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UROPATHOGENIC *CANDIDA* SPECIES AND THEIR SENSITIVITY TO ANTIFUNGAL AGENTS

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The present study included 400 patients admitted to Department of Urology, Assiut University Hospitals during the period from September 2015 to June 2016. The patients' ages ranged from 20- 84 years. The mycological analysis of their urine samples revealed that samples of 75 patients produced *Candida* colonies after culturing on Sabouraud`s dextrose agar and the rate of candiduria was highest in patients above 60 years of age (46.7%). Females were more affected than males (65.3% versus 34.7% of positive samples). Phenotypic (growth on Hicrome agar, microscopic examination, germ tube test, chlamydospore formation) and genotypic (sequencing ITS region of rDNA) allowed identification of four *Candida* species. *Candida albicans* was the most common species (41% of strains), followed by *C. glabrata* (34%), *C. tropicalis* (19%) and *C. krusei* (6%). Varying degrees of proteolytic and lipolytic activities was expressed by 65.12% and 90% of *candida* strains respectively. All strains of *C. albicans, C. krusei* and *C. tropicalis* were sensitive to Amphitericin-B and Nystatin. However these strains (except *C. krusei*) were resistant to each of Clotrimazole, Fluconazole and itraconazole Ketoconazole, Voriconazole where the percentage of resistant strains ranged from 47% to 100%. The majority of *C. glabrata* strains exhibited higher sensitivity to nystatin and fluconazole than to other tested antifungals. The higher incidence of non albicans *candida* (59% of strains) and resistance to the commonly used drug fluconazole is a

matter of concern. It is recommended to perform routine mycological analysis for species identification of *Candida* isolates along with their antifungal susceptibility pattern to help the clinicians in better treating patients with candiduria.

Keywords: Candiduria, proteolytic and lipolytic activity, antifungal susceptibility

INTRODUCTION

Urinary tract infections (UTI) are commonly encountered in clinical practice. They can be caused by bacteria, fungi, parasites, and viruses. However, among the fungal agents Candida species are the most frequent causes of UTI (**Esmailzadeh *et al*., 2018**) Although UTI by Candida species (candiduria) is reported to be rare in healthy adults. The disease is usually common in the elderly and neonates, especially those receiving prolonged antibiotic therapy (**Achkar and Fries, 2010**). Candiduria is very common in patients exposed to risk factors such as hospitalization, diabetic mellitus and admission in Intensive Care Units (ICUs). Catheterization process increases chances of UTI by allowing migration of the organisms into the bladder from external periurethral surface. Candida species can form drug resistant bio films on Foleys catheter which may be a constant source of nidus of infection **(Febre *et al*., 1999).** As estimated by **Shay and Miller (2004),** the incidence of candiduria in the United States was 25,000 cases per year and approximately one third of hospitalized patients yielding Candida with urine cultures were in the Intensive care unit (ICU) where bladder catheter use was high. However, as early as **1986 Platt and colleagues** found that 26.5% of cases with bladder catheter usage were due to Candida species. This observation was later substantiated by others who found that 90% of Candida UTIs in a large tertiary care centre in the United States were related to bladder

catheters (**Berrouane *et al.*, 1999**). On a national scale, surveillance studies have indicated that 25% of all UTI in ICUs are caused by Candida species (**Banerjee *et al.*, 1991 and Jarvis *et al.*, 1999**) and the length of stay in such units influences the incidence significantly. According to **Jang *et al.*, (2005)** and **Jain *et al.*, (2007)**, the isolation rate of Candida ranged from 10-15% of positive urine cultures in tertiary care centers. Fungal UTI may cause serious complications that influence graft success and patient survival **(Säemann and Hörl (2008). Valera *et al.*,(2006)** expressed that fungus microorganisms caused 3% of whole UTIs. Candiduria incidence was 8.5% based on results of **Shams *et al.*, (2017).** *Candida albicans* accounted for the majority of candiduria reported (about 50-70%).Whereas, non-albicans Candida especially *C. glabrata and C. tropicalis* were emerging pathogens (**Achkar and Fries, 2010 and Gharaghani *et al.*, 2018). Wiwanitkit (2008)** reviewed three cases of acute renal failure due to *C. tropicalis* obstruction and patients responded well to therapy with oral fluconazole and percutaneous amphotericin B. Other reports stated that candiduria can be an early marker of disseminated fungal infections in critically ill patients and is associated with higher mortality **(Knoke *et al.*, 2000 and Vidigal *et al.*, 2011)**. It was also documented that secretion of hydrolytic enzymes, such as proteases, phospholipases, and lipases have an important role in the pathogenesis of Candida species **(Sardi *et al.*, 2013)**. Adherences to host tissue, heat shock proteins and thigmotropism have also been demonstrated to share to the subsequent Urinary tract infection **(Fisher *et al.*, 2011 and Mayer *et al.*, 2012).** In addition, some of these factors such as biofilm formation can interfere with antifungal therapy. On the other hand, routine antifungal prophylaxis may increase the pathogenicity of

Candida and resistance to antifungal drugs **(Badiee and Alborzi, 2011 and Mahmoudabadi *et al.*, 2013)**. Several studies considered that Fluconazole is effective in the short-term eradication of candiduria. However, the optimal therapy for candiduria is becoming complicated by the emerging resistance to antifungal agents such as newer azoles and echinocandins **(Chapeland-Leclerc *et al.*, 2010 and Pfaller *et al.*, 2011).** In Egypt, **Alhussaini *et al.* (2013)** studied the prevalence of candiduria in renal failure patients of different ages (27- 80 years old). They isolated Candida species from 20% of patients and the disease was more common in females than males (64% versus 36%). **Alkilani *et al.*, (2016)** studied the incidence of UTI caused by candida species in patients admitted to intensive care unit in Menoufia university hospitals, Egypt and tried to determine their antifungal susceptibility. They isolated Candida species from urine of 38 (19%) patients and identified *C. albicans* in 18 (47.3%) of positive samples. Antifungal susceptibility showed that Flucytosine, Amphotericin B, Voriconazole were the most effective drugs against Candida species. However, 72.2% of the tested strains *C. albicans*, were resistant to Fluconazole. The present work was designed to study the prevalence of fungal infections in patients admitted to Nephron`s Tropical Department at Assiut University Hospitals.

SUBJECTS, MATERIALS AND METHODS

Study subjects:

This is a hospital based descriptive study carried out in microbiology unit, Clinical Pathology Department at Assuit University Hospitals in collaboration with Department of Botany and Microbiology, Faculty of Science, Assuit University. The study included 400 patients

admitted to Department of Urology, Assiut University Hospitals during the period from September 2015 to June 2016.

Inclusion criteria:

Eligible participants were patients who had chronic kidney diseases with manifestations of urinary tract infection (UTI, i.e. burning sensation, lower abdominal pain, fever, turbid or bloody urine…etc.)

Exclusion criteria:

Patients who received antifungal or antibiotic therapy within 3 days prior to sample collection and patients who refused to participate in the study.

Ethical aspects:

The study did not include any additional intervention to the standard treatment regimens the patients receive in the hospital. Patient's consent had been obtained before sample collection. Patient's

confidentiality has been respected. Patients' refusal of participation in the study did not affect the quality of treatment they had received in the hospital.

Samples collection, handling and transport:

Urine samples were collected from patients with urinary tract infection under complete aseptic precisions in sterile containers as freshly voided mid-stream urine or by means of a catheter and immediately transferred to the laboratory. All specimens were immediately subjected to:

Physical and chemical urine analyses: (Bartlett, 1974)

1- Physical examination: Turbidity, odor and color.
2- Chemical examination (dipstick urinalysis): The strips had reagent pads for semiquantitative assessment of pH, specific gravity, leukocyte esterase, nitrite, and protein, as a predictive parameter for UTI.
3- Direct microscopical examination (DME) was done after centrifugation of approximately (10 mL) of urine at 2000 r.p.m. for 2 minutes. The supernatant layer was poured off and the deposit was shaken then one drop of it was placed on a slide and examined by the microscope using the high power magnification for presence of :

a- Pus cells{pyuria means >5 pus cells/HPF}(**Chessbrough, 2007**), red blood cells (RBCs), crystals, casts, microorganisms.

Culturing of collected specimens

Urine specimens(200 micro-liter) were individually inoculated on the surface of Sabouraud dextrose agar (SDA, **Ellis *et al*., 2007**). The inoculated plates were incubated at 28ºC for 3-7 days after which pure colonies were picked for identification and subsequent studies.

Phenotypic identification of fungal isolates:

Phenotypic identification was performed using Hicrome Candida Differential Agar (Himedia Company, India) which contained a chromogenic mixture that represents enzyme substrates linked to chromogenic substances. Color variations of yeast colonies (Table 1) are useful for the presumptive identification of the isolated *Candida* species within 24-48 hours. Peptone and yeast extract contained in the medium provided nitrogenous, carbonaceous compounds and other essential growth nutrients. Phosphate buffers in the medium and chloramphenicol suppresses the accompanying bacterial contaminants. Microscopic examination was done to ensure the presence of fungal units (budding

cells and pseudohyphae, chlamydospores). Germ tube test was also performed for rapid screening of *C. albicans* and *C. dubliniensis* **(Ellis *et al.*, 2007).** Chlamydospore production on corn meal agar with tween 80 (McGinnis, 1980) was also tested.

Table (1): Colony colour of yeast isolates on HiChrom agar *Candida* at 37ºC

Yeast species	Colony colour
C. albicans	light green
C. glabrata	cream to white smooth colonies
C. tropicalis	blue to metallic blue raised colonies
C. krusei	purple fuzzy colonies
C. parapsilosis	pale to pink colonies

Molecular identification of fungal isolates:

Seven-day old culture on SDA was sent to the Molecular Biology Research Unit, Assiut University for DNA extraction using Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. The fungal DNA was then sent to SolGent Company, Daejeon South Korea for polymerase chain reaction (PCR) and gene sequencing. PCR was performed using ITS1 (forward) and ITS4 (reverse) primers which were incorporated in the reaction mixture. Primers 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'). The purified PCR product (amplicons) was sequenced with the same primers (**White *et al.*, 1990)**. The obtained sequences were analyzed using Basic Local Alignment Search Tool (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.

Screening of yeast isolates for extra-cellular enzymes production:

Protease detection: (Paterson and Bridge, 1994)

The tested yeast isolates were individually inoculated in test tubes containing casein hydrolysis medium and incubated at 28 ºC for 10 days. After incubation, protease producing yeasts resulted in complete degradation of milk protein that was seen as a clear depth in the tube. The clear depth below the colony was measured in mm.

Lipase detection: (Ullman and Blasins, 1994)

Detection of lipase production by isolated yeasts was carried out in test tubes . Each tube was inoculated with atested yeast isolate and incubated at 28 ºC for 10 days. After incubation, the lipolytic ability of each yeast isolate 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 was measured (in mm).

Antifungal susceptibility testing of isolated yeasts:

In vitro susceptibility test of the different yeast strains (75 strain) was performed using the disc diffusion method according to the procedure described in the CLSI M44-A **(CLSI, 2011).** Colonies were suspended in 2ml of sterile saline (0.85%). The resulting suspension was homogenized by vortex and the turbidity was adjusted to yield 1×10^5 - 5×10^5 cells/ml (0.5 McFarland standard). Mueller- Hinton agar (MHA) (HiMedia, India) supplemented with 2% glucose and 0.5 µg/ml methylene blue (MHA) was used to perform the antifungal susceptibility testing. Eight different antifungal therapeutic drugs belonging to **polyenes** (Amphotericin B and Nystatin), **azoles** (Fluconazole, Ketoconazole, Clotrimazole, voriconazole

and Itraconazole) and allylamines (terbinafine) were incorporated in this test. All antifungals discs were obtained from HiMedia Company in India. Results were read as the diameter (mm) of inhibition zone exhibited by each fungal strain. The response to the antimicrobial agents was determined via the interpretive breakpoints (Table 2).

Table (2): Interpretative breakpoints of antifungal agents against isolated yeasts (CLSI, 2011).

Antifungal	Disc content	Inhibition Zone diameter in mm		
		S	I	R
Amphotericine B (AP)	100 U	≥ 15	10 - 14	< 10
Clotrimazole (CLO)	10 µg	≥ 20	12 - 19	≤ 11
Fluconazole (FU)	25 µg	≥ 19	15 - 18	≤ 14
Itraconazole (IT)	10 µg	≥ 23	14 - 22	≤ 13
Ketoconazole (KT)	10 µg	≥ 28	21- 27	≤ 20
Nystatin (NS)	100 U	≥ 15	10 - 14	< 10
Terbinafine	25 µg	≥20	12 - 19	≤11
Voriconazole (VOR)	1 µg	≥17	14 - 16	≤ 13

S: Susceptible, I: Intermediate, R: Resistant.

RESULTS AND DISSCTION

Prevalence of candiduria in relation to age, gender and clinical picture:

The patients' ages ranged from 20- 84 years, and the rate of candiduria was highest in patients above 60 years of age (35 patients representing 46.7%) followed the age group of 46-60 (21 cases, % 28%). Patients with chronic kidney diseases (CKD) and acute kidney injury (AKI) were the commonest (31 cases for each). The remaining patients were marked as end stage renal disease (ESRD, 11 cases) and pyelonephritis (2 cases) as

shown in table (4). The age and sex-wise distribution of candiduria among males and females are shown in table (3). Females markedly outnumbered males (49 versus 26 patients matching 65.3% and 34.6 % , respectively) as shown in table (4). The higher prevalence of candiduria in females than in males confirms the work of **Passos *et al*. (2005)** who found that the percent of candiduria in females was 61.6% compared to 38.4% in males. **Rashwan *et al*. (2010)** observed candiduria in 34.4% females versus 14.9% in males. A plausible explanation of this phenomenon is the presence of *Candida* in the genital tract of women complaining of vaginal candidiasis. During his study of the incidence of candiduria in 200 diabetic patients in health care centers the Northern area of Saudia Arabia**, Alenezy (2014)** found that 90 (45%) were males and110 (55%) were females. the ages ranged from 16 years to 68 years (mean = 43 ± 3.12 years). *Candida* was detected in 12% (24 out of 200) of diabetic patients, compared to 4% (2 out of 50) in control group. In Zagazig Governorate, Egypt **Hassaneen *et al.* (2014)** reported that the prevalence of candiduria in 300 hospitalized patients was 42 (14%) including 26 cases of females and 16 males. The most common isolates were *C. albicans* (61.9%) followed by *C. glabrata* (21.4%), *C. krusei* (9.5%) and C. tropicalis (7.5%). In Menofiya Governorate, Egypt, **Alkilani *et al.* (2016)** reported that candiduria affected 52.6 % of females compared to 47.5 % of males and the disease was more common in persons above 50 years of age (57.9%). In Iran, **Esmailzadeh *et al.*(2018)** candiduria had a significantly higher rate in females (87.5%) than males (12.5%). **Reports from Cameroon (Diesse *et al.*, 2017)** showed that 22% of patients had *Candida* spp in their urine. *C. albicans* had a proportion of 37% against 63% for non-albicans *Candida*. The conflicting results of

these studies may be due to differences in the sample size, population studied, and even the location and geographical area. Some reasons for higher prevalence of candiduria in females compared with males may be their shorter urethral length, transmission from the genital tract to the urinary tract, and the anti-*Candida* activity of prostatic fluid in male.

Table (3): Prevalence of candiduria cases in relation to clinical diagnosis

Clinical diagnosis	No. of patients	%	M	F
AKI	30	40	5	25
CKD	32	42.7	15	17
ESRD	11	14.7	7	4
Pyelonephritis	2	2.7	1	1
Total	75	100	28	47

AKI: Acute kidney injury **CKD:**Chronic kidney injury **ESRD:**End stage renal disease

Table (4): Age and percentage (% out of 75 cases) and sex of patients tested for candidurea.

Age group by years	No. of patients	%	M	F
<15	0	0	0	0
16-30	5	6.7	0	5
31-45	14	18.7	4	10
46-60	23	30.6	7	16
>60	33	44	15	18
TOTAL	75	100	26	49

Yeasts detected by Direct microscopic examination DME and culture based method

Results in tables (5) revealed that only 60 out of 400 cases (15%) were positive for DME showing budding yeast cells and in some cases associated with pseudo-hyphae in urine samples. The positivity of culturing was slightly higher than that of DME (18.75% versus 15% of samples) as shown in Table (6). The majority of cases (61 patients) were found to be caused by a single fungal species. Mixed fungal infections (with two species of yeasts) were observed in 14 patients.

Table (5): Number and percentage (%) of yeasts detected by DME and culture

Method of detection (n= 400 samples)	**Yeast infection (%)**	
	Positive	Negative
DME	60 (15%)	340 (85%)
Culturing on SDA	75 (18.75%)	325 (81.25%)

Yeasts identified by phenotypic and genotypic characteristics:

A total of 89 isolates of yeasts were identified from 75 patients after culturing on Hichrome Candida medium and performing germ tube test and production of chlamydospores on corn meal agar. From an etiological standpoint, *Candida albicans* was the most frequent species (37 isolates out of 89) representing 41% of total cultures. *C. glabrata* came second sharing with 30 isolates matching 34% of cultures. The remaining two species; *C. tropicalis* and *C. krusei* accounted for 19% and 5% of total *Candida* isolates. Total non albicans *Candida* accounted for 52 strains (59%) as shown in table (6). Since *C. glabrata* appear as white colored colonies on Hicrome agar, molecular identification was performed to confirm identification of this species .Sequences of rDNA of a representative isolate (AUMC 14225) showed 100% similarity with

several strains of *Candida glabrata* and 99.29% with the type strain NRRL Y-65 (GenBank accession No. NR 130691) as shown in Fig (1).

Table (6): *Candida* species identified from urine samples

Yeast species	Colour on HiCrome Agar	Germ tube test	Chlamydospore production	No. of isolates	%
C. albicans	**Light Green**	**+**	**+**	**37**	**41%**
C. glabrata	**White**	**-**	**-**	**30**	**34%**
C. tropicalis	**Dark blue**	**-**	**-**	**17**	**19%**
C. krusei	**Pink fuzzy**	**-**	**-**	**5**	**6%**
Total Non albicans Candida	**Not green**	**-**	**-**	**52**	**59%**
Total				**89**	**100**

(**GenBank Accession Number of *Candida glabrata* :MN699325)**

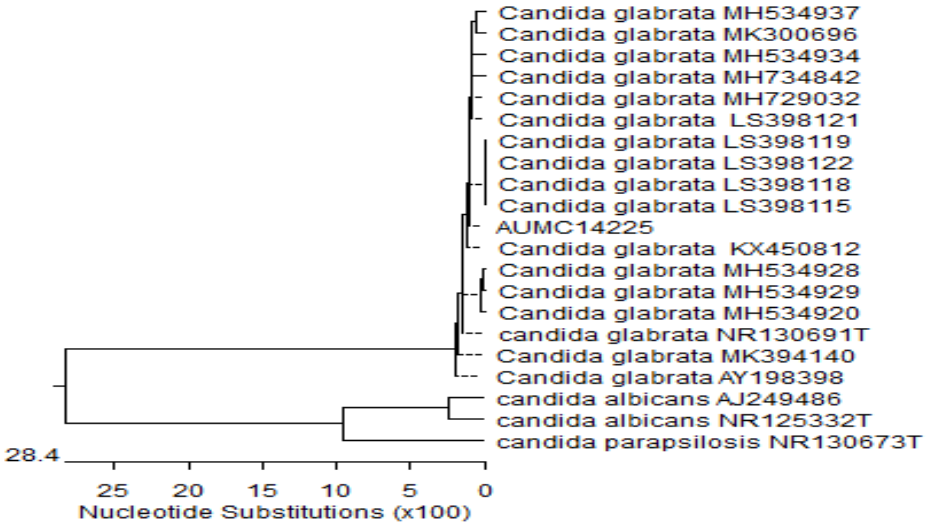

Fig. (1): Phylogenetic tree based on ITS region of the yeast strain isolated in the present study (AUMC 14225) aligned with closely related sequences accessed from the GenBank *(*Accession No. :MN699325).

A wide range of reported data shows that *C. albicans* ranks first for causing candiduria among more than 200 *Candida* species. In Saudia Arabia, **Alenezy (2014)** reported that the most common isolated strain was *C. albicans* (41.7% of cases), followed by *C. glabrata* (29.2%), and *C. tropicalis* (16.7%). In Iran, **Ghiasian *et al.*, (2014)** mentioned that Candiduria was confirmed in 50 out of 155 (32.26 %) patients and *Candida albicans* (60.0% of isolates)was the most frequently isolated species followed by *C. glabrata* (14.0%), C. parapsilosis (12.0%), C. krusei (10.0%), and C. tropicalis (4.0%). Most patients (58%) were females with a mean age of 46.7 years old. **Esmailzadeh *et al.* (2018)** reported that of the 400 urine specimens, 40 (10%) had positive cultures for Candida species with a colony count of ≥1 × 103 colony forming units (CFU)/mL. The frequencies of the Candida species were as follows: *C. albicans* (47.5%), *C. glabrata* (37.5%), *C. kefyer* (10%) and *C. krusei* (5%). A recent report from Iran (**Gharaghani *et al.*, 2018)** showed that common etiologic agents of candiduria were *C. albicans* (58.53%), followed by *C. glabrata* (15.39%), *C. tropicalis* (5%), *C. krusei* (2.72%), *C. parapsilosis* (1.53%), *C. kefyr* (1.03%), *C. lusitaniae* (0.42%) and *Candida* species (14.72%). Furthermore, uncommon yeast / yeast-like microorganisms such as *C. albidus* (0.23%), *C. laurentii* (0.07%), *Geotrichum* (0.12%) and unidentified yeasts (0.22%) were also isolated from urine samples. Furthermore, rarely *Trichosporum* and *Saccharomyces* were isolated from patients' urine cultures. Reports from India **(Singhal *et al.*, 2015)** revealed that Candida species isolation was 10.2% (112/1092) and the commonest was *Candida tropicalis* (54.5%), followed by *C. glabrata* (25%), and *C. albicans*, (11.6%). On the other hand, **Bisane and Basak (2018)** isolated 55 Candida strains from 630 urine samplesfrom Indian patients and found that *C. tropicalis* was the commonest species (36.4%) followed by *C. albicans* (29.1%), *C. parapsilosis* (16.4%), *C. glabrata* and *C. pelliculosa* (7.3% each). Also, **Goyal *et al.* (2016)** who reported that 180 (2.36%) samples showed the growth of Candida species out of 7627 urine samples. Among them non albicans Candida species 120 (66.7 %), were predominant compared to *C. albicans* 60 (33.3%). Non albicans Candida species included *C. tropicalis* (20.6%), *C. gullermondi* (15.5%), *C. intermedia* (15%), *C. krusei* (11.1%), *C. pseudotropicalis* (3.9%) and *C. stelloidia* (0.5%). The rate of isolation of Candida species were more in females, 101 (56.1%) than in males 79 (43.9%). The highest isolation rates of Candida among uropathogens were found in age group above 60 years. As mentioned by **Prakash *et al.* (2018)** *Candida* was isolated in 113(2.7%) out of total 4192 urine samples and 16.8% of these isolates were *Candida albicans* as

compared to 83.2% non albicans Candida. Amongst the non albicans Candida, *C. tropicalis* was seen to be the most common species, with 35.8%, incidence, followed by *C. glabrata* 32.7% and *C. krusei* 8.0%, while *C. parapsilosis* and *C. guilliermondii* accounted for 1.8% and 0.9% respectively.

Extra-cellular enzymes produced by *Candida* strains:

Testing of the proteolytic activity of *Candida* strains as shown in table (7) revealed that, out of 89 strains, 56 (65.12%) were protease producers. Only 13 strains had high proteolytic activity (9 of them were *C. albicans*, 3 of them were *C. glabrata* and only one was *C. tropicalis*), 15 strains had intermediate proteolytic activity (8 *C. albicans*, 3 *C. glabrata* and 4 *C. tropicalis*) while 28 strains had low proteolytic activity (13 *C. albicans*, 8 *C. glabrata* and 7 strains of C. *tropicalis*). On the other hand *C. krusei* did not exhibit any detectable proteolytic activity.

Extra-cellular lipase produced by *Candida* strains:

Considering the lipolytic activity of the isolated Candida spp. , results in table (8) showed that 90% of the yeast isolates showed lipolytic activities but with varying capabilities. Out of 89 *Candida* strains , 15 (16.9%) exhibited high lipolytic activity. These included *C. albicans* (8 strains), *C. glabrata* (5) and *C. tropicalis* (2). Intermediate Lipolytic activity was expressed by *C. albicans* (4 strains), *C. glabrata* (6) and *C. tropicalis* (one strain). Low lipolytic activity was expressed by other strains of *C. albicans* (10), *C. glabrata* (9), *C. tropicalis* (11) and *C. krusei* (2).

Table (7): Number (N) and percentage (%) of fungal strains showing proteolytic activities.

Yeast species	No. of tested strains	High		Moderate		Low		Total positive	
		N	%	N	%	N	%	N	%
C. albicans	37	9	24.3	8	21.6	13	35.1	30	81
C. glabrata	30	3	10	3	10	8	26.7	14	46.6
C. tropicalis	17	1	5.88	4	23.5	7	41.2	12	70.5
C. krusei	5	0	0	0	0	0	0	0	0
Total	89	13	14.6	15	16.9	28	31.5	56	62.9

High enzyme producers: Depth of clear media ≥ 18 mm., **Moderate** enzyme producers: Depth of clear 15-17 mm., **L**ow enzyme producers: Depth of clear 0-14 mm.

Candida species employ a repertoire of virulence factors, including phenotypic switching, dimorphism, galvano - and thigmotropism, and hydrolytic enzymes, to colonize and then invade the urinary tract (**Fisher *et al.*, 2011**). *C. albicans* is capable of producing a range of hydrolytic enzymes that facilitate adherence to host tissue, rupture of cell membranes, invasion of mucosal surfaces and blood vessels, and evasion of the host's immune response (Haynes, 2001). Secreted proteinases are principal among such enzymes and degrade proteins related to structural and immunologic defenses, such as collagen, keratin, mucin, antibodies,

complement, and cytokines, during tissue invasion. Although *C. albicans* was the highest producer, these proteinases are present in *C. tropicalis*, *Candida parapsilosis*, and *Candida dubliniensis* but not in *C. glabrata* (**Kaur *et al.*, 2005**). *C. glabrata* was considered a relatively nonpathogenic commensal fungal organism of human mucosal tissues. However, with the increased use of immunosuppressive agents, mucosal and systemic infections caused by *C. glabrata* have increased significantly, especially in the human immunodeficiency virus-infected population (**Fidel *et al.*, 1999**)

Table (8): Number (N) and percentage (%) of *Candida* spp. showing proteolytic activities.

Yeast species	No.oftested strains	High		Moderate		Low		Total positive	
		N	%	N	%	N	%	N	%
C.albicans	37	8	21.6	4	10.8	10	27	22	59.4
C.glabrata	30	5	16.7	6	20	9	30	20	66.6
C.tropicalis	17	2	11.8	1	5.88	11	64.7	14	82.3
C. krusei	5	0	0	0	0	2	40	2	40
Total	89	15	16.9	11	12.4	32	36	58	65.1

High enzyme producers: Depth of visible precipitate ≥ 15 mm., **Moderate** enzyme producers: Depth of visible precipitate 6-14 mm., **L**ow enzyme producers: Depth of visible precipitate 0-5 mm.

Sensitivity of *Candida* isolates to antifungal agents:

Data in table (9) showed that the most active drugs were nystatin, amphotericine B and Clotrimazole affecting 100%, 94% and 58% of strains**.** All strains of *C. albicans*, *C. krusei* and *C. tropicalis* were sensitive to Amphiterici-B and Nystatin. With the exception of *C. krusei* these strains were resistant to each of Voriconazole, Ketoconazole, Clotrimazole, fluconazole and itraconazole where the percentage of resistant strains ranged from 47% to 100%. The majority of *C. glabrata* strains exhibited higher sensitivity to nystatin and fluconazole than to other tested antifungals. In agreement with our results**. Singhal *et al.* (2015)** tested the antifungal susceptibility of 112 *Candida* isolates to 5 antifungal therapeutic agents and found that *C. albicans* showed 84.6% susceptibility to Amphotericin B. However, the non-albicans group showed a lower susceptibility to this drug (60.6%). The sensitivity of *C. albicans* was low to all of the azoles tested. Sensitivity to both fluconazole and Voriconazole was seen in 46.2% of *C. albicans* isolates while only 15.4% were sensitive to itraconazole**.** In Cameroon**, Diesee *et al.* (2017)** reported that of the 53 isolates tested, ketoconazole had the highest percentage of resistance (88.6%) followed by fluconazole (64.1.6%), amphotericin B (56.6%) and nystatin (49.0%). The highest sensitivity was observed with nystatin (33.9%) while the lowest was found with ketoconazole (5.6%). According to **Prakash *et al.* (2018)** the resistance to fluconazole is of great concern, because it is the most common azole used for the treatment of candiduria, and also in disseminated candidiasis. It is available in both intravenous and oral formulation with high bio availability and is more cost effective than other antifungal agents. Although, Amphotericin-B is effective against

most strains of Candida spp. , it is not the first drug of choice for the treatment of candidemia because of nephrotoxicity associated with it. The increase in resistance to fluconazole is a matter of great concern as it is the most commonly used azole for the treatment of candiduria. The mechanism of azole resistance could be a structural or functional change at the azole-binding site, expression or over-expression of efflux pumps or change in biosynthetic pathway of ergosterols (**Klesper, 2001**).

Table (9): Degree of sensitivity (DS) of *Candida* strains to tested antifungal agent

Yeast isolates	DS	AP100		NS100		VRC1		KT10		CC10		FLC10		IT10	
		N	%	N	%	N	%	N	%	N	%	N	%	N	%
C. albicans **(n=37)**	**S**	37	100	37	100	0	0	0	0	15	41	0	0	8	22
	I	0	0	0	0	0	0	0	0	0	0	0	0	17	46
	R	0	0	0	0	37	100	37	100	22	59	37	100	12	32
C.glabrata **(n=30)**	**S**	25	83	30	100	7	23	8	27	25	83	27	90	0	0
	I	5	17	0	0	0	0	2	6.7	5	17	0	0	1	3.3
	R	0	0	0	0	23	77	20	67	0	0	3	10	29	97
C.tropicalis **(n=17)**	**S**	17	100	17	100	1	5.9	0	0	7	41	4	23.5	3	18
	I	0	0	0	0	1	5.9	1	5.9	2	12	2	11.8	9	53
	R	0	0	0	0	15	88	16	94	8	47	11	64.7	5	29
C. krusei **(n=5)**	**S**	5	100	5	100	1	20	0	0	5	100	3	60	2	40
	I	0	0	0	0	0	0	1	20	0	0	0	0	0	0
	R	0	0	0	0	4	80	4	80	0	0	2	40	3	60
TOTAL	**S**	84	94	89	100	9	10	8	9	52	58	34	38.2	13	15
	I	5	6	0	0	1	1.1	4	4.5	7	8	2	2	27	30
	R	0	0	0	0	79	89	77	87	30	34	53	59.6	49	55

DS: Degree of sensitivity. **S:** Sensitive, **I:** intermediate, **R:** Resistant, **AP:** Amphotericine-B, **CC:** Clotrimazole, **NS:** Nystatin, **FU:** Fluconazole, **KT:** Ketoconazole, **IT:** Itraconazole, **VRC:** Voriconazol

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أنواع الكانديدا الممرضة للجهاز البولي وحساسيتها للمضادات الفطرية

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أجري هذا البحث على أربعمائة من المرضى بقسم المسالك البولية بمستشفيات جامعة أسيوط في الفترة من سبتمبر ٢٠١٥ حتى يونيو ٢٠١٦. تراوحت أعمار المرضى بين ٢٠ – ٨٤ سنة. أظهرت التحاليل الميكولوجية لعينات البول وجود فطرة كانديدا في عينات البول ل٧٥ مريض ، وخاصة الذين تجاوزوا الستين عاماً حيث بلغت نسبة الإصابة ٤٦.٧% من الحالات الإيجابية، وكان إنتشار الفطر في الإناث أعلى منه في الذكور (٦٥.٣% مقابل ٣٤.٧%).وقد تم تعريف أنواع الكانديدا مورفولوجياً بالتنمية على البيئات المنتجة للألوان وتكوين أنابيب الإنبات والجراثيم الكلاميدية وتم تأكيد تعريف أحد أنواع الكانديدا بالطرق الجزيئية. أمكن تشخيص أربعة أنواع من جنس كانديدا هي كانديدا ألبيكانس (٤١%)، كانديدا جلابراتا (٣٤%) كانديدا تروبيكالس (١٩%) وكانديدا كروسياي (٦%). وقد استطاعت هذه الأنواع إنتاج الإنزيمات المحللة للبروتينات والدهون بدرجات مختلفة، وقد أظهرت سلالات كانديدا ألبيكانس وكانديدا كروسياي وكانديدا تروبيكالس حساسية لنوعين من المضادات الفطرية هما أمفوتيرسين ب ونستاتين، بينما كانت هذه الأنواع بإستثناء (كانديدا كروسياي) مقاومة لكل من كلوتريمازول، فلوكونازول، اتراكونازول، كيتو كونازول وفوريكونازول وذلك بنسبة ٧٤% - ١٠٠%. أما السلالات التابعة لنوع كانديدا جلابراتا فقد أظهرت استجابة أفضل لتأثير كل من نستاتين وفلوكونازول عن بقية المضادات الفطرية. وقد تبين من هذه الدراسة إرتفاع نسبة الكانديدا غير ألبيكانس (٥٩%) وأنها مقاومة للمضادات الفطرية خاصة فلوكونازول مما يؤكد أهمية إجراء الفحص الميكولوجي لعينات البول من مرضى إلتهاب الجهاز البولي وإختبار تأثير المضادات الفطرية على السلالات المعزولة مما يساعد الأطباء لتحديد العلاج الأمثل للمرضى.