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Vulvovaginal Candidiasis In Pregnant Women

Nawal Abdulwahhab Alfaytouri[1], Mohamed Ahmed Al-Ryani[2*], Mahmoud F. Gaballa[3], Idress Hamad Attitalla[4]

[1]*Department of Botany, Faculty of Science, Sabratha University.*

[2*]*Medicine Faculty, Zintan University.*

[3,4,] *Department of Microbiology, Science Faculty, Omar Al-Mukhtar University AL-Bayda, Libya*

***Corresponding author E-mail:** profdrnani@gmail.com

Abstract:

This study aims to analyzing demographic data of patients, isolate and identify *Candida* species, which causes vaginal infections, and a study of its prevalence among pregnant women in Sorman city, Libya.210 specimens collected from patients admitted to the Maternity Care Center in the combined clinic. carried immediately to the Microbiology Laboratory in the National Cancer Institute, Subrata, Libya for direct microscopy, culturing, and characterization. Each participant was given an interview questionnaire and asked about their age, educational level, employment position, and history of recurrent vaginal yeast infection. Chronic diseases were also listed on the data collecting form. Identification of *Candida* species using Chrome agar: A total of 100 isolates have been recovered in this study, of which 72isolates were obtained as pure cultures on Chrome agar medium. According to their color on Chrome agar, these 72 colonies were categorized to 5 main species namely *Candida albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis*. Genotypic identification of *Candida* species in this investigation was validated by the ITS tree. Eight strains from this investigation were found in the *Candida albicans* clade, which had a high bootstrap value of 99 percent ML/99 percent MP. These were therefore identified as *Candida albicans*., Within the *Candida glabrata* clade, three isolates were grouped together, demonstrating a strong support value of 99% ML/99%MP. These strains were recognized as belonging to the *C. glabrata* species, while one isolate was recognized as belonging to the *C. tropicalis* species, with a high support value of 99% ML/99%MP.

Keywords:

VVC infections, candidemia patients, genotypic identification, and *Candida albicans*

Introduction:

The vagina contains a variety of microbe species (microbiota) that are beneficial to vaginal health. A diversity of species that develop into aerobic and anaerobic microbes make up the vaginal ecosystem. (**Mendling., 2016.; Amabebe and Anumba., 2018.; Kovachev., 2018**). The vaginal microbiome (VMB), which consists of both aerobic and anaerobic microbes, is crucial for maintaining vaginal health and avoiding STDs (STIs). The vaginal canal is home to more than 50 different types of bacteria, according to recent studies (**Mansour *et al.*, 2011**).Numerous endogenous and exogenous factors, including those brought on by hormones, menstrual cycles, social interactions, antibiotic therapy, antifungal use, contraceptive use, vaginal douching, menopause, pregnancy, and lactation, diabetes mellitus, stress, and immunosuppressive conditions, can alter the composition of VMB. It was shown that, the integrity of the mucosal surface protective layer was significantly impacted by variations in the quantity of normal VMB colonies throughout the course of the preceding rounds (**Blekhman *et al.*, 2015.; Petrova *et al.*, 2015**). Imbalance of VMB colonies and their effects on sexually transmitted diseases (STIs) such as bacterial vaginosis, vulvovaginal candidiasis, gonorrhea, trichomoniasis, Chlamydia trachomatis infection, herpes simplex virus (HSV), human papillomavirus (HPV), and HIV-related vaginosis (HIV) (**Mansour *et al.*, 2011.; Mendling., 2016.; Amabebe and Anumba., 2018**). The VMB is essential for maintaining vaginal health. The investigation of VMB colonization and infectious diseases is still in its early stages. These investigations show a dysbiosis of VMB, which can lead to STDs (STIs). Therefore, deepening our understanding of it can provide us with essential knowledge about VMB and its effects on STI disease transmission (**Mendling., 2016.; Amabebe and Anumba 2018.; Kencana and Yenny., 2022**).Yeasts are part of the normal microbiota of the vagina **(Underhill and Iliev., 2014)**. However, some species display an opportunistic behavior shifting from a non-pathogenic commensal microorganism to a tissue colonizing fungus leading to a variety of clinical manifestations in patients with predisposing factors **(Littman and Pamer., 2011)**. A vaginal yeast infection usually commences when a change in the delicate balance in a woman's system occurs, as a result of pregnancy, diabetes or drug use like antibiotic or corticosteroid treatment **(Parker and Parker., 2002)**.Vaginal thrush has been documented for almost a century, when Wilkinson in (1849) described the first infection with VVC and contrasted the prevalence of fungus and vaginal secretions, observing that vaginitis was the most prevalent disease in women of reproductive age **(Vazquez and Sobel., 2002.; Kamath *et al.*, 2014)**.Pathogenic *Candida* species are responsible for a wide range of superficial, systemic, and profound clinical symptoms that comprise candidiasis. Recent epidemiologic alterations in causative *Candida* species reflect the influence of many antimycotic medication groups or classes, as well as a rise in at-risk and immune compromised hosts **(`Sobel., 2015)**. The distribution of species has shifted during the last few decades. Whereas *Candida albicans* was formerly the dominant pathogen, it now accounts for barely half of the isolates reported in several studies. *C. glabrata* has developed as a significant pathogen in northern Europe, the United States, and Canada, whereas *C. parapsilosis* is more common in southern Europe, Asia, and South America. Given the variations in sensitivity to azoles and echinocandins across various species, changes in species distribution may influence treatment recommendations. The pathogenicity of *Candida* species varies greatly. *C. parapsilosis* and *C. krusei* are less pathogenic than *Candida albicans*, *Candida tropicalis*, and *Candida glabrata* (**Arendrup *et al.*, 2002**). This diversity is reflected in the low death rate among *C. parapsilosis* candidemia patients, as well as the fact that infection with *C. krusei* is extremely rare, especially in individuals with severe immunodeficiency and prior azole exposure (**Arendrup *et al.*, 2011**).

Candida albicans, an opportunistic human pathogenic fungus, is part of the natural microbiota on mucosal surfaces in humans (oral cavity, gastrointestinal system, and genitals) **(Odds., 1987.; Naglik *et al.*, 2011)**. *C. albicans*, on the other hand, is one of the most prevalent causes of

fungal infections, including oral or vulvovaginal candidiasis (VVC)**(Calderone and Fonzi., 2001.; Mayer *et al.*, 2013)**.There are several reports recording differences in the types of *Candida* causing VVC and their antifungal drug susceptibility patterns from different geographic locations **(Richter *et al.*, 2005)**. *C.albicans* has been reported to be responsible even for 90% of VVC infections **(Wang *et al.*, 2016)**. In the past decades, the incidence of infections caused by NCA species has increased significantly, especially recurrent infections **(Salari *et al.*, 2016)**.

In this study we aims toanalyzing demographic data of patients, isolate and identify *Candida* species, which causes vaginal infections, and a study of its prevalence among pregnant women in Sorman city, Libya.

Patients and Methods:

Collection of samples: This study included 210 specimens from vaginal yeast infections collected from patients admitted to the Maternity Care Center in the combined clinic, Surman, Libya, during the period from June to December2021.Samples were taken by sterile swabs under complete aseptic precautions in sterile containers from the patient's vagina. These samples were handled with caution to avoid sample contamination or dissemination of infectious agents and carried immediately to the Microbiology Laboratory in the National Cancer Institute, Subrata, Libya for direct microscopy, culturing, and characterization. Each participant was given an interview questionnaire and asked about their age, educational level, employment position, and history of recurrent vaginal yeast infection. Chronic diseases were also listed on the data collecting form.

Direct microscopic examination (DME): From each vaginal swab, two slides were prepared, one stained with Gram stain (GS) and the other with lactophenol cotton blue (LPCB) stain. The light microscope was used to examine the slides at 40x and 100x magnifications. Positive yeast infection findings were obtained when samples showed budding yeast cells with or without pseudohyphae.

Culturing of specimens: To identify mixed infections, swabs were streaked over the surface of Sabouraud's dextrose agar (SDA) and HiCrome*Candida* agar (HiMedia Company, India). For 24-96 hours, cultures were incubated at 37^{o}C. A microscopic inspection of colony development was performed to establish the existence of fungal units. The developing fungi were kept in SDA slants for further study.

Identification methods: The identification scheme was created utilizing both conventional and genetic approaches for all isolated yeasts from specimens.

Traditional methods: Traditional methods used included germ tube testing, chlamydospore production on corn meal agar, and yeast growth on special chromogenic medium.

Culturing on HiCrome*Candida* agar: HiCrome*Candida* differential agar (HiMedia Company, India) is a selective and differential medium that allows for the rapid isolation of yeasts from mixed cultures and the differentiation of *Candida* species such as *Candida albicans*, *Candida krusei* (*Issatchenkiaorientalis*), *Candida tropicalis*, and *Candida glabrata* based on coloration and colony morphology. The results are received in 48 hours and are useful for the quick and preliminary identification of common yeasts in Mycology and Clinical Microbiology Laboratories.*C. albicans* has light green-colored smooth colonies, while *C. tropicalis* has blue to metallic blue-colored elevated colonies, according to the manufacturer. Colonies of *C. glabrata* have a creamy to white smooth appearance, whilst those of *Issatchenkiaorientalis* have a purple fuzzy appearance.

Genotypic identification: A number of yeast isolates were identified using genotypic identification based on sequencing of the internal transcribed spacer (ITS) region. Molecular identification of isolated yeasts was done with the help of SolGent Company, South Korea. Some yeast isolates were selected and individually grown on SDA and incubated at 28°C for three days.

DNA extraction: A small amount of 48-hour-old yeast culture grown on SDA was scraped and suspended in 200 µl of sterile distilled water in 2ml sterile vials and boiled at 100°C for 15 minutes **(Deak *et al.*, 2000**). A 800 µl CTAB buffer composed of 3 % CTAB, 1.4 M NaCl, 0.2 % Mercaptoethanol, 20 mM EDTA, 100 mM TRIS-HCl pH 8.0 and 1 % PVP-40, were added to each tube. After incubation at 65 °C for 30 min, 800 µl of CI Mix with the composition of 24 ml chloroform and 1 ml isoamyl alcohol, were gently added and mixed with the tube contents. A clear supernatant was obtained by centrifugation at 10000 xg for 10 min. For DNA precipitation 2/3 volume of isopropanol (precooled at -20 °C) was added and mixed gently. The samples were incubated at 4 °C overnight, thereafter centrifugation at 13000 xg for 10 min. The supernatant was discarded and the pellet was pooled and washed with 200 µl washing buffer composed of 76 % ethanol and 10 mM ammonium acetate. The washing buffer was carefully decanted and the pellet was suspended in 200 µl TE buffer supplemented with 10 mg/ml RNase. After incubation at 37 °C for 30 min, 100 µl of 7.5 M ammonium acetate and 750 µl ethanol were added and mixed gently. Samples were centrifuged at 13000 xg for 10 min at room temperature. The supernatant was completely discarded and the pellet was suspended in 100 µl sterile distilled water.

PCR for rDNA and sequencing using ITS1 and ITS4 primers: The universal primers ITS1 and ITS4 (**White *et al.* 1990**) were used for DNA amplification. In the PCR tubes 1µl of DNA template, 1 µl 2.5 mMdNTP mix, 0.2 unit of Taq polymerase, 5 µl of 10x complete buffer and 40 µl of sterile ddH2O, 10 pmol of ITS1 (5´ TCC GTA GGT GAA CCT TGC GG 3´) and ITS4 (5´ TCC TCC GCT TAT TGA TAT GC 3´) were added. Then the PCR amplification was carried out using the following sequence: one round of amplification consisting of denaturation at 95 °C for 15 min followed by 30 cycles of denaturation at 95 °C for 20 sec, annealing at 50 °C for 40 sec and extension at 72 °C for 1 min, with a final extension step of 72 °C for 5 min. The PCR products were then purified with the SolGent PCR Purification Kit-Ultra (SolGent, Daejeon, South Korea) prior to sequencing. The purified PCR products were confirmed on 1% agarose gel by electrophoresis using size marker. The bands were eluted and sequenced in the forward and reverse directions.

Alignment and phylogenetic analysis: Contiguous sequences of *Candida* speciesin this study were uploaded to GenBank and accession numbers were given. Sequences of the nearest species belonging to genus *Candida*were downloaded from GenBank including sequences of the available type specimens. Sequence of *Nematosporavalgi*CBS 12562 was used as an out group. All sequences in this analysis were aligned together using MUSCLE with the default options (**Edgar., 2004**).

Alignment gaps and parsimony uninformative characters were optimized manually. Maximum-likelihood (ML) and Maximum parsimony (MP) phylogenetic analyses were performed using MEGA X version 10.2.6 **(Kumar *et al.*, 2018**). The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (**Felsenstein., 1985**).The best optimal model of nucleotide substitution for the ML analyses wasdetermined using akaike information criterion (AIC) as implemented in Modeltest 3.7 (**Posada and Crandall., 1998**). The phylogenetic tree was drawn and visualized using MEGA X(**Kumar *et al.*, 2018**). The resulting tree was edited using Microsoft Power Point (2016) and saved as TIF file(Al-Bedak et al.,2020).

Results:

Demographic data analysis: Patients varied in age from 17 to 53, with a 35-year average. All of the patients were from Surman, Libya, and two of them were illiterate and 208 were educated. 101 of the patients were teachers, making up 48.1% of the total, followed by 68 housewives, who made up 32.4% of the total, and the remaining (41 women) worked as students (20), doctors (4), employees (4), lab technicians (4), nurses (3), professors (2), dentists (2), coiffeurs (1), and pharmacists (1). From 1-9 months, all patients became pregnant.152 women do not used the vaginal douche, compared to 52 patients who have, and 63 individuals underwent one to four abortions, whereas 147 did not.The great majority of patients (183) do not have any chronic conditions, but 27 did, including those with chest allergies (7), hyperthyroidism (7),diabetes (4), high blood pressure during pregnancy (3), heart disease (1), asthma (1), rheumatism (1), gluten allergy (1), gestational diabetes (1), and milk allergy (1). (Table 1).

Isolation of *Candida* species: During the present study, it was possible to examine 210 patients complaining of yeast vaginal infections. Mycological analysis of all collected samples revealed the isolation of 100 strains belonging to genus *Candida*.

Identification of *Candida* species using Chrome agar: A total of 100 isolates have been recovered in this study, of which 41isolates were obtained as pure cultures on Chrome agar medium. According to their color on Chrome agar, these 41 colonies were categorized to 4 main species namely *Candida albicans, C. glabrata, C. krusei,* and*C. parapsilosis*,While 59 positive cases were of mixed cultures (Table 2).

Molecular identification of *Candida* species: In the ITS analysis, 26 sequences were used, including 12 sequences from the study's *Candida* species, 13 sequences from the most comparable *Candida* species in GenBank, and one sequence from *Nematodosporavalgi* CBS 12562, which was used as the outgroup. The resulting dataset had 513 characters in total, 376 of which could be aligned without any ambiguity, 156 of which were classified as variable characters, and 125 of which were classified as informative characters. The identification of *Candida* species in this investigation was validated by the ITS tree. Eight strains from this investigation were found in the *Candida albicans* clade, which had a high bootstrap value of 99 percent ML/99 percent MP. These were therefore identified as *Candida albicans*. They were deposited as *Candida albicans* AUMC 15689, AUMC 15690, AUMC 15693, AUMC 15694, AUMC 15697, AUMC 15698, AUMC 15699, and AUMC 15700 in the culture collection of the Assiut University Mycological Centre (AUMC). Their ITS region sequences were submitted to GenBank with the accession codes ON626464, ON626465, ON626466, ON626470, ON626475, ON626476, ON626477, and ON626478, respectively (Figure 1). Within the *Candida glabrata* clade, three isolates were grouped together, demonstrating a strong support value of 99% ML/99%MP. These strains were recognized as belonging to the *C. glabrata*species, while one isolate was recognized as belonging to the *C. tropicalis* species, with a high support value of 99% ML/99% MP (Figure 1).

Discussion:

In the current study, 210 women, from Surman, Libya were clinically diagnosed with VVC, of which 71 revealed positive direct microscopic examination (DME) accounting for 33.8 % of the total. Budding cells and/or pseudohyphae in the examined samples were considered as indications of the positive results. Culturing on specific media demonstrated positive results in 100 cases constituting 47.62 % of the total cases.

In this investigation, the most common species isolated from women with VVC, was *C. albicans*. *Candida albicans* predominated in most cases. The development of *C. albicans* may be explained by the presence of vaginal cell receptors, which make it easier for this particular species of *Candida* to attach to the vaginal mucosa, allowing them to germinate and change from pathogenic blastospores to filamentous forms **(Grigoriou *et al.*, 2006; Sobel., 2014)**.Additional virulence factors that contribute to *C. albicans* are the exoenzymes including lipase and protease that help in making *C. albicans*the most prevalent types in vaginal mycobiota**(Deorukhkar *et al.*, 2014a)**.Additionally, non-*Candida albicans* (NCA) contributed to roughly 20.0 % of all cases, according to the data from the current study. As a result, several investigations in recent decade have noted a gradual shift in the prevalence of yeast infections away from *C. albicans* and towards NCA species **(Bajwa and Kulshrestha., 2013)**. Accordingly, comparable investigations carried out in Egypt found that the frequency of NCA ranged from 21.7 % to 54.8 % **(Moharram *et al.*, 2013; Shaaban *et al.*, 2015; El-Feky *et al.*, 2016)**, and reached 53.0% in India **(Ahmad and Khan., 2009)**. *Candida glabrata*, *C. krusei*, *C. parapsilosis*, and *C. tropicalis* were isolated from VVC less often after *C. albicans*in the current investigation. In this regard, numerous investigations have demonstrated that these species coexisted with *C. albicans* in vaginal yeast infections, which is consistent with the present findings **(Mohanty *et al.*, 2007; Rad *et al.*, 2011; Seifi *et al.*, 2015)**. The incidence of VVC varies amongst nations in general, with a range of incidence between 20.0 % and 75.0 % **(Costa *et al.*, 2004; Ahmad and Khan 2009; Nwadioha *et al.*, 2010; Guzel *et al.*, 2011; Mahmoudabadi *et al.*, 2012)**. However, according to many studies conducted in Egypt, the prevalence of VVC infections ranged from 49 % to 64 % **(Moharram *etal.*, 2013; Shaaban *et al.*, 2015; El-Feky *et al.*, 2016)**. According to reports, one of the reasons of recurring VVC and antifungal drug resistance is NCA infection **(Shaaban *et al.*, 2015)**. In Egypt, samples taken from long-term steroid users who complained of vulvovaginal candidiasis included 48.0 % of NCA**(Shriefet *al.*, 2019)**. Nearly the same percentage of NCA (47.1%) was observed among HIV-positive women in Brazil **(Oliveira *et al.*, 2011)**. This high incidence of NCA may be explained by the fact that chronic steroid users typically have more frequent VVC episodes that need for more frequent empirical antifungal therapy, which contributes to the switch from *C. albicans* to NCA forms of yeast infections **(Bassetti *et al.*, 2006; dos Santos Abrantes *et al.*, 2014)**. Additionally, frequent use of antifungal medication alters colonization patterns, enabling a rise in the prevalence of NCA species in appreciable numbers **(Sobel., 2006)**.

Table 1:Demographic data analysis of specimens collected from patients having vaginal yeast infections

No.	Age	Job	Education	DME		Culture	Pregnant (month)	Number of children	Abortion	Vaginal douche	Chronic disease
				GS	CB						
1	47	Teacher	Diploma	-	-	-	4	6	-	-	-
2	43	Teacher	License	-	-	+	7	4	-	-	-
3	35	Doctor	Bachelor	+	+	+	1	2	-	-	-
4	30	Teacher	License	-	-	-	2	1	-	-	-
5	43	Teacher	License	-	-	+	7	5	-	-	-
6	38	Teacher	License	+	-	-	9	5	-	-	-
7	21	Student	University	-	-	-	7	0	-	-	-
8	36	Nurse	Diploma	-	-	-	4	2	1	-	-
9	25	Housewife	Diploma	+	-	-	9	0	-	-	-
10	38	Lab technician	Bachelor	-	-	-	9	7	-	-	-
11	25	Teacher	License	-	-	-	9	6	3	+	-
12	21	Student	University	-	-	-	7	0	1	-	-
13	36	Teacher	Bachelor	-	-	-	4	5	1	-	-
14	26	Housewife	Illiterate	+	+	+	2	1	-	-	-
15	22	Student	University	+	+	+	7	0	-	-	-
16	35	Teacher	Bachelor	+	+	+	7	3	-	-	-
17	22	Student	University	-	-	+	6	1	-	-	-
18	34	Doctor	Bachelor	-	-	-	4	2	-	-	-
19	28	Housewife	License	+	+	+	8	1	-	-	Diabetes
20	21	Student	University	-	-	-	4	0	-	-	-
21	30	Teacher	License	-	-	-	1	3	4	-	-
22	32	Teacher	Bachelor	-	-	-	7	4	1	+	-
23	24	Housewife	Diploma	-	-	-	1	2	1	-	-
24	20	Student	University	-	-	+|	3	1	-	-	-
25	34	Teacher	License	+	+	+	8	4	-	-	-
26	31	Teacher	License	-	-	-	3	1	-	-	-
27	27	Teacher	Bachelor	-	-	-	1	1	-	-	-
28	21	Student	University	-	-	-	5	1	-	-	-
29	32	Teacher	License	+	+	+	7	3	-	-	-

No.	Age	Job	Education	DME		Culture	Pregnant (month)	Number of children	Abortion	Vaginal douche	Chronic disease
				GS	CB						
30	22	Housewife	Diploma	-	-	-	2	0	-	-	-
31	34	Teacher	Bachelor	-	-	-	7	3	2	+	-
32	30	Teacher	Bachelor	-	-	+	9	2	-	-	-
33	27	Teacher	Diploma	-	-	-	5	1	-	-	-
34	24	Housewife	License	-	-	-	6	0	-	-	-
35	34	Teacher	License	-	-	-	2	3	-	-	-
36	22	Student	University	+	+	+	7	0	-	+	-
37	34	Teacher	Bachelor	+	+	+	4	4	1	-	-
38	23	Housewife	Bachelor	-	-	-	4	0	-	-	-
39	19	Student	Secondary	+	+	+	9	0	-	+	-
40	24	Housewife	Bachelor	-	-	-	6	3	-	+	-
41	23	Housewife	Bachelor	-	-	+	6	2	1	+	-
42	29	Housewife	Bachelor	+	+	+	9	2	1	-	-
43	27	Dentist	Doctor	-	-	-	9	1	1	-	-
44	26	Teacher	License	-	-	+	9	0	-	+	-
45	40	Housewife	Bachelor	+	+	+	6	3	1	-	-
46	40	Teacher	Bachelor	-	-	-	1	1	1	-	Heart disease
47	31	Housewife	Diploma	-	-	-	9	1	1	-	-
48	32	Housewife	Bachelor	-	-	-	5	1	1	+	-
49	42	Teacher	License	-	-	-	8	4	-	-	-
50	39	Teacher	Bachelor	-	-	-	7	4	-	+	-
51	34	Teacher	Bachelor	-	-	-	8	1	-	+	-
52	30	Professor	Master	-	-	-	2	1	1	-	-
53	34	Teacher	Bachelor	-	-	-	6	4	-	-	Hyperthyroidism
54	32	Doctor	Bachelor	-	-	-	9	1	-	-	Chest allergy
55	38	Teacher	License	-	-	-	2	3	-	-	-
56	38	Teacher	Bachelor	+	+	+	8	5	-	-	Chest allergy
57	28	Teacher	Diploma	-	+	+	9	0	-	-	-
58	27	Housewife	Preparatory	-	-	-	5	2	-	+	Chest allergy
59	24	Student	Diploma	+	+	+	5	1	2	+	-
60	35	Housewife	Bachelor	-	-	-	2	3	-	-	-
61	35	Housewife	Illiterate	-	-	-	5	6	2	-	Hyperthyroidism

No.	Age	Job	Education	DME		Culture	Pregnant (month)	Number of children	Abortion	Vaginal douche	Chronic disease
				GS	CB						
62	45	Teacher	License	-	-	+	1	3	2	-	-
63	27	Teacher	Bachelor	+	+	+	7	3	-	-	-
64	44	Coiffeur	Primary	-	-	-	2	3	-	+	High pressure pregnancy
65	27	Housewife	Bachelor	+	+	+	2	2	-	-	-
66	39	Teacher	License	-	-	-	9	1	-	-	-
67	30	Housewife	Bachelor	+	-	+	8	2	-	+	-
68	35	Teacher	Bachelor	+	+	+	4	1	-	-	-
69	27	Housewife	Secondary	+	+	+	3	3	-	-	-
70	32	Teacher	Diploma	-	-	+	4	2	1	+	-
71	28	Teacher	Bachelor	-	-	-	4	1	-	+	-
72	31	Teacher	License	-	-	-	8	4	-	+	-
73	31	Teacher	Bachelor	+	+	+	4	2	-	+	-
74	27	Housewife	Diploma	-	-	-	5	2	3	+	-
75	24	Teacher	Diploma	-	-	-	4	3	-	-	-
76	23	Housewife	License	-	-	-	1	0	-	-	-
77	27	Housewife	Bachelor	-	-	-	6	2	-	+	-
78	30	Housewife	Bachelor	-	-	+	8	1	-	-	-
79	35	Teacher	Bachelor	-	-	+	2	4	3	-	-
80	29	Teacher	Diploma	-	-	-	1	0	-	-	-
81	34	Teacher	License	-	-	-	9	2	-	-	-
82	27	Teacher	License	-	-	+	7	2	1	+	-
83	34	Housewife	Bachelor	-	-	-	5	6	1	-	-
84	29	Housewife	Bachelor	-	-	-	7	3	2	-	-
85	36	Teacher	License	-	-	-	7	3	1	-	-
86	40	Teacher	Bachelor	-	-	+	7	5	-	+	-
87	30	Housewife	Diploma	+	+	+	9	2	-	-	-
88	33	Teacher	Bachelor	+	-	+	9	2	-	-	-
89	40	Housewife	Diploma	+	+	+	8	4	-	+	-
90	36	Pharmacist	Master	-	-	-	7	4	3	-	-
91	30	Teacher	License	-	-	-	4	3	-	-	Asthma
92	28	Housewife	License	-	-	-	6	2	-	+	Chest allergy

No.	Age	Job	Education	DME		Culture	Pregnant (month)	Number of children	Abortion	Vaginal douche	Chronic disease
				GS	CB						
93	30	Teacher	License	-	-	+	2	2	3	-	Hyperthyroidism
94	32	Teacher	Bachelor	-	+	+	4	5	-	-	-
95	34	Teacher	License	+	+	+	1	3	1	-	-
96	33	Housewife	Diploma	-	-	+	6	3	2	-	-
97	26	Housewife	Bachelor	+	+	+	5	2	-	-	-
98	31	Employee	Diploma	-	-	+	5	2	1	-	-
99	30	Housewife	Diploma	+	+	+	1	3	2	+	-
100	27	Housewife	Diploma	+	-	+	4	2	1	-	High pressure pregnancy
101	30	Housewife	Bachelor	-	-	-	3	3	4	+	-
102	24	Teacher	Bachelor	-	-	-	8	0	-	+	-
103	35	Teacher	License	+	+	+	4	6	-	-	-
104	34	Professor	Master	+	+	+	9	3	-	-	Hyperthyroidism
105	36	Teacher	License	+	+	+	6	3	2	-	-
106	51	Teacher	Diploma	-	-	+	4	6	-	-	Rheumatism
107	26	Housewife	Bachelor	+	+	+	1	1	1	-	Chest allergy
108	42	Teacher	Bachelor	+	+	+	7	6	-	+	Hyperthyroidism
109	28	Teacher	License	+	+	+	5	2	-	-	-
110	26	Housewife	Diploma	-	-	-	7	0	-	-	-
111	39	Housewife	Diploma	+	+	+	9	0	1	+	-
112	33	Lab technician	Diploma	-	-	-	4	4	-	+	-
113	30	Lab technician	Bachelor	-	-	-	5	3	-	-	-
114	23	Housewife	Preparatory	+	+	+	3	4	-	+	-
115	38	Teacher	License	-	-	-	3	3	-	-	-
116	39	Teacher	Diploma	+	+	+	3	3	1	-	-
117	39	Teacher	Bachelor	+	+	+	8	3	4	-	-
118	40	Teacher	License	+	+	+	8	3	2	+	-
119	30	Housewife	Secondary	-	-	-	4	4	1	-	-
120	27	Teacher	License	+	+	+	7	4	-	-	-
121	53	Housewife	Secondary	-	-	+	2	6	1	-	-
122	21	Housewife	License	+	+	+	2	0	-	-	-
123	36	Teacher	Bachelor	-	-	-	1	3	1	-	-

No.	Age	Job	Education	DME		Culture	Pregnant (month)	Number of children	Abortion	Vaginal douche	Chronic disease
				GS	CB						
124	33	Employee	Bachelor	+	+	+	6	3	-	-	-
125	27	Teacher	License	+	+	+	5	1	-	-	Chest allergy
126	17	Student	Secondary	-	-	-	3	0	-	+	-
127	45	Housewife	Preparatory	+	+	+	9	6	-	-	Hyperthyroidism
128	23	Housewife	License	-	-	-	5	4	-	-	-
129	38	Housewife	Secondary	+	+	+	8	8	-	-	-
130	30	Employee	Bachelor	+	+	+	4	2	-	-	-
131	37	Teacher	Diploma	+	+	+	4	5	-	-	-
132	40	Housewife	Diploma	-	-	+	5	4	2	-	-
133	40	Teacher	Diploma	-	-	-	5	5	1	-	-
134	26	Housewife	License	-	-	-	6	3	-	-	-
135	28	Housewife	Bachelor	+	+	+	5	2	-	-	-
136	24	Housewife	Diploma	-	-	-	3	1	-	+	-
137	28	Teacher	Bachelor	-	-	-	1	6	-	-	-
138	38	Teacher	Bachelor	+	+	+	9	2	1	-	Diabetes
139	25	Employee	Bachelor	+	+	+	2	3	-	-	-
140	25	Housewife	License	-	-	+	4	1	-	-	-
141	35	Teacher	Bachelor	-	-	-	7	4	-	-	-
142	32	Teacher	Bachelor	-	-	-	7	3	-	-	-
143	25	Housewife	Bachelor	+	+	+	1	1	-	+	-
144	23	Student	University	+	+	+	4	0	-	-	-
145	30	Teacher	Diploma	+	+	+	5	5	-	-	-
146	32	Teacher	Diploma	-	-	-	6	3	-	-	-
147	28	Housewife	Bachelor	-	-	-	8	2	-	-	-
148	32	Teacher	Bachelor	-	+	+	2	3	1	-	-
149	36	Teacher	Bachelor	-	-	-	3	4	-	-	-
150	27	Housewife	License	-	-	-	7	2	-	-	-
151	25	Housewife	Bachelor	+	+	+	7	1	2	-	-
152	31	Housewife	Bachelor	-	-	-	6	5	-	-	-
153	18	Student	University	-	-	-	7	0	-	+	-
154	45	Teacher	Diploma	+	+	+	8	6	2	-	-
155	35	Teacher	Bachelor	-	-	-	2	4	-	-	-

No.	Age	Job	Education	DME		Culture	Pregnant (month)	Number of children	Abortion	Vaginal douche	Chronic disease
				GS	CB						
156	27	Teacher	Diploma	+	+	+	5	2	-	-	-
157	33	Teacher	Bachelor	+	+	+	1	4	-	-	-
158	29	Dentist	Bachelor	-	-	-	2	2	-	-	-
159	25	Housewife	License	-	-	-	9	1	-	+	Gluten allergy
160	28	Housewife	Bachelor	-	+	+	1	2	-	-	-
161	21	Student	University	-	-	-	6	0	-	-	-
162	27	Housewife	Bachelor	-	-	-	6	1	-	-	-
163	18	Housewife	Preparatory	-	-	-	8	0	-	+	Gestational diabetes
164	20	Student	University	+	+	+	5	0	-	-	-
165	32	Teacher	License	-	-	-	7	2	-	-	-
166	28	Teacher	Bachelor	-	-	-	8	2	-	-	-
167	26	Teacher	Bachelor	-	-	+	9	3	-	-	-
168	28	Housewife	License	-	-	-	7	4	1	-	-
169	21	Student	University	-	-	-	1	1	-	+	-
170	17	Student	Secondary	-	+	+	7	0	-	+	Chest allergy
171	37	Teacher	Bachelor	-	-	-	7	5	1	-	-
172	30	Teacher	License	-	-	+	3	2	1	-	-
173	26	Teacher	License	-	-	-	5	3	-	+	-
174	23	Housewife	Diploma	+	+	+	2	0	-	+	-
175	23	Housewife	Diploma	-	-	-	4	1	-	+	-
176	45	Teacher	Bachelor	-	-	-	8	5	3	-	High pressure pregnancy
177	30	Teacher	Bachelor	-	-	-	3	2	-	-	-
178	25	Housewife	License	+	+	+	2	2	-	+	-
179	38	Teacher	Bachelor	-	-	-	9	4	2	-	-
180	30	Doctor	Bachelor	+	+	+	7	4	-	-	Hyperthyroidism
181	38	Lab technician	Bachelor	+	+	+	5	6	1	-	-
182	40	Teacher	Diploma	+	+	+	8	4	-	-	Diabetes
183	36	Teacher	License	-	-	-	4	4	2	-	-
184	37	Teacher	License	-	-	-	3	5	-	-	-
185	40	Nurse	Diploma	-	-	-	7	6	-	-	-

No.	Age	Job	Education	DME		Culture	Pregnant (month)	Number of children	Abortion	Vaginal douche	Chronic disease
				GS	CB						
186	33	Teacher	Diploma	+	+	+	9	5	-	-	-
187	34	Teacher	Bachelor	-	-	-	2	3	-	-	-
188	42	Teacher	License	-	+	+	6	7	1	-	-
189	33	Teacher	License	-	-	-	6	2	3	-	-
190	40	Teacher	Diploma	-	+	+	3	5	1	-	-
191	17	Student	Secondary	-	-	-	1	0	-	+	Milk allergy
192	29	Housewife	Bachelor	+	+	+	9	4	-	-	-
193	37	Teacher	Diploma	-	-	-	8	5	-	+	-
194	21	Student	University	-	-	-	6	0	-	+	-
195	36	Teacher	License	-	-	-	8	4	1	-	-
196	31	Teacher	Bachelor	-	-	-	7	3	-	-	-
197	18	Student	University	-	-	-	1	0	-	+	-
198	30	Teacher	Bachelor	+	+	+	6	4	-	-	-
199	30	Teacher	Bachelor	+	+	+	8	5	1	-	-
200	18	Housewife	Diploma	+	+	+	1	0	-	-	-
201	20	Housewife	Diploma	-	-	-	2	0	-	+	-
202	28	Teacher	Bachelor	-	+	+	5	3	-	-	-
203	23	Nurse	Diploma	-	-	-	1	0	-	-	-
204	23	Housewife	Diploma	-	-	-	8	0	-	-	-
205	28	Housewife	License	-	-	-	9	2	-	+	-
206	23	Teacher	Diploma	-	-	-	1	1	-	+	-
207	34	Teacher	License	-	-	+	4	5	-	-	-
208	20	Housewife	Diploma	+	-	+	1	0	-	-	-
209	28	Teacher	Bachelor	+	+	+	4	4	1	-	-
210	25	Housewife	Bachelor	-	-	-	3	3	-	-	-

Table 2:*Candida* species isolated in the present study.

Yeast species(Pure culture)	Authors	No. of isolates
Candida albicans	(Robin) Berkhout	26

Candida glabrata	(Anderson) Meyer & Yarrow	2
Candida krusei	(Castell.) Berkhout	12
Candida parapsilosis	(Ashford) Langeron&Talice	1
Total		**41**

Yeast species(Mixed culture)	**Authors**	**No. of isolates**
C. albicans + C. krusri		41
C. albicans + C. glabrata		5
C. albicans + C. parapsilosis		1
C. glabrata + C. krusri		8
C. glabrata + C. tropicalis	(Anderson) Meyer & Yarrow + (Castell.) Berkhout	2
C. albicans +C. glabrata + C. krusri		2
Total		**59**

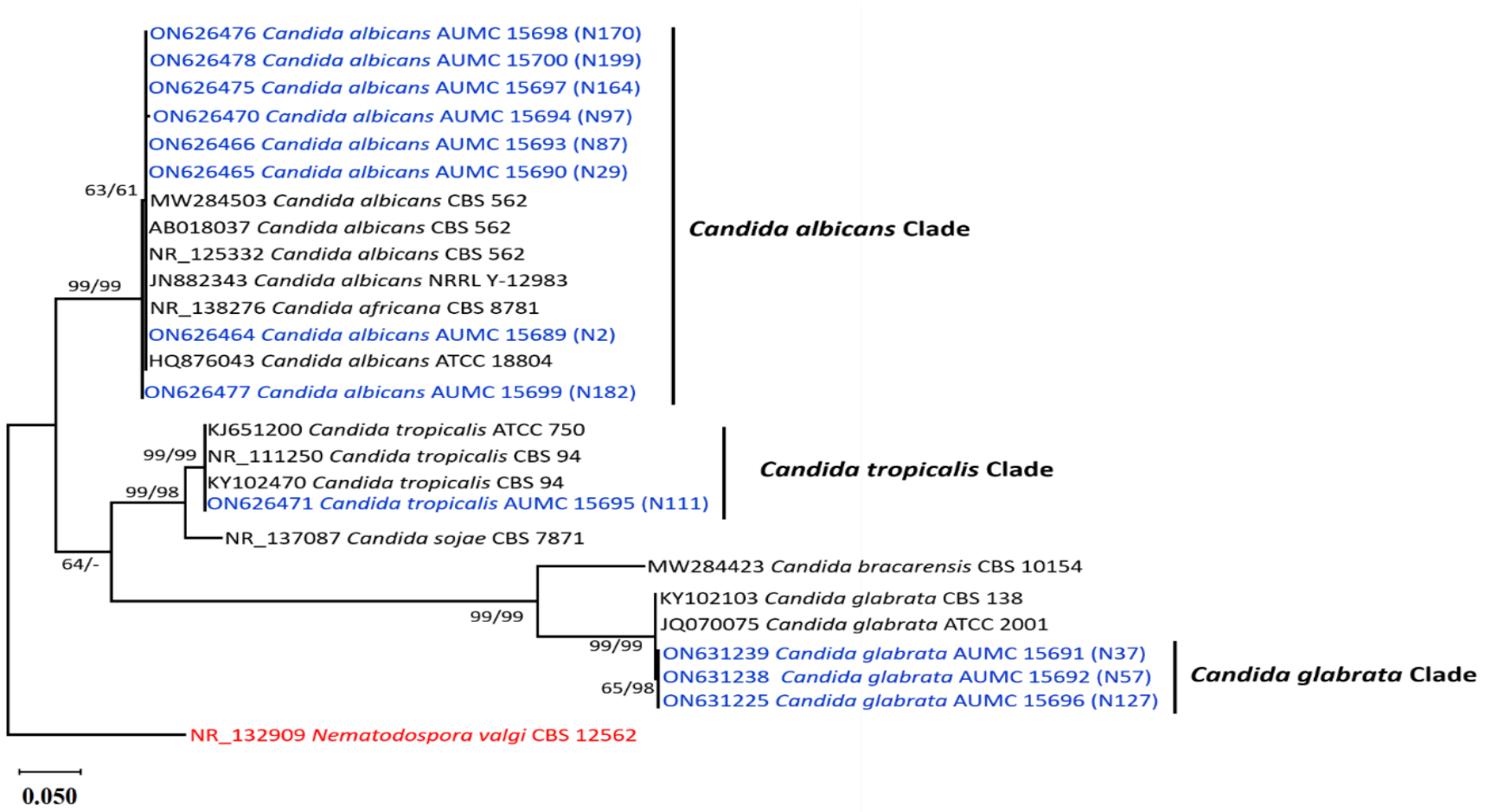

Figure 1: Maximum likelihood phylogenetic tree based on ITS sequences of *Candida* species in this study (in blue color) compared to the most similar *Candida* species in GenBank. ML/MP support value (1000 replications) are indicated above/below the respective nodes. The tree is rooted to *Nematodosporavalgi* CBS 12562 (in red color).

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References:

Ahmad A. *and* Khan AU. (2009): Prevalence of *Candida* species and potential risk factors for vulvovaginal candidiasis in Aligarh, India. Eur J Obstet Gynecol Reprod Biol. 144 (1):68-71.

Al-Bedak OA.; Teama EA.; Ali E.; Said M.; Shalaby E. *and* Moharram ZA. (2020): Impact of fumigation with phosphine on viability of wheat grains stored for six months at two levels of moisture content, in addition to description of four new records of associated fungi and assessment of their potential for enzymatic production. Journal of Basic & Applied Mycology (Egypt). 11:77-97.

Amabebe E. *and* Anumba DO. (2018): The vaginal microenvironment: the physiologic role of lactobacilli. Frontiers in medicine. 5:181.

Arendrup M.; Horn T. *and* Frimodt-Møller N. (2002): In vivo pathogenicity of eight medically relevant *Candida* species in an animal model. Infection. 30 (5):286-291.

Arendrup MC.; Sulim S.; Holm A.; Nielsen L.; Nielsen SD.; Knudsen JD.; Drenck NE.; Christensen JJ. *and* Johansen HK. (2011): Diagnostic issues, clinical characteristics, and outcomes for patients with fungemia. Journal of clinical microbiology. 49 (9):3300-3308.

Bajwa S. *and* Kulshrestha A. (2013): Fungal infections in intensive care unit: challenges in diagnosis and management. Ann Med Health Sci Res. 3 (2):238-244.

Bassetti M.; Righi E.; Costa A.; Fasce R.; Molinari MP.; Rosso R.; Pallavicini FB. *and* Viscoli C. (2006): Epidemiological trends in nosocomial candidemia in intensive care. BMC Infect Dis. 6:1-6.

Blekhman R.; Goodrich JK.; Huang K.; Sun Q.; Bukowski R.; Bell JT.; Spector TD.; Keinan A.; Ley RE. *and* Gevers D. (2015): Host genetic variation impacts microbiome composition across human body sites. Genome biology. 16 (1):1-12.

Calderone RA. *and* Fonzi WA. (2001): Virulence factors of *Candida albicans*. Trends in microbiology. 9 (7):327-335.

Costa M.; Passos XS.; Miranda ATB.; de Araújo RSC.; Paula CR. *and* Silva MdRR. (2004): Correlation of in vitro itraconazole and fluconazole susceptibility with clinical outcome for patients with vulvovaginal candidiasis. Mycopathologia. 157 (1):43-47.

Deak T.; Chen J. *and* Beuchat L. (2000): Molecular characterization of *Yarrowia lipolytica* and *Candida zeylanoides* isolated from poultry. Applied and environmental microbiology. 66 (10):4340-4344.

Deorukhkar SC.; Saini S. *and* Mathew S. (2014a): Virulence factors contributing to pathogenicity of *Candida tropicalis* and its antifungal susceptibility profile. International Journal of Microbiology https://doi.org/10.1155/2014/456878

dos Santos Abrantes PM.; McArthur CP. *and* Africa CWJ. (2014): Multi-drug resistant oral *Candida* species isolated from HIV-positive patients in South Africa and Cameroon. Diagnostic microbiology and infectious disease. 79 (2):222-227.

Edgar RC. (2004): MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic acids research. 32 (5):1792-1797.

El-Feky DS.; Gohar NM.; El-Seidi EA.; Ezzat MM. *and* AboElew SH. (2016): Species identification and antifungal susceptibility pattern of *Candida* isolates in cases of vulvovaginal candidiasis. Alexandria Journal of Medicine. 52 (3):269-277.

Felsenstein J. (1985): Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 39 (4):783-791.

Grigoriou O.; Baka S.; Makrakis E.; Hassiakos D.; Kapparos G. *and* Kouskouni E. (2006): Prevalence of clinical vaginal candidiasis in a university hospital and possible risk factors. Eur J Obstet Gynecol Reprod Biol. 126 (1):121-125.

Guzel AB.; Ilkit M.; Akar T.; Burgut R. *and* Demir SC. (2011): Evaluation of risk factors in patients with vulvovaginal candidiasis and the value of chromID Candida agar versus CHROMagar *Candida* for recovery and presumptive identification of vaginal yeast species. Med Mycol. 49 (1):16-25.

Kamath P.; Pais M.; Nayak MG. *and* D'souza CP. (2014): An awareness program on prevention of vaginal candidiasis among pregnant women. Nitte University Journal of Health Science. 4 (2):86-89.

Kencana I. *and* Yenny SW. (2022): The vaginal microbiota. Journal of General-Procedural Dermatology and Venereology Indonesia. 52-57.

Kovachev S. (2018): Defence factors of vaginal lactobacilli. Critical reviews in microbiology. 44 (1):31-39.

Kumar S.; Stecher G.; Li M.; Knyaz C. *and* Tamura K. (2018): MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution. 35 (6):1547-1549.

Littman DR. *and* Pamer EG. (2011): Role of the commensal microbiota in normal and pathogenic host immune responses. Cell Host Microbe. 10 (4):311-323.

Mahmoudabadi AZ.; Zarrin M. *and* Fard MB. (2012): Antifungal susceptibility of *Candida* species isolated from candiduria. Jundishapur J Microbiol. 6 (1):24-28.

Mansour A.; Hamed G.; Ziba O.; Sakineh F. *and* Maasomeh J. (2011): Isolation and identification of *Lactobacillus* species from the vagina and their antimicrobial properties. African Journal of Microbiology Research. 5 (20):3300-3304.

Mayer FL.; Wilson D. *and* Hube B. (2013). *Candida albicans* pathogenicity mechanisms. Virulence. 4 (2):119-128.

Mendling W (2016) Vaginal microbiota. Microbiota of the Human Body. 83-93.

Mohanty S.; Xess I.; Hasan F.; Kapil A.; Mittal S. *and* Tolosa JE. (2007): Prevalence & susceptibility to fluconazole of *Candida* species causing vulvovaginitis. Indian J Med Res. 126 (3):216-224.

Moharram A.; Abdel-Ati MG. *and* Othman E. (2013): Vaginal yeast infection in patients admitted to Al-Azhar University Hospital, Assiut, Egypt. Journal of Basic and Applied Mycology (Egypt). 4:21-32.

Naglik JR.; Moyes DL.; Wächtler B. *and* Hube B. (2011): *Candida albicans* interactions with epithelial cells and mucosal immunity. Microbes and Infection. 13 (12-13):963-976.

Nwadioha S.; Egesie JO.; Emejuo H. *and* Iheanacho E. (2010): A study of female genital swabs in a Nigerian Tertiary Hospital. Asian Pac J Trop Med. 3 (7):577-579.

Odds FC. (1987): *Candida* infections: an overview. CRC Critical Reviews in Microbiology. 15 (1):1-5.

Oliveira PM.; Mascarenhas RE.; Lacroix C.; Ferrer SR.; Oliveira RPC.; Cravo EA.; Alves APR. *and* Grassi MFR. (2011): *Candida* species isolated from the vaginal mucosa of HIV-infected women in Salvador, Bahia, Brazil. Braz J Infect Dis. 15 (3):239-244.

Petrova MI.; Lievens E.; Malik S.; Imholz N. *and* Lebeer S. (2015): *Lactobacillus* species as biomarkers and agents that can promote various aspects of vaginal health. Frontiers in physiology. 6: 81.

Posada D. *and* Crandall KA. (1998): Modeltest: testing the model of DNA substitution. Bioinformatics (Oxford, England). 14 (9):817-818.

Rad MM.; Zafarghandi S.; Abbasabadi B. *and* Tavallaee M. (2011): The epidemiology of *Candida* species associated with vulvovaginal candidiasis in an Iranian patient population. Eur J Obstet Gynecol Reprod Biol. 155 (2):199-203.

Salari S.; Bakhshi T.; Sharififar F.; Naseri A. *and* Almani PGN. (2016): Evaluation of antifungal activity of standardized extract of *Salvia rhytidea* Benth.(Lamiaceae) against various Candida isolates. J Mycol Med. 26 (4):323-330.

Seifi Z.; Mahmoudabadi AZ. *and* Zarrin M. (2015): Extracellular enzymes and susceptibility to fluconazole in *Candida* strains isolated from patients with vaginitis and healthy individuals. Jundishapur J Microbiol. 8 (3):1-5.

Shaaban OM.; Abbas AM.; Moharram AM.; Farhan MM. *and* Hassanen IH. (2015): Does vaginal douching affect the type of candidal vulvovaginal infection? Med Mycol. 53 (8):817-827.

Shrief R.; Zaki M.; El-Sehsah E. M.; Ghaleb S. *and*Mofreh M. (2019): Study of Antifungal Susceptibility, Virulence Genes and Biofilm Formation in *Candida albicans*. The Open Microbiology Journal Content list available at: https://openmicrobiologyjournal.com

Sobel JD. (2006): The emergence of non-albicans *Candida* species as causes of invasive candidiasis and candidemia. Curr Infect Dis Rep. 8 (6):427-433.

Sobel JD. (2014): Genital candidiasis. Medicine. 42 (7):364-368. doi: https://doi.org/ 10. 1016/j.mpmed. 2014.04.006

Sobel JD. (2015): Candidiasis. In: Diagnosis and Treatment of Fungal Infections. Springer, pp. 101-117.

Underhill DM. *and* Iliev ID. (2014): The mycobiota: interactions between commensal fungi and the host immune system. Nat Rev Immunol. 14 (6):405-416. doi:10.1038/nri3684

Vazquez JA. *and* Sobel JD. (2002): Mucosal candidiasis. Infectious disease clinics of North America. 16 (4):793-820.

White TJ.; Bruns T.; Lee S. *and* Taylor J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: A guide to methods and applications. 18 (1):315-32