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Vaginal yeast infection in patients admitted to Al-Azhar University Hospital, Assiut, Egypt

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Abstract: In the present study, 145 women were clinically examined during the period from December 2011 to July 2012 for vaginal yeast infection. Direct microscopy and culturing of vaginal swabs revealed that only 93 cases (64.1 %) were confirmed to be affected by yeasts. The majority of patients were 21-40 years old representing 70% of the positive cases. Yeast infection was more encountered in women receiving oral contraceptives (40%) than in those complaining of diabetes mellitus (25%) or treated with corticosteroids (17%). Phenotypic and genotypic characterization of yeast isolates showed that *Candida albicans* was the most prevalent species affecting 45.2% of patients, followed by *C. krusei* and *C. tropicalis* (20.4 % and 10.8% respectively). *C. glabrata* and *C. parapsilosis* were rare (3.3% and 1.1% respectively). *Rhodotorula mucilaginosa* and *Geotrichum candidum* occurred in 18.3% and 1.1% of vaginal samples respectively. Protease was produced by 83 out of 93 isolates tested (89.2%) with active isolates belonging to *C. albicans* and *C. krusei*. Lipase was produced by 51.6% of isolates with active producers related to *C. albicans*, *C. tropicalis*, *C. glabrata* and *C. krusei*. All isolates of *Candida* and *Geotrichum* were unable to produce urease enzyme, but those of *R. mucilaginosa* produced this enzyme. In-*vitro* sensitivity test showed that *C. albicans* isolates were sensitive to all antifungal agents (12 types) with the most effective drugs being Nystatin and Terbinafine affecting 100% of strains. Cetrimide, Fluconazole, Clotrimazole, Miconazole and Amphotericin-B affected 59.5.8% - 85.7% of the tested isolates.

Key words: vaginal candidiasis, antifungal agents, enzymes.

Introduction

Candida species are commensals of diseased skin and mucosal surfaces of the gastrointestinal, genitourinary, and respiratory tracts. Vulvovaginal candidiasis (VVC) occurs most commonly in post-pubertal women who have diabetes mellitus, have been taking systemic antibacterial agents, or in the third trimester of pregnancy, or those who are sexually active (Odds 1994). Although the evidence is contradictory, estrogen contraceptive therapy probably predisposes women to the infection. Although an intestinal reservoir or a sexual-partner with *Candida* balanitis has been said to be the source of recurrent vulvovaginitis, the best current evidence indicates that relapse comes from organisms persisting in the vagina (O'Connor *et al.* 1986 and Reed 1992). Sobel (1985) mentioned that *Candida* vaginitis is the second most common vaginal infection. During the childbearing years, 75% of women experience at least one episode of VVC, and 40%-50% of these women experience a second attack. *Candida* is isolated from the genital tract of approximately 10%-20% of asymptomatic, healthy women of childbearing age.

The present work was designed to study the prevalence of vaginal yeast infection in women admitted to Al-Azhar University Hospital in Assiut City. Possible risk factors for infection as well as the ability of isolated fungi to produce some enzymes were studied. Sensitivity or resistance of yeasts to common antifungal agents was also tested.

Materials and methods

1. Patients and sample collection: Eligible participants were women admitted to the outpatient clinic of Gynecology of Al-Azhar University Hospital, Assiut city, during the period from December 2011 to July 2012. Vaginal swabs were taken from women with proven clinical diagnosis of vaginal yeast infection. A questionnaire was also prepared to get information on age of patients as well as on possible risk factors.

2. Mycological analyses

a- Direct microscopic examination (DME): Wet and dry smears stained with lactophenol cotton blue (LPCB) were prepared. Appearance of budding cells with or without pseudohyphae under microscope indicates positive results (Ellis *et al.* 2007).

b- Culturing of specimens: Samples were streaked on the surface of Sabouraud glucose agar (SGA) supplemented with chloramphenicol (0.5 gm/l) to suppress bacterial growth. Cultures were incubated at 37°C for 24-96 h or until the

appearance of colonies. The growing fungi were kept in SGA slants for further investigation (Ellis *et al*. 2007).

c- Phenotypic identification of yeasts: CHROMagar *Candida* medium kindly supplied by the CHROMagar Company, Paris, France, has been recommended for rapid identification of many common *Candida* species (Pfaller *et al*. 1996). Yeast cultures were streaked on the medium surface and incubated at 37ºC for 48 hours. Chemical colorimetric reaction on agar allows distinction between *C. albicans* (green colonies), *C. tropicalis* (metallic blue colonies), *C. krusei* (pink-fuzzy colonies), *C. glabrata* (mauve-dark pink colonies), and *C. parapsilosis* (white-pale pink colonies) (Odds and Bemaerts 1994). Yeasts were also cultured on corn meal agar (CMA) amended with Tween 80 for 4-7 days to ensure production of chlamydospores (Ellis *et al*. 2007). The ability of *Candida* species to grow on SGA at 45°C was studied in an attempt to differentiate the *C. albicans* isolates from the *C. dubliniensis* isolates (Pinjon *et al.* 1998). Germ tube (GT) production was also tested by inoculating yeasts in small tubes containing 0.5 ml of human serum (male AB plasma, Sigma-Aldrich, Steinem, Germany) containing 0.5 % glucose and incubated at 37ºC for 2-3 hours (Ellis *et al.* 2007). API *Candida* strips (Biomuriex) which allow the performance of 12 identification tests were used to confirm identification of *Candida* species (Bernal *et al.* 1998).

d- Genotypic identification of yeast isolates: Nucleotide sequencing of rRNA genes of some yeast species was done with the help of Solgent Company, Daejeon, South Korea. Primers used for gene amplification have the following composition: ITS1 (5' - TCC GTA GGT GAA CCT GCG G - 3'), and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC - 3'). Then the amplification was carried out in a thermal cycler under the following conditions: one round of denaturation at 95°C for 15 sec 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 at 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. Purified PCR products were reconfirmed (using size marker) by electrophoreses on 1% agarose gel. Then the bands were eluted and sequenced with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture. Each sample was sequenced in the sense and antisense directions using ITS1 and ITS4 primers (White *et al.* 1990). Sequences were further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

3. Extracellular enzyme production

a- Protease production was tested on the medium of Paterson & Bridge (1994) containing skimmed milk. The medium was dispensed aseptically in 15 cm test tubes (10 ml/tube) which were inoculated on the surface of agar by 25 µl of yeast cell suspension and incubated at 37°C for 7 days. Formation of clear zone around yeast colonies indicates hydrolysis of skimmed milk by proteolytic enzymes.

b- Lipase production was tested using the medium of Ullman & Blasins (1974) which incorporated Tween 80 as a substrate. The medium was dispensed aseptically in 15 cm test tubes (10 ml/tube), inoculated on the surface of agar by 25 µl of yeast cell suspension and incubated at 37ºC for 10 days. The lipolytic ability was observed as a visible precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme. The depth of each visible precipitate (in mm) was measured.

c- Urease production was tested in Difco urea broth suspended into tubes,in aliquots of 0.5 ml. A loopful of cells from 1-2-old-day culture was suspended in the broth and incubated at 37^0C. A change of the color to bright pink or red indicates urease activity (Barnett *et al*. 2000).

4. Antifungal susceptibility test

The disk diffusion method adopted by the Clinical and Laboratory Standards Institute (CLSI 2004) was used to evaluate the in-*vitro* sensitivity of yeasts to 12 antifungal agents and to clove oil. The antifungal agents, producing companies and the interpretative breakpoints of antifungal agents given by Ellis (2011) and Al- Hussaini *et al*. (2013) are shown in Table (1).

Table 1: Interpretative breakpoints of antifungal agents (Ellis 2011 and Al-Hussaini *et al* 2013).

Antifungal agents	Abbr.	Producing Company	Dose/ disc	Zone diameter in mm		
				Susceptible	Intermediate	Resistan
Amphotericin B	AP	Hi-Media	100 units	≥ 15	10 – 14	≤ 10
Cetrimide	CET	Pharco Pharm	5.6 µg	≥ 15	14 – 9	≤ 8
Clotrimazole	CC	Hi-Media	10 µg	≥ 20	12 – 19	≤ 11
Clove oil	C. Oil	Philo Pharm	15 µl	≥ 15	14 – 9	≤ 8
Fluconazole	FLU	Hi-Media	10 µg	≥ 19	15 – 18	≤ 14
Itaconazole	IT	Hi-Media	10 µg	≥ 23	14 – 22	≤ 13
Ketoconazole	KT	Hi-Media	10 µg	≥ 28	27 – 21	≤ 20
Miconazole	MIC	Hi-Media	30 µg	≥ 20	19 - 12	≤ 11
Nystain	NS	Hi-Media	100 units	≥ 15	10 – 14	< 10
Sertaconazole	SER	October Pharm	450 µg	≥ 23	14 – 22	≤ 13
Terbifene HCL	TER	Novartis Pharm	300 µg	≥ 23	14 – 22	≤ 13
Tioconazole	TIO	Pfizer Egypt Pharm	300 µg	≥ 23	14 – 22	≤ 13
Voriconazole	VRC	Hi-Media	1 µg	≥17	16 – 14	≤ 13

Results and discussion

Prevalence of yeast infection in relation to age of patients

During the present work, 145 women were clinically examined for vaginal yeast infection. The direct microscopic examination (DME) revealed that only 64 out of 145 cases (44.1%) were positive showing budding yeast cells either alone or in combination with pseudohyphae. The number of positive culture on SGA cases was higher (93 patients representing 64.1% of total samples) with the majority of cases being affected by a single yeast species. Mixed fungal infections were only observed in few numbers of patients. Results of the present work showed that yeast vaginitis was more common in the age group 21 - 40 years (65 patients representing 69.8% of total positive cases) as shown in Figure (1). These results are nearly similar to those reported from the United States (Sobel 1985) where 75% of women experience at least one episode of vulvovaginal candidiasis (VVC), and 40%-50% of these women experience a second attack. Sobel (1985) suggested that *Candida* organisms gain access to the vagina from the adjacent perianal area and then adhere to vaginal epithelial cells, but *Candida albicans* adheres to vaginal epithelial cells in significantly greater numbers than non-*albicans Candida* species. In Nigeria, Jombo *et al*. (2010) found that the prevalence of *Candida* infection in women was 29.1% (n=2458); no isolate was recovered from persons less than 10 years of age, while the peak age group of infection was 30 - 39 years (11.8%, n=997); the age-group 20 - 49 years accounted for over 25% of the entire infections. In the same country, Nwadioha *et al*. (2010) mentioned that VC was a leading cause of abnormal vaginal discharge in the study constituting 60% (n=420) of the 700 female genital discharge of microbial causes. The result was almost similar to some earlier studies (Sobel *et al*. 1998 and Nwokedi and Anyiam 2003) which recorded 52.5% and 60% respectively.

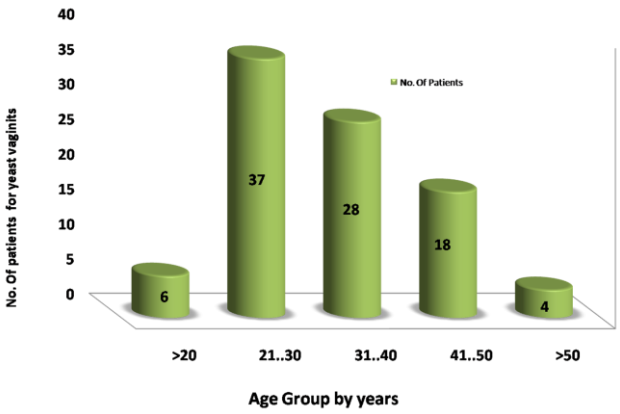

Fig 1: Incidence of vaginal yeast infection in relation to age.

Predisposing factors for vaginal yeast infection

Major risk factors included treatment with oral contraceptives, corticosteroids or diabetes mellitus (Fig 2). As mentioned by Reed (1992), several factors were associated with increased rates of asymptomatic vaginal colonization with *Candida* as well as *Candida* vaginitis including pregnancy (30%-40%), oral contraceptives with a high estrogen content, and uncontrolled diabetes mellitus. The hormonal dependence of VVC is illustrated by the fact that *Candida* is seldom isolated from premenarchial girls, and the

prevalence of *Candida* vaginitis is lower after the menopause, except in women taking hormone replacement therapy. Other predisposing factors include corticosteroids, antimicrobial therapy, intrauterine devices, and high frequency of coitus. As mentioned by Vazquez and Sobel (2003), vaginal colonization with *Candida* is more common in diabetic patients, and poorly controlled diabetes predisposes to symptomatic vaginitis. Glucose tolerance tests have been recommended for women with recurrent vulvovaginal candidiasis (RVVC); however, the yield is low and testing is not justified in otherwise healthy premenopausal women. Diets high in refined sugar may precipitate symptomatic *Candida* vaginitis.

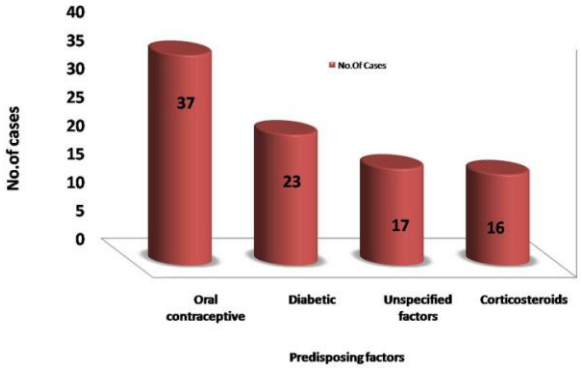

Fig 2: Incidence of vaginal yeast infection in relation to predisposing factors.

Yeasts identified in the present study

Candida was the most prevalent genus being recovered from 75 out of 93 cases representing 80.6% of positive vaginal yeast infections. Five species of *Candida* were identified of which *C. albicans* was the most prevalent (isolated from 45.2% of cases) (Table 2). Colonies of *C. albicans* appeared green on CHROMagar with positive germ tube test and production of chlamydospores (Fig 3). Biochemical reactions using API *Candida* strips as well as molecular characterization based on rRNA gene sequencing and establishment of the phylogenetic tree (Fig 4) confirmed the phenotypic identification of *Candida* species. *C. krusei* and *C. tropicalis* were obtained from 20.4 % and 10.8% of positive cases respectively. *C. glabrata* and *C. parapsilosis* were rarely recovered from vaginal dischange (3.3% and 1.1% of cases respectively). *Rhodotorula mucilaginosa* was found in 18.3% of vaginal samples whereas *Geotrichum candidum* was only obtained from one case (1.1% of the total cases). As mentioned by Ferrer (2000), *Candida albicans* is by far the most common species in gynecology (80-90% of cases) and *Candida glabrata* is the second most common species, causing approximately 5-15% of cases of vaginal candidiasis. In Brazil, Ribeiro *et al.* (2001) found that the prevalence of vaginal yeast isolates was 60% among patients with symptoms of vulvovaginitis and *C. albicans* was the most frequently isolated species followed by *C. glabrata*. According to Nyirjesy *et al.* (1995) an increase of non-*albicans* species has been observed, particularly in recurrent cases. These species are found in approximately 20-30% of cases of recurrent vaginal candidiasis. Among them *C. glabrata* is the most common. Reports from Iran showed that the commonest species was *C. albicans* (78.75%). While other species such as *C. glabrata* (8.75%), *C. krusei* (6.25%) and *C. tropicalis* (2.5%) were less frequent (Pakshir *et al.* 2007). Our results are also in agreement with those of Van Dyck *et al*. (1999) who reported that vulvovaginal candidiasis is created by the fungus *C. albicans* in approximately 85% of cases, with *C. glabrata* being responsible for the remaining 15%. An explanation for the predominance of *C. albicans* in the previous study may be ascribed to its predominance over other *Candida* species in the environment and more importantly, is its favourable survival ability in a depressed human immune system. More recently, Monjaraz-Rodriguez *et al*. (2012) identifying *Candida* species by conventional culturing on chromogenic media as well as by biochemical tests and PCR analysis, found that the most frequent agent of vaginal infection was *C. glabrata* whereas other yeasts as *C. albicans*, *C. krusei* and *C. parapsilosis* were less common.

Table 2: Incidence of yeast species isolated from 93 different vaginal swabs.

Fungal species	Number of cases
Candida (total species)	75 (80.6%)
Candida albicans (Robin) Berkhout	42 (45.2%)
Candida glabrata (Anderson) Meyer and Yarrow	3 (3.3%)
Candida krusei (Castell.) Berkhout	19 (20.4%)
Candida parapsilosis (Ashford) Langeron and Talice	1 (1.1%)
Candida tropicalis (Castell.) Berkhout	10 (10.8%)
Geotrichum candidum (Butler and Peterson) Redhead and Malloch	1 (1.1%)
Rhodotorula mucilaginosa (Jorgensen) Harrison	17 (18.3%)

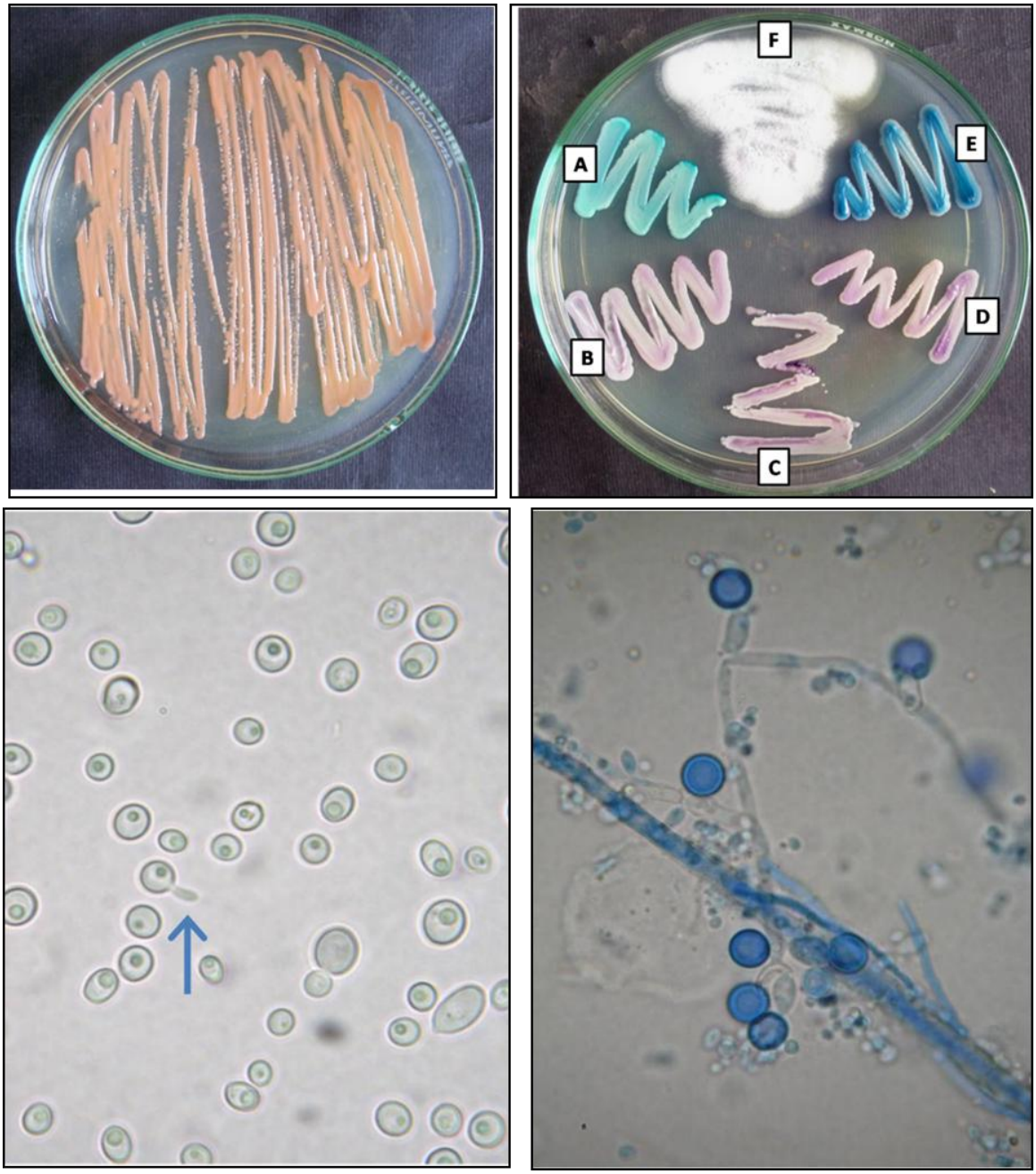

Figure 3: Colony colour of *Rhodotorula mucilaginosa* (top left), *Candida* and *Geotrichum* (top right) as shown on CHROMagar *Candida:* A) Green *Candida albicans*, B) Dark pink *Candida glabrata*, C) Pale pink fuzzy *Candida krusei*, D) Pale pink *Candida parapsilosis*, E) Blue *Candida tropicalis*, F) Chalky white *Geotrichum candidum;* Germ tube (bottom left) and Chlamydospores (bottom right) of *Candida albicans.*

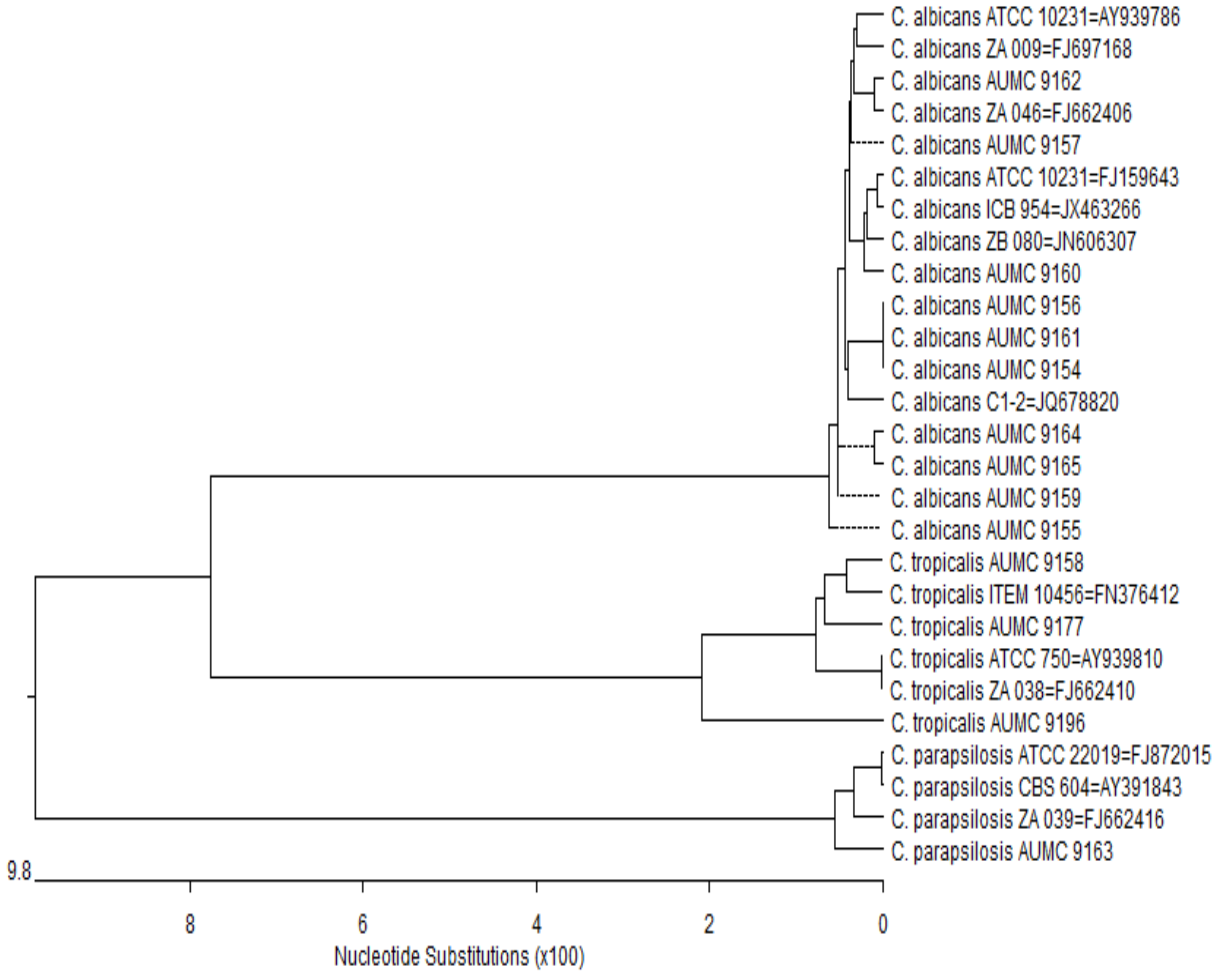

Figure 4: Phylogenetic tree for some *Candida* species isolated (C = *Candida*, given AUMC No.). The scale indicates the number of nucleotide substitutions per site. Reference strains of corresponding fungi are involved in the tree

Extracellular enzymes produced by the isolated yeasts

Since phospholipids and proteins represent the major chemical constituents of the host cell membranes (outer and inner membranes) and the cell contents are mainly of proteins, it was necessary to shed light on the ability of the isolated fungi to produce lipolytic and proteolytic enzymes. Results of the present study showed that protease was produced by 83 (89.2%) of the 93 isolates tested and most isolates of *C. albicans* and *C. krusei* produced moderate to high levels of this enzyme as shown in Table (3). *C. tropicalis* produced variable levels of the proteolytic and lipolytic enzymes. On the other hand, the tested isolate of *C. parapsilosis* yielded low level of protease but the lipolytic activity was not detectable. According to Chakrabarti *et al.* (1992) isolates of *C. glabrata* have shown to be capable of proteinase production. High rates of proteinase in *C. albicans* have also been reported by other workers (Wu *et al.*, 1996 and Koelsch *et al.*, 2000). Kantarcioglu and Yucel (2002) reported that the positivity rate for protease activity from *Candida* was 78.9%. Oksuz *et al.* (2007) reported protease production in 64 (52.4%) isolates. Protease-positive isolates consisted of 46 (56.7%) *C. albicans* and 18 (43.9%) non-*albicans Candida* isolates, namely *C. parapsilosis* and *C. tropicalis* isolates. More recently, Sachin *et al* (2012) detected proteinase in 65 (59.1%) of *Candida* isolates and maximum production was seen in *C. albicans* and *C. tropicalis*. The same authors ascribed the pathogenicity of *Candida* to several putative virulence factors, including germination, adherence to host cells, phenotypic switching and production of extracellular hydrolytic enzymes.

Table 3: Number of yeast isolates showing positive enzymatic activities.

Fungal species	No of isolates tested	Proteolytic			Lipolytic		
		Low	Moderate	High	Low	Moderate	High
C. albicans	42	5	20	16	15	20	3
C. glabrata	3	0	2	0	1	1	0
C. krusei	19	5	8	2	1	0	0
C. parapsilosis	1	1	0	0	0	0	0
C. tropicalis	10	2	8	0	2	2	3
G. candidum	1	0	0	0	0	0	0
R. mucilaginosa	17	14	0	0	0	0	0
Total Isolates	93	27	38	18	19	23	6

Results of the present investigation showed also that lipase was produced by most isolates of *C. albicans*, *C. tropicalis* and *C. glabrata.* On the other hand, the majority of *C. krusei* did not show detectable lipase (Table 3). Phospholipase activity was reported in 30 to 100% of *Candida* isolates tested by Price *et al*. (1982). According to Samaranayake *et al.* (1984) 73% of *C. albicans* isolates showed phospholipase activity. Thangam *et al* (1989) also reported high phospholipase activity in *C. tropicalis*. Kantarcioglu and Yucel (2002) reported that the positivity rate for phospholipase activitiy was 62.1%, in samples from patients with invasive *Candida* infection and phospholipase activity was found to be higher in *C. albicans* isolates than in non-*albicans Candida*. Oksuz *et al*. (2007) found that the phospholipase positive isolates comprised 43 *C. albicans* and 7 non-*albicans Candida* isolates. In their review on microbial lipases, Stehr *et al*. (2003) focused on the virulent traits of these enzymes with special emphasis on lipases from *C. albicans.* They suggested the following roles i) during microbial infections lipolysis might provide carbon sources that the micro-organism could use for growth, ii) the released free fatty acids (FFA) due to lipolytic activity could support cell-to-cell and/or cell-to-host tissue adhesion, iii) a lipase might work hand in hand with another enzyme or it might optimize conditions for other enzymes, iv) lipases might possess additional phospholipolytic activity, v) lipases and their catalytical end products may have an effect on different immune cells and might initiate inflammatory processes, and vi) micro-organisms that secrete lipolytic enzymes might have a selection advantage by lysing competing microflora. Lipolytic enzymes have also been implicated in the virulence of fungal pathogens; the contribution of lipases in fungal pathogenesis has been extensively characterized in *Candida* spp. *C. albicans* possesses at least 10 lipase-encoding genes, the expression of which is largely influenced by the stage of infection (Hube *et al*. 2000). In *C. parapsilosis*, lipases are responsible for the destruction of epidermal and epithelial tissues (Gacser *et al*. 2007). Phospholipase enzyme digests the phospholipid constituents of the host cell membrane leading to cell lysis and alterations of surface characteristics that facilitate adherence and subsequent infection and hence phospholipase production may be used as one of the parameters to distinguish virulent invasive strains from non-invasive colonizers (Sachin *et al*. 2012).

Urease test in the present work was negative for all isolates of *Candida* and *Geotrichum* but those belonging to *Rhodotorula* were urease positive. According to Ellis *et al.* (2007), *R. mucilaginosa* is urease positive and the fungus is a known cause of fungal peritonitis in patients on continuous ambulatory peritoneal dialysis.

Sensitivity of yeasts to antifungal agents

In-*vitro* sensitivity test of vaginal yeast isolates to 12 different antifungal therapeutic agents and clove oil revealed that Nystatin and Terbinafine inhibited all isolates of *C. albicans*, *C. glabrata* and *C. parapsilosis.* Cetrimide, Fluconazole, Clotrimazole, Miconazole and Amphotericin-B inhibited 59.5.8%-85.7% of yeast isolates. *R. mucilaginosa* showed intermediate sensitivity to the majority of antifungal agents (Table 4). Data of the present work showed also that some fungal isolates were resistant to one or more of the frequently prescribed antifungal agents such as Tioconazole (24% of isolates), Ketoconazole (23%), Fluconazole (20%), Clotrimazole (17%), and Sertaconazole (13.9%). There are reports on limited clinical response following fluconazole treatment of *C. glabrata* (Horowitz *et al*. 1985 and Redondo-Lopez *et al*. 1990). Houang *et al*. (1990) reported that after oral therapy of administration of a single dose of 150 mg of Fluconazole, concentrations of Fluconazole above the MIC that inhibited the growth of 90% of *Candida* isolates were achieved for 72-96 h in vaginal tissue (2.43 ng/gm) and secretions. However, no clinical correlation was observed between the in-*vitro* Fluconazole susceptibility profile and clinical response both in diabetic and non-diabetic subjects.

Table 4: Sensitivity of yeast isolates to antifungal agents.

Yeast species (No. of isolates)	DS	AP		CC		CET		FU		IT		KT		MIC	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%
C. albicans (42)	S	25	59.5	31	73.8	36	85.7	34	80.9	20	47.6	9	30.9	21	61.8
	I	17	40.4	5	11.9	1	2.3	1	2.3	20	47.6	23	41.8	21	38.2
	R	0	0.0	6	14.2	5	11.9	7	16.6	2	4.7	10	27.3	0	0.0
C. glabrata (3)	S	2	75	2	75	3	100	3	100	2	75	2	75	1	50
	I	1	25	0	0	0	0.0	0	0.0	1	25	1	25	2	50
	R	0	0	1	25	0	0.0	0	0.0	0	0	0	0	0	0.0
C. krusei (19)	S	4	21	9	47.3	9	47.3	9	47.3	3	15.7	8	37.5	7	62.5
	I	10	52.6	8	42.1	4	21	4	21	8	42.1	3	37.5	12	37.5
	R	5	26.3	2	10.5	6	31.5	6	31.5	8	42.1	8	25.0	0	0.0
C. parapsilosis (1)	S	1	100	1	100	0	0.0	1	100	1	100	1	100	1	100
	I	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	R	0	0.0	0	0.0	1	100	0	0.0	0	0.0	0	0.0	0	0.0
C. tropicalis (10)	S	4	40.0	4	40	8	80	8	80	2	20	0	0	4	40
	I	4	40.0	2	20	2	20	2	20	4	40	6	60.0	6	60
	R	2	20.0	4	40	0	0.0	0	0.0	4	40	4	40.0	0	0.0
G.candidum (1)	S	0	0.0	0	0.0	0	0.0	1	100	0	0.0	0	0.0	0	0.0
	I	1	100	1	100	0	0.0	0	0.0	1	100	0	0.0	1	100
	R	0	0.0	0	0.0	1	100	0	0.0	0	0.0	1	100	0	0.0
R. mucilaginosa (17)	S	2	11.7	0	0.0	10	58.8	10	58.8	4	23.5	0	0.0	1	4.5
	I	15	88.2	13	76.4	0	0.0	0	0.0	9	52.9	17	100.0	8	54.6
	R	0	0.0	4	23.5	7	41.1	7	41.1	4	23.5	0	0.0	8	40.9
Non- *Candida albicans* (33)	S	11	33.30	16	48.48	20	60.60	21	63.63	8	24.24	13	39.39	13	39.39
	I	15	45.4	10	30.30	6	18.18	6	18.18	13	39.39	10	30.30	20	60.60
	R	7	21.21	7	21.21	7	21.21	6	18.18	12	36.36	10	30.30	0	0.0
Total (93)	S	38	40.8	47	50.5	66	70.9	66	70.9	32	34.4	20	21.5	35	37.6
	I	48	51.6	29	31.1	7	7.5	7	7.5	40	43.1	50	53.7	50	53.8
	R	7	7.5	17	18.2	20	21.5	20	21.5	18	19.3	23	24.7	8	8.6

Table 4: Continued

Yeast species (No. of isolates)	DS	NS		SER		TER		TIO		VRC		Clove oil	
		N	%	N	%	N	%	N	%	N	%	N	%
C. albicans (42)	S	42	100	32	76.1	42	100	17	40.4	12	28.5	10	23.8
	I	0	0	8	19	0	0	10	23.8	30	71.4	29	69
	R	0	0	2	4.7	0	0	15	35.7	0	0.0	3	7.1
C. glabrata (3)	S	3	100	3	100	3	100	3	100	1	33.3	3	100
	I	0	0.0	0	0	0	0.0	0	0	1	33.3	0	0
	R	0	0.0	0	0	0	0.0	0	0	1	33.3	0	0.0
C. krusei (19)	S	14	73.6	2	10.5	7	36.8	3	15.7	8	42.1	17	89.4
	I	5	26.3	15	78.9	5	26.3	12	63.1	5	26.3	2	10.5
	R	0	0.0	2	10.5	7	36.8	4	21	6	31.5	0	0.0
C. parapsilosis (1)	S	1	100	0	0.0	1	100	0	0.0	1	100	0	0.0
	I	0	0.0	1	100	0	0.0	1	100	0	0.0	1	100
	R	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
C. tropicalis (10)	S	10	100	6	60	10	100	2	20	6	60	0	0.0
	I	0	0.0	2	20	0	0.0	4	40	2	20	8	80
	R	0	0.0	2	20	0	0.0	4	40	2	20	2	20
G.candidum (1)	S	1	100	0	0.0	0	0.0	0	0.0	0	0.0	1	100
	I	0	0.0	0	0.0	0	0.0	1	100	1	100	0	0.0
	R	0	0.0	1	100	1	100	0	0.0	0	0.0	0	0.0
R. mucilaginosa (17)	S	0	0.0	7	41.1	0	0.0	0	0.0	0	0	0	0.0
	I	17	100	4	23.5	17	100	17	100	17	100	13	76.4
	R	0	0.0	6	35.2	0	0.0	0	0.0	0	0.0	4	23.5
Non- *Candida albicans* (33)	S	28	84.84	11	33.33	21	63.63	8	24.24	16	48.48	20	60.60
	I	5	15.15	18	54.55	5	15.15	17	51.51	8	24.24	11	33.33
	R	0	0.0	4	12.12	7	21.21	8	24.24	9	27.27	2	6.06
Total (93)	S	71	76.3	50	53.7	63	67.7	25	26.8	28	30.1	31	33.3
	I	22	23.6	30	32.2	22	23.6	45	48.3	56	60.2	53	56.9
	R	0	0.0	13	13.9	8	8.6	23	24.7	9	9.7	9	9.6

DS: Degree of sensitivity. **S**: Sensitive, **I: I**ntermediate, **R:** Resistant,
AP: Amphotericine-B, **CC**: Clotrimazole, **CET:** Cetrimide**, FU:** Fluconazole, **IT:** Itraconazole**, KT**: Ketoconazole, **MIC:** Miconazole, **NS:** Nystatin, **SER**: Sertaconazole, **TER**: Terbinafine, **TIO**: Tioconazole, **VRC**: voriconazole**.**

In Turkey, Nawrat *et al*. (2000) found Amphotericin-B effective against 100% of *Candida* isolates. They also noticed that 72.1% of *Candida* strains were sensitive to Ketoconazole, 61.4% to Fluconazole and 47.1% to Itraconazole. The study of Kronvall and Karlsson (2001) showed that all *Candida* isolates were susceptible to Fluconazole except *C. glabrata* and *C. Krusei*. According to Sobel *et al.* (2004), treatment with Fluconazole has been successful based on moderate evidence from a randomized clinical trial. As stated by Khan and Baqai (2010), Clotrimazole was more effective as compared to Fluconazole and Nystatin. Working with 270 isolates of *Candida*, Ellis (2011) recorded that all isolates of *C. albicans* were susceptible to Fluconazole and Clotrimazole but resistance to Fluconazole was manifested by 20% of *C. glabrata* isolates. The in-*vitro* drug sensitivity test performed by Goswami *et al*. (2000) showed no significant difference in the Fluconazole susceptibility pattern between *C. albicans* and non-*C. albicans* candidiasis The same authors mentioned that certain strains of *C. glabrata* are genetically tolerant to Fluconazole.

Conclusion It can be concluded that correct diagnosis of yeast isolates based on phenotypic and genotypic methods is necessary. Antifungal susceptibility testing is also of great value to exclude ineffective antifungal agents and allows better selection of the most active drugs.

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