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RESEARCH ARTICLE

PMQR Determinants Expression in Gram-negative Uropathogens Clinically Isolated from Hospitalized Patients with Pyelonephritis in Kharkiv, Ukraine

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Abstract:

Background:

Resistance to beta-lactams and fluoroquinolones has been increasing in the treatment of urinary tract infections (UTIs), worldwide. Recent studies in Europe and the United States have demonstrated that steady increase in the rate of uropathogen's resistance to commonly prescribed antibiotics is associated with plasmid-mediated resistance genes existence. According to the published data, acquired resistance to quinolones is predominantly mediated by plasmid-mediated quinolone resistance determinants (PMQR) that compromise the efficacy of the first, second and third generation quinolones.

The objective of this study was to determine the prevalence of PMQR genes among uropathogens from hospitalized patients with pyelonephritis and to identify the presence of genes involved in the resistance, specifically - aac(6')-Ib-cr, QnrA and efflux pump QepA.

Methods:

A cross-sectional study of 105 patients with pyelonephritis, treated in Kharkiv City Clinical Emergency Hospital, Ukraine was carried out. Bacterial isolates were collected, antimicrobial susceptibility of isolates was determined by the Kirby Bauer disk diffusion method and screening for the presence of aac(6')-Ib-cr, QnrA and efflux pump QepA PMQR genes was performed by polymerase chain reaction.

Results:

Among 81 isolated gram negative bacterial strains, 39 (48.1%) were identified to carry different types of plasmid-mediated resistance determinants, among which 27 (69.2%) were found to be extended spectrum beta-lactamases producers, and 12 (30.8%) – were positive for plasmid-mediated quinolone resistance genes. Most of the identified genes were found in *P. mirabilis, E. coli* and *Serratia* spp. strains with its prevalence of 62.5%, 52.8% and 50%, respectively. Most common isolated gene was efflux pump QepA. In this study, 100% of the PMQR producing isolates are identified as meropenem susceptible. Global resistance to fluoroquinolones was ≥ 20% among isolated gram-negative strains. Treatment by fluoroquinolones demonstrated the best favorable clinical response in the patients infected with ESBL-producing organisms, whereas cephalosporins were the most effective in patients infected with fluoroquinolone resistance uropathogens.

Conclusion:

Therapeutic alternatives for the treatment of UTI patients with resistant uropathogens, particularly in hospitalized patients, are

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limited. Rational use of antibiotics in practice and/or the proper detection of plasmid-mediated resistance genes among the bacteria in communities are crucial for further prevention of antimicrobial resistance development.

Keywords: Quinolones, Pyelonephritis, Uropathogens, Plasmids, Resistance, Genes.

INTRODUCTION

Urinary tract infections (UTIs) are thought to be the most common group of bacterial infections, worldwide. It is estimated that the proportion of UTIs remains at a high level reaching 150 million episodes per year worldwide and accounting for $6 billion in health care expenditures [1, 2]. In Ukraine, among the UTI, pyelonephritis is a leading cause of end-stage chronic kidney disease (CKD) [3]. In the USA, more than 100,000 hospital admissions per year are the UTIs patients, among them, the patients with pyelonephritis are the most frequent. For the US outpatients, approximately 15% of antibiotics used are prescribed for UTIs [4].

Meanwhile, beta-lactams and fluoroquinolones are well-known as the main therapeutics effective to treat such infections [4]. However, recent studies in Europe and the United States have demonstrated a steady increase in the rate of uropathogen resistance to commonly prescribed antibiotics, and this obviously will lead to a reduction in therapeutic possibilities of UTI [2, 5]. The most recent worldwide estimates of global antibiotic resistance, published by the World Health Organization (WHO) in 2014, list *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus* as the three agents of greatest concern, associated with both hospital – and community acquired infections. In five of the six WHO regions, some countries reported *E. coli* resistance of more than 50 percent to fluoroquinolones and third-generation cephalosporins [6]. In the United States, CDC has estimated that more than 2 million infections and 23,000 deaths are due to antibiotic resistance each year [7]. In Europe, an estimated 25,000 deaths are attributable to antibiotic-resistant infections [5].

Recent studies in Europe and the United States have demonstrated that steady increase in the rate of uropathogen resistance to commonly prescribed antibiotics is associated with plasmid-mediated extended spectrum β-lactamases and fluoroquinolone resistance determinants [2, 5]. According to the published data, acquired resistance to beta-lactams is predominantly mediated by extended spectrum beta-lactamases (ESBLs) that compromise the efficacy of all known beta-lactams, except cephamycine and carbapenems [8]. ESBLs are often encoded by genes located on large plasmids, which also carry genes related with resistance to other antimicrobial agents such as aminoglycosides, trimethoprim, sulphonamides, tetracyclines and chloramphenicol [9]. This type of resistance is basically encoded by plasmids derived from TEM, SHV and CTX-M family. In addition, it has been reported by the European Antimicrobial Resistance Surveillance Studies (EARSS) that the level of ESBL-positivity among *Escherichia coli* and *K. pneumoniae* isolates resistant to the third-generation cephalosporins fluctuates from 71.1% to 100%, with a majority of the countries reporting percentages above 90%. For 2014, 29 countries reported 82 815 *E. coli* isolates with AST information for fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin, norfloxacin or ofloxacin). The number of isolates reported per country ranged from 141 to 10 307. The national percentages of resistant isolates ranged from 7.8% (Iceland) to 46.4% (Cyprus) in 2014. A majority of the countries reporting resistance percentages of 25% or higher were located in southern and south-eastern Europe. The EU/EEA population-weighted mean percentage for fluoroquinolone resistance was 22.4% in 2014 [5].

Three mechanisms for plasmid-mediated quinolone resistance (PMQR) have been discovered since 1998. Plasmid genes qnrA, qnrB, qnrC, qnrD, qnrS, and qnrVC code for proteins of the pentapeptide repeat family that protects DNA gyrase and topoisomerase IV from quinolone inhibition. The second plasmid-mediated mechanism involves acetylation of quinolones with an appropriate amino nitrogen target by a variant of the common aminoglycoside acetyltransferase AAC(6′)-Ib. The third mechanism is enhanced efflux produced by plasmid genes for pumps QepA and OqxAB. Genes for PMQR have been found on plasmids varying in size, incompatibility and specificity, indicating that the spread of multiple plasmids has been responsible for the dissemination of this resistance around the world. AAC(6′)-Ib-cr is a bifunctional variant of a common acetyltransferase active on such aminoglycosides as amikacin, kanamycin, and tobramycin but also able to acetylate those fluoroquinolones with an amino nitrogen on the piperazinyl ring, such as ciprofloxacin and norfloxacin. QepA is a plasmid-mediated efflux pump in the major facilitator (MFS) family that decreases susceptibility to hydrophilic fluoroquinolones, especially ciprofloxacin and norfloxacin [10 - 12]. Qnr genes are usually found in multi-resistance plasmids linked to other resistance determinants. Many studies have reported fluoroquinolone resistance mediated by co-transfer of the qnr determinant on ESBL-producing plasmids [13 - 15].

The data about the prevalence of plasmid-mediated quinolone resistance genes in our country is quite limited.

Moreover we did not find any publications regarding the prevalence of plasmid-mediated resistance genes to fluoroquinolones and its association with ESBL-producing plasmids in Ukraine as well as in contiguous countries, like Belarus, Moldova or Russia.

Therefore, the aim of our cross-sectional study was to determine the prevalence of plasmid-mediated quinolone resistance genes among urinary strains clinically isolated from hospitalized patients with pyelonephritis, treated in the Kharkiv City Clinical Emergency Hospital, Ukraine and to identify the presence of genes involved in the resistance, specifically – aac(6')-Ib-cr, qnrA and efflux pump QepA.

SUBJECTS AND METHODS

A cross-sectional study of 105 adult patients with pyelonephritis (the diagnosis pyelonephritis has been stated in accordance with the criteria established by European Association of Urologists), who were admitted in Kharkiv City Clinical Emergency Hospital, Ukraine, was carried out. The study has been carried out between April 2013 and February 2014. The patients were considered as those who have chronic kidney disease (CKD) G1-G4 stage, where the stage was evaluated using glomerular filtration rate (GFR) index calculated by formula CKD-EPI (KDIGO 2012) [16].

Our study is a prospective one. All the patients were discharged from the hospital; no mortality case was reported during hospitalization.

Bacterial Isolates

Midstream urine from the patients with pyelonephritis collected in a sterile container and processed in the medical biology department of the Kharkiv City Clinical Emergency Hospital within 2 h of collection. Urine samples were inoculated on blood agar or chromogenic media ChromID CPS (bioMerieux, France) then positive cases were incubated at 37°C for 24 hours, while negative cases – at 37°C for 48 hours. The samples were considered as significant if the number of colony forming units (CFU) was $\geq 10^5$ CFU/ml of urine. Identification of Gram positive catalase negative cocci (*Streptococci, Enterococci* and related genera) was performed using test systems ID 32 STREP production of bio Mérieux, France. Identification of Gram positive catalase positive cocci (*Staphylococcus, Micrococcus* and related genera) was performed using test systems ID 32 STAPH production of bio Mérieux, France. Identification of Gram negative bacillus - Enterobacteriaceae and other non-fastidious Gram-negative Bacillus was performed using test systems ID 32 GN production of bio Mérieux, France. Commercial identification kits were used according to the manufacturer's instructions.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of isolates was determined by the Kirby Bauer disk diffusion method on Mueller–Hinton agar-containing plates. The size of zone around each antimicrobial disk was interpreted as sensitive, intermediate or resistant according to the CLSI criteria. The following antibiotics had been tested: ampicillin, amoxicillin/clavulanate, cefotaxime, ceftriaxone, cefepime, ciprofloxacin, levofloxacin, nitrofurantoin, amikacin, gentamicin, nitroxolinum, meropenem ("Limited Liability Company ASPECT" Kyiv, Ukraine) co-trimoxazole, furazidinum, fosfomycin (HIMEDIA Laboratories, Pvt. Ltd., Mumbai, India) [17].

Detection of Plasmid-mediated Resistance Genes

The total DNA extraction was performed for all samples using the heat-shock technique. Screening for the presence of plasmid-mediated resistance genes was performed by polymerase chain reaction (PCR) sequencing assays. The following primers used for extended spectrum beta-lactamases and genes involved in the resistance to fluoroquinolones are shown in Table **1**. PCR products were analyzed by agarose gel electrophoresis and stained with ethidium bromide. Plasmid DNA, used as a molecular weight marker, was hydrolyzed by the enzyme puc19 HpaII [18].

Statistical Analysis

Statistical analysis was performed using the statistical the Statistical Package for the Social Sciences (SPSS), version 20. Categorical data (sex, setting and susceptibility to antibiotics) were presented as the number and percentage. The percentages in different categories were compared using Chi square test. The statistical analysis was done by using the proportions of sensitive, resistant and intermediates. A difference was considered to be significant if the probability that chance would explain the results, was reduced to less than 5% ($p \leq 0.05$). For each potential risk factor, odds ratios and 95% confidence intervals were calculated using multivariate analysis.

Table 1. Primers used for detection of plasmid-mediated resistance genes.

Resistance genes	Primers	Amplicon size (bp)
blaTEM	5'- ATG AGT ATT CAA CAT TTC CG 5'- CCA ATG CTT AAT CAG TGA GG	858
blaSHV	5'- ATG CGT TAT ATT CGC CTG TG 5'- AGC GTT GCC AGT GCT CGA TC	862
blaCTX-M	5'-SCS ATG TGC AGY ACC AGT AA 5'-ACC AGA AYV AGC GGB GC	585
Qnr A	qnrAF ATT TCT CAC GCC AGG ATT TG qnrAR GAT CGG CAA AGG TTA GGT CA	516
aac(6')-Ib-cr	aacIbF TTG CGA TGC TCT ATG AGT GGC TA aacIbR CTC GAA TGC CTG GCG TGT TT	482
QepA	qepAF AAC TGC TTG AGC CCG TAG AT qepAR GTC TAC GCC ATG GAC CTC AC	596

Ethics

Each patient was aware about the data collection, and written informed consent was obtained from each subject. The study protocol has been approved by the ethics committee at the Kharkiv Academy for Postgraduate Education, Kharkiv, Ukraine (No. 2, 22.02.2013). Anonymity was guaranteed during and after the study. The study is not invasive to be fully consistent with the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects approved by The World Medical Association.

RESULTS

From 105 observed patients, 14 (13.3%) were male and 91 (86.7%) were female, the mean age was 56.9 ± 1.7 years with an age range of 21 to 86 years. Among those, 21 (20%), 28 (26.7%), 27 (25.7%) and 29 (27.6%) were identified to have CKD G1, CKD G2, CKD G3 and CKD G4, respectively. The patients were hospitalized when they had the following symptoms: fever, nausea, vomiting, pain sensation in a costovertebral angle. Patients were divided into two treatment groups: group 1 (n=56) patients who were treated by cephalosporins (ceftriaxone, cefepim), and group 2 (n=49) patients who received fluroquinolones (ciprofloxacin, levofloxacin).

Out of 105 adult patients, 84 (80%) had positive urine cultures. From them, 115 different microorganisms were isolated, where 34 (29.6%) were gram positive and 81 (70.4%) were gram negative bacterial strains. The majority of the isolates (n = 73) were retrieved from the patients between ages 18–65 years, while 42 isolates were obtained from those aged more than 65 years. Overall, *Escherichia coli* was the most common microorganism isolated (53/115, 46.1%), while, among the gram-positive bacterial, *Enterococcus* spp. and *Staphylococcus* spp. were the dominant pathogens strains Table (**2**).

Table 2. Distribution of pathogens in urine isolates according to age and gender.

Organism	Total (n=115)	Male (n=6)	Female (n=78)	≤ 65 yrs (n=54)	≥ 65 yrs (n=30)
E. coli	53 (46.1%)	1 (16.7%)	52 (66.7%)	31 (57.4%)	22 (73.3%)
K.pneumoniae	9 (7.8%)	3 (50%)	6 (7.7%)	6 (11.1%)	3 (10%)
P.mirabilis	8 (6.9%)	0 (0.0)	8 (10.3%)	4 (7.4%)	4 (13.3%)
P.aeruginosa	8 (6.9%)	2 (33.3%)	6 (7.7%)	8 (14.8%)	0 (0.0)
E.cloacae	1 (0.9%)	0 (0.0)	1 (1.3%)	0 (0.0)	1 (3.3%)
***Serratia* spp.**	2 (1.7%)	1 (16.7%)	1 (1.3%)	0 (0.0)	2 (6.7%)
***Enterococcus* spp.**	17 (14.8%)	2 (33.3%)	15 (19.2%)	12 (22.2%)	5 (16.7%)
***Staphylococcus* spp.**	12 (10.4%)	0 (0.0)	12 (15.4%)	11 (20.4%)	1 (3.3%)
Corynebacterium	4 (3.5%)	0 (0.0)	4 (5.1%)	0 (0.0)	4 (13.3%)
***Streptococcus* spp.**	1 (0.9%)	0 (0.0)	1 (1.3%)	1 (1.9%)	0 (0.0)

Beside this, among 81 gram negative bacterial isolates, 39 (48.1%) were identified to carry different types of plasmid-mediated resistance determinants, among which 27 (69.2%) were found to be extended spectrum beta-lactamases producers, and 12 (30.8%) were positive for plasmid-mediated quinolone resistance genes. Most of the identified genes were found in *P.mirabilis, E. coli* and *Serratia* spp. strains with the prevalence of 62.5%, 52.8% and 50%, respectively. Beside this, among 28 (52.8%) plasmid positive *E. coli* isolates, 20 (71.4%) and 8 (28.5%) were

identified to carry ESBLs genes and PMQR determinants respectively. Out of five *P.mirabilis* strains, 4 (80%) and 1 (20%) were positive for extended spectrum β-lactamases and PMQR genes, respectively. Two of eight *P. aeruginosa* strains (25%) were observed carrying plasmid-mediated quinolone resistance determinants. The frequencies of ESBLs and PMQR genes in *K.pneumoniae* isolates were 66.7% and 33.3%, respectively. Most common isolated genes were TEM-type ESBLs and efflux pump QepA. According to our results, plasmid-mediated quinolone resistance determinants were present in 12 gram negative urinary isolates (12/81, 14.8%), among which, the efflux pump QepA and proteins Qnr predominated (6/12, 50% and 5/12, 41.7%, respectively). The prevalence of aminoglycoside acetyltransferase aac(6')-Ib-cr was 25% (3/12). Out of 53 *E. coli* isolates, 2 (3.8%), 2 (3.8%) and 4 (7.5%) were identified to carry QnrA, aac(6')-Ib-cr, and QepA determinants respectively. Of note, out of eight *P. aeruginosa*, two isolates (25%) were carrying QnrA. While, *P. mirabilis* strain and *K. pneumoniae* strain were positive for one aac(6')-Ib-cr and QepA, respectively. The prevalence of plasmid-mediated ESBL and PMQR determinants in the isolates of urine is presented in Table **3**.

Table 3. Prevalence of plasmid-mediated ESBL and PMQR determinants in isolates of urine.

Uropathogens	Total n (%)	ESBLs-producers	plasmid-mediated quinolone resistance genes n (%)		
			QnrA	AAC(6')-Ib-cr	QepA
E. coli	28/53 (52.8%)	20 (71.4%)	2 (25)	2 (25)	4 (50)
K. pneumoniae	3/9 (33.3%)	2 (66.7%)	0 (0.0)	0 (0.0)	1 (100)
P. mirabilis	5 (62.5%)	4 (80%)	0 (0.0)	1 (100)	0 (0.0)
P. aeruginosa	2 (25%)	0 (0)	2 (100)	0 (0.0)	0 (0.0)
***Serratia* spp.**	1 (50%)	1 (100%)	-	-	-

Overall, out of 27 ESBL-producing isolates, 7 (25.9%) coharboured at least two different ESBLs and plasmid-mediated quinolone resistance genes. Out of 20 ESBL producers *E. coli* isolates, 2 (10%) coharboured two different bla genes (CTX-M and blaTEM); 2 (10%) were positive for combination of QepA, blaTEM and blaCTX-M; 1 (5%) was positive for both blaSHV and QnrA. Two *P. mirabilis* (2/8 25%) strains carried both bla(TEM) and bla(CTX-M) genes. TEM-type ESBLs and efflux pump QepA were the most common isolated ESBLs and PMQR genes respectively.

In the treatment group 1, the prevalence of patients infected with ESBLs and PMQR positive urinary strains was 23.2% (13/56) and 10.7% (6/56), respectively. In the treatment group 2, the prevalence of patients infected with ESBLs and PMQR positive urinary strains was 14.3% (7/49) and 6.1% (3/49), respectively and 2 patients (4.1%) were infected with both ESBLs and PMQR producing urinary strains.

Antimicrobial testing of isolated gram negative uropathogens revealed the following susceptibility rates: ampicillin (19/81, 23.5%), amoxicillin/clavulanate (48/81, 59.3%), cefotaxime (48/81, 59.3%), ceftriaxone (58/81, 71.6%), cefepime (66/81, 81.5%), ciprofloxacin (57/81, 70.4%), levofloxacin (62/81, 76.5%), co-trimoxazole (65/81, 80.2%), nitroxolinum (74/81, 91.4%), furazidinum (64/81, 79%), amikacin (62/81, 76.5%), gentamicin (57/81, 70.4%), nitrofurantoin (64/81, 79%), meropenem (81/81, 100%), and fosfomycin (66/81, 81.5%). Accordingly, the global resistance to fluoroquinolones was ≥ 20% for ciprofloxacin and levofloxacin. The most active antimicrobial agents against isolated strains were meropenem, nitroxolinum, fosfomycin and cefepime.

Table **4** demonstrates the susceptibility of isolated strains against a spectrum of 15 selected antimicrobial agents of different classes. The highest resistance among QnrA-positive strains was observed against ampicillin (100%), amoxicillin/clavulanate (100%), fosfomycin (100%), nitrofurantoin (100%), nitroxolinum (100%) ciprofloxacin (75%), third generation cephalosporins (75%), fluoroquinolones (75%) and aminoglycosides (75%). Beside this, only meropenem (100% susceptibility) exhibited a good enough activity against PMQR-producing urinary strains. The highest resistance among QepA-positive strains was observed against ampicillin (100%), amoxicillin/clavulanate (60%), cephalosporins (60%), fluoroquinolones (60%) and aminoglicosides (60%). Only meropenem, nitroxolinum, nitrofurantoin, furazidinum, co-trimoxazole and fosfomycin exhibited a good enough activity against QepA-producing urinary strains. The most active antimicrobial agent against AAC(6')-Ib-cr-positive urinary strains was meropenem (100%).

Table 4. *In vitro* activity of several antimicrobial agents against PMQR producing urinary isolates.

Drug	PMQR-negative bacterial strains	PMQR-positive bacterial strains			
		Total number n (%)	QnrA (n=4)	QepA (n=5)	AAC(6')-Ib-cr (n=3)
Ampicillin	16/42 (38.1%)	0/12 (0%)	0%	0%	0%
Amoxicillin/clavulanate	31/42 (73.8%)	4/12 (33.3%)	0%	2/5 (40%)	2/3 (66.7%)
Cefotaxime	32/42 (76.2%)	6/12 (50%)	1/4 (25%)	3/5 (60%)	2/3 (66.7%)
Ceftriaxone	34/42 (81%)	5/12 (41.7%)	1/4 (25%)	3/5 (60%)	1/3 (33.3%)
Cefepime	40/42 (95.2%)	6/12 (50%)	1/4 (25%)	3/5 (60%)	2/3 (66.7%)
Ciprofloxacin	38/42 (90.5%)	4/12 (33.3%)	1/4 (25%)	3/5 (60%)	0%
Levofloxacin	40/42 (95.2%)	5/12 (41.7%)	1/4 (25%)	3/5 (60%)	1/3 (33.3%)
Co-trimoxazole	38/42 (90.5%)	8/12 (66.7%)	1/4 (25%)	5/5 (100%)	2/3 (66.7%)
Nitroxolinum	42/42 (100%)	7/12 (58.3%)	0%	5/5 (100%)	2/3 (66.7%)
Furazidinum	42/42 (100%)	5/12 (41.7%)	0%	5/5 (100%)	0%
Amikacin	36/42 (85.7%)	5/12 (41.7%)	1/4 (25%)	2/5 (40%)	2/3 (66.7%)
Gentamicin	35/42 (83.3%)	6/12 (50%)	1/4 (25%)	3/5 (60%)	2/3 (66.7%)
Nitrofurantoin	42/42 (100%)	6/12 (50%)	0%	5/5 (100%)	1/3 (33.3%)
Meropenem	42/42 (100%)	12/12 (100%)	100%	100%	100%
Fosfomycin	39/42 (92.9%)	6/12 (50%)	0%	4/5 (80%)	2/3 (66.7%)

It is clear in the table that, *in vitro* activity of cephalosporins, fluoroquinolones and aminoglycosides against PMQR-negative bacterial strains was significantly higher. However, the overall rate of uropathogen's resistance to commonly prescribed antibiotics was high, and this obviously will lead to a reduction in therapeutic possibilities of UTI, particularly in hospitalized patients. Beside this, we have established antimicrobial susceptibility of ESBLs-producing urinary strains with combination of different bla genes and PMQR determinants. Nitroxolinum and meropenem exhibited a good enough activity against ESBLs-producers with expression of different bla genes, whereas the most active antimicrobial agents against ESBLs-producing urinary strains with expression of PMQR genes were meropenem (100%), fosfomycin (100%), furazidinum (100%), nitroxolinum (100%), and amoxicillin/clavulanate (100%).

Out of 105 patients, 20 (19%) and 9 (9%) had ESBLs and PMQR positive urine cultures, respectively. Two patients (2%) were infected with both ESBLs and PMQR producing urinary strains. Clinically, the vast majority of the patients with resistance genes had flank pain (92.6% vs. 85.2% P = 0.045), symptoms of lower urinary tract infection (59.8% vs. 45.6% P = 0.002), and costovertebral angle tenderness (72.2% vs. 64.9% P = 0.015). The mean body temperature in patients infected with resistance bacteria was 38.3±0.1 °C, whereas in patients without resistance gene was 37.9±0.3°C. In both the treatment groups, the prevalence of patients infected with ESBLs producing urinary strains was significantly higher, than the prevalence of patients, infected with PMQR producers. Treatment by fluoroquinolones demonstrated the best favorable clinical response in the patients infected with ESBL-producing organisms, whereas cephalosporins were the most effective in patients infected with fluoroquinolone resistance uropathogens. Of note, that 12 (44.4%) of the ESBL-producers were isolated on the fifth day after the beginning of antibiotic therapy. However, the hospitalization term was longer for the patients with plasmid-mediated genes compared to the patients without this gene (median 10.4 days vs. 8.4 days, P < 0.05). All the patients were discharged from the hospital; no mortality case was reported during hospitalization. In the treatment group 1, a favorable clinical response was seen in 6 of 13 patients (46.2%) infected with ESBL-producing organisms compared with 4 of 6 patients (66.7%) infected with PMQR-

producing organisms ($p<0.05$). In the treatment group 2, a favorable clinical response was seen in 5 of 7 patients (85.7%) infected with ESBL-producing organisms compared with 1 of 3 patients (33.3%) infected with PMQR-producing organisms ($p<0.05$). In contrast, clinical efficacy of antibiotic therapy was higher in patients without plasmid-mediated genes existence and favorable clinical response was seen in 30 of 37 patients (81.1%) and 35 of 37 patients (94.6%) treated by cephalosporins and fluoroquinolones, respectively.

According to multivariate analysis, age > 55 years (OR 3.05; 95% CI: 1.12-8.32), atrial hypertension (OR 2.57; 95% CI: 0.94-7.04), chronic kidney disease stage III (OR 2.03; 95% CI: 0.80-5.10) and stage IV ст. (OR 1.1; 95% CI: 0.40-2.60), hospital admission (OR 2.02; 95% CI: 0.78-5.23), and the use of a beta-lactam antibiotic in the preceding year (OR 1.41; 95% CI: 0.60-3.33) were found to be associated with plasmid-mediated genes existence.

DISCUSSION

It was concluded from other papers that the prevalence of PMQRs producers among clinical isolates varies from country to country. The most recent worldwide estimates of global antibiotic resistance, published by the World Health Organization (WHO) in 2014, list *Escherichia coli, Klebsiella pneumoniae*, and *Staphylococcus aureus* as the three agents of greatest concern, associated with both hospital and community acquired infections. In five of the six WHO regions, some countries reported *E. coli* resistance of more than 50 percent to fluoroquinolones and third-generation cephalosporins [6]. The ARESC (Antimicrobial Resistance Epidemiological Survey on Cystitis) study, which was performed in nine European countries including Russia and in Brazil during 2003-2006, showed that the ciprofloxacin resistance for *E. coli* isolates in the healthy women having uncomplicated lower UTIs was 8.3% [19]. Higher resistance rates, however, were found in several countries, including Brazil (10.8%), Spain (10.7%), Italy (12.5%), and Russia (13.6%). A recent surveillance study for gram-negative pathogens causing UTIs in Asia-Pacific regions, the SMART (the Study for Monitoring Antimicrobial Resistance Trends) study, showed 48.6% resistance to ciprofloxacin with wide range among different countries, from 10.0% in New Zealand to as high as 76.2% in Vietnam and 72.0% in China [20]. In a recent prospective Korean nationwide surveillance during 2010-2012, the ciprofloxacin resistance in *E. coli* isolates in women having community-acquired acute pyelonephritis was 20.0% [21]. In Ukraine, the prevalence of extended spectrum beta-lactamases among urinary pathogens according to the published data, was 25.2%. In this cross-sectional study, the highest resistance among ESBLs-producing urinary strains was observed against ampicillin (75.9%), ciprofloxacin (48.3%), levofloxacin (41.4%) and gentamicin (41.4%) [22]. However, the prevalence of PMQRs among resistant strains has never been studied.

This is the first study demonstrating the prevalence of PMQR's genes among the uropathogens isolated from pyelonephritis patients in Ukraine. In this cross-sectional study, we have shown that 12 (12.2%) of urinary isolates were PMQR producers. Efflux pump QepA was the most common isolated genes. According to Piekarska K *et al*. 2015, PMQR determinants were present in 49 urinary isolates (22.8%), among which aac(6')-Ib-cr and proteins Qnr predominated (85.7% and 26.5%, respectively) [15]. But Marchisio M *et al*. did not find any plasmid-mediated quinolone resistance determinants among *Enterobacteriaceae* isolated from urinary tract infections patients [23].

In this study, only meropenem exhibited a good enough activity against PMQR-producing urinary strains. Nitroxolinum and fosfomycin were also found to be highly effective drugs *in vitro*. The resistance rates of PMQR-produces were significantly higher compared to non-PMQR-producing urinary strains. Moreover, the resistance rates have been substantially growing up among all isolated uropathogens to commonly prescribed antibiotics, such as third generation cephalosporins and fluoroquinolones, which are the main therapeutics effective to treat such infections. Out of 105 patients, 31 (29.5%) were infected with resistance bacteria. Clinical efficacy of antibiotic therapy was lower in these patients. Moreover, the hospitalization term was longer for the patients with plasmid-mediated genes compared to the patients without this gene (median 10.4 days vs. 8.4 days, $P < 0.05$). Longhi C *et al*. suggest that the prevalence of PMQR genes among uropathogenic Escherichia coli was 11% in outpatients and 21% inpatients [24].

It has been clearly observed in other papers that therapeutic options are limited in patients infected with resistance bacteria. Moreover, antimicrobials activity *in vitro* and *in vivo* may be different in the case of such infections. According to Paterson and colleagues, who published a series that included 32 patients (age ≥ 16 years) with *Klebsiella* bacteremia where the organism was confirmed by phenotypic tests to produce an ESBL. Despite ESBL production, the MICs of all of these organisms were in the susceptible or intermediate range to the cephalosporins used for treatment. Out of 32 study patients, 19 (59%) experienced clinical failure despite the lack of laboratory-determined resistance to the drug used [25]. Kim *et al*. examined patients with bloodstream infections due to *E. coli* or *K. pneumoniae*. Among patients treated with a cephalosporin to which the organism was susceptible, a favorable clinical response was seen in 9

out of 17 patients (52.9%) infected with ESBL-producing organisms compared with 47 out of 50 patients (94.0%) infected with non–ESBL-producing organisms ($p<0.001$) [26]. In our study, treatment by fluoroquinolones demonstrated the best favorable clinical response in the patients infected with ESBL-producing organisms, whereas cephalosporins were the most effective in patients infected with fluoroquinolone resistance uropathogens. In the treatment group 1, a favorable clinical response was seen in 6 out of 13 patients (46.2%) infected with ESBL-producing organisms compared with 5 of 6 patients (83.3%) infected with PMQR-producing organisms ($p<0.05$). In the treatment group 2, a favorable clinical response was seen in 5 out of 7 patients (85.7%) infected with ESBL-producing organisms compared with 1 out of 3 patients (33.3%) infected with PMQR-producing organisms ($p<0.05$). Besides, 12 (44.4%) of the ESBL-producers were isolated on the fifth day after the beginning of antibiotic therapy.

Thus, *in vitro-in vivo* differences in resistance bacteria became an established problem, and therefore, ESBL and PMQR producing organisms pose a major challenge for clinicians, limiting therapeutic options.

The known risk factors for quinolone resistance in uropathogenic *E. coli* are prior exposure to quinolones, previous hospitalization, recurrent UTIs, previous invasive procedures, the presence of complicated UTIs, chronic diseases including neurologic diseases, age over 50 years, and the presence of a urinary catheter in the past 6 months [27 - 29]. In our study, the main factors related to the appearance of plasmid-mediated resistance genes were Chronic Kidney Disease stage III (OR 2.03) and IV (OR 1.1), hypertension (OR 2.57), age range above 55 years (OR 3.05), in-patient treatment history (OR 2.02), and the history of using antibiotics last year (OR 1.41).

CONCLUSION

The prevalence of PMQR among uropathogens, isolated from hospitalized patients with pyelonephritis was 12.2%. Efflux pump QepA was observed to be the most common isolated gene. Out of 105 patients, 31 (29.5%) were infected with resistance bacteria. Global resistance to fluoroquinolones was ≥ 20% among isolated gram-negative strains. In this study, 100% of the PMQR producing isolates were identified as meropenem susceptible. Fosfomycin, nitroxolinum and co-trimoxazole were also found to be highly effective drugs *in vitro*. Treatment by fluoroquinolones demonstrated the best favorable clinical response in the patients with ESBL-producing organisms, whereas cephalosporins were the most effective in patients infected with fluoroquinolone resistance uropathogens. The main risk factors related to the appearance of plasmid-mediated resistance genes were Chronic Kidney Disease stage III (OR 2.03) and IV (OR 1.1), arterial hypertension (OR 2.57), age range above 55 years (OR 3.05), in-patient treatment history (OR 2.02), and the history of using antibiotics last year (OR 1.41).

Taking all these things into consideration, we can assume that isolation and detection of ESBLs and PMQR-producing urinary strains are essential for the selection of the most effective antibiotic for the empiric treatment. Since, the most of ESBLs and PMQR genes are carried by plasmids, these genes could be easily transferred among hospitalized patients. This is a major factor responsible for increasing the spread of both ESBL and PMQR producers. Moreover, therapeutic alternatives for the treatment of UTI patients with resistant uropathogens, particularly in hospitalized patients, are limited. Further clinical studies are needed to establish the guideline for the management of patients with plasmid-mediated resistance.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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