Original Article

A Retrospective Analysis of Antifungal Susceptibilities of Candida Bloodstream Isolates From Singapore Hospitals

Thean Yen Tan,[1] *MB.BCh, MRCPath*, Ai Ling Tan,[2] *MBBS, Dip Bact, FRCPA*, Nancy WS Tee,[3] *MBBS, FRCPA*, Lily SY Ng,[1] *DQE (Bacteriology), SpDip (Microbiology)*

Abstract

Introduction: Worldwide, *Candida albicans* is the most common *Candida* species implicated in bloodstream infections. However, the proportion of non-albicans bloodstream infections is increasing. Fluconazole resistance is known to be more common in non-albicans species, but is also reported in *C. albicans*. This retrospective study was performed to determine the species epidemiology of *Candida* bloodstream infections in Singapore hospitals, and to perform susceptibility testing to a range of antifungal drugs. Materials and Methods: *Candida* spp. isolated from bloodstream infections from October 2004 to December 2006 were collected from 3 participating hospitals: a tertiary referral hospital (Singapore General Hospital), a secondary referral hospital (Changi General Hospital) and an obstetrics/paediatric hospital [KK Women's and Children's Hospital (KKWCH)]. Isolate collection was also retrospectively extended to January 2000 for KKWCH because of the limited number of cases from this hospital. Isolates were identified by a common protocol, and antifungal susceptibility testing was performed by microbroth dilution (Sensititre One, Trek Diagnostics, United Kingdom). Results: The most common isolates were *C. albicans* (37%), *C. tropicalis* (27%) and *C. glabrata* (16%). There were differences in species distribution between institutions, with *C. parapsilosis* and *C. albicans* predominant in KKWCH, and *C. albicans* and *C. tropicalis* predominant in the other 2 institutions. Fluconazole resistance was detected in 3.2% of all *Candida* spp., and 85.3% were classified as susceptible. All *C. albicans* and *C. parapsilosis* were susceptible to fluconazole and voriconazole, while susceptibility to fluconazole was much more variable for *C. glabrata* and *C. krusei*. Conclusion: This study shows that *C. albicans* remains the predominant *Candida* species isolated from bloodstream infections in the 3 participating hospitals. However, non-albicans species accounted for nearly two-thirds of all cases of candidaemia. Resistance to fluconazole was uncommon, and was generally confined to *C. krusei* and *C. glabrata*.

Ann Acad Med Singapore 2008;37:835-40

Key words: Antifungal agents, Antifungal drug resistance, Fungaemia

Introduction

Nosocomial infection with *Candida* species is increasing in significance worldwide. A recent review of positive blood cultures noted the relative increase in importance of fungal bloodstream infections (BSI),[1] and *Candida* was reported as the fourth most common blood stream pathogen in the United States.[2] *Candida* BSI is associated with a very high crude mortality of over 60%,[3] while the attributable mortality may be as high as 49%.[4]

C. albicans remains the predominant pathogen associated with BSI caused by *Candida* spp. Population-based studies in Europe[5] and the United States[6] demonstrate that approximately 95% of candidaemia is caused by 4 species: *C. albicans*, *C. glabrata*, *C. parasilosis* and *C. tropicalis*. There has been a documented increase in the proportion of infections caused by other non-*albicans* sp.,[7] particularly *C. glabrata*. In addition to this shift in species distribution, geographic variations are apparent in the distribution of *Candida* species implicated in BSI,[7] emphasising the importance of knowing local epidemiological patterns.

[1] Division of Laboratory Medicine, Changi General Hospital, Singapore
[2] Department of Pathology, Singapore General Hospital, Singapore
[3] Laboratory, KK Women and Children's Hospital, Singapore
Address for Correspondence: Dr Thean Yen Tan, Division of Laboratory Medicine, Changi General Hospital, 2 Simei Street 3, Singapore 529889.
Email: thean_yen_tan@cgh.com.sg

Fluconazole has remained the mainstay of empiric antifungal therapy since its introduction in the early 1990's, because of favourable pharmacokinetic and side effect profiles. However, *C. glabrata* and *C. krusei* exhibit reduced susceptibilities or frank resistance to fluconazole.[8] In addition, there is documented increasing fluconazole resistance in all *Candida* spp., including *C. albicans*,[9] *C. lusitaniae*, *C. tropicalis* and *C. dubliniensis*.[5,10] The gold standard of antifungal therapy for fluconazole resistant species and for severe infections has remained Amphotericin B. Amphotericin resistance in *Candida* spp. has been documented, particularly in *C. lusitaniae*[11] and therapeutic failures with this drug have been documented.[12] The recent introduction of new azole derivatives and the echinocandins has offered increased choices for the treatment of resistant or refractory fungal infections.[13]

Antifungal susceptibility is a rapidly changing area of knowledge. Current internationally accepted standards of testing require broth dilution methods[14,15] which are labour intensive, time consuming and expensive. An alternative commercially available equivalent method (Sensititre YeastOne, Trek Diagnostics, UK) has been demonstrated to show good correlation and reproducibility with the reference CLSI M27-A method[16-18] for the azoles, amphotericin B and the newer antifungal agents.

There is patchy information available on the spectrum of *Candida* BSI in Singapore. A 6-month study performed in 2001 was based on *Candida* species isolated from most body sites, including a limited number of BSI isolates, and documented a lower prevalence of *C. albicans* and a different distribution of non-*albicans* species.[19] A more recent study performed at a single institution reported that *C. tropicalis* was the predominant species isolated from BSI.[20] This study was implemented as the first systematic multi-centre survey of *Candida* BSI infections in Singapore. The participating hospitals were chosen to include a pediatric, an acute care and a tertiary referral hospital in order to sample a representative patient population.

Materials and Methods

Collection of Isolates

All strains of *Candida* spp. isolated from BSI between October 2004 and December 2006 from 3 participating Singapore hospitals were included in the study. The 3 participating hospitals were Singapore General Hospital (SGH), Changi General Hospital (CGH) and KK Women's and Children's Hospital (KKWCH). Due to the low incidence of Candida BSI in paediatric patients, archived bloodstream isolates dating from 2000 from KKWCH were also included in the study. The 3 study hospitals represent 28% of all hospital beds available in Singapore, and 56% of acute-care beds available in the 6 public acute-care hospitals.

Identification of Yeast Isolates

Species determination was initially performed at each participating laboratory using a standardised protocol. Isolates were identified based on API 20C AUX (bioMérieux, France) and morphology expression on cornmeal agar. Isolates were then forwarded to CGH for further testing.

All isolates were sub-cultured on to CHROMagar Candida (Becton Dickinson, USA).

All isolates submitted as *C. albicans* with concordant colonial morphology on CHROMagar Candida were screened for growth at 42°C in order to differentiate possible *C. dubliniensis* isolates. The species identification of strains with poor growth at 42°C was confirmed using ID-YST panels (Vitek 2 Compact, bioMerieux, France).

For non-*albican* isolates, species identification was accepted as accurate if the submitted identification by participating laboratories was concordant with colonial morphology on CHROMagar Candida.

Isolates with discordant colonial morphologies were further tested using ID-YST panels and a repeat test to check morphology expression on cornmeal agar supplemented with Tween 80.

Minimum Inhibitory Concentration Testing

Antifungal susceptibility testing was performed at CGH using the Sensititre YeastOne YO-5/6 panels. MIC values were obtained for the following antifungals: fluconazole, itraconazole, ketoconazole, voriconazole, amphotericin and 5-flucytosine. Posaconazole and caspofungin MIC were available for a subset of tested isolates. *C. krusei* ATCC 6258 and *C. parapsilosis* ATCC 22019 were used as controls for each batch of testing. The accuracy of inoculum density was checked by quantitative plating.

Test panels were read following 24 hours incubation in ambient condition. Following the manufacturer's guidelines, the MIC was interpreted as the lowest concentration of antifungal that produced a colour change from blue to red. For isolates with trailing colorimetric endpoints for flucytosine and the azole drugs, the MIC was interpreted as the first well showing a less intense colour change compared to the positive growth well.

Susceptibility breakpoints and categorical interpretations (sensitive, resistant and susceptible-dose-dependent) for fluconazole, itraconazole and flucytosine were interpreted according to those set by the Clinical Laboratory Standards Institute (CLSI).[15] Proposed susceptibility breakpoints were used for voriconazole.[21] There is insufficient data at present for interpretive breakpoints for amphotericin, caspofungin and posaconazole.

Results

Two hundred seventy-nine isolates of *Candida* spp. were collected from the 3 participating institutions over the study period: 49 from KKWCH (February 2000 to December 2006), 53 from CGH (October 2004 to December 2006) and 165 from SGH (October 2004 to December 2006). The most common isolates were *C. albicans* (37%), *C. tropicalis* (27%), *C. glabrata* (16%) and *C. parapsilosis* (14%). If isolates collected prior to Oct 2004 were excluded from analysis, there was no change in the relative frequency ranking for each *Candida* spp. The distribution of *Candida* spp. isolated from each institution is shown in Table 1.

There were some differences in species distribution between the institutions. *C. parapsilosis* was significantly more frequently isolated in KKWCH as compared with SGH (relative risk RR 3.2; 95%CI 2.0-5.0) and CGH (RR 2.4, 95%CI 1.7-3.3), and was the most common species isolated from *Candida* BSI in this institution. *C. albicans* remained the most common species for BSI infection in both CGH and SGH (45.3% and 35.2% respectively). *C. glabrata* was significantly more common in CGH compared with KKWCH (RR 1.6; 95%CI 1.1-2.3), but there were no significant differences in isolation rate for this species between the other 2 institutions.

Antifungal Susceptibility

85.3% of *Candida* isolates were susceptible to fluconazole based on CLSI breakpoints, and a further 11.5% were classified as susceptible-dose-dependant (S-DD). Fluconazole resistance was detected in 3.2% of all isolates, predominantly in *C. glabrata* but also for *C. krusei* and *C. tropicalis*.

Susceptibilities to the other azoles varied: only 62.5% of isolates were susceptible to itraconazole, while 98.0% were considered susceptible to voriconazole, using suggested susceptible breakpoints of ≤1 μg/mL.[21] As in other studies, susceptibilities to the azoles could be predicted according by species, as shown in Table 2. *C. albicans* and *C. parapsilosis* were uniformly susceptible to fluconazole and voriconazole, and resistance to flucytosine was low. 2.7% of *C. tropicalis* were resistant to fluconazole and voriconazole. Susceptibility to fluconazole was much more variable for *C. glabrata*, with 66.7% of isolates categorised as S-DD, and 20% categorised as resistant. Voriconazole retained in-vitro activity in *C. glabrata*, with 97.8% of isolates being classified as susceptible.

Amphotericin MIC for the study isolates ranged from 0.0625 μg/ml to 1 μg/mL, with most isolates clustering around 0.25 μg/ml to 1 μg/mL. The mean amphotericin MIC was significantly higher for *C. tropicalis* and *C. glabrata* when analysed by analysis of variance.

MIC distributions for the other tested antifungals showed a wider distribution of results, including caspofungin (0.008 -1 μg/mL), posaconazole (0.008-16 μg/mL) and ketoconazole (0.008-32 μg/mL). The MIC distributions for these drugs against the 4 commonest *Candida* species are shown in Table 3.

Discussion

This study provides the first 2 years of data from a 3-year surveillance program for *Candida* spp isolated from BSI in 3 Singapore hospitals. In line with other published studies, *C. albicans* was the most common species isolated from BSI. However, in contrast to other population-based studies where *C. glabrata* is ranked as the second most commonly reported species, *C. tropicalis* was the second most common species isolated, comprising 27% of bloodstream isolates. Although there is limited regional data available, *C. tropicalis* appears to predominate in Latin America, with a higher prevalence in South East Asia.[22] Data available from 2 previous studies performed in Singapore confirmed the

Table 1. Distribution of *Candida* spp. by Institution

Organism	All hospitals		CGH		KKWCH		SGH	
	n	%	n	%	n	%	n	%
Candida albicans	104	(37.3)	24	(45.3)	16	(32.7)	58	(35.2)
Candida tropicalis	75	(26.9)	12	(22.6)	9	(18.4)	53	(32.1)
Candida glabrata	45	(16.1)	12	(22.6)	4	(8.2)	29	(17.6)
Candida parapsilosis	40	(14.3)	2	(3.8)	18	(36.7)	15	(9.1)
Candida dubliniensis	8	(2.9)	0	(0)	0	(0)	8	(4.8)
Candida sp. (others)	3	(1.1)	1	(1.9)	1	(2)	1	(0.6)
Candida krusei	2	(0.7)	1	(1.9)	0	(0)	1	(0.6)
Candida guilliermondii	1	(0.4)	0	(0)	1	(2)	0	(0)
Candida rugosa	1	(0.4)	1	(1.9)	0	(0)	0	(0)

Table 2. Susceptibilities to Fluconazole, Voriconazole and Flucytosine of the Four Most Common Species From BSI

Antifungal	Species	%S	%S-DD	%R	MIC90
Fluconazole	*C. albicans*	100	0	0	1
	C. tropicalis	97.3	0	2.7	4
	C. glabrata	20	66.7	13.3	64
	C. parapsilosis	97.5	2.5	0	8
Voriconazole	*C. albicans*	100	0	0	0.016
	C. tropicalis	97.3	0	2.7	0.25
	C. glabrata	97.8	2.2	0	1
	C. parapsilosis	100	0	0	0.125
Flucytosine	*C. albicans*	98.1	0	1.9	0.25
	C. tropicalis	98.7	0	1.3	0.125
	C. glabrata	20	66.7	13.3	0.125
	C. parapsilosis	97.5	0	2.5	0.25

relative higher incidence of *C. tropicalis* from BSI,[19, 23] while a more recent study by Chai and colleagues report that a predominant clonal strain of *C. tropicalis* accounted for 36% of *Candida* BSI in their institution.[20]

There were also other subtle inter-hospital differences: *C. parapsilosis* was the predominant species from BSI in KKWCH, followed by *C. albicans*, whereas in SGH, *C. albicans* and *C. tropicalis* predominated. CGH showed the highest proportion of *C. albicans* and *C. glabrata* BSI when compared with the other 2 institutions. The predominance of *C. parapsilosis* in KKWCH specimens is probably due to the fact that 64% of isolates from this hospital were isolated from patients ≤2 years of age. The predominance of *C. parapsilosis* infections in paediatric patients has previously been noted in other surveillance programs.[24]

Antifungal susceptibilities to fluconazole were consistent with previously reported data, and could be reliably predicted by speciation. *C. albicans* was uniformly susceptible to fluconazole. Fluconazole resistance was detected in only 3% of all *Candida* isolates, although 12% of isolates (mainly *C. glabrata*) were categorised as S-DD. MIC data for fluconazole showed a trend towards higher fluconazole MIC for non-albicans species, even when the data for *C. glabrata* and *C. krusei* were excluded. Voriconazole retained activity against *Candida* sp. (including those with reduced susceptibility to fluconazole), and in-vitro resistance was rare.

Similarly, resistance to flucytosine was uncommon across all *Candida* spp.

There are no accepted susceptibility breakpoints for amphotericin against *Candida* spp. A susceptibility breakpoint of ≤1 μg/ml has been suggested,[25] based on predicted microbiologic failure. Based on this breakpoint, none of the study isolates were resistant to amphotericin. However, it has been apparent that amphotericin MIC testing, when performed by the current reference susceptibility methods from the CLSI, results in a narrow spread of MIC values, and may poorly differentiate resistant from susceptible isolates.[26] As the Sensititre panels are based on CLSI methods, amphotericin MIC for our study isolates were similarly constrained. Alternative testing methods such as Etest or the use of other susceptibility testing media (e.g. antibiotic medium 3) may better elucidate amphotericin resistance within *Candida* spp.[25, 27]

Antifungal susceptibility testing remains a rapidly changing field. Standardised testing methods (such as those from the CLSI or EUCAST) are labour-intensive, but have improved the reproducibility of testing results. Existing issues such as the interpretation of trailing endpoints, poor discrimination of amphotericin-resistant yeasts and correlation with clinical endpoints remain to be resolved. The availability of commercial testing systems[28-30] and disc diffusion guidelines[31] has enabled wider adoption of antifungal susceptibility testing, though it has to be emphasised that limitations in each method, when compared to the reference methods, should always be considered.

The Sensititre panel used in this study is based on the CLSI microbroth dilution methodology. Evaluations of this test method have shown good reproducibility of categorical interpretations when compared with the reference method,[16-18] although some investigators report that the Sensititre panel tended to yield higher MIC for most tested drugs, with lower rates of categorical agreement for *C. glabrata* when tested against fluconazole.[28]

This study demonstrates that *C. albicans* remains the predominant species isolated from Candida BSI, although inter-institutional differences were apparent. Non-*albicans*

Table 3. MIC Distributions for Amphotericin B, Caspofungin, Ketoconazole and Posaconazole of the Four Most Common Species From BSI

Antifungal	*Candida* spp.	n	MIC90	MIC range	% MIC (µg/mL)													
					≤0.05	0.01	0.02	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	≥32
Amphotericin B	*C. albicans*	107							0.9	0.9	33.6	52.3	12.1					
	C. glabrata	45	1	0.25 - 1							13.3	37.8	48.9					
	C. tropicalis	75	1	0.25 - 1							5.3	44	50.7					
	C. parapsilosis	40	1	0.06 - 1					2.5	2.5	35	35	25					
Caspofungin	*C. albicans*	56	0.125	0.03 - 0.25				1.8	69.6	26.8	1.8							
	C. glabrata	28	0.125	0.03 - 0.25				3.6	14.3	75	7.1							
	C. tropicalis	46	0.125	0.015 - 0.25			6.5	52.2	21.7	15.2	4.3							
	C. parapsilosis	18	1	0.12 - 1						11.1	22.2	44.4	22.2					
Posaconazole	*C. albicans*	55	0.016	0.008 - 0.12		47.3	45.5	3.6	1.8	1.8								
	C. glabrata	28	1	0.008 - 2		3.6	3.6			3.6		46.4	35.7	7.1				
	C. tropicalis	45	0.5	0.03 - 16				11.1	6.7	37.8	33.3	8.9					2.2	
	C. parapsilosis	18	0.064	0.015 - 1			16.7	61.1	16.7				5.6					
Voriconazole	*C. albicans*	107	0.016	0.008 - 0.12		79.4	17.8	0.9	0.9	0.9								
	C. glabrata	45	1	0.008 - 2		4.4		2.2	2.2		31.1	44.4	13.3	2.2				
	C. tropicalis	75	0.25	0.015 - 16			1.3	9.3	28	41.3	12	5.3			1.3		1.3	
	C. parapsilosis	40	0.125	0.008 - 0.5		12.5	20	35	20	10		2.5						

species now account for nearly two-thirds of BSI. The main non-*albicans* species isolated were *C. tropicalis* in the hospitals serving mainly adult populations, and *C. parapsilosis* in the hospital serving mainly a paediatric and obstetric population. Other than for *C. krusei*, and to a lesser extent, *C. glabrata*, resistance to fluconazole was uncommon.

Acknowledgements

We would like to thank Ms Chloe Wang Jun Chee for her unflagging technical assistance and to all staff members involved in each participating institution for making this study possible. This study was funded by a grant from the National Medical Research Council, Singapore.

REFERENCES

1. Weinstein MP, Towns ML, Quartey SM, Mirrett S, Reimer LG, Parmigiani G, et al. The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis 1997;24:584-602.
2. Pfaller MA, Jones RN, Messer SA, Edmond MB, Wenzel RP. National surveillance of nosocomial blood stream infection due to *Candida albicans*: frequency of occurrence and antifungal susceptibility in the SCOPE Program. Diagn Microbiol Infect Dis 1998;31:327-32.
3. Lark RL, Chenoweth C, Saint S, Zemencuk JK, Lipsky BA, Plorde JJ. Four year prospective evaluation of nosocomial bacteremia: epidemiology, microbiology, and patient outcome. Diagn Microbiol Infect Dis 2000;38:131-40.
4. Gudlaugsson O, Gillespie S, Lee K, Vande Berg J, Hu J, Messer S, et al. Attributable mortality of nosocomial candidemia, revisited. Clin Infect Dis 2003;37:1172-7.
5. Cuenca-Estrella M, Rodriguez D, Almirante B, Morgan J, Planes AM, Almela M, et al. In vitro susceptibilities of bloodstream isolates of *Candida* species to six antifungal agents: results from a population-based active surveillance programme, Barcelona, Spain, 2002-2003. J Antimicrob Chemother 2005;55:194-9.
6. Ostrosky-Zeichner L, Rex JH, Pappas PG, Hamill RJ, Larsen RA, Horowitz HW, et al. Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. Antimicrob Agents Chemother 2003;47:3149-54.
7. Pfaller MA, Diekema DJ. Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida*. Clin Microbiol Infect 2004;10 Suppl 1:11-23.
8. Rex JH, Rinaldi MG, Pfaller MA. Resistance of *Candida* species to fluconazole. Antimicrob Agents Chemother 1995;39:1-8.
9. Hajjeh RA, Sofair AN, Harrison LH, Lyon GM, Arthington-Skaggs BA, Mirza SA, et al. Incidence of bloodstream infections due to *Candida* species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J Clin Microbiol 2004;42:1519-27.
10. Pfaller MA, Diekema DJ. Rare and emerging opportunistic fungal pathogens: concern for resistance beyond *Candida albicans* and *Aspergillus fumigatus*. J Clin Microbiol 2004;42:4419-31.
11. Pappagianis D, Collins MS, Hector R, Remington J. Development of resistance to amphotericin B in *Candida lusitaniae* infecting a human. Antimicrob Agents Chemother 1979;16:123-6.
12. Minari A, Hachem R, Raad I. *Candida lusitaniae*: a cause of breakthrough fungemia in cancer patients. Clin Infect Dis 2001;32:186-90.
13. Boucher HW, Groll AH, Chiou CC, Walsh TJ. Newer systemic antifungal agents: pharmacokinetics, safety and efficacy. Drugs 2004;64:1997-2020.
14. Cuenca-Estrella M, Moore CB, Barchiesi F, Bille J, Chryssanthou E, Denning DW, et al. Multicenter evaluation of the reproducibility of the proposed antifungal susceptibility testing method for fermentative yeasts of the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST). Clin Microbiol Infect 2003;9:467-74.
15. National Committee for Clinical Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard—Second Edition. NCCLS document M27-A2. 2 ed. Wayne, PA, USA.: NCCLS; 2002.
16. Espinel-Ingroff A, Pfaller M, Messer SA, Knapp CC, Holliday N, Killian SB. Multicenter comparison of the Sensititre YeastOne colorimetric antifungal panel with the NCCLS M27-A2 reference method for testing new antifungal agents against clinical isolates of *Candida* spp. J Clin Microbiol 2004;42:718-21.
17. Espinel-Ingroff A, Pfaller M, Messer SA, Knapp CC, Killian S, Norris HA, et al. Multicenter comparison of the sensititre YeastOne Colorimetric Antifungal Panel with the National Committee for Clinical Laboratory standards M27-A reference method for testing clinical isolates of common and emerging *Candida* spp., *Cryptococcus* spp., and other yeasts and yeast-like organisms. J Clin Microbiol 1999;37:591-5.
18. Pfaller MA, Espinel-Ingroff A, Jones RN. Clinical evaluation of the Sensititre YeastOne colorimetric antifungal plate for antifungal susceptibility testing of the new triazoles voriconazole, posaconazole, and ravuconazole. J Clin Microbiol 2004;42:4577-80.
19. Yang CW, Barkham TM, Chan FY, Wang Y. Prevalence of Candida species, including *Candida dubliniensis*, in Singapore. J Clin Microbiol 2003;41:472-4.
20. Chai YA, Wang Y, Khoo AL, Chan FY, Chow C, Kumarasinghe G, et al. Predominance of *Candida tropicalis* bloodstream infections in a Singapore teaching hospital. Med Mycol 2007;45:435-9.
21. Pfaller MA, Diekema DJ, Rex JH, Espinel-Ingroff A, Johnson EM, Andes D, et al. Correlation of MIC with outcome for *Candida* species tested against voriconazole: analysis and proposal for interpretive breakpoints. J Clin Microbiol 2006;44:819-26.
22. Pfaller M, Pappas P, Wingard J. Invasive Fungal Pathogens: Current Epidemiological Trends. Clin Infect Dis 2006;43:S3-S14.
23. Oon LL, Yeo MG. Fluconazole susceptibility of *Candida* species in Singapore by disc diffusion test. Ann Acad Med Singapore 2002;31: 497-501.
24. Pfaller MA, Diekema DJ, Jones RN, Messer SA, Hollis RJ. Trends in antifungal susceptibility of *Candida* spp. isolated from pediatric and adult patients with bloodstream infections: SENTRY Antimicrobial Surveillance Program, 1997 to 2000. J Clin Microbiol 2002;40:852-6.
25. Nguyen MH, Clancy CJ, Yu VL, Yu YC, Morris AJ, Snydman DR, et al. Do in vitro susceptibility data predict the microbiologic response to amphotericin B? Results of a prospective study of patients with Candida fungemia. J Infect Dis 1998;177:425-30.
26. Peyron F, Favel A, Michel-Nguyen A, Gilly M, Regli P, Bolmstrom A. Improved detection of amphotericin B-resistant isolates of *Candida lusitaniae* by Etest. J Clin Microbiol 2001;39:339-42.
27. Sterling TR, Merz WG. Resistance to amphotericin B: emerging clinical and microbiological patterns. Drug Resist Updat 1998;1:161-5.
28. Cuenca-Estrella M, Gomez-Lopez A, Mellado E, Rodriguez-Tudela JL. Correlation between the procedure for antifungal susceptibility testing for *Candida* spp. of the European Committee on Antibiotic Susceptibility Testing (EUCAST) and four commercial techniques. Clin Microbiol Infect 2005;11:486-92.
29. Alexander BD, Byrne TC, Smith KL, Hanson KE, Anstrom KJ, Perfect JR, et al. Comparative evaluation of Etest and sensititre yeastone panels against the Clinical and Laboratory Standards Institute M27-A2 reference broth microdilution method for testing Candida susceptibility to seven antifungal agents. J Clin Microbiol 2007;45:698-706.
30. Pfaller MA, Diekema DJ, Procop GW, Rinaldi MG. Multicenter comparison of the VITEK 2 yeast susceptibility test with the CLSI broth microdilution reference method for testing fluconazole against *Candida* spp. J Clin Microbiol 2007;45:796-802.
31. National Committee for Clinical Laboratory Standards Institute. Method for antifungal disk diffusion susceptibility testing of yeasts: approved guideline M44-A. 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.: NCCLS; 2005.