Vaginal candidiasis among women of reproductive age in Dnipro city (Ukraine): Species spectrum of Candida and their susceptibility to antymycotics

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Candidiasis is one of the most common pathological processes, especially among women of reproductive age, especially pregnant. It can be expressed as Candida carriage and as an active form of infection. Although candidiasis is caused by opportunistic microorganisms, its effects can be quite significant, so it is necessary to treat it with the use of antymycotic drugs. In view of the spread among these microorganisms of resistance to antimicrobial drugs, the determining factor for the success of therapeutic measures, it is important to make a preliminary assessment of the susceptibility to antymycotics of strains isolated from persons with Candida carriage or active candidiasis. The aim of the research was to study the species range of fungi of the genus Candida isolated from the reproductive tract in women with dysbiosis and to study their susceptibility to antymycotics. Total of 227 samples of biological material from women aged 16 to 56 years have been investigated. Microscopic and cultural methods of detecting fungi in the primary material were used for research. For the identification of species of Candida fungi, a polymerase chain reaction method in real-time was used. It was determined that the frequency of detection of Candida fungi in the composition of vaginal microbiome during inflammatory pathological processes was 76.7%. It was shown that the incidence of Candida carriage – 51.7% – slightly exceeded the frequency of detection of the active form of infection – 48.3%. The species spectrum of pathogens included: C. albicans (57.1%), C. krusei (12.6%), C. glabrata (19.5%) and Candida spp. (16.1%). In the case of active form of infection, C. albicans was prevalent – 64 (76.2%) cases. In the case of Candida carriage, other species were prevalent – 58 (64.4%) cases. The most effective drug against all isolates was amphotericin B – more than 87% of isolates were susceptible. The least effective drug was nystatin: less than 60% of isolates were susceptible. There is a rather high level of resistance to certain antymycotic drugs among the Candida species, which requires a prior preliminary study of the susceptibility of isolated strains to antymycotics for the purpose of choosing a rational and effective treatment scheme.

Keywords: Candida; candidiasis; carriage; species spectrum; incidence rates; antymycotic susceptibility.

Introduction

Vulvovaginal candidiasis is one of the most common pathological processes in the structure of inflammatory diseases of female genital organs. It is known that among the infections of the lower reproductive system up to 50% of cases of lesions are caused by Candida (Richter et al., 2005; Rathod et al., 2012). As described by Rathod & Buffler (2014), 75% of women will experience an episode of vulvovaginal candidiasis in their lifetimes, 50% of whom will experience at least a second episode, and 5–10% of all women will experience recurrent vulvovaginal candidiasis (≥4 episodes/year) (Rathod et al., 2012). In recent years there has been a tendency towards an increase of the number of vaginal candidiasis (≥4 episodes/year) (Rathod et al., 2012). In recent years there has been a tendency towards an increase of the number of vaginal candidiasis (≥4 episodes/year) (Rathod et al., 2012).

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Candidiasis is one of the most common pathological processes, especially among women of reproductive age, especially pregnant. It can be expressed as Candida carriage and as an active form of infection. Although candidiasis is caused by opportunistic microorganisms, its effects can be quite significant, so it is necessary to treat it with the use of antymycotic drugs. In view of the spread among these microorganisms of resistance to antimicrobial drugs, the determining factor for the success of therapeutic measures, it is important to make a preliminary assessment of the susceptibility to antymycotics of strains isolated from persons with Candida carriage or active candidiasis. The aim of the research was to study the species range of fungi of the genus Candida isolated from the reproductive tract in women with dysbiosis and to study their susceptibility to antymycotics. Total of 227 samples of biological material from women aged 16 to 56 years have been investigated. Microscopic and cultural methods of detecting fungi in the primary material were used for research. For the identification of species of Candida fungi, a polymerase chain reaction method in real-time was used. It was determined that the frequency of detection of Candida fungi in the composition of vaginal microbiome during inflammatory pathological processes was 76.7%. It was shown that the incidence of Candida carriage – 51.7% – slightly exceeded the frequency of detection of the active form of infection – 48.3%. The species spectrum of pathogens included: C. albicans (57.1%), C. krusei (12.6%), C. glabrata (19.5%) and Candida spp. (16.1%). In the case of active form of infection, C. albicans was prevalent – 64 (76.2%) cases. In the case of Candida carriage, other species were prevalent – 58 (64.4%) cases. The most effective drug against all isolates was amphotericin B – more than 87% of isolates were susceptible. The least effective drug was nystatin: less than 60% of isolates were susceptible. There is a rather high level of resistance to certain antymycotic drugs among the Candida species, which requires a prior preliminary study of the susceptibility of isolated strains to antymycotics for the purpose of choosing a rational and effective treatment scheme.

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of the enzyme 1,3-β glucan synthase (Morris & Villmann, 2006). They can influence a wide spectrum of Candida yeasts, and microorganisms of this genus are not broadly resistant to them. Therapeutic concentration of representatives of group are smaller compared to other antifungal drugs (Perlin, 2014; Boikov et al., 2017). But at the same time, echi-nocandins have some significant disadvantages: they were found to be embryotoxic in animal studies (category C) thus should be avoided if possible in pregnancy, need dose adjustment in cases of liver disease and have poor ocular penetration in fungal endophthalmitis (Long, 2003).

Among the Candida yeasts, there is a rather high level of resistance to certain antifungal drugs, in particular to fluconazole, which requires a preliminary study of the sensitivity of isolated strains to antifungotics for the development of the rational and effective treatment regimen (Deorakkar et al., 2014; Liu et al., 2014; Monroy-Pérez et al., 2016; Rezaei-Matehkolaei et al., 2016; Zazhanskyi et al., 2019).

In view of this, the objective of the research was studying the species range of fungi of the genus Candida isolated from the reproductive tract during dysbiosis and to study their susceptibility to antifungotics.

Materials and methods

The research was conducted in the laboratory of PE “Center of laboratory medicine “VIS-MEDIC” (Dnipro, Ukraine). The samples of biological material (secretion of vaginal mucosa) was obtained from women with pathological processes of the genital tract, the etiology of which had to be established. Materials were obtained in 2018 from women aged 16 to 56 years (n = 227). The specimens were taken in accordance to the standards of biomedical ethics.

The study of the material taken from the mucous membrane included microscopic analysis and determination of the Candida species by the polymerase chain reaction (PCR) method. For all isolated strains of Candida the definition of susceptibility to antifungotics was also carried out. Preparations for microscopy were prepared by swirling of a tampon with material on the surface of an object glass. Three preparations were made:

– unstained – for seeing the presence of pseudomycocytes, chlamydomyces and blastospores (microscopy by ×200);
– stained by Giemsa method: yeast-like fungal cells are pinkish-purple, chromatin is red, and voluminous granules are dark violet. Separate cells (their shape, size, and status) were studied (microscopy by ×900);
– stained by Gram method – to assess the presence of other microorganisms for the diagnosis of dysbiosis according to the criteria of Nugent score. In smears stained by Gram method, the dark-purple or un-even colouration of cells of yeast morphology was determined (microscopy by ×900).

Positive results of microscopy are the detection of round and oval cells, many of which are found in the process of budding. Besides the cellular elements, the presence of pseudomyceum was determined (the chain of cells were not separated from each other), often with bloating due to budding. The location of yeast cells by clusters in the form of pockets along the pseudomyceum in the places where it joins is typical.

Detection of a large number of cells budding on the pseudomyceum is an important diagnostic symptom, indicating precisely the infectious process. When acute lesions on the vaginal mucosa take place, there are accumulation of large numbers of budded blastospores and pseudomyceum. The presence of single budding cells indicates candidiasis. Candidiasis was also determined in the absence of pseudomyceum, but by the presence of blastospores only (Mendling et al., 2015).

The identification of fungi was carried out using the RealBest DNA Candida albicans/Fungi and RealBest DNA Candida krusei/Candida glabrata test kits (JSC Vector-Best, Russia) by the methods of molecular diagnostics using a polymerase chain reaction (PCR) in real-time. This was done by iCycler IQ™ 5 (Bio-Rad, USA) for performing PCR with real-time results detection in accordance with the manufacturer’s instructions. Qualitative and quantitative indexes of the presence of Candida species in the samples were obtained. The criteria of candidiasis was the detection of fungi in more than 10³ CFU/mL.

Material for identification was obtained by growing isolated colonies of fungi on Saburo medium (HiMedia, India). The surface of colonies (smooth, rough, wrinkled), pigment, consistency and the presence of chlamydospores and pseudomyceum were taken into account. The colonies which were later tested for the species C. albicans, on the Saburo medium had a colour from whitish-cream to snow-white, and were smooth and shiny. As non-albicans Candida species pre-determined rough colonies, this material was tested for belonging to the species C. krusei and C. glabrata. Cultures with a typical morphotype of Candida that did not give a positive response when determining the species by PCR were marked as Candida spp.

After identifying the isolates and determining their significance as an etiological factor of vaginal dysbiosis, the study of the susceptibility of the culture to antifungotics was carried out. The susceptibility of the isolated strains was determined by a disk diffusion method (Method..., 2009) using standard disks with amphotericin B, ketoconazole, itraconazole, clotrimazole, nystatin and fluconazole (HiMedia, India).

Results

A total of 227 samples of biological material from women with reproductive tract pathology and complaints of itching, burning, purulent edema in the perineum were investigated. At the first stage, microscopy of the material was performed. According to the results of microscopic analysis, the presence of yeast-like fungi Candida in 174 samples of the material was determined, which was 76.7%. In the study of microscopic dyes stained by the Gram method, the presence of other microorganisms was established, which prevents the identification of fungi as a monoculture, which determines the dysbiosis of the female vagina. The predominance of Candida presence was determined for 38 (21.8%) samples of biological material.

Microscopy of non-stained preparations allowed visualization of the presence of pseudomyceum, chlamydomyces and blastospores. The presence of these objects allowed us to pre-determine the infectious process in 76 (43.7%) women. For other specimens, these signs were not marked, which made it possible to make a preliminary conclusion about carriage of Candida in 98 (56.3%) cases. On the Gram-stained preparations, vaginal epithelium cells and consequent microraflora were present. In 115 (66.1%) cases a large number of epithelial cells, surrounded by small gram negative microraflora (“key cells”) was noted, which allowed us to confirm dysbiosis. By the analysis of the obtained data it was established that the incidence of Candida carriage (56.3%) exceeds the frequency of detection of active Candida infection (43.7%). Such distribution is probably due to the fact that Candida infection, even in the absence of treatment, often occurs asymptotically and women do not seek medical assistance, which leads to chronic infection.

Microscopic diagnostics in questionable and in doubtful cases should be confirmed by laboratory isolation of pure culture or by means of molecular genetic analysis. The real-time PCR method is used to establish species diversity. All the cultures grown on Saburo medium were identified as belonging to C. albicans, C. glabrata, C. krusei or Candida spp. (Fig. 1).

Fig. 1. Species composition of Candida fungi, isolated from vagina (n = 174)
It was found that *C. albicans* was the predominant species, whose frequency of detection was more than half of cases – 90 (51.7%). Other *Candida* species were *C. glabrata* – 34 (19.5%) and *C. krusei* – 22 (12.6%) cultures. 28 other cultures were identified as *Candida* spp. (16.1%).

The quantitative studies allowed us to confirm the infectious process (number of fungal cells in the vaginal secretion exceeded 10^9 CFU/mL) in all cases of positive microscopic analysis, as well as in 8 cases, where microscopy revealed carriage of fungi. Consequently, the final frequency of *Candida* carriage was 51.7%, and the frequency of infection was 48.3%. In this case, *C. albicans* prevailed in infectious lesions, which were detected in excess of 10^4 CFU/mL: 64 (76.2%) cases (Fig. 2). Other *Candida* species were found predominantly in carriage – 58 (64.4%) cases of 90. In the infectious process, *C. glabrata*, *C. krusei* and *Candida* spp. were determined predominantly in the amount of 10^2–10^6 CFU/mL. Excess in the index of more than 10^8 CFU/mL. For these microorganisms was noted in exceptional cases.

![Fig. 2. The number of cases of isolation of various Candida species in the carriage and infectious process: white < 10^3 CFU/mL; grey 10^3–10^6 CFU/mL; black > 10^6 CFU/mL](image)

For all *Candida* cultures, the amount of which in the material exceeded 10^4 CFU/mL, an antibiotic susceptibility study was performed, which is necessary for further development of the scheme of rational antibiotic therapy. The study of susceptibility to antifungal drugs has made it possible to determine that resistance is not typical for the surveyed species (Fig. 3). The exception was *C. krusei*, which has natural resistance to fluconazole.

It is evident from data the presented in Figure 3 that the percentage of susceptible isolates with the exception of nystatin is quite large (more than 60–70%) and is a good predictor for the treatment of vulvovaginal candidiasis. The exception is the only drug – nystatin, for which the number of susceptible strains of fungi does not reach 60%. The least number of susceptible isolates were among *Candida* spp. – 40% and *C. krusei* – 50%. Consequently, the use of this drug for the treatment of women will not be effective and other options should be considered. All isolates were the most susceptible to amphotericin B: more than 87%, but this antifungal is not used in gynecology. For other strains, virtually all drugs have been effective, which allows a wide reserve among the drugs of choice. For *C. krusei*, the drug of choice is itraconazole, to which 98.5% of isolates were sensitive.

**Discussion**

Yeast-like fungi *Candida* are opportunistic microorganisms that cause diseases in carriers in the event of damage to their immune system (Sobel, 2016). Vulvovaginal candidiasis is important in obstetric and gynecological practice: more than 70% of women at least once in their life suffer from this illness (Hedayati et al., 2015). It is known that most common pathogens which provoke candidiasis are *C. albicans*, *C. krusei* and *C. glabrata*. But we found many other species that we described as *Candida* spp. It should be noted that in the latter group it is possible to detect a sufficiently large number of *Candida* species, in particular *C. tropicalis*, *C. parapsilosis*, *C. famata*, *C. lusitaniae*, *C. kefyr*, *C. guilliermondii*. However, in most cases, their presence does not exceed 3–5% in the general structure of pathogens causing vulvovaginal candidiasis (Fornari et al., 2012; Bitew & Abebaw, 2018). Indeed, vulvovaginal candidiasis is predominantly caused by *C. albicans*, and other *Candida* species occur much less frequently. According to various data (Bradford & Ravel, 2017; Bitew & Abebaw, 2018), the detection rate of *C. albicans* is at least 35–45%, which is confirmed by the results obtained by us. Accordingly, the total frequency of detection of non-*Candida* *Candida* species is more than 50% (Liu et al., 2014; Khan et al., 2018). Separately, species such as *C. glabrata* and *C. krusei* are quite common. The frequency of their detection is 10–20% (Hedayati et al., 2015; Wang et al., 2016; Bradford & Ravel, 2017). Most other species are often combined in *Candida* spp., whose share is formed by species, the frequency of which does not exceed 2–3% (Gamarna et al., 2014; Wang et al., 2016).

The disease occurs in women of all age groups, often during pregnancy. However, the urgency of the problem is due not only to its significant prevalence (Consello et al., 2003). Vulvovaginal candidiasis is often combined with other disorders of the microbiocenosis of the vagina and is associated with the threat of abortion, involuntary miscarriage, premature delivery, chorioamnionitis, untimely discharge of amniotic fluid, the birth of children with low body weight, chronic hypoxia or signs of intrauterine infection, the onset of wound infection of the birth canal, endometritis in the postpartum period. Healthy pregnant women and pregnant women with vaginal vaginal candidiasis can be a source of both intrauterine and postnatal infections in newborns (Bitew & Abebaw, 2018).

The use of antifungal drugs is a mandatory step in the treatment of vulvovaginal candidiasis. Under conditions of successful prescription of drugs it is possible to remove yeast from the vaginal biotope. However, in case of non-compliance with the prescribed course of treatment or in case of unsuccessful use of the drug, carriage is formed, which is much more difficult to get rid of than active infection. The best therapy to use is local, but the success of therapy will be guaranteed only if the sensitivity of the selected culture to the available antifungal drugs is determined (Gamarna et al., 2014; Fornari et al., 2016). Nowadays there is a tendency towards the spread of candidiasis, and therefore the problem of its effective treatment needs special attention. Taking into account the spread of resistance to medicines among the *Candida* strains, the treat-
ment of candidiasis is becoming a difficult task, which cannot be based solely on canonical ideas about the mechanism of action of drugs (Deorukhkar et al., 2014; Liu et al., 2014). There is a need to study the level of sensitivity to the antifungal susceptibility pattern of vaginal iso-
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