

Susceptibility of *Candida albicans* from different clinical samples to fluconazole

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ABSTRACT

Candida are an opportunistic human fungal pathogens. The last two decades witnessed a rapid spread of candidiasis. *Candida* classified as the fourth causative agent of nosocomial infections. Resistance to azole among *Candida* spp. clinical isolates has been increased, especially to fluconazole, which is the most common prescribed drug used. Samples collected during the period from December 2016, and March 2017, from different biological samples of patients aged between 8-70 years old. Out of 100 samples, 48 were *Candida albicans*, *C. glabrata* 14, *C. tropicalis* 9, *C. krusei*, and *C. sphaerica* 7, *C. parapsilosis*, and *C. kefyr* 4, *C. guilhermondii* 3; and *C. norvegenesis*, and *C. famata* 2. Infections were predominant at age group 20-39 in the area of study. Fluconazole susceptibility of *Candida albicans* isolates were tested using disc diffusion method. *C. albicans* isolates were susceptible to Fluconazole with high significant differences ($P < 0.01$). Out of 48 isolates, 39 were susceptible, 5 were S-DD, and 4 were resistant. Non-*albicans* infections' rate was exceeding that of *Candida albicans*, and *Candida albicans* showed a high susceptibility to fluconazole.

Keywords: *Candida albicans*, *Candida* species, Candidiasis, Fluconazole, drug resistance

1. INTRODUCTION

Candida are an opportunistic human fungal pathogens causing mild to severe life-threatening infections. *Candida albicans* is most common human opportunistic pathogen among other *Candida* species [1]. *Candida* species are ranked as the fourth causative agent of nosocomial infections after resistant *Staphylococcus*, *Acinetobacter*, and *Pseudomonas* species. Candidemia responsible for 7–10% of nosocomial bloodstream infections [2]. It is the most common infection among women, 75% of healthy women experience at least one symptomatic episode of vulvovaginal candidiasis (VVC) during their life [3]. In Iraq candidiasis ranked as the fourth among the sexually transmitted diseases, with incidence of 76.73 per 100,000 population [4]. Candidiasis accounted for 26.2% of skin mycosis in Iraq [5], and 38% of women with vaginal discharge diagnosed with VVC [6].

The use of antifungals, especially fluconazole which is the drug of choice in many countries caused the declining of *Candida albicans* from the majority species to cause less than 50% of infections, and the incidence of infection has been shifted towards the resistant non-*albicans* *Candida* species [7]. *C. albicans*, together with *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, and *C. krusei* are responsible for 50–90% of candidiasis in humans [8, 9].

Resistance to azoles among *Candida* spp. clinical isolates has been increased in the last two decades, especially to fluconazole. Immunosuppression and long term therapy with azoles are the main risk factor for the development of azole resistance. Azole resistance is public health problem in patients receiving fungistatic azole and in immunocompromised patients, thus the global burden of azole resistance to *Candida albicans* increased with the increasing immunocompromised patients [7].

2. MATERIALS AND METHODS

2.1 Samples collection

Sample collection was performed between December 2016 and March 2017, 100 Samples were collected from patients suffering from fungal infection diagnosed by consultant physicians in Baghdad; Madinat AL-tib teaching hospital. Samples were taken as: sputum from patients with respiratory tract infections, swabs from vulvovaginal infected women, and patients with oral thrush; and as urine. All samples were cultured on SDA.

2.2 Identification of *Candida* isolates

The isolates identified as *Candida* depending on morphological features on culture medium, germ tube formation, and API *Candida* system.

2.3 Antifungal susceptibility test

Antifungal susceptibility test performed according to M44-A which is a global guideline developed by CLSI for disk diffusion testing of *Candida* spp. [10]. *Candida albicans* subcultured on SDA at 37 °C for 24 h, inoculum prepared by picking five distinct colonies from 24 hour old culture, suspended in 5 mL of sterile 0.145 mol/L saline (8.5 g/L NaCl; 0.85% saline), the suspension then vortexed for 15 seconds and the turbidity adjusted visually by either adding more saline or more colonies to adjust the transmittance to that produced by

McFarland 0.5 turbidity standard. For obtaining yeast stock solution of 1×10^6 to 5×10^6 cells per mL. Mueller Hinton + 2% glucose+ 0.5 methylene blue dye (GMB) agar plate was inoculated by sterile cotton swab dipped in the inoculum suspension. Fluconazole discs was dispensed onto the inoculated agar surface, finally the plates were incubated at 37 °C for 24 hours.

2.4 Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to study the effect of difference factors in study parameters. Chi-square test was used to significantly compare between percentages.

3. RESULTS AND DISCUSSION

3.1 Identification of *Candida* spp. isolates

A total of 100 isolates 39 from vagina, 32 sputa, 15 oral swabs, 10 were urine, and 4 were ear swabs. *C. albicans* represented the most isolated species 48 (48%). Non-albicans candida contributed to (52%) of the samples (Table 1). Among non-albicans spp. *C. glabrata* 14 (14%), followed by *C. tropicalis* 9 (9%), *C. krusei*, and *C. sphaerica* 7 (7%), *C. parapsilosis*, and *C. kefyr* 4 (4%), *C. guiliermondii* 3(3%); and *C. norvegenesis*, and *C. famata* 2 (2%) (Table 2) *C. albicans* was the most isolated species with high significant differences ($P < 0.01$).

Table 1: Isolated *C. albicans* and non- albicans

Species	No. (%)
<i>Candida albicans</i>	48 (48%)
Non-albicans	52 (52%)
Chi-Square	1.394 NS

Table 2: Identification of *Candida* isolated, and their distribution to samples.

Species	Quantity %	Isolation site				
		Vaginal swab	Sputum	Oral swab	Urine	Ear swab
<i>C. albicans</i>	48 (48%)	18	17	8	5	---
<i>C. glabrata</i>	14 (14%)	7	4	1	1	---
<i>C. tropicalis</i>	9 (9%)	3	4	1	1	---
<i>C. krusei</i>	7 (7%)	4	2	1	---	---
<i>C. sphaerica</i>	7 (7%)	2	2	2	1	---
<i>C. parapsilosis</i>	4 (4%)	2	--	--	2	--
<i>C. kefyr</i>	4 (4%)	1	---	2	---	1
<i>C. guiliermondii</i>	3 (3%)	1	1	---	---	1
<i>C. norvegenesis</i>	2 (2%)	1	1	---	---	---
<i>C. famata</i>	2 (2%)	1	---	---	---	1
Chi-Square (χ^2)	11.762					
	($P < 0.01$)					

The present study showed that *Candida albicans* was predominant among other pathogenic *Candida* species, and non- albicans *Candida* represented more than half of the samples. Due to the use of azole, non-albicans *Candida* species infections has been increased gradually, and exceeded the infection rate of *C. albicans* [11]. The present results were supported by Kim *et al.* (2016) who revealed that *C. albicans* is the most frequently isolated species (50.5%) [12]. Also

Deorukhkar *et al.* (2014) who reported the same trend, non-albicans infections have an isolation ratio (63.3%) higher than *Candida albicans* ratio (36.7%) [13].

3.2 Distribution of *Candida* infections according to age groups

The majority of *Candida* infections have been at the age groups 20-30 (27%), and 30-40 (25%), followed by age groups 40-50 (17%), 50-60 (12%), and the lowest

infections rate showed in very young people 10-20 (8%), and very old people more than 60 (11%) (Table 3). Statistical analysis showed a highly significant

differences ($P < 0.01$) in infection's rate between age groups.

Table 3: Distribution of *Candida* infections according to age groups.

Age group (year)	No. (%)
10-20	8
20-30	27
30-40	25
40-50	17
50-60	12
More than 60	11
Chi-Square (χ^2)	9.447
	($P < 0.01$)

Candida is an opportunistic pathogen infection with candidiasis depend on the immunological state, and social activities, especially people with age of 20-49 have more outdoor activities than teenagers with vigorous immune system and elderly with low outdoor activities [12]. A study by Fernandes *et al.* (2009) also reported that age groups between 20-49 years has the highest rate of infections, and the age groups younger than 20 year has the lowest infection rate, which agree with the present study results [14].

3.3 Fluconazole susceptibility results

Out of 48 isolates of *Candida albicans* 4 isolates were fluconazole (FLC) resistant, 5 were S-DD, and 39 were susceptible to FLC (Table 4). Statistical analysis showed *Candida albicans* isolates were susceptible to FLC with high significant differences ($P < 0.01$). The present results agree with AL-Maliki *et al.* (2011) who revealed the high susceptibility of *Candida albicans* against fluconazole with (78.37%) of isolates being sensitive, and (21.2 %) resistant [15].

Table 4: Fluconazole susceptibility among *Candida albicans* isolates.

Susceptibility	No. (%)
S	39 (81.25%)
R	4 (8.33%)
S-DD	5 (10.42%)
Chi-Square (χ^2)	13.519
	($P < 0.01$)

4. CONCLUSION

Candida albicans was most frequently isolated species among other pathogenic *Candida* species. Non-*albicans* infections' rate exceeding that of *Candida albicans*. The majority of infections are in the age group of 20-39. It was observed that *Candida albicans* was highly susceptible to fluconazole.

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