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Analysis of Plasmid-Mediated Quinolone Resistance (PMQR) Genes Among Escherichia coli Strains Isolated from Patients with Urinary Tract Infection in Eastern Turkey

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Abstract

Aim: Urinary tract infections are a common occurrence worldwide, with females being more susceptible than males. Infections can manifest as asymptomatic bacteriuria or more severe forms such as sepsis. This study aims to analyse the plasmid-mediated quinolone resistance genes of *Escherichia coli (E. coli)* responsible for causing urinary tract infections.

Material and Method: Microbiological evaluation was conducted using conventional culture method, API 20E (API-bioMérieux, France), antibiotic susceptibility tests, and polymerase chain reaction (PCR).

Results: During the study period, 145 *E. coli* strains were collected from 96 (66.2%) outpatients and 49 (33.8%) hospitalised patients with urinary tract infections (UTI). The resistance rates of *E. coli* strains to quinolone antibiotics were as follows: nalidixic acid in 102 strains (70.3%), ciprofloxacin in 96 strains (66.2%), ofloxacin in 73 strains (50.3%), gatifloxacin in 47 strains (32.4%), levofloxacin in 41 strains (28.3%) and moxifloxacin in 32 (22%) strains. Based on the PCR test results, it was found that 80% (116) of the isolates carried at least one PMQR gene. Furthermore, 31.9% (37/116) of the isolates were found to carry two or three PMQR markers simultaneously.

Discussion and Conclusion: The objective of this study was to establish the prevalence of quinolone resistance among *E. coli* strains isolated from patients with UTI. To control the high rate of spread of antibiotic-resistant microorganisms, it is crucial for all healthcare facilities to implement more careful management of antibiotic use and adhere to procedures set by infection control programmes.

Keywords: *E. coli*, PMQR, Urinary tract infections

Introductıon:

Urinary tract infections (UTIs) are among the most common bacterial infections, which can cause morbidity and mortality (1). Urinary tract infections (UTIs) can manifest in various forms, ranging from asymptomatic bacteriuria and cystitis to more severe conditions such as pyelonephritis and sepsis (2, 3). Urinary tract infections (UTIs) affect between 150 and 250 million people worldwide every year. They are more prevalent in females, with an estimated 50% of women experiencing this type of infection at some point in their lives (4). Although UTIs can be caused by many microorganisms, the majority of cases are caused by *E. coli*. This bacterium is recognised as an opportunistic pathogen in hospitals and is considered one of the most serious therapeutic challenges due to its acquisition of plasmids encoding genes resistant to different antibiotics, including beta-lactams and quinolones (3, 5). It is important to consider *E. coli* in the empirical treatment of community-acquired UTIs. Beta-lactams and fluoroquinolones are commonly used to treat urinary tract infections due to their efficacy, broad spectrum of action, oral bioavailability and patient compliance (6). However, the emergence of multidrug-resistant (MDR) bacteria has recently made the use of these antibiotics ineffective.

The emergence of *E. coli* isolates that contain extended-spectrum lactamases (ESBLs) posed a

significant problem in the treatment of urinary tract infections (UTIs) with beta-lactams. ESBLs were first observed in hospitals in the early 1980s (7). ESBLs are enzymes that degrade beta-lactam antibiotics, causing resistance to various antibiotics, including third-generation cephalosporins such as cefotaxime, ceftazidime, and ceftriaxone. Additionally, bacteria were found to have plasmid-mediated quinolone resistance (PMQR) caused by proteins called Qnr determinants. These Qnr proteins protect from quinolone inhibition by binding to bacterial DNA gyrase and topoisomerase IV. Several qnr genes (qnrA, qnrB, qnrC, qnrD, qnrS, qnrE and qnrVC) have been identified in bacteria. Additionally, other PMQR determinants for drugs include aac (6')-Ib-cr, qepA and oqxAB (6, 8).

This study was designed to investigate the occurrence rates of quinolone resistance and PMQR genes in ESBL-producing isolates in eastern Turkey. The resistance patterns of *E. coli* strains causing UTIs are constantly changing, and there is insufficient data on this topic.

Materials ve Methods

Assessment of clinical samples

In January and December 2021, *E. coli* isolates were collected from urine samples of outpatients and hospitalised patients diagnosed with UTI in Van city, district and training research hospital in eastern Turkey. Urine samples were obtained using a clean capture mid-flow protocol. A sample was considered positive for UTI if the count of a single microorganism was 10^5 CFU/ml or higher in a urine sample from all patients with UTI symptoms (9). The study excluded patients who had taken antibiotics within two weeks prior to sampling. To isolate organisms in UTI, conventional microbiological methods were used, including MacConkey (MAC) and eosin methylene blue (EMB) agar (Merck, Germany) (10). The strains were confirmed using the identification kit API 20E protocol (API-bioMérieux, France). All identified isolates were stored at -20°C in tryptic soya broth with 20% glycerol.

Antimicrobial susceptibility tests (AST) of *E. coli* isolates

The Kirby-Bauer disc diffusion test method, recommended by the Clinical and Laboratory Standards Institute (CLSI), was used to perform AST on both ESBL-positive and ESBL-negative isolates (11). The antibiotic discs used in the test included ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), gatifloxacin (5 µg), and moxifloxacin (5 µg) (Mast Group, Bootle, UK). The test employed *E. coli* ATCC 25,922 as a quality control strain. The minimum inhibitory concentration (MIC) of ESBL isolates against ciprofloxacin and levofloxacin antibiotics was determined using the E-test method (AES, AB Biodisk, Solna, Sweden).

ESBL verification test

Phenotypic confirmatory testing for extended-spectrum beta-lactamase (ESBL) was conducted using a combination disc test with ceftazidime (30 µg) and cefotaxime (30 µg), with or without clavulanic acid (10 µg) (Mast Diagnostics, UK). Organisms were considered ESBL-positive when the zone of inhibition around the antimicrobial agents in combination with clavulanic acid was ≥ 5 mm (11). Klebsiella pneumoniae ATCC 700,603 was used as positive control strain.

Molecular analysis of PMQR genes of *E. coli* isolates

The EcoSpin Bacterial Genomic DNA kit (Echotech Biotechnology, Turkey) protocol was used to extract DNA from a pure colony of *E. coli* isolates. PCR screening was performed on all isolates using the specific primers listed in Table I. The amplification conditions were set according to the following thermal cycling profile: 5 minutes at 96°C, followed by 30 cycles of 30 seconds at 96°C, annealing for 30 seconds (Table I), 1 minute at 72°C, and 5 minutes at 72°C for the final extension (17). To distinguish the aac(6')-Ib-cr variant, the amplified region was deleted using BseGI restriction endonuclease, as previously described (15). PCR amplification for the studied genes was performed using a C1000 Bio-Rad

Thermal Cycler (Bio-Rad Laboratories, Inc.). The May Taq™ DNA Polymerase (Bioline, Bio-21105) kit protocol was used for bacterial DNA amplification. For Polymerase Chain Reaction (mPCR), a set of chemical solutions and substances were used. The reaction mixture consisted of 10µL 5x MyTaq reaction buffer (5 mM dNTPs, 15 mM MgCl2), 5µL template DNA, 1µL of each primer (20uM), 1µL MyTaq DNA polymerase, and 8µL nuclease-free water, making a total of 25µl of the final solution. The resulting amplicons were electrophoresed on a 1.5% agarose gel containing ethidium bromide (0.5 mg/mL) at 100 V for 2 hours.

Table I. Reference primers used in this study

Target gene	Primer sequences	Product size (bp)	Annealing temperature (^{o}C)	References
qnrA	F-5'-TTTCTCACGCCAGGATTTG -3' R-3'-GATCGGCAAAGGTTAGGTCA-5'	516	53	12
qnrB	F-5'-GATCGTGAAAGCCAGAAAGG-3' R-3'-ACGATGCCTGGTAGTTGTCC-5'	469	53	12
qnrC	F-5'-GGGTTGTACATTTATTGAATC-3' R-3'-TCCACTTTACGAGGTTCT-5'	447	50	13
qnrD	F-5'-CGAGATCAATTTACGGGGAATA-3' R-3'-AACAAGCTGAAGCGCCTG-5'	582	50	14
qnrS	F-5'-ACGACATTCGTCAACTGCAA-3' R-3'-TAAATTGGCACCCTGTAGGC-5'	417	53	12
aac(6')Ib	F-5'-TTGCGATGCTCTATGAGTGGCTA-3' R-3'-CTCGAATGCCTGGCGTGTTT-5'	482	54	15
qepA	5'-GCAGGTCCAGCAGCGGGTAG-3' 3'-CTTCCTGCCCGAGTATCGTG-5'	199	60	16

Results

During the study period, 145 confirmed *E. coli* strains were collected from patients with UTI. Out of the 145 strains, 96 (66.2%) were from outpatients and 49 (33.8%) were from hospitalised patients. The majority of the patients (80%) were female, and the age range of the volunteers was between 6 and 68 years (mean 43 ± 20.2). The resistance rates of *E. coli* strains to quinolone antibiotics were as follows: 102 (70.3%) for nalidixic acid, 96 (66.2%) for ciprofloxacin, 73 (50.3%) for ofloxacin, 47 (32.4%) for gatifloxacin, 41 (28.3%) for levofloxacin, and 32 (22%) for moxifloxacin. The results of the ESBL screening test revealed that 39.3% (57/145) of the strains analysed were ESBL-producing. Of these, 76.9% (45/57) were isolated from female patients, and the majority were obtained from outpatients. The analysis of the AST showed that both the ESBL-positive and ESBL-negative groups had the highest resistance rates to nalidixic acid (87.6% and 75.4%, respectively) and ciprofloxacin (67.3% and 64.5%, respectively). Additionally, the quinolone antibiotic with the lowest resistance rate was moxifloxacin, with rates of 44.3% in ESBL positive isolates and 23% in ESBL negative isolates. Out of the 45 ESBL-producing isolates, 27 (60%) had high MICs (≥1 µg/ml for ciprofloxacin and ≥2 µg/ml for levofloxacin) and 15 (33.3%) had high MICs for both antibiotics. The PCR test results showed that 116 (80%) of the isolates harboured at least one PMQR gene. Specifically, 66/116 (56.9%) had the acc(6')-Ib-cr variant (34 ESBL-positive and 32 ESBL-negative), while 35/116 (30%)... Of the isolates tested, 9 ESBL-positive and 26 ESBL-negative were found to carry qnrB (2%), 5 ESBL-positive and 19 ESBL-negative carried qnrS (20.7%), and 3 ESBL-positive and 8 ESBL-negative carried qnrA (14.7%). None of the isolates were found to carry qnrC, qnrD, or qepA genes. Additionally, 31.9% (37/116) of isolates carried two or three PMQR markers simultaneously. There was no coexistence of plasmid-mediated fluoroquinolone

resistance determinants among ESBL-negative strains.

Discussion

The increased use of quinolones and beta-lactams in treating various diseases has been linked to the emergence of drug resistance (18). To date, PMQR determinants have been extensively reported worldwide. However, no such survey has been conducted in Van, eastern Turkey. In this study, we examined quinolone resistance and PMQR genes in ESBL-positive *E. coli* strains in Van for the first time. The aim was to inform clinicians about the local antimicrobial resistance and contribute to global data to advance surveillance programmes. ESBL production of bacteria varies geographically and even within hospitals. For instance, in Iran, ESBL-producing *E. coli* isolates have an overall prevalence of 43.2% (19). Furthermore, ESBL rates have been reported in various regions across the globe. For instance, in Colombia, the rate was 11.7% (20), while in Portugal, it was 67.9% (21), and in Japan, it was 20.4% (22). The prevalence rate of ESBL in our study was 39.3%. Variations in ESBL production may be attributed to differences in sample type and volume used in different geographical areas, methodology, and treatment protocol changes. Our study found that *E. coli* isolates exhibited high levels of resistance to nalidixic acid (88%), ciprofloxacin (65.4%), and ofloxacin (53%), which is consistent with previous studies (23, 24). However, resistance to nalidixic acid was higher compared to ciprofloxacin and ofloxacin. This may be due to the prolonged use of nalidixic acid in the studied region for over thirty years (25). Furthermore, the high rates of quinolone resistance in different regions may be related to the widespread use of oral types, which are often available over-the-counter. Quinolone resistance is caused by mutations in quinolone target gene regions. Recently, plasmid-dependent quinolone resistance genes have also played a role in the spread of quinolone-resistant isolates due to their high horizontal transferability (26). The percentage of *E. coli* isolates containing qnr genes in our study was 80%, which is significantly higher than the percentages reported in other studies (12, 27, 28). Furthermore, previous studies have demonstrated that the prevalence of qnr genes in ESBL-producing *E. coli* strains is similar to our findings (23, 29). Zhou et al., (30) reported the frequencies of these genes (qnrA, qnrB and qnrS) in *E. coli* isolates as 2 (0.4%), 6 (1.2%) and 14 (2.7%), respectively. Mansouri et al., (31) reported that the highest frequency of qnr genes was reported for qnrB (56.5%), followed by qnrA (31.8%) and qnrS (28.9%) genes and this rate was significantly higher than our study. Furthermore, the prevalence of qnr genes in ESBL-positive isolates was found to be 6.8%, which is notably lower than the rate obtained in this study. The variation in the prevalence of qnr genes may be attributed to differences in the geographical regions studied, material characteristics, and public health levels in communities. The second group of PMQR genes is a variant of aminoglycoside acetyltransferase (aac(6')-Ib-cr), which reduces sensitivity to ciprofloxacin by N-acetylation of piperazinyl amine (32). The frequency of the aac(6')-Ibcr gene among ESBL-positive *E. coli* isolates has been reported in several studies, such as 23.3% in Egypt (33) and 59.2% in Hungary (34). In this study, the aac(6')-Ib-cr variant gene was detected in 56.9% of PMQR-positive strains and 51.5% of ESBL producers. Quinolone resistance genes are commonly plasmid-mediated and spread rapidly through gene transfer mechanisms, especially conjugative plasmids with high molecular weight, according to studies (35, 36). According to a study, the high incidence of the transconjugation mechanism of the gene may be related to the hot and humid weather of south-western Iran (27).

Conclusions

The objective of this study was to determine the prevalence of quinolone resistance among *E. coli* isolates obtained from patients with UTI. The study reports a significant prevalence of PMQR determinants in ESBL-producing isolates from Van. However, these genes do not appear to be the main mechanism of quinolone resistance. To control the high rate of spread of antibiotic-resistant microorganisms, it is crucial for all healthcare facilities to implement careful antibiotic use management and follow infection control programmes.

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