SPECIES DISTRIBUTION AND ANTIFUNGAL SUSCEPTIBILITY OF VAGINAL *CANDIDA* ISOLATES

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ABSTRACT

Introduction: Vulvovaginal candidiasis (VVC) is the most common *Candida* infection in females of reproductive age. Data concerning the species identification and antifungal susceptibility of vaginal *Candida* isolates are of great importance for the infection management.

Aim: The aim of the present study was to determine the species distribution and antifungal susceptibility of *Candida* isolates obtained from vaginal samples of women with VVC.

Material and methods: A total of 125 vaginal *Candida* isolates were collected and tested. The definitive species identification was performed by VITEC2 Compact (*Bio Merieux, France*) using YST ID cards. The minimum inhibitory concentrations (MICs) of nine antifungal agents were determined by the commercial system Micronaut-AM (*Merlin Diagnostika GmbH, Germany*).

Results: Overall, 8 *Candida* species were established among the vaginal yeast isolates. The most common was *C. albicans* (77.6%), followed by *C. glabrata* (12%), *C. krusei* (4%), *C. kefyr* (2.4%), *C. spherica* (1.6%), *C. lusitaniae*, *C. utilis*, and *C. sake* (each one 0.8%). All *C. albicans* and 20 non-*albicans Candida* (NAC) were susceptible to nine antifungal agents. In the group of NAC, 8 isolates were resistant to fluconazole – 5 *C. krusei* with intrinsic resistance, 2 *C. spherica*, and 1 *C. sake*. The fluconazole MICs of *C. spherica* and *C. sake* were 32 - 128 μg/mL, and 16 μg/mL, respectively.

Conclusions: *C. albicans* was the main causative agent of VVC. Among NAC, *C. glabrata* was the predominant species. In general, vaginal *C. albicans* and non-*albicans Candida* were susceptible to azoles as well as echinocandins, amphotericin B and 5-fluorocytosine. Of particular interest was the detection of rare non-*albicans Candida* isolates with acquired resistance to azoles.

Key words: vulvovaginal candidiasis, antifungal agents, *Candida* species

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INTRODUCTION

Candida albicans and some other *Candida* species are common colonizers of the female genital system. The colonization is a predisposing factor for development of vulvovaginal candidiasis (VVC). VVC is a widespread disease in otherwise healthy women, predominantly during the reproductive years. The potential risk factors for VVC include pregnancy, use of antibiotics, cytostatics, corticosteroids, oral contraceptives, and the presence of underlying diseases such as diabetes, AIDS, etc. (1- 8). Although not life-threatening illness, its global rate and negative effect on life quality warrant a thorough study of the problem (9).

In the majority of cases, the causative agent of VVC is *C. albicans* but a share of non-*albicans Candida* (NAC) species is also increased (5, 10, 11, 12). The treatment of VVC involves application of topical or oral azoles but as is known, some NAC possess natural or acquired resistance to azole antifungals. Because of that, the precise species identification and antifungal susceptibility testing are of great importance for the proper therapy.

The aim of the present study was to determine the species distribution and antifungal susceptibility of *Candida* isolates obtained from vaginal samples of women with VVC.

MATERIALS AND METHODS

Yeast isolates

The study covers 125 vaginal *Candida* isolates collected from hospitalized female patients and outpatients with vaginal candidiasis in Pleven, Bulgaria, for the period 2018 – 2022. All yeasts were isolated in the routine laboratory work. The pure cultures of yeast strains were stored in skim-milk

media at -20°C before testing *in vitro*. Since personal data were not used, this study was not under ethics approval.

Species identification

The identification of Candida species was carried out by microscopic, cultural and biochemical methods. The preliminary identification was based on the growth with different color on CHROMagar *Candida* (*BD, UK*), and the microscopic characteristics on a cornmeal agar (*BD, UK*). The CHROMagar *Candida* is used for differentiating *Candida albicans* from non-*albicans Candida*. On the cornmeal agar, the specific chlamydospores of *C. albicans* were observed. The germ tube test which is not routinely done in laboratories was used for rapid detection of *C. dubliniensis* and *C. albicans*. Both yeasts form germ tubes after inoculation in sera and incubation at 37º C for 2-4 hours. The definitive species determination was done by the automatic system for biochemical identification VITEK 2 Compact (*Bio Merieux, France*) using YST ID cards. The results were generated automatically by the apparatus database and these with a confidence level more than 89% probability were accepted as correct.

Antifungal susceptibility testing

The susceptibility to nine antifungal agents was established by detection of minimum inhibitory concentrations (MICs) using the commercial system Micronaut-AM (*Merlin Diagnostika GmbH, Germany*). The procedure was performed according to the manufacturer's instruction. The antifungals tested were amphotericin B (APH), 5-fluorocytosine (FCY), fluconazole (FCA), voriconazole (VOR), posaconazole (POS), itraconazole (ITR), micafungin (MIF), anidulafungin (ANF), and caspofungin (CAS). The results were presented in µg/mL and interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, 2023 (13). The MIC breakpoints in µg/mL were as follows: for *C. albicans, C. glabrata*, and *C. krusei* – APH susceptibility (S) ≤ 1 and resistance (R) > 1; for *C. albicans* – FCA S ≤ 2 and resistance R > 4, ITR and POS S ≤ 0.06 and R > 0.06, VOR S ≤ 0.06 and R > 0.25, ANF S ≤ 0.03 and R > 0.03, MIF S ≤ 0.016 and R > 0.016; for *C. glabrata* and *C. krusei* – ANF S ≤ 0.06 and R > 0.06; for *C. glabrata* – FCA S ≤ 0,001 and R > 16, MIF S ≤ 0.03 and R > 0.03. Because no EUCAST breakpoints for CAS, isolates which are ANF-S and MIF-S have to be interpreted as CAS-S. For interpretation of FCY, the manufacturer's instruction was used: S ≤ 4 µg/mL, R > 32 µg/mL and intermediate (I) 8-32 µg/mL.

RESULTS

The 125 tested vaginal *Candida* isolates belonged to a total of 8 species (**Figure 1**). The most frequently identified was *C. albicans* (n=97; 77.6%), followed by *C. glabrata* (n=15; 12%) and *C. krusei* (n=5; 4%). The remaining species – *C. kefyr*, *C. spherica*, *C. lusitaniae*, *C. utilis*, and *C. sake* were detected in single cases.

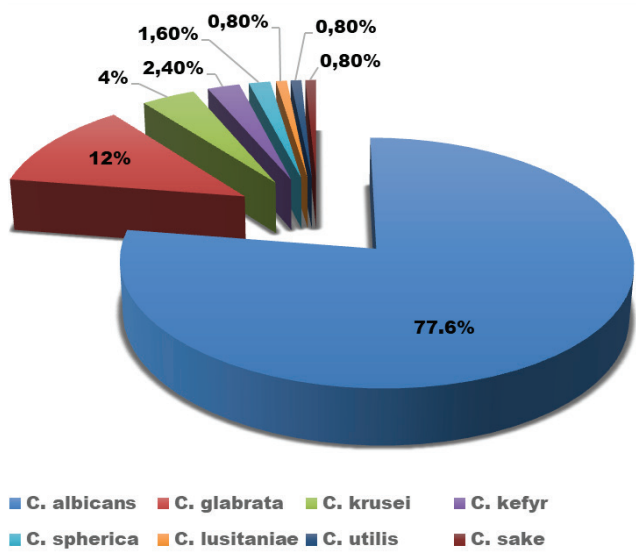

Figure 1 Species distribution of 125 vaginal *Candida* isolates

The results of antifungal susceptibility are shown in Table 1. They reveal high susceptibility of all *C. albicans* isolates to the nine antifungal agents. The MICs of fluconazole ranged from 0.25 - 2 µg/mL and the calculated MIC_{50} and MIC_{90} were 0.5 and 1 µg/mL, respectively. The fifteen *C. glabrata* strains were also susceptible to all antifungals with fluconazole MICs between <0.002 and 0.5 µg/mL.

Overall, non-albicans Candida kept their antifungal susceptibility with the exception of the naturally resistant to fluconazole species *C. krusei* (fluconazole MICs: 32 - 128 µg/mL). *C. spherica* and *C. sake* showed a high level of resistance to fluconazole with MICs: 32 - 128 µg/mL and MIC - 16 µg/mL, respectively. These isolates also expressed resistance to other azoles (voriconazole, posaconazole, and itraconazole) with

Table 1. Susceptibility of 125 vaginal *Candida* isolates to nine antifungal agents

Candida species	Antifungal agents (*MIC ranges in µg/mL)*								
	FCA	**ITR**	**POS**	**VOR**	**ANF**	**CAS**	**MIF**	**FCY**	**APH**
***C. albicans* (n=97)**	0.25-2	<0.031-0.125	<0.0078-0.0625	<0.0078-0.0625	<0.002-0.031	<0.002-0.0625	<0.002-0.015	0.125-1	0.0625-1
***C. glabrata* (n=15)**	<0.002-0.5	0.125-1	<0.0078-0.25	0.031-0.25	<0.002-0.0625	<0.13-0.031	<0.002-0.031	<0.0625-0.25	0.031-1
***C. krusei* (n=5)**	32-128	1-4	0.125-0.5	0.125-1	0.031-0.125	0.031-0.125	0.031-0.125	1-4	0.125-1
***C. kefyr* (n=3)**	0.25-0.5	<0.031-0.0625	<0.0078	<0.0078	0.0625-0.25	0.0625-0.125	0.0625-0.125	<0.0625-0.125	0.5-1
***C. spherica* (n=2)**	32-128	4	8	4-8	0.25-0.5	0.5	0.125-0.25	0.0625-0.125	1-2
***C. lusitaniae* (n=1)**	0.5	<0.031	<0.0078	<0.0078	0.0625	0.0625	0.0625	4	0.5
***C. utilis* (n=1)**	0.5	0.0625	0.0625	0.0625	0.0625	0.125	0.031	0.5	1
***C. sake* (n=1)**	16	1	2	1	0.0625	0.031	0.031	2	1

Abbreviation: MIC - minimum inhibitory concentration, FCA - fluconazole, ITR - itraconazole, POS - posaconazole, VOR - voriconazole, ANF - anidulafungin, CAS - caspofungin, MIF - micafungin, FCY - 5-fluorocytosine, and APH - amphotericin B.

MICs 4 – 8 µg/mL for *C. spherica*, and 1 - 2 µg/mL for *C. sake*.

All *C. albicans* and non-*albicans* species were susceptible to amphotericin B (MICs: 0.031 - 1 µg/mL), 5-fluorocytosine (MICs: 0.0625 - 4 µg/mL), and the MICs of echinocandins were within susceptible breakpoints.

DISSCUSION

Herein we presented information concerning the species identification and antifungal susceptibility of *Candida* spp. isolated from women with vulvovaginal candidiasis. Our results are in line with the concept of the global prevalence of *C. albicans* as a cause of VVC. It is generally accepted that *C. albicans* is responsible for 70 to 90% of the fungal vulvovaginitis (14).

Some publications pay attention on the molecular analyses to distinguish *C. albicans* from its closely related species *C. dubliniensis* and *C. africana*. Using the *hwp1* gene amplification, Klesiewicz et al. (15) detected 94.17% *C. albicans* and 5.22% *C. dubliniensis* among vaginal yeasts, phenotypic identified as *C. albicans*. The same authors revealed 97.85% compliance between the VITEK2 system and the molecular assay, and correct identification of all *C. albicans* sensu stricto with the VITEK2. In our study, *C.albicans* strains were also identified by the VITEK2 Compact with excellent and very good confidence levels with more than 93% probability.

A report of genital tract candidiasis in Poland showed *C. albicans* in 78.3% of the cases (16). These data are very close to our findings. Similar rates of *C. albicans* vulvovaginal candidiasis were published in Iran, Taiwan and Australia – 67%, 67.3% and 73%, respectively (17, 18, 7). Holland et al. (5) detected *C. albicans* in 89% of a total of 1221 vaginal isolates, and others published an even higher rate – 92.3% (19). *C. albicans* was also found as a dominant species of VVC in China and it was confirmed in 84.71% of overall 543 vaginal isolates (20). Hedayati et al. (10) determined three main species – *C. albicans* (42.5%), *C. glabrata* (21.9%) and *C. dubliniensis* (16.4%) among 66 yeasts obtained from females with VVC. In contrast, *C. glabrata* (57.4%) and *C. albicans* (25.9%) were detected as the primary and secondary species among pregnant women in Ghana (12).

In our study, *C. glabrata* was the second leading causal pathogen of VVC. This observation is consistent

with many other publications (1, 5, 7, 10, 16, 17, 20). However, some researchers presented *C. tropicalis* (11) or *C. krusei* (8) as the second most important species in this target group. The clinical importance of non-*albicans Candida* is based on the presence of species such as *C. krusei* and *C. glabrata* which respond poorly to azole antifungal agents.

According to the current requirements of EUCAST, the antifungal susceptibility testing of yeasts has to be performed by determining MIC values using microdilution in a liquid culture medium RPMI 1640 (13), but it is a difficult to perform and time-consuming method. Automatic systems (e.g. VITEK2 Compact, *Bio Merieux, France*) or commercial manual kits (e.g. Fungitest, *BioRad, France* or Micronaut-AM, *Merlin Diagnostika GmbH, Germany*) can be applied, but these are expensive and rarely used in diagnostic microbiology labs.

Many studies have revealed that *C. albicans* strains obtained from the female genital tract are usually susceptible to antifungal agents. Our results confirmed these finding and showed that all vaginal *C. albicans* were susceptible to azoles and other antifungals. Data on 100% sensitivity to amphotericin B, 5-fluorocytosine and voriconazole in *C. albicans*, isolated from pregnant women, were reported in Bulgaria (21). In a recent publication, among 307 vaginal isolates of *C. albicans* no one was resistant to fluconazol , while susceptibility to ketoconazole and miconazole was 99.35% and 96.74%, respectively (15). On the other hand, Mohammmadi et al. (17) found for genital *C. albicans* strains fluconazole and itraconazole resistance rates of 23.4% (MIC ≥ 64 μg/mL) and 10.6% (MIC ≥ 1 μg/mL), whereas susceptibility to amphotericin B and voriconazole was 100%. Other studies of this species showed fluconazole resistance of 53.7%, and 14.7% (8, 18).

Published data on antifungal susceptibility of NAC strains recovered from VVC varies widely. Among NAC isolates from pregnant women, Waikhom et al. (12) observed 17.5% susceptibility and 47.5% resistance to fluconazole. Fluconazole resistance of vaginal *C. glabrata* species varied from 7.7% to 38.7% (17, 12). According to some researchers (5), only 7 from 29 *C. glabrata* strains were susceptible to fluconazole with MICs: 0.25- 0.5 mg/L, but other reported opposite data and 100% susceptibility (8).

Our findings demonstrated a good sensitivity of almost all NAC with the exception of *C. krusei*, which is naturally resistant to fluconazole, and single isolates of *C. spherica* and *C. sake*, which expressed a high level of resistance to all tested azoles. In addition, vaginal *C. albicans* and non-*albicans* species were susceptible to amphotericin B, 5-fluorocytosine and echinocandins. However, these agents are not recommended for treatment of VVC (9).

CONCLUSION

The knowledge of species composition, and antifungal susceptibility patterns of vaginal *Candida* are important for management of VVC as some *Candida* isolates can fail first-line antifungal therapy.

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