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Research article

Low prevalence of antifungal resistant *Candida africana,* in the *C. albicans* complex causing vulvovaginal candidiasis

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ABSTRACT

The *Candida* (*C.*) *albicans* complex includes *C. albicans*, *C. dubliniensis*, *C. stellatoidea,* and *C. africana*, with the last mentioned as an important emerging agent of vulvovaginal candidiasis (VVC). The aim of the study was to identify *C. africana* and *C. dubliniensis* and assess their drug susceptibility in vaginitis. One-hundred *Candida* isolates of the *C. albicans* complex from women diagnosed with vaginitis and from vaginal samples in the culture collection of a medical mycology laboratory were examined. Species of the *C. albicans* complex were identified with conventional and molecular methods using polymerase chain reaction (PCR) for amplification and sequencing of the internal transcribed spacer (ITS) region, PCR for partial amplification of hyphal wall protein 1 (*HWP1*) gene and duplex PCR. The effects of antifungal drugs were evaluated according to standard broth microdilution protocols.

Ninety-*seven C. albicans* (97%) and three *C. africana* (3%) isolates were identified. Results of susceptibility testing revealed one isolate of *C. africana* to be resistant to both clotrimazole and fluconazole, and one showed reduced susceptibility to itraconazole.

Identification of *Candida* species especially *C. africana* in vaginitis is crucial, there are varying levels of resistance to antifungal drugs.

1. Introduction

Species in the *C. albicans* complex can colonize in the vaginal tract. Through changes in the relationship between the host and this commensal yeast, an invasive form may occur (Goncalves et al., 2016) causing mucosal infection and vulvovaginal candidiasis (VVC) (Hedayati et al., 2015). In 2001, *C. albicans* isolates of African and German patients with atypical phenotypes were described as a novel species, *C. africana* (Tietz et al., 2001). *C. africana* produces a germ tube but not chlamydospores. *C. albicans* remains the most common pathogenic yeast isolated from vaginal specimens of VVC patients, but the closely-related *C. africana* and *C. dubliniensis* have often been mistaken for *C. albicans*. *C. africana* has been isolated from candidal balanoposthitis in China (Hu et al., 2015), and clinical isolates have been reported as the cause of VVC in Germany, China, Italy, Iran, Nigeria, Spain, the United Kingdom, Argentina, Colombia, among other countries (Tietz et al., 2001; Alonso-Vargas et al., 2008; Romeo and Criseo, 2009; Dieng et al., 2012; Nnadi et al., 2012; Borman et al., 2013; Shan et al., 2014; Rodriguez-Leguizamon et al., 2015; Yazdanparast et al., 2015; Theill et al., 2016). The vaginal candidiasis studies in Iran have been identified susceptible (Yazdanparast et al., 2015) and resistant (Majdabadi et al., 2018) antifungal drugs *C. africana* isolates.

Molecular methods are suitable for identification of *Candida* species. The use of the ribosomal DNA (rDNA) ITS region for sequence-analysis appears to be the most sensitive and specific method for accurate and reliable molecular identification of *Candida* species (Merseguel et al.,

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2015; Zarrinfar et al., 2016). The sequencing of a short fragment of the ITS2 region has been shown effective in detecting the presence of *C. africana* in clinical *Candida* infections (Borman et al., 2013). Duplex PCR is a suitable method for identification of isolates such as *C. albicans* and *C. dubliniensis* (Ahmad et al., 2012). Amplification of the *HWP1* gene was the first molecular method for discriminating *C. albicans*, *C. dubliniensis*, and *C. africana* (Romeo and Criseo, 2008). Many antifungal agents are effective treating VVC, but some vaginal *Candida* isolates have developed resistance to antifungal drugs. Assessment of antifungal susceptibility of pathogenic yeasts is essential to proper therapy and will allow evaluation of their efficacy and treatment results as well as prevention of drug resistance (Mukasa et al., 2015; Fornari et al., 2016). However, there is limited information about the prevalence and antifungal susceptibility of *C. africana* in VVC in Iran. The aim of this study was to identify *C. africana* and *C. dubliniensis* in vaginal samples from VVC patients and to evaluate their antifungal susceptibility pattern.

2. Materials and methods

2.1. Patients, sample collection and definitions

The present study analyzed clinical isolates of vaginal discharge from 300 non-pregnant patients aged 18–57 years with suspected VVC admitted to the Shahid Akbar Abadi hospital and Shahriar health centers from June through December 2016, along with culture samples from our laboratory. Patients had not used antifungal drugs within the preceding four weeks. Samples were collected with sterile cotton swabs, and microscopic examination was done to identify yeast forms or pseudohyphae. In addition, 30 *Candida* spp. of the *C. albicans* complex obtained from VVC patients were selected from the culture collection of the medical mycology laboratory in our department. All specimens were cultured on CHROMagar *Candida* medium (CHROMagar, France) at 37 °C for 24–48 hrs for detection of mixed *Candida* spp. infections. A single colony of each *Candida* isolate on CHROMagar *Candida* medium was selected and cultured on Sabouraud dextrose agar (SDA) medium with chloramphenicol at 30 °C for 48–72 hrs for further use. The germ tube test, chlamydospore-forming assay on corn meal agar (CMA) with 1% Tween 80 and growth on SDA medium with chloramphenicol at 42 °C and 45 °C was conducted.

2.2. DNA extraction

A single colony of each clinical isolate from CHROMagar *Candida* medium was cultured on yeast extract peptone dextrose (YEPD) agar and incubated at 37 °C for 24–48 hrs. Genomic DNA was extracted from yeast cultures using the Qiagen DNA tissue kit (Germany). The extracted DNA was stored at -20 °C until use.

2.3. PCR amplification and sequencing of ITS region

The universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCC GCTTATTGATATGC-3′) were used to amplify the ITS1-5.8S-ITS2 region (Fujita et al., 2001; Ciardo et al., 2006) under the following conditions: 98 °C at 5 min; 35 cycles of 30 s at 98 °C, annealing for 30 s at 56 °C, 30 s at 72 °C, and a final extension of 5 min at 72 °C. The PCR products were sequenced by Macrogen (Korea). Sequences were compared with reference data available from the GenBank database using the BLAST sequence search tool (http://www.ncbi.nlm.nih.gov/BLAST).

2.4. Duplex PCR

C. albicans and *C. dubliniensis* strains were analyzed by duplex PCR with paired primers CALF (5′-TGGTAAGGCGGGATCGCTT-3′) and CALR (5′-GGTCAAAGTTTGAAGATATAC-3′) for *C. albicans* and CDUF (5′-AAACTTGTCACGAGATTATTTTT-3′) and CDUR (5′-AAAGTTTGAAG AATAAAATGGC-3′) for *C. dubliniensis* (Ahmad et al., 2012). Specific primers used the following PCR protocol: 95 °C for 5 min; 30 cycles of 1 min at 95 °C, 30 s at annealing temperature 55 °C, and 1 min at 72 °C; and a final extension of 10 min at 72 °C.

2.5. PCR amplification of HWP1 gene

The PCR primers (CR-f 5′-GCTACCACTTCAGAATCATCATC-3′ and CR-r 5′-GCACCTTCAGTCGTAGAGACG-3′) used to amplify the *HWP1* gene for detection *C. albicans, C. africana* and *C. dubliniensis*, as previously described by Romeo and Criseo (2008).

Reference strains used as control were *C. albicans* (ATCC10231) and *C. dubliniensis* from the archives of department of Medical Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences. *C. dubliniensis* was 100% confirmed by sequencing to species level.

2.6. In vitro antifungal susceptibility testing

The *in vitro* susceptibility was determined by the Clinical and Laboratory Standards Institute (CLSI) (CLSI M27-S3 2008; CLSI M27-S4 2012). Briefly, final inocula of 0.5×10^3 to 2.5×10^3 colony-forming units/ml were distributed in 96 well microtiter plates in RPMI 1640 medium buffered to pH 7.0 with 0.165M morpholinepropanesulfonic acid with diluted antifungal drugs and incubated at 35 °C. The antifungals used were amphotericin B, itraconazole, fluconazole, and clotrimazole (Sigma, Germany), and all tests were duplicated. *C. glabrata* CBS 138 was used as quality control strain. According to CLSI M27-S3 criteria for fluconazole, the sensitivity profile is classified as sensitive (≤8 μg/ml), dose-dependent sensitive (16–32 μg/ml), and resistant (≥64 μg/ml), and breakpoints for itraconazole is sensitive (≤0.125 μg/ml), dose-dependent sensitive (0.25–0.5 μg/ml), and resistant (≥1 μg/ml).

2.7. Ethical approval

This research was approved by Ethics Committee of Iran University of Medical Sciences, under Ethics Committee number 95-01-30-27842.

2.8. Informed consent

Written informed consent was obtained from all patients.

3. Results

3.1. Patients

All patients diagnosed with VVC (23%) were in the 18–50 years age range. None exhibited diabetes, immunodeficiency, or other chronic disease. The archival information on VVC patients indicated a very similar age range, 18–50 years, and without disease.

3.2. Yeast isolates

One hundred isolates belonging to the *C. albicans* complex were identified, 97 as *C. albicans* (data not shown) 97% and three (3%) as *C. africana. C. africana* produced small turquoise-green colonies on CHROMagar *Candida* and did not produce chlamydospores on CMA medium. *C. albicans* isolates proliferated at 42 °C and 45 °C, while the *C. africana* isolates did not.

3.3. Duplex PCR assay

The results of amplification of isolates with CALF/CALR and CDUF/CDUR specific primers for *C. albicans* and *C. dubliniensis*, respectively, for duplex PCR assay, were determined for a ~100 bp fragment of *C. albicans* and a ~325 bp fragment of *C. dubliniensis* (Figure 1). No *C. dubliniensis* isolates were found in this study.

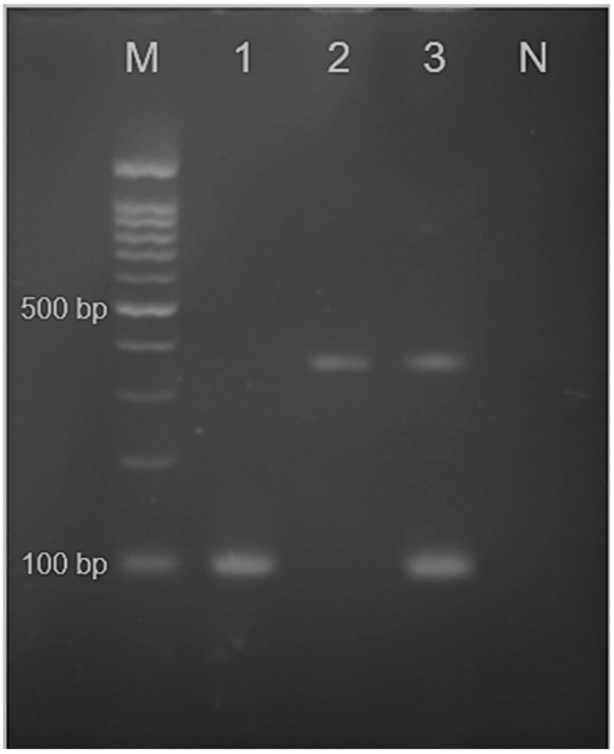

Figure 1. Agarose gel of duplex PCR using primers CALF, CALR, CDUF andC-DUR. Lane 1: *C. albicans* clinical isolate (~100 bp); Lane 2: positive control *C. dubliniensis* (~325 bp) from the archives of the Department of Medical Parasitology and Mycology; Lane 3: positive controls *C. albicans* (ATCC10231) and *C. dubliniensis*. N: negative control. M: marker 100 bp.

3.4. PCR amplification and sequencing of ITS region

Amplification of all clinical isolates of *C. albicans* complex with ITS1 and ITS4 primers yielded fragments of 530 bp for both *C. albicans* and *C. africana* (Figure 2). The ITS region sequences of *Candida* spp. clinical isolates were compared in the GenBank database using BLAST. All clinical isolates showed 100% and 99% similarity to *C. albicans* or *C. africana*. The ITS sequences of *C. africana* isolates were deposited in GenBank under accession numbers MG757669, MG757670, and MG757671.

3.5. PCR amplification with specific primers of HWP1 gene

The results of partial amplification of isolates with specific primers of *HWP1* gene yielded fragments of ~900 bp, ~700 bp and ~560 bp for *C. albicans*, *C. africana* and *C. dubliniensis*, respectively (Figure 3).

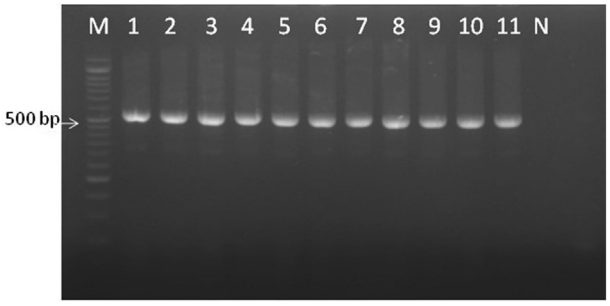

Figure 2. Clinical isolates of *C. albicans* complex analyzed with ITS1 and ITS4 universal primers; 530 bp fragment produced. Lanes 1–8: *C. albicans*; Lanes 9–11: *C. africana*; N: negative control. M: marker 50 bp.

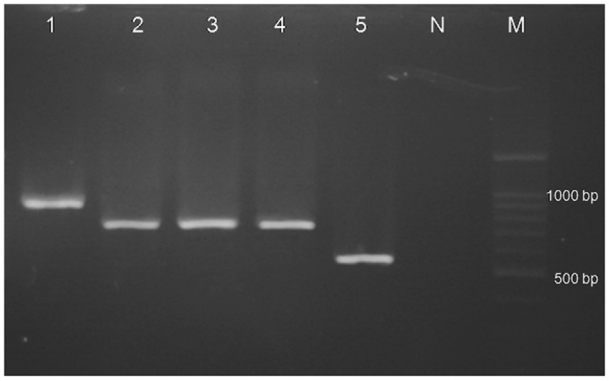

Figure 3. Agarose gel of PCR amplification with specific primers of *HWP1* gene; Lane 1: *C. albicans* (ATCC10231) ~900 bp; Lanes 2–4: *C. africana* (~700 bp); Lane 5: positive control of *C. dubliniensis* (~560 bp) from the archives of the Department of Medical Parasitology and Mycology; N: negative control. M: marker 100 bp.

3.6. Antifungal susceptibility testing

Candida africana isolates were classified as sensitive, dose-dependent sensitive, or resistant to antifungal agents based on their minimum inhibitory concentration (MIC) according to the M27-S3 protocol (Table 1). One isolate of *C. africana* isolates showed resistance to both clotrimazole (MIC $\geq$ 1 μg/ml) (Pelletier et al., 2000) and fluconazole (MIC $\geq$ 64 μg/ml). One isolate showed susceptibity in a dose-dependent manner to itraconazole (0.25–0.5 μg/ml). Clinical *C. africana* isolates were susceptible to amphotericin B.

4. Discussion

The female genital tract is a common site of pathogenic growth of *Candida* spp., and VVC is a common infection. *C. albicans* is reported to be the most common yeast species involved in VVC (Alizadeh et al., 2017; Kord et al., 2017; Khorsand et al., 2015). Species of *C. albicans* complex include *C. albicans*, *C. dubliniensis*, *C. africana* and *C. stellatoidea* that are important in vaginitis, with *C. albicans* the primary agent of *Candida* infections. *C. dubliniensis* was identified as the most common cause of oral candidiasis in HIV-infected individuals (Sullivan et al., 2005). Accurate identification of these closely related yeasts is facilitated by the use of both conventional and molecular methods. *C. africana* (6%) has been reported to be more prevalent than *C. dubliniensis* (Borman et al., 2013), with worldwide distribution identified from vaginal discharge, candidal balanoposthitis, and blood culture (Odds et al., 2007; Romeo and Criseo, 2011; Romeo et al., 2013; Hu et al., 2015). The present study confirmed *C. africana* (3%), in the *C. albicans* complex, as an important agent of VVC. In order to administer effective treatment, the species must be identified, since some show resistance to antifungal drugs. The *HWP1* gene amplification is adequate to identify species of this complex (Hu et al., 2015), and sequencing of the ITS region has proven to be a feasible method for the reliable identification of clinically important yeasts. *C. dubliniensis* and *C. stellatoidea* were not detected in our study. Other studies of VVC have also not reported *C. dubliniensis* isolate (Nnadi et al., 2012; Shan et al., 2014; Hu et al., 2015; Ngouana et al., 2015) while some have found it to have higher prevalence than *C. africana* (Borman et al., 2013; Theill et al., 2016). Romeo et al. (Romeo and Criseo, 2009) studied isolates of *C. albicans* (338), *C. africana* (27), *and C. dubliniensis* (11) from several anatomical sites and found *C. albicans* (89.9%) to be the most common species, followed by *C. africana* (7.2%) and *C. dubliniensis* (2.9%), with *C. africana* isolated only from vaginal secretions. An investigation from China (Hu et al., 2015) found five *C. africana* isolates (6.3%) involved in candidal balanoposthitis to be susceptible to fluconazole, itraconazole,

Table 1. *In vitro* susceptibility profile of three *C. africana* vaginal isolates using the breakpoints of CLSI M27- S3[a].

Isolates	Antifungal agents	Dosage Range µg/ml	Number of isolates		
			S[b]	S-DD[c]	R[d]
C. africana	Fluconazole	0.25–64	2	-	1
	Clotrimazole	0.06–16	2	-	1
	Itraconazole	0.06–16	2	1	-
	Amphotericin B	0.06–2.0	3	-	-

[a] CLSI document M27- S3 (2008).
[b] S: sensitive.
[c] S-DD: susceptible dose-dependent.
[d] R: resistant.

voriconazole, posaconazole, caspofungin, flucytosine, micafungin, and amphotericin B. Shan et al. (2014) reported that *C. africana* isolates (1.5%) from vaginal specimens from China were susceptible to nystatin, fluconazole, itraconazole, miconazole, and clotrimazole, while another study reported five (4.38 %) *C. africana* VVC isolates to be susceptible to tested antifungal agents (Yazdanparast et al., 2015). Theill et al. (2016) showed low MIC values of nystatin, fluconazole, itraconazole, voriconazole, clotrimazole, and terbinafine to *C. africana* and *C. dubliniensis* vaginal isolates. Borman et al. (2013) showed *C. africana* isolates to be susceptible to tested antifungal agents suitable for VVC treatment. Ngouana et al. (2015) reported a *C. africana* isolate in vaginal samples from HIV infected patients to be resistant to ketoconazole and exhibit reduced susceptibility to amphotericin B. Majdabadi et al., isolated two *C. africana* of vaginal candidiasis in which one species was resistant to fluconazole and itraconazole, the other one to itraconazole (Majdabadi et al., 2018). We found one *C. africana* vaginal isolate resistant to both clotrimazole and fluconazole, and another one showed dose-dependent susceptibility to itraconazole.

5. Conclusion

Our finding of *C. africana* as a rare agent of vaginitis resistant to both clotrimazole and fluconazole with reduced susceptibility to itraconazole is important in VVC treatment. Identification of *C. africana* as an uncommon agent of VVC is critical, since this yeast showed a range of susceptibility to antifungal agents.

Declarations

Author contribution statement

S. Farahyar: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

S. Izadi: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

M. Falahati: Conceived and designed the experiments.

E. Razmjou: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Z. Ghahri-Mobaser: Performed the experiments; Contributed reagents, materials, analysis tools or data.

M. Ashrafi-Khozani, M. Roudbary, M. Rahimi: Performed the experiments.

S. Ansari: Contributed reagents, materials, analysis tools or data.

A. Fattahi: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

Data associated with this study has been deposited at GenBank under the accession numbers MG757669, MG757670, and MG757671.

References

Ahmad, S., Khan, Z., Asadzadeh, M., Theyyathel, A., Chandy, R., 2012. Performance comparison of phenotypic and molecular methods for detection and differentiation of *Candida albicans* and *Candida dubliniensis*. BMC Infect. Dis. 12, 230.

Alizadeh, M., Kolecka, A., Boekhout, T., Zarrinfar, H., Ghanbari Nahzag, M.A., Badiee, P., Rezaei-Matehkolaei, A., Fata, A., Dolatabadi, S., Najafzadeh, M.J., 2017. Identification of *Candida* species isolated from vulvovaginitis using matrix assisted laser desorption ionization-time of flight mass spectrometry. Curr. Med. Mycol. 3 (4), 21–25.

Alonso-Vargas, R., Elorduy, L., Eraso, E., Francisco Cano, J., Guarro, J., Pontón, J., Quindós, G., 2008. Isolation of *Candida africana*, probable atypical strains of *Candida albicans,* from a patient with vaginitis. Med. Mycol. 46 (2), 167–170.

Borman, A.M., Szekely, A., Linton, C.J., Palmer, M.D., Brown, P., Johnson, E.M., 2013. Epidemiology, antifungal susceptibility, and pathogenicity of *Candida africana* isolates from the United Kingdom. J. Clin. Microbiol. 51 (3), 967–972.

Ciardo, D., Schär, G., Böttger, E., Altwegg, M., Bosshard, P., 2006. Internal transcribed spacer sequencing versus biochemical profiling for identification of medically important yeasts. J. Clin. Microbiol. 44 (1), 77–84.

Clinical and Laboratory Standards Institute (CLSI), 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Third Informational Supplement. CLSI Document M27-S3. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.

Clinical and Laboratory Standards Institute (CLSI), 2012. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Fourth Informational Supplement. CLSI Document M27-S4. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.

Dieng, Y., Sow, D., Ndiaye, M., Guichet, E., Faye, B., Tine, R., Lo, A., Sylla, K., Ndiaye, M., Abiola, A., Dieng, T., Ndiaye, J.L., Le Pape, P., Gaye, O., 2012. Identification of three *Candida africana* strains in Senegal. J. Mycol. Med. 22 (4), 335–340.

Fornari, G., Vicente, V.A., Gomes, R.R., Muro, M.D., Pinheiro, R.L., Ferrari, C., Herkert, P.F., Takimura, M., Carvalho, N.S., Queiroz-Telles, F., 2016. Susceptibility and molecular characterization of *Candida* species from patients with vulvovaginitis. Braz. J. Microbiol. 47 (2), 373–380.

Fujita, S.I., Senda, Y., Nakaguchi, S., Hashimoto, T., 2001. Multiplex PCR using internal transcribed spacer 1 and 2 regions for rapid detection and identification of yeast strains. J. Clin. Microbiol. 39 (10), 3617–3622.

Goncalves, B., Ferreira, C., Alves, C.T., Henriques, M., Azeredo, J., Silva, S., 2016. Vulvovaginal candidiasis: epidemiology, microbiology and risk factors. Crit. Rev. Microbiol. 42 (6), 905–927.

Hedayati, M.T., Taheri, Z., Galinimoghadam, T., Aghili, S.R., Yazdani Cherati, J., Mosayebi, E., 2015. Isolation of different species of *Candida* in patients with vulvovaginal candidiasis from Sari, Iran. Jundishapur J. Microbiol. 8 (4), e15992.

Hu, Y., Yu, A., Chen, X., Wang, G., Feng, X., 2015. Molecular characterization of *Candida africana* in genital specimens in Shanghai, China. BioMed Res. Int. 2015, 185387.

Khorsand, I., Ghanbari Nehzag, M.A., Zarrinfar, H., Fata, A., Naseri, A., Badiee, P., Najafzadeh, M.J., 2015. Frequency of variety of *Candida* species in women with *Candida* vaginitis referred to clinical centers of Mashhad, Iran. Iran. J. Obstet. Gynaecol. Infertil. 18 (168), 15–22.

Kord, Z., Fata, A., Zarrinfar, H., 2017. Molecular Identification of *Candida* species isolated from patients with vulvovaginitis for the first time in Mashhad. Iran. J. Obstet. Gynaecol. Infertil. 20 (4), 50–57.

Majdabadi, N., Falahati, M., Heidarie-Kohan, F., Farahyar, S., Rahimi-Moghaddam, P., Ashrafi-Khozani, M., Razavi, T., Mohammadnejad, S., 2018. Effect of 2-phenylethanol as antifungal agent and common antifungals (amphotericin B,

fluconazole, and itraconazole) on *Candida* species isolated from chronic and recurrent cases of candidal vulvovaginitis. Assay Drug Dev. Technol. 16 (3), 141–149.

Merseguel, K.B., Nishikaku, A.S., Rodrigues, A.M., Padovan, A.C., e Ferreira, R.C., de Azevedo Melo, A.S., Briones, M.R., Colombo, A.L., 2015. Genetic diversity of medically important and emerging *Candida* species causing invasive infection. BMC Infect. Dis. 15, 57.

Mukasa, K.J., Herbert, I., Daniel, A., Sserunkuma, K.L., Joel, B., Frederick, B., 2015. Antifungal susceptibility patterns of vulvovaginal *Candida* species among women attending Antenatal clinic at Mbarara Regional Referral Hospital. South West. Uganda 5 (4), 322–331.

Ngouana, T.K., Krasteva, D., Drakulovski, P., Toghueo, R.K., Kouanfack, C., Ambe, A., Reynes, J., Delaporte, E., Boyom, F.F., Mallie, M., 2015. Investigation of minor species *Candida africana, Candida stellatoidea* and *Candida dubliniensis* in the *Candida albicans* complex among Yaoundé (Cameroon) HIV-infected patients. Mycoses 58 (1), 33–39.

Nnadi, N.E., Ayanbimpe, G.M., Scordino, F., Okolo, M.O., Enweani, I.B., Criseo, G., Romeo, O., 2012. Isolation and molecular characterization of *Candida africana* from Jos, Nigeria. Med. Mycol. 50 (7), 765–767.

Odds, F.C., Bougnoux, M.E., Shaw, D.J., Bain, J.M., Davidson, A.D., Diogo, D., Jacobsen, M.D., Lecomte, M., Li, S.-Y., Tavanti, A., 2007. Molecular phylogenetics of *Candida albicans*. Eukaryot. Cell 6 (6), 1041–1052.

Pelletier, R., Peter, J., Antin, C., Gonzalez, C., Wood, L., Walsh, T.J., 2000. Emergence of resistance of *Candida albicans* to clotrimazole in human immunodeficiency virus-infected children: in vitro and clinical correlations. J. Clin. Microbiol. 38 (4), 1563–1568.

Rodriguez-Leguizamon, G., Fiori, A., Lopez, L.F., Gomez, B.L., Parra-Giraldo, C.M., Gomez-Lopez, A., Suarez, C.F., Ceballos, A., Van Dijck, P., Patarroyo, M.A., 2015. Characterising atypical *Candida albicans* clinical isolates from six third-level hospitals in Bogota, Colombia. BMC Microbiol. 15, 199.

Romeo, O., Criseo, G., 2008. First molecular method for discriminating between *Candida africana, Candida albicans*, and *Candida dubliniensis* by using hwp1 gene. Diagn. Microbiol. Infect. Dis. 62 (2), 230–233.

Romeo, O., Criseo, G., 2009. Molecular epidemiology of *Candida albicans* and its closely related yeasts *Candida dubliniensis* and *Candida africana*. J. Clin. Microbiol. 47 (1), 212–214.

Romeo, O., Criseo, G., 2011. *Candida africana* and its closest relatives. Mycoses 54 (6), 475–486.

Romeo, O., Tietz, H.-J., Criseo, G., 2013. *Candida africana*: is it a fungal pathogen? Curr. Fungal Infect. Rep. 7 (3), 192–197.

Shan, Y., Fan, S., Liu, X., Li, J., 2014. Prevalence of *Candida albicans*-closely related yeasts, *Candida africana* and *Candida dubliniensis*, in vulvovaginal candidiasis. Med. Mycol. 52 (6), 636–640.

Sullivan, D.J., Moran, G.P., Coleman, D.C., 2005. *Candida dubliniensis*: ten years on. FEMS Microbiol. Lett. 253 (1), 9–17.

Theill, L., Dudiuk, C., Morano, S., Gamarra, S., Nardin, M.E., Méndez, E., Garcia-Effron, G., 2016. Prevalence and antifungal susceptibility of *Candida albicans* and its related species *Candida dubliniensis* and *Candida africana* isolated from vulvovaginal samples in a hospital of Argentina. Rev. Argent. Microbiol. 48 (1), 43–49.

Tietz, H.J., Hopp, M., Schmalreck, A., Sterry, W., Czaika, V., 2001. *Candida africana* sp. nov., a new human pathogen or a variant of *Candida albicans*? Mycoses 44 (11-12), 437–445.

Yazdanparast, S.A., Khodavaisy, S., Fakhim, H., Shokohi, T., Haghani, I., Nabili, M., Gholami, H., Ahmadi, I., Badali, H., 2015. Molecular characterization of highly susceptible *Candida africana* from vulvovaginal candidiasis. Mycopathologia 180 (5-6), 317–323.

Zarrinfar, H., Kaboli, S., Dolatabadi, S., Mohammadi, R., 2016. Rapid detection of *Candida* species in bronchoalveolar lavage fluid from patients with pulmonary symptoms. Braz. J. Microbiol. 47 (1), 172–176.