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Antifungal Susceptibility Patterns Among *Candida* Species Isolated from Blood at Universiti Kebangsaan Malaysia Medical Centre

(Corak Kerentanan Antikulat di Kalangan Spesies *Candida* yang dipencil daripada Darah di Pusat Perubatan Universiti Kebangsaan Malaysia)

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ABSTRACT

Many challenges arise in candidaemia treatment which involves emergence of antifungal resistance. New species have been identified due to improved methods of detection and some are resistant to commonly prescribed antifungal agents such as fluconazole and amphotericin B. Therefore, the objective of the study was to observe any changes in the susceptibility patterns and distribution of Candida species. *This cross sectional study was conducted at the Department of Medical Microbiology and Immunology in UKMMC, a tertiary teaching hospital. One hundred and fifty one data were collected from the department's laboratory records from January 2008 to December 2010. The yeasts were identified using ID32C carbohydrate assimilation tests whilst the antifungal susceptibility test was performed using Sensititre® YeastOne® broth microdilution method. Antifungal agents tested included amphotericin B, fluconazole, itraconazole, voriconazole, 5-flucytosine and caspofungin. Out of 151 blood isolates, 47 (31.1%) were* Candida albicans *and 104 (68.9%) were non-albicans* Candida species. Candida tropicalis *has surpassed* C. albicans *as the most commonly isolated Candida species from blood. Overall susceptibility (as compared to 2005-2006 data in brackets) to caspofungin was 99.3% (n/a), 5-flucytosine 97.4% (98%), amphotericin B 94.7% (100%), voriconazole 92.7% (98%), fluconazole 86.8% (90%) and itraconazole 39.1% (40%). In conclusion, although the isolates were generally still susceptible to amphotericin B and fluconazole, resistance to these drugs is increasing.*

Keywords: Antifungal resistance; antifungal susceptibility; Candida*; candidaemia*

ABSTRAK

Banyak cabaran timbul dalam rawatan kandidemia melibatkan kemunculan kerintangan antikulat. Spesies baru telah dikenal pasti selaras dengan penambahbaikan kaedah pengenalpastian dan sebahagiannya adalah rintang terhadap antikulat yang biasa diberi seperti flukonazol dan amfoterisin B. Oleh itu, tujuan kajian ini adalah untuk memerhati sebarang perubahan dalam corak-corak kerentanan dan taburan spesies Candida. *Kajian keratan rentas ini telah dilakukan di Jabatan Mikrobiologi dan Imunologi Perubatan di PPUKM, sebuah hospital pengajar tertier. Data telah dikumpul daripada rekod makmal jabatan dari Januari 2008 hingga Disember 2010. Yis-yis telah dikenal pasti dengan menggunakan ujian asimilasi karbohidrat ID32C manakala ujian kerentanan antikulat telah dilakukan dengan kaedah pencairan kaldu mikro Sensititre® YeastOne®. Agen-agen antikulat yang diuji termasuk amfoterisin B, flukonazol, itrakonazol, vorikonazol, 5-flusitosin dan kaspofungin. Daripada 151 isolat darah, 47 (31.1%) adalah* Candida albicans *dan 104 (68.9%) adalah spesies bukan-albicans* Candida. Candida tropicalis *telah mengatasi* C. albicans *sebagai spesis* Candida *yang paling kerap dipencil daripada darah. Kerentanan keseluruhan (dibandingkan dengan data 2005-2006 dalam kurungan) terhadap kaspofungin adalah 99.3% (n/a), 5-flukitosin 97.4% (98%), amfotericin B 94.7% (100%), vorikonazol 92.7% (98%), flukonazol 86.8% (90%) dan itrakonazol 39.1% (40%). Kesimpulannya, walaupun isolat-isolat secara umumnya masih rentan terhadap amfoterisin B dan flukonazol, kerintangan terhadap ubatan ini semakin bertambah.*

Kata kunci: Candida*; Kandidemia; kerentanan antikulat; kerintangan antikulat*

INTRODUCTION

Candida species is the fourth most common organism associated with bloodstream infections (BSIs) which represent the eighth leading cause of death in the world (Wenzel & Edmond 2001). In many studies, *C. albicans* had been reported to be the most common *Candida* species responsible for candidaemia (Cuenca-Estrella et al. 2002; Takakura et al. 2003; Tortorano et al. 2003; Tzar & Shamim 2009; Xess et al. 2007). However, non-albicans *Candida* species (NACs) were also noted to be increasingly common especially *C. tropicalis*, *C. parapsilosis* and *C. glabrata* (Cuenca-Estrella et al. 2002; Kalkanci et al. 2007; Takakura et al. 2003; Tortorano et al. 2003; Zepelin et al. 2007).

Since these NACs often have variable susceptibilities to antifungal agents, it is recommended that every healthcare institution have their own local data on antifungal susceptibility (Xess et al. 2007). In many countries including Malaysia, antifungal susceptibility testing is not routinely offered. However, it is becoming more relevant nowadays due to various issues such as amphotericin B nephrotoxicity, emergence of fluconazole-resistant isolates and a wider choice of antifungal agents. Therefore, this study aimed to investigate changes in the local epidemiology and susceptibility patterns of *Candida* species at Universiti Kebangsaan Malaysia Medical Centre (UKMMC).

Materials and Methods

Study Populations

A cross-sectional study was conducted at the Department of Medical Microbiology and Immunology, UKMMC. This medical centre is a 1054-bedded, tertiary level teaching hospital that provides various medical, surgical and intensive care services. The study was carried out from January 2008 to December 2010.

Data Collection

Data were collected from the Mycology Laboratory Records, UKMMC. Information collected included month, year, laboratory number, medical record number, location (ward / clinic), age, sex, race, yeast isolated and antifungal susceptibility results. All *Candida* species isolated from the blood cultures from January 2008 to December 2010 were included in this study with the exception of *Candida* species from the same patient with identical species and susceptibility pattern.

Yeast Identification

ID 32C carbohydrate assimilation tests (bioMerieux, Inc., USA) were used to identify the yeasts isolates according to the manufacturer's instructions as described previously (Tzar & Shamim 2009).

Antifungal Susceptibility Testing

Antifungal susceptibility test was performed on all *Candida* species isolated using commercially–prepared Sensititre YeastOne YO-8 broth microdilution kit by Trek Diagnostic (UK) according to the manufacturer's instructions as described previously (Tzar & Shamim 2009). The minimal inhibitory concentration (MIC) was taken as the lowest antifungal concentration that inhibits fungal growth (the first blue well). The MICs were interpreted according to the breakpoints provided by the Clinical Laboratory and Standards Institute (CLSI). The antifungal agents were categorized as susceptible, susceptible but dose-dependent (SDD), intermediate or resistant. Antifungal agents used were amphotericin B, fluconazole, itraconazole, voriconazole, flucytosine and caspofungin.

Ethical Considerations

This study was approved by the Research and Ethics Committee of Medical Faculty, Universiti Kebangsaan Malaysia (research code FF-363-2010).

Results

A total of 151 *Candida* species were identified. More *Candida* isolates were obtained from blood samples taken from male patients (95, 63.8% versus 54, 36.2%), with a ratio of 1.8:1 (2 missing data). Majority of the isolates came from adult patients (18-64 years old), which accounted for 105 (69.5%) isolates. The mean age was 46 years old and the median age was 52. The most common age group affected was 51-60 years old (40, 26.5%). Out of 151 isolates, 28 (18.5%) were from the elderly patients (aged 65 and above) and only 18 (11.9%) came from the paediatrics group (aged below 18). Four (2.6%) isolates came from infants. There were 90 (59.6%) isolates from the Malays, 40 (26.5%) from the Chinese, 11 (7.3%) from the Indians and 10 (6.6%) from other races. *Candida albicans* showed predilection towards the younger age groups while the reverse is true for non-albicans *Candida* species (NAC). *Candida albicans* accounted for 55.6% of candidaemia cases among infants, 38.9% among all paediatrics (less than 18 years old), 30.7% among adults (18-64 years old) and only 25% among elderly (65 years and above). Most isolates of *Candida* were from the Intensive Care Unit 41 (27.2%), surgical wards 38 (25.2%), medical wards 34 (22.5%), High Dependency Unit 13 (8.6%), Paediatrics wards 6 (4.0%) and Paediatrics Intensive Care Unit 5 (3.3%). Three isolates were obtained from Bone Marrow Transplant Unit, Paediatrics High Dependency Unit and Cardiac Care Unit. Two isolates from Obstetrics & Gynaecology wards and Burns unit and one isolate from the Neonatal Intensive Care Unit. Out of 151 blood isolates, 47 (31.1%) were *Candida albicans* and 104 (68.9%) were NAC species. Fifty-five (36.4%) isolates were *C. tropicalis*, 47 (31.1%) *C. albicans*, 26 (17.2%) *C. parapsilosis*, 13 (8.6%) *C. glabrata*, 4 (2.6%) *C. pelliculosa*, 2 (1.3%) *C. sake*, 1 (0.7%) *C. krusei*, 1 (0.7%) *C. globosa*, 1 (0.7%) *C. melibiosica* and 1 (0.7%) *C. famata*. From the total 151 isolates, 46 (30.5%) were reported in 2008, 67 (44.4%) in 2009 and 38 (25.1%) in 2010. In 2008, *C. albicans* was still the most common *Candida* species isolated from blood followed by *C. tropicalis*. However, in 2009, *C. tropicalis* has surpassed *C. albicans* by almost 20%. This trend continued in 2010 although the gap now has reduced to about 3%. The trend of *Candida* species according to year is shown in Figure I.

Among antifungal agents, the overall susceptibility (according to MIC_{90}) was highest to caspofungin 99.3%, followed by flucytosine 97.3%, amphotericin B 94.7%,

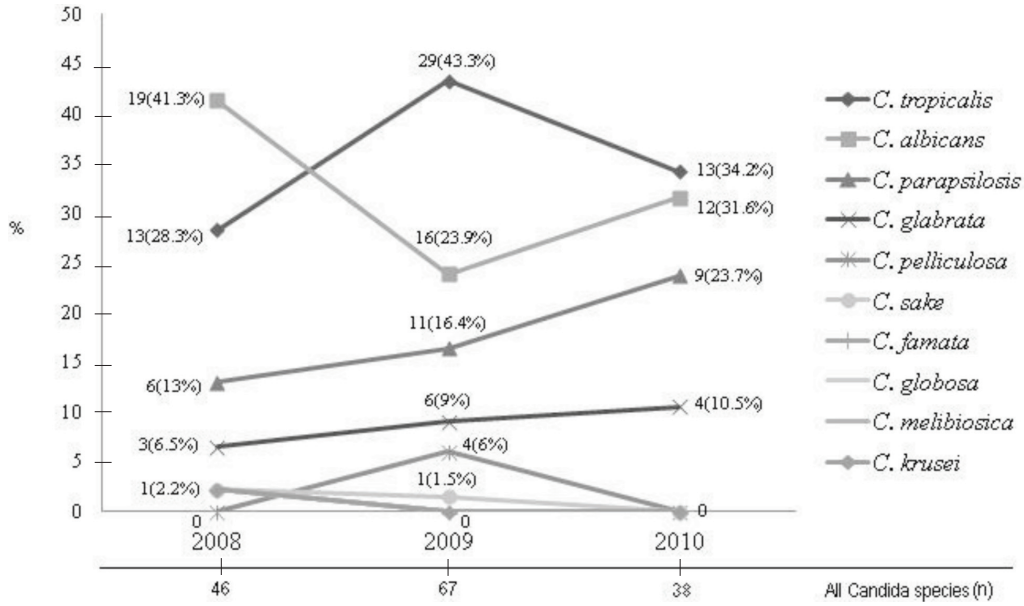

FIGURE 1. Distribution of *Candida* species isolated form blood (2008 to 2010)

voriconazole 92.7%, fluconazole 86.7% and itraconazole 39.1% (Table 1). Antifungal susceptibility patterns were interpreted according to the breakpoints provided by the Clinical Laboratory and Standards Institute (CLSI 2008) (Table 2). Of all nine fluconazole-resistant isolates, 100% of them were still susceptible to caspofungin and flucytosine and 88.9% to amphotericin B. There was marked cross-azole resistance noted in this group where none were susceptible to voriconazole, only 25% were SDD and 75% were resistant. Similarly for itraconazole; none were susceptible, 50% SDD and 50% resistant.

DISCUSSION

Since the introduction of fluconazole in 1990, the management of candidaemia has changed tremendously resulting in the extensive use of the drug in most countries worldwide. The increased fluconazole usage is thought to cause a corresponding increase in NAC proportion since NACs in general, are relatively less susceptible to fluconazole than *C. albicans* (Playford et al. 2008). More than 65% of the candidaemia cases in this study were caused by the NACs, compared with 57.5% in our previous study (Tzar & Shamim 2009).

Majority of (95.7%) *C. albicans* were still susceptible to fluconazole. However lower susceptibility patterns were noted in NACs especially in *C. tropicalis* (87.3%), *C. parapsilosis* (88.5%) and *C. glabrata* (53.8%). *Candida albicans* showed an MIC_{90} of 2 μg/mL, which is in the susceptible range. Conversely, *C. tropicalis*, *C. parapsilosis* and *C. glabrata* showed MIC_{90} values of 32, 16, and 32 μg/mL, respectively. These values fell within the susceptible dose-dependent range. Therefore, it is advisable to give high-dose fluconazole if one wants to treat NAC candidaemia, particularly those caused by *C. glabrata, C. tropicalis* and *C. parapsilosis*. Apart from *C. krusei* which is known to be intrinsically resistant to fluconazole, the overall frequency of resistance to fluconazole was still low (6%). The highest fluconazole resistance rate was noted with *C. tropicalis* (9.1%). This finding was consistent with other reports from European studies (Tortorano et al. 2003; Zepelin et al. 2007). We found that of all nine fluconazole-resistant isolates, none of them were susceptible to voriconazole or itraconazole. Only 25% and 50% of these isolates were SDD to voriconazole and itraconazole, respectively. However, about 90 to 100% of them were still susceptible to amphotericin B, caspofungin and flucytosine. Therefore, we would advise against azoles for treatment if the isolate is known to be fluconazole-resistant.

All four major *Candida* species, viz. *C. tropicalis, C. albicans, C. parapsilosis* and *C. glabrata* (Table 1), showed reduced susceptibility to itraconazole, which is consistent with the previous study in our institution (Tzar & Shamim 2009). The MIC_{90} of *C. tropicalis* and *C. glabrata* were highly elevated that they were categorized under the resistant category. Hence from our finding, itraconazole is not recommended for treatment of candidaemia. It can be used as an alternative agent for invasive aspergillosis, histoplasmosis or penicilliosis marneffei.

Although the majority of *Candida* species (94.7%) was still susceptible to amphotericin B with an MIC_{90} of 1μg/mL, resistance towards amphotericin B was noted in several species including *C. tropicalis*, *C. parapsilosis* and *C. glabrata* (Table 2). No amphotericin B resistance was reported previously in UKMMC (Tzar & Shamim 2009). Thus, the fact that amphotericin resistance has emerged in our institution must be borne in mind whenever treatment failures occur with this drug.

In our findings, flucytosine was found to be efficacious against all *Candida* isolates, with 97.4% of isolates were susceptible to it. This is probably due to lack of flucytosine

TABLE 1. Antifungal MIC_{90}[a] against commonly isolated *Candida* species

Candida species (total n)		All *Candida* species (n=151)				*C. tropicalis* (n=55)				*C. albicans* (n=47)			
Year (n)		2008 (n=46)	2009 (n=67)	2010 (n=38)	2008 - 2010	2008 (n=13)	2009 (n=29)	2010 (n=13)	2008 - 2010	2008 (n=19)	2009 (n=16)	2010 (n=12)	2008- 2010
Antifungal agent[b] MIC_{90} (μg/mL)	AMB	2	1	1	1	2	1	1	1	1	1	0.5	1
	FLU	32	16	8	16	4	64	8	32	1	2	2	2
	ITR	1	0.5	0.5	0.5	0.5	0.5	1	1	0.25	0.25	0.25	0.25
	5FC	1	0.5	0.25	0.25	0.06	0.125	0.125	0.125	0.125	0.125	0.5	0.25
	VOR	1	0.5	0.5	0.5	0.5	4	4	2	2	0.064	0.016	0.125
	CAS	1	0.5	0.5	0.5	0.25	0.125	0.125	0.25	0.125	0.125	0.25	0.125

cont.

C. parapsilosis (n=26)				*C. glabrata* (n=13)			
2008 (n=6)	2009 (n=11)	2010 (n=9)	2008 - 2010	2008 (n=3)	2009 (n=6)	2010 (n=4)	2008 -2010
2	1	2	1	1	1	2	1
2	8	32	16	32	16	32	32
0.25	0.25	0.5	0.25	4	0.5	2	2
0.25	0.5	2	0.5	0.06	0.06	1	0.06
0.064	0.125	0.5	0.125	0.5	0.25	16	1
2	1	2	2	0.125	0.5	0.5	0.5

[a]MIC_{90}, minimum inhibitory concentration that will inhibit 90% of the isolates

[b]AMB, Amphotericin B; FLU, Fluconazole; ITR, Itraconazole; 5FC, Flucytosine; VOR, Voriconazole; CAS, Caspofungin

Susceptibility breakpoints according to CLSI 2008 (all in ug/mL): AMB, S≤1.0, R≥2; FLU, S≤8, 16≤SDD≤32, R≥64; ITR, S≤0.125, 0.25≤SDD≤0.5, R≥1; 5FC, S≤4, 8≤I≤16, R≥32; VOR, S≤1, SDD=2, R≥4; CAS, S≤2, R≥4 (Clinical and Laboratory Standards Institute document M27-A3); S, susceptible; S-DD, susceptible-dose dependent; I, intermediate; R, resistant

TABLE 2. Susceptibility patterns of *Candida* species to various antifungal agents (values in percentage)

Antifungal agent[a]	Susceptibility[b]	All *Candida* sp. (n = 151)	*C. tropicalis* (n = 55)	*C. albicans* (n = 47)	*C. parapsilosis* (n = 26)	*C. glabrata* (n = 13)	*C. pelliculosa* (n = 4)	*C. sake* (n = 2)	*C. famata* (n = 1)	*C. globosa* (n = 1)	*C. melibiosica* (n = 1)	*C. krusei* (n = 1)
AMB	S	94.7	96.4	100	92.3	92.3	100	100	0	0	0	100
	R	5.3	3.6	0	7.7	7.7	0	0	100	100	100	0
FLU	S	86.7	87.3	95.7	88.5	53.8	100	100	100	0	100	0
	SDD	7.3	3.6	0	11.5	46.2	0	0	0	0	0	0
	R	6.0	9.1	4.3	0	0	0	0	0	100	0	100
ITR	S	39.1	10.9	85.1	42.3	0	0	50	100	0	0	0
	SDD	53.0	78.2	10.6	57.7	76.9	100	50	0	0	100	0
	R	7.9	10.9	4.3	0	23.1	0	0	0	100	0	100
5FC	S	97.3	96.4	100	96.2	100	100	100	100	100	100	0
	I	0.7	0	0	0	0	0	0	0	0	0	0
	R	2.0	3.6	0	3.8	0	0	0	0	0	0	100
VOR	S	92.7	89.1	93.6	100	92.3	100	100	100	100	100	0
	SDD	2.0	1.8	2.1	0	0	0	0	0	0	0	0
	R	5.3	9.1	4.3	0	7.7	0	0	0	0	0	100
CAS	S	99.3	98.2	100	100	100	100	100	100	100	100	0
	R	0.7	1.8	0	0	0	0	0	0	0	0	100

cont.

[a]AMB, Amphotericin B; FLU, Fluconazole; ITR, Itraconazole; 5FC, Flucytosine; VOR, Voriconazole; CAS, Caspofungin

[b]S, susceptible; S-DD, susceptible-dose dependent; I, intermediate; R, resistant

exposure to our *Candida* isolates because it is mainly used to treat cryptococcosis. It is rarely used as monotherapy since it may lead to treatment failure and development of resistance (Hospenthal & Bennett 1998). In our study, flucytosine resistance that was seen in *C. tropicalis* and *C. parapsilosis* is consistent with a previous study done in Europe (Tortorano et al. 2003).

The newer drugs, caspofungin and voriconazole, were also found to be efficacious against all *Candida* species. Although 100% susceptible, *C. parapsilosis* had shown an elevated MIC_{90} of 2 µg/mL to caspofungin which was just one-dilution away from being resistant. Only one species was noted to show resistance to caspofungin which was *C. tropicalis* (1.8%) and three species to voriconazole; *C. albicans* (4.3%), *C. glabrata* (7.7%) and *C. tropicalis* (9.1%). Even so, the overall prevalence of caspofungin resistance among our *Candida* isolates was low (0.7%) and it remains the recommended drug against NAC.

In comparison to the previous study done in UKMMC, this study showed that the commonest age group with candidaemia had shifted from 61-70 to 51-60 years old (Tzar & Shamim 2009). However, the mean age remained the same at 46 years old. Infants were mostly affected by *C. albicans*, whereas the older age group by NACs. This trend was consistent with the results shown by the SENTRY Antimicrobial Surveillance Programme (1997-2000), in which the incidence of candidaemia caused by *C. albicans* decreased with age (Pfaller et al. 2002).

Our results showed that most candidaemia cases were from the ICU, followed by surgical wards and medical wards. This result is similar to previous studies done in UKMMC (Tzar & Shamim 2009) and in Italy (Luzzaro et al. 2011). Patients in these wards usually have risk factors for candidaemia such as debilitating underlying conditions, prolonged hospital stay, exposure to many invasive procedures and various broad-spectrum antibiotics. Therefore, more stringent infection control measures should be in place in these locations. Interestingly, the number of *Candida* isolates from our ICU had decreased from 30.6% (2005-2006) to 19.9% in current study (Tzar & Shamim 2009). This could be attributed to intensive infection control programmes such as hand hygiene surveillance, catheter care bundle and antibiotic stewardship that had taken place in our ICU since 2006. Another study in Latin America and North America also showed the same trend of decreasing frequency of candidaemia in their ICU settings (Pfaller et al. 2011). This trend was thought to be due to a decrease in the incidence of candidaemia caused by *C. albicans;* most probably as a result of increased use of fluconazole and preventive measures to curb bloodstream infections (Trick et al. 2002).

In our study, *C. tropicalis* has surpassed *C. albicans* as the commonest species by 19.4% in 2009 and 2.6% in 2010 (Figure 1). In comparison, *C. albicans* was the predominant species in years 2005-2006, followed by *C. tropicalis, C. parapsilosis* and *C. glabrata* (Tzar & Shamim 2009). This trend is worrying because the morbidity and mortality rates were reported to be higher in *C. tropicalis* infection than *C. albicans* infection (Kothavade et al. 2010). The authors hypothesized that this might be due to additional *C. tropicalis* secretion, which could be highly cytotoxic in immunocompromised patients. In view of this, referral centres including UKMMC must be vigilant and administer fast and accurate treatment of candidaemia, especially the ones caused by *C. tropicalis*.

Conclusion

Candida tropicalis, C. albicans, C. parapsilosis and *C. glabrata* were the four most commonly isolated species causing candidaemia in our study. Generally, all our *Candida* species showed a trend of reducing *in vitro* susceptibility towards all antifungal agents tested. Although resistance towards caspofungin was noted in this study, the percentage was still low. Thus, from the study, as reports of candidaemia and its geographical variations are becoming more common, local data on *Candida* epidemiology and susceptibility patterns are vital in management of candidaemia.

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