

# Antifungal susceptibility of oral *Candida* species isolated from patients with thalassemia major

## Abstract

**Background:** Invasive fungal infections are the primary cause of morbidity and mortality in patients with thalassemias. This high mortality rate stems, in part, from the prevalence of resistance to antifungal agents. *Candida* species remain the most common cause of invasive fungal infection in patients with thalassemia. Nevertheless, other *Candida* species become increasingly spreading and need to be identified to a species level.

**Objectives:** The objectives of the study were to estimate the prevalence of *Candida* infection among Thalassemia major patients compared to healthy controls and to identify the resistance patterns among the recovered species.

**Methods:** A total of 60 oral samples were collected from two groups: Group I: 40 oral swabs were collected from patients clinically diagnosed with thalassemia major from the out-patient clinics of Pediatric department in Ain Shams University Hospital. Group II: 20 oral swabs were collected from healthy subjects matched in age and sex with the patients' group. The swabs were cultured onto both CHROM and Sabaroud's agar media. The recovered colonies on Sabaroud's agar were identified using Gram stain and Germ tube test while the colonies on CHROM agar were self-identified through color produced according to manufacturer instructions. For all identified colonies API 20 C AUX was used as a confirmatory test. Antifungal Susceptibility testing using Fluconazole (25µg), Voriconazole (1µg), Nystatin (100 units) was used to identify the resistance strains.

**Results:** *C. albicans*, were isolated from 69.2% of cases while 30.8% were non *C. albicans*. (23% *C. tropicalis* and 7.8% *C. krusei*). Susceptibility testing for all identified colonies revealed that (13.8%) of all isolates were resistant to fluconazole, (17.3%) to voriconazole and (89.7%) to nystatin.

**Conclusion:** The use of CHROM agar media offers multiple advantages without increasing costs in comparison to other procedures. CHROM agar media have equal or greater sensitivity of detection, superior detection of mixed cultures as a result of better visualization of different species, and enable shorter times to report results. Antifungal susceptibility tests are now required to optimize antifungal treatment to avoid emergence of new resistant strains. The structural analysis with X-rays, as the research method of the thin superficial layers.

**Keywords:** *Candida* species, CHROM agar media, *C. albicans*, voriconazole

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## Introduction

Thalassemias are a diverse group of genetic blood diseases characterized by absence or decreased production of either  $\alpha$  or  $\beta$ -globulin protein chains, resulting in microcytic anemia of varying degrees and referred to as  $\alpha$  or  $\beta$ -thalassemia.<sup>1</sup>

In developing countries, diligent efforts have been devised to screen and monitor the patients closely and have helped preventing morbidity and mortality due to iron overload. However, infection-related deaths in developing and developed countries still occur.<sup>2</sup> Invasive fungal infections are the major cause of infections in patients with thalassemias. This high mortality rate stems, in part, from the prevalence of resistance to antifungal agents, characteristics of the population at risk and the standard of the health care facilities available.<sup>3</sup>

*Candida* species remain the most common cause of invasive fungal infection in patients with thalassemia. Nevertheless, other *Candida* species become increasingly spreading and need to be identified to a species level.<sup>4</sup> One of the methods that widely used is the Cornmeal

agar (CHROM agar) especially when Germ tube failed to identify the recovered *Candida* species. This special medium yield microbial colonies with varying colors secondary to chromogenic substrates that react with enzymes secreted by microorganisms.<sup>5</sup>

Treating infections caused by *Candida* species warrant the use of specific antifungal agent due to rapid emergence of resistant strain of *Candida* species. Antifungal susceptibility testing deemed mandatory for patients with thalassemia suspiciously infected with *Candida* species.<sup>6</sup> The aim of the study was to estimate the prevalence of *Candida* infection among Thalassemia major patients compared to healthy controls and to identify the resistance patterns among the recovered species.

## Materials and methods

During the period of April 2011 to January 2012; a total of 60 oral samples were collected from two groups:

Group I: 40 oral swabs were collected in the morning from patients clinically diagnosed with thalassemia major within the onset of

symptoms and before initiation of antifungal therapy when possible. The patients were selected from the out-patient clinics of Pediatric department in Ain Shams University Hospital.

Group II: 20 oral swabs were collected from healthy subjects matched in age and sex with the patients' group.

Once collected swabs were sent immediately to the Medical Microbiology and Immunology department, Faculty of Medicine, Ain Shams University and cultured onto both CHROM and Sabaroud's agar media (Oxoid Brilliance *Candida* Agar - England). The recovered colonies on Sabaroud's agar were identified using Gram stain and Germ tube test while the colonies on CHROM agar were self-identified through color produced according to manufacturer instructions.

*Candida tropicalis* and *C. albicans* possess hexosaminidase which results in green coloured colonies, however, other metabolic reactions of *C. tropicalis* produce a localized drop in pH which results in dark blue colonies. Alkaline phosphatase activity in *C. krusei* results in a brown or pink pigmentation. For all identified colonies API 20 CAUX (BioMérieux - France) was used as a confirmatory test. Antifungal Susceptibility testing using Fluconazole (25µg), Voriconazole (1µg), Nystatin (100units) was used to identify the resistance strains.

### Statistical analysis

Analysis of data was done by using SPSS (statistical program for social science version 16). Quantitative data were described by mean and standard deviation.

Qualitative data were be described by frequency and percentage using Chi-square test and student t-test. A p-value less than 0.05 will be considered significant.

### Results

The age range of the whole population was between 6 to 14years with mean age of (9.4±2.08years) and were 19 boys and 21 girls. 65% of *Candida* species were isolated from clinical cases of thalassemia while 15% were isolated from healthy controls and the difference was statistically significant (Table 1).

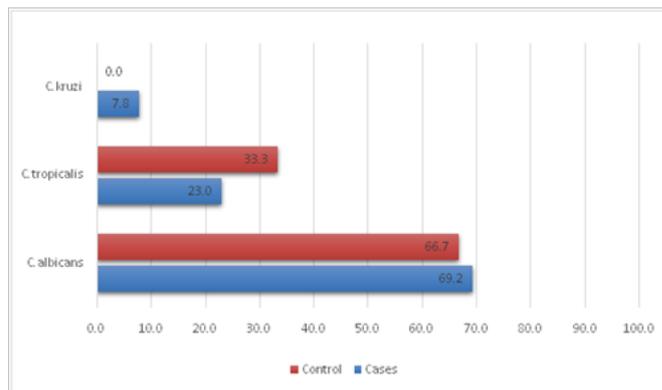
**Table 1** Culture results on CHROM agar

	Culture results (CHROM Agar)			p-value
	Cases n(%)	Controls n(%)	Total n(%)	
<i>Candida</i> species	26(65%)	3(15%)	29(48.3%)	0.001
No growth	14(35%)	17(85%)	31(51.7%)	0.001
Total	40(100%)	20(100%)	60(100%)	0.001

However, both groups showed similar patterns of distribution of *Candida* species where *Candida albicans* constitutes the majority of isolated species in both cases and controls (69.2% and 66.7% respectively) and *Candida tropicalis* constituted (23% and 33.3% respectively), *Candida krusei* was only recovered from cases (7.8%) (Figure 1).

The data indicated that 69% of positive cases and 66.7% of controls identified on Chrom agar were also positive by Germ tube test with no statistical difference between results in both groups (Table 2).

All isolates identified by Chrom agar were confirmed by API *Candida*. Chrom agar showed 100% sensitivity and specificity (Table 3).



**Figure 1** Distribution of *Candida* Species Identified on Chrom Agar.

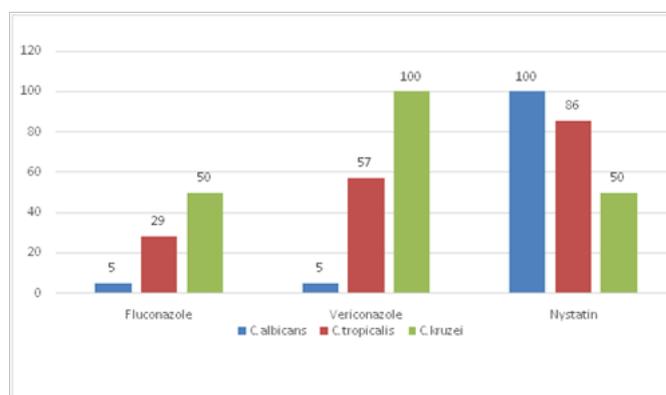
**Table 2** Comparison between Germ tube test and CHROM agar culture results

Germ tube test	Positive culture samples on (CHROMAagar)			
	Cases(n=26)		Controls(n=3)	
	No.	%	No.	%
Positive germ tube test	18	69	2	66.7
Negative germ tube test	8	31	1	33.3
Total	26	100	3	100

**Table 3** Comparison between CHROM agar culture results and API results

<i>Candida</i> Spp.	Chrom agar identification results		API <i>Candida</i> results		p-Value
	N	(%)	N	(%)	
<i>Candida albicans</i>	20	69	20	69	1
<i>Candida tropicalis</i>	7	24.1	7	24.1	1
<i>Candida krusei</i>	2	6.9	2	6.9	1
Total	29	(100)	29	(100%)	

All *C. albicans* strains were resistant to Nystatin but more susceptible to Fluconazole and Voriconazole (95% and 75% respectively). Similarly, most *C. tropicalis* strains were resistant to Nystatin (85.7%) and more susceptible to Fluconazole and Voriconazole (57% and 14% respectively). On the other hand all *C. krusei* strains showed 100% susceptibility to Nystatin and (50%) susceptibility to Fluconazole and Voriconazole (Figure 2).



**Figure 2** Proportion of *Candida* spp. resistance to antifungal agents

History of splenectomy was a significant predisposing factor for *Candida* colonization among thalassemic patients (p-value<0.05) 28 cases had history of splenectomy, 22 of them (78.6%) were colonized with *Candida* species (Table 4).

Serum ferritin was also a significant predisposing factor in developing *candida* colonization among cases and controls (p-value<0.05) (Table 5).

**Table 4** Proportion of *Candida* species isolated from splenectomized and non splenectomized patients

History of splenectomy	Culture results on (CHROM Agar)				Total	p-Value
	Positive		Negative			
Splenectomized patients						
Non-splenectomized patients	22	-78.60%	6	-21.40%	28(100%)	0.002
Total	4	-33.30%	8	-66.60%	12(100%)	0.02
	26		14		40	0.01

**Table 5** Proportion of *Candida* species isolated in relation to serum ferretin level

Ferretin level	Culture results (CHROM Agar)				Total	p-Value
	Positive (No) (%)		Negative (No) (%)			
Ferretin level lower than 1000	1	-50.00%	38	-50.00%	28(100%)	0.5
Ferretin level higher than 1000	25	-65.90%	40	-34.10%	12(100%)	0.002
Total	26		14		2	0.01

## Discussion

Our results showed that (65%) of clinically confirmed cases of thalassemia major were colonized with *Candida* species, which were significantly higher than healthy subjects (15%). Similar rate was reported by Hazza'a et al.,<sup>3</sup> who isolated *Candida* species from 74% and 69% of thalassemic patients respectively. They attributed the high rate of colonization by *candida* to the immune abnormalities that may have increased the predisposition to oral fungal colonization in these thalassemic patients compounded by iron overload and anemia.

*C. albicans* was the most commonly isolated species from both thalassemic and healthy groups (69%, 66.7 % respectively). A similar finding was reported in another study.<sup>7</sup> Our results showed that non-albicans *Candida* species (NAC) which were isolated from the thalassemic group (31%) were nearly similar to that of healthy group (33.3%). Nweze et al.,<sup>7</sup> reported an increase in the prevalence of non albicans species isolated from periodontal lesions of HIV-infected patients (45%).

*C. albicans* was isolated from (69%) of cases and (35%) were NAC. A comparable rate were obtained by Dimopoulos et al.,<sup>8</sup> where *C. albicans* was recovered from (64.3%) of candidemia episodes in intensive care unit patients compared to NAC from (35.7%). On the contrary Rajendran et al.,<sup>9</sup> reported that forty one percent of candidaemia cases were associated with *Candida albicans*, while 59% were associated with NAC.

The present study showed that (24.1%) of *candida* species were *C. tropicalis*, this rate was nearly similar to the rate reported by Nweze et al.,<sup>7</sup> who reported that (18.3%) of the isolates were *C. tropicalis* isolates. However different rates were reported by other studies with rates (10.7%), (9%) and (32%) respectively.<sup>8,10</sup>

*C. krusei* represented (6.9%) of isolated *Candida* species in our study and this finding was nearly similar to the rate (13%) reported by other studies.<sup>9,11</sup> Certain systemic factors like serum ferritin had increase the predisposition for oral *Candida* colonization due to their effects in immune system.<sup>3</sup> This study showed that (65.9%)

of thalassemic patients who had high serum ferretin levels were colonized with *Candida* species compared to (34.1%) who had lower serum ferretin levels.

All results were confirmed by API system to identify three *Candida* species (*C. albicans*, *C. tropicalis* and *C. krusei*). These results were in agreement with other studies<sup>12,13</sup> who reported that the identification rates were 100%.

However, Sultan et al.,<sup>14</sup> observed less sensitivity for *C. albicans* on CHROM agar (92.1%). This was attributed to the direct inoculation of clinical samples on CHROM agar. Results of identification with the API 20 C AUX (BioMérieux - France) system using inocula directly from CHROMagar medium gave the same biochemical profile and morphology as inocula obtained from Nutrient agar. These findings demonstrate the suitability of using CHROM agar for confirmatory or supplementary testing without time-consuming subculture on sabouraud agar.

In the present study antifungal susceptibility tests using disc diffusion method was done for all positive isolates of different *Candida* species (13.8%) of all isolates were resistant to Fluconazole, (17.3%) to Voriconazole and (89.7%) were resistant to Nystatin. These rates were similar to results reported by Nweze et al.,<sup>7</sup> who found that (11.7%) of all *Candida* isolates were resistant to Fluconazole and (17%) to Voriconazole.

The low Fluconazole-resistance rate (5%) of *C. albicans* in this study is consistent with other research findings. A study in Nigeria by Akortha et al.,<sup>15</sup> reported (4.3%) of *C. albicans* resistance to Fluconazole. Another study by Sobel et al.,<sup>16</sup> reported Fluconazole resistance in (3.6%) of *C. albicans* isolates.

Several studies concluded that the prophylactic use of Fluconazole may permit intrinsically resistant *Candida* species to emerge as many patients who received Fluconazole found to be infected with azole resistant *C. krusei*, and *C. glabrata*.<sup>17</sup>

In the present study, *C. albicans* isolates were totally resistant (100%) to Nystatin only however, *C. tropicalis* were (85.7%),

but *C. krusei* isolates were sensitive (100%) to Nystatin. Nystatin prophylaxis significantly reduces *Candida* colonization, but no definite conclusions about Nystatin impact on oral candidiasis.<sup>18</sup>

In resource-limited countries, clinicians have little confidence in the accuracy and quality of laboratory test results. They continue to prescribe costly antifungal drugs without knowing the exact antifungal profile of the infectious agent; thereby they increase the economic burden of the society.

## Conclusion

The use of CHROM agar media offers multiple advantages without increasing costs in comparison to other procedures. Using CHROM agar media have equal or greater sensitivity of detection, superior detection of mixed cultures as a result of better visualization of different species, and enable shorter times to report results. The life expectancy of patients with thalassemia has improved dramatically over the past 50 years owing Improved survival has mainly been achieved by early diagnosis of the disease and proper treatment through optimized antifungal therapy.<sup>2,19</sup>

## Acknowledgements

None.

## Conflict of interest

The author declares no conflict of interest.

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