Assiut Univ. J. of Botany and Microbiology ***Printed* ISSN:** *1687-4927*
48(1), pp1-16 (2019)

RELATIONSHIP BETWEEN ANTIFUNGAL RESISTANCE AND ENZYMATIC ACTIVITIES OF YEASTS CAUSING ORAL AND VAGINAL MYCOSIS

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Received: 29/1/2019 **Accepted:** 28/2/2019 **Available Online:** 7/7/2019

This study was designed to highlight the relationship between resistance of yeast strains, isolated from oral and vaginal infections, to polyene and azole compounds and their abilities to produce protease and lipase enzymes. A total of 194 patients admitted to Assiut University Hospitals, Egypt from January 2015 to December 2017 were clinically diagnosed with candidiasis. Most patients (129 cases) were immunocompromised (ICPs) receiving chemotherapy, radiotherapy or corticosteroids, whereas 65 of patients were non-ICPs. Yeast colonies were purified and identified using traditional methods including germ tube test, chlamydospore formation, and plating on CHROMagar *Candida*. Identification of some isolates was confirmed using API 20C AUX strips and rDNA sequencing. Also, the yeast strains were tested for their sensitivity to antifungal agents as well as for their abilities to produce proteolytic and lipolytic enzymes. A total of 146 yeast strains were recovered from patients and classified into 10 species belonging to 7 genera. *Candida albicans* was the most common species being represented by 87 (59.6%) strains. *C. albicans* showed higher statistically significant proteolytic and lipolytic activity than other yeast strains. There was a highly significant statistical correlation between enzymatic production and resistance of yeasts to azole antifungal agents. Proteolytic activity was detected in 58.7 - 79.8% of azole-resistant yeasts compared with 20.2 - 41.3% of sensitive ones. Similarly, 56.8 - 76.0% of lipolytic yeasts exhibited resistance to azole drugs compared to 24.0 - 43.2% of azole sensitive yeasts. In case of polyene antifungal compounds (nystatin and amphotericin-B), almost all yeast strains were sensitive and could produce both protease and lipase enzymes.

Keywords: Protease, Lipase, Antifungal resistance, Yeast, Enzymatic activity.

INTRODUCTION

Opportunistic fungi can produce hydrolytic enzymes, which play an important role in their virulence and pathogenicity. Of these hydrolases, proteases and lipases are the most highly recognized extracellular enzymes (Park *et al.*, 2013). External digestion of proteins or lipids, by proteases and lipases, is required for survival and growth of both saprophytic and pathogenic fungi (Yike, 2011). Proteases can increase the capacity of the fungus to colonize and penetrate the tissues causing the degradation of an important number of proteins in the host to provide a source of nitrogen to the pathogen, in addition to the tissue adhesion (Hube, 1996; Kantarcioğlu & Yücel, 2002). Lipases have an active role in the invasion of the host's tissue in lesions, causing rupture of the epithelial cell membrane and allowing penetration of the hyphae into the cytoplasm (Kantarcioğlu & Yücel, 2002). Proteases and lipases have been shown to contribute to *Candida albicans* morphological transition, colonization, cytotoxicity, and penetration to the host tissue (D'Eça Júnior *et al.*, 2011; Park *et al.*, 2013; Sharma *et al.*, 2017).

Many pathogenic *Candida* species have been shown to produce, *in vitro*, active extracellular proteases (Gilfillan *et al.*, 1998), whereas less pathogenic or nonpathogenic *Candida* species do not appear to produce significant amounts of proteases, even though they may possess proteases genes. It was demonstrated that all secreted proteases by *Candida* species belong to the same class of aspartyl proteases (Naglik *et al.*, 2003). In Italy, Agatensi *et al.* (1991) observed that isolates of *C. albicans* and *C. parapsilosis* isolated from patients could produce proteases, *in vitro*, in significantly higher levels than those isolated from carriers. They suggested that proteases production could be a reliable factor for distinguishing clinically active infection from asymptomatic fungal carriage. As mentioned by Copping *et al.* (2005) the antifungal agents generated a rise in expression of secreted aspartyl proteinase gene (*SAP2*) and the activity of the *SAP2* gene product; a known virulence factor in most isolates of *C. albicans*.

The relationship between production of proteases and lipases and resistance of yeasts to antifungal agents is still requiring more clarification. Therefore, this study was designed to highlight the relationship between resistance of yeast strains isolated from oral and vaginal infections to polyene and azole compounds and their abilities to produce protease and lipase enzymes.

MATERIALS AND METHODS

Collection of clinical samples

A total of 194 patients admitted to Assiut University Hospitals, Assiut Governorate, Egypt during the period from January 2015 to December 2017 were included in this study. Oral swabs were taken from 106 patients clinically diagnosed with oral candidiasis. Most patients (88 cases) were immunocompromised (ICPs) receiving chemotherapy and/or radiotherapy. Vaginal swabs were taken from women clinically diagnosed with vaginal candidiasis (41 ICPs due to treatment with corticosteroids and 47 non-ICPs).

Identification of yeast isolates

Swabs were streaked on the surface of Petri-dishes containing Sabouraud's glucose agar medium of the following composition (g/l): peptone, 15; glucose, 40 and agar, 20. Plates were incubated at 37 °C for 3-7 days. The developing yeast colonies were purified and identified in Assiut University Mycological Centre (AUMC) using traditional methods such as germ tube test, chlamydospore formation, and plating on CHROMagar *Candida* (Kidd *et al.*, 2016). Some isolates were confirmed by sugar assimilation test using API 20C AUX strips according to the manufacturer's instructions. For more confirmation of some doubtful yeast isolates, molecular identification based on sequencing of internal transcribed spacer (ITS) region of rDNA was also performed with the help of Solgent Company, South Korea. After purification and identification, the sensitivity of yeast strains to antifungal agents as well as their abilities to produce protease and lipase enzymes were conducted.

Extracellular assay of protease

Proteolytic activity was carried out on casein hydrolysis medium with the composition (g/l): KH_2PO_4, 1.0; KCL, 0.5; $MgSO_47H_2O$, 0.2; $CaCI_2.2H_2O$, 0.1; 15 % skimmed milk, 25 ml; glucose, 10; and agar, 15 (Paterson & Bridge, 1994). After autoclaving at 121 °C for 15 minutes, casein medium was poured into sterile 16 ml-test tubes (~10 ml/ tube) and allowed to solidify. A

25 µl from cell suspensions (0.5 McFarland) of yeast strains were pipetted into each test tube followed by incubation at 37 °C for 10 days. 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). Yeast strains were classified into three categories: low enzyme producers (depth ≤ 15 mm), moderate (depth >15-30 mm), and high (depth > 30 mm).

Extracellular assay of lipase

Lipolytic activity was performed according to Ullmann & Blasius (1974). The medium has the composition (g/l): peptone, 10; $MgSO_4 .7H_2O$, 0.2; $CaCI_2.2H_2O$, 0.2; Tween 20, 10 ml; and agar, 15. The medium was sterilized at 121 °C for 15 minutes. Tween 20 was autoclaved separately and added to the sterile and cooled basal medium. The medium was then dispensed, aseptically, in 16 ml-test tubes (~10 ml/tube). The tubes were inoculated with 25 µl of yeast cell suspension (0.5 McFarland) and then incubated at 37 °C for 10 days. The lipolytic ability of the fungal strains was observed as a visible precipitate below the colony surface due to the formation of calcium salt crystals of the liberated free fatty acid. The depth of the precipitate was measured (in mm) and the yeast strains were classified into three categories: low enzyme producers (depth ≤ 15 mm), moderate (depth >15-30 mm), and high (depth > 30 mm).

Antifungal susceptibility test

In vitro disk diffusion method adopted by Clinical and Laboratory Standards Institute (CLSI) M44-A2 protocol (CLSI, 2009) was used to evaluate the degree of fungal sensitivity for eight common antifungal agents. All antifungal discs were obtained from HiMedia Company (India). The Interpretative breakpoints of the tested antifungal agents were determined according to Espinel-Ingroff (2007) and Ellis (2011) as shown in Table 1.

Table 1: Interpretative breakpoints of antifungal agents.

Antifungal agents	**Dose/disc**	**Zone diameter (mm)**		
		S	**I**	**R**
Nystatin (NS)	100 U	≥ 15	10 – 14	≤ 9
Amphotericin B (AP)	100 U	≥ 15	10 – 14	≤ 9
Fluconazole (FLC)	25 µg	≥ 19	15 – 18	≤ 14
Itraconazole (IT)	10 µg	≥ 23	14 – 22	≤ 13
Clotrimazole (CC)	10 µg	≥ 20	12 – 19	≤ 11
Ketoconazole (KT)	10 µg	≥ 28	21 – 27	≤ 20
Miconazole (MIC)	10 µg	≥ 20	12 – 19	≤ 11
Voriconazole (VRC)	1 µg	≥ 17	14 – 16	≤ 13

S: Sensitive. **I:** Intermediate. **R:** Resistant. **mm:** Millimeter.

Statistical analysis

The statistical analysis was performed using the Statistical Program for Social Science (SPSS) version (24.0). A χ^2 test or Fisher exact test was used to determine differences in the proportion of categorized variables. Continuous variables with an approximately normal distribution were tested using the Student's test. A *P*-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Yeast strains identified in the current study

A total of 146 yeast strains including 92 from oral- and 54 from vaginal candidiasis were identified and classified into 10 species belonging to 7 genera. *C. albicans* was the most common species being represented by 87 strains comprising 59.6% of total yeast strains followed by *C. glabrata*, *C. tropicalis,* and *Issatchenkia orientalis* (20.5%, 8.9%, and 6.2% of total strains respectively). The remaining yeasts were less frequently recovered (Table 2)**.** Consistent with the present results, many reports have shown that these three species were next to *C. albicans* in oral and vaginal infections (Mohanty *et al.*, 2007; Rad *et al.*, 2011; Bashir & Ahmad, 2014; Seifi *et al.*, 2015). The predominance of *C. albicans* may be explained by the presence of host cell receptors which facilitate the adherence of this type of *Candida* to the oral and vaginal mucosa allowing their germination and transformation from blastospores to pathogenic filamentous form (Grigoriou *et al.*, 2006; Sobel, 2014). Other virulence factors of *C. albicans* include the production of various exoenzymes (Deorukhkar *et al.*, 2014). *C. albicans* was also the most common species in both oral and vaginal candidiasis whatever the patients were immunocompromised or not.

Table 2: Number of yeast strains isolated from oral and vaginal candidiasis.

Yeast species	**Oral candidiasis (*n*= 92 strains)**		**Vaginal candidiasis (*n*= 54 strains)**		**Total *n* (%)**
	ICPs	**NICPs**	**ICPs**	**NICPs**	
Candida albicans	42	8	17	20	87 (59.5)
Candida glabrata	15	5	8	2	30 (20.5)
Candida tropicalis	7	2	1	3	13 (8.9)
Issatchenkia orientalis	6	1	2	0	9 (6.2)
Clavispora lusitaniae	2	0	0	0	2 (1.4)
Candida dubliniensis	1	0	0	0	1 (0.7)
Dipodascus geotrichum	1	0	0	0	1 (0.7)
Filobasidium magnum	1	0	0	0	1 (0.7)
Pichia norvegensis	0	1	0	0	1 (0.7)
Saccharomyces cerevisiae	0	0	1	0	1 (0.7)
Total	**75**	**17**	**29**	**25**	**146**

ICPs: Immunocompromised patients. **NICPs:** Non-Immunocompromised patients.

Results of gene sequencing (ITS region of rDNA) revealed the identification of 37 yeast strains belonging to 6 genera and 9 species namely *C. albicans* (18 strains), *C. glabrata* (6), *C. tropicalis* (6), *Clavispora lusitaniae* (two), *Issatchenkia orientalis, C. dubliniensis*, *Filobasidium magnum*, *Pichia norvegensis*, and *Saccharomyces cerevisiae* (one for each). The sequences of these strains were uploaded to the GenBank and accession numbers were given as shown in Table 3. The phylogenetic tree based on ITS sequencing data is illustrated in Figure 1.

Table 3: Sequencing results of yeast strains isolated from oral and vaginal candidiasis.

AUMC	GenBank accession number	Length (bp)	Closely related strains accessed from GenBank		
			Fungal species	Accession number	Similarity (%)
13405	MH534905	344	*Clavispora lusitaniae*	LS999912	100
13412	MH534934	778	*Candida glabrata*	NR_130691 T	98
13415	MH534935	488	*Candida albicans*	KP765021	100
13420	MH534936	466	*Issatchenkia orientalis*	AB467300T	98
13421	MH534906	493	*Candida albicans*	NR_125332 T	99
13426	MH534907	491	*Candida albicans*	NR_125332 T	99
13429	MH534908	383	*Candida tropicalis*	NR_111250 T	100
13439	MH534909	483	*Candida tropicalis*	NR_111250 T	99
13440	MH534910	494	*Candida albicans*	NR_125332 T	99
13446	MH534937	345	*Candida glabrata*	NR_130691 T	99
13448	MH534911	338	*Candida tropicalis*	NR_111250 T	100
13449	MH534912	839	*Candida glabrata*	NR_130691 T	99
13468	MH534913	458	*Pichia norvegensis*	NR_138242 T	99
13475	MH534914	348	*Clavispora lusitaniae*	LS999912	100
13477	MH534915	502	*Candida albicans*	NR_125332 T	99
13482	MH534916	501	*Candida albicans*	NR_125332 T	99
13483	MH534917	502	*Candida albicans*	NR_125332 T	98
13489	MH534918	591	*Filobasidium magnum*	NR_130655 T	99
13495	MH534919	503	*Candida albicans*	NR_125332 T	99
13498	MH534920	847	*Candida glabrata*	NR_130691 T	99
13501	MH534921	505	*Candida dubliniensis*	NR_119386 T	99
13515	MH534922	806	*Saccharomyces cerevisiae*	KM029995	97
13527	MH534938	501	*Candida albicans*	NR_125332 T	99
13529	MH534923	498	*Candida albicans*	NR_125332 T	99
13531	MH534924	503	*Candida albicans*	NR_125332 T	99
13532	MH534925	490	*Candida albicans*	NR_125332 T	99
13534	MH534926	491	*Candida tropicalis*	NR_111250 T	99
13535	MH534927	475	*Candida albicans*	NR_125332 T	98
13536	MH534928	844	*Candida glabrata*	NR_130691 T	99
13537	MH534929	843	*Candida glabrata*	NR_130691 T	99
13538	MH534930	492	*Candida tropicalis*	NR_111250 T	99
13540	MH534931	486	*Candida albicans*	NR_125332 T	99
13542	MH534932	486	*Candida tropicalis*	NR_111250 T	99
13543	MH534933	496	*Candida albicans*	NR_125332 T	99
13544	MH534943	488	*Candida albicans*	KP674921	99
13545	MH534944	488	*Candida albicans*	KP675403	98
13546	MH534945	490	*Candida albicans*	KP674921	98

AUMC: Assiut University Mycological Centre accession number. **T:** Type strain. **bp:** Base pair.

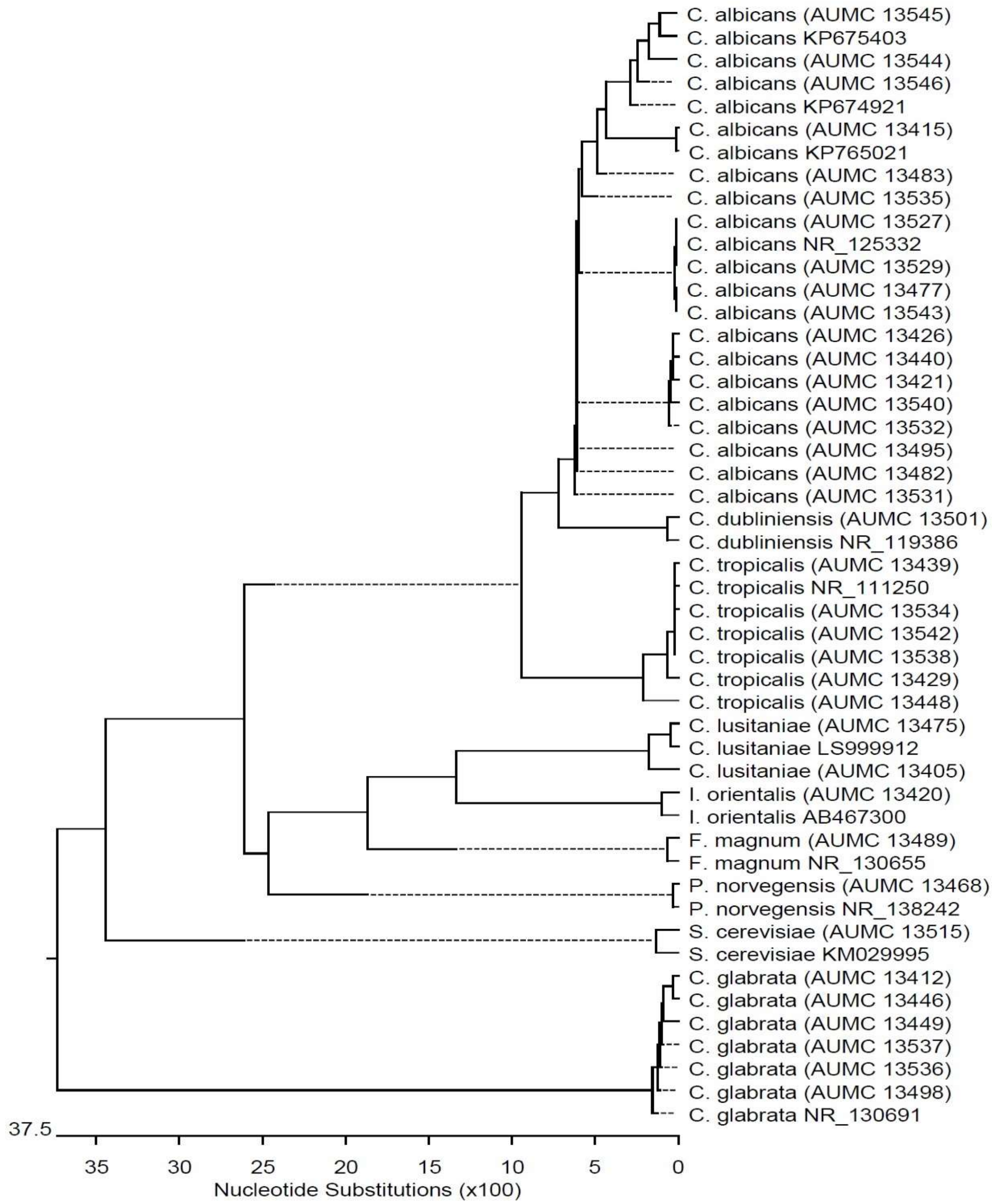

Figure 1: Phylogenetic tree derived from analysis of ITS sequences of some yeast strains isolated from oral and vaginal candidiasis combined with sequences of other yeast strains in GenBank.

Extracellular enzymatic activities

In the current study, protease enzyme was produced by 109 out of 146 (74.7%) strains tested. High protease production was exhibited by 44 strains (43 of *C. albicans* and one *Filobasidium magnum*). Moderate production of protease was shown by 37 strains (34 of *C. albicans*, two *C. tropicalis*, and one *C. dubliniensis*), while the remaining 38 strains (7 of *C. albicans*, 15 of *C. glabrata*, and 6 of *C. tropicalis*) were low producers. Thirty-seven strains (3 of *C. albicans*, 15 of *C. glabrata*, 5 of *C. tropicalis*, 9 of *Issatchenkia orientalis*, two of *Clavispora lusitaniae*, and one of each of *Pichia norvegensis*, *Dipodascus geotrichum*, and *Saccharomyces cerevisiae*) comprising 25.3% of total strains could not produce protease enzyme (Table 4). In agreement with the current results, Kantarcioğlu & Yücel (2002) reported that 78.9 % of *Candida* species were positive to produce of protease enzyme. However, a low percentage (52.4 %) was reported by Oksuz *et al.* (2007). In the current study, 96.6 % of *C. albicans* and 50.0 % of *C. glabrata* could release protease enzyme while *I. orientalis* and *S. cerevisiae* have failed to produce this enzyme. These findings were somewhat consistent with those reported by Al-Hedaithy (2002) who revealed that all strains of *C. albicans* produced protease but other strains of *C. glabrata, C. krusei* (*Issatchenkia orientalis*)*,* and *S. cerevisiae* could not release this enzyme.

In the present investigation, lipase enzymes were detected in cultures of 125 out of 146 (85.6%) strains tested. Active strains included *C. albicans* (53 strains), *C. tropicalis* (13), *C. glabrata* (4), *Filobasidium magnum,* and *C. dubliniensis* (one for each). Low lipolytic activity was exhibited by 22 strains (3 of *C. albicans* and 19 of *C. glabrata*) while the remaining 21 (14.4%) strains could not produce lipase enzyme (Table 4). Our results showed also that all strains of *C. albicans* were positive for lipase test. This observation comes in complete agreement with the results of Kumar *et al.* (2006) who found that 100% of clinical isolates of *C. albicans* isolated from HIV positive and cancer patients produced a pronounced lipase activity. In Scotland, Samaranayake *et al.* (1984) screened 41 *Candida* isolates for lipase activity by using a plate assay method and found that 79% of *C. albicans* strains produced extracellular lipases whereas strains of *C. tropicalis*, *C. glabrata*, and *C. parapsilosis* could not produce this enzyme. Report from Turkey Kantarcioğlu & Yücel (2002) showed a lower degree of lipase activity (62.1%) produced by yeast strains from clinical samples. They also noticed that 93.3% of *C. albicans* isolates were lipase producers, and only a few strains of *C. glabrata* and *C. kefyr* behaved in the same way. In India, Sachin *et al.* (2012) reported that 60.9% of 110 *Candida* isolates obtained from various clinical specimens (from blood, vaginal swab, oral swabs, and urine) were lipase producers. Also, Udayalaxmi *et al.* (2014) found that lipase production was better in *C. albicans* (52.5%) than in *C. tropicalis* (15.8%) and *C. krusei* (22.2%). Previous reports from Egypt (Moharram *et al.*, 2013) showed that 51.6% of yeast strains isolated from vaginal candidiasis were able to produce lipase.

Table 4: Proteolytic and lipolytic activity of yeast strains.

Yeast species	No. of tested strains	Proteolytic activity				Lipolytic activity			
		Positive			Neg.	Positive			Neg.
		L	M	H		L	M	H	
Candida albicans	**87**	7	34	43	3	3	53	31	0
Candida glabrata	**30**	15	0	0	15	19	4	0	7
Candida tropicalis	**13**	6	2	0	5	0	0	13	0
Issatchenkia orientalis	**9**	0	0	0	9	0	0	0	9
Clavispora lusitaniae	**2**	0	0	0	2	0	0	0	2
Candida dubliniensis	**1**	0	1	0	0	0	1	0	0
Pichia norvegensis	**1**	0	0	0	1	0	0	0	1
Filobasidium magnum	**1**	0	0	1	0	0	0	1	0
Dipodascus geotrichum	**1**	0	0	0	1	0	0	0	1
Saccharomyces cerevisiae	**1**	0	0	0	1	0	0	0	1
Total	**146**	**28**	**37**	**44**	37	**22**	**58**	**45**	**21**
		109				**125**			

L: Low (depth ≤ 15 mm). **M:** Moderate (depth >15-30 mm). **H:** High (depth > 30 mm). **Neg.:** Negative.

In the current findings, *C. albicans* showed higher statistically significant proteolytic (96.6%) and lipolytic (100%) activities than other yeast strains (42.4% and 64.4% respectively) ($P < 0.001$) as shown in Table 5. In consistence with the present results, Ramos *et al.* (2015) found that strains of *C. albicans* could produce protease and lipase enzymes at a higher level (100% each) than other yeast strains (53.1% and 4.1% respectively). They also observed that the protease and lipase enzyme profiles provide some data about the potential virulence factors produced by yeast strains. Increasing the proportion of these enzymes produced by *C. albicans* makes the yeast strains more virulent and more capable of causing disease than others (Karkowska-Kuleta *et al.*, 2009; Zarei Mahmoudabadi *et al.*, 2010). In contrast, Sharma *et al.* (2017) reported that non-*C. albicans* were more active producers of lipase than *C. albicans* (63% versus 37% respectively).

Table 5: Relationship between types of yeasts and their enzymatic activities.

Extracellular enzymes	Types of yeasts: *C. albicans* (*n*= 87)		Other yeast strains (*n*= 59)		Total (*n*= 146)		*P*- value
	n	%	*n*	%	*n*	%	
Protease							< 0.001
Positive	84	96.6	25	42.4	109	74.7	
Negative	3	3.4	34	57.6	37	25.3	
Lipase							< 0.001
Positive	87	100	38	64.4	125	85.6	
Negative	0	0.0	21	35.6	21	14.4	

Response of yeast strains to antifungal agents

The present results showed that polyene antifungals were highly effective on tested yeasts where 97.9% of strains were sensitive to nystatin and 35% to amphotericin B (Table 6). Shaik *et al.* (2016) found that 98.7% of *Candida* isolates from various clinical specimens were susceptible to amphotericin B and about 97.3% to nystatin. As for azole compounds, more than 50% of strains were resistant to these compounds. Many researchers indicated that prolonged therapy and increased use of antifungals for prophylaxis or treatment of recurrent candidiasis are the most common risk factors to azoles resistance (Ehrström *et al.*, 2006; Paulitsch *et al.*, 2006). In addition, the inappropriate use of antifungal drugs and introduction of over-the-counter antimycotics worldwide predispose development of antifungal resistance (Aalei & Touhidi, 2000). The susceptibility of *Candida* species to antifungal agents varies widely, while *C. albicans* is sensitive to azoles, other species are resistant to them (Nagashima *et al.*, 2016), and the resistant strains are increasing in number, particularly to the azoles (Smith *et al.*, 2015; Farhan *et al.*, 2018). In Nigeria, Ejike *et al.* (2018) tested the antifungal susceptibility of *Candida* species recovered from vaginal candidiasis patients in a secondary Hospital. They recorded higher MICs to azole antifungals among species of non-*C. albicans*. They concluded that efficacious treatment of vaginal candidiasis requires an adequate knowledge of the causative agents and more importantly the antifungal agents to which yeasts exhibit high susceptibility.

In the current research, there was a significant statistical correlation between enzymatic production and resistance of yeasts to azole antifungal agents. Proteolytic enzymes were detected in 58.7 - 79.8% of azole-resistant yeasts compared to 20.2 - 41.3% of sensitive ones. Similarly, 56.8 - 76.0% of lipolytic yeasts exhibited resistance to azole drugs compared to 24.0 - 43.2% of azole sensitive ones. In case of polyene antibiotics (nystatin and amphotericin B), almost all yeast strains were sensitive and could produce both proteolytic and lipolytic enzymes (Table 7). In this respect, the proteolytic activity is more intense in *Candida* isolates resistant to amphotericin B than in those susceptible to the same drug (Kumar & Shukla, 2010).

Table 6: Response of 146 yeast strains to antifungal agents.

Yeast species (*n*)		Antifungal agents, *n* (%)							
		NS	AP	FLC	IT	CC	KT	MIC	VRC
C. albicans **(87)**	**S**	87	35	12	5	21	12	12	8
	I	0	51	0	21	12	2	18	0
	R	0	1	75	61	54	73	57	79
Candida glabrata **(30)**	**S**	27	7	22	22	23	19	22	23
	I	2	23	1	1	1	4	3	0
	R	1	0	7	7	6	7	5	7
Candida tropicalis **(13)**	**S**	13	2	3	0	2	3	1	3
	I	0	11	0	2	0	0	2	0
	R	0	0	10	11	11	10	10	10
Issatchenkia orientalis **(9)**	**S**	9	4	6	8	8	1	1	9
	I	0	5	0	1	1	7	7	0
	R	0	0	3	0	0	1	1	0
Clavispora lusitaniae **(2)**	**S**	2	2	2	2	2	2	2	2
	I	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0
Candida dubliniensis **(1)**	**S**	1	1	1	1	1	1	1	1
	I	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0
Dipodascus geotrichum **(1)**	**S**	1	0	1	0	0	0	1	1
	I	0	1	0	0	1	1	0	0
	R	0	0	0	1	0	0	0	0
Filobasidium magnum **(1)**	**S**	1	0	0	0	0	0	0	1
	I	0	1	0	1	0	0	1	0
	R	0	0	1	0	1	1	0	0
Pichia norvegensis **(1)**	**S**	1	0	0	1	1	1	0	1
	I	0	1	0	0	0	0	0	0
	R	0	0	1	0	0	0	1	0
Saccharomyces cerevisiae **(1)**	**S**	1	1	1	1	1	1	1	1
	I	0	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0	0
Total (146)	**S**	**143 (97.9)**	**52 (35.6)**	**48 (32.9)**	**40 (27.4)**	**59 (40.4)**	**40 (27.4)**	**41 (28.1)**	**50 (34.2)**
	I	**2 (1.4)**	**93 (63.7)**	**1 (0.7)**	**26 (17.8)**	**15 (10.3)**	**14 (9.6)**	**31 (21.2)**	**0 (0.0)**
	R	**1 (0.7)**	**1 (0.7)**	**97 (66.4)**	**80 (54.8)**	**72 (49.3)**	**92 (63.0)**	**74 (50.7)**	**96 (65.8)**

NS: Nystatin. **AP:** Amphotericin B. **FLC:** Fluconazole. **IT:** Itraconazole. **CC:** Clotrimazole. **KT:** Ketoconazole. **MIC:** Miconazole. **VRC:** Voriconazole. **S:** Sensitive. **I:** Intermediate. **R:** Resistant.

Table 7: Relationship between production of protease and lipase enzymes and resistance of yeast strains to antifungal agents.

Antifungal agents	Proteolytic yeast strains					Lipolytic yeast strains				
	P. (*n*=37)		N. (*n*=109)		*P*-value	P. (*n*=21)		N. (*n*=125)		*P*-value
	n	%	*n*	%		*n*	%	*n*	%	
Azoles										
Fluconazole					< 0.001					< 0.001
Resistant	84	77.1	13	35.1		92	73.6	5	23.8	
Not resistant	25	22.9	24	64.9		33	26.4	16	76.2	
Itraconazole					< 0.001					< 0.001
Resistant	70	64.2	10	27.0		78	62.4	2	9.5	
Not resistant	39	35.8	27	73.0		47	37.6	19	90.5	
Clotrimazole					< 0.001					< 0.001
Resistant	64	58.7	8	21.6		71	56.8	1	4.8	
Not resistant	45	41.3	29	78.4		54	43.2	20	95.2	
Ketoconazole					< 0.001					< 0.001
Resistant	82	75.2	10	27.0		90	72.0	2	9.5	
Not resistant	27	24.8	27	73.0		35	28.0	19	90.5	
Miconazole					< 0.001					< 0.001
Resistant	66	60.6	8	21.6		72	57.6	2	9.5	
Not resistant	43	39.4	29	78.4		53	42.4	19	90.5	
Voriconazole					< 0.001					< 0.001
Resistant	87	79.8	9	24.3		95	76.0	1	4.8	
Not resistant	22	20.2	28	75.7		30	24.0	20	95.2	
Polyenes										
Nystatin					0.253					0.856
Resistant	0	0.0	1	2.7		1	0.8	0	0.0	
Not resistant	109	100	36	97.3		124	99.2	21	100	
Amphotericin B					0.747					0.856
Resistant	1	0.9	0	0.0		1	0.8	0	0.0	
Not resistant	108	99.1	37	100		124	99.2	21	0.0	

P.: Positive. **N.:** Negative.

In agreement with these observations, Lyon & de Resende (2006) found an increase in MIC values of antifungal agents with the higher lipolytic activity of the tested fungi. They also demonstrated that lipase activity was stronger in *Candida* strains whose MICs for fluconazole were below 2 μg/ml. Ying & Chunyang (2012) noticed a correlation between high lipase activity and resistance of *C. albicans* to antifungal drugs and they suggested that lipase may play an important role in the emergence of azole resistance where the gene expression of lipase was higher in fluconazole-resistant strains than in susceptible strains. In addition, Wu *et al.* (2000) found a decrease in extracellular protease activity in susceptible strains of *C. albicans* exposed to fluconazole and an increase in resistant strains. Furthermore, Silva *et al.* (2014) observed a dose-dependent reduction of protease activity in isolates susceptible to fluconazole whereas resistant isolates showed increased protease activity depending on the dose of fluconazole to which they were subjected. Accordingly, exposure to sub-inhibitory concentrations of antifungal agents promotes the development of resistant strains with an increased expression of the target genes. A study conducted by Seneviratne *et al.* (2011) revealed that *Candida* isolates resistant to azoles and caspofungin showed a higher protease activity than the susceptible isolates. In contrast, the results obtained by Schulz *et al.* (2011) revealed no significant differences in the secretion of protease between isolates of *Candida* whether they are susceptible or resistant to fluconazole.

CONCLUSION

The tested yeast strains have responded well to nystatin and amphotericin B, while a high percentage of them have shown resistance to azole compounds. The resistant yeast strains exhibited higher capabilities of secreting protease and lipase enzymes than the sensitive strains.

Acknowledgment

The authors acknowledge the great contribution of all staff members of the AUMC.

Declaration of interest

All authors declare no conflict of interest.

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العلاقة بين المقاومة للمضادات الفطرية والنشاط الإنزيمي للخمائر المسببة لالتهابات الفم والمهبل

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تهدف هذه الدراسة إلى القاء الضوء على العلاقة بين مقاومة الخمائر للمضادات الفطرية التابعة لمركبات الـ Azoles وPolyenes وقدرتها على انتاج الإنزيمات المحللة للبروتينات والدهون. وقد عزلت الخمائر من ١٩٤ مريضا من المترددين على مستشفيات جامعة أسيوط خلال الفترة من يناير ٢٠١٥م حتى ديسمبر ٢٠١٧م، وكان معظمهم (١٢٩ مريضا) منقوصي المناعة ممن يتلقون العلاج الكيميائي أو الإشعاعي أو يتناولون الستيرويدات القلوية. تم اخذ مسحات من المرضى الذين يعانون من التهاب الفم او المهبل بالخمائر لعمل مزارع فطرية على بيئة سبارود جلوكوز اجار والتي حضنت عند درجة حرارة ٣٧°م لمدة ٣-٧ أيام. كما أجريت خطوات التنقية للعزلات الفطرية وتعريفها بالطرق التقليدية متضمنة اختبار انبات الخلايا وتكوين الجراثيم الكلاميدية وتلون المستعمرات على البيئات المنتجة للألوان (CHROMagar *Candida*). وقد تم تأكيد التعريف لعدد من السلالات بالطرق البيوكيميائية (API 20C AUX) وأيضا بالطرق الجزيئية بدراسة تتابع النيوكلتيدات في الحمض النووي الريبوسومي (rDNA). اسفرت الدراسة عن تعريف ١٤٦ سلالة فطرية تنتمي الى عشرة أنواع وسبعة اجناس من الخمائر وكانت *Candida albicans* هي السائدة حيث شاركت بعدد ٨٧ سلالة بنسبة ٥٩,٦٪ من اجمالي عدد الخمائر المعزولة، كما أظهر هذا النوع من الخمائر نشاطا عاليا في انتاج الإنزيمات المحللة للبروتينات والدهون بالمقارنة بالأنواع الأخرى. كما اثبتت النتائج وجود علاقة معنوية بين انتاج السلالات للإنزيمات المحللة للبروتينات والدهون ومقاومتها لمركبات الـ Azole المضادة للفطريات، وقد كانت نسبة السلالات الفطرية المقاومة لمركبات الـ Azoles (٥٦,٨ - ٧٩,٨٪) أكثر نشاطا في تحليل البروتينات عن السلالات الحساسة لهذه المركبات (٢٠,٢ - ٤١,٣٪)، وكذلك كانت ٥٦,٨ - ٦٧,٠٪ من سلالات الخمائر المحللة للدهون مقاومة لمركبات الـ Azoles مقارنة بالسلالات الحساسة (٢٤,٠ - ٤٣,٢٪) وكانت الفروقات ذات دلالات إحصائية عالية. اما في حالة المضادات الفطرية التابعة لمجموعة الـ Polyenes (Nystatin وAmphotericin B) فلم تظهر الدراسة علاقة معنوية بين مقاومة السلالات الفطرية لهذه المركبات مع قدرتها على انتاج الانزيمات حيث كانت جميع سلالات الخمائر حساسة لهذه المركبات وكانت غالبية هذه السلالات منتجة للإنزيمات المحللة للبروتينات والدهون.