In vitro antifungal susceptibilities of *Candida* isolates from patients with invasive candidiasis in Kuala Lumpur Hospital, Malaysia

Fairuz Amran,¹ M. Nazri Aziz,² Hishamshah M. Ibrahim,³ N. H. Atiqah,¹ S. Parameswari,¹ M. R. Hafiza¹ and M. Ifwat¹

Correspondence

Fairuz Amran
fairuz@imr.gov.my

¹Bacteriology Unit, Institute for Medical Research, Kuala Lumpur, Malaysia

²Pathology Department, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia

³Paediatric Department, Kuala Lumpur Hospital, Kuala Lumpur, Malaysia

The *in vitro* antifungal susceptibilities of 159 clinical isolates of *Candida* species from patients with invasive candidiasis in Kuala Lumpur Hospital, Malaysia, were determined against amphotericin B, fluconazole, voriconazole, itraconazole and caspofungin. The most common species were *Candida albicans* (71 isolates), *Candida parapsilosis* (42 isolates), *Candida tropicalis* (27 isolates) and *Candida glabrata* (12 isolates). The susceptibility tests were carried out using an E-test. The MIC breakpoints were based on Clinical Laboratory Standards Institute criteria. Amphotericin B and voriconazole showed the best activities against all the isolates tested, with MIC₉₀ values of $\leq 1 \mu\text{g ml}^{-1}$ for all major species. Only one *Candida lusitanae* isolate was resistant to amphotericin B, and all the isolates were susceptible to voriconazole. In total, six isolates were resistant to fluconazole, comprising two isolates of *C. albicans*, two of *C. parapsilosis*, one *C. tropicalis* and one *C. glabrata*, and all of these isolates showed cross-resistance to itraconazole. The MIC₉₀ of itraconazole was highest for *C. glabrata* and *C. parapsilosis*. Caspofungin was active against most of the isolates except for five isolates of *C. parapsilosis*. The MIC₉₀ of caspofungin against *C. parapsilosis* was $3 \mu\text{g ml}^{-1}$. In conclusion, amphotericin B remains the most active antifungal agent against most *Candida* species except for *C. lusitanae*. Voriconazole is the best alternative for fluconazole- or itraconazole-resistant isolates. Although five of the *C. parapsilosis* isolates showed *in vitro* resistance to caspofungin, more clinical correlation studies need to be carried out to confirm the significance of these findings. Currently, despite the increase in usage of antifungals in our hospitals, especially in the management of febrile neutropenia patients, the antifungal-resistance problem among clinically important *Candida* isolates in Kuala Lumpur Hospital is not yet worrying. However, continued antifungal-susceptibility surveillance needs to be conducted to monitor the antifungal-susceptibility trends of *Candida* species and other opportunistic fungal pathogens.

Received 3 November 2010

Accepted 30 March 2011

INTRODUCTION

Candida species have emerged as a significant cause of morbidity and mortality worldwide over the past few decades, accounting for approximately 15 % of all hospital-acquired infections, >72 % of all nosocomial fungal infections and 8–15 % of all nosocomial bloodstream infections (BSIs) (Blot *et al.*, 2008; Edmond *et al.*, 1999; Wenzel & Edmond, 2001). Between 25 and 50 % of nosocomial candidaemia cases occur in critical care units (Rangel-Frausto *et al.*, 1999). Among the major risk factors for developing systemic candidiasis in cancer patients are

neutropenia and chemotherapy-induced gut-wall injuries, whilst in non-cancer patients the most significant risk factors are the presence of catheters, gut-wall surgery and prematurity (Bodey *et al.*, 2002; Pfaller *et al.*, 1998a; Wenzel & Edmond, 2001). Analysis of secular trends in epidemiology and microbiology of nosocomial BSIs in the USA was made using nationwide surveillance data from the SCOPE (Surveillance and Control of Pathogens of Epidemiological Importance) survey, and *Candida* species were shown to be the fourth most common cause of nosocomial BSIs, accounting for 9 % of all cases of BSI in hospitals in the USA (Pfaller *et al.*, 1998b). The crude mortality rate of BSIs due to *Candida* species was 39.2 %, the highest among all other blood isolates (Wisplinghoff *et al.*, 2004). There are few reports of *Candida* species

Abbreviations: BSI, bloodstream infection; SDD, susceptible dose-dependent.

distribution in Malaysia and their biotypes and genetic relatedness (Chong *et al.*, 2003; Ng *et al.*, 1998), and reports on antifungal susceptibilities of *Candida* species towards the commonly used antifungal are not readily available.

In this study, we analysed the antifungal susceptibilities of *Candida* species isolated from sterile clinical samples of hospitalized patients admitted to Kuala Lumpur Hospital from January 2006 to December 2008.

METHODS

Candida isolates. *Candida* spp. isolated from sterile clinical samples of patients with invasive candidiasis were collected. The isolates were from patients admitted to Kuala Lumpur Hospital from January 2006 to December 2008. Most of the samples were from blood (77 %) and peritoneal dialysis fluid (10 %). Other samples (13 %) were urine, bronchoalveolar lavage and cerebrospinal fluid. All isolates were identified to the species level using conventional as well as a commercial biochemical identification kit (API 20C AUX; bioMérieux).

Antifungal-susceptibility tests. *In vitro* antifungal-susceptibility tests were conducted on all the *Candida* isolates using an E-test (AB Biodisk). Instructions provided by the manufacturer were adhered to throughout the tests. The antifungals tested in this study were amphotericin B (range 0.002–32 mg ml⁻¹), fluconazole (0.016–256 mg ml⁻¹), itraconazole (0.002–32 mg ml⁻¹), voriconazole (0.002–32 µg ml⁻¹) and caspofungin (0.002–32 µg ml⁻¹).

Yeast suspensions were prepared in 0.85 % NaCl. The turbidity of each suspension was adjusted to a 0.5 McFarland standard. Using cotton-tipped swabs, each organism suspension was inoculated onto agar plates containing RPMI 1640 supplemented with 2 % glucose and buffered to pH 7.0 with MOPS. After application of the E-test strips, the plates were incubated for 48 h at 35 °C. The MIC of each isolate against each antifungal tested was read after 24 and 48 h.

Reference isolates *Candida albicans* ATCC 90028 and *Candida parapsilosis* ATCC 22019^T were used as controls. Interpretive susceptibility criteria recommended by the Clinical and Laboratory Standards Institute (CLSI) were used to evaluate the susceptibilities of isolates against fluconazole, itraconazole, voriconazole and caspofungin. For fluconazole, isolates showing an MIC of ≤ 8.0 µg ml⁻¹ were

regarded as susceptible, 16–32 µg ml⁻¹ as susceptible dose-dependent (SDD) and ≥ 64 µg ml⁻¹ as resistant. For itraconazole, isolates with an MIC of ≤ 0.125 µg ml⁻¹ were regarded as susceptible, 0.25–0.5 µg ml⁻¹ as SDD and ≥ 1 µg ml⁻¹ as resistant. For voriconazole, isolates with an MIC of ≤ 1.0 µg ml⁻¹ were regarded as susceptible, 2.0 µg ml⁻¹ as SDD and ≥ 4 µg ml⁻¹ as resistant (CLSI, 2008; Ostrosky-Zeichner *et al.*, 2003). Isolates with an MIC of ≤ 2.0 µg ml⁻¹ were considered susceptible to caspofungin. Due to the lack of defined breakpoint for amphotericin B, isolates showing an MIC of ≤ 1.0 µg ml⁻¹ were taken as susceptible and those with an MIC of > 2 µg ml⁻¹ were considered resistant (Ostrosky-Zeichner *et al.*, 2003; Rex *et al.*, 1997).

RESULTS

A total of 159 *Candida* isolates were collected and tested. They comprised 71 isolates of *C. albicans*, 42 isolates of *C. parapsilosis*, 27 isolates of *Candida tropicalis*, 12 isolates of *Candida glabrata*, two isolates of *Candida guilliermondii*, two isolates of *Candida famata*, one strain of *Candida inconspicua*, one strain of *Candida valida* and one strain of *Candida lusitanae*. The distribution of the isolates by wards is shown in Table 1. Most of the *C. albicans* isolates (66/71, 92 %) were isolated from non-neutropenic patients.

Antifungal-susceptibility tests were performed on all the clinical isolates collected. All the organisms tested produced detectable growth by 48 h. The MIC ranges and MIC₉₀ values of the five antifungal drugs against *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* are summarized in Table 2. The percentage susceptibilities of *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* are shown in Table 3. For the other *Candida* species, the number of isolates was too low for full analyses to be performed.

The MIC₉₀ values of amphotericin B against *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* were all ≤ 1 µg ml⁻¹. All isolates except for the single isolate of *C. lusitanae* (MIC 4 µg ml⁻¹) were susceptible to amphotericin B. The MIC₉₀ values of fluconazole for *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* were 8, 4, 0.75 and 16 µg ml⁻¹, respectively. The percentage susceptibilities

Table 1. Distribution of *Candida* isolates by hospital ward

Ward	No. (%) of <i>Candida</i> isolates				
	<i>C. albicans</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	<i>C. glabrata</i>	Other
Adult medical and surgical	23 (32.4)	8 (19.0)	4 (14.8)	5 (41.7)	3 (42.9)
General intensive-care unit	11 (15.5)	4 (9.5)	3 (11.1)	4 (33.3)	1 (14.3)
Adult haematology	2 (2.8)	4 (9.5)	6 (22.2)	1 (8.3)	–
Paediatrics medical and surgical	6 (8.5)	5 (11.9)	3 (11.1)	–	2 (28.6)
Paediatrics intensive-care unit	5 (7.0)	5 (11.9)	2 (7.4)	–	–
Neonatal intensive-care unit	8 (11.3)	5 (11.9)	3 (11.1)	–	1 (14.3)
Paediatrics haematology, oncology and bone-marrow transplant unit	3 (4.2)	7 (16.7)	5 (18.5)	–	–
Urology and nephrology unit	13 (18.3)	4 (9.5)	1 (3.7)	2 (16.7)	–
Total	71 (100)	42 (100)	27 (100)	12 (100)	7 (100)

Table 2. MIC₉₀ values and MIC ranges ($\mu\text{g ml}^{-1}$) of amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin against 159 isolates of *Candida* species

ND, Not done (the number of isolates was too low for full analyses to be performed).

<i>Candida</i> species (n)	Antifungal agent									
	Amphotericin B		Fluconazole		Itraconazole		Voriconazole		Caspofungin	
	MIC ₉₀	MIC range	MIC ₉₀	MIC range	MIC ₉₀	MIC range	MIC ₉₀	MIC range	MIC ₉₀	MIC range
<i>C. albicans</i> (71)	0.094	0.003–0.38	8	0.047–64	0.75	0.003–1	1	0.118–2	0.5	0.004–2
<i>C. parapsilosis</i> (42)	0.094	0.004–0.19	4	0.047–64	1	0.006–1	0.094	0.002–0.094	3	0.064–>32
<i>C. tropicalis</i> (27)	0.125	0.003–0.5	0.75	0.047–64	0.125	0.003–0.13	0.094	0.003–0.13	1	0.016–2
<i>C. glabrata</i> (12)	0.019	0.004–0.25	16	0.094–64	>2	0.004–>2	0.125	0.004–0.13	0.38	0.064–0.75
<i>C. guilliermondii</i> (2)	ND	0.008–0.03	ND	0.75–1.5	ND	0.25–1	ND	0.032–0.13	ND	0.75–2
<i>C. famata</i> (2)	ND	0.004–0.06	ND	0.13–0.25	ND	0.094–0.13	ND	0.006–0.03	ND	0.03–0.064
<i>C. lusitanae</i> (1)	ND	4	ND	1	ND	0.75	ND	0.094	ND	0.25
<i>C. inconspicua</i> (1)	ND	0.004	ND	4	ND	0.032	ND	0.032	ND	0.5
<i>C. valida</i> (1)	ND	0.064	ND	0.38	ND	>2	ND	0.012	ND	0.25

of *C. albicans*, *C. parapsilosis* and *C. tropicalis* to fluconazole were >90 %, but only 83.4 % of *C. glabrata* isolates were susceptible to fluconazole. The MIC₉₀ of itraconazole for *C. albicans*, *C. parapsilosis* and *C. tropicalis* was $\leq 1 \mu\text{g ml}^{-1}$. Two of the isolates that showed resistance to fluconazole also demonstrated cross-resistance to itraconazole. More than 90 % of the *C. albicans* and *C. tropicalis* isolates were susceptible to itraconazole, and 85.7 % of *C. parapsilosis* and 83.4 % of *C. glabrata* isolates were susceptible. The MIC₉₀ values of voriconazole against *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* were all $\leq 1 \mu\text{g ml}^{-1}$. The

MIC₉₀ values of caspofungin against *C. albicans*, *C. tropicalis* and *C. glabrata* were all $\leq 1 \mu\text{g ml}^{-1}$. However, for *C. parapsilosis*, the MIC₉₀ of caspofungin was $3 \mu\text{g ml}^{-1}$. Five of the *C. parapsilosis* isolates and one *C. tropicalis* isolate were resistant to caspofungin.

DISCUSSION

During the study period, a total of 159 *Candida* isolates from systemic candidiasis cases were collected and their

Table 3. Antifungal-susceptibility patterns of *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata* to amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin

Antifungal agent susceptibility pattern*	Percentage (n) of isolates			
	<i>C. albicans</i>	<i>C. parapsilosis</i>	<i>C. tropicalis</i>	<i>C. glabrata</i>
Amphotericin B				
Susceptible	100 (71)	100 (42)	100 (27)	100 (12)
Resistant	0 (0)	0 (0)	0 (0)	0 (0)
Fluconazole				
Susceptible	94.4 (67)	92.9 (39)	92.6 (25)	83.4 (10)
SDD	2.8 (2)	2.4 (1)	3.7 (1)	8.3 (1)
Resistant	2.8 (2)	4.7 (2)	3.7 (1)	8.3 (1)
Itraconazole				
Susceptible	97.2 (69)	85.7 (36)	96.3 (26)	83.4 (10)
SDD	0 (0)	2.4 (1)	3.7 (1)	0 (0)
Resistant	2.8 (2)	11.9 (5)	0 (0)	16.6 (2)
Voriconazole				
Susceptible	98.6 (70)	100 (42)	100 (27)	100 (12)
SDD	1.4 (1)	0 (0)	0 (0)	0 (0)
Resistant	0 (0)	0 (0)	0 (0)	0 (0)
Caspofungin				
Susceptible	100 (71)	88.1 (37)	96.3 (26)	100 (12)
Resistant	0 (0)	11.9 (5)	3.7 (1)	0 (0)

*Definitions of the interpretive susceptibility criteria used, as recommended by the CLSI, are available in Methods.

in vitro antifungal susceptibilities were analysed. Almost half (44.7%) of the isolates were *C. albicans*, followed by *C. parapsilosis* (26.4%). This is consistent with most published reports, where *C. albicans* has been the predominant species isolated from cases of systemic candidiasis (Kao *et al.*, 1999; Mokaddas *et al.*, 2007; Morgan, 2005). The emergence of infections due to non-*albicans* *Candida* species has been noted particularly among neutropenic patients (Kao *et al.*, 1999). In this study, 82% of all *Candida* isolates from patients admitted to the adult and paediatric haematology, oncology and bone-marrow transplant wards were non-*albicans* *Candida* species.

Antimicrobial-surveillance programmes have provided useful information regarding resistant trends and can be used as guidance for empirical therapy. However, most surveillance programmes focus only on bacterial pathogens and seldom emphasize fungal pathogens. In Malaysia, for example, surveillance does not include surveys on the antifungal resistance of opportunistic fungal pathogens. The present study was conducted to obtain basic data on the distribution of systemic candidiasis and the antifungal-susceptibility patterns of the clinically important *Candida* species in Kuala Lumpur Hospital, Malaysia. The *in vitro* activities of *Candida* species against amphotericin B, fluconazole, itraconazole, voriconazole and caspofungin have been reported in several studies (Anaissie *et al.*, 1996; Pfaller *et al.*, 2005, 2008).

In this study, amphotericin B showed good activity against all the isolates, with MIC₉₀ values of $\leq 1 \mu\text{g ml}^{-1}$ for all major isolates. In fact, all the isolates were susceptible except for *C. lusitaniae*. The level of fluconazole resistance among the isolates tested was relatively low, similar to that found in other studies (Pfaller *et al.*, 2005; Ostrosky-Zeichner *et al.*, 2003; Ruan *et al.*, 2008). Only a few isolates of *C. albicans* (two), *C. parapsilosis* (two), *C. tropicalis* (one) and *C. glabrata* (one) were resistant to fluconazole and all of these isolates showed cross-resistance to itraconazole. Therefore, fluconazole can still be used as a first-line therapy for infections caused by *Candida* species other than *C. glabrata* and *C. krusei*. The percentage susceptibility of *C. glabrata* isolates to fluconazole in this study was 83.4%. However, because of the low number of isolates ($n=12$), it is not justifiable to say that there is high prevalence of fluconazole-resistant *C. glabrata* in this hospital. It is interesting to note that, despite the wide usage of fluconazole over the past decade, emergence of *C. krusei* infections due to selective pressure has not occurred, in contrast to the findings observed in other reports (Ruan *et al.*, 2008). The MIC₉₀ values for itraconazole were highest for *C. glabrata* and *C. parapsilosis*.

Voriconazole had the best activity (MIC₉₀ $\leq 1 \mu\text{g ml}^{-1}$) against all the isolates tested. Cross-resistance was not demonstrated between the older azoles and voriconazole, making it a good alternative agent against isolates that are resistant to the older azoles.

In vitro resistance of *C. parapsilosis* to caspofungin has been reported in several studies (Mora-Duarte *et al.*, 2002;

Forrest *et al.*, 2008). However, Pfaller *et al.* (2006) reported that the caspofungin MIC distribution remains unchanged for *Candida* isolates, including *C. parapsilosis* collected before and after the introduction of caspofungin into clinical use (Anaissie *et al.*, 1996). Because of the slightly higher prevalence of resistance among *C. parapsilosis* to caspofungin, there have been reports of breakthrough infection of *C. parapsilosis* whilst patients were on caspofungin (Forrest *et al.*, 2008; Cheung *et al.*, 2006). In another study, there was a significant increase in *C. parapsilosis* infections following extensive usage of caspofungin as an empirical and prophylactic antifungal agent (Forrest *et al.*, 2008). In the present study, the MIC₉₀ of caspofungin against *C. parapsilosis* was $3 \mu\text{g ml}^{-1}$. Five isolates were resistant and three of them had MICs of $>32 \mu\text{g ml}^{-1}$.

In conclusion, amphotericin B remains the most active antifungal agent against most *Candida* species except for *C. lusitaniae*. Fluconazole can still be used as a first-line empirical antifungal agent except in settings where the majority of isolates are *C. glabrata*. Voriconazole is the best alternative for fluconazole- and itraconazole-resistant isolates. The findings of *in vitro* resistance of *C. parapsilosis* against caspofungin need to be studied further to define its clinical significance. Antifungal-susceptibility testing should be carried out routinely for all invasive infections caused by *Candida* species and other opportunistic fungal pathogens to monitor epidemiological trends and to detect the emergence of antifungal resistance.

ACKNOWLEDGEMENTS

The authors would like to thank the Director of the Institute for Medical Research and the Director General of the Ministry of Health Malaysia for permission to publish this article. We would also like to acknowledge the contributions of all staff of the Bacteriology Unit, Institute for Medical Research; the Microbiology Unit, Kuala Lumpur Hospital; and the clinicians of the Paediatric Department, Kuala Lumpur Hospital, for their kind support and contributions to this study.

REFERENCES

- Anaissie, E. J., Paetznick, V. L., Ensign, L. G., Espinel-Ingroff, A., Galgiani, J. N., Hitchcock, C. A., LaRocco, M., Patterson, T., Pfaller, M. A. & other authors (1996). Microdilution antifungal susceptibility testing of *Candida albicans* and *Cryptococcus neoformans* with and without agitation: an eight-center collaborative study. *Antimicrob Agents Chemother* **40**, 2387–2391.
- Blot, S., Vandewoude, K. & Vogelaers, D. (2008). Discriminating invasive fungal infection from colonization. *Crit Care* **12**, 412, author reply 412.
- Bodey, G. P., Mardani, M., Hanna, H. A., Boktour, M., Abbas, J., Gorgawy, E., Hachem, R. Y., Kontoyiannis, D. P. & Raad, I. I. (2002). The epidemiology of *Candida glabrata* and *Candida albicans* fungemia in immunocompromised patients with cancer. *Am J Med* **112**, 380–385.
- Cheung, C., Guo, Y., Gialanella, P. & Feldmesser, M. (2006). Development of candidemia on caspofungin therapy: a case report. *Infection* **34**, 345–348.

- Chong, P. P., Lee, Y. L., Tan, B. C. & Ng, K. P. (2003). Genetic relatedness of *Candida* strains isolated from women with vaginal candidiasis in Malaysia. *J Med Microbiol* **52**, 657–666.
- CLSI (2008). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts*, 3rd edn. Approved standard M27–A3. Wayne, PA: Clinical and Laboratory Standards Institute.
- Edmond, M. B., Wallace, S. E., McClish, D. K., Pfaller, M. A., Jones, R. N. & Wenzel, R. P. (1999). Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clin Infect Dis* **29**, 239–244.
- Forrest, G. N., Weekes, E. & Johnson, J. K. (2008). Increasing incidence of *Candida parapsilosis* candidemia with caspofungin usage. *J Infect* **56**, 126–129.
- Kao, A. S., Brandt, M. E., Pruitt, W. R., Conn, L. A., Perkins, B. A., Stephens, D. S., Baughman, W. S., Reingold, A. L., Rothrock, G. A. & other authors (1999). The epidemiology of candidemia in two United States cities: results of a population-based active surveillance. *Clin Infect Dis* **29**, 1164–1170.
- Mokaddas, E. M., Al-Sweih, N. A. & Khan, Z. U. (2007). Species distribution and antifungal susceptibility of *Candida* bloodstream isolates in Kuwait: a 10-year study. *J Med Microbiol* **56**, 255–259.
- Mora-Duarte, J., Betts, R., Rotstein, C., Colombo, A. L., Thompson-Moya, L., Smietana, J., Lupinacci, R., Sable, C., Kartsonis, N. & other authors (2002). Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Engl J Med* **347**, 2020–2029.
- Morgan, J. (2005). Global trends in candidemia: review of reports from 1995–2005. *Curr Infect Dis Rep* **7**, 429–439.
- Ng, K. P., Madasamy, M., Saw, T. L., Baki, A., He, J. & Soo-Hoo, T. S. (1998). *Candida* biotypes isolated from clinical specimens in Malaysia. *Mycopathologia* **144**, 135–140.
- Ostrosky-Zeichner, L., Rex, J. H., Pappas, P. G., Hamill, R. J., Larsen, R. A., Horowitz, H. W., Powderly, W. G., Hyslop, N., Kauffman, C. A. & other authors (2003). Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. *Antimicrob Agents Chemother* **47**, 3149–3154.
- Pfaller, M. A., Jones, R. N., Doern, G. V., Sader, H. S., Hollis, R. J. & Messer, S. A. for The SENTRY Participant Group (1998a). International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and antifungal susceptibilities of isolates collected in 1997 in the United States, Canada, and South America for the SENTRY Program. *J Clin Microbiol* **36**, 1886–1889.
- Pfaller, M. A., Jones, R. N., Messer, S. A., Edmond, M. B. & Wenzel, R. P. (1998b). National surveillance of nosocomial blood stream infection due to species of *Candida* other than *Candida albicans*: frequency of occurrence and antifungal susceptibility in the SCOPE Program. SCOPE Participant Group. Surveillance and Control of Pathogens of Epidemiologic Importance. *Diagn Microbiol Infect Dis* **30**, 121–129.
- Pfaller, M. A., Diekema, D. J., Rinaldi, M. G., Barnes, R., Hu, B., Veselov, A. V., Tiraboschi, N., Nagy, E. & Gibbs, D. L. (2005). Results from the ARTEMIS DISK Global Antifungal Surveillance Study: a 6.5-year analysis of susceptibilities of *Candida* and other yeast species to fluconazole and voriconazole by standardized disk diffusion testing. *J Clin Microbiol* **43**, 5848–5859.
- Pfaller, M. A., Boyken, L., Hollis, R. J., Messer, S. A., Tendolkar, S. & Diekema, D. J. (2006). *In vitro* susceptibilities of *Candida* spp. to caspofungin: four years of global surveillance. *J Clin Microbiol* **44**, 760–763.
- Pfaller, M. A., Boyken, L., Hollis, R. J., Kroeger, J., Messer, S. A., Tendolkar, S. & Diekema, D. J. (2008). *In vitro* susceptibility of invasive isolates of *Candida* spp. to anidulafungin, caspofungin, and micafungin: six years of global surveillance. *J Clin Microbiol* **46**, 150–156.
- Rangel-Frausto, M. S., Wiblin, T., Blumberg, H. M., Saiman, L., Patterson, J., Rinaldi, M., Pfaller, M., Edwards, J. E., Jr, Jarvis, W. & other authors (1999). National Epidemiology of Mycoses Survey (NEMIS): variations in rates of bloodstream infections due to *Candida* species in seven surgical intensive care units and six neonatal intensive care units. *Clin Infect Dis* **29**, 253–258.
- Rex, J. H., Pfaller, M. A., Galgiani, J. N., Bartlett, M. S., Espinel-Ingroff, A., Ghannoum, M. A., Lancaster, M., Odds, F. C., Rinaldi, M. G. & other authors (1997). Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of in vitro-in vivo correlation data for fluconazole, itraconazole, and *Candida* infections. *Clin Infect Dis* **24**, 235–247.
- Ruan, S.-Y., Chu, C.-C. & Hsueh, P.-R. (2008). In vitro susceptibilities of invasive isolates of *Candida* species: rapid increase in rates of fluconazole susceptible-dose dependent *Candida glabrata* isolates. *Antimicrob Agents Chemother* **52**, 2919–2922.
- Wenzel, R. P. & Edmond, M. B. (2001). Severe sepsis – national estimates. *Crit Care Med* **29**, 1472–1474.
- Wisplinghoff, H., Bischoff, T., Tallent, S. M., Seifert, H., Wenzel, R. P. & Edmond, M. B. (2004). Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* **39**, 309–317.