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Inna Ponomarova, Tamara Lisyana, Svitlana Kryshchuk, Anastasia Timofeeva

State Institution "Institute of Paediatrics, Obstetrics and Gynaecology named after academician OM Lukyanova, National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine

Features of vaginal microbiota in women with vulvovaginal candidiasis

Corresponding author:

Inna Georgiivna Ponomaryova, PhD
State Institution Institute of Paediatrics, Obstetrics and Gynaecology named after academician OM Lukyanova, National Academy of Medical Sciences of Ukraine,
Platona Maiborody 8 St.
04050, Kyiv, Ukraine
tel. +380679712056
e-mail: microbiki@gmail.com

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ABSTRACT

Introduction: Dysbiotic changes in the microbiota of the genital tract in candidal vulvovaginitis are manifested by the formation of associations of *Candida* fungi with various representatives of opportunistic microflora against the background of *Lactobacillus spp*. deficiency. In the vast majority of cases, the causative agent of the infection is *Candida albicans*, with *Candida non-albicans* being less frequently registered.

Material and methods: To assess the species and quantitative composition of the vaginal microflora in women, bacteriological studies were conducted. The research group consisted of 60 women with candidal vulvovaginitis. The control group consisted of 30 healthy women.

Results: The results indicate that in 95% of women with vulvovaginal candidiasis, the vaginal microbiota is characterized by the formation of 2–4 component associations of *Candida* fungi with various representatives of facultatively anaerobic and obligately anaerobic conditionally pathogenic microflora. The species spectrum of fungi isolated from the genital tract of women with candidal vulvovaginitis mainly included *C. albicans* (75%) and, less frequently, *Candida non-albicans* (25%). In healthy women, *C. albicans* was recorded in 10% of those examined, and *Candida non-albicans* in 6.6%.

Conclusions: An increase in the frequency of detection of *Candida non-albicans* species in the spectrum of fungi isolated from the vagina of women with candidal vulvovaginitis, an increase in the frequency of fungal-bacterial associations of microorganisms, as well as a deficiency or absence of *Lactobacillus spp*., confirms the importance of constant bacteriological monitoring to identify changes in the composition of the vaginal microbiota and prescribe adequate therapy.

Keywords: vulvovaginal candidiasis, vaginal microbiota, *Candida,* conditionally pathogenic microorganisms

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Introduction

In recent years, the prevalence of candidiasis vulvovaginitis among women has ranged from 8% to 32%, and these rates are steadily increasing [1, 2]. The relevance of this problem today is explained by both the high prevalence and the tendency of this pathology to become chronic and recurrent [3, 4]. The reasons for the high prevalence of vulvovaginal candidiasis, including its recurrent forms, are numerous: diseases in the host organism that weaken the immune system or disrupt the secretory link of immunity, diabetes, hormonal imbalance, and the irrational use of antibacterial drugs. These changes create favourable conditions for the spread and reproduction of yeast-like fungi of the genus *Candida* [5–7].

Currently, more than 196 biological species of yeast-like fungi have been described, among which *Candida albicans* is the causative agent of infection in the vast majority of cases (about 70–90%). Other types of fungi of the genus *Candida—Candida tropicalis, Candida krusei, Candida parapsilosis, Candida glabrata* — are isolated in 10–30% of cases [8–10].

Diseases caused by *C. albicans* are associated with pathogenicity factors such as morphological transformation, adhesion, invasion, formation of biofilms, and secretion of hydrolases, which are determined by the genome of the fungus. One of the main pathogenicity factors of *C. albicans* is a mannose-containing ingredient, asparagine proteinases. Mannoproteins of the cell walls of this type of yeast fungus function as surface antigens, mainly providing adhesion and invasion into the

vaginal epithelium. This is the initial stage of the pathogenesis of candidiasis, which allows fungi to remain on the surface of the multilayered squamous epithelium of a healthy mucous membrane. Factors that increase the adhesive properties of *Candida* fungi include drugs often used in obstetrics and gynaecology: synthetic progestins, corticosteroids, and cytostatics [11–13].

It is known that the ability to adhere directly correlates with the prevalence of the strain and is strongest in *C. albicans* and to a lesser extent in *C. tropicalis, C. parapsilosis,* and *C. glabrata* [14, 15].

During electron microscopic examination of vaginal secretions in women with candidiasis vulvovaginitis, intracellular parasitism of *Candida* fungi was detected, particularly through the invasion of pseudo mycelia into epithelial cells with the formation of phagosomes, which serve as reservoirs of infection. The invasion of *Candida* fungi into epithelial cells leads to their parasitism in the cytoplasm, forming lysis zones. Under favourable conditions, fungal blastospores transform into pseudomycelium threads, which grow invasively into epithelial cells towards the nucleus, causing its destruction and the rarefaction of the cytoplasm [16, 17].

A characteristic of modern genital candidiasis is the formation of associations of *Candida* fungi with various representatives of opportunistic microflora: Gram-positive and Gram-negative aerobic, facultatively anaerobic, and obligately anaerobic microorganisms [18]. An important factor in their active growth is the deficiency of *Lactobacillus spp*., which produces H_2O_2 [19–22].

This work aimed to study the qualitative and quantitative indicators of vaginal colonization by facultatively anaerobic and obligately anaerobic microorganisms in women with candidiasis vulvovaginitis.

Material and methods

Bacteriological studies were carried out to assess the species and quantitative composition of the facultatively anaerobic and obligately anaerobic microflora of the vagina in 30 women of childbearing age with candidiasis vulvovaginitis. The diagnosis of candidiasis vulvovaginitis was verified according to clinical and laboratory research methods. The International Classification of Diseases (ICD-10), created in accordance with WHO recommendations and adopted in Ukraine, was used for establishing the gynaecological diagnosis.

The age of the study participants ranged from 24 to 30 years, with a BMI of 18.5–24.9. At the time of the examination, the women were not pregnant and did not take hormonal contraception or other medications. According to the gynaecological history, 48% of the women had previously been treated for colpitis of various aetiologies, 22.5% had menstrual cycle disorders, and 15.5% had a history of cervical erosion.

There was a somatic history burden in 35% of the women: 15% had been diagnosed with cardiovascular pathology, 10% suffered from chronic pyelonephritis, and 10% had gastrointestinal diseases (gastritis, cholecystitis). Data obtained from the examination of 30 healthy women were used as a control.

Microbiological studies and recording of the results were carried out in accordance with Order No. 1614 of the Ministry of Health of Ukraine dated 03.08.2021, "Procedure for Epidemiological Surveillance and Keeping Records of Infectious Diseases Related to the Provision of Medical Care" and other regulatory documents. Differential diagnostic media were used to culture vaginal mucus: blood agar, yolk-salt agar, Endo medium, Lactoagar for lactobacilli, chromogenic medium for group B streptococci (bioMerieux, France), Saburo agar, and chromogenic medium for *Candida* (SANIMED-M LLC, Ukraine), which allows differentiation of *Candida* fungi into species.

Sowing was carried out by the method of sectoral sowing on dense nutrient media, which allows for determining the degree of microbial insemination and revealing the maximum possible spectrum of aerobic and facultatively anaerobic microflora. The anaerobic microflora of the genital tract was studied with strict adherence to the technique of anaerobic cultivation. The material obtained without access to oxygen was sown on a dense nutrient medium with subsequent spreading; then, the material was immersed in the bottom of a test tube with a storage medium. Solid and liquid nutrient environments were used for research (thioglycolic medium, blood agar with glucose, liver broth, Kitt-Tarozzi medium).

All cultures were placed in an anaerostat at 37°C for 7 days. In parallel, cultures were cultivated in aerobic conditions. To create anaerobic conditions, the Anaerocult system (Merck, Germany) and Anaerogaz gas packages were used. When signs of bacterial growth appeared, smears were made, stained by Gram, and microscopied.

The identification of the selected microorganisms was carried out according to their cultural, morphological, staining, and biochemical properties on the automatic microbiological analyser Vitek 2 Compact (bioMerieux, France).

Statistical processing of the obtained research results was carried out using the standard "Data Analysis"

package of Microsoft Excel for Windows 2007. The arithmetic mean (M), the standard error of the mean (m), and the probability value (p) were calculated. The reliability of the obtained data was assessed using the Student's t-test. Reliability was considered established if the probability was at least 95% ($p < 0.05$).

Results

Microscopy of vaginal contents in most women with candidiasis vulvovaginitis indicates negative changes in microbiota indicators, which manifest as vaginitis. In 93.3% of patients, a significant increase in the number of neutrophils and leukocytes (30–100 in the field of vision), increased desquamation of the epithelium, degenerative changes in the nuclei, vacuolization of the cytoplasm, and a significant increase in the number of Gram-positive and Gram-negative microorganisms (cocci, streptococci, bacilli) were observed. Microflora morphotypes corresponding to Lactobacillus spp. were found with insignificant frequency or were absent. In 46.7% of those examined, fungi were found in the form of yeast cells, and in 53.3%, yeast and pseudomycelium were found.

As is known, the morphogenesis of yeast into hyphal elements is considered a factor in the pathogenicity of *Candida* fungi because it contributes to their migration through the affected mucous membrane into healthy tissues.

Using the cultural method of research, it was established that the species spectrum of fungi isolated from the genital tract of women with candidiasis vulvovaginitis mainly included *C. albicans* (75%) and less frequently *Candida non-albicans* (25%) (Table 1). Among *Candida non-albicans, Candida glabrata* (10%) and *Candida tropicalis* (6.7%) were detected with the highest frequency, and their quantitative indicators exceeded the diagnostic level [according to lg 5.3 CFU/mL and lg 4.3 CFU/ml ($p < 0.05$)].

Lactobacillus spp. in the composition of the vaginal microflora is of essential importance for the realization of the functions of the vaginal microbiota. *Lactobacillus spp*. deficiency was found in 78.3% of patients, and their absence was noted in 21.7%. A decrease in the quantitative level of *Lactobacillus spp.* seeding was also found — lg 3.9 CFU/mL (Table 2).

The study of the vaginal microflora using the cultural bacteriological method allowed the establishment of active proliferation of representatives of the phylum *Firmicutes* and phylum *Proteobacteria,* in particular, representatives of Gram-negative bacteria of the family *Enterobacteriaceae*, in women with candidiasis vulvovaginitis. Changes in indicators of contamination of the vagina with anaerobic Gram-positive coccal microflora and representatives of the genera *Bacteroides spp.* and *Prevotella spp*. were also found. The level of the latter significantly decreased compared to healthy women.

In 95% of patients, the formation of fungal associations was found between *Candida* and various representatives of conditionally pathogenic microflora. Two-component bacterial-fungal associations were found in 28.3% of cases, three-component in 48.3%, and four-component in 18.3% of patients. Monocultures of *Candida* fungi were registered in 6% of women.

As a result of bacteriological examination of women with candidiasis vulvovaginitis, an increase in the composition of bacterial and fungal associations in vaginal contents was established, particularly cocci with pathogenic properties (haemolysis, plasma coagulation). For example, the frequency of the presence of *Staphylococcus aureus* in the composition of associations in the studied group of women reached 18.3%, *Staphylococcus epidermidis* with haemolysis — 23.3%, *Enterococcus spp.* — 20.0%.

Also, the composition of associations in sick women with significant frequency included various types of enterobacteria. A significant proportion of *Escherichia coli* with haemolytic properties was established (16.7%), *Klebsiella spp*. — 15%, *Enterobacter spp*. — 13.3%.

Quantitative indicators of seeding of various representatives of facultatively anaerobic conditionally pathogenic microflora of the vagina in patients exceeded the diagnostic level (lg 4.2 — lg 5.8 CFU/mL).

Thanks to the development of modern microbiological diagnostic methods, information about the role of anaerobic microflora in the vaginal microbiome has significantly increased. One of the tasks of this work was to assess the degree of insemination of the vagina by representatives of obligate anaerobic microflora in women with candidiasis vulvovaginitis.

According to the obtained results, the vagina of sick women was most frequently contaminated with *Peptostreptococcus spp.* (28.3%), *Fusobacterium spp.* (16.7%), and *Veillonella spp*. (16.7%). Quantitative indicators of seeding of these representatives of obligate anaerobic microflora exceeded the diagnostic level and were lg 5.4 CFU/mL — lg 4.4 CFU/mL. The presence of *Bacteroides spp.* (13.3%) and *Prevotella spp.* (10.0%) was less frequent. The quantitative level of vaginal contamination by *Bacteroides spp.* and *Prevotella spp.* was also reduced compared to healthy women.

Examination of vaginal contents in healthy women revealed the presence of endogenous stabilizing

Table 1. Candida fungi in patients with candidiasis vulvovaginitis (%, lg CFU/mL)

Types of mushrooms	Women from candida vulvovaginitis n = 30		Healthy women n = 30	
	%	lg CFU/mL	%	lg CFU/mL
Candida albicans	75.0	5.9 ± 0.04*	10.0	3.4 ± 0.02
Candida tropicalis	6.7	4.3 ± 0.02*	3.3	2.5 ± 0.04
Candida glabrata	10.0	5.3 ± 0.08	–	–
Candida parapsilosis	5.0	3.7 ± 0.06*	3.3	2.8 ± 0.06
Candida krusei	3.3	3.5 ± 0.02	–	–

* — statistically significant difference between indicators of sick and healthy women ($p < 0.05$)

Table 2. Microflora of the vagina in patients with candidiasis vulvovaginitis (%, lg CFU/mL)

Microflora	Patients with candidiasis vulvovaginitis N = 30		Healthy women N = 30	
	%	lg CFU/mL	%	lg CFU/mL
S. epidermidis	21.7	5.6 ± 0.06*	20.0	3.3 ± 0.04
S. epidermidis (hem+)	23.3	5.8 ± 0.02*	6.7	2.5 ± 0.02
S. aureus	18.3	5.0 ± 0.04*	3.3	2.0 ± 0.04
E. faecalis	20.0	4.8 ± 0.08*	13.3	3.5 ± 0.05
S. agalactiae	15.0	4.2 ± 0.02*	6.7	2.5 ± 0.02
S. pyogenes	8.3	4.2 ± 0.04	–	–
Corynebacterium spp.	13.3	4.0 ± 0.06	10.0	3.3 ± 0.04
E. coli	18.3	5.4 ± 0.02*	13.3	3.2 ± 0.02
E. coli (hem+)	16.7	5.0 ± 0.04*	6.7	3.0 ±0.03
Klebsiella spp.	15.0	4.8 ± 0.08*	3.3	2.0 ± 0.04
Enterobacter spp.	13.3	4.4 ± 0.02	6.7	3.2 ± 0.06
Candida fungi	100.0	5.8 ± 0.04*	10.0	3.3 ± 0.04
Lactobacillus spp.	90.0	3.8 ± 0.02*	100.0	6.2 ± 0.08
Bifidumbacterium spp.	11.7	2.4 ± 0.06*	13.3	4.0 ± 0.06
Bacteroides spp.	13.3*	2.6 ± 0.04*	43.3	5.2 ± 0.06
Peptostreptococcus spp.	28.3	5.2 ± 0.02	20.0	4.0 ± 0.04
Fusobacterium spp.	16.7	4.4 ± 0.06	13.3	4.2 ± 0.02
Prevotella spp.	10.0	2.8 ± 0.04*	40.0	5.0 ± 0.08
Veilonella spp.	16.7	5.4 ± 0.02*	10.0	4.2 ± 0.06

* — statistically significant difference between indicators in sick and healthy women ($p > 0.05$)

microflora and transient microorganisms. The dominant genus of the vaginal biotope is *Lactobacillus spp*. in association with obligately anaerobic and facultatively anaerobic types of microorganisms. Coagulase-negative staphylococci (*S. epidermidis*), *Corynebacterium spp., Streptococcus spp., Bacteroides spp., Prevotella spp., Peptostreptococcus spp., Fusobacterium spp., E. coli*, and other coliform bacteria were recorded less often. *Candida* fungi were determined with a low frequency (10.0%). Quantitative indicators of opportunistic microflora isolated from the vagina of healthy women did not reach the diagnostic level (< lg 4.0 CFU/mL). *Lactobacillus spp.* concentration was lg 6.2 CFU/mL.

Discussion

The data from the present research indicating *C. albicans* as the dominant species is consistent with previous studies [23, 24]. However, there is a growing tendency to increase the specific weight of *Candida non-albicans* in the general spectrum of fungal microflora in candidiasis vulvovaginitis. This is evidenced by the present study, which found the level of isolation of *Candida non-albicans* species to be 25%. Notably, species such as *C. glabrata* and *C. tropicalis* are quite common, with a detection frequency of 10–15% [25, 26].

Results of bacteriological examination of women with vulvovaginal candidiasis indicate negative changes in the composition of the vaginal microbiota. These changes include the formation of various variants of aerobic-anaerobic bacterial imbalance, a decrease in protective microflora, and a significant frequency of associative forms of bacterial-fungal contamination of the genital tract. The significant frequency of bacterial and fungal associations is problematic. Associative forms of vaginal dysbiosis differ from monomicrobial ones in greater aggressiveness, significant resistance to antibacterial drugs, and severe structural changes in the vaginal mucosa [27].

This study has several limitations. The study was designed as a pilot study, so sample size and funds were limited. A formal power calculation is usually not necessary for a pilot study [28]. The analysis of a pilot study can focus on descriptive statistics (e.g., means, standard deviations, and frequencies and percentages for categorical variables).

Additionally, the sample size was reduced due to the exclusion of women using hormonal contraception. In most epidemiological studies, the use of hormonal contraception is associated with an increased risk of vulvovaginal candidiasis [29].

The present study did not test the correlation between the types and quantities of microorganisms detected and the clinical characteristics of patients and the severity of vulvovaginal candidiasis. To establish the cause-and-effect relationship between vulvovaginal candidiasis, its chronicity and recurrence, a larger study is planned to examine the composition of the microbiota not only of the vagina but also of the intestine in women with vulvovaginal candidiasis and compare the strains of isolated fungi, as there is evidence that the digestive tract is a constant reservoir of fungi and a source of vaginal reinfection in vulvovaginal candidiasis [30].

Conclusions

1. The high prevalence of vulvovaginal candidiasis in women and the increased frequency of detection of *Candida non-albicans* species in the spectrum of isolated fungi confirm the importance of constant bacteriological monitoring to detect changes in the distribution of fungal species from *C. albicans* to *Candida non-albicans*.
2. In patients with candidiasis vulvovaginitis, there is a high frequency of fungal-bacterial associations (95%), with a significant presence of *Firmicutes* associations with pathogenic properties and *Proteobacteria* against the background of *Lactobacillus spp*. deficiency or absence.
3. Further studies are needed to determine the causes of relapses and chronicity of vulvovaginal candidiasis and to develop differentiated approaches to correcting the detected dysbiotic changes in the vagina.

Article information

Data availability statement: *Data supporting this study are included in the article.*
Ethics statement: *This study was approved by the local bioethics committee, which recognized that there was no need to sign an informed consent. The study was conducted in accordance with the Declaration of Helsinki. This study did not involve animal experiments.*
Author contributions: *IP — conceptualization; reviewing and editing; TL — conceptualization; writing: reviewing and editing; SK — setting up research software; AT— analysis of received data, writing: preparation of the initial project.*
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References

1. Nyirjesy P, Brookhart C, Lazenby G, et al. Vulvovaginal Candidiasis: A Review of the Evidence for the 2021 Centers for Disease Control and Prevention of Sexually Transmitted Infections Treatment Guidelines. Clin Infect Dis. 2022; 74(Suppl_2): S162–S168, doi: 10.1093/cid/ciab1057, indexed in Pubmed: 35416967.
2. Yano J, Sobel JD, Nyirjesy P, et al. Current patient perspectives of vulvovaginal candidiasis: incidence, symptoms, management and

post-treatment outcomes. BMC Womens Health. 2019; 19(1): 48, doi: 10.1186/s12905-019-0748-8, indexed in Pubmed: 30925872.
3. Rosati D, Bruno M, Jaeger M, et al. Recurrent Vulvovaginal Candidiasis: An Immunological Perspective. Microorganisms. 2020; 8(2), doi: 10.3390/microorganisms8020144, indexed in Pubmed: 31972980.
4. Cooke G, Watson C, Smith J, et al. Treatment for recurrent vulvovaginal candidiasis (thrush). Cochrane Database of Systematic Reviews. 2011, doi: 10.1002/14651858.cd009151.
5. Farhan MA, Moharram AM, Salah T, et al. Types of yeasts that cause vulvovaginal candidiasis in chronic users of corticosteroids. Med Mycol. 2019; 57(6): 681–687, doi: 10.1093/mmy/myy117, indexed in Pubmed: 30544194.
6. Gaziano R, Sabbatini S, Monari C. The Interplay between Vaginal Mucosa, Host Immunity and Resident Microbiota in Health and Disease: An Overview and Future Perspectives. Microorganisms. 2023; 11(5), doi: 10.3390/microorganisms11051211, indexed in Pubmed: 37317186.
7. Chen X, Lu Y, Chen T, et al. The Female Vaginal Microbiome in Health and Bacterial Vaginosis. Front Cell Infect Microbiol. 2021; 11: 631972, doi: 10.3389/fcimb.2021.631972, indexed in Pubmed: 33898328.
8. Willems HME, Ahmed SS, Liu J, et al. Vulvovaginal Candidiasis: A Current Understanding and Burning Questions. J Fungi (Basel). 2020; 6(1), doi: 10.3390/jof6010027, indexed in Pubmed: 32106438.
9. Donders GGG, Ravel J, Vitali B, et al. Role of Molecular Biology in Diagnosis and Characterization of Vulvo-Vaginitis in Clinical Practice. Gynecol Obstet Invest. 2017; 82(6): 607–616, doi: 10.1159/000478982, indexed in Pubmed: 29017160.
10. Aguirre-Quiñonero A, Castillo-Sedano IS, Calvo-Muro F, et al. Accuracy of the BD MAX™ vaginal panel in the diagnosis of infectious vaginitis. Eur J Clin Microbiol Infect Dis. 2019; 38(5): 877–882, doi: 10.1007/s10096-019-03480-8, indexed in Pubmed: 30685805.
11. Rodríguez-Cerdeira C, Gregorio MC, Molares-Vila A, et al. Biofilms and vulvovaginal candidiasis. Colloids Surf B Biointerfaces. 2019; 174: 110–125, doi: 10.1016/j.colsurfb.2018.11.011, indexed in Pubmed: 30447520.
12. Bertolini M, Ranjan A, Thompson A, et al. Candida albicans induces mucosal bacterial dysbiosis that promotes invasive infection. PLoS Pathog. 2019; 15(4): e1007717, doi: 10.1371/journal.ppat.1007717, indexed in Pubmed: 31009520.
13. d'Enfert C, Kaune AK, Alaban LR, et al. The impact of the Fungus-Host-Microbiota interplay upon Candida albicans infections: current knowledge and new perspectives. FEMS Microbiol Rev. 2021; 45(3), doi: 10.1093/femsre/fuaa060, indexed in Pubmed: 33232448.
14. Galocha M, Pais P, Cavalheiro M, et al. Divergent Approaches to Virulence in and : Two Sides of the Same Coin. Int J Mol Sci. 2019; 20(9), doi: 10.3390/ijms20092345, indexed in Pubmed: 31083555.
15. Malinovská Z, Čonková E, Váczi P. Biofilm Formation in Medically Important Species. J Fungi (Basel). 2023; 9(10), doi: 10.3390/jof9100955, indexed in Pubmed: 37888211.
16. Swidsinski A, Guschin A, Tang Q, et al. Vulvovaginal candidiasis: histologic lesions are primarily polymicrobial and invasive and do not contain biofilms. Am J Obstet Gynecol. 2019; 220(1): 91.e1–91.e8, doi: 10.1016/j.ajog.2018.10.023, indexed in Pubmed: 30595144.
17. Maza PK, Bonfim-Melo A, Padovan ACB, et al. : The Ability to Invade Epithelial Cells and Survive under Oxidative Stress Is Unlinked to Hyphal Length. Front Microbiol. 2017; 8: 1235, doi: 10.3389/fmicb.2017.01235, indexed in Pubmed: 28769876.
18. Chen Z, Jin J, Chen H, et al. The bacterial communities in vagina of different Candida species-associated vulvovaginal candidiasis. Microb Pathog. 2023; 177: 106037, doi: 10.1016/j.micpath.2023.106037, indexed in Pubmed: 36842517.
19. Tortelli BA, Lewis WG, Allsworth JE, et al. Associations between the vaginal microbiome and Candida colonization in women of reproductive age. Am J Obstet Gynecol. 2020; 222(5): 471.e1–471.e9, doi: 10.1016/j.ajog.2019.10.008, indexed in Pubmed: 31654610.
20. Zheng N, Guo R, Wang J, et al. Contribution of to Vaginal Health and Diseases: A Systematic Review. Front Cell Infect Microbiol. 2021; 11: 792787, doi: 10.3389/fcimb.2021.792787, indexed in Pubmed: 34881196.
21. Ouarabi L, Drider D, Taminiau B, et al. Vaginal Microbiota: Age Dynamic and Ethnic Particularities of Algerian Women. Microb Ecol. 2021; 82(4): 1020–1029, doi: 10.1007/s00248-020-01606-6, indexed in Pubmed: 32975677.
22. Saraf VS, Sheikh SA, Ahmad A, et al. Vaginal microbiome: normalcy vs dysbiosis. Arch Microbiol. 2021; 203(7): 3793–3802, doi: 10.1007/s00203-021-02414-3, indexed in Pubmed: 34120200.
23. Seyoum E, Bitew A, Mihret A. Distribution of Candida albicans and non-albicans Candida species isolated in different clinical samples and their in vitro antifungal suscetibity profile in Ethiopia. BMC Infect Dis. 2020; 20(1): 231, doi: 10.1186/s12879-020-4883-5, indexed in Pubmed: 32188422.
24. Zuo XS, Liu Y, Cai X, et al. Association of different Candida species with catheter-related candidemia, and the potential antifungal treatments against their adhesion properties and biofilm-forming capabilities. J Clin Lab Anal. 2021; 35(4): e23738, doi: 10.1002/jcla.23738, indexed in Pubmed: 33608902.
25. Surain P, Aggarwal N. Candida, a human pathogen and major types of candidiasis . Int J Pharm Sci & Res. 2019; 11(1): 41–67.
26. Sun Z, Ge X, Qiu Bo, et al. Vulvovaginal candidiasis and vaginal microflora interaction: Microflora changes and probiotic therapy. Front Cell Infect Microbiol. 2023; 13: 1123026, doi: 10.3389/fcimb.2023.1123026, indexed in Pubmed: 36816582.
27. Ceccarani C, Foschi C, Parolin C, et al. Diversity of vaginal microbiome and metabolome during genital infections. Sci Rep. 2019; 9(1): 14095, doi: 10.1038/s41598-019-50410-x, indexed in Pubmed: 31575935.
28. Kunselman AR. A brief overview of pilot studies and their sample size justification. Fertil Steril. 2024; 121(6): 899–901, doi: 10.1016/j.fertnstert.2024.01.040, indexed in Pubmed: 38331310.
29. Rabi S, Solafa A, Reem A. Prevalence of vulvovaginal Candidiasis and its association with Contraceptives. Archivos Venezolanos de Farmacología y Terapéutica. 2021; 20(4): 373–376, doi: 10.5281/zenodo.5224567.
30. Mesquida A, Machado M, Dávila-Cherres L, et al. The Gastrointestinal Tract Is Pinpointed as a Reservoir of , , and Genotypes Found in Blood and Intra-Abdominal Samples. J Fungi (Basel). 2023; 9(7), doi: 10.3390/jof9070732, indexed in Pubmed: 37504721.