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# Plasmid profiles of antibiotic-resistant opportunistic strains of the Enterobacteriaceae family in the microbiota of men with urogenital tract inflammatory pathology

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Bacterial resistance to antimicrobial drugs is a serious public health problem. Plasmids represent one of the most difficult problems in combating the spread of antibiotic resistance. The article presents the results of a real-time PCR study of the microbiota of 257 men with inflammatory processes of the urogenital tract. The role of representatives of the opportunistic microbiota in the occurrence of infectious and inflammatory processes of the urogenital system in men was established. The composition of the microbiota was represented by bacteria of the Enterobacteriaceae family in 39.3% of cases, *Enterococcus* spp. – 10.9%, *Haemophilus* spp. – 3.1% and *Pseudomonas aeruginosa* – in 0.4% of cases. Analysis of the antibiotic sensitivity spectra of the bacterial intestinal group allowed us to establish that among the isolated enterobacteria monoresistance to ampicillin (21–23%) and resistance to ampicillin and amoxicillin/clavulanate (11–14%) were the most common. The work also revealed multiple resistance of clinical strains of enterobacteria to antibiotics: 8% of strains were found to be resistant to cephalosporins of the 3rd and 4th generations and ampicillin, i.e. to β-lactams (phenotype ApCpmCtaCrdCtx); 5% of strains were resistant to 7 and 9 drugs. For *Escherichia*, *Klebsiella* and *Proteus* (resistance to Ap and Ap Am/Cl) only two spectra were characteristic. At the same time, seven spectra were common to *Escherichia coli* and *Klebsiella* sp., five – to *E. coli* and *Proteus* sp., three – to *Proteus* sp. and *Klebsiella* sp. Plasmids of different sizes were isolated from clinical polyresistant strains of *E. coli*, *Proteus mirabilis*, *Klebsiella oxytoca*. However, some multiresistant strains didn't contain plasmids. Direct relationship between antibiotic resistance and the plasmid profile of *E. coli* wasn't found. In experimental clinical strains of enterobacteria the presence of such plasmid spectrum opens up prospects for studying the widespread distribution of multidrug-resistant strains.

*Keywords*: enterobacteria; *Escherichia coli*; *Proteus mirabilis*; *Klebsiella oxytoca*; plasmids; antibiotic resistance; multidrug-resistant strains; electrophoregram.

## Introduction

Microorganisms are becoming resistant to antibiotics faster than scientists can replace the arsenal of antimicrobial agents. The incidence of infections caused by antibiotic-resistant microorganisms is increasing worldwide. This leads to amplification of morbidity and mortality, as well as healthcare costs (Larsson & Flach, 2022, Zhu et al., 2024).

There are many reasons for this trend, but in the spectrum of antibiotic-resistant bacteria the main factor behind the increase is the increase in use of antimicrobial drugs, especially broad-spectrum ones (San Millan, 2018). Today treatment of infections is becoming less effective, as the number and prevalence of penicillin-resistant strains is constantly growing and the resistance of microorganisms to other antibiotics, such as macrolides, cephalosporins and other newer antibacterial drugs, is rapidly strengthening (Alekshun & Levy, 2007; Aminul et al., 2021). An important trigger contributing to the emergence and spread of nosocomial infections is the formation of hospital strains of antibiotic resistant microorganisms. Their emergence is due to the selective pressure of antimicrobial drugs on the bacterial population, as well as the transfer of plasmids and migratory genetic elements, which can occur between bacterial cells belonging to different species, genera and even families (Akter et al., 2021; Aminul et al., 2021; Sklyar et al., 2021). This ensures high rates of evolution of the biological properties of pathogenic microbiota, primarily resistance to antimicrobial drugs, the formation and spread of the strains that have adaptive mechanisms to environment objects in hospitals (Ahmetagic & Pemberton, 2011; Jia et al., 2022, Pusparajah et al., 2022, Sklyar et al., 2022). Antibiotic resistance plasmids are widely distributed among clinical strains of bacteria, but their distribution among host bacteria isn't random (David et al., 2020). Certain associations between clinically important bacterial clones and antibiotic resistance plasmids are particularly successful and lead to the widespread spread of antibiotic resistance. For example, *Klebsiella pneumoniae* harboring pOXA-48-like plasmids confers carbapenem resistance (San Millan, 2018; David et al., 2019), *Escherichia coli* ST131 harboring IncF plasmids encode the extended-spectrum β-lactamase CTX-M (Palkovicova et al., 2022). Colonization with one of these strains poses a serious threat to hospitalized patients (Tischendorf et al., 2016). While antibiotic resistance plasmids allow bacteria to survive in the presence of antibiotics, they can also affect the phenotype of bacteria in the absence of the antibiotic (San Millan & MacLean, 2017). The phenotypes of bacteria containing plasmids are variable among different bacteria (Hall et al., 2016; Cairns et al., 2018; Bethke et al., 2020; Li et al., 2020; Alonso del Valle et al., 2021). For example, the widespread antibiotic resistance plasmid pOXA-48 confers different resistance spectra of clinical strains of Enterobacteriaceae (Alonso del Valle et al., 2021; Fernández-Calvet et al., 2023). These interactions between the plasmid and the genetic background of the host bacterium are key factors in the success of plasmid-bacterial associations (Benz & Hall, 2023; Kosterlitz et al., 2023). Therefore, the aim of the work was to study the antibiotic resistance of clinical strains of enterobacteria and its relationship with the plasmid profile of these bacteria in the microbiota of men with inflammatory processes of the urogenital tract.

## Materials and methods

The studies were conducted in accordance with the principles of bioethics set out in the WMA Declaration, Helsinki "Ethical Principles for Medical Research Involving Human Subjects" and the "Universal Declaration on Bioethics and Human Rights" (UNESCO).

The microbiota spectrum of the pathological urogenital tract was studied in 257 men aged 25 to 62 years. A scraping from the urethra was taken as clinical material. Sample selection was carried out in accordance with the current Order of the Ministry of Health No. 234 dated May 10, 2007 on the basis of Kryvyi Rih City Hospital No. 11. The obtained biological samples were studied by the polymerase chain reaction method on the DT-96/DT-322 amplifier in real-time mode (Q-PCR) using standard sets of reagents and primers, which allows detection of the DNA of microorganisms (normal flora, residents and transient opportunistic microbiome, obligate pathogens), and determination of the number of genomic equivalents (GE/mL) of microbial cells in 1 mL of clinical sample, expressed as lg X copies of DNA/sample.

Isolation of bacteria from biomaterial was performed on selective nutrient media. Identification of microorganisms isolated during primary culture on solid nutrient media was performed based on their cultural, morphological and biochemical characteristics on classical differential diagnostic media using standard methods.

All isolated cultures of enterobacteria had characteristic morphological and biochemical features. Morphotinctorial, biochemical properties, and antibiotic sensitivity of the isolated cultures were studied. The biochemical properties were assessed by the ability of the isolated cultures to ferment carbohydrates and other substrates. Given that the genetic control of these properties of bacteria may be associated with plasmids (Paitan, 2018), extrachromosomal DNA was isolated from typical representatives of different groups of antibiotic-resistant strains of *Escherichia coli*, *Proteus mirabilis*, *Klebsiella oxytoca*, and *Klebsiella pneumonia* obtained from the urogenital tract of the men.

*Determination of the spectrum of antibiotic resistance*. For bacterial strains identified as *E. coli*, *P. mirabilis*, *K. oxytoca* and *K. pneumonia*, susceptibility to antibacterial drugs was determined by the disk diffusion method according to the CLSI/NCCLS criteria (CLSI, 2018, 2020). The spectrum of antibacterial drugs included: azithromycin, amikacin, ampicillin, amoxicillin/clavulanate, gentamicin, imipenem, co-trimoxazole, levofloxacin, nitroxoline, fosfomycin, furagin, cefepime, cefotaxime and ceftazidime. The sensitivity degree of the isolated cultures was assessed according to the system on which the test object belonged to one of the categories: sensitive (S), intermediately resistant (IR) or resistant (R), and multidrug-resistant strains (MDR) were defined as insensitive to one in three categories of antibiotics; extensively resistant strains (XDR) – insensitive to one antibiotic in all but one or two antimicrobial drug categories; pan-resistant strains (PDR) – insensitive to all antibiotics in all antimicrobial drug categories (Mohapatra, 2018).

Plasmid DNA was isolated by the alkaline lysis method (Kado & Liu, 1981) in its own modification. For this, bacterial cultures were grown on solid nutrient medium at the optimal temperature (37 °C) for 24 hours. 2–3 mm size colonies were carefully resuspended in 100 µL of TAE buffer (40 mM Tris-acetate, pH 7.9; 2 mM sodium EDTA). A double volume (200 µL) of lysis buffer (3% SDS; 50 mM Tris, pH 12.6) was added to the resulting suspension. The samples were incubated at 58 °C for 60 min. 300 µL of a mixture of acidic phenol and chloroform (1:1) was added to the obtained lysate, gently mixed to a homogeneous suspension form and centrifuged in an ELMI microcentrifuge at 11,000 rpm for 5 min. For better phase separation, centrifugation was performed twice or three times (if necessary), alternating with mixing. The upper aqueous phase with the located plasmid DNA was collected. The obtained DNA samples were analyzed by agarose gel electrophoresis.

Electrophoretic separation of DNA was performed in horizontal agarose gels with a concentration of 1% agarose and TAE buffer×1. The electric field strength was 6–8 V/cm. Separation time was 3–5 h. After electrophoresis, the gel plate was stained with ethidium bromide (1 µg/mL). To visualize DNA, the gel was viewed under direct ultraviolet light and photographed. As a control of the plasmid molecular weights the *E. coli* strains were used: C600 (P1), lysogenic by phage P1 (molecular weight of the phage plasmid 93.601 kb), K12 containing the F plasmid (molecular weight of 99.159 kb), J5 (RP4) (molecular weight of the RP4 plasmid 60.099 kb) and plasmid pBR322 (4.4 kb) strains.

Statistical processing of the experimental results using the Statistica 10 software (StatSoft Inc., USA) was performed. Standard deviation and mean values were calculated. Differences between group values on Student's t-test with considered significance at $P < 0.05$ were determined.

## Results

Currently, the problem of drug resistance development among microorganisms colonizing the urogenital system is becoming especially relevant due to the widespread dysbiotic conditions of the reproductive systems of men and women. Previously, we showed that 82.7% of the total number of medical care requests were diagnosed with the development of dysbiotic disorders in the urogenital tract. In the work, a targeted identification of the microbiome of the urogenital tract was carried out using real-time PCR. This analysis allows us to determine the balance of bacterial colonization of the urogenital tract: normal state – a small number of several species of commensal bacteria, which are in equilibrium concentrations with each other; abnormal state – excessive reproduction of opportunistic bacteria groups, which shift the balance until the complete displacement of all other bacteria.

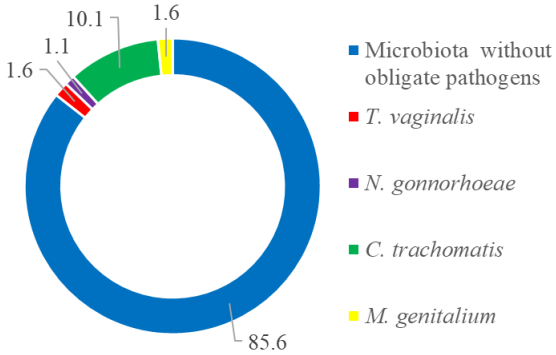



**Fig. 1.** Distribution of samples containing representatives of the obligate pathogenic microbiota of the microbiome from the urogenital tract of men (%, n = 257)

The results of the study showed that samples obtained from 220 (85.6%) examined men didn't contain obligate pathogenic bacteria, and the pathogenic flora was represented by *T. vaginalis*, *N. gonnorhoeae*, *Ch. trahomatis*, and *M. genitalium* (Fig. 1).

It turned out that representatives of the Enterobacteriaceae family were most often involved in the infectious process of the men's urogenital tract. Thus, in 202 (78.6%) clinical samples, DNA of the Enterobacteriaceae family representatives, 28 (10.9%) – DNA of *Enterococcus* spp., 8 (3.1%) *Haemophilus* spp. was detected, and one sample contained DNA of *P. aeruginosa* (Fig. 2).

The next stage of the work was the isolation of the Enterobacteriaceae family representatives in pure culture on appropriate differential diagnostic media and subsequent identification to the species. From 202 clinical samples, 128 strains (63.4%) were isolated and identified as *E. coli*, 30 strains (14.9%) of *Proteus mirabilis*, 12 strains (5.9%) of *Proteus vulgaris*, 22 strains (10.9%) of *Klebsiella pneumoniae* and 10 strains (4.9%) of *Klebsiella oxytoca* were detected (Fig. 3).

Analysis of antibiotic sensitivity spectra of isolated intestinal bacteria allowed us to establish that among *Escherichia* monoresistance to ampicillin (21.1%) and resistance to ampicillin and amoxicillin/clavulanate (12.3%) were the most common. Strains with the ApCpmCtaCrdCtx phenotype, resistant to cephalosporins of the III and IV generations and ampicillin, i.e. to β-lactams, were determined quite often (8.3%). Strains with resistance to 7 and 9 drugs were established with a frequency of 5.0%. *E. coli* strains resistant only to

ampicillin accounted for 21.1%, strains resistant to 9 antibiotics (phenotype ApCpmCtaCtxCzdAm/ClGmCipMox) – 5.0%, other combinations of sensitivity to different antibiotics were represented by single strains, which accounted for less than a third of the studied cultures (26.1%, Fig. 4).

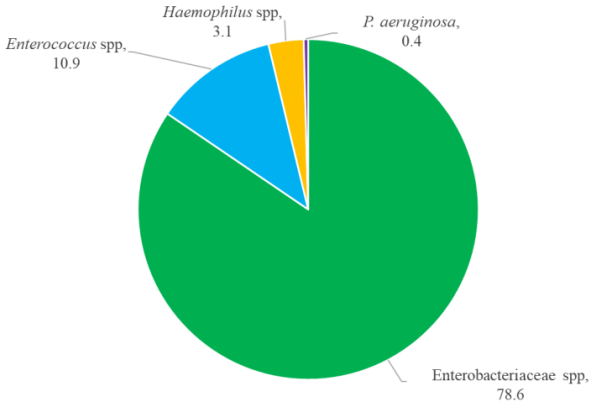



**Fig. 2.** Distribution of samples containing representatives of transient microbiota in clinical samples from the urogenital tract of men (%, n = 257)

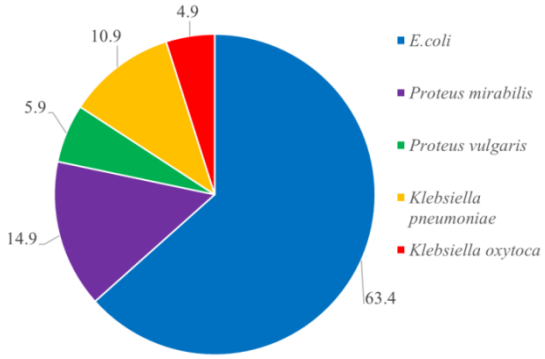



**Fig. 3.** Species composition and ratio of strains of the Enterobacteriaceae family bacteria isolated from clinical samples from the urogenital tract of men (%, n = 202)

23.4% of *Klebsiella* strains were resistant to ampicillin alone and 14.1% to ampicillin and amoxicillin/clavulanic acid. The phenotype with resistance to eight antibiotics was determined with a frequency from 5.1% to 9.2% (Fig. 4).

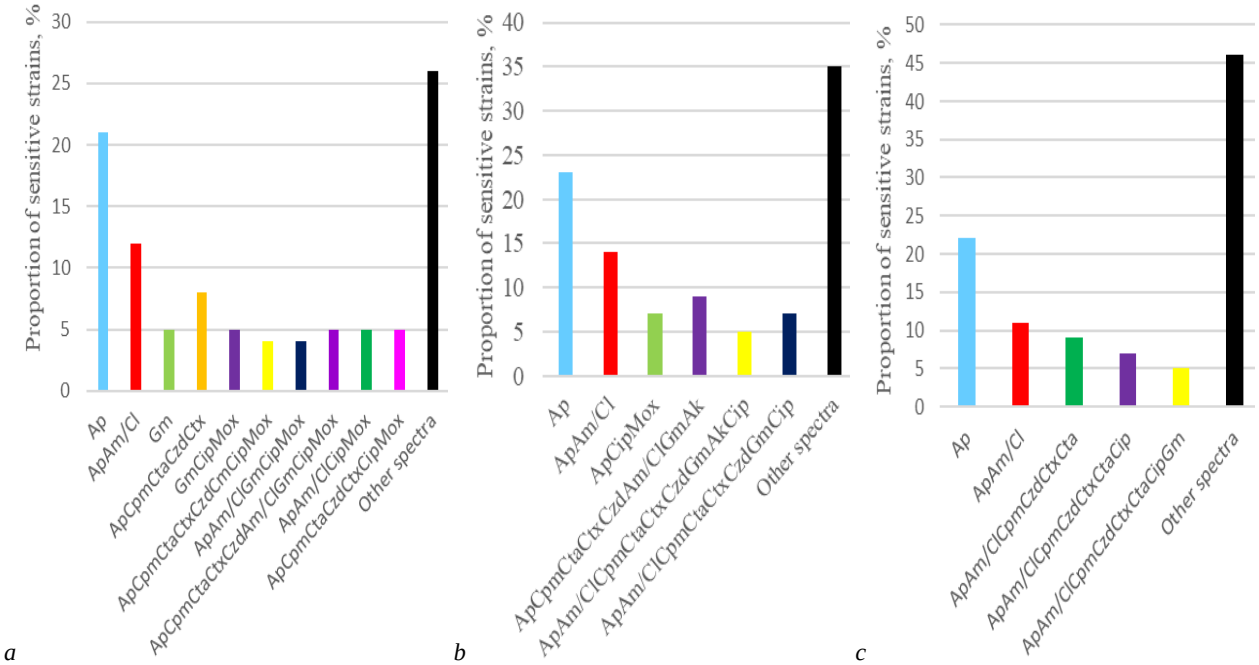
*Proteus*, as other enterobacteria, the most often had resistance to ampicillin (22,0%) and the combination of ampicillin and amoxicillin/clavulanate (11.3%). A phenotype with resistance to β-lactams – ApAm/ClCpmCzdCtxCta was found with a frequency of 9.2%. Phenotypes with combined resistance to β-lactams and ciprofloxacin and gentamicin – ApAm/ClCpmCzdCtxCtaCip – 7.2% and ApAm/Cl CpmCzdCtxCtaCipGm – 5.1%, were determined, respectively (Fig. 4). Thus, only two spectra were characteristic for all three genera of enterobacteria (resistance to Ap and Ap Am/Cl). At the same time, seven spectra were common to *Escherichia* and *Klebsiella*, five – to *Escherichia* and *Proteus*, and three – to *Klebsiella* and *Proteus*.

Then the studies with clinical strains of *E. coli* were conducted. To study the multidrug resistance of strains and its relationship with the presence of extrachromosomal genetic determinants, 11 *E. coli* strains were selected from 128 isolated strains. According to international criteria the selected strains were classified into the following groups: multidrug-resistant strains (MDR), extremely drug-resistant strains (XDR) and pandrug-resistant strains (PDR).

From the eleven strains, one was classified as PDR, four as XDR, and six as MDR (Table 1).

The next stage of the work was the isolation of plasmid DNA from resistant clinical strains of *E. coli*. For comparison of plasmid profiles, 12 polyresistant strains of *P. mirabilis*, *K. pneumonia*, and *K. oxytoca* were selected.

The plasmid profiles of the selected multidrug-resistant bacterial strains were ambiguous. Thus, plasmid DNA was detected in three multidrug-resistant strains – PEC15/1, PEC47/2 and PEC47/4, as well as in one extremely resistant strain – PEC04/7, PEC51/9 and one panresistant strain PEC08/11 (lanes 4, 7, 10, 12 and 14 in Fig. 5). Strains PEC15/1 and PEC47/2 attracted attention. According to the analysis of the electrophoresis, these strains have the same plasmid profile and contain one large plasmid (about 54 kb) and two much smaller ones (lanes 4 and 5, respectively in Fig. 5). Despite the fact that strains PEC47/4, PEC04/7, PEC51/9, and PEC08/11 belong to different groups in terms of antibiotic resistance (Table 1), they have the same plasmid profiles and, like other strains, contain a large plasmid (lanes 7, 10, 12, and 14, respectively in Fig. 5).




*a* *b* *c*

**Fig. 4.** Antibiotic resistance spectra of isolated strains of enterobacteria: *a* – *E. coli*, *b* – *Klebsiella* spp., *c* – *Proteus* spp.; Ak – amikacin, Ap – ampicillin, Am/Cl – amoxiclav, Cfp/sb – cefoperazone/sulbactam, Cip – ciprofloxacin, Cpm – cefepime, Cta – ceftriaxone, Ctx – cefotaxime, Czd – ceftazidime, Gm – gentamicin, Mox – moxifloxacin

**Table 1**

Antibiotic resistance of typical strains to various antibiotics and their classification into groups (MDR, XDR, PDR)

| Strains | | Amoxicillin/ clavulonate | Meropenem | Cefuroxime | Cefepim | Ceftriaxone | Ceftibuten | Azithromycin | Clarithromycin | Ofloxacin | Moxifloxacin | Levofloxacin | Tetracycline | Gentamicin | Amikacin | Fosfomycin | Chloramphenicol | Trimethoprim |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Multidrug-resistant strains (MDR) | PEC15/1 | S | R | S | R | R | R | S | R | R | R | R | S | R | R | R | R | R |
| | PEC28/2 | S | R | R | R | S | R | S | R | R | R | R | S | S | R | R | R | R |
| | PEC46/3 | R | R | R | R | R | S | R | R | S | R | R | S | S | R | R | R | R |
| | PEC47/4 | S | R | S | R | R | R | R | S | R | R | R | R | S | R | R | R | R |
| | PEC55/5 | R | R | R | R | S | R | R | R | S | R | R | R | R | R | R | S | R |
| | PEC68/6 | S | R | R | R | R | S | S | R | R | R | R | S | R | R | R | R | R |
| Extremely drug-resistant strains (XDR) | PEC04/7 | R | S | R | R | R | R | R | R | R | R | R | R | R | R | R | R | S |
| | PEC49/8 | R | S | R | R | R | R | R | R | R | S | R | R | R | R | R | R | R |
| | PEC51/9 | R | R | R | R | R | R | R | R | R | S | R | R | R | R | S | R | R |
| | PEC05/10 | R | R | S | R | R | R | R | R | R | S | R | R | R | R | S | R | R |
| Pandrug-resistant strain (PDR) | PEC08/11 | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R | R |

*Note*: S – sensitive strain; R – resistant strain.

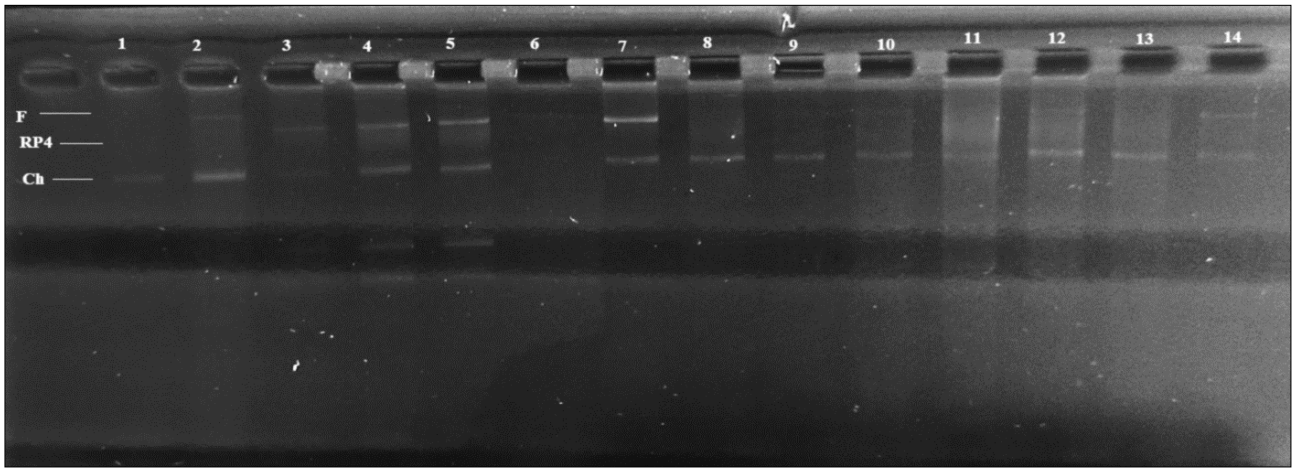



**Fig. 5.** Electrophoregram of plasmid profiles of *E. coli* multidrug-resistant experimental strains: *1* – *E. coli* C600; *2* – *E. coli* K12 (F); *3* – *E. coli* J53 (RP4); *4* – 14 experimental strains of *E. coli*; sizes of marker plasmids: F – 99.159 kb, RP4 – 60.099 kb

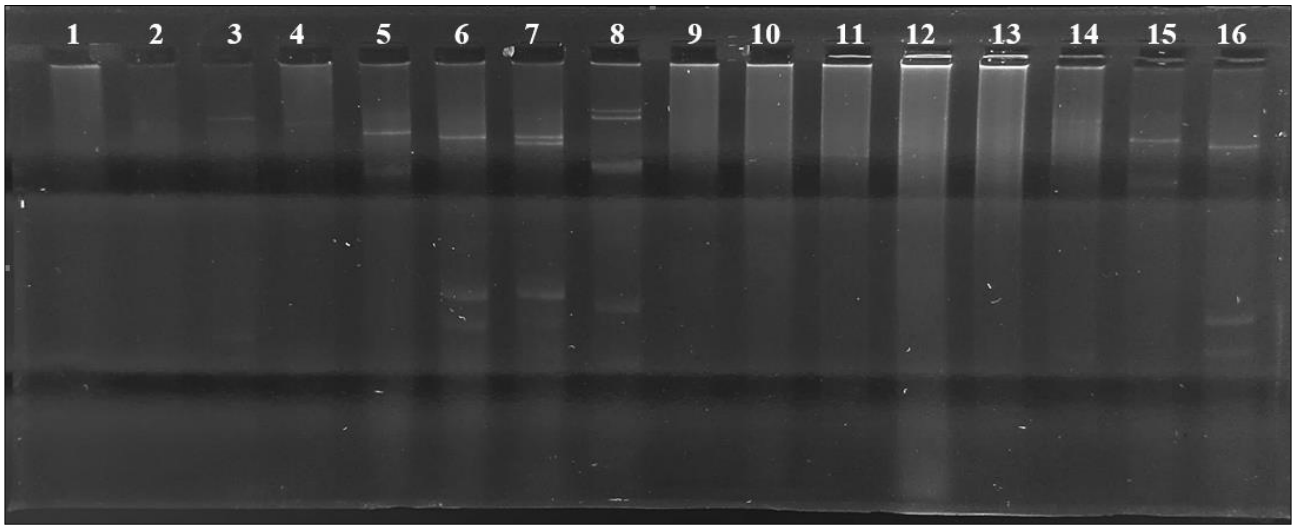



**Fig. 6.** Electrophoregram of plasmid profiles of experimental multidrug-resistant strains of *E. coli* (lanes *1–4*), *Proteus mirabilis* (lanes *5–8*), *Klebsiella pneumonia* (lanes *9–12*), *Klebsiella oxytoca* (lanes 13–16)

For determining the molecular weight of the isolated DNA, marker plasmids with a known mass as phage plasmid P1, isolated from the lysogenic culture of C600 (P1), F plasmid, isolated from strain K12 and RP4, isolated from strain J53 were used. Plasmids with different molecular masses were found in *E. coli* experimental strains (Table 3). Only three multiresistant strains as PEC46/3, PEC55/5 and PEC68/6 didn't contain plasmids. Two strains of *E. coli* had the same plasmid set. All other variants had a very diverse spectrum, which didn't coincide either in their set or in molecular mass (Table 2). The presence of plasmids didn't correlate with antibiotic resistance of clinical strains. It is evident that the resistance of these strains is associated with both the presence of extrachromosomal DNA and other mechanisms. This provides an incentive for the study

of plasmids themselves and other possible pathways of antibiotic resistance in *E. coli* clinical isolates (Table 2).

**Table 2**
Characterization and number of plasmids of resistant clinical strains of *E. coli*

| Strain of *E. coli* | Presence of plasmids (quantity) | Molecular weight of plasmids, kb | Resistance |
|---|---|---|---|
| PEC15/1 | 3 | 50.0; 4.4; 1.5 | Multidrug-resistant strains (MDR) |
| PEC28/2 | 3 | 50.0; 4.4; 1.5 | |
| PEC46/3 | – | – | |
| PEC47/4 | 1 | 50.0 | |
| PEC55/5 | – | – | |
| PEC68/6 | – | – | |
| PEC04/7 | 1 | 50.0 | Extremely drug-resistant strains (XDR) |
| PEC49/8 | – | – | |
| PEC51/9 | 1 | 50.0 | |
| PEC05/10 | – | – | |
| PEC08/11 | 1 | 50.0 | Pandrug-resistant strain (PDR) |

Plasmids were also detected in *Proteus mirabilis*: some multidrug-resistant strains contained 3–4 plasmids of different molecular weights (lanes 5–8, Fig. 6). It is very difficult to isolate plasmid DNA in *Klebsiella pneumonia* and *K. oxytoca* (Delaney et al., 2018). However, one small plasmid was visualized on the electrophoresis in *K. pneumonia* (lane 11, Fig. 6) and several plasmids of different sizes in *K. oxytoca* (lanes 14–16, Fig. 6).

## Discussion

β-Lactam antibiotics, such as penicillins, cephalosporins, monobactams, and carbapenems, are among the most commonly prescribed antibiotics for the treatment of clinical infections (Bush & Bradford, 2020). In recent years bacteria with multidrug resistance (MDR) and extensive drug resistance (XDR) have emerged due to the widespread use and even misuse of antibiotics. CTX-M, the most common type of ESBL, has been distributed worldwide. Plasmids, mobile elements, and bacterial sources are important interrelated factors in the study of the global spread of CTX-M (Bevan et al., 2017). CTX-M-type ESBLs have created enormous problems for public health. Therefore, there is an urgent need to understand the mechanisms of antibiotic resistance spread and the involvement of extrachromosomal genetic elements in this process (Keyi et al., 2024).

The rise of antimicrobial resistance, particularly from Enterobacteriaceae (ESBL-E) producing extended-spectrum β-lactamases, poses a major global public health challenge as it often results in the ineffectiveness of empirical antibiotic therapy, leading to morbidity and mortality (Husna A et al., 2023). Extended-spectrum β-lactamases (ESBLs) are a group of enzymes that can hydrolyze a variety of β-lactams, including fourth generation cephalosporins, and compromise the efficacy of all β-lactams except cephamycins and carbapenems. The ESBL group of enzymes is widespread worldwide and causes severe infection. β-lactamases CTX-M (class A) are more effective against cefotaxime compared to other oxyimino-β-lactam substrates such as ceftazidime, ceftriaxone or cefepime (Saravanan et al., 2018). In our work, we showed that the frequency of detection of enterobacterial resistance to cephalosporins of the 3rd-4th generation strains was 48%. This is a low index, since according to other scientists this index can reach 85% (Bevan et al., 2017; Madrazo et al., 2021). However, in our studies, this resistance was associated with resistance to other antibiotics such as fluoroquinolones (ciprofloxacin, moxifloxacin) and aminoglycosides (gentamicin). The CTX-M genotype, derived from *E. coli* and *K. pneumoniae*, is one of the major types of ESBLs (Husna et al., 2023). They are also examples of plasmid acquisition of β-lactamase genes, which are normally found in the chromosomes of *Kluyvera* species, as group of rarely pathogenic, commensal organisms. Furthermore, a recent study shows that the CTX-M enzyme contains more than 80 types, and most of the types are currently known. Apparently, they have been found mainly in *Salmonella enterica* serovar typhimurium and *E. coli* strains but have also been described in other Enterobacteriaceae species (Saravanan et al., 2018). Among bacterial populations mobile genetic elements (MGEs) are involved in the spread of ESBL genes. Plasmid-mediated transfer of ESBLs is an important route of resistance acquisition (Husna et al., 2023; Keyi et al., 2024). Resistance determinants can have different localizations, and it has been noted that very often antibiotic resistance genes are localized in plasmids (Alekshun & Levy, 2007; Sklyar et al., 2021; Jia et al., 2022; Meng et al., 2022). Thus, our results indicate that among the studied *Escherichia* strains, almost all typical *E. coli* strains were carriers of antibiotic resistance determinants.

In multidrug-resistant *E. coli* strains, we detected extrachromosomal genetic elements, but plasmid profiles didn't show a connection with the spectrum of bacterial resistance to various antibiotics. The results of *Escherichia* study demonstrated a significant diversity of plasmids with different molecular weights and plasmid types in their combination in one strain of *E. coli*. This feature has also been noted in other studies (Johnson & Nolan, 2009; Burova et al., 2012; Pusparajah et al., 2022). The identified plasmids were characterized by different molecular weights (from 1.5 to 50 kb) and could have contained from 1 to 3 plasmids per cell. Some multidrug-resistant strains didn't contain plasmids.

In other isolated members of the Enterobacteriaceae family the presence of plasmids was also assessed. Four plasmids of different molecular weights were identified in *Proteus mirabilis*. Despite the difficulty of isolating plasmids in *Klebsiella* spp. (Delaney et al., 2018), we isolated one small plasmid in *K. pneumonia* and several plasmids in *K. oxytoca* of different sizes. All tested strains of *Proteus* and *Klebsiella* were multidrug-resistant. In the selected strains of *K. pneumonia* the absence of plasmids may indicate the need to use a different plasmid isolation method for these clinical strains.

## Conclusion

Thus, real-time PCR study of the microbiota of men with inflammatory processes of the urogenital tract indicates the dominant role of transient bacteria of the Enterobacteriaceae family as an etiological factor. The concomitant pathognomonic microbiome is represented by *Enterococcus* spp., *Haemophilus* spp. and *P. aeruginosa* in trace amounts. Among the studied strains of enterobacteria, almost all typical strains were carriers of determinants of multiple antibiotic resistance. The relationship of antibiotic resistance of clinical strains of *E. coli, P. mirabilis, K. oxytoca* with the presence of extrachromosomal DNA was shown. However, direct dependence of antibiotic resistance on the plasmid profile of *E. coli* wasn't found. In the studied clinical strains of enterobacteria the detection of such a wide range of plasmids encourages a detailed study of the mechanisms of antibiotic resistance to identify the localization of resistance genes, and the wide distribution of multiresistant strains.

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