The genetic background of antibiotic resistance among clinical uropathogenic *Escherichia coli* strains

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Abstract

The spreading mechanisms of antibiotic resistance are related to many bacterial and environment factors. The overuse of antibiotics is leading to an unceasing emergence of new multidrug resistant strains. This problem also concerns uropathogenic *Escherichia coli* strains, which is the most common pathogen causing urinary tract infections. The aim of this study was the genetic analysis of antibiotic resistance in comparison to the phenotypic background of *E. coli* strains. The characterized collection of *E. coli* strains isolated 10 years ago from the urine samples of patients with urinary tract infections was used for antimicrobial susceptibility testing (the disc diffusion method) and analysis of antibiotic resistance genes (PCR reaction, sequencing). Additionally, the presence of ESBL strains was analyzed. Fourteen genes were associated with resistance to beta-lactams, aminoglycosides, sulfonamides and quinolones. The genetic analysis revealed that *bla*TEM-1 and *sul2* were present in almost all of the studied strains. Other drug-resistance genes were very rare or non-existent. Otherwise, the phenotypic resistance to fluoroquinolones was well correlated with the genotypic background of the studied bacteria. The presence of particular genes and specific mutations indicate a high bacterial potential to multidrug resistance. On the other hand, it needs to be emphasized that the standard disk diffusion test for the routine antimicrobial susceptibility analysis is still the best way to estimate the current situation of bacterial drug-resistance.

Keywords  UPEC · Antibiotic resistance · Beta-lactamases · Quinolones

Introduction

Molecular analyses are increasingly being introduced into routine diagnostics. The identification of the genetic determinants of pathogenicity and antibiotic resistance is very important for prevention against the widespread of hazardous bacteria, especially Multiple Drug Resistant (MDR) strains [1]. In the case of urinary tract infections, where uropathogenic *Escherichia coli* strains (UPEC) are the main causative agent [2], the most attention is paid to beta-lactam antibiotics [3]. Among resistance genes that are often located on plasmids are those coding for multiple types of β-lactamases (*bla* genes) [4, 5]. In a significant part, it refers to extended-spectrum β-lactamases (ESBLs) that are one of the main problems in the epidemiology of infections caused by organisms from the family *Enterobacteriaceae*. ESBLs usually confer resistance to all penicillins, cephalosporins (except for cephamycins), and monobactams, being inhibited by β-lactam inhibitors [6, 7], and they are the predominant source of enterobacterial resistance to 3rd- and 4th-generation cephalosporins [8, 9]. Among ESBLs observed in *E. coli*, the most commonly identified are enzymes from the family CTX-M (mostly CTX-M-1 lineage), followed by SHVs, and more rarely TEMs [10–13]. Another group of acquired β-lactamases responsible for resistance to newer generation β-lactams are cephalosporinases of the AmpC type [14]. These are derivatives of enzymes specific for organisms like *Enterobacter cloacae*, *Citrobacter freundii*...
or Morganella morganii [15]. In general they confer a similar resistance profile to ESBLs, except for resistance to cephamycins but not 4th-generation cephalosporins, and resistance to β-lactam-inhibitor combinations, mainly those with clavulanic acid [8]. Among the several families of the acquired AmpC-type enzymes identified so far, the group deriving from C. freundii is the largest, including CMY-2 which is the most common enzyme of this kind [16, 17].

Similarly, the E. coli resistance to other antibiotics like sulfonamides and aminoglycosides applied during UTI treatment is often associated with the presence of specific plasmids. Resistance to sulfonamides is determined by three genes (sul1, sul2 and sul3) [18]. Gene sul1 has usually been identified on large conjugative plasmids, opposite sul2 has mainly been detected on small non-conjugative plasmids. Recently, sul2 has been observed also on a large conjugative plasmids related to the streptomycin resistance [18–20]. sul3 is the least known and also the least frequently detected plasmid gene in E. coli [19]. At present, the knowledge about various sul genes and their carriers is poor and diverse depending on reservoirs (e.g. animals and human) [21]. Similarly, aminoglycosides resistance is also connected with few genes carried by plasmids. The aadB, aac(3)-II and aac(3)-IV genes are related to the gentamycin, tobramycin, neomycin resistance and other aminoglycosides. They belong to the most frequent genes detected in Escherichia coli strains and other Gram-negative bacteria [22].

The frequent antibiotic resistance of UPEC strains is also associated with fluoroquinolones. Primarily, they induce the mutation in DNA gyrase (gyrA) and topoisomerase IV (parC) genes. In the literature, the mutations of genes controlling fluoroquinolones accumulation are also describing [23]. Additionally, resistance to fluoroquinolones can also be facilitated by plasmids producing the Qnr protein (QnrA, QnrB, QnrS), which protects the antibiotic targets from quinolone treatment. Qnr plasmids induce resistance on low level, but it was observed also that their presence strongly enhance the quinolone resistance determined by other mechanisms [24, 25].

The uninterrupted increasing of resistance and the emergence of MDR strains are still monitored among UPEC [26]. Therefore, there is a need for periodic screening of common bacterial pathogens such as UPEC to control their antibiotic susceptibility profiles in different communities [27, 28]. It seems to be important to also monitor the distribution of genes associated with antibiotic resistance. This knowledge can allow us to prevent the spreading of strains with a high risk of MDR expression. In reference to this, the aim of the study was to investigate the prevalence of the genes encoding the resistance to the most popular antibiotics class (beta-lactams, aminoglycosides, sulfonamides and quinolones) used during therapy of UTI against E. coli [24, 26, 29].

Materials and methods

Bacterial strains

A previously characterized collection of 127 clinical Escherichia coli strains [30, 31] isolated from the urine of patients in different hospital wards in Lodz (Poland) in the years 2005–2007 was used. Additionally, reference E. coli strains producing different beta-lactamases (No. 3272/96–TEM-1, No. 3290/96–CTX-M-3, No. 3274/96–SHV-5, No. 394/06–CMY-2, No. 348/04–OXA-1) from the National Medicines Institute (Poland) and E. coli ATCC 25922 (Argenta) were used as a control during the antimicrobial disc diffusion test.

Susceptibility testing and phenotypic ESBL detection

Antimicrobial susceptibility testing was performed using the disk-diffusion method on Mueller–Hinton agar, using commercial disks (Oxoid, Wesel, Germany). The isolates were tested against 16 antimicrobials: amoxicillin (AMX, 25 µg), amoxicillin/clavulanate (AMC, 30 µg), piperacillin (PIP, 30 µg), cefoxitin (FOX, 30 µg), ceftazidime (CTX, 5 µg), cefotaxime (CTX, 5 µg), imipenem (IMP, 10 µg), amikacin (AMK, 30 µg), tobramycin (TOB, 10 µg), gentamicin (GEN, 10 µg), netilmicin (NET, 10 µg), norfloxacin (NOR, 10 µg), ciprofloxacin (CIP, 5 µg), ofloxacin (OFX, 5 µg), trimethoprim (TMP, 5 µg), trimethoprim-sulfamethoxazole (STX, 25 µg). The results of susceptibility testing were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [32]. E. coli ATCC 25922 was used as a quality control strain. Resistance to newer generation cephalosporins was also confirmed on chromID® ESBL plates (bioMérieux). Additionally, all isolates were tested for ESBL production by the double-disk synergy test (DDST) with disks containing cefotaxime, cefazidime, and amoxicillin with clavulanate [33, 34].

PCR detection of resistance genes

Bacterial DNA was purified with the Gen Elute™ Bacterial Genomic DNA kit (Sigma Aldrich, Germany). The identification of the genes was carried out by PCR using previously described primers. The specific PCR parameters for all primers used in the study and their references have been shown in Table 1. In this study bla<sub>CTX-M-1</sub>, bla<sub>TEM</sub>, bla<sub>SHV</sub>,
**Results**

The collection of uropathogenic *Escherichia coli* strains was re-characterized based on antimicrobial susceptibility testing in order to verify the resistance profiles published previously [31]. Additionally, all isolates were tested for ESBL production. The whole collection of *E. coli* strains was analyzed based on the genetic conditioning of antibiotic resistance. The 5 genes encoding beta-lactamases (*bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>CMY</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>) were detected at first in the reference group of *E. coli* ESBL strains and next, their presence was defined in the entire collection of clinical *E. coli* strains. Additionally, the presence of *aac(3)-II* determines resistance to aminoglycosides and three genes (*sul1*, *sul2*, *sul3*) encoding resistance to sulfonamides were studied. Further, the strains resistant to quinolones [55] and the control representative group of strains [33]—intermediate sensitive and sensitive to quinolones—were analyzed based on the mutations in *parC* and *gyrA* genes and based on the presence of *qnr* genes.

### β-Lactamase contents of *E. coli* isolates

The strains were selected based on the most beta-lactams resistant (Table 2). They were resistant to antibiotics from at least three different class of beta-lactams or/and resistant to III’rd generation of cephalosporins. Based on the phenotypic

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### Table 1 Oligonucleotides used in the study

| Primer  | Sequence (5′ → 3′) | Locus                          | Ta [°C] | PCR [bp] | Ref. |
|---------|-------------------|--------------------------------|---------|----------|------|
| TEM-A   | ATAAAATCTTGAAGAC  | Flank of *bla*<sub>TEM</sub>-like genes | 42      | 1181     | [12] |
| TEM-B   | TTACCAATGTTAATCA  |                                 |         |          |      |
| P1C     | TTAATTCGCTCTTCCAGA| Flank of *bla*<sub>CTX-M</sub>-like genes | 55      | 1042     | [11] |
| P2D     | CAGCCGTGTCTCTGCTAG|                                 |         |          |      |
| SHV-A   | ACTGAATGGACCGCTTC| Flank of *bla*<sub>SHV</sub>-like genes | 55      | 329      | [12] |
| SHV-B   | ATCCGGAGATAAATCAC|                                 |         |          |      |
| OXA-1/F  | ATGAAAAAACAAATACTAAATAACAC | Internal fragment of *bla*<sub>OXA-1</sub>-related genes | 48      | 755      | [35] |
| OXA-1/R  | TTTTCTGTAATGCGGACAC |                                 |         |          |      |
| CF-1     | ATGATGAAAAACATCGATATG| Flank of *bla*<sub>CMY</sub>-like genes | 45      | 1146     | [15] |
| CF-2     | TTAATTCGATTATTCGAGATTG |                                 |         |          |      |
| aac(3)-IF| TGAACGCTGACCGGACCTTAC | aac(3)-II | 55      | 369      | [36] |
| aac(3)-II| GTCGAAACAGGACAGTGAG |                                 |         |          |      |
| sul1-F   | TGGTACGCGGTTCCGCACTTAC | sulI | 56      | 790      | [37] |
| sul1-R   | GCGAAGGGTTCCGAGAAAGTG |                                 |         |          |      |
| SUL2F    | CGCAGCTGCAATACATAACCT| sulIII | 55      | 721      | [38] |
| SUL2R    | TGTCGCGAATGAGCTAGCTC|                                 |         |          |      |
| SUL3F    | CAACGGAGAAGCTGGCGTCTGGA | sulIV | 57      | 244      | [38] |
| SUL3R    | GCTGCACCAATTCGGAAGCG |                                 |         |          |      |
| gyrA-P1  | TGGTCCAGAAGATGAGCAGA | QRDR gyrA | 58      | 374      | [37] |
| gyrA-P3  | TGCCGATCATGATACAAAGA |                                 |         |          |      |
| parC-3   | CGGTGGCTCTGCTGCTGCTGCTGC | QRDR parC | 58      | 368      | [37] |
| parC-4   | AATGCCTCGCGAAGGCGCTGCTGCTGCTGC |                                 |         |          |      |
| qnrA-1   | ATTCTTCAGCGCAGGATTG | qnrA | Gradient | 516      | [39, 40] |
| qnrA-2   | GATCCGCAAGAGGGTACGGTCA |                                 |         |          |      |
| qnrB-1   | GATCGTGAAAGACCGCAAGAACGAGAAGG | qnrB | 469      |          |      |
| qnrB-2   | ACGATGCTTGTGATGGTCTTCCTT |                                 |         |          |      |
| qnrS-1   | ACGATCCTCGTCAACTGCA | qnrS | 417      |          |      |
| qnrS-2   | TAAATGCGACCTCGTAGG |                                 |         |          |      |

Ta annealing temperature of PCR, Ref references, bp base pair

bla<sub>OXA-1</sub>- and bla<sub>CMY-2</sub>-like β-lactamase-encoding genes and also aac(3)-II, sul1, sul2, and sul3 were detected. The identification of the quinolones resistance was performed by PCR reaction for the *qnrA*, *qnrB*, *qnrS* genes detection and DNA sequencing of the PCR products of the *gyrA* and *parC* genes. The individual adjusted conditions of DNA amplification were carried out for each gene. After PCR amplification, products were visualized under the gel documentation system. The nucleic acid sequences of PCR products (Macrogen Europe) were compared to the original gene sequences accessed in the GenBank of the National Center for Biotechnology Information (NCBI) database. Nucleotide and amino-acid sequences were analyzed by searching the GenBank database of the NCBI with the Basic Local Alignment Search Tool (BLAST network service).
The correlation between phenotypic resistance to beta-lactams (the strains were selected based on the resistance to antibiotics from at least 3 different class of beta-lactams or/and resistant to III’rd generation of cephalosporins) and ESBL-connected genes identified in the studied collection of E. coli strains

| E. coli strains | Penicillins | Penicillins inhibitor | Cephalosporins | Carbapenems | Identified β-lactamases | Gene profiles |
|----------------|-------------|-----------------------|---------------|-------------|------------------------|--------------|
|                | AMX         | PIP                   | AMC           | FOX         | CAZ                    | CTX          | IMP          |                |              |
| 3              | R           | R                     | R             | S           | S                      | R            | S            | Not observed | blatem          |
| 7              | R           | R                     | R             | S           | R                      | AmpC         | S            | Not observed | blatem, blacmy2 |
| 23             | R           | R                     | R             | S           | I                      | S            | S            | Not observed | blatem         |
| 27             | R           | R                     | R             | R           | I                      | S            | S            | AmpC         | blatem         |
| 39             | R           | R                     | S             | I           | R                      | S            | S            | Not observed | blatem         |
| 44             | R           | R                     | R             | I           | S                      | S            | Not observed | blatem       |
| 50             | S           | I                     | R             | R           | S                      | S            | Not observed | blatem       |
| 87             | R           | S                     | R             | R           | S                      | S            | Not observed | blatem, blaxa1 |
| 91             | R           | R                     | R             | I           | S                      | Not observed | blatem       |
| 97             | R           | R                     | S             | R           | S                      | ESBL         | S            | Not observed | blatem         |
| 122            | R           | R                     | R             | I           | S                      | Not observed | blatem, blacmy2 |
| 127            | R           | R                     | R             | R           | I                      | S            | S            | AmpC         | blacmy2         |

AMX amoxicillin, PIP piperacillin, AMC amoxicillin/clavulanate, FOX cefoxitin, CAZ cefazidime, CTX cefotaxime, IMP imipenem, R resistance, S sensitive, I intermediate

The most popular gene in the studied collection of E. coli strains was sul2—96% of the studied strains were positive. Also, sul1 was popular in 86% of these strains, but only 33% of the studied strains possessed sul3. Sensitive strains were positive for at least one sul gene (sul1, sul2, sul3). These results also did not correlate with resistance to sulfonamides.

**Genetic association with quinolone resistance in E. coli strains**

The mutations in parC and gyrA were also investigated among the studied E. coli strains. The point mutations correlated with phenotypic resistance to fluoroquinolones are well known as Ser/80/Ile in parC and also Ser/83/Leu and Asp/87/Asn in gyrA [37, 41, 42]. They were likewise recognized in this study (Fig. 3). The strains were divided into two groups: R3—strains resistant to ciprofloxacin, norfloxacin, ofloxacin, and strains with decreasing resistance to fluoroquinolones (R < I < S). The strains from the second group were arranged on the basis of changing susceptibility: from resistance to 2 or 1 quinolones (R), intermediate sensitivity (I) to sensitivity to all three quinolones (S). The most common were silent point mutations in different codons—transversion and transition in parC and mainly transition in gyrA. All strains from R3 group were positive for specific missense mutations mentioned above. Ser/80/Ile mutation in parC and then Ser/83/Leu and Asp/87/Asn mutations in gyrA were the most frequently identified. The parC gene had more frequent silent mutations in comparison to gyrA (Fig. 1). Additionally, silent mutations in codon 80 of parC were typical in the second group of strains (R < I < S). In contrast, the silent mutation in the hot spot of gyrA was observed only in one E. coli strain No 9 (Asp/87/Asp). On the other hand, generally
**Fig. 1** Characteristics of identified mutations in the *parC* gene of the studied uropathogenic *E. coli* strains. The diagram was made using GraphPad Prism6.

**Fig. 2** Characteristics of identified mutations in the *gyrA* gene of the studied uropathogenic *E. coli* strains. The diagram was made using GraphPad Prism6.

**Fig. 3** Comparison of occurrence of specific mutations in the *parC* and *gyrA* genes of the studied uropathogenic *E. coli* strains. The diagram was made using GraphPad Prism6.
there were more mutations in the gyrA gene compared to the parC gene (Fig. 2). Further, in case of gyrA gene—the occurrence of the Asp/87/Asn missense mutation always correlated with the occurrence of the Ser/83/Leu mutation. Ser/83/Leu mutation occurred alone in the case of strains from R < 1 < S group. Strains which were the most sensitive to fluoroquinolones possessed only missense and/or silent mutations in other codons.

Additionally, the strains were analyzed based on the presence of qnr genes and despite many attempt of optimizing PCR procedures, the results remained negative.

Discussion

The problem of bacterial antibiotic resistance belongs to the priorities of World Health Organization concerning threat to human health. The widespread use of antibiotics often without the antibiotic susceptibility testing is one of the reasons for the emergence of multidrug resistant pathogens, which seriously impedes therapeutic activities [43, 44]. This can also hinder other therapeutic successes as infectious complications appearing in patients undergoing chemotherapy for cancer or dialysis for renal failure. The effectiveness of secondary infections treatment is crucial also in surgery, especially organ transplantation [1, 45, 46]. Instead, urinary tract infections (UTIs) belong to the most common human infections in both hospital and community settings, where antibiotics are also usually applied [47]. Approximately every second woman and every twentieth man will suffer from UTI in their lifetime. Uropathogenic Escherichia coli (UPEC) is the primary agent causing uncomplicated and complicated UTIs [2, 47, 48]. Therapeutic difficulties result largely from the quick spread of multidrug resistance (MDR) among them. This problem concerns the large group of beta-lactam antibiotics but also other compounds, such as fluoroquinolones, aminoglycosides, etc., which are often used in UTI treatment [30]. The mechanisms of bacterial antibiotic resistance are often associated with the mobile properties of a genome, especially with horizontal gene transfer [6, 7, 49]. The genetic markers of bacterial antibiotic resistance are often described in the literature. The prevalence and differentiation of these genetic profiles vary depending on the countries, antibiotic policy, source and year of bacteria isolation.

In this study, we analyzed 14 genes related to resistance to antibiotics belonging to four different classes. We would like to check the relationships between genotypic and phenotypic resistance among uropathogenic Escherichia coli strains isolated from central Poland (Łódź). The obtained results represent the characteristic of the bacterial population from 10 years ago, which allowed us to look at the potential changes currently observed in a similar bacterial population. At present, the hot topic is ESBL strains. Among the studied bacterial collection we identified approx. 1.5% of ESBL-producing strains and 1.5% AmpC-producing strains, which was the standard level in those days (2005–2007). The situation in Poland seems to have been relatively stable for 10 years (at present approx. 2% ESBL of UPEC strains), but in many other countries the situation is much worse [3, 50–53]. However, the increase in bacterial antibiotic resistance is still observed. Considering the genetic background, these strains (ESBL- and AmpC-producing) carrying blatem gene and additionally blacMY-2 was present only in one AmpC-producing E. coli strain, although blacMY-2 belongs to a small family of plasmid-mediated AmpC-like enzymes [54]. The remaining blacMY-2 positive strains did not correlate with the production of AmpC beta-lactamase. The mechanisms of resistance against beta-lactam antibiotics seem to be the most complicated and differential. This is also clearly visible in the case of genetic background analysis. Considering that the vast majority of the isolates were ESBL-negative despite the presence blatem in most strains (91%), it may be assumed that the blatem genes encoded broad-spectrum enzymes, most likely TEM-1. TEM-1 is the major determinant of E. coli resistance to amino-penicillins and the most common plasmid-encoded β-lactamase; it is estimated that this enzyme occurs in approximately 50% all of E. coli clinical isolates [12, 13, 55]. As it was mentioned above, the blacMY-2 was the next most common gene in the studied bacterial collection (19.5%). The blacMY-2 has been also often identified in E. coli, Klebsiella sp. and Salmonella spp. from different sources in the United States, Greece and Algeria [54, 56–58]. The prevalence of this gene did not correlate with the resistance to β-lactams in our study. In the studied E. coli collection the other genes were sought, too. Only one strain possessed blaxxa1 and despite its resistance to all analyzed beta-lactam antibiotics (except amoxicillin/clavulanate and imipenem), it was not detected as ESBL-positive. A similar prevalence of blaxxa1 was observed at present by other authors [59, 60]. Also, one other strain possessed blasHV (E. coli No 108) and it was resistant to only one beta-lactam antibiotic (ceftazidime), which may suggest the presence of only broad-spectrum β-lactamase type SHV-1 or SHV-11 [61]. The SHV-2, SHV-5, SHV-7 or SHV-18 belong to the common ESBL variants that have been often observed in Poland [12, 13]. The blactxM-1 gene was not detected in any strains, despite the first strain producing β-lactamase, CTX-M-3 was identified originally in Poland in 1996 [11], being the far predominant ESBL type in the country [11, 13]. Analyzing the occurrence of the blactxM-1 gene, as reported in 2006, is not one of the most common genes in Poland like in the case of the genes encoding beta-lactamase from the CTX-M group: CTX-M-3, CTX-M-15, CTX-M-2 [62]. According to this, the lack of the blactxM-1 gene in the collection of E. coli strains is not
surprising. However, the distribution of the studied genes is very varied depending on the region, country, and the year of the strains isolation. The literature shows that CTX-M enzymes were identified in different locations in the second half of the twentieth century, among others, in Argentina, Israel, and Paraguay [62]. In Europe, \( \text{bla}_{\text{CTX-M}} \) genes were first identified in 1989 in Germany [62]. Results of antibiotic resistance from 2014 presented by Ojdana et al. [13] have shown that \( \text{bla}_{\text{CTX-M15}} \) genes were identified in all of the 12 analyzed \( E. \text{coli} \) strains from a Polish patient (Białystok) and only two strains were positive for \( \text{bla}_{\text{TEM1}} \) and \( \text{bla}_{\text{SHV}} \) genes. Bailey et al. [55] presented antibiotic resistance among the collection of commonal \( E. \text{coli} \) strains. 35% of these strains were ampicillin-resistant and containing the \( \text{bla}_{\text{TEM}} \) gene. In 2012, Korzeniewska et al. [63] published studies about the antibiotic resistance of \( E. \text{coli} \) strains from different sources (hospital and communal wastewater, river, air) and thereabouts 30% of studied strains were ESBL positive. In these strains genes \( \text{bla}_{\text{CTX-M1}}, \text{bla}_{\text{CTX-M3}}, \text{bla}_{\text{CTX-M5}}, \text{bla}_{\text{CTX-M15}} \) were identified as the most common. Winokur et al. [14] analyzed the presence of the \( \text{bla}_{\text{CMY2}} \) gene in \( E. \text{coli} \) strains isolated from people and animals in the USA. In the case of strains resistant to cephalosporins, \( \text{bla}_{\text{CMY2}} \) was identified in 33% of human isolates and 94.8% of animal isolates [14]. It must be noted that the occurrence of antibiotic resistance genes usually differ between people and animals and they are determined by many other factors. It is very well known that bacteria can induce a lot of mechanisms against drugs, so the specific genes often do not correlate with the phenotypic antibiotic resistance, for example, structural changes at the site of the drug’s action or change of the action point for the antibiotic [43, 44]. Additionally, the efflux pumps system AcrAB-TolC, AcrEF-TolC, AcrABC-TolC is also often described in case of \( E. \text{coli} \) phenotypic resistance to beta-lactam antibiotic [64]. As we can see, the distribution of the analyzed genes is not correlated with the year in a global coverage, but in the Polish view we can see the rise of the number of antibiotic resistance genes in \( E. \text{coli} \) strains [10].

Taking into consideration the resistance to other antibiotics, aminoglycosides and sulfonamides also play a significant role during UTI treatment. The \( \text{aac(3)-II} \) is described as the most correlated gene with aminoglycosides resistance, which was not confirmed in our results. Both group of strains, resistant or sensitive, have or have not carried the \( \text{aac(3)-II} \) gene. Therefore, it should be stated that \( \text{aac(3)-II} \) cannot play the role of a marker for resistance to aminoglycosides or at least for one of them. A similar high prevalence of that gene was observed in other studied collection of \( E. \text{coli} \) isolated from Europe [36, 65, 66]. We also analyzed the occurrences of \( \text{sul} \) genes which are responsible for resistance to sulfonamides by changed activity of DHPS (dihydropteroate synthase). This enzyme shows affinity to PABA and when it is encoded by \( \text{sul} \) it remains insensitive to sulfonamides. Trimethoprim, which we used in our study, binds to dihydrofolate reductase and inhibits the reduction of dihydrofollic acid (DHF) further upstream in the same pathway [67]. We wanted to check if there is any correlation between the phenotypic resistance to trimethoprim and the occurrences of \( \text{sul} \) genes—there was none. Furthermore, the presence of the studied \( \text{sul} \) genes also correlated with resistance to cotrimoxazole. These genes were very common for the studied \( E. \text{coli} \) strains, even in the case of strains sensitive to trimethoprim or cotrimoxazole, which can only mean that sulfonamides may enhance the expression of resistance to these antibiotics. The results presented by other authors show a similar distribution of these genes [68, 69]. Completely different results were presented by Mazurek et al. [70] where the presence of the studied genes was significantly lower. However, those \( E. \text{coli} \) strains were isolated from animals in Poland which could probably be an important reason for the differences [70]. The rare occurrence of \( \text{sul3} \) may prove that the synthesis of modified DHPS due to the presence of \( \text{sul3} \) gene has recently appeared in resistant bacteria strains [71]. This confirms the high incidence in this group of questionable and negative results obtained by PCR. Sulfonamides therapy is used in UTI as a combination of sulfamethoxazole with trimethoprim. This was the dominant therapy for UTI between 1995 and 1996. At present, due to the increasing prevalence of resistance to trimethoprim-sulfamethoxazole among \( E. \text{coli} \) strains, it should not be the first method of choice in the treatment of \( E. \text{coli} \) infection [47].

The most obvious results were obtained by analyzing the point mutations in \( \text{parC} \) and \( \text{gyrA} \) genes. Ser/80/Ile in \( \text{parC} \) and also Ser/83/Leu and Asp/87/Asn in \( \text{gyrA} \) give a strong correlation with phenotypic resistance to quinolones [37, 41, 42]. Our results confirm these findings (Fig. 1); however, there can be some other possible mechanisms involved in quinolones resistance, like the permeability effect, efflux pumps, and the decreased availability of quinolones at the target site can also be involved [9, 72, 73]. Looking more closely at the results we observed a specific tendency. The group of strains with reduced susceptibility to quinolones (R < I < S) carried a lot of atypical mutations (Figs. 2, 3). The silent mutations in the hot spot were characteristic for \( \text{parC} \), and missense mutations in other codons were characteristic for \( \text{gyrA} \). It can play a predictive role of imminent phenotypic resistance to fluoroquinolones and makes it suitable for epidemiological studies [74]. Silent mutation have also been identified by other authors [73, 75–77]. It could be interesting to observe the genetic background during the process of acquiring resistance to fluoroquinolones. This can be related to the accumulation of mutations because of the low specificity of fluoroquinolones action. Also, the antibiotics, especially fluoroquinolones can induce the response of SOS systems, which can be responsible for DNA changes in bacteria genome [78, 79]. There is some evidence that
silence mutations might cause a phenotypic effect, they can especially have an influence on the regulation of transcription [80–82]; possibly they can also change the affinity of the hot spot to fluoroquinolones. Nevertheless, we can conclude that the hot spot of parC is more specific but less sensitive to fluoroquinolones (more silent mutations), whereas gyrA conversely—a lot of missense mutations give the phenotypic effect but not in the hot spot of gyrA. So, in this case, two hot spots were evolved. We have not identified more other specific mutations, but in the literature other mutations correlated with phenotypic resistance to quinolones were identified. These mutations were described generally in gyrA and parC genes, for example as new and rare mutations in gyrA—Ser/83/stop, Asp/82/Asn, Gly/81/Asp, Asp/82/Gly, Ser/431/Pro in resistant E. coli strains [83].

Additionally, none of the strains possessed the qnr genes. This similar low prevalence is still relevant in most cases [84–87]. Mammeri et al. in 2005 [72] published an analysis of the qnr gene in a collection of 297 of nalidixic-acid resistant E. coli strains. In this collection, only 1 strain with the qnr gene was identified. The low level of identification of the qnr gene can be due to the weak expression of the Qnr determinant [72]. Some authors present dissimilar results [88–90]. It probably depends on the local distribution of Qnr plasmid.

To conclude, the genetic background is not sufficient for identifying bacterial antibiotic resistance. This kind of analysis can play a role for predicting resistance and it may mark the high or low risk of the emergence of resistance. However, quinolones resistance is very strongly dependent on the specific mutations. As we can see, the distribution of the analyzed genes is very differentiated and shows a high adaptive potential of bacteria to a toxic (antibiotic) environment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval For this type of study formal consent is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study formal consent is not required.

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