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Frequency of detection of antibiotic-resistant strains among microorganisms causing urogenital diseases in women of reproductive age, pregnant women in particular

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Urogenital tract infections are among the most common diseases in women of reproductive age and represent a significant part of obstetrics and gynecology pathologies. These infections can lead to serious complications that negatively affect the health of both mother and fetus. The problem of antibiotic resistance is increasing annually due to the widespread and irrational use of antibiotics. The etiological structure of these infections is often polymicrobial. Monitoring the frequency of antibiotic-resistant strains is crucial for adjusting treatment strategies.
The objective: **to monitor the state of the urogenital system microbiota and identify antibiotic-resistant pathogens of urogenital tract infectious diseases in women of reproductive age, including pregnant women, during 2022–2024.**
***Materials and methods*. Over the period 2022–2024, 677 women were examined, including 320 women aged 15–49 years, 137 pregnant women whose pregnancies ended in childbirth, and 45 pregnant women whose pregnancies ended in miscarriage or stillbirth. Microbiological analysis was performed using bacteriological methods, and species identification was conducted with STAPHYtest, ANAEROtest, ENTEROtest, and NEFERMtest kits. Polymerase chain reaction diagnostics were used to detect *Mycoplasma* and *Ureaplasma*. Antibiotic sensitivity was determined by the disk diffusion method according to EUCAST 2023 recommendations.**
***Results*. Infectious processes were identified in 175 women (20.62%). The most common pathogens isolated from non-pregnant women and pregnant women who delivered were *Candida albicans*, *Ureaplasma* spp., and *Escherichia coli*. In pregnant women with miscarriage/stillbirth, the predominant pathogens were *Ureaplasma* spp., *Mycoplasma hominis*, *Candida albicans*, *Escherichia coli*, and *Streptococcus agalactiae*. Overall, 83 out of 254 isolated cultures of microorganisms were found to be resistant to antibiotics. Antimicrobial susceptibility testing revealed high resistance to tetracycline (29.31% in 2022, 32.32% in 2023, and 32.98% in 2024) and to fluoroquinolones (12.07% in 2022, 13.13% in 2023, and 12.07% in 2024), indicating the emergence of multidrug-resistant strains among urogenital microbiota. Moderate resistance was observed to erythromycin, clindamycin, chloramphenicol and cotrimoxazole, while resistance to amikacin, vancomycin, meropenem and linezolid remained rare.**
***Conclusions*. The results of the study indicate the need for regular microbiological monitoring to improve the effectiveness of treatment for patients with inflammatory diseases of the genitourinary system. Treatment should be prescribed taking into account the susceptibility of pathogens to antimicrobial drugs.**
Keywords: *urogenital tract, microbiota, antibiotic resistance, pregnancy.*

Частота виявлення антибіотикорезистентних штамів серед мікроорганізмів, що спричиняють урогенітальні захворювання у жінок репродуктивного віку, зокрема вагітних

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Інфекції сечостатевого тракту є одними з найпоширеніших захворювань серед жінок репродуктивного віку й становлять значну проблему в акушерстві та гінекології. Ці патології можуть призводити до серйозних ускладнень, що негативно впливають на здоров'я матері й плода. Поширена та ірраціональна антибіотикотерапія сприяє зростанню антибіотикорезистентності, що ускладнює лікування. Етіологічна структура урогенітальних інфекцій часто є полімікробною. Моніторинг чутливості збудників до антибіотиків є критично важливим для розробки ефективних стратегій лікування.
Мета дослідження: провести моніторинг стану мікробіоти сечостатевої системи та визначити стійких до антибіотиків збудників інфекційних захворювань сечостатевого тракту в жінок репродуктивного віку, включно з вагітними, протягом 2022–2024 рр.
***Матеріали та методи*.** Упродовж 2022–2024 рр. було обстежено 677 жінок, з яких 320 були віком 15–49 років, 137 – вагітні, в яких вагітність завершилася пологами, і 45 жінок – вагітні, в яких вагітність завершилася викиднем або мертвонародженням. Для вивчення мікробіоти піхви використовували бактеріологічні методи, а видову ідентифікацію здійснювали за допомогою тест-систем STAPHYtest, ANAEROtest, ENTEROtest, NEFERMtest. Для виявлення штамів *Mycoplasma* та *Ureaplasma* застосовували полімеразну ланцюгову реакцію. Чутливість до антибіотиків визначали методом диско-дифузії згідно з рекомендаціями EUCAST 2023.
***Результати*.** Інфекційні процеси були виявлені у 175 жінок (20,62%). Найчастіше виділеними збудниками були *Candida albicans*, *Ureaplasma* spp. та *Escherichia coli* у невагітних і вагітних, в яких вагітність завершилася пологами. У вагітних, чия вагітність завершилася викиднем/мертвонародженням, домінуючими були *Ureaplasma* spp., *Mycoplasma hominis*, *Candida albicans*, *Escherichia coli* та *Streptococcus agalactiae*. Загалом 83 з 254 виділених культур виявилися резистентними до антибіотиків. Дослідження антимікробної чутливості виявило високий рівень резистентності до тетрацикліну (29,31% у 2022 р., 32,32% у 2023 р. та 32,98% у 2024 р.) та до фторхінолонів (12,07% у 2022 р., 13,13% у 2023 р.

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та 12,07% у 2024 р.), що свідчить про появу мультирезистентних штамів серед урогенітальної мікробіоти. Помірна стійкість спостерігалася до еритроміцину, кліндаміцину, хлорамфеніколу і котримоксазолу, тоді як резистентність до амікацину, ванкоміцину, меропенему та лінезоліду залишалася поодинокою.
Висновки. Результати дослідження підтверджують необхідність регулярного мікробіологічного моніторингу для підвищення ефективності лікування пацієнтів із запальними захворюваннями сечостатевої системи. Призначення лікування має враховувати чутливість збудників до протимікробних препаратів.
Ключові слова: *урогенітальний тракт, мікробіота, антибіотикорезистентність, вагітність.*

Urogenital tract (UGT) infections are one of the most common diseases in women of reproductive age, including pregnant women, and constitute a significant part of diseases in obstetrics and gynecology. UGT pathologies can cause serious complications that negatively affect the health of the mother and fetus. At the same time, the problem of antibiotic resistance of typical infections pathogens arises, as the number of strains that show resistance to "reference" antibiotics increases every year.

It has been confirmed that the etiological structure of urogenital infections is polymicrobial in nature and often occurs in the form of associations of various microorganisms [1–4]. However, the issue of safe diagnosis of inflammatory processes using modern laboratory methods remains unresolved. The main purpose of such studies is to prevent reproductive losses, the development of opportunistic infections and dysbiosis [5–9]. The ability to monitor and detect the frequency of antibiotic-resistant strains of UGT pathogens is of great importance for adjusting treatment and prevention strategies. Studying of this problem allows collecting current data about the level of bacterial resistance, which, in turn, contributes to the implementing of new approaches in treatment and preventing the complications in women [10–15].

The emergence of antibiotic resistance in microorganisms is a biological process caused by the widespread irrational use of antibiotics by both healthcare professionals and patients [16]. This situation contributes to the formation of antibiotic-resistant populations, which often leads to the development of recurrent infections and pathological changes in the urogenital system of the macroorganism. Therefore, the increase resistance of microorganisms to the main groups of antimicrobial drugs dictates the need for a dynamic monitoring process in all countries, including Ukraine, since this concern has grown from a medical to a significant socio-economic in recent years [17, 18].

The objective of this study was to monitor the state of urogenital system microbiota and identify antibiotic-resistant pathogens of UGT infectious diseases in women of reproductive age, pregnant women including, during 2022–2024.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology of Danylo Halytsky Lviv National Medical University (LNMU) in collaboration with the obstetrics and gynecology department and the women's consultation unit of the MNPE "Zhovkva Hospital" (Zhovkva, Lviv region).

A total of 677 women of reproductive age were included in the study during the period of 2022–2024, of whom 175 had vaginal microbiocenosis disorders.

The participants were divided into three groups:
- women aged 15–49 years (n = 320);
- pregnant women whose pregnancies ended in childbirth (n = 137);
- pregnant women whose pregnancies ended in miscarriage or fetal demise (n = 45).

Inclusion criteria: women of reproductive age (15–49 years), presence of complaints or clinical signs of inflammatory diseases of the UGT, and voluntary informed consent.

Exclusion criteria: antibacterial therapy within the last 30 days, severe systemic diseases, oncopathology, or lack of consent to participate.

Sample collection was performed at different time periods depending on the study group:
- in non-pregnant women – during a visit to the obstetrician-gynecologist with signs or suspicion of inflammatory UGT diseases;
- in pregnant women whose pregnancies ended in childbirth – during the II–III trimester (28–37 weeks), when routine microbiological screening was performed to prevent intrauterine infection, chorioamnionitis, and premature rupture of membranes;
- in women up to 22 weeks of gestation whose pregnancies ended in miscarriage or fetal demise, samples were collected during hospitalization – after the diagnosis was confirmed but before any surgical intervention and initiation of antibiotic therapy, as well as after the procedure and completion of treatment, to evaluate the effectiveness of therapy and restoration of the vaginal microbiocenosis.

For all groups, standard methods were used to collect vaginal swabs from the posterior fornix under sterile conditions, followed by transportation to the laboratory within no more than two hours after collection.

The study of the vaginal microbiota composition by qualitative and quantitative indicators was carried out using bacteriological analysis methods. Species identification of the isolated clinical strains was performed using the STAPHYtest, ANAEROtest, ENTEROtest, and NEFERMtest kits to identify members of the families *Enterobacteriaceae (Escherichia coli (E. coli), Klebsiella pneumoniae), Streptococcaceae (Streptococcus agalactiae (S. agalactiae)), Staphylococcaceae (Staphylococcus* spp.*),* as well as *Enterococcus faecalis* and *Candida albicans (C. albicans)*. Polymerase chain reaction (PCR) diagnostics have been used to detect important human pathogens from the genera Mycoplasma *(Mycoplasma hominis* (*M. hominis*)*)* and *Ureaplasma* spp., performed on the CFX96 Real-Time PCR System (Bio-Rad Laboratories, USA) using diagnostic reagents from the Anyplex™ II STI-7 Detection Assay (Seegene Inc., South Korea). Differentiation between *Ureaplasma urealyticum* and *Ureaplasma parvum* was not performed; therefore, results are presented as *Ureaplasma* spp. The sensitivity and resistance of UGT pathogens to the main groups of antibiotics were determined by disk diffusion method in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2023). The results were recorded and processed using Microsoft Office Excel.

RESULTS AND DISCUSSION

According to the results of the comprehensive study, infectious lesions were detected in 175 individuals: in 2022 – 20.62% (n = 40), with 58 isolates obtained (considering that more than one pathogen was isolated in some patients); in 2023 – 28.81% (n = 68), with 99 isolates obtained; and in 2024 – 27.13% (n = 67), with 97 isolates obtained (Table 1).

When analyzing the species composition of microorganisms isolated from the urogenital system of non-pregnant women aged 15–49 years, it was found that the microbiota had a polymicrobial character (Table 2).

The most common representatives were *C. albicans, Ureaplasma* spp., and *E. coli*.

The proportion of *C. albicans* in the overall isolate structure increased from 26.92% in 2022 to 32.61% in 2024, which may indicate a trend toward a higher frequency of vaginal fungal infections.

The prevalence of *Ureaplasma* spp. also showed an upward trend (from 15.38% in 2022 to 21.74% in 2024), whereas the proportion of *M. hominis* remained relatively stable (approximately 9–11%).

The observed dynamics may indicate the persistent leading role of conditionally pathogenic bacteria, along with an increasing frequency of *Candida* species isolation.

As a result of diagnosing urogenital infections in pregnant women whose pregnancies ended in childbirth during the study period, similar statistical patterns were observed.

An analysis of the microbiological composition of the urogenital system in these women revealed the predominance of conditionally pathogenic bacteria and fungi of the genus *Candida* (Table 3).

The most frequently isolated microorganism throughout the three-year study was *C. albicans* – its proportion was 30.77% in 2022, 31.58% in 2023, and 31.82% in 2024, indicating a stable frequency of vaginal colonization by yeast-like fungi in pregnant women with a physiological course of pregnancy.

Among bacterial isolates, *E. coli* (18.18–23.08%), *S. agalactiae* (7.69–13.63%), and *Staphylococcus* spp. (4.55–15.39%) occupied leading positions.

Table 1

Indicators of disturbances in the microbiocenosis of the UGT in women of reproductive age, including pregnant women, during 2022–2024

Year	Examination results		Women aged 15–49	Pregnant women (pregnancy ended in childbirth)	Pregnant women (pregnancy ended in miscarriage/stillbirth)	Total
2022	Examined	Abs.	98	44	12	154
		%	63.63	28.57	7.80	79.38
	with disturbance of the vaginal microbiota	Abs.	26	9	5	40
		%	65.00	22.50	12.50	20.62
2023	Examined	Abs.	104	48	16	168
		%	61.91	28.57	9.52	71.19
	with disturbance of the vaginal microbiota	Abs.	42	19	7	68
		%	61.76	27.94	10.30	28.81
2024	Examined	Abs.	118	45	17	180
		%	65.56	25	9.44	72.87
	with disturbance of the vaginal microbiota	Abs.	46	12	9	67
		%	68.66	17.91	13.43	27.13
Total examined for the period 2022–2024						677
Total infected for the period 2022–2024						175

A moderate frequency of *Ureaplasma* spp. isolation (7.69–13.63%) and occasional detection of *M. hominis* (5.26–9.09%) were noted.

Overall, the structure of the microbiota in pregnant women with a physiological course of pregnancy remained relatively stable over the three-year observation period, without significant changes in species composition.

In the group of pregnant women whose pregnancies ended in miscarriage or fetal demise, significant changes

Table 2

Species spectrum of microorganisms isolated from the urogenital system of non-pregnant women aged 15–49 years (proportion of obtained isolates)

Microorganisms	Number of obtained isolates (%) by year		
	2022 (n = 26)	2023 (n = 42)	2024 (n = 46)
E. coli	5 (19.23)	7 (16.67)	8 (17.39)
S. agalactiae	2 (7.69)	3 (7.15)	3 (6.52)
Staphylococcus spp.	3 (11.54)	4 (9.52)	3 (6.52)
Enterococcus faecalis	1 (3.85)	2 (4.76)	2 (4.35)
Klebsiella pneumoniae	1 (3.85)	2 (4.76)	2 (4.35)
C. albicans	7 (26.92)	12 (28.57)	15 (32.61)
Ureaplasma spp.	4 (15.38)	8 (19.05)	10 (21.74)
M. hominis	3 (11.54)	4 (9.52)	3 (6.52)

Table 3

Species spectrum of microorganisms isolated from the urogenital system of pregnant women whose pregnancies resulted in childbirth

Microorganisms	Number of obtained isolates (%) by year		
	2022 (n = 13)	2023 (n = 19)	2024 (n = 22)
E. coli	3 (23.08)	4 (21.05)	4 (18.18)
S. agalactiae	1 (7.69)	2 (10.53)	3 (13.63)
Staphylococcus spp.	2 (15.39)	2 (10.53)	1 (4.55)
Enterococcus faecalis	1 (7.69)	1 (5.26)	1 (4.55)
Klebsiella pneumoniae	0 (0.00)	1 (5.26)	1 (4.55)
C. albicans	4 (30.77)	6 (31.58)	7 (31.82)
Ureaplasma spp.	1 (7.69)	2 (10.53)	3 (13.63)
M. hominis	1 (7.69)	1 (5.26)	2 (9.09)

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were observed in the species composition of microorganisms in the UGT compared to women with a physiological course of pregnancy (Table 4).

The most frequently isolated microorganisms in this group were *Ureaplasma* spp. (21.06–24.14%) and *M. hominis* (15.78–20.69%), indicating the important role of conditionally pathogenic mycoplasmas in the pathogenesis of complicated pregnancy outcomes.

Among other bacterial isolates, *E. coli* (13.16–15.78%) and *S. agalactiae* (10.34–10.53%) were more commonly detected.

The detection rate of *C. albicans* remained relatively stable (10.53–13.79%); however, in combination with the high colonization rates of *Ureaplasma* spp. and *M. hominis*, this may indicate the formation of mixed infectious associations.

The obtained data confirm an increased infectious burden of the UGT in women with adverse pregnancy outcomes.

Based on the conducted research, it is possible to identify the priority pathogens most frequently causing lesions of the UGT in women of reproductive age.

In non-pregnant women and in pregnant women whose pregnancies ended in childbirth, the main pathogens were *C. albicans, Ureaplasma* spp., and *E. coli*.

In pregnant women whose pregnancies ended in miscarriage or fetal demise, the predominant pathogens were *Ureaplasma* spp., *M. hominis, C. albicans, E. coli,* and *S. agalactiae*.

Analysis of the dynamics of antibiotic resistance in urogenital microorganism isolates from women of reproductive age during 2022–2024 revealed a steady trend toward increasing resistance rates across most groups of antibacterial agents (Table 5).

The highest resistance level throughout all study years was observed in isolates resistant to tetracyclines (tetracycline) – 29.31% in 2022, 32.32% in 2023, and 32.98% in 2024 – confirming the persistently high frequency of resistance to this class of drugs.

The obtained results are consistent with studies showing that among conditionally pathogenic agents of urogenital infections (*Ureaplasma* spp., *M. hominis, E. coli*), the frequency of resistance to tetracyclines exceeds 30–40% [19, 20].

This is explained by the extensive and prolonged use of tetracyclines in clinical practice for empirical therapy of urogenital infections.

Resistance to fluoroquinolones (ciprofloxacin) also remained consistently high: 12.07% in 2022 and 13.13% in 2023.

This indicator corresponds to the findings of Ann E. Stapleton et al., who reported 10–20% resistance among *E. coli* urogenital isolates in women of reproductive age [21].

Aztreonam and chloramphenicol demonstrated moderate resistance levels (6.90–9.27%), which tended to increase gradually.

This may be associated with cross-resistance between β-lactams and amphenicols, which develops under conditions of prolonged antibacterial pressure.

Macrolides (erythromycin) maintained a moderate level of resistance – from 8.62% in 2022 to 10.30% in 2024 – consistent with other studies reporting resistance rates among *Ureaplasma* spp. within 8–10% [22].

Moderate resistance levels were also observed to lincosamides (clindamycin) – 5.17–7.21%, cotrimoxazole – 3.45–5.15%, and ceftriaxone – 1.72–5.15%, which may indicate the preservation of clinical efficacy of these agents when used rationally.

Table 4

Species spectrum of microorganisms isolated from the urogenital system of pregnant women whose pregnancies ended in miscarriage or fetal demise

Microorganisms	Number of obtained isolates (%) by year		
	2022 (n = 19)	2023 (n = 38)	2024 (n = 29)
E. coli	3 (15.78)	5 (13.16)	4 (13.79)
S. agalactiae	2 (10.53)	4 (10.53)	3 (10.34)
Staphylococcus spp.	2 (10.53)	3 (7.89)	2 (6.90)
Enterococcus faecalis	1 (5.26)	2 (5.26)	1 (3.45)
Klebsiella pneumoniae	2 (10.53)	3 (7.89)	2 (6.90)
C. albicans	2 (10.53)	5 (13.16)	4 (13.79)
Ureaplasma spp.	4 (21.06)	9 (23.69)	7 (24.14)
M. hominis	3 (15.78)	7 (18.42)	6 (20.69)

Table 5

Resistance of clinical strains of UGT infection pathogens to antimicrobial agents

Antimicrobial agents	Number of resistant strains (%) by year		
	2022 (n = 53)	2023 (n = 100)	2024 (n = 107)
Ampicillin	6 (10.34)	7 (7.07)	7 (7.21)
Amikacin	2 (3.45)	2 (2.02)	4 (4.12)
Erythromycin	5 (8.62)	9 (9.09)	10 (10.30)
Clindamycin	3 (5.17)	7 (7.07)	7 (7.21)
Meropenem	0 (0.00)	1 (1.01)	1 (1.03)
Ceftriaxone	1 (1.72)	3 (3.03)	5 (5.15)
Linezolid	0 (0.00)	1 (1.01)	1 (1.03)
Aztreonam	4 (6.90)	9 (9.09)	9 (9.27)
Chloramphenicol	3 (5.17)	8 (8.08)	9 (9.27)
Cotrimoxazole	2 (3.45)	5 (5.05)	5 (5.15)
Tetracycline	17 (29.31)	32 (32.32)	32 (32.98)
Ciprofloxacin	7 (12.07)	13 (13.13)	13 (12.07)
Furadonin	2 (3.45)	2 (2.02)	2 (2.06)
Vancomycin	1 (1.72)	1 (1.01)	2 (2.06)

Vaginal isolates of *Staphylococcus* spp. and *Enterococcus* spp. showed rare resistance to aminoglycosides (amikacin) and glycopeptides (vancomycin), amounting to about 2–4%.

Minimal resistance was observed to meropenem and linezolid (around 1%), which can be attributed to the limited use of carbapenems and oxazolidinones in gynecological practice.

Overall, the study results indicate a persistently high level of resistance to tetracyclines and fluoroquinolones, as well as a gradual increase in resistance to aztreonam, macrolides, and third-generation cephalosporins.

This trend is unfavorable, as these groups of drugs are widely used to treat urogenital infections in women of reproductive age.

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Compared with other studies, the obtained data reflect a pan-European trend toward the rise of multidrug resistance among urogenital microbiota isolates, particularly due to *Ureaplasma* spp. and *M. hominis*, highlighting the need to revise approaches to antibiotic therapy and ensure the rational use of reserve drugs [23, 24].

The obtained findings also correlate with the results of E. T. Amin, C. Njumkeng, and B. T. Kika, who reported the highest resistance levels among clinical isolates of uropathogenic microorganisms to antibiotic classes as follows: tetracyclines – up to 50.0%, cephalosporins – up to 45.0%, fluoroquinolones – up to 30.0%, monobactams – up to 25.0%, macrolides – up to 25.0%, amphenicols – up to 25.0%, lincosamides – up to 20.0%, sulfonamides – up to 15.0%, penicillins – up to 15.0%, aminoglycosides – up to 10.0%, and the lowest resistance rates to nitrofurans, oxazolidinones, glycopeptides, and carbapenems – up to 5.0% of cases [25].

These findings have practical significance for the development of regional protocols for empirical therapy and for optimizing laboratory monitoring of antibiotic resistance in women with vaginal microbiocenosis disturbances.

CONCLUSIONS

1. The microbiological analysis of urogenital isolates obtained from women of reproductive age revealed significant diversity of pathogens and confirmed the polymicrobial nature of urogenital infections. The most frequently isolated microorganisms were *C. albicans*, *Ureaplasma* spp., *E. coli*, and *M. hominis*.

2. In non-pregnant women and in pregnant women with a physiological course of pregnancy, *C. albicans* and *Ureaplasma* spp. predominated.

In contrast, in women with missed miscarriage or spontaneous abortion, the detection rates of *Ureaplasma* spp. and *M. hominis* were significantly higher. These findings suggest a possible etiological role of mycoplasmas in the development of infectious and inflammatory complications of pregnancy.

3. Antimicrobial susceptibility testing demonstrated consistently high resistance to tetracycline (32.98%) and ciprofloxacin (13.13%), indicating the emergence of multidrug-resistant strains among the predominant representatives of the urogenital microbiota. Moderate resistance levels were observed to erythromycin, clindamycin, chloramphenicol, and cotrimoxazole, whereas resistance to amikacin, vancomycin, meropenem, and linezolid remained rare.

4. The observed increase in resistance to β-lactams and third-generation cephalosporins highlights the necessity of rational antibiotic use, continuous monitoring of urogenital isolate susceptibility, and regular updates to local antimicrobial therapy protocols.

5. The obtained results confirm the relevance of the antimicrobial resistance problem within the structure of urogenital infections in women of reproductive age and emphasize the need for a personalized approach to antibiotic therapy, considering local microbiological and epidemiological characteristics.

Presented data indicate the need for regular microbiological monitoring to improve the effectiveness of treatment the patients with genitourinary system inflammatory diseases. The appointment of syndromic treatment of urogenital infections should be carried out taking into account the susceptibility of pathogens to antimicrobial drugs. The results obtained are the basis for improving prophylactic and therapeutic measures to prevent the occurrence and spread of urogenital infections and pathologies of the reproductive system.

Ethics approval and data availability. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board. The permission to conduct experiments on animals and to isolate/work with cultures of microorganisms was approved by the Commission on Ethics of Scientific Research, Experimental Development, and Scientific Works of Danylo Halytsky LNMU, Ukraine, under protocol No. 13 on December 15, 2023. Written informed consent was obtained from the patients for publication and any accompanying images. The trial was registered at ClinicalTrials.gov under ID: NCT06616168.

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