 115 quinolone-resistant *E.coli* strains isolated from various clinical samples at Microbiology Laboratory of Selcuk University Medical School Hospital, and of which 88 (76.5 %) were ESBL-positive. *qnrA*, *qnrC* and *qepA*-type genes were not found in any of the 115 *E.coli* strains, whereas *qnrB* was discovered in three (2.6 %) strains, *qnrS* was found in nine (7.8%) strains and *aac (6')-1b-cr* was found in 50 (43.5%) strains. When we compare the results of the studies from Turkey *aac (6')-1b-cr* gene is the most common gene responsible from PMQR as we found. There are two limitations in this study; firstly, it is the lack of characterization of quinolone resistance-determining regions, and the latter the strains were not tested for clonality.

In conclusion, we determined that *aac(6')-1b-cr* gene was responsible for most of the quinolone-resistant *E. coli* strains from Konya, Turkey. Although 77.4% of the *E.coli* isolates were from uncomplicated UTIs, in our study and quinolones have been widely used for the treatment of UTI because of their *in vitro* activity and high efficacy widely use of quinolones has contributed in increasing resistance. The resistance limits the use of these useful antimicrobials, so surveillance of local and national resistance is very important on using quinolones carefully.

Acknowledgement

This study was supported by Selcuk University Scientific Research Projects Coordinatorship with project number 14202010.

This study was presented as poster in 1st Turkish Congress of One Health, Konya, Turkey, 9-10 April 2015.

REFERENCES

1. Murray PR, Rosenthal KS, Pfaller MA. *Escherichia coli*: Medical Microbiology. Philaedelphia, Elsevier, 2016.
2. Croxen MA, Finlay BB. Molecular mechanisms of *Escherichia coli* pathogenicity. Nat Rev Microbiol. 2010;8:26-38.
3. Fàbrega A, Madurga S, Giralt E, Vila J. Mechanism of action of and resistance to quinolones. Microb Biotechnol. 2009;2:40–61.
4. Heeb S, Fletcher MP, Chhabra SR, Diggle SP, Williams P, Cámara M. Quinolones: from antibiotics to autoinducers. FEMS Microbiol Rev. 2011;35:247–74.
5. Poirel L, Cattoir V, Nordmann P. Plasmid-mediated quinolone resistance; interactions between human, animal, and environmental ecologies. Front Microbiol. 2012;3:24.
6. Jacoby GA, Strahilevitz J, Hooper DC. Plasmid-mediated quinolone resistance. Microbiol Spectr. 2014;2:PLAS-0006-2013.
7. Rodriguez-Martinez JM, Cano ME, Velasco C, Martinez-Martinez L, Pascual A. Plasmid-mediated quinolone resistance: an update. J Infect Chemother. 2011;17:149–82.
8. Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A. Plasmid-mediated quinolone resistance: a multifaceted threat. Clin Microbiol Rev. 2009;22:664–89.
9. Yamane K, Wachino J, Suzuki S, Kimura K,Sahipata N, Kato H et al. New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate. Antimicrob Agents Chemother. 2007;51:3354-60.
10. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-third informational supplement. CLSI Document M100-S23. Wayne, PA, Clinical and Laboratory Standards Institute,. 2013.
11. Aktepe OC, Asık G, Cetinkol Y, Bicmen M, Gulay Z. Investigation of plasmid-mediated quinolone resistance in *Escherichia coli* strains. Mikrobiyol Bul. 2012;46:9-16.
12. Mavroidi A, Miriagou V, Liakopoulos A, Tzelepi E, Stefos A, Dalekos GN et al. Ciprofloxacin-resistant Escherichia coli in Central Greece: mechanisms of resistance and molecular identification. BMC Infect Dis. 2012;12:371.
13. Zhao L, Chen X, Zhu X, Yang W, Dong L, Xu X et al. Prevalence of virulence factors and antimicrobial resistance of uropathogenic *Escherichia coli* in Jiangsu province (China). Urology. 2009;74:702–7.
14. Yilmaz N, Agus N, Yurtsever SG, Pullukcu H, Gulay Z, Coskuner A et al. Prevalence and antimicrobial susceptibility of *Escherichia coli* in outpatient urinary isolates in Izmir, Turkey. Med Sci Monit. 2009;15:161–5.
15. Dogru A, Karatoka B, Ergen P, Sen Aydın O, Tigen ET. The resistance rates of urinary tract infections: our data from year 2010. Turk J Urol. 2013;39:237-43.
16. Nazik H, Poirel L, Nordmann P. Further identification of plasmid-mediated quinolone

resistance determinant in *Enterobacteriaceae* in Turkey. Antimicrob Agents Chemother. 2005;49:2146-7.
17. Nazik H, Öngen B, Kuvat N. Investigation of plasmid-mediated quinolone resistance among isolates obtained in a Turkish intensive care unit. Jpn J Infect Dis. 2008;61:310-2.
18. Poirel L, Gür D, Minarini L, Arslan U, Nordmann P. Molecular epidemiology of plasmid mediated quinolone resistance determinants in extended spectrum beta-lactamase producing *E.coli* and *K.pneumoniae* isolates from Turkey. 18th European Congress of Cilinical Microbiology and Infectious Diseases Abstract CD, 19-22 April 2008, Barcelona 2008; P 1527.