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Azole Antifungal Resistance in *Candida albicans* and *Candida glabrata* Isolated from Vulvovaginal Candidiasis Patients

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Abstract

Background: Vulvovaginal candidiasis (VVC) is the most frequent fungal disorder in healthy and normal women.
Objectives: The aim of this study was to evaluate the in vitro antifungal susceptibility of clinical isolates *Candida albicans* and *Candida glabrata*, the two most common *candida* species in Iranian patients with VVC.
Methods: One hundred and eight clinical isolates of *candida*, including; *C. albicans* (n = 77) and *C. glabrata*: (n = 31) were isolated from the 108 patients with VVC. The in vitro activity of caspofungin (CAS), amphotericin B (AMB), voriconazole (VRC), itraconazole (ITC), fluconazole (FLC), and nystatin (NYS) were determined according to the CLSI M27-A3 and CLSI M27-S4.
Results: Our results were shown 8 (25.8 %) and 6 (7.8 %) *C. glabrata* and *C. albicans* isolates resistance to FLU, respectively. Furthermore, resistance to VRC and ITC were observed in 8.4%, and 3.7% of all isolates, and six isolates (5.6%) had intermediate MIC to CAS.
Conclusions: We reported 8 (25.8 %) and 6 (7.8 %) *C. glabrata* and *C. albicans* isolates resistance to FLU, respectively. Furthermore, resistance to VRC and ITC were observed in 8.4% and 3.7% of all isolates, respectively.

Keywords: Antifungal Susceptibility, *Candida albicans*, *Candida glabrata*, Vulvovaginal Candidiasis

1. Background

In healthy individuals, the capacity of *Candida*, as a commensal, to produce either systemic or superficial infections is depended on the host immune system and different risk factors (1). Bacterial vaginosis, vulvovaginal candidiasis (VVC), and trichomoniasis are the most frequent types of vaginitis, among which VVC is the second most frequent form after bacterial vaginosis (2). This infection is one of the most common gynecological disorder among women who are in their childbearing age. About 75% of sexually active women have at least once experienced symptomatic VVC, which is usually presented as itching, soreness, burning, and abnormal curd-like vaginal discharge (1). According to several studies, non-albicans *candida* species are increasing as causative agents of RVVS in the world. (3). *Candida albicans*, followed by *C. glabrata*, are the most frequent causative agents of VVC, and they are also a part of the indigenous flora of the gastrointestinal tract and skin (1-5). Furthermore, in local and global antifungal surveillance studies, a growing prevalence of fungal resistance has been reported (1-4). The growing incidence of VVC, RVVC, and drug resistance led to an essential public health issue, and it is a challenge for the treatment strategy of clinicians (3). Although there are many effective antifungal drugs, both oral and topical use for treating VVC decreased susceptibility of some vaginal yeast isolates to some antifungal agents (4). One of the main points in guiding appropriate therapy selection for mycoses is the antifungal susceptibility patterns of pathogenic fungi (2-4, 6, 7). An estimation of antifungal effectiveness, monitoring of the development of drug resistance, ensuring beneficial treatment outcome, and therapeutic potential of unproven compounds may also be provided by antifungal susceptibility testing (4, 6, 7).

2. Objectives

The present investigation aimed to define in vitro antifungal susceptibility profile of 108 clinical isolates of *C. albicans* and *C. glabrata* against itraconazole (ITC), voriconazole (VRC), fluconazole (FLC), amphotericin B (AMB), nystatin (NYS), and caspofungin (CAS).

3. Methods

3.1. Clinical Isolates

Seventy-seven strains of *C. albicans* and 31 strains of *C. glabrata* used in this study were isolated in 2017 in Tehran. The test strains were sub-cultured on potato dextrose agar (PDA, Merck, Germany) with chloramphenicol (Merck, Germany) and incubated at 37°C for 48 h. The species-level identification of clinical isolates was achieved using the previously described PCR- restriction fragment length polymorphism (RFLP) method. Briefly, PCR products of the ITS1-5.8S-ITS2 region were subject to digestion with the restriction enzyme MspI, and the strains were identified by comparing the electrophoretic RFLP patterns with those profiles obtained previously (8).

3.2. In vitro Antifungal Susceptibility Testing

Antifungal susceptibility testing (AFST) was performed according to the recommendations proposed by the Clinical and Laboratory Standards Institute (CLSI) M27-A3 and M27-S4 documents (9, 10). The antifungal agents tested were itraconazole (ITC), voriconazole (VRC), fluconazole (FLC), amphotericin B (AMB), nystatin (NYS) and caspofungin (CAS) (Sigma, St. Louis, MO, USA). The drug dilution ranges tested were 0.063 - 64 mg/L for FLC and NYS, 0.016 – 16mg/L for ITC, VRC and, AMB, and 0.008 - 8 mg/L for CAS. *C. parapsilosis (ATCC 22019)* were used as quality control strains. Briefly, homogeneous conidial suspensions were spectrophotometrically measured at the 530 nm wavelength and a percent transmission within the range of 75 - 77%. The final inocula suspension adjusted to 0.5 - 2.5 $\times$ 10^3 conidia/mL in RPMI-1640 with L-glutamine and without sodium bicarbonate (Sigma-Aldrich, St. Louis, MO, USA) buffered at pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS, Sigma-Aldrich, St. Louis, MO, USA). The 96-well microplates were incubated at 35°C and examined visually after 24 and 48 h to determine MIC values. Minimum inhibitory concentration (MIC) was determined according to the recommendations proposed by the CLSI M27-A3 and M27-S4 documents. The geometric mean (GM) MICs, MIC50, MIC90, and MIC ranges were calculated. Following the CLSI M27-S4, resistance to FLC for *C. albicans* was defined as MIC $\geq$ 8 mg/L and MIC $\geq$ 64 mg/L for *C. glabrata*, for VRC, MIC of $\geq$ 1mg/L was defined as resistant for *C. albicans* and *C. glabrata* and CAS, MIC of $\geq$ 1mg/L was defined as resistant for *C. albicans* and MIC of $\geq$ 0.5 mg/L *C. glabrata*. Isolates with MIC $\geq$ 2 mg/L for AMB were considered resistant.

3.3. Statistical Analysis

The statistical differences between the mean values were evaluated with SPSS (version 17.0) using student t-test. Statistical significance was set at 0.05.

4. Results

Our results showed 8 (25.8 %) and 6 (7.8 %) *C. glabrata* and *C. albicans* isolates resistance to FLU, respectively. Furthermore, resistance to VRC and ITC were observed in 8.4% and 3.7% of all isolates, respectively, and six isolates (5.6%) had intermediate MIC to CAS.

Table 1 lists the geometric mean (GM) MIC, MIC ranges, MIC50, and MIC90 of six antifungal agents against 108 *candida* strains (*C. albicans*; n: 77 and *C. glabrata*; n: 31). CAS (GM: 0.05 mg/L), AMB (GM: 0.12 mg/L), VRC (GM: 0.13 mg/L), and ITC (GM: 0.15 mg/L) had low MIC against all tested strains, whereas FLC (GM: 3.2 mg/L) and NYS (GM: 0.7 mg/L) had higher MIC. The MIC90 for all isolates were CAS: 0.25 mg/L. AMB: 0.5 mg/L, ITC: 0.5 mg/L, VRC: 1 mg/L, NYS: 2 mg/L and FLC: 32 mg/L. The MIC ranges across all isolates were as follows: CAS: 0.016-0.5 mg/L, AMB: 0.032 - 0.5 mg/L, ITC: 0.063 - 1 mg/L, VRC: 0.016 - 2 mg/L, NYS: 0.125 - 2 mg/L and FLC: 0.25 - 64 mg/L. The GM MICs of *C. glabrata* strains were as follows: CAS (GM: 0.08 mg/L), AMB (GM: 0.16 mg/L), ITC (GM: 0.34 mg/L), VRC (GM: 0.38 mg/L), FLC (GM: 16.53 mg/L) and NYS (GM: 0.82 mg/L), whereas for *C. albicans* strains the GM MICs were: AMB (GM: 0.11 mg/L), CAS (GM: 0.05 mg/L), ITC (GM: 0.10 mg/L), VRC (GM: 0.08 mg/L), FLC (GM: 1.56 mg/L) and NYS (GM: 0.59 mg/L). Our results indicated that no statistically significant differences existed in the patterns of susceptibility to each compound within species.

5. Discussion

Several previous studies were reported that *C. albicans* (76 - 89%) was the most common causative agent of infection in women with VVC, followed by *C. glabrata* (7 - 16%) from different countries (1, 2, 11-15). The rise of VVC incidence and drug resistance leads to an important public health issue and challenges clinician's treatment strategies (1). Antifungal susceptibility surveillance investigation was exerting an increasingly essential role in pursuing the progress of antifungal resistance and beginning primary antifungal therapy. With the growing application

Table 1. Geometric Mean of MIC, MIC Ranges, MIC50, and MIC90 Values Obtained by Testing the Susceptibility of 108 *Candida* isolates Obtained from 108 Vulvovaginal Candidiasis Patients to the Antifungal Agents

Strains/Antifungals	GM	Range	MIC50	MIC90	R (%)	SSD (%)	I (%)	S (%)
***C. albicans* (n:77)**								
AMB	0.11	0.032 - 0.5	0.125	0.5				77 (100)
CAS	0.05	0.016 - 0.5	0.063	0.25			4 (5.2)	73 (94.8)
FLC	1.56	0.25 - 16	2	8	6 (7.8)	11 (14.2)		60 (78)
VRC	0.08	0.016 - 1	0.125	0.5	4 (5.2)	8 (10.4)		65 (84.4)
ITC	0.10	0.063 - 1	0.063	0.25	3 (4)	7 (9)		67 (87)
NYS	0.59	0.125 - 2	0.5	2				
***C. glabrata* (n:31)**								
AMB	0.16	0.063 - 0.5	0.125	0.5				
CAS	0.08	0.032 - 0.5	0.063	0.25			2 (6.5)	29 (93.5)
FLC	16.53	2 - 64	16	64	8 (25.8)	5 (16.1)		18 (58)
VRC	0.38	0.063 - 1	0.5	1	5 (16.1)			26 (80.6)
ITC	0.34	0.125 - 1	0.5	1	2 (6.5)	5 (16.1)		24 (77.4)
NYS	0.82	0.25 - 2	0.5	2				
Total (n:108)								
AMB	0.12	0.032 - 0.5	0.125	0.5				
CAS	0.06	0.016 - 0.5	0.063	0.25			6 (5.6)	102 (94.4)
FLC	3.2	0.25 - 64	4	32	14 (13)	16 (14.8)		78 (72.2)
VRC	0.13	0.016 - 2	0.125	1	9 (8.4)	8 (7.4)		91 (84.2)
ITC	0.15	0.063 - 1	0.125	0.5	4 (3.7)	9 (8.3)		95 (88)
NYS	0.7	0.125 - 2	0.5	2				

Abbreviations: MIC, minimum inhibitory concentration; GM, geometric mean; MIC50, minimal concentration that inhibits 50% of isolates; MIC90, minimal concentration that inhibits 90% of isolates; CAS, caspofungin; AMB, amphotericin B; ITC, itraconazole; VRC, voriconazole; FLC, fluconazole; NYS, nystatin; R, resistance; SSD, susceptible dose-dependent; I, intermediate; S, susceptible.

of antimicrobial agents, the susceptibilities of the *candida* might be altering now and then (1-4). In the present study, the drug susceptibility profile of 108 *C. albicans* and *C. glabrata* isolates, as the two most frequent causative agents of VVC, was tested against six antifungal drugs. Based on the results in the present study, CAS (GM: 0.06 mg/L), AMB (GM: 0.12 mg/L), VRC (GM: 0.13 mg/L) and ITC (GM: 0.15 mg/L) had low MICs against all tested species, while NYS (GM: 0.7 mg/L) and FLC (GM: 3.2 mg/L) had higher MICs. As Table 1 shows, CAS had the lowest GM MIC with 0.05 mg/L, 0.08 mg/L against *C. albicans* and *C. glabrata* clinical strains, respectively. Azole drugs, especially FLC, are a major group of antifungals drugs that have been used for the treatment of VVC and RVVC (16). Azole drugs disrupt the biosynthesis of the fungal-specific membrane ergosterol by inhibiting the 14α-sterol demethylase enzyme encoded by the ERG11 gene (17). Fluconazole is the most commonly prescribed azole drug that is used for *C. albicans* infections (18). Our results showed 7.8% of *C. albicans* strains were resistant to FLC. According to our results, resistance to FLC among *C. albicans* strains was reported in several studies (19-25). However, in several reports, all *C. albicans* strains were sensitive to FLC (26-28). It was shown that almost all women diagnosed with FLC-resistant *C. albicans* had experienced previous exposure to FLC (29). The rates of azole drug resistance are variable, and they can be affected by the prescribing patterns of clinicians for both prophylaxis and treatment (16). Our results showed that 25.8% of *C. glabrata* strains were resistant to FLC. In line with our findings, resistance to FLC in *C. glabrata* isolates was reported in several studies (1, 21, 30-32). One important reason for the development of azole resistance in clinical strains of *C. glabrata* is the presence of mutations in some genes, especially the PDR1 gene (33). In the present study, 4.6% of all *Candida* strains (*C. albicans*: 4% and *C. glabrata*: 6.5%) were resistant to ITC. Resistance to ITR was detected in the previous studies (20, 32, 34, 35), and in contrast, some researchers did not observe any resistance to ITC (36). In the study conducted by Gross

et al. in Costa Rica, 100% of *C. albicans* isolates were susceptible to ITC (37). Voriconazole has a wide range of activity against filamentous, dimorphic fungi and yeasts (including fluconazole-resistant *Candida* spp.) (38). Our results were shown 16.1% of *C. glabrata* and 5.2% of *C. albicans* strains were resistant to VRC. Although VRC was approved for the treatment of invasive aspergillosis in 2002 by the US Food and Drug Administration (FDA), this antifungal drug was prescribed for the treatment of esophageal candidiasis, candidemia, disseminated *Candida* infections in viscera and skin (39). Mukasa et al. and El-Feky et al. reported that 6.6% and 10.5% of *C. albicans* isolates were resistant to VRC (4, 22). Nystatin is a member of polyene drugs, and in the form of an ointment or vaginal pessary, it has been used for four decades (40). In the previous study, Carrillo- Munoz et al. reported the NYS MIC90 values for 11 *C. glabrata* and 55 *C. albicans* clinical isolates as 4 mg/L and 2 mg/L, respectively (41). Furthermore, in South Africa, in patients with human immunodeficiency virus (HIV) and healthy individuals, NYS MICs for the 589 oral isolates ranged from 2 to 16 mg/L with reported MIC90 of 8 mg/L for the 466 *C. albicans* species (42). Our results showed that NYS had lower MIC in comparison to Carrillo-Munoz et al. (41) and Blignaut et al. (42) findings. Caspofungin, as a member of echinocandins, acts by inhibiting 1,3-ß-D-glucan synthase has good activity against *candida* species (43). As exhibited in Table 1, CAS had the lowest MIC with GM of 0.05 mg/L and 0.08 mg/L for *C. albicans* and *C. glabrata*, respectively. Our findings showed that 5.6% of *Candida* strains had an intermediate MIC range to CAS. Several other investigations also confirmed that CAS was excellent antifungal activity against *Candida* spp. (2, 43, 44). In the study conducted by Castanheira et al., the MIC90 value for AMB was reported as 1 mg/L in all *Candida* species, except for *C. krusei* that had a MIC of 2 mg/L (21). Furthermore, in another study, the MIC 90 value for AMB in the 1,310 isolated *C. albicans* was detected 1 mg/L that is higher than the present study (0.5 mg/L) (45).

5.1. Conclusions

Based on the results of the present study, CAS, AMB, and VRC had the lowest MIC against all *Candida* isolates, respectively. However, recently, resistance to azole among the *Candida* isolates is constantly increasing worldwide. Therefore, antifungal susceptibility testing can be used to predict clinical responses to malfunctions in the management.

Footnotes

Authors' Contribution: Study concept and design: S.A.; analysis and interpretation of data: B. A., and GR. SH.; drafting of the manuscript: N. D., M. F., SH. Y, K. V.; critical revision of the manuscript for important intellectual content: P. P.; statistical analysis: E. L.

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References

1. Khan M, Ahmed J, Gul A, Ikram A, Lalani FK. Antifungal susceptibility testing of vulvovaginal Candida species among women attending antenatal clinic in tertiary care hospitals of Peshawar. *Infect Drug Resist*. 2018;**11**:447–56. doi: 10.2147/IDR.S153116. [PubMed: 29628769]. [PubMed Central: PMC5878663].
2. Bitew A, Abebaw Y. Vulvovaginal candidiasis: species distribution of Candida and their antifungal susceptibility pattern. *BMC Womens Health*. 2018;**18**(1):94. doi: 10.1186/s12905-018-0607-z. [PubMed: 29902998]. [PubMed Central: PMC6003188].
3. Wang FJ, Zhang D, Liu ZH, Wu WX, Bai HH, Dong HY. Species distribution and in vitro antifungal susceptibility of vulvovaginal Candida isolates in China. *Chin Med J (Engl)*. 2016;**129**(10):1161–5. doi: 10.4103/0366-6999.181964. [PubMed: 27174323]. [PubMed Central: PMC4878160].
4. Mukasa KJ, Herbert I, Daniel A, Sserunkuma KL, Joel B, Frederick B. Antifungal susceptibility patterns of vulvovaginal Candida species among women attending antenatal clinic at Mbarara Regional Referral Hospital, South Western Uganda. *Br Microbiol Res J*. 2015;**5**(4):322–31. doi: 10.9734/BMRJ/2015/13804. [PubMed: 26594637]. [PubMed Central: PMC4652954].
5. Ghajari A, Lotfali E, Ahmadi NA, Nazer Fassihi P, Shahmohammadi N, Ansari S, et al. Isolation of Different Species of Candida in Patients with Vulvovaginal Candidiasis from Damavand, Iran. *Arch Clin Infect Dis*. 2018;**13**(6). doi: 10.5812/archcid.59291.
6. Aslani N, Shokohi T, Ataollahi MR, Ansari S, Gholampour Y, Khani Jeihooni A, et al. In vitro activity of four triazole antifungal drugs against clinically common and uncommon yeast species. *Curr Med Mycol*. 2019;**5**(4):14–9. doi: 10.18502/cmm.5.4.1949. [PubMed: 32104739]. [PubMed Central: PMC7034789].
7. Hedayati MT, Tavakoli M, Zakavi F, Shokohi T, Mofarrah R, Ansari S, et al. In vitro antifungal susceptibility of Candida speciesisolated from diabetic patients. *Rev Soc Bras Med Trop*. 2018;**51**(4):542–5. doi: 10.1590/0037-8682-0332-2017. [PubMed: 30133642].
8. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A one-enzyme PCR-RFLP assay for identification of six medically important Candida species. *Nihon Ishinkin Gakkai Zasshi*. 2006;**47**(3):225–9. doi: 10.3314/jjmm.47.225. [PubMed: 16940958].
9. Clinical And Laboratory Standards Institute. *Reference method for broth dilution antifungal susceptibility testing of yeasts; approved standard-third edition M27-A3*. CLSI; 2008. Available from: https://webstore.ansi.org/standards/clsi/clsim27a3.
10. Clinical And Laboratory Standards Institute. *Reference method for broth dilution antifungal susceptibility testing of yeasts: fourth informational supplement M27-S4*. CLSI Wayne, PA, USA; 2012. Available from: https://webstore.ansi.org/standards/clsi/clsim27s4.
11. Mutua F, Revathi G, Machoki JM. Species distribution and antifungal sensitivity patterns of vaginal yeasts. *East Afr Med J*. 2010;**87**(4):156–62. doi: 10.4314/eamj.v87i4.62202. [PubMed: 23057291].

12. Nelson M, Wanjiru W, Margaret MW. Prevalence of vaginal candidiasis and determination of the occurrence of Candida species in pregnant women attending the antenatal clinic of Thika District Hospital, Kenya. *Open J Med Microbiol*. 2013;**3**(4):264–72. doi: 10.4236/ojmm.2013.34040.
13. Oyewole OA, Okoliegbe IN, Alkhalil S, Isah P. Prevalence of vaginal candidiasis among pregnant women attending Federal University of Technology, Minna, Nigeria, Bosso Clinic. *Res J Pharm Biol Chel Sci*. 2013;**4**:113–20.
14. Corsello S, Spinillo A, Osnengo G, Penna C, Guaschino S, Beltrame A, et al. An epidemiological survey of vulvovaginal candidiasis in Italy. *Eur J Obstet Gynecol Reprod Biol*. 2003;**110**(1):66–72. doi: 10.1016/s0301-2115(03)00096-4.
15. Shokoohi G, Rasekh-Jahromi A, Solhjoo K, Hasannezhad A, Nouripour-Sisakht S, Ahmadi B, et al. Molecular Characterization and Antifungal Susceptibility of Candida Species Isolated From Vulvovaginitis in Jahrom City, South of Iran. *Jundishapur J Microbiol*. 2020;**13**(10). doi: 10.5812/jjm.106825.
16. Bulik CC, Sobel JD, Nailor MD. Susceptibility profile of vaginal isolates of Candida albicans prior to and following fluconazole introduction - impact of two decades. *Mycoses*. 2011;**54**(1):34–8. doi: 10.1111/j.1439-0507.2009.01752.x. [PubMed: 19563489].
17. Oxman DA, Chow JK, Frendl G, Hadley S, Hershkovitz S, Ireland P, et al. Candidaemia associated with decreased in vitro fluconazole susceptibility: is Candida speciation predictive of the susceptibility pattern? *J Antimicrob Chemother*. 2010;**65**(7):1460–5. doi: 10.1093/jac/dkq136. [PubMed: 20430790].
18. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Ellis D, Tullio V, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of Candida Species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *J Clin Microbiol*. 2010;**48**(4):1366–77. doi: 10.1128/JCM.02117-09. [PubMed: 20164282]. [PubMed Central: PMC2849609].
19. Katiraee F, Khosravi AR, Khalaj V, Hajiabdolbaghi M, Khaksar AA, Rasoulinejad M. [In vitro antifungal susceptibility of oral candida species from Iranian HIV infected patients]. *Tehran University Medical Journal*. 2012;**70**(2):96–103. Persian.
20. Badiee P, Alborzi A, Davarpanah MA, Shakiba E. Distributions and antifungal susceptibility of Candida species from mucosal sites in HIV positive patients. *Arch Iran Med*. 2010;**13**(4):282–7. [PubMed: 20597560].
21. Castanheira M, Messer SA, Rhomberg PR, Dietrich RR, Jones RN, Pfaller MA. Isavuconazole and nine comparator antifungal susceptibility profiles for common and uncommon Candida species collected in 2012: application of new CLSI clinical breakpoints and epidemiological cutoff values. *Mycopathologia*. 2014;**178**(1-2):1–9. doi: 10.1007/s11046-014-9772-2. [PubMed: 24952015].
22. ElFeky DS, Gohar NM, El-Seidi EA, Ezzat MM, AboElew SH. Species identification and antifungal susceptibility pattern of Candida isolates in cases of vulvovaginal candidiasis. *Alexandria J Med*. 2019;**52**(3):269–77. doi: 10.1016/j.ajme.2015.10.001.
23. Fothergill AW, Sutton DA, McCarthy DI, Wiederhold NP. Impact of new antifungal breakpoints on antifungal resistance in Candida species. *J Clin Microbiol*. 2014;**52**(3):994–7. doi: 10.1128/JCM.03044-13. [PubMed: 24403302]. [PubMed Central: PMC3957792].
24. Dorrell L, Edwards A. Vulvovaginitis due to fluconazole resistant Candida albicans following self treatment with non-prescribed triazoles. *Sex Transm Infect*. 2002;**78**(4):308–9. doi: 10.1136/sti.78.4.308-a. [PubMed: 12181480]. [PubMed Central: PMC1744499].
25. Sobel JD, Zervos M, Reed BD, Hooton T, Soper D, Nyirjesy P, et al. Fluconazole susceptibility of vaginal isolates obtained from women with complicated Candida vaginitis: clinical implications. *Antimicrob Agents Chemother*. 2003;**47**(1):34–8. doi: 10.1128/AAC.47.1.34-38.2003. [PubMed: 12499165]. [PubMed Central: PMC148960].
26. El-Din SS, Reynolds MT, Ashbee HR, Barton RC, Evans EG. An investigation into the pathogenesis of vulvo-vaginal candidosis. *Sex Transm Infect*. 2001;**77**(3):179–83. doi: 10.1136/sti.77.3.179. [PubMed: 11402224]. [PubMed Central: PMC1744307].
27. Ribeiro MA, Dietze R, Paula CR, Da Matta DA, Colombo AL. Susceptibility profile of vaginal yeast isolates from Brazil. *Mycopathologia*. 2001;**151**(1):5–10. doi: 10.1023/a:1010909504071. [PubMed: 11502064].
28. Arzeni D, Del Poeta M, Simonetti O, Offidani AM, Lamura L, Balducci M, et al. Prevalence and antifungal susceptibility of vaginal yeasts in outpatients attending a gynecological center in Ancona, Italy. *Eur J Epidemiol*. 1997;**13**(4):447–50. doi: 10.1023/a:1007366722235. [PubMed: 9258552].
29. Marchaim D, Lemanek L, Bheemreddy S, Kaye KS, Sobel JD. Fluconazole-resistant Candida albicans vulvovaginitis. *Obstet Gynecol*. 2012;**120**(6):1407–14. [PubMed: 23168767].
30. Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. Antifungal susceptibilities of Candida species causing vulvovaginitis and epidemiology of recurrent cases. *J Clin Microbiol*. 2005;**43**(5):2155–62. doi: 10.1128/JCM.43.5.2155-2162.2005. [PubMed: 15872235]. [PubMed Central: PMC1153777].
31. Abbasi Nejat Z, Farahyar S, Falahati M, Ashrafi Khozani M, Hosseini AF, Faiazy A, et al. Molecular identification and antifungal susceptibility pattern of non-albicans Candida species isolated from vulvovaginal candidiasis. *Iran Biomed J*. 2017;**22**(1):33–41. doi: 10.22034/ibj.22.1.33. [PubMed: 28688376]. [PubMed Central: PMC5712382].
32. Mohamadi J, Havasian MR, Panahi J, Pakzad I. Antifungal drug resistance pattern of Candida. spp isolated from vaginitis in Ilam-Iran during 2013-2014. *Bioinformation*. 2015;**11**(4):203–6. doi: 10.6026/97320630011203. [PubMed: 26124561]. [PubMed Central: PMC4479047].
33. Whaley SG, Berkow EL, Rybak JM, Nishimoto AT, Barker KS, Rogers PD. Azole antifungal resistance in Candida albicans and emerging non-albicans Candida species. *Front Microbiol*. 2016;**7**:2173. doi: 10.3389/fmicb.2016.02173. [PubMed: 28127295]. [PubMed Central: PMC5226953].
34. Haddadi P, Zareifar S, Badiee P, Alborzi A, Mokhtari M, Zomorodian K, et al. Yeast colonization and drug susceptibility pattern in the pediatric patients with neutropenia. *Jundishapur J Microbiol*. 2014;**7**(9). e11858. doi: 10.5812/jjm.11858. [PubMed: 25485060]. [PubMed Central: PMC4255375].
35. Brandolt TM, Klafke GB, Goncalves CV, Bitencourt LR, Martinez AM, Mendes JF, et al. Prevalence of Candida spp. in cervical-vaginal samples and the in vitro susceptibility of isolates. *Braz J Microbiol*. 2017;**48**(1):145–50. doi: 10.1016/j.bjm.2016.09.006. [PubMed: 27756539]. [PubMed Central: PMC5220630].
36. Paul L, Rejitha IM, Revathy C. Azole resistance in Candida species isolated from diabetic women with vulvovaginal candidiasis in a tertiary care centre. *Int J Curr Microbiol Appl Sci*. 2019;**8**(2):738–44. doi: 10.20546/ijcmas.2019.802.085.
37. Gross NT, Arias ML, Moraga M, Baddasarow Y, Jarstrand C. Species distribution and susceptibility to azoles of vaginal yeasts isolated prostitutes. *Infect Dis Obstet Gynecol*. 2007;**2007**:82412. doi: 10.1155/2007/82412. [PubMed: 18273407]. [PubMed Central: PMC2216416].
38. Espinel-Ingroff A. In vitro activity of the new triazole voriconazole (UK-109,496) against opportunistic filamentous and dimorphic fungi and common and emerging yeast pathogens. *J Clin Microbiol*. 1998;**36**(1):198–202. doi: 10.1128/JCM.36.1.198-202.1998. [PubMed: 9431946]. [PubMed Central: PMC124833].
39. Gonzalez GM, Portillo OJ, Uscanga GI, Andrade SE, Robledo M, Rodriguez C, et al. Therapeutic efficacy of voriconazole against a fluconazole-resistant Candida albicans isolate in a vaginal model. *J Antimicrob Chemother*. 2009;**64**(3):571–3. doi: 10.1093/jac/dkp228. [PubMed: 19570754].
40. Martins HP, da Silva MC, Paiva LC, Svidzinski TI, Consolaro ME. Efficacy of fluconazole and nystatin in the treatment of vaginal Candida

species. *Acta Derm Venereol*. 2012;**92**(1):78–82. doi: 10.2340/00015555-1194. [PubMed: 21918792].

41. Carrillo-Munoz AJ, Quindos G, Tur C, Ruesga MT, Miranda Y, del Valle O, et al. In-vitro antifungal activity of liposomal nystatin in comparison with nystatin, amphotericin B cholesteryl sulphate, liposomal amphotericin B, amphotericin B lipid complex, amphotericin B desoxycholate, fluconazole and itraconazole. *J Antimicrob Chemother*. 1999;**44**(3):397–401. doi: 10.1093/jac/44.3.397. [PubMed: 10511410].
42. Blignaut E, Messer S, Hollis RJ, Pfaller MA. Antifungal susceptibility of South African oral yeast isolates from HIV/AIDS patients and healthy individuals. *Diagn Microbiol Infect Dis*. 2002;**44**(2):169–74. doi: 10.1016/s0732-8893(02)00440-6. [PubMed: 12458124].
43. Pappas PG, Rotstein CM, Betts RF, Nucci M, Talwar D, De Waele JJ, et al. Micafungin versus caspofungin for treatment of candidemia and other forms of invasive candidiasis. *Clin Infect Dis*. 2007;**45**(7):883–93. doi: 10.1086/520980. [PubMed: 17806055].
44. Lyon GM, Karatela S, Sunay S, Adiri Y. Antifungal susceptibility testing of Candida isolates from the Candida surveillance study. *J Clin Microbiol*. 2010;**48**(4):1270–5. doi: 10.1128/JCM.02363-09. [PubMed: 20129963]. [PubMed Central: PMC2849617].
45. Castanheira M, Deshpande LM, Davis AP, Rhomberg PR, Pfaller MA. Monitoring antifungal resistance in a global collection of invasive yeasts and molds: Application of CLSI epidemiological cutoff values and whole-genome sequencing analysis for detection of azole resistance in Candida albicans. *Antimicrob Agents Chemother*. 2017;**61**(10). e00906. doi: 10.1128/AAC.00906-17. [PubMed: 28784671]. [PubMed Central: PMC5610521].