New Microbiologica, 39, 3, 210-215, 2016, ISSN 1121-7138

FULL PAPER

Fluoroquinolone-resistance mechanisms and phylogenetic background of clinical *Escherichia coli* strains isolated in south-east Poland

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SUMMARY

Fluorochinolones are a class of broad-spectrum antimicrobials in the treatment of several infections, including those caused by *Escherichia coli*. Due to the increasing resistance of bacteria to antimicrobials, an understanding of fluoroquinolone resistance is important for infection control. The aim of this study was to determine susceptibility of clinical *E. coli* strains to fluoroquinolones and characterize their mechanisms of quinolone resistance. Totally, 79 non-duplicate clinical *E. coli* isolates included in this study were mainly from skin lesion -36 (45.6%) isolates; 54 (68.4%) isolates were assigned to phylogenetic B2 group. Resistance to ciprofloxacin was found in 20 isolates. In the quinolone resistance-determining region (QRDR) region of *gyr*A and *par*C, 4 types of point mutations were detected. Mutations in *parC* gene were found in all strains with *gyrA* mutations. Predominance of double mutation in codon 83 and 87 of *gyrA* (90%) and in codon 80 of *parC* (90%) was found. Moreover, plasmid-mediated quinolone resistance (PMRQ) determinants (*qnrA* or *qnrB* and/or *aac(6')-Ib*-cr) were present in 5 (25%) out of 20 fluoroquinolone-resistant isolates. Resistance to fluoroquinolones in all of the tested clinical *E. coli* isolates correlated with point mutations in both *gyr*A and *par*C. The majority of fluoroquinolone-resistant strains belonged to D and B2 phylogenetic groups.

Received December 30, 2015 *Accepted* June 4, 2016

INTRODUCTION

Escherichia coli is one of the most prevalent commensal inhabitant of the gastrointestinal tract of humans as well as one of the most frequent causes of several infections, including enterititis, urinary tract infections, septicaemia and other infections, such as neonatal meningitis (Allocati *et al.,* 2013). Fluoroquinolones are a class of broad-spectrum antimicrobials effective in the treatment of selected community-acquired and nosocomial infections with excellent broad-spectrum activity both against Gram-positive and Gram-negative bacteria, including *E. coli*. These antimicrobials are characterized by good oral absorption and tissue penetration, and a low incidence of serious side effects (Hooper, 2000). The bactericidal effect of fluoroquinolones results from the poisoning of type II topoisomerases. The DNA gyrase, which is composed of GyrA and GyrB subunits, and topoisomerase IV, which is composed of ParC and ParE subunits. DNA gyrase is an enzyme essential for catalysing the negative supercoiling of DNA, while topoisomerase IV separates the DNA chains after replication. The primary quinolone targets are basically different for Gram-negative bacteria (DNA gyrase) and Gram-positive bacteria (topoisomerase IV) (Ruiz, 2003, Soni, 2012).

The most important mechanism of fluoroquinolone resistance is alteration in the quinolone resistance-determining region (QRDR) within the subunits constituting topoisomerases II (GyrA and GyrB) and IV (ParC and ParE). Most of the quinolone-resistant clinical isolates of *E. coli* have mutations in the QRDRs of both the *gyrA* and *parC*, encoding the GyrA of DNA gyrase and ParC of topoisomerase IV. Alterations in GyrB and ParE are of minor importance and are rare contributors to quinolone resistance (Hooper, 1999). Additional chromosomally-mediated mechanisms causing decreased accumulation of these antimicrobials due to impermeability of the membrane and/or overexpression of efflux pump systems have been established (Ruiz, 2003). Moreover, fluoroquinolone resistance genes associated with plasmids have also been described; the *qnr* genes encode a pentapeptide repeat protein, which blocks the action of quinolones on the DNA gyrase and topoisomerase IV, *aac*(6')-*Ib*-cr gene encodes an acetylase which modifies the amino group of the piperazin ring of some fluoroquinolones, while *qepA* and *oqxAB* genes encoded an efflux pumps that decreases intracellular drug levels (Nordman and Poirel, 2005; Hansen *et al*., 2007).

Recently, the situation was aggravated worldwide due to the appearance and spread of invasive *Enterobacteriaceae* strains, including *E. coli*, that have acquired resistance to ciprofloxacin (EARS-Net, 2011; Van der Donk *et al*., 2012). There is limited information on the frequency of plasmid-mediated quinolone resistance (PMQR) determinants and the diversity of DNA gyrase and topoisomerase

Key words:
Escherichia coli, Fluorochinolone resistance, QRDR mutations, PMQR determinants, Phylogenetic groups

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IV mutations among clinical isolates of *E. coli* in Poland. The aim of this study was to determine the susceptibility of clinical *Escherichia coli* strains to fluoroquinolones (norfloxacin, ciprofloxacin, levofloxacin) and to assess the presence of mutations in the QRDR of *gyr*A and *par*C genes in fluoroquinolone-resistant strains as well as PMQR determinants such as *qnrA, qnrB, qnrC, qnrD, qnrS* and *aac(6')-Ib*-cr among clinical isolates of *E. coli* collected during one year study in the clinical hospital in Lublin situated in south-east Poland.

MATERIALS AND METHODS

Ethics statement

During 2012, the 79 non-duplicate isolates of *E. coli* (i.e. one isolate with the defined biochemical phenotype obtained from each single patient specimen) were recovered from clinical samples taken as a part of standard care of patients admitted to the Independent Public Teaching Hospital No. 1 in Lublin (Poland). Informed consent was obtained from all patients with respect to the use of their samples for scientific purposes. All bacterial isolates in this study were collected and analysed anonymously. The study protocol was approved by the Ethical Committee of the Medical University of Lublin (no. KE-0254/75/2011).

Bacterial isolates and fluoroquinolone susceptibility testing

Samples were inoculated on Mueller-Hinton agar with 5% sheep blood and MacConkey agar (Biocorp) for selective cultivation of Gram-negative rods and incubated for 18-24 hours at 35°C. Identification of *E. coli* strains was based on the appearance of colonies, negative Gram staining, an oxidase test (TaxoN; BectonDickinson) and biochemical tests (API20E - BioMerieux). The isolated strains were frozen and stored for further analysis at -70°C in Trypticase Soy Broth (Biocorp) with glycerol (POCH).

Bacterial susceptibility to norfloxacin, ciprofloxacin and levofloxacin was determined on Mueller-Hinton agar with the use of Kirby-Bauer method. For isolates resistant to the tested antimicrobials (exhibiting the growth inhibition zone of <19 mm) the minimal inhibitory concentrations (MIC) were determined by the broth microdilution method using concentration of tested antimicrobials ranging from 0.015 mg/L to 256 mg/L). The results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing recommendations (EUCAST, 2013). *E. coli* ATCC 25922 was used as a control strain in the antimicrobial susceptibility tests.

Determination of phylogenetic groups, Sanger DNA sequence analysis of gyrA and parC fragments and detection of plasmid-mediated quinolone resistance (PMQR) determinants

DNA was extracted from pure 24-hour *E. coli* cultures with the use of the Genomic Mini AX BACTERIA isolation kit (A&A Biotechnology), according to manufacturer's recommendations. The major phylogenetic groups (A, B1, B2, D) were determined by PCR amplification of three gene DNA fragments of the scheme (*chuA, yjaA* and TSPE4. C2). Phylogroups were determined as described previously (Clermont *et al.,* 2000). PCR and DNA sequencing was performed using previously described primers (Gharib *et al*., 2013) for all 20 fluoroquinolone-resistant isolates. The *gyrA* and *parC* gene fragments were amplified, then PCR products were purified. DNA sequences were determined by the dideoxy-chain termination method using an automatic DNA analyser (LICOR 4300), the USB Thermo Sequenase Cycle Sequencing Kit (Affymetrix), and IRD 800- and IRD700-labelled custom sequencing primers. Sequences were determined on both strands using denatured double-stranded DNA templates. Sequences of fragments of *gyr*A and *par*C genes were analysed and compared with sequences of *gyr*A and *par*C genes available in the National Center for Biotechnology Information (NCBI) database (GenBank accession nos. AF052254 for *gyrA* and KF550122 for *parC*). Data were analysed with the use of BLAST tool (www.ncbi.nlm.nih.gov). PMQR determinants (*qnrA, qnrB, qnrC, qnrD, qnrS* and *aac(6')-Ib*-cr) were screened for all of 20 fluoroquinolone-resistant isolates by PCR amplification using primers and conditions as described previously (Robicsek *et al*., 2006; Wang *et al*., 2009; Cavaco *et al*., 2009; Park *et al*., 2006).

ERIC-PCR fingerprinting

Typing of *E. coli* strains by ERIC-PCR fingerprinting was performed as described by Versalovic *et al*. (1991). DNA banding patterns were analysed using BIO-GENE analysis software according to the instructions of the manufacturer. Genetics tree was constructed after the gel pictures were normalized on the basis of the presence of the molecular size marker – Gene Ruler 100 bp DNA Ladder Mix (Fermentas, Lithuania). Detailed comparison of the different gels was performed by the unweighted pair group method with arithmetic averages (UPGMA) clustering method, with the Dice coefficient. A band tolerance setting of 3% was applied. A homology level of at least 95% was set as the definition of a separate genotype.

Statistical analysis

The two-sided Fisher's exact test was used for analysing categorical data. Differences at p values of <0.05 were considered statistically significant.

RESULTS

In all, 79 non-duplicate clinical *E. coli* isolates included in this study were obtained from various clinical specimens; mainly from skin lesions - 36 (45.6%) isolates (wound and ulcer swabs); respiratory tract - 13 (16.5%) isolates (swabs from nasopharynx, pharyngx, tracheal tube, tracheostomy tube, pleural cavity as well as sputum); gastrointestinal tract - 12 (15.2%) isolates (swabs from rectum, abdominal cavity, peritoneal cavity); cardiovascular system - 5 (6.3%) isolates (blood, swabs from drain); genitourinary system - 6 (7.6%) isolates (urine, swabs from cervix, vagina); other - 7 (8.9%) isolates (swabs from abscess, fistula).

Table 1 summarises the distribution of the MICs of each fluoroquinolone tested (ciprofloxacin, norfloxacin, levofloxacin) against clinical *E. coli* isolates. According to the breakpoints reported by EUCAST (2013), 19 isolates were resistant to all fluoroqinolones used, while 1 isolate was resistant to ciprofloxacin and norfloxacin.

Distribution of phylogenetic groups among clinical *E. coli* isolates was shown in *Table 2*. Predominance of phylogenetic group B2 assigned to 54 (68.4%) isolates was apparent as compared to the prevalence of the other phylogroups (D - 17 (21.5%) isolates, A - 6 (7.6%) isolates and B1 - 2 (2.5%) isolates). B2 group *E. coli* isolates were the

Table 1 - *Fluoroquinolone susceptibility of 79 clinical E. coli isolates.*

Antimicrobial agent	*No. of isolates at the MIC (mg/L)*										*MIC_{50} (mg/L)*	*MIC_{90} (mg/L)*	*No. of resistant isolates (%)*
	≤0.5	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*≥256*			
Ciprofloxacin	58	1	2	-	4	4	3	4	2	1	0.06	32	20 (15.6)
Norfloxacin	55	4	-	1	1	3	4	5	2	4	0.25	64	20 (15.6)
Levofloxacin	55	2	3	1	8	4	4	2	-	-	0.12	16	19 (15.0)

Table 2 - *Distribution of the 79 clinical E. coli strains among the phylogenetic groups.*

Origin of strains	*No. of strains in phylogenetic group (%)*			
	A	*B1*	*B2*	*D*
Skin lesion (n=36)	3 (8.3)	1 (2.8)	23 (63.9)	9 (25.0)
Respiratory tract (n=13)	3 (23.1)	-	10 (76.9)	-
Gastrointestinal tract (n=12)	-	1 (8.3)	7 (58.3)	4 (33.3)
Cardiovascular system (n=5)	-	-	3 (60.0)	2 (40.0)
Genitourinary system (n=6)	-	-	5 (83.3)	1 (16.7)
Others (n=7)	-	-	6 (85.7)	1 (14.3)
Total (n=79)	6 (7.6)	2 (2.5)	54 (68.4)	17 (21.5)

Table 3 - *Amino acid substitutions in the quinolone resistance-determining region (QRDR) of GyrA and ParC and plasmid-mediated quinolone resistance (PMQR) determinants in the resistant Escherichia coli strains and the corresponding phylogenetic groups and ciprofloxacin (Cip), norfloxacin (Nor), and levofloxacin (Levo) minimal inhibitory concentrations (MICs).*

No. of strain	*Origin of strain*	*Amino acid substitutions in the QRDR*						*PMQR*	*MIC (mg/L)*			*Phylogenetic group*	*ERIC group*
		GyrA			*ParC*				*Cip*	*Nor*	*Levo*		
		Ser83	*Asp87*	*Leu98*	*Ser80*	*Glu84*	*Gly78*						
64	Respiratory tract	Leu			Arg				2	2	4	A	1
72	Skin lesion	Leu			Ile				2	16	4	D	9
17	Gastrointestinal tract	Leu	Tyr		Ile				128	128	8	D	9
51	Gastrointestinal tract	Leu	Tyr		Ile			*aac(6')-Ib-cr*	32	64	32	D	9
11	Skin lesion	Leu	Asn		Ile				32	32	16	D	7
29	Skin lesion	Leu	Asn		Ile			*qnrA*	8	128	16	B2	1
39	Cardiovascular system	Leu	Asn		Ile				16	32	8	D	7
54	Cardiovascular system	Leu	Asn		Ile				128	>256	32	B2	1
55	Respiratory tract	Leu	Asn		Ile				64	64	32	B2	1
58	Skin lesion	Leu	Asn		Arg				16	16	8	D	7
73	Gastrointestinal tract	Leu	Asn		Ile				>256	>256	64	D	12
74	Gastrointestinal tract	Leu	Asn		Ile			*qnrB, aac(6')-Ib-cr*	64	64	32	B2	3
75	Gastrointestinal tract	Leu	Asn		Ile			*qnrB, aac(6')-Ib-cr*	32	32	8	B1	1
80	Skin lesion	Leu	Asn		Ile				64	>256	64	B1	3
1	Respiratory tract	Leu	Asn		Ile			*qnrA, aac(6')-Ib-cr*	8	64	8	B2	1
36	Respiratory tract	Leu	Asn		Ile				8	16	8	A	1
46	Respiratory tract	Leu	Asn		Ile				16	64	16	B2	1
56	Gastrointestinal tract	Leu	Asn		Ile	Val			8	8	8	B2	Unique
37	Respiratory tract	Leu	Asn		Ile				64	>256	16	B2	12
38	Respiratory tract	Leu	Asn	Pro	Ile		Ala		16	32	8	A	1

most frequently cultured from all specimen types (*Table 2*). Among the fluoroquinolone-resistant strains, the frequencies of phylogenetic groups A, B1, B2 and D were 15%, 10%, 40% and 35%, respectively. The isolates belonging to B2 group were found to be significantly more prevalent among quinolone sensitive strains in comparison to D group isolates (p=0.037) and B1 group isolates (p=0.029). Conversely, the prevalence of isolates belonging to group B1 (100%) and D (41.2%) was significantly higher amongst fluoroquinolone-resistant isolates as compared to B2 group isolates (14.8%) with p=0.029 and p=0.037, respectively.

DNA sequencing of the QRDRs of *gyr*A and *par*C genes of the quinolone-resistant *E. coli* isolates revealed point mutations involving amino acid substitutions as well as silent mutations (*Table 3*). In the QRDR region of *gyr*A 4 types of point mutations were detected, responsible for 4 types of amino acid substitutions: Ser83→Leu in 20 (100%) isolates, Asp 87→Asn in 16 (80%) isolates, Asp 87→Tyr in 2 (10%) isolates and Leu 98→Pro in 1 (5%) isolate. In the QRDR region of *par*C gene, 4 types of point mutations were detected, responsible for 4 types of amino acid substitutions: Ser 80→Ile in 18 (90%) isolates, Ser 80→Arg in 2 (10%) isolates, Glu 84→Val in 1 (5%) isolate and Gly 78→Ala in 1 (5%) isolate.

Seven different kinds of mutations were observed in the tested *E. coli* isolates (*Table 3*). Sixteen (80%) out of 20 *E. coli* isolates with altered QRDRs, carried 3 substitutions (2 in GyrA and 1 in ParC). One isolate (5%) carried 4 amino acid substitutions (2 in GyrA and 2 in ParC) and one isolate carried 5 amino acid substitutions (3 in GyrA and 2 in ParC). The ciprofloxacin MIC for these isolates was ≥8 mg/L. One substitution in GyrA and one in ParC was detected in 2 (10%) isolates with MIC 2 mg/L.

PMRQ determinants were present in 5 (25%) of the fluoroquinolone-resistant *E. coli* isolates (*Table 3*). The *qnr* genes were found in 4 isolates, including *qnrA* (2 isolates) and *qnrB* (2 isolates). The *aac(6')-Ib*-cr was detected in 4 isolates. One isolate possessed *aac(6')-Ib*-cr as a single gene and 3 isolates carried *qnr* and *aac(6')-Ib*-cr in combination. None of the tested isolates was positive in *qnrC, qnrD* and *qnrS* genes.

Using the ERIC-PCR technique, from 66 distinct ERIC patterns, 13 groups of similarity were identified when 60% level of similarity was used for grouping the isolates and 6 isolates had unique genotype. There was no significant association of ERIC groups with origin of isolation as well as with phylogenetic groups. However, it was shown that fluoroquinolone-resistant *E. coli* isolates belonged to five ERIC groups represented: 9 out of 16 isolates from ERIC group 1, 2 out of 2 isolates from ERIC group 3, 3 out of 5 isolates from ERIC group 7 and ERIC group 9 as well as 2 out of 6 isolates from ERIC group 12 (*Table 3*).

DISCUSSION

An initial phylogeny, obtained through cluster analysis of MLEE data defined six major phylogenetic groups, designated A, B1, B2, C, D and E (Selander *et al*., 1987). Although relationships between *E. coli* strains have evolved with the increasing availability of molecular data and the application of sophisticated methods, the phylogenetic groups A, B1, B2 and D are still evident in whole genome analyses of independently isolated *E. coli* strains. The virulent extra-intestinal strains belong mainly to B2 group and, to a lesser extent, to D group (Mosquito *et al*., 2015). Most of commensal strains belong to A and B1 groups (Diurez *et al*., 2001). In our study, clinical *E. coli* strains isolated mainly from extra-intestinal samples belonged mostly to phylogenetic B2 group - 68% and to D group - 21%. Additionally, it was observed that the proportion of phylogenetic B2 group strains may differ among geographically different populations. Differences in the distribution of the phylogenetic groups among *E. coli* strains, may be due to climatic conditions, dietary factors and the use of antibiotics as well as host genetic factors (Diurez *et al*., 2001). In Europe, antimicrobial resistance in Gram-negative bacteria is on the rise, particularly in *E. coli* strains which constitute a majority of invasive Gram-negative isolates in European countries (EARS-Net, 2011; Van der Donk *et al*., 2012). According to the European Antimicrobial Resistance Surveillance Network (EARS-Net, 2011), 27.3% of *E. coli* isolates from blood and cerebrospinal fluid collected in Poland during 2005-2011 were resistant to fluoroquinolones. In our study, 25% of *E. coli* strains isolated from various specimens and wards during a one-year study were resistant to ciprofloxacin. A recent study by Piekarska *et al*. (2015) revealed 11.8% of ciprofloxacin-resistant strains among clinical *E. coli* strains isolated in Poland, mainly from urine and wounds.

According to our results, QRDR mutations in chromosomal genes encoding GyrA and ParC play an essential role in fluoroquinolone resistance in the clinical *E. coli* strains studied. It has been established that amino acid alterations and their accumulation in DNA gyrase and topoisomerase IV predominantly occur in *E. coli* between amino acid positions 67 and 106 of QRDR region (Hooper, 1999). Alterations in both GyrA and ParC often show high level resistance and are reported more frequently than alterations in GyrB or ParE. In *E. coli*, substitutions at codon 83 (Ser→Phe, Ala, or Tyr) and codon 87 (Asp→Asn, Gly, or Tyr) in GyrA as well as at codon 80 (Ser→Arg or Ile) and codon 84 (Glu→Lys) in ParC are the most common mutations observed among quinolone-resistant strains (Hooper, 1999).

The majority of quinolone-resistant *E. coli* strains in our study (85%) showed QRDR region double mutations in codons 83 and 87 of the *gyrA* gene. All of the tested strains had mutations both at Ser83 in the GyrA QRDR, which is the most frequently described mutation in literature (Ruiz, 2003; Minarini and Darini, 2012; Hopkins *et al.,* 2005) and substitutions in *parC* gene. The most frequent amino acid substitutions in our study: Ser83→Leu, Asp87→Asn in GyrA and Ser80→Ile in ParC were also observed by other authors with high prevalence (Minarini and Darini, 2012; Lim *et al*., 2010; Chen *et al*., 2001).

In the present study, at ciprofloxacin MIC ≥8 mg/L we were able to identify *E. coli* isolates harboring 4, 5 or 6 QRDR mutations. Sole substitution in GyrA (Ser83) and one in ParC (Ser 80) were found alterations in two strains with lower MICs for ciprofloxacin (2 mg/L).

Different amino acid substitutions at the same position result in different quinolone susceptibility levels indicating that the final MIC is a function of the specific substitution (Ruiz, 2003; Tavio *et al*., 1999). Recently, it was determined that the serine and acidic amino acid residues act as anchor points which coordinate the water-metal ion bridge between clinically relevant quinolones and bacterial II type topoisomerases. *E. coli* GyrA Ser83 and Asp87 form hydrogen bridge with $Mg2+$ ion that is chelated by the quinolone keto acids. Thus, mutations in these amino acid residues cause resistance by interfering with bridge-enzyme interactions (Aldred *et al.,* 2014). This model also applies to amino acids 80 and 84 of ParC by analogy. Thus, different amino acid substitutions at these points would affect the affinity for the quinolone molecule in different ways (Ruiz, 2003).

A robust relationship between quinolone resistance and the number of mutations in the QRDRs of *gyrA* and *parC* was shown (Aoike *et al*., 2013; Morgan-Linnell and Zechiedrich, 2007). However, in the present study, there was no apparent correlation between ciprofloxacin MIC and the number of amino acid replacements in the QRDRs. One strain with MIC for ciprofloxacin 64 mg/L had an accumulation of amino acid changes in GyrA (codon 83 and 87) and in ParC (codon 80 and 84) whereas MIC of the strain with 5 substitutions: in GyrA (codon 83, 87 and 98) and in ParC (codon 80 and 78) was 16 mg/L. This is in line with the view that not only the number of amino acid replacements in the QRDRs influence the level of resistance but the specific amino acid substitution is more important. All of *E. coli* strains tested in our study had mutations observed at codon 83 of *gyrA*, which is the most frequently detected in quinolone-resistant *E. coli* (Ruiz, 2003) and higher MICs of quinolones in strains with double mutation at codons 83 and 87 were observed (Aldred *et al*., 2014). It is noteworthy that in *E. coli*, GyrA is the primary site of action of fluoroquinolones and ParC is the secondary site (Ruiz, 2003). Moreover, accumulation of amino acids changes in GyrA (codons 83 and 87) and simultaneous alterations of ParC (codons 80 and 84) have been reported to play a key role in the development of the high level resistance (MIC ≥32 mg/L) to fluoroquinolones (Hooper, 1999; Fu *et al*., 2013).

Most of the GyrA and ParC substitution observed in our study have already been observed (Hooper, 1999; Piekarska *et al*., 2015; Minarini and Darini, 2012). However, to the best of our knowledge amino acid substitution of Gly by Ala at position 78 in ParC has not previously been reported.

In the present study, *aac(6')-Ib-cr* was the most frequent PMQR determinant detected among clinical quinolone-resistant *E. coli* isolates. A similar prevalence was observed by Piekarska *et al*. (2015), where in 85.7% of clinical *Enterobacteriaceae* isolates *aac(6')-Ib*-cr gene was detected. The predominance of this PMQR determinant was also shown in a study from Spain (Briales *et al*., 2012). Due to the small number of PMQR positive isolates obtained in the present study, it is hard to explain the importance of these determinants in increasing MIC value. However, our results confirm the different but still active role of PMQR determinants in the development of quinolone resistance. Until recently, PMQR determinants were observed at low prevalence in most reported screenings and further genes and variants might still be discovered, increasing the pool of determinants conferring resistance to quinolones and contribute to better understanding the background of resistance. QRDR mutations and PMQR determinants are two resistance mechanisms often found together in clinical strains of *Enterobacteriacae* with a high level of quinolone resistance (Briales *et al*., 2012; Yang *et al*., 2008), that was confirmed in the present study.

From a genetic point of view, there was a shift in phylogroups when resistant and susceptible strains were compared. When we analysed the relation between the phylogenetic group and antibiotic resistance we found that the isolates belonging to groups D and B1 were more related with quinolone resistance than those belonging to other groups. A link between genetic background of *E. coli* and the pattern of antibiotic resistance has been reported (Basu *et al*., 2013; de Lastours *et al*., 2014). Strains belonging to phylogroup A and some D group strains are more often resistant to third-generation cephalosporins and quinolones (Bukh *et al*., 2009; Horcajada *et al*., 2005). Conversely, B2 strains are less resistant than the remaining strains, regardless of the molecular mechanism involved in resistance (Johnson *et al*., 2002; Johnson and Stell, 2000; Moreno *et al*., 2006). However, phylogenetic studies have also revealed a significant association of B2 group with quinolone-resistant clinical *E. coli* strains (Mosquito *et al.,* 2015; Basu *et al.,* 2013; Piatti *et al.,* 2008; Katouli *et al.,* 2005). The reason for these discrepancies can arise/appear from various numbers of sample analysed, geographical variations, or various origin of clinical samples.

CONCLUSIONS

The present study supplied baseline information on the prevalence and types of point mutations within the QRDRs of *gyrA* and *parC* as well as the prevalence of PMQR determinants in clinical *E. coli* strains isolated from various specimens obtained from patients from south-east Poland. The resistance to fluoroquinolones in clinical *E. coli* was correlated with phylogenetic background indicating a higher prevalence of resistant strains belonging to D and B2 groups. Escalating fluoroquinolone resistance in clinical *E. coli* strains requires further studies to elucidate the relationship between phylogroups, specific virulence factors and mechanisms of resistance.

Acknowledgements

The paper was developed using the equipment purchased within the agreement No. POPW.01.03.00-06-010/09-00 Operational Program Development of Eastern Poland 2007-2013, Priority Axis I, Modern Economy, Operations 1.3. Innovations Promotion.

We wish to thank Grazyna Zdzienicka, Jadwiga Zajaczkowska, Grazyna Kuciapa, Anna Blajerska, and Katarzyna Matuska for their contribution to this project.

Author Disclosure Statement

The authors have declared that no competing interests exist.

References

Aldred K.J., Kerns R.J., Osheroff N. (2014). Mechanism of quinolone action and resistance. *Biochemistry.* **53**, 1565-1574.

Allocati N., Masulli M., Alexeyev M.F., Di Ilio C. (2013). *Escherichia coli* in Europe: an overview. *Int. J. Environ. Res. Public Health.* **10**, 6235-6254.

Aoike N., Saga T., Sakata R., Yoshizumi A., Kimura S., Iwata M., et al. (2013). Molecular characterization of extraintestinal *Escherichia coli* isolates in Japan: relationship between sequence types and mutation patterns of quinolone resistance-determining regions analyzed by pyrosequencing. *J. Clin. Microbiol*. **51**, 1692-1698.

Basu S., Mukherjee S.K., Hazra A. Mukherjee M. (2013). Molecular characterization of uropathogenic *Escherichia coli*: nalidixic acid and ciprofloxacin resistance, virulent factors and phylogenetic background. *J. Clin. Diagn. Res*. **7**, 2727-2731.

Briales A., Rodríguez-Martínez J.M., Velasco C., de Alba P.D., Rodríguez-Baño J., Martínez-Martínez L., et al. (2012). Prevalence of plasmid-mediated quinolone resistance determinants *qnr* and *aac(6')-Ib*-cr in *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases in Spain. *Int. J. Antimicrob. Agents*. **39**, 431-434.

Bukh A.S., Schønheyder H.C., Emmersen J.M., Søgaard M., Bastholm S., Roslev P. (2009). *Escherichia coli* phylogenetic groups are associated with site of infection and level of antibiotic resistance in community-acquired bacteraemia: a 10 year population-based study in Denmark. *J. Antimicrob. Chemother*. **64**, 163-168.

Cavaco L.M., Hasman H., Xia S., Aarestrup F.M. (2009). *qnr*D, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob. Agents Chemother*. **53**, 603-608.

Chen J.Y., Siu L.K., Chen Y.H., Lu P.L., Ho M., Peng C.F. (2001). Molecular epidemiology and mutations at gyrA and parC genes of ciprofloxacin-resistant *Escherichia coli* isolates from a Taiwan Medical Center. *Microb. Drug Res*. **7**, 47-53.

Clermont O., Bonacrosi S., Bingen E. (2000). Rapid and simple determination of the *E. coli* phylogenetic group. *Appl. Environ. Microbiol*. **66**, 4555-4558.

de Lastours V., Bleibtreu A., Chau F., Burdet C., Duval X., Denamur E., et al. (2014). Quinolone-resistant *Escherichia coli* from the faecal microbiota of healthy volunteers after ciprofloxacin exposure are highly adapted to a commensal lifestyle. *J. Antimicrob. Chemother.* **69**, 761-768.

Diurez P., Clermont O., Bonacorsi S., Bingen B., Chaventre A., Elion J., Picard B., Denamur E. (2001). Commensal *Esherichia coli* isolates are phylogenetically distributed among geographically distinct human populations. *Microbiology.* **147**, 1671-1676.

European Centre for Disease Prevention and Control. European Antimicrobial Surveillance Network (EARS-Net) annual report. Antimicrobial resistance surveillance in Europe 2011. http:/www.ecdc.europa.eu

Fu Y., Zhang W., Wang H., Zhao S., Chen Y., Meng F., Zhang Y., Xu H., Chen X., Zhang F. (2013). Specific patterns of *gyrA* mutations determine the resistance difference to ciprofloxacin and levofloxacin in *Klebsiella pneumoniae* and *Escherichia coli*. *BMC Infect. Dis*. **7**, 8.

Gharib A.A., El-Aziz N.K.A. (2013). Molecular Analysis of Quinolone Resistance-Determining Regions in Avian Pathogenic *Escherichia coli*. *Inter. J. Adv. Res.* **1**, 145-157.

Hansen L.H., Jensen L.B., Sorensen H.I., Sorensen S.J. (2007). Substrate specificityof the OqxAB multidrug resistance pump I *Escherichia coli* and selected enteric bacteria. *J. Antimicrob. Chemother.* **60**, 147-147.

Hooper DC. (1999). Mechanisms of fluoroquinolone resistance. *Drug Resist. Updat*. 2, 38-55.

Hooper DC. (2000). Mechanisms of action and resistance of older and newer fluoroquionolones. *Clin. Infect. Dis.* **31** (Suppl. 2), S24-S28.

Hopkins K.L., Davies R.H., Threlfall E.J. (2005). Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *J. Antimicrob. Agents.* **25**, 358-373.

Horcajada J.P., Soto S., Gajewski A., Smithson A., Jiménez de Anta M.T.,

Mensa J., et al. (2005). Quinolone-resistant uropathogenic *Escherichia coli* strains from phylogenetic group B2 have fewer virulence factors than their susceptible counterparts. *J. Clin. Microbiol*. **43**, 2962-2964.

Johnson J.R., Stell A.L. (2000). Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *J. Infect. Dis*. **181**, 261-272.

Johnson J.R., van der Schee C., Kuskowski M.A., Goessens W., van Belkum A. (2002). Phylogenetic background and virulence profiles of fluoroquinolone-resistant clinical *Escherichia coli* isolates from the Netherlands. *J. Infect. Dis*. **186**, 1852-1856.

Katouli M., Brauner A., Haghighi L.K., Kajser B., Muratov V., Ilby M. (2005). Virulence characteristicd of *Escherichia coli* strains causing acute cystitis in young adults in Iran. *J. Infect. Immun*. **50**, 312-322.

Lim S.K., Lim K.G., Lee H.S., Jung S.C., Kang M.I., Nam H.M. (2010). Prevalence and molecular characterization of fluoroquinolone-resistant *Escherichia coli* isolated from diarrheic cattle in Korea. *J. Vet. Med. Sci*. **72**, 611-614.

Minarini L.A.R., Darini A.L.C. (2012). Mutations in the quinolone resistance-determining regions of *gyrA* and *parC* in *Enterobacteriaceae* isolates from Brazil. *Braz. J. Microbiol*. **43**, 1309-1314.

Moreno E., Prats G., Sabaté M., Pérez T., Johnson J.R., Andreu A. (2006). Quinolone, fluoroquinolone and trimethoprim/sulfamethoxazole resistance in relation to virulence determinants and phylogenetic background among uropathogenic *Escherichia coli*. *J. Antimicrob. Chemother*. **57**, 204-211.

Morgan-Linnell S.K., Zechiedrich L. (2007). Contributions of the combined effects of topoisomerase mutations toward fluoroquinolone resistance in *Escherichia coli*. *Antimicrob. Agents Chemother*. **51**, 4205-4208.

Mosquito S., Pons M.J., Riveros M., Ruiz J., Ochoa1 T.J. (2015). Diarrheagenic *Escherichia coli* phylogroups are associated with antibiotic resistance and duration of diarrheal episode. *Scient. World J*. 2015; Article ID 610403, 6 pages.

Nordman P., Poirel L. (2005). Emergence plasmid mediated resistance to chinolones in *Enterobacteriaceae*. *J. Antimicrob. Chemother*. **56**, 463-469.

Park C.H., Robicsek A., Jacoby G.A., Sahm D., Hooper D.C. (2006). Prevalence in the United States of *aac(6')*-Ib-cr encoding a ciprofloxacin-modifying enzyme. *Antimicrob. Agents Chemother*. **50**, 3953-3955.

Piatti G., Mannini A., Balistreri M., Schito A.M. (2008). Virulence factors in urinary *Escherichia coli* strains: phylogenetic background and quinolone and fluoroquinolone resistance. *J. Clin. Microbiol*. **46**, 480-487.

Piekarska K., Wołkowicz T., Zacharczuk K., Rzeczkowska M., Chróst A., Bareja E., Olak M., Gierczyński R. (2015). Co-existence of plasmid-mediated quinolone resistance determinants and mutations in *gyrA* and *parC* among fluoroquinolone-resistant clinical *Enterobacteriaceae* isolated in a tertiary hospital in Warsaw, Poland. *Int. J. Antimicrob. Agents*. **45**, 238-243.

Robicsek A., Strahilevitz J., Sahm D.F., Jacoby G.A., Hooper D.C. (2006) *qnr* Prevalence in Ceftazidime-Resistant *Enterobacteriaceae* isolates from United States. *Antimicrob. Agents Chemother*. **50**, 2872-2874.

Ruiz J. (2003). Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J. Antimicrob. Chemother*. **51**, 1109-1117.

Selander R.K., Caugant D.A., Whittam T.S. (1987). Genetic structure and variation in natural populations of *Escherichia coli. Escherichia coli* and *Salmonella typhimurium. Cell Mol. Biol*. **2**, 1625-1648

Soni K. (2012). Fluoroquinolones: Chemistry & Action - A Review. *Indo. Glob. J. Pharm. Sci*. **2**, 43-53.

Tavío M.M., Vila J., Ruiz J., Martín-Sánchez A.M., Jiménez de Anta M.T. (1999). Mechanisms involved in the development of resistance to fluoroquinolones in *Escherichia coli* isolates. *J. Antimicrob. Chemother*. **44**, 735-742.

Van der Donk C.F.M., van de Bovenkamp J.H.B., De Brauwer E.I.G.B., De Mol P., Feldhoff K.H., Kalka-Moll W.M., et al. (2012). Antimicrobial resistance and spread of multi drug resistance *Escherichia coli* isolates collected from nine urology services in Euregion Meuse-Rhine. *PLOS One*. 7, e7707.

Versalovic J., Koeuth T., Lupski J.R. (1991). Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res*. **19**, 6823-6831.

Wang M., Guo Q., Xu X., Wang X., Ye X., Wu S., Hooper D., Wang M. (2009). New plasmid-mediated quinolone resistance gene, *qnr*C, found in a clinical isolate of *Proteus mirabilis*. *Antimicrob Agents Chemother.* **53**, 1892-1897.

Yang H., Chen H., Yang Q., Chen M., Wang H. (2008). High prevalence of plasmid-mediated quinolone resistance genes *qnr* and *aac(6')-Ib*-cr in clinical isolates of *Enterobacteriaceae* from nine teaching hospitals in China. *Antimicrob. Agents Chemother.* **52**, 4268-4273.