Candidaemia and Antifungal Susceptibility Testing in a Teaching Hospital

M N Tzar, MPath(Microbiology), A S Shamim, PhD

Department of Medical Microbiology & Immunology, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia

SUMMARY

We reviewed cases of candidaemia at Universiti Kebangsaan Malaysia Medical Centre from 1st January 2005 to 30th June 2006. All blood cultures positive for *Candida* species or its teleomorphs within the study period were identified and antifungal susceptibility testing was performed. Out of 50 blood isolates, 20 (40%) were identified as *Candida albicans*, 16 (32%) *C. tropicalis*, five (10%) *C. parapsilosis*, three (6%) *C. famata*, two (4%) *C. glabrata*, two (4%) *Pichia ohmeri*, one (2%) *C. krusei* and one (2%) *P. etchell/carsonii*. Susceptibility to amphotericin B was 100%, fluconazole 90%, itraconazole 40%, ketoconazole 88%, 5-flucytosine 98% and voriconazole 98%.

KEY WORDS:
Antifungal susceptibility, Candida, Candidaemia, Fungaemia

INTRODUCTION

Candida has been ranked as the fourth most common cause of nosocomial bloodstream infections (BSI) with *C. albicans* being the most common species isolated[1]. However, a previous study in Malaysia has shown that *C. parapsilosis*, rather than *C. albicans*, was the most common species isolated from blood specimens[2]. Our laboratory record in 2002 also showed similar findings (unpublished data). The difference in the distribution patterns does not really affect antifungal treatment options as the three most common species (*C. albicans*, *C. parapsilosis* and *C. tropicalis*) are usually susceptible to either amphotericin B or fluconazole. Antifungal susceptibility testing is not routinely done at our centre or any other centre in Malaysia, mainly due to limited resources and low demand. However, as invasive fungal infections are becoming more common, there is a need for tertiary centres like our hospital for antifungal susceptibility testing because a substantial number of immunosuppressed patients are predisposed to opportunistic fungal infections. Subjecting these patients to empiric antifungal therapy without ever knowing the true susceptibility of the invading *Candida* pathogen may result in increased morbidity and mortality as well as the total cost of hospital care. By performing antifungal susceptibility testing, unnecessary usage of antifungal agents can be avoided and more importantly, the patients do not have to bear the unnecessary side effects or toxicity of these antifungal agents. The changing epidemiology of *Candida* infections, the emergence of resistant *Candida* strains and the availability of new antifungal agents, may influence the choice of antifungal agents for the patients. The objectives of this study are therefore to determine the epidemiology of candidaemia and the antifungal susceptibility patterns of candidal isolates in our institution.

MATERIALS AND METHODS

Study populations

We conducted a retrospective study of 50 blood-culture-positive isolates of *Candida* spp. or its teleomorphs from 49 patients between 1st January 2005 and 30th June 2006. Candidaemia was defined as presence of at least one isolate of *Candida* spp. or its teleomorphs in the blood.

Data collection

Patient data were obtained from the Medical Records Department, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) and from the hospital's computer information system. The data obtained included name, medical record number, age, gender, ward, laboratory request number, date of blood culture taken, organism isolated, patients' underlying conditions (diabetes mellitus, HIV, lung disease, malignancy, organ transplant, prematurity, renal insufficiency, SLE, splenic disease, surgery); patients' predisposing factors for candidal bloodstream infection (prior antibacterial therapy, empirical antifungal therapy, steroids, chemotherapy, presence of central venous catheter, total parenteral nutrition, dialysis, mechanical ventilation, urinary catheter, neutropaenia, length of hospital stay, intensive care unit stay); and patient outcome.

Organism identification

All yeasts isolated from blood culture were identified using a yeast identification kit, ID 32C (bioMérieux, Lyon, France). Briefly, fresh yeast colonies (24 to 48 hours) from pure cultures on Sabouraud Dextrose Agar (SDA) were used to make a uniform colony suspension in sterile distilled water. The turbidity was adjusted visually to 2 McFarland. Two hundred and fifty microlitre of this suspension was transferred into an ampoule of API® C Medium and homogenized. One hundred and thirty five microlitre of this mixture was used to fill each of the 32 cupules that contained different carbohydrate substrates. The final inoculum was incubated at 30°C for 24 to 48 hours to promote growth of the yeasts. The differentiation of growth (turbid) or absence of growth (clear) was depending on whether the yeast can assimilate or ferment these sugars. A numeric code generated by the carbohydrate assimilation pattern was analyzed using

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Corresponding Author: Tzar Mohd Nizam, Department of Medical Microbiology & Immunology, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia Email: tzarmohdnizam@yahoo.com

a computer program for species identification of the yeast. Yeasts other than *Candida* spp. or its teleomorphs were excluded from the study. *Candida* isolates were stored as stock cultures on SDA slants and kept at room temperature until needed.

Antifungal susceptibility testing

Antifungal susceptibility testing of the yeast isolates was performed using Sensititre® YeastOne® kit by Trek Diagnostic System (Cleveland, OH, USA). Briefly, fresh yeast colonies (24 to 48 hours) from pure culture on SDA were used to make a uniform colony suspension in sterile water. The turbidity was adjusted visually to 0.5 McFarland. Twenty microlitre of this suspension was transferred into 11 mL of YeastOne inoculum broth to give a final organism density of approximately 1.5 – 8 x 10^3 colony-forming units per milliliter (CFU/mL). Using a manual pipette, 100 µL of this mixture were inoculated into microtitre wells containing six different antifungal agents (amphotericin B, fluconazole, itraconazole, ketoconazole, 5-flucytosine and voriconazole). A plastic adhesive seal supplied with the kit was used to cover the surface of the microtitre plate to reduce evaporation and prevent drying. The plates were then incubated at 35°C for 24 hours in a non-CO_2 incubator. The validity of this test was checked by using standard quality control strains; *Candida krusei* ATCC 6258 (American Type Culture Collection, Manassas, VA) and *Candida parapsilosis* ATCC 22019. After 24 hours of incubation, the microtitre plates were read under normal laboratory lighting using a reading mirror, which displayed the underside of the wells. A colour changed from blue to red, rather than turbidity, was taken as an indicator of yeast growth. End-point reading was taken if there was positive growth in control well. Otherwise, the plates were reincubated for another 24 hours at 35°C for a total incubation of 48 hours. The minimum inhibitory concentrations (MICs) were read only once, at 24 hours or at 48 hours. The lowest concentration of antifungal agent that inhibited the growth of yeast in the well (i.e. the first blue well) was taken as the MIC. The susceptibility was interpreted according to the breakpoints suggested by other published studies [3-7].

Ethical considerations

The study was approved by the Research and Ethics Committee of Medical Faculty, Universiti Kebangsaan Malaysia.

RESULTS

Fifty *Candida* species and its teleomorphs were isolated from the blood specimens of 49 patients (one patient had a mixed infection with *C. albicans* and *C. parapsilosis*). Twenty (40%) were identified as *Candida albicans*, 16 (32%) *C. tropicalis*, five (10%) *C. parapsilosis*, three (6%) *C. famata*, two (4%) *C. glabrata*, two (4%) *Pichia ohmeri*, one (2%) *C. krusei* and one (2%) *P. etchell/carsonii*. The distribution of gender, race and age groups of the patients are summarized in Table I. The mean and median ages of all patients were 46 and 50 years, respectively. Cases of candidaemia from Intensive Care Unit (ICU) and Medical (Haematology and General) wards represented 63.2% of the total number of candidaemia cases in this study. The rest of the wards are shown in Table II. The most common underlying medical conditions were lung disease (57.1%), renal insufficiency (46.9%), haematological malignancies (30.6%) and diabetes mellitus (24.5%). The most frequent predisposing factors were antibacterial therapy (93.9%), central venous catheters (75.5%) and prolonged hospital stay (75.5%). Prolonged hospital stay is defined as hospitalization of more than ten days[8]. The duration of hospital stay was in the range of 1 to 59 days with a mean of 24 days. Other underlying conditions and predisposing factors are listed in Table III. The clinical outcomes of the affected patients were not favourable. Almost two-thirds (32 out of 49 patients; 65.3%) died, while only 17 (34.7%) patients were still alive when discharged from the hospital. Among all six antifungal agents tested, amphotericin B was shown to be the most effective antifungal agent, followed by voriconazole (98% susceptibility), 5-flucytosine (98%), fluconazole (90%), ketoconazole (88%) and itraconazole (40%). The susceptibility patterns to various antifungal agents are shown in Table IV.

Table I: Demographic data of patients with candidaemia

Characteristic	No. of Patients (%)
Gender (n = 49)	
Male	21 (42.9)
Female	28 (57.1)
Race (n = 49)	
Malay	28 (57.1)
Chinese	18 (36.7)
Indian	1 (2.0)
Other	2 (4.1)
Age in years (n = 49)	
< 1	4 (8.2)
1 – 10	4 (8.2)
11 – 20	1 (2.0)
21 – 30	3 (6.1)
31 – 40	6 (12.2)
41 – 50	8 (16.3)
51 – 60	6 (12.2)
61 – 70	12 (24.5)
> 70	5 (10.2)

Table II: Distributions of wards with candidaemic patients

Ward	No. of Patients (%)	Cumulative %
Intensive care unit	15 (30.6)	30.6%
Medical (Haematology)	11 (22.4)	53.0%
Medical (General)	5 (10.2)	63.2%
Burns unit	3 (6.1)	69.3%
High dependency ward	3 (6.1)	75.4%
Neonatal intensive care unit	3 (6.1)	81.5%
Paediatrics	3 (6.1)	87.6%
Surgical	3 (6.1)	93.7%
Bone marrow transplant unit	1 (2.1)	95.8%
Paediatric intensive care unit	1 (2.1)	97.9%
Spinal	1 (2.1)	100.0%
Total	49 (100.0)	

Table III: Frequency of patients with identified risk factors[a]

Underlying condition	No. of Patients (%)	Predisposing factor	No. of Patients (%)
Lung disease	28 (57.1)	Antibacterial therapy	46 (93.9)
Renal insufficiency	23 (46.9)	Central venous catheter	37 (75.5)
Malignancy (haematological)	15 (30.6)	Prolonged hospital stay[b]	37 (75.5)
Diabetes mellitus	12 (24.5)	Urinary catheter	28 (57.1)
Surgery (other)	9 (18.4)	Mechanical ventilation.	23 (46.9)
Surgery (abdominal)	7 (14.3)	Steroid use	23 (46.9)
Malignancy (other)	5 (10.2)	Parenteral nutrition	19 (38.8)
Prematurity	3 (6.1)	Antifungal therapy	18 (36.7)
HIV infection	2 (4.1)	ICU stay	15 (30.6)
SLE	2 (4.1)	Dialysis	13 (26.5)
Organ transplant	1 (2.0)	Chemotherapy	12 (24.5)
Splenic disease	1 (2.0)	Neutropaenia	11 (22.4)

[a]Risk factors include underlying medical conditions and other predisposing factors [8, 9].
[b]Prolonged hospital stay is defined as hospital length of stay of more than ten days [8].

Table IV: Susceptibility patterns to various antifungal agents (values in percentage)

Antifungal	Susceptibility	*C.albicans* (n=20)	*C.famata* (n=3)	*C.glabrata* (n=2)	*C.krusei* (n=1)	*C.parapsilosis* (n=5)	*C.tropicalis* (n=16)	*P.etchell/carsonii* (n=1)	*P.ohmeri* (n=2)
Amphotericin B	S	100	100	100	100	100	100	100	100
	R	0	0	0	0	0	0	0	0
Fluconazole	S	100	100	0	0	100	87.50	100	100
	S-DD	0	0	100	0	0	12.50	0	0
	R	0	0	0	100	0	0	0	0
Itraconazole	S	85	0	0	0	20	6.25	100	0
	S-DD	15	33	0	100	80	81.25	0	100
	R	0	67	100	0	0	12.50	0	0
Ketoconazole	S	100	100	0	0	80	87.50	100	100
	S-DD	0	0	0	100	20	6.25	0	0
	R	0	0	100	0	0	6.25	0	0
5-Flucytosine	S	100	100	100	0	100	100	100	100
	I	0	0	0	100	0	0	0	0
	R	0	0	0	0	0	0	0	0
Voriconazole	S	100	100	100	100	100	93.75	100	100
	S-DD	0	0	0	0	0	6.25	0	0
	R	0	0	0	0	0	0	0	0

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

DISCUSSION

The pattern of candidal bloodstream infection (BSI) in various ethnic groups in our hospital does not reflect any racial predilection. Rather it reflects the distribution of the ethnic groups in Malaysia. Patients from all age groups were affected, although the elderly were more frequently and severely affected. This was probably due to the elderly being more likely to have debilitating underlying medical conditions and other predisposing factors than younger patients. The majority of candidaemic cases came from the Intensive Care Unit (ICU) and haematological wards, suggesting the presence of many treatment-related factors and debilitating underlying conditions that contribute to BSI. Our most common underlying diseases (lung disease, renal insufficiency and malignancy) however, are not the same as shown by another study done in Chile[9] which reported that HIV infection, lung disease and surgery as the most common underlying diseases. This could be explained by the fact that our hospital is not a referral centre for HIV patients. Almost all HIV patients are referred to Kuala Lumpur Hospital or Sungai Buloh Hospital. The overall mortality of candidaemia and invasive candidiasis in other controlled studies over the past 10 years remains at 30-40%[10]. The global crude mortality rate observed in the community is higher and exceeds 50% in many cases. In our study, almost two-thirds (32 out of 49 patients; 65.3%) died, however it was difficult to say whether the deaths were directly attributed to candidaemia without proper post-mortem diagnosis. Isolation of *Candida* from blood does not necessarily signify direct involvement in the patients' eventual death.

The distribution pattern of *Candida* species in this study differs from our previous laboratory record for candidaemia in 2002, where *C. parapsilosis* was the most common species isolated (33%); followed by *C. albicans* (28.2%), *C. tropicalis* (17.9%), *C. glabrata* (7.7%), *C. famata* (7.7%), *C. lusitaniae* (2.6%) and *Candida* spp. (2.6%) (unpublished data). Similar finding was also reported at University Malaya Medical Centre (UMMC) between 1997 and 1999, where *C. parapsilosis* was the most common species isolated (51.0%), followed by *C. tropicalis* (25.5%) and *C. albicans* (11.8%)[2]. Despite the slight variations, it appears that the majority (79.1 to 88.3%) of candidal BSI in Klang Valley hospitals were caused by *C. albicans, C. tropicalis* and *C. parapsilosis*. With increasing use of fluconazole, there is an increasing trend of candidaemia caused by non-albicans *Candida* (NAC) species. The two most common NAC species were *C. tropicalis* and *C. parapsilosis*. In our study, NAC species constituted 60% of all yeast isolates. This is in agreement with a study in the United Kingdom[11] which showed the increasing trend of NAC candidaemia to up to 65%. However, this trend could be due to the reduction in the number of *C. albicans* isolated, not reflecting a true increase in NAC cases. One patient in our study had mixed infections with *C. albicans* and *C.*

parapsilosis. Polymicrobial candidaemia is quite uncommon. It is usually seen in hospitalized patients with multiple co-morbidities and heavy candidal colonization. Patients with polymicrobial candidaemia tend to be sicker, non-oncologic patients with frequent concomitant bacterial infections, as compared to those with monomicrobial candidaemia. However, the crude mortality rate of polymicrobial candidaemia was reported to be 43%, which was similar to that seen with monomicrobial candidaemia [12].

All *Candida* isolates in this study were susceptible to amphotericin B. Most isolates (72%) demonstrated MIC values of 0.5 µg/mL. This is in contrast to a study in Taiwan[13] which reported a significant rate of high MICs to amphotericin B among their *Candida* isolates including *C. albicans*. All *C. albicans* isolates in our study were found to be susceptible to fluconazole. As previously reported in other studies [13-16], *C. albicans* tended to be more susceptible to fluconazole compared to NAC species such as *C. tropicalis, C. parapsilosis* and *C. famata*. Ninety percent of *C. albicans* showed MIC values of 0.25 – 1 µg/mL, whilst the latter yeasts were mostly in the MIC range of 1 – 2 µg/mL. Reduced susceptibility to fluconazole was seen mainly in *C. glabrata* and *C. krusei*. It is therefore not advisable to use fluconazole to treat infections caused by these species. It is interesting to note that 60% of our isolates, mostly represented by *C. tropicalis, C. parapsilosis, C. famata, C. glabrata, C. krusei* and *P. ohmeri*; showed reduced susceptibility to itraconazole. Similar to our findings, researchers in Taiwan also reported high rates of reduced susceptibility to itraconazole among their NAC species [13]. It is possible that the common use of itraconazole for yeasts and mould infections could have caused this trend. It is not known however, whether this trend also affects itraconazole activity against moulds, especially *Aspergillus* species. There are no official breakpoints for ketoconazole produced by the Clinical Laboratory Standards Institute (CLSI) but some authors have suggested that ketoconazole MICs would be very close to that of itraconazole [5]. Based on the suggested breakpoints, 88% of all 50 isolates were susceptible to ketoconazole. However, ketoconazole is no longer used in the treatment of invasive candidiasis or candidaemia due to its adverse effects and the availability of other less toxic, more effective antifungal agents. Voriconazole is a relatively new drug to be licensed by United States Food and Drug Administration (USFDA) for invasive candidiasis [8]. In our study, voriconazole showed a very good activity against most of the isolates including *C. krusei* and *C. glabrata*. Only one isolate of *C. tropicalis* showed slightly reduced susceptibility. However, a study by The European Confederation of Medical Mycology (ECMM) from 1997 – 1998 [16] showed presence of voriconazole resistance in 2.7% of 375 *C. albicans* isolates. The emergence of resistance to this drug in our institution is yet to be seen. Most of our strains of *C. albicans* and NAC species were highly susceptible to 5-flucytosine except for *C. krusei* (intermediate). On the contrary, other studies reported 1-2% resistance among *C. albicans* and 6-15% among NAC species [15-17].

CONCLUSION

The main candidal isolates from BSI in our study were *C. albicans*, *C. tropicalis* and *C. parapsilosis,* and they were generally still susceptible to amphotericin B and fluconazole. However, the distribution and susceptibility patterns of the candidal isolates from one centre to another can be different. Therefore, knowledge of local distribution and susceptibility patterns is essential in formulating treatment guidelines for candidaemia. Although the sample size of the study was relatively small, this pilot study has gained an important preliminary data for more extensive studies to be carried out in the future on yeasts isolates in Malaysia.

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