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Original Article

Distribution and Antifungal Susceptibility Pattern of *Candida* species at a Tertiary Hospital in Malaysia

Nurul Azmawati Mohamed[1], Siva Gowri Pathmanathan[1], Hazilawati Hussin[2], Adilahtul Bushro Zaini[3]

[1] Department of Basic Medical Sciences II, Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia (USIM), Kuala Lumpur, Malaysia
[2] Microbiology Unit, Department of Pathology, Hospital Ampang, Selangor, Malaysia
[3] Microbiology Unit, Department of Pathology, Hospital Sungai Buloh, Selangor, Malaysia

Abstract

Introduction: Invasive *Candida* infections cause significant mortality and morbidity worldwide. Information on recent trends in species distribution and antifungal resistance in local settings is essential.

Methodology: Yeast isolates identified through standard culture methods throughout 2014 and 2015 from Hospital Ampang, Malaysia were retrospectively studied. The antifungal susceptibility of *Candida* species was determined using colorimetric broth microdilution method and MIC values interpreted according to CLSI breakpoints.

Results: Out of all the 149 yeast cultures collected, most were from blood (55.7%) and respiratory specimens (33.6%). *Candida tropicalis* was the most common (28.9%), followed by *C. albicans* (26.2%), *C. parapsilosis* (15.4%), *C. glabrata* (14.1%), *Crytococcus neoformans* (6.7%), *Trichosporon asahi* (3.4%), *C. krusei* (2.0%), *C. famata*, *C. rugose*, *C. guilliermondii*, *C. dublinensis* and *Trichosporon* spp. (0.7% each). Occurrence of *C. tropicalis* in candidaemia cases was significantly associated to presence of an underlying haematological disorder, while *C. albicans* isolates in blood were significantly found in absence of such disorders. The four most common *Candida* species isolated showed high susceptibility to amphotericin B (100%), anidulafungin (100%), micafungin (100%), caspofungin (98.4%), flucytosine (98.4%) and voriconazole (84.1%). However, drug susceptibility to itraconazole and fluconazole was comparatively lower (57.9% and 72.2%, respectively). *C. glabrata* and *C. tropicalis* were the least susceptible to these azoles.

Conclusion: Prevalence of the high number of non-*albicans Candida* species with slight predominance of *C. tropicalis* over *C. albicans* was observed. Low susceptibility to itraconazole among *C. glabrata* and *C. tropicalis* isolates and to fluconazole among *C. glabrata* isolates warrants for continued surveillance to monitor emerging antifungal resistance.

Key words: Yeast, *Candida*, Antifungal Susceptibility, Malaysia.

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Introduction

The incidence of invasive yeast infections worldwide continues to rise mainly due to increased use of immunosuppressive therapy and invasive devices [1]. *Candida* spp. are the most common cause of invasive yeast infections yet alone the most common cause of fungal infections [2]. *Candida* spp. are part of the normal flora of healthy hosts that are responsible for various superficial and systemic, opportunistic infections [3]. Although *Candida albicans* remains the major cause of invasive candidiasis, increasing trends in infections caused by non-*albicans* species have been observed in recent years worldwide [4-6] including Malaysia [7-9]. Next to candidiasis, other yeast infections are usually caused by *Cryptococcus neoformans* followed by the less common *Trichosporon* spp., *Rhodotorula* spp and others [3;6;10].

Invasive candidiasis is associated with significant morbidity and mortality and therefore appropriate and timely antifungal therapy is essential [11]. The Infectious Diseases Society of America (IDSA) recommends the newest class of antifungal agents, the echinocandins i.e. caspofungin, micafungin or anidulafungin as initial therapy for candidaemia in both neutropenic and non-neutropenic patients [12]. However, fluconazole is still widely used due to its low cost and availability for both parenteral and enteral administration. Reduced susceptibility of *Candida,* especially non-*albicans* species, to fluconazole and the overall cost effectiveness with echinocandins, especially in the critically ill patients, favours echinocandins [13;14]. While fluconazole resistance differs between countries, resistance to echinocandins has also emerged in some countries [15]. Updated

knowledge of local antifungal resistance patterns is hence deemed essential. Limited number of studies have reported the antifungal susceptibility of *Candida* species isolated in Malaysian clinical settings between 2005 to 2009 [7;9;16]. Herein we report distribution of yeast isolates especially *Candida* spp. and their susceptibility to antifungal drugs at a tertiary hospital in Malaysia throughout 2014 and 2015.

Methodology

Study Design

This is a retrospective study analysing yeast infections among patients at Hospital Ampang, Selangor, Malaysia from 1st January 2014 to 31st December 2015. The Hospital Ampang is a tertiary government hospital and the national reference centre for the treatment of haematological disorders. It is a 500-bed hospital with a total of 12 general wards and four haematology wards. This study was approved by the Medical Research and Ethics Committee, Ministry of Health, Malaysia [KKM/NIHSEC/800-4/4/1 Jld. 48(58)].

Yeast Isolation and Identification

Data on yeast cultures obtained from normally sterile sites (i.e. blood; respiratory, such as broncho-alveolar lavage and nasopharyngeal aspirate; tissue; cerebrospinal fluid; bone; peritoneal fluid and bile) and detailed information of the patients were gathered from the Laboratory and Hospital Information System. Repetitive cultures from the same patient that yielded same yeast species (within three months) were excluded. The isolation and identification of fungi were performed using standard methods in the microbiology laboratory within the hospital; all specimens were inoculated on Sabouraud's dextrose agar, brain heart infusion agar and mycosel agar and incubated at 30ºC for 48 hours. Identification was done by germ tube technique, inoculation on chromogenic agar and biochemical identification tests. Germ tube test was performed by inoculating isolated yeast cells into serum and incubating at 37ºC for 2 hours. Yeast isolates were subcultured on chromogenic *Candida* medium (Oxoid, Basingstoke, UK) at 37ºC for 48 hours to distinguish *Candida* species based on specific colony colours produced by the chromogenic substrates in the medium. All isolates were then subjected to the API 20C AUX biochemical identification kit (BioMérieux, Marcy I'Etoile, France) for confirmatory identification of the yeast isolates.

Antifungal Susceptibility Testing

Antifungal susceptibility test was performed at the reference laboratory in Hospital Sungai Buloh, Selangor, Malaysia using colorimetric microdilution susceptibility test (Sensititre YeastOne) by Trek Diagnostic System (Cleveland, USA). Briefly, a 20µL volume of suspension of 24-hour yeast colony adjusted to 0.5 Mc Farland density was transferred to 11mL of YeastOne inoculum broth to obtain a final density of 1.5 – 8.0 $\times 10^3$ CFU/mL. A 100µl volume of this mixture was transferred into each well of the Sensititre plate containing serial dilutions of amphotericin B, fluconazole, itraconazole, voriconazole, anidulafungin, micafungin, caspofungin and 5-flucytosine. The wells were covered using an adhesive seal and incubated at 35ºC in a non-CO_2 incubator for 24 hours.

The plate was then read under normal laboratory lighting using a reading mirror that displayed the underside of the wells. The MIC was recorded as the lowest concentration of antifungal agent inhibiting the development of colour change from the initial blue to red or purple (indicating yeast growth). MIC results were interpreted according to species-specific clinical breakpoints established by Clinical and Laboratory Standards Institute (CLSI) [17]. MIC50 and MIC90 values, defined as the concentration of antifungal agent that inhibited 50% and 90% of the isolates, respectively, were determined.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 24.0 (IBM Corporation, New York, USA). Pearson's Chi-squared test was performed to investigate statistical significance between variables. All statistical analysis were two tailed and a p-value < 0.05 in a two-sided test was considered significant.

Results

A total of 149 cases of yeasts infection were identified during this 2-year- period, 85 of which were from male patients (57%) and 64 from female patients (43%). Only 53 of these patients had an underlying haematological disorder (35.5%). The underlying haematological disorders were lymphoma (17.4%), acute myeloid leukaemia (10.7%), acute lymphoblastic leukaemia (3.4%), chronic lymphocytic leukaemia (2.0%), multiple myeloma (1.3%) and aplastic anaemia (0.7%). Most of the isolates obtained were from blood (55.7%) and respiratory (33.6%) specimens. Respiratory specimens included broncho-alveolar lavage and nasopharyngeal aspirate. Table 1 shows the distribution of the different yeast species according to

Table 1. Distribution of yeast species according to type of specimen.

Species	No. (%) of yeast isolates							Total
	Blood	Respiratory (BAL/ NPA)	Tissue	CSF	Bone	Peritoneal fluid	Bile	
C. tropicalis	28 (33.7)	10 (20)	3 (60)	0	1 (25)	1 (50)	0	43 (28.9)
C. albicans	17 (20.5)	18 (36)	2 (40)	0	1 (25)	0	1 (100)	39 (26.2)
C. parapsilosis	13 (15.7)	8 (16)	0	0	1 (25)	1 (50)	0	23 (15.4)
C. glabrata	12 (14.5)	9 (18)	0	0	0	0	0	21 (14.1)
Cryptococcus neoformans	6 (7.2)	0	0	4 (100)	0	0	0	10 (6.7)
Trichosporon asahi	3 (3.6)	1 (2)	0	0	1 (25)	0	0	5 (3.4)
C. krusei	1 (1.2)	2 (4)	0	0	0	0	0	3 (2.0)
C. famata	1 (1.2)	0	0	0	0	0	0	1 (0.7)
C. rugosa	1 (1.2)	0	0	0	0	0	0	1 (0.7)
C. guilliermondii	0	1 (2)	0	0	0	0	0	1 (0.7)
C. dublinensis	0	1 (2)	0	0	0	0	0	1 (0.7)
Trichosporon spp	1 (1.2)	0	0	0	0	0	0	1 (0.7)
Total	**83 (55.7)**	**50 (33.6)**	**5 (3.4)**	**4 (2.7)**	**4 (2.7)**	**2 (1.3)**	**1 (0.7)**	**149**

BAL: broncho-alveolar lavage, NPA: nasopharyngeal aspirate, CSF: cerebrospinal fluid.

type of specimen. *Candida* spp. accounted to a total of 133 (89.3%) isolates. *C. tropicalis* (28.9%) was the most commonly isolated yeast, followed by *Candida albicans* (26.2%), *Candida parapsilosis* (15.4%) *Candida glabrata* (14.1%) and others. Among the non-*Candida* yeasts ten (6.7%), were *Cryptococcus neoformans*, five were *Trichosporon asahi* and one *Trichosporon* species.

C. tropicalis was the yeast most commonly isolated from blood (33.7% of all blood isolates), while *C. albicans* predominated in the respiratory specimens (36% of all respiratory isolates). The distribution of the different *Candida* species isolated from blood according to age group and presence of an underlying haematological disorder is shown in Table 2. While the 73 candidaemia isolates included seven from infants, the most number of isolates were equally found in the 40-59 and 60 & above age groups. No significant association was found between patient age group and species of *Candida*. However, the occurrence of *C. tropicalis* in candidaemia cases was significantly associated to presence of an underlying haematological disorder (p = 0.002). *C. albicans* on the other hand was mostly found in the absence of haematological disorders (p = 0.009).

The antifungal susceptibility results of the four most commonly isolated *Candida* species are summarised in Table 3. Table 4 summarizes the MIC ranges as well as MIC_{50} and MIC_{90} values of the eight antifungal drugs tested against these *Candida* species. Overall, high susceptibility to amphotericin B (100%), anidulafungin (100%), micafungin (100%), caspofungin (98.4%) and flucytosine (98.4%) was observed. *C. albicans* and *C. parapsilosis* also showed high susceptibility to the three azoles i.e. voriconazole, itraconazole and fluconazole. *C. tropicalis* however had reduced susceptibility to voriconazole (76.7%), itraconazole (39.5%) and fluconazole (76.7%). *C. glabrata* on the other hand, although was highly susceptible to voriconazole (81.0%), was only 9.5% susceptible to itraconazole and fluconazole.

Table 2. Distribution of *Candida* species in blood specimens according to age groups and presence of underlying hematological disorders.

Patient characteristics	No. (%) of *Candida* isolates					Total
	C. tropicalis	*C. albicans*	*C. parapsilosis*	*C. glabrata*	Other *Candida*	
Age (yr.)						
Below 1	1 (3.6)	3 (17.6)	3 (23.1)	0	0	7 (9.6)
1-19	1 (3.6)	1 (5.9)	2 (15.4)	0	1 (33.3)	5 (6.8)
20-39	5 (17.9)	2 (11.8)	2 (15.4)	1 (8.3)	1 (33.3)	11 (15.1)
40-59	11 (39.3)	6 (35.3)	4 (30.8)	4 (33.3)	0	25 (34.2)
60 & above	10 (35.7)	5 (29.4)	2 (15.4)	7 (58.3)	1 (33.3)	25 (34.2)
Hematological disorder						
Yes	19 (67.9)*	3 (17.6)	6 (46.2)	3 (25.0)	2 (66.7)	33 (45.2)
No	9 (32.1)	14 (82.4)*	7 (53.8)	9 (75.0)	1 (33.3)	40 (54.8)

Table 3. Antifungal susceptibility pattern of the four most common *Candida* spp. isolated.

Antifungal agent		Percentage (n) of isolates susceptible, resistant, intermediate or SDD				
		C. tropicalis	*C. albicans*	*C. parapsilosis*	*C. glabrata*	All 4 *Candida* spp
Amphotericin B	S	100 (43)	100 (39)	100 (23)	100 (21)	100 (126)
Anidulafungin	S	100 (43)	100 (39)	100 (23)	100 (21)	100 (126)
Micafungin	S	100 (43)	100 (39)	100 (23)	100 (21)	100 (126)
Caspofungin	S	97.7 (42)	100 (39)	95.7 (22)	100 (21)	98.4 (124)
	R	2.3 (1)	0	0	0	0.8 (1)
	I	0	0	4.3 (1)		0.8 (1)
Flucytosine	S	97.7 (42)	100 (39)	100 (23)	95.2 (20)	98.4 (124)
	R	0	0	0	0	0
	SDD	0	0	0	4.8 (1)	0.8 (1)
	na	2.3 (1)	0	0	0	0.8 (1)
Voriconazole	S	76.7 (33)	87.2 (34)	95.7 (22)	81.0 (17)	84.1 (106)
	R	10.8 (8)	12.8 (5)	0	14.3 (3)	12.7 (16)
	I	0.6 (1)	0	0	4.8 (1)	1.6 (2)
	SDD	0.6 (1)	0	4.3 (1)	0	1.6 (2)
Itraconazole	S	39.5 (17)	87.2 (34)	87.0 (20)	9.5 (2)	57.9 (73)
	R	16.3 (7)	12.8 (5)	4.3 (1)	38.1 (8)	16.7 (21)
	I	16.3 (7)	0	0	9.5 (2)	7.1 (9)
	SDD	8.4 (12)	0	4.3 (1)	42.9 (9)	17.5 (22)
	na	0	0	4.3 (1)	0	0.8 (1)
Fluconazole	S	76.7 (33)	87.2 (34)	95.7 (22)	9.5 (2)	72.2 (91)
	R	18.6 (8)	12.8 (5)	4.3 (1)	23.8 (5)	15.1 (19)
	I	0	0	0	4.8 (1)	0.8 (1)
	SDD	4.7 (2)	0	0	61.9 (13)	11.9 (15)

S: susceptible; R: resistant; I: intermediate; SDD: susceptible dose dependent; na: not available.

Table 4. MIC ranges, MIC50 and MIC90 values (μg/mL) of the antifungal agents against the four most common *Candida* spp. isolated.

Antifungal agent		*C. tropicalis*	*C. albicans*	*C. parapsilosis*	*C. glabrata*	All 4 *Candida* spp
Amphotericin B	MIC range	0.25-25	0.12-1	0.12-1	0.25-1	0.12-25
	MIC_{50}	1	0.5	0.25	0.5	0.5
	MIC_{90}	1	1	1	1	1
Anidulafungin	MIC range	0.015-1	0.015-0.25	0.12-2	0.015-1	0.015-2
	MIC_{50}	0.06	0.015	0.5	0.03	0.06
	MIC_{90}	0.12	0.06	2	0.06	1
Micafungin	MIC range	0.008-2	0.008-0.25	0.06-2	0.008-0.5	0.008-2
	MIC_{50}	0.03	0.008	0.5	0.015	0.015
	MIC_{90}	0.05	0.03	2	0.015	0.5
Caspofungin	MIC range	0.008-8	0.015-0.12	0.06-0.5	0.015-0.5	0.008-8
	MIC_{50}	0.06	0.03	0.25	0.06	0.06
	MIC_{90}	0.25	0.06	0.5	0.12	0.25
Flucytosine	MIC range	0.012-0.25	0.06-1	0.06-0.25	0.06-16	0.012-16
	MIC_{50}	0.06	0.06	0.06	0.06	0.06
	MIC_{90}	0.12	0.12	0.12	0.12	0.12
Voriconazole	MIC range	0.008-8	0.008-8	0.008-0.12	0.12-8	0.008-8
	MIC_{50}	0.12	0.015	0.015	0.25	0.06
	MIC_{90}	8	8	0.06	2	8
Itraconazole	MIC range	0.025-16	0.012-16	0.015-0.5	0.25-16	0.012-16
	MIC_{50}	0.25	0.06	0.06	0.5	0.12
	MIC_{90}	0.5	16	0.12	1	1
Fluconazole	MIC range	0.05-256	0.05-256	0.05-4	4-256	0.05-256
	MIC_{50}	2	0.25	0.25	16	1
	MIC_{90}	128	128	1	64	128

Discussion

Candida spp. are the most common cause of invasive yeast infections. At least 15 distinct species of *Candida* cause human diseases, although 95% of infections are caused by the five most common pathogens, *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis*, and *C. krusei* [18]. In the present study, non-*albicans Candida* accounted to 70.7% of the total *Candida* isolates and 76.7 % of the candidaemia causing isolates obtained throughout year 2014 and 2015. The incidence of non-*albicans Candida* worldwide is generally observed to be increasing. Still, the proportion of non-*albicans Candida* among *Candida* species in hospital-based studies done in various parts of the world seem to largely vary from 18.3% to 83.0% [19]. This variability between different geographical regions is caused by several factors, mostly due to different antifungal treatment practices, use of indwelling catheters, patient demographic features and chronic underlying diseases [18].

In this study*, C. tropicalis* was found to be the most common yeast isolated, specifically in blood specimens. The organism outnumbered *C. albicans* by 2.7%, a trend not observed before in other Malaysian studies. A study on candidaemia cases from 2005 to 2006 at another tertiary hospital in Malaysia by Tzar and Shamim [7] also found *C. tropicalis* to be the most frequent non-*albicans Candida*, however *C. albicans* isolates were higher by 8%. Similarly, Ng and colleagues [8] also found *C. tropicalis* to be the most common *Candida* species isolated from blood next to *C. albicans*, among isolates from various specimens obtained between 2000 and 2013. *C. tropicalis* is indeed the leading non-*albicans* species in Asia and more so in tropical areas [20], when compared to other regions such as Europe, UK and USA [19]. Increase in number of *C. glabrata* isolates on the other hand have been reported in Europe and USA, owing to the extensive use of fluconazole as prophylaxis and treatment [19].

In this study, blood and respiratory cultures accounted for 55.7% and 33.6%, respectively of all yeast isolates. It is important to note that isolation of *Candida* from respiratory tract should not be considered as a marker of lung infection. A study reported that no cases of *Candida* pneumonia was identified among 135 autopsies with histopathological evidence of bacterial infection and high rate of *Candida* isolation from endotracheal aspirate and/ or bronchalveolar lavage [21].

Although not significant, most (65.1%) of the *C. tropicalis* isolates found herein, were from blood specimens, a trend also reported elsewhere [6]. On the other hand, more than half of the *C. albicans* isolates (56.4%) were obtained from non-blood samples. *C. tropicalis* isolation from blood specimens was found to be significantly associated to an underlying haematological disorder, while *C. albicans* was significantly negatively related. Similarly, a study revealed significantly lower occurrence of *C. albicans* in patients with haematological disorders when compared to patients with solid tumours; while non-*albicans Candida*, namely *C. krusei* and *C. glabrata* were more prevalent among the former [22].

This study is the first of such, reporting on yeast isolates from Hospital Ampang, Malaysia. The hospital being a national reference centre for haematological disorders in Malaysia, and significant association between *C. tropicalis* isolation and underlying haematological disorders (and vice versa with *C. albicans*) justifies the increased incidence of *C. tropicalis* as compared to *C. albicans* noted herein.

Risk factors of invasive *Candida* infections vary between species. Risk factors for C. *tropicalis* includes older age, cancer, neutropenia and presence of biofilms in urinary catheters [23]. Duration of central venous catheter use, use of fluconazole, gastrointestinal surgery, older age, intravenous drug use and glucocorticoid therapy are significant risk factors for *C. glabrata* and *C. krusei*; while younger age and prior exposure to echinocandin antifungals have been associated with *C. parapsilosis* infections [18]. Information on recent trends in antifungal susceptibility patterns is important in determining best prophylactic and therapeutic strategies. In the present study, the antifungal susceptibility pattern of the four most common *Candida* spp. isolated herein were analysed. All the isolates were either completely and/ or highly susceptible to amphotericin B, the echinocandins and flucytosine. Antifungal resistance among *C. albicans* is generally uncommon [12] and the same was noted herein. *C. parapsilosis* was also highly susceptible to all antifungals tested including the azoles. Relatively higher MIC_{90} values for the echinocandins were noted in *C. parapsilosis*. Four of the 23 *C. parapsilosis* isolates had an MIC value of 2µg/mL for anidulafungin and micafungin. Although resistance to echinocandins is rare among *Candida* species, *C. parapsilosis* is known to be innately less active to the echinocandins [12; 24].

Only 9.5% of *C. glabrata* isolates were susceptible to fluconazole and itraconazole. This is a huge difference compared to an 83.4% (n=12; year 2006-2008) susceptibility of *C. glabrata* to these azoles reported by Amran and colleagues at another tertiary

hospital in Malaysia [9]. However, they also noted that MIC_{90} value of itraconazole was comparatively high for *C. glabrata*. Although only 9.5% of *C. glabrata* isolates were susceptible to fluconazole and itraconazole, the resistance level was about similar to *C. tropicalis* and *C. albicans* because a large percentage of *C. glabrata* isolates were susceptible-dose dependant (SDD) to these two drugs (42.9% and 61.9% respectively). The low susceptibility of *C. glabrata* to fluconazole and itraconazole is in accordance to findings from other tertiary hospitals in Malaysia [7; 16]. Furthermore, the total susceptibility of all candidaemia isolates to itraconazole was reported to be as low as 40% (of total 50 *Candida* isolates) by Tzar and Shamim [7]. Santhanam and colleagues reported a susceptibility of 56.1% among a total of 82 *Candida* isolates obtained in 2009 at Hospital Kuala Lumpur, Malaysia, further confirming this low susceptibility of *Candida* species to itraconazole as a recent trend in Malaysia [16]. In agreement to that, the present study found the overall susceptibility of the four most common *Candida* species to itraconazole to be 57.9%. Herein, a slightly low susceptibility to fluconazole (72.2%) was also noted, as compared to 90% and 82.9% reported by the other two studies, respectively [7; 16]. High reduction in fluconazole susceptibility rate over the years among *C. glabrata* isolates is not uncommon [20; 25] owing to long term and routine use of the drug. It is hence important to monitor the resistance pattern in Malaysia to provide useful information on therapeutic approaches.

Voriconazole was the most active azole against *C. glabrata*, as also noted in another study [6]. Due to increase in azole resistance among *C. glabrata* isolates worldwide, antifungal susceptibility testing is highly recommended prior to treatment of this species with azoles [12]. Next to *C. glabrata*, *C. tropicalis* showed lowest susceptibility to the azole group of antibiotics, especially itraconazole. This is not encouraging since the prevalence of both species accounts to about 43% of the total isolates. Furthermore these two species are reportedly more virulent than *C. albicans* [22].

Conclusions

The yeast isolates, mainly being *Candida* spp, isolated in the year 2014 and 2015 at Hospital Ampang in Selangor, Malaysia, were predominated by non-*albicans Candida* species. *C. tropicalis* was the most common species isolated and more so in blood samples. The antifungal resistance observed in the present study was mainly restricted to the azoles. All *Candida* species were highly susceptible to amphotericin B, the echinocandins and flucytosine. Lowest drug susceptibility was noted to itraconazole especially among *C. glabrata* and C. *tropicalis* isolates and to fluconazole among *C. glabrata* isolates. These findings emphasize the trend seen worldwide on increasing resistance to the azoles especially among the non-*albicans Candida* species. The opposite correlation seen in the present study between prevalence of *C. tropicalis* and *C. albicans* with underlying haematological disorders may have caused a higher incidence of azole-resistance in this clinical setting which warrants for a multicenter study in the future, for confirmation.

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Corresponding author

Dr. Siva Gowri Pathmanathan
Faculty of Medicine and Health Sciences,
Universiti Sains Islam Malaysia, Menara B
Persiaran MPAJ, Jalan Pandan Utama,
Pandan Indah, 55100 Kuala Lumpur, Malaysia
Phone: +603 4289 2400
Fax: +603 4289 2477
Email: gowri@usim.edu.my

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