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Original

In vitro susceptibilities of bloodstream isolates of *Candida* spp.: results from a multicenter active surveillance program in Andalusia

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ABSTRACT

Objectives: The aim of this study was to determine the antifungal drug susceptibilities of *Candida* bloodstream isolates in Andalusia, obtained through a multicenter active laboratory-based surveillance between October 2005 and September 2006.

Methods: One hundred and ninety-seven *Candida* isolates were collected. The MICs of amphotericin B, fluconazole, itraconazole and voriconazole were established using the Sensititre YeastOne panel. The MICs of posaconazole and caspofungin were determined by Etest.

Results: C. albicans was the most frequently isolated species (49.2%), followed by *C. parapsilosis* (17.3%), *C. tropicalis* (15.2%), *C. glabrata* (13.7%) and *C. krusei* (3.6%). All strains were inhibited at MICs of $\leq$1 mg/L of amphotericin B and 98.5% of isolates were inhibited at MICs of $\leq$ 1 mg/L of posaconazole. A total of 8 isolates (4.1%) were classified as resistant to fluconazole (MIC$\geq$64 mg/L) and 7 (3.6%) were considered resistant to itraconazole (MIC$\geq$1 mg/L). All the isolates were susceptible to voriconazole and caspofungin.

Conclusion: In our study *C. krusei* and *C. glabrata* were identified in over 18% of cases of candidemia. Most clinical isolates of these species are resistant or susceptible-dose-dependent to fluconazole but susceptible to voriconazole and caspofungin. These agents must be used in the empiric treatment of candidemia rather than fluconazole.

Sensibilidad "in vitro" de las cepas de *Candida* spp. aisladas en sangre: resultados de un estudio multicéntrico realizado en Andalucía

Palabras clave:
Candidemia
Candida spp.
Sensibilidad antifúngica

RESUMEN

Objetivos: Determinar la sensibilidad antifúngica de las cepas de *Candida* spp. aisladas en sangre en Andalucía, obtenidas en un estudio prospectivo multicéntrico realizado entre octubre de 2005 y septiembre de 2006.

Métodos: Se aislaron 197 cepas de *Candida* spp. Las CMIs de anfotericina B, fluconazol, itraconazol y voriconazol se realizaron usando el panel de Sensititre YeastOne. Las CMIs de posaconazol y caspofungina se determinaron por Etest.

Resultados: C. albicans fue la especie más frecuentemente aislada (49,2%), seguida de *C. parapsilosis* (17,3%), *C. tropicalis* (15,2%), *C. glabrata* (13,7%) y *C. krusei* (3,6%) Todas las cepas tuvieron CMIs $\leq$ 1mg/l de anfotericina y 98,5% de los aislamientos fueron inhibidos por 1mg/l de posaconazol. Ocho aislamientos

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(4,1%) fueron identificados como resistentes a fluconazol (CMI ≥ 64 mg/l) y 7 (3,6%) como resistentes a itraconazol (CMI ≥ 1 mg/L). Todos los aislamientos fueron sensibles a voriconazol y caspofungina.
Conclusión: En nuestro estudio *C. Krusei* y *C. glabrata* se aislaron en el 18% de los casos de candidemia. La mayor parte de los aislamientos de estas especies son resistentes a fluconazol o sensibles dependiendo de la dosis, pero sensibles a voriconazol y caspofungina. Por ello creemos que el tratamiento empírico de la candidemia debe ser realizado con estos antifúngicos y no con fluconazol.

Introduction

Candidemia is the fourth most common hospital-acquired bloodstream infection in the USA[1] and is associated with high morbidity and mortality.[2] Within the rising incidence of bloodstream infections caused by yeasts, there has been a sharp increase in the percentage caused by *Candida* species other than *Candida albicans*, such as *Candida glabrata* and *Candida krusei*.[3] *C. krusei* is innately resistant to fluconazole,[4] and *C. glabrata* often develops acquired resistance to this agent.[5] Previous investigations have suggested that prior exposure to fluconazole may be a risk factor for subsequent infection with *C. glabrata* and *C. krusei*.[6,7] The increase in infections caused by these *Candida* species presents a particular challenge for the clinical efficacy of triazole antifungal agents. Among several new triazole and echinocandin agents, voriconazole, posaconazole, and caspofungin appear to be highly active against all *Candida* species, including those that are less susceptible or resistant to fluconazole. There are certain differences in the distribution of species and antifungal drug susceptibilities between countries, and this fact underscores the need for continuing surveillance to monitor the trends related to these factors.[8–10]

To date, there is little data on *Candida* bloodstream infection in Andalusia. Thus, we conducted a prospective multicenter surveillance study from 2005 to 2006 for *Candida* bloodstream infection in Andalusian adults to determine the distribution of the species involved in these infections and the percentages of antifungal drug resistance among the isolated strains. Herein, we report the antifungal drug susceptibility profiles of the strains found to amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole, and caspofungin.

Materials and methods

Isolates

Between 1 October 2005 and 30 September 2006, 197 *Candida* isolates from adults with bloodstream infection were collected as part of a multicenter active surveillance program conducted in the region of Andalusia (Spain) by SAEI-SAMPAC (Andalusian Infectious Diseases Society-Andalusian Society of Clinical Microbiology and Parasitology). Seventeen hospitals participated (6 university tertiary care centers and 11 secondary care hospitals). Among the total of isolates collected, 115 were from tertiary hospitals and 82 from secondary care hospitals. Candidemia detection and identification of the species involved were performed at the participating laboratories and confirmed by the Valme laboratory, using the Vitek 2 ID-YST card (bioMerieux Vitek, Inc., Hazelwood, MO).[11] *Candida parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as quality control organisms for antifungal drug susceptibility testing.

Susceptibility testing

The minimum inhibitory concentrations (MICs) of amphotericin B, fluconazole, itraconazole, and voriconazole were determined at Valme Laboratory using the Sensititre YeastOne panel (Trek Diagnostic Systems, East Grinstead, UK),[12] which incorporates alamarBlue as the oxidation-reduction colorimetric indicator (fungal growth changes the indicator from blue to pink). Susceptibility testing, reading, and interpretation of the results were performed in accordance with the manufacturer's instructions, as follows: 20 μL of inoculum suspension was added to 11 mL of RPMI 1640 broth to obtain a working suspension (approximately $1.5–5 \times 10^3$ cells/mL), and 100 μL of this suspension was added to each well. The panels were sealed and incubated in air at 37 °C. The azoles were read after 24 h, and amphotericin B was read after 48 h; The MIC was the first well that did not show a color change from blue to pink.

The MICs of posaconazole and caspofungin were determined at the Valme Laboratory by Etest (AB BIODISK, Solna, Sweden)[12] on RPMI 1640 agar plates with 2% glucose according to the manufacture's instructions. Etest strips containing concentrations ranging from 0.002 mg/L to 32 mg/L were used. MICs were read where the edge of the inhibition ellipse intersected the MIC scale on the Etest strip after 24 h of incubation in air at 37 °C.

The interpretive breakpoints were those proposed in the Clinical and Laboratory Standards Institute (CLSI) M44-S1 reference method for fluconazole, itraconazole and voriconazole[13] and in the CLSI M27-A3 reference method for caspofungin.[14] Isolates showing fluconazole MICs of ⩽8.0 mg/L were considered susceptible (S), those with MICs of 16 to 32 mg/L were considered susceptible dose dependent (S-DD), and isolates with fluconazole MICs of ⩾64 mg/L were considered resistant (R). These breakpoints applied to all *Candida* spp. with the exception of *C. krusei*, which is considered inherently resistant to fluconazole, regardless of the MIC value.[4] The interpretive breakpoints defined for itraconazole were the following: S, ⩽0.12 mg/L; S-DD, 0.25 to 0.5 mg/L; and R, ⩾1.0 mg/L. Isolates showing voriconazole MICs of ⩽1 mg/L were classified as susceptible, those with MICs of 2 mg/L as S-DD, and those with MICs of ⩾4 mg/L as resistant. The interpretive breakpoints defined for caspofungin were S, <2 mg/L; and R, >4 mg/L. Although interpretive breakpoints for amphotericin B have not been established, *Candida* isolates showing MICs of >1 mg/L are likely to be resistant to amphotericin B.[4] Interpretive criteria have not been established for posaconazole.

Results

During the 12-month study period, a total of 197 *Candida* bloodstream infections in adults were reported by the 17 SAEI-SAMPAC Candidemia Program participants. The species distribution and *in vitro* susceptibilities of the 197 Candida strains to amphotericin B, fluconazole, itraconazole, voriconazole, posaconazole and caspofungin are shown in Table 1. The results are reported as MIC ranges, and the MICs at which 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates tested were inhibited by each drug. *C. albicans* was the most common isolate (49.2%), followed by *C. parapsilosis* (17.3%), *Candida tropicalis* (15.2%), *C. glabrata* (13.7%), and *C. krusei* (3.6%).

Amphotericin B MICs were in the range 0.01–1 mg/L, with *C. krusei* isolates demonstrating the highest MIC_{90} values (1 mg/L).

Table 1
Susceptibility test results for *Candida* bloodstream isolates

Species	No./%[a]	Agent	MIC (mg/L)			No. S-DD (%)	No. R (%)
			Range	MIC_{50}	MIC_{90}		
C. albicans	97/49.2	Amphotericin B	0.01–1	0.12	0.5		
		Fluconazole	⩽ 0.12–8	0.12	0.5	0	0
		Itraconazole	⩽0.008–0.5	0.03	0.06	2 (2.1)	0
		Voriconazole	⩽0.008–0.5	⩽0.008	0.03	0	0
		Posaconazole	0.002–0.12	0.02	0.06		
		Caspofungin	0.002–0.12	0.06	0.12	0	0
C. parapsilosis	34/17.3	Amphotericin B	0.01–0.5	0.12	0.5		
		Fluconazole	0.25–8	1	4	0	0
		Itraconazole	0.01–0.25	0.06	0.25	5 (14.7)	0
		Voriconazole	⩽0.008–0.5	0.03	0.12	0	0
		Posaconazole	0.002–0.5	0.03	0.09		
		Caspofungin	0.01–1.5	0.5	0.75	0	0
C. tropicalis	30/15.2	Amphotericin B	0.03–1	0.25	0.5		
		Fluconazole	0.25–16	1	2	1 (3.3)	0
		Itraconazole	0.01–2	0.12	0.25	9 (30)	1 (3.3)
		Voriconazole	⩽0.008–0.5	0.06	0.12	0	0
		Posaconazole	0.002–0.12	0.03	0.12		
		Caspofungin	0.002–0.5	0.19	0.25	0	0
C. glabrata	27/13.7	Amphotericin B	0.12–1	0.25	0.5		
		Fluconazole	0.5–>64	8	32	11 (40.7)	1 (3.7)
		Itraconazole	0.03–>2	0.25	1	15 (55.6)	5 (18.5)
		Voriconazole	⩽0.008–1	0.06	1	0	0
		Posaconazole	0.047–8	0.12	1.5		
		Caspofungin	0.047–0.5	0.19	0.25	0	0
C. krusei	7/3.6	Amphotericin B	0.06–1	0.25	ND		
		Fluconazole	16–64	32	ND	0	7 (100)
		Itraconazole	0.12–1	0.25	ND	5 (71.4)	1 (14.3)
		Voriconazole	0.06–0.25	0.12	ND	0	0
		Posaconazole	0.12–0.25	0.19	ND		
		Caspofungin	0.25–0.38	0.25	ND	0	0
C. famata	1/0.5	Amphotericin B	1	ND	ND		
		Fluconazole	2	ND	ND	0	0
		Itraconazole	0.06	ND	ND	0	0
		Voriconazole	0.03	ND	ND	0	0
		Posaconazole	0.06	ND	ND		
		Caspofungin	0.75	ND	ND	0	0
C. guilliermondii	1/0.5	Amphotericin B	1	ND	ND		
		Fluconazole	16	ND	ND	1 (100)	0
		Itraconazole	0.5	ND	ND	1 (100)	0
		Voriconazole	⩽0.008	ND	ND	0	0
		Posaconazole	0.75	ND	ND		
		Caspofungin	1	ND	ND	0	0
Total	197/100	Amphotericin B	0.01–1	0.12	0.5		
		Fluconazole	⩽0.12–>64	0.5	16	13 (6.6)	8 (4.1)
		Itraconazole	⩽0.008–>2	0.06	0.5	37 (18.8)	7 (3.6)
		Voriconazole	⩽0.008–1	0.16	0.25	0	0
		Posaconazole	0.002–8	0.04	0.19		
		Caspofungin	0.002–1.5	0.09	0.5	0	0

S-DD = susceptible dose dependent; R = resistant. MIC breakpoints are according to the CLSI M44-S1 and CLSI M27-A3 documents. ND = not determined.
[a] Number of isolates and percentage. MIC_{50} and MIC_{90} are the concentrations required to inhibit 50% and 90% of isolates, respectively.

C. krusei and *C. glabrata* showed the highest MICs of fluconazole, itraconazole, voriconazole, and posaconazole. A total of 8 isolates (4.1%) were classified as resistant to fluconazole (MIC ⩾64 mg/L) and 13 (6.6%) were S-DD (16–32 mg/L). Seven isolates (3.6%) were classified as resistant to itraconazole (MIC ⩾1 mg/L) and 37 (18.8%) were S-DD (MIC 0.25–0.5 mg/L). All isolates were classified as susceptible to voriconazole (⩽1 mg/L). Posaconazole MICs were in the range 0.002–8 mg/L, with a MIC_{50} and MIC_{90} of 0.04 and 0.19 mg/L, respectively; *C. glabrata* showed the highest MICs. Posaconazole MICs of >1 mg/L were demonstrated for only 3 *C. glabrata* isolates (1.5%), 1 of which was resistant and 2 S-DD to fluconazole. All isolates were susceptible to caspofungin, with *C. parapsilosis* and *Candida guilliermondii* showing the highest MICs. *C. albicans* was the most susceptible species to all the antifungal drugs studied.

Discussion

We present the species distribution and in vitro susceptibility data obtained in a multicenter active surveillance program for *Candida* spp. isolates causing bloodstream infection in adults in the south of Spain between 1 October 2005 and 30 September 2006. Although *C. albicans* remains the predominant species in these infections, the frequency with which it occurs varies worldwide from as low as 37% in the USA[15] to a high of 70% in Norway.[16] *C. albicans* was the predominant species in our geographic area, accounting for 49% of all *Candida* bloodstream infections, a value similar to reported rates in Spain and other countries in Europe.[7,8,17] In our study, *C. parapsilosis* accounted for 17% of all Candida bloodstream infections in adults and was the second most common species causing these infections, as has

been reported in other studies from Spain,[7,8] although at a lower incidence than the 29% and 23% described in these studies. It is, however, higher than the 1% to 6% reported for *C. parapsilosis* from Switzerland, Denmark and Norway.[9,16,18] These differences might be attributable to differences in the population studied and healthcare practices. In other reports, *C. parapsilosis* was isolated in 45% to 67% of neonatal candidemias,[8,19] and was associated with nosocomial spread.[20,21].

In the present study, *C. glabrata* was the fourth most common species isolated, whereas in the United States and Europe it was the second most common.[17,22,23] The low percentage of *Candida* bloodstream infections due to *C. krusei*, *C. guilliermondii* and *C. famata*, are consistent with reports from Spain, other European countries, and the USA.[8,19,24,25]

Our susceptibility results are in keeping with those of other studies: resistance is uncommon among *C. albicans* and *C. parapsilosis,* and there is a high level of reduced azole susceptibility among *C. glabrata.*[26,27] Overall resistance to fluconazole was documented in 4.1% of the tested strains, a percentage similar to other reported rates,[7,24] but lower than the 15% seen in Portugal.[28] Complete resistance to itraconazole was recorded in 3.6% of isolates. This finding is consistent with a report from the USA,[24] but is lower than the 12.6% to 19.4% described in Belgium, Germany, and Spain.[7,29,30]

In this study, amphotericin B MICs were in the range of 0.01–1 mg/L, with a MIC_{90} of 1 mg/L for *C. krusei*. The decreased susceptibility of *C. krusei* to amphotericin B is consistent with previous reports for this microorganism.[24,26] In other studies, 0.4% to 9% of isolates demonstrated potential resistance, with amphotericin B MICs of $\geqslant$2 mg/L.[7,19,24]

Fluconazole resistance was not always associated with resistance to the other azoles studied; in some cases, only an increase in the MIC endpoint was observed. Moreover, intrinsically fluconazole-resistant *C. krusei* showed no cross resistance with other azoles. The new azoles, posaconazole and voriconazole, displayed potent antifungal activity against all *Candida* spp. including *C. glabrata* and *C. krusei*. In this study, all the isolates were susceptible to voriconazole and 98.5% of the strains were inhibited at MICs of $\leq$1 mg/mL of posaconazole. Our results are consistent with those of other studies.[10,29,31]

All the isolates studied were susceptible to caspofungin. Of note are the relatively high MICs that were seen for *C. parapsilosis* and *C. guilliermondii*. These results are consistent with other reports.[5,26,32]

In conclusion, this study identified *C. krusei* and *C. glabrata* in 17.3% of candidemia cases. Most clinical isolates of these species were resistant or susceptible dose dependent to fluconazole, but were susceptible to voriconazole and caspofungin. Hence, these latter agents should be used in empirical treatment for candidemia rather than fluconazole.

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References

1. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39: 309–17.
2. Viudes A, Pemán J, Cantón E, Úbeda P, López-Ribot JL, Gobernado M. Candidemia at a tertiary-care hospital: epidemiology, treatment, clinical outcome and risk factors for death. Eur J Clin Microbiol Infect Dis. 2002;21: 767–74.
3. 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.
4. National Committee for Clinical Laboratory Standards. Reference Methods for Broth Dilution Antifungal Susceptibility Testing of Yeasts-Second Edition: Approved Standard M27-A2. NCCLS, Wayne, PA, USA, 2002.
5. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007;20:133–63.
6. Hope W, Morton A, Eisen DP. Increase in prevalence of nosocomial non-*Candida albicans* candidaemia and the association of *Candida krusei* with fluconazole use. J Hosp Infect. 2002;50:56–65.
7. Peman J, Cantón E, Gobernado M. Epidemiology and antifungal susceptibility of *Candida* species isolated from blood: results of a 2-year multicentre study in Spain. Eur J Clin Microbiol Infect Dis. 2005;24:23–30.
8. Almirante B, Rodriguez D, Park BJ, Cuenca-Estrella M, Planes AM, Almela M, et al. Epidemiology and predictors of mortality in cases of *Candida* bloodstream infection: results from population-based surveillance, Barcelona, Spain, from 2002 to 2003. J Clin Microbiol. 2005;43:1829–35.
9. Arendrup MC, Fuursted K, Gahrn-Hansen B, Schonheyder HC, Knudsen JD, Jensen IM, et al. Semi-national surveillance of fungaemia in Denmark 2004–2006: increasing incidence of fungaemia of isolates with reduced azole susceptibility. Clin Microbiol Infect. 2008;14:487–94.
10. Tortorano AM, Kibbler C, Pemán J, Bernhardt H, Klingspor L, Grillot R. Candidaemia in Europe: epidemiology and resistance. Int J Antimicrob Agents. 2006;27:359–66.
11. Hazen KC, Howell SA. *Candida*, *Cryptococcus*, and other yeasts of medical importance. In: Murray PR, Baron EJ, Jorgensenn JH, Landry ML, Pfaller MA, editors. Manual of Clinical Microbiology. 9th ed. Washington: ASM Press; 2007. p. 1777–8.
12. Espinel-Ingroff AV, Pfaller MA. Susceptibility test methods: yeasts and filamentous fungi. In: Murray PR, Baron EJ, Jorgensenn JH, Landry ML, Pfaller MA, editors. Manual of Clinical Microbiology. 9th ed. Washington: ASM Press; 2007. p. 1978–9.
13. Clinical and Laboratory Standards Institute. Quality Control Minimal Inhibitory Concentration (MIC) Limits for Broth Microdilution and MIC Interpretative Breakpoints. Supplement M44-S1. CLSI, Wayne, PA, USA, 2006.
14. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts: Approved Standard M27-A3. CLSI, Wayne, PA, USA, 2007.
15. Sofair AN, Marshall G, Huie-White S, Reiss E, Harrison LH, Thomson-Sanza L, et al. Epidemiology of a community-onset candidemia in Connecticut and Maryland. Clin Infect Dis. 2006;43:32–9.
16. Sandven P, Bevanager L, Digranes A, Haukland HH, Mannsaker T, Gausted P, et al. Candidemia in Norway, 1991 to 2003: results from a nationwide study. J Clin Microbiol. 2006;44:1977–81.
17. Tortorano AM, Peman J, Bernhardt H, Klingspor L, Kibbler CC, Faure O, et al. Epidemiology of candidemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. Eur J Clin Microbiol Infect Dis. 2004;23:317–22.
18. Marchetti O, Bille J, Fluckiger U, Eggimann P, Ruef C, Garbino J, et al. Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991–2000. Clin Infect Dis. 2004;38:311–20.
19. Kao AS, Brandt ME, Pruitt WR, Conn LA, Perkins BA, Stephens DS, et al. The epidemiology of candidemia in two United States cities: results of a population-based active surveillance. Clin Infect Dis. 1999;29:1164–70.
20. Sarvikivi E, Lyytikainen O, Soll DR, Pujol C, Pfaller A, Richardson M, et al. Emergence of fluconazole resistance in a *Candida parapsilosis* strain that caused infections in a neonatal intensive care unit. J Clin Microbiol. 2005;43: 2729–35.
21. San Miguel LG, Cobo J, Sánchez-Sousa A, Abraira V, Moreno S. Secular trends of candidemia in a large tertiary-care hospital from 1988 to 2000: emergence of *Candida parapsilosis*. Infect Control Hosp Epidemiol. 2005;26: 548–52.
22. Pappas PG, Rex JH, Lee J, Hamill RJ, Larsen A, Powderly W, et al. A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. Clin Infect Dis. 2003;37:634–43.

23. Pfaller MA, Diekema DJ. Role of sentinel surveillance of candidemia: trends in species distribution and antifungal susceptibility. J Clin Microbiol. 2002;40:3351–7.
24. 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.
25. Ásmundsdóttir LR, Erlendsdóttir H, Gottfredsson M. Increasing incidence of candidemia: results from a 20-year nationwide study in Iceland. J Clin Microbiol. 2002;40:3489–92.
26. 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.
27. Odds FC, Hanson MF, Davidson AD, Jacobsen MD, Wrigth P, Whyte JA, et al. One year prospective survey of *Candida* bloodstream infection in Scotland. J Med Microbiol. 2007;56:1066–75.
28. Costa-de-Oliveira S, Pina-Vaz C, Mendonça D, Gonçalves Rodrigues A. A first Portuguese epidemiological survey of fungaemia in a university hospital. Eur J Clin Microbiol Infect Dis. 2008;27:365–74.
29. Borg-von Zepelin M, Kunz L, Rüchel R, Reichard U, Weig M, Gross U. Epidemiology and antifungal susceptibilities of *Candida* spp. to six antifungal agents: results from a surveillance study on fungaemia in Germany from July 2004 to August 2005. J Antimicrob Chemother. 2007;60:424–8.
30. Lagrou K, Verhaegen J, Peetermans WE, De Rijdt T, Maertens J, Van Wijngaerden E. Fungemia at a tertiary care hospital: incidence, therapy, and distribution and antifungal susceptibility of causative species. Eur J Clin Microbiol Infect Dis. 2007;26:541–7.
31. Quindós G, Sánchez-Vargas LO, Villar-Vidal M, Eraso E, Alkorta M, Hernández-Almaraz JL. Activities of fluconazole and voriconazole against bloodstream isolates of *Candida glabrata* and *Candida krusei*: a 14-year study in a Spanish tertiary medical centre. Int J Antimicrob Agents. 2008;31:266–71.
32. Dannaoui E, Lortholary O, Raoux D, Bougnoux ME, Galeazzi G, Lawrence C, et al. Comparative in vitro activities of caspofungin and micafungin, determined using the method of the European Committee on Antimicrobial Susceptibility Testing, against yeast isolates obtained in France in 2005–2006. Antimicrob Agents Chemother. 2008;52:778–81.