Impact Evaluation of Gender and Age on Percentage Distribution of Candidiasis and their Control by Medicinal Mushroom in Laboratory

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Abstract

Biological control represents an important approach for controlling many pathogenic fungi. *Pleurotus ostreatus* is the most promising and effective bioagents against many pathogenic fungi. In this paper seventy clinical specimens were collected from hospitalized patients, who were aged between (1 and 71) years old for both genders. The results of this study showed that out of 70 specimens that collected from urine, Skin, Vaginal and Oral specimens, (61) specimens (87.14%) were positive for fungal infections and (9) specimens (12.86%) were negative. Out of (61) positive specimens, (50) specimens (82%) were positive for *Candida* spp, (7) specimens (11%) were *Trichophyton* spp and (4) specimens (7%) were *Microsporum* spp. Out of (50) positive specimens for candidal infections, *Candida albicans* was the more prevalence fungus (66%) followed by the fungus *Candida glabrata* (14%). The maximum infection incidence has been recorded in the age group of (21- 40) years and the women have been considerably more than the male in all tested age groups. *Pleurotus ostreatus* culture filtrate affected the growth of *Candida* spp and the effect had been increased with the increasing of the filtrate concentrations. The more sensitive species was *C. tropicalis* and C. *krusei* than the other species.

Key words: *Candidiasis, Biocontrol, Medicinal Mushroom, Pleurotus ostreatus.*

Introduction

Fungi were ubiquitous and might grow on the skin, in intestinal tracts and mucous membranes[1]. *Candida* species are normal microbiota of the respiratory tract, gastrointestinal tract, mouth and vagina, and cause opportunistic infections when altered host conditions enable the fungus to proliferate. Although genitourinary candidiasis can occur in both immunocompetent and immunocompromised persons, it is a cause of morbidity and mortality in immunocompromised patients in particular[2]. Gender and age, could be advanced as possible factors in community and hospital acquired infections[3]. Also, the life style of an individual or a group of people within a given community can primarily be a risk factor[4] (Aaron *et al*., 2017). The incidence of candidiasis varies in relation to gender, host susceptibility and hospital settings. The incidence is higher in women and among hospitalized patients[5]. Antimicrobial compounds played a necessary role in the treatment of these diseases. Antifungal resistance has been suggested with long-term antifungal use and with recurrent infections, such as those with persistent mucocutaneous candidiasis or recurrent oropharyngeal candidiasis in patients with uncontrolled human immunodeficiency virus infection[6].

Antimicrobial agent from natural biological supply such as fungal sources is needed. *Pleurotus ostreatus* is a mushroom that used now not for meals consumption since historical instances due to their dietary values and taste but for their medicinal properties. The bioactive substances contained in both the mycelium and fruiting bodies of *P. ostreatus* have been the major sources of exhibit antiarthritis, anti-inflammatory, antidiabetic, immunostimulatory, antioxidative properties, antifungal and antibacterial[7,8]. Emoghene and Onwudinjo[9] proved the antifungal activity of ethanol and aqueous extract of *P. ostreatus* against *C. albicans*. Similar studies were conducted about this field and revealed that the extract of *P. ostreatus* affect the growth of *Candida* species[10,11]. This study is intended for the evaluation of the distribution of *Candida species* isolated from patients in hospital along gender and age divides and

for the evaluation the anticandidal effect of *Pleurotus ostreatus* extract.

Materials and Methods

Collection of samples

Seventy clinical specimens were collected from hospitalized patients who were aged between (1 and 71) years old for both genders, suspected have infection with candidiasis and suffering from symptoms of stomatitis, dermatitis, endometriosis and Pain when urinating. The specimens taken from different sources include (Skin, Urine, Oral and Vaginal thrux). The pathogenic fungal isolate which grown on SDA medium has been identified morphologically according to the microscopic examination, cultural characteristics, growth rate, texture, colony size, germ tube test and Vitek 2 system.

Preparation of *Pleurotus ostreatus* extracts

Twenty grams (20g) of *Pleurotus ostreatus* dried fruiting bodies powder were soaked in 180ml of absolute ethanol for 24hrs and stirred every 6hrs and then filtered through Whatman No.1 filter paper, then concentrated using the rotary evaporator at 40°C until a paste was formed, solutions produced by dissolution of (0.5g) of the extracts in (10ml) of the normal saline to prepare stock solution (S) [12].

Biochemical test (VITEK 2 system)

Vitik-2 system was used in this study in order to diagnose the *Candida* spp to species level according to Sariguzel *et al*., [13].

Effect of *Pleurotus* extracts on Candidal growth

Effect of *Pleurotus* extracts by disc diffusion Method.

Sterilized agar plates have been utilized based on the disc diffusion assays[14], filter paper discs (5.0 mm in diameter) have been impregnated with a variety of the concentration values [50 (S), 25 (S/2) and 12.5 mg/ml (S/4)] of *Pleurotus* extracts. Impregnated disc have been placed at periphery of *Candida* culture plates, the activity has been assessed through the measuring of the inhibition zones of the fungal growth.

Effect of ***Pleurotus*** extracts by poisoned food technique.

The effect was performed according to Kumar *et al*.,[15] different volumes of *Pleurotus* extracts (stock solution) were mixed with the molten SDA media for obtaining the ultimate concentration values of 50%, 25% and 10%, the medium has been put in Petri-plate and inoculated with candidal inoculums, the Petri-plate were incubated at 30ºC. Colony forming unit were calculated by using haemocytometer.

Results and Discussion

Isolation and identification of *Candida* species

The *Candida* species have been characterized by the morphology of the colony, cultures which have dry, white-creamy color, opaque, pinpoint and fluffy colony morphology on the Sabouraud dextrose agar have been suspected as species of the *Candid.* The identification of the species-specific has been made by germ tube test and Vitek 2 system.

Germ tube testing

The results showed that 68% (n=34/50) species have been germ tube positive and have been identified as *C. albican*s. Approximately 32% (n=16/50) species produced no germ tubes and have been identified to be non albican *Candida* species. Two *Candida tropicalis* strains have given false positive results for the *Candida albicans*. The false negative results have been noticed for 1 *C. albicans* species. The sensitivity of this approach for the *Candida albicans* has been 97%.

Biochemical test (VITEK 2 system)

The results of this test confirmed the identification of *Candida* species by morphological features and showed that all *Candida* spp. strain has been successfully identified by Vitek 2 YST card. The diagnosis of *Candida* species gave the diagnostic results of (98%) unequivocal identification, (2%) low discrimination correct results and there was no false identification. *Candida albicans* has been the most common amongst all species of *Candida* which was 33(66%) isolates , *C. glabrata, C. Tropicalis* and *C. krusei* were 7(14%), 8(16%) and 2(4%) strains, respectively.

The result agree with the result of Sanguinetti *et al*.[16] who found that out of 750 isolates, (98.2 %) were identified correctly to the species level by using VITEK 2 system, including those isolates identified with low discrimination but resolved by additional tests, two isolates (0.3 %) were identified with low discrimination and not resolved by additional tests, eight isolates (1.0

%) were misidentified, and four isolates (0.5 %) could not be identified by the VITEK 2 system.

Distribution of fungal species according to the type of specimen and gender

The results of this study showed that out of 70 specimens were collected from different sources include (skin, urine, oral and vaginal thrux), 61(87%) specimens were positive for fungal infections and 9(13%) specimens were negative. Out of (61) positive specimens, 50(82%) specimens were identified as *Candida* spp, 7(11%) specimens were *Trichophyton* spp and 4(7%) specimens were *Microsporum* spp (Table 1).

Table (1): Distribution of fungal species according to type of specimen

Fungal spp.		Clinical specimens (n = 61)				Total	Total percentage
		Oral	Skin	Vaginal	Urine		
Candida spp.	C. albicans	4 (100%)	1 (8%)	12 (75%)	16 (55%)	33	50 (82%)
	C. tropicalis			1 (6%)	7 (24%)	8	
	C. glabrata			3 (19%)	4 (14%)	7	
	C. krusei				2 (7%)	2	
Trichophyton spp.			7 (58%)			7	7 (11%)
Microsporum spp.			4 (34%)			4	4 (7%)
Total		4 (100%)	12 (100%)	16 (100%)	29 (100%)	61	61 (100%)

The result was agree with the results of Al-Oebady[17] that found the most prevalent yeast isolates from vaginal swabs, urine samples and oral thrush samples are *C. albicans* 58/92 (63%), *C. glabrata* 13/92 (14.2%), *Trichosporon* sp. 5/92 (5.43%) and *Geotrichum* sp. 1/92 (1.08).

Sheevani *et al*.[18] referred that the infections caused by *Candida* species was the most significant opportunistic mycosis worldwide, in addition to being amongst the leading nosocomial infections causes.

The result also showed that out of (50) positive *Candida* specimens, 17(34%) specimens and 33(66%) isolated from male and female respectively (Table 2).

Table (2): *Candida* species gender wise distribution (n = 50)

Gender	Candida spp.				Total
	C. albicans	C. glabrata	C. tropicalis	C. krusei	
Male	11 (22%)	2 (4%)	3 (6%)	1 (2%)	17 (34%)
Female	22 (44%)	5 (10%)	5 (10%)	1 (2%)	33 (66%)
Total	33 (66%)	7 (14%)	8 (16%)	2 (4%)	50 (100%)
* P - Value = 0.0050 significant					

The result was compatible with the results of Heidrich *et al*.[19] who found that *Candida* genus has been of a higher prevalence in the females (5.84% males and 15.90% of the females), moreover, it had an impact over the females who have been older than the males (54 vs. 47 years, respectively).

Distribution of *Candida* infection in various age groups

The results also revealed that the *Candida* species distribution in the males and females has been different with the difference of age, it has been observed that the *C. albicans* has been the prevalent one amongst *Candida* species and in male and female and in all age groups. The result had been revealed that the maximum infection incidence has been recorded in the age group of (21 - 40) years, followed by the age group of (41 - 60) years and the minimum infection incidence has been recorded in the age group of (61 - 71) years (Table 3).

Table (3): Gender wise *Candida* species distribution in different ages

Age (Years)/Gender		Candida spp. (n = 50)				Total
		C. albicans	C. glabrata	C. tropicalis	C. krusei	
1 - 20	M	3	1	0	0	4 (8%)
	F	5	1	0	0	6 (12%)
21 - 40	M	4	1	1	1	7 (14%)
	F	9	2	2	1	14 (28%)
41 - 60	M	3	0	1	0	4 (8%)
	F	5	2	2	0	9 (18%)
61 - 71	M	1	0	1	0	2 (4%)
	F	3	0	1	0	4 (8%)
Subtotal	M	11	2	3	1	17 (34%)
	F	22	5	5	1	33 (66%)
Total	M+F	33 (66%)	7 (14%)	8 (16%)	2 (4%)	50 (100%)
* P - Value = 0.0427 significant						

The result was agree with the results of Loster *et al*.[20] who found that the maximum occurring of candidiasis has been recorded in age group of less than (50) years which was (61.4%) followed by the age group (50 – 60) years which was (43.7%).

The effect of *Pleurotus* culture filtrate

The effect of culture filtrate by poison food technique

The results showed different sensitivities of *Candida* species to the *Pleurotus* filtrate and the more sensitive species are *C. tropicalis* and C. *krusei* than the other species. Lowest cell account was recorded in case of *C. tropicalis* at the concentration of (50%) of the *Pleurotus* filtrate stuck solution on SDA which was (1.51 * 10^8 cell/ml) and the highest one was recorded at the concentration (10%) which was (1.95 * 10^8 cell/ml) for species *C. glabrata* which was more resistant to *Pleurotus* filtrate than the other species (Table 4).

Table (4): Effects of *P. ostreatus* culture filtrate on the growth of *Candida* spp. by poison food technique

Treatments	Concentration %	Cell account (cell/ml)			
		C. albicans	C. glabrata	C. tropicalis	C. krusei
Pleurotus ostreatus	50	1.56 * 108	1.58 * 108	1.51 * 108	1.52 * 108
	25	1.75 * 108	1.79 * 108	1.72 * 108	1.72 * 108
	10	1.93 * 108	1.95 * 108	1.92 * 108	1.93 * 108
Control	-	2.06 * 108	2.10 * 108	2.02 * 108	2.04 * 108

The result was agree with the study achieved by Phan *et al.*[21] to evaluate the anti-Candidial activities of the ethyl acetate, methanol and the aqueous extracts of the *Pleurotus giganteus* against the *Candida* species and revealed that the aqueous extract had minimum activity while the extract of the ethyl acetate entirely inhibited all *Candida* spp.

The effect of culture filtrate by disk diffusion method

The results showed that the highest inhibition zone that has been recorded for the *C. tropicalis* at the concentration (50 mg/ml) has been 8 mm, and the smallest inhibition zone was recorded at a concentration of (25 mg/ml) which has been 6 mm, there is no any inhibition at the concentration (12.5 mg/ml) (Table 5).

Table (5): Activity of *P. ostreatus* extracts against *Candida* species by disk diffusion method

Treatments	Concentration	Zone inhibition (mm)			
		C. albicans	C. glabrata	C. tropicalis	C. krusei
Pleurotus ostreatus	50 mg/ml	7	7	8	7
	25 mg/ml	6	6	7	6
	12.5 mg/ml	0	0	0	0
Control	-	0	0	0	0

The medicinal types of mushroom are rather less researched for the anti-fungal characteristics they have, none-the-less, in the past 5 years, there was an increased level of the interest in their use as antimicrobial agents, the oyster mushroom (*Pleurotus ostreatus*) as well as other medicinal mushroom types have been exhibiting anti-fungal activities against the *C. albicans*[22].

Conclusions

Candida albicans was the more prevalence fungus. The maximum infection incidence has been recorded in the age group of (21- 40) years and the women have been considerably more than the male in all tested age groups. *Pleurotus ostreatus* culture filtrate affected the growth of *Candida* species.

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