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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

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***Objectives*: The antifungal drug susceptibilities of 351 isolates of *Candida* species, obtained through active laboratory-based surveillance in the period January 2002–December 2003, were determined (*Candida albicans* 51%, *Candida parapsilosis* 23%, *Candida tropicalis* 10%, *Candida glabrata* 9%, *Candida krusei* 4%).**

***Methods*: The MICs of amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and caspofungin were established by means of the broth microdilution reference procedure of the European Committee on Antibiotic Susceptibility Testing.**

***Results and conclusions*: Amphotericin B and flucytosine were active *in vitro* against all strains. A total of 24 isolates (6.8%) showed decreased susceptibility to fluconazole (MIC ≥ 16 mg/L) and 43 (12.3%) showed decreased susceptibility to itraconazole (MIC ≥ 0.25 mg/L). Voriconazole and caspofungin were active *in vitro* against the majority of isolates, even those that were resistant to fluconazole.**

Keywords: fluconazole resistance, caspofungin, voriconazole, EUCAST

Introduction

Candida species now rank as one of the most common causes of hospital-acquired bloodstream infections (BSI).[1] Although *Candida albicans* remains the most frequent cause of *Candida* BSI, longitudinal surveillance programmes in the USA and Europe have detected an increase in the prevalence of BSI caused by *Candida* species other than *Candida albicans*, such as *Candida glabrata*, *Candida krusei* and *Candida parapsilosis*.[2–6] Moreover, it appears that marked differences exist in species distributions and antifungal drug susceptibilities between different countries, underscoring the need for continued surveillance, to monitor trends in pathogen distribution and drug susceptibilities.[3,7,8]

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Table 1. *In vitro* susceptibilities of 351 *Candida* bloodstream isolates to six antifungal agents

Species	*n*/%[a]	Amphotericin B			Flucytosine			Fluconazole			Itraconazole			Voriconazole			Caspofungin		
		range	GM[b]	MIC_{90}[c]	range	GM	MIC_{90}	range	GM	MIC_{90}	range	GM	MIC_{90}	range	GM	MIC_{90}	range	GM	MIC_{90}
C. albicans	178/51	0.03–0.25	0.06	0.12	0.125–4	0.17	0.25	0.125–8	0.16	0.25	0.01–1	0.01	0.03	0.01–0.5	0.01	0.01	0.01–1	0.06	0.25
C. parapsilosis	81/23	0.03–0.25	0.19	0.25	0.125–1	0.15	0.25	0.125–>64	0.41	1	0.01–0.25	0.02	0.06	0.01–0.5	0.02	0.03	0.125–2	0.48	1
C. tropicalis	36/10	0.06–0.5	0.09	0.12	0.125–0.5	0.15	0.25	0.125–>64	0.28	0.5	0.01–8	0.03	0.06	0.01–8	0.03	0.06	0.01–2	0.12	0.25
C. glabrata	31/9	0.03–0.5	0.19	0.25	0.125–0.25	0.14	0.25	2–>64	6.3	16	0.06–2	0.40	1	0.06–2	0.25	0.5	0.06–0.5	0.15	0.5
C. krusei	14/4	0.25–0.5	0.3	0.5	2–4	2.43	4	32–>64	52	64	0.12–0.25	0.18	0.25	0.25–1	0.32	0.5	0.50–2	0.51	1
Others[d]	11/3	0.03–0.25	0.08	0.25	0.125–4	0.41	2	0.125–16	1.06	16	0.01–0.25	0.05	0.125	0.01–0.25	0.03	0.25	0.03–16	0.57	2
Total	351/100	0.03–0.5	0.08	0.25	0.125–4	0.18	0.5	0.125–>64	0.39	8	0.01–8	0.03	0.25	0.01–8	0.02	0.25	0.01–16	0.11	0.50

Susceptibility data in mg/L.
[a]Number of isolates and percentage.
[b]Geometric mean.
[c]MIC including 90% of isolates tested.
[d]Others are three *Candida kefyr*, two *Candida lusitaniae*, two *Candida guilliermondii*, two *Candida inconspicua*, one *Candida norvegensis* and one *Candida dubliniensis*.

Over the last decade, treatment of *Candida* BSI has been enhanced by the introduction of fluconazole. Because of its extensive usage in many countries, concern has arisen about the possible development of fluconazole resistance. This has been documented among *Candida* species isolated from human immunodeficiency virus-infected persons with recurrent oropharyngeal candidiasis,[9] but it appears to be uncommon among North American and European bloodstream isolates.[3,8,10–13] Of several new triazole and echinocandin agents, voriconazole and caspofungin appear to be highly active against all *Candida* species, including those that are less susceptible or resistant to fluconazole and/or itraconazole.[8,14–16] To date, however, few prospective surveillance studies of the activity of these agents against *Candida* bloodstream isolates have been reported.

During 2002–2003, we conducted prospective population-based surveillance for *Candida* BSI in Barcelona, Spain, to determine the distribution of species involved in these infections and the proportion of antifungal drug resistance among the isolates. This report describes the antifungal drug susceptibility profiles of these isolates to various antifungal agents including voriconazole and caspofungin.

Materials and methods

Isolates

A total of 351 incident bloodstream isolates of *Candida* species were obtained as part of a population-based active surveillance programme conducted in Barcelona and the greater Barcelona area in the period 1 January 2002–31 December 2003. These organisms had been recovered from 345 cases; in six cases, two different *Candida* species were identified. In 16 cases, a second isolate was recovered after antifungal therapy was started and in four cases, a third consecutive isolate was obtained. In each case, the time interval between the serial isolates was at least 5 days.

Species identification was performed at the participating laboratories and confirmed by the Mycology Reference Laboratory, National Center for Microbiology, Madrid, Spain, using standard morphological and physiological methods, including fermentation of and growth on carbon sources, growth on nitrogen sources and growth at various temperatures.[17] *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as quality control organisms for antifungal drug susceptibility testing.

Antifungal drugs

Standard powders of amphotericin B (Sigma Aldrich Quimica S.A., Madrid, Spain), flucytosine (Sigma Aldrich Quimica S.A.), fluconazole (Pfizer S.A., Madrid, Spain), itraconazole (Janssen S.A., Madrid, Spain), voriconazole (Pfizer S.A.) and caspofungin (Merck & Co., Inc., Rahway, NJ, USA) were used. The final concentrations were in the range 16–0.03 mg/L for amphotericin B and caspofungin, 64–0.12 mg/L for flucytosine and fluconazole and 8–0.015 mg/L for itraconazole and voriconazole.

Broth microdilution susceptibility testing method

The MICs of amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and caspofungin were determined by the reference procedure proposed by the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antibiotic Susceptibility Testing for testing fermentative yeasts (AFST-EUCAST, discussion document 7.1).[18] These recommendations are based on the National

Committee for Clinical Laboratory Standards (NCCLS) reference procedure described in document M27-A2,[19] but include some modifications to allow for automation of the method and to permit the incubation period to be shortened from 48 to 24 h.[20] Briefly, testing was performed with RPMI 1640 medium supplemented with 2% glucose; an inoculum size of 10^5 cfu/mL was used, as were flat-bottom microdilution plates. MIC endpoints were determined spectrophotometrically at 24 and 48 h. For amphotericin B, the MIC endpoint was defined as the lowest drug concentration that resulted in a reduction in growth of 90% or more, compared with that of a drug-free growth control well. For flucytosine and azoles, the MIC endpoint was defined as a 50% reduction in optical density. For caspofungin, the endpoint was defined according to reports recently published (50% or greater inhibition relative to the control), which analysed influences of methodological variables on susceptibility testing of caspofungin against *Candida* species.[21,22]

Analysis of results

Interpretive breakpoints proposed by the NCCLS for flucytosine, fluconazole and itraconazole were used.[19] Isolates were classified as susceptible or as showing decreased susceptibility. The latter category included the susceptible dose-dependent (SDD), intermediate and resistant categories of the NCCLS. An analysis of the SDD or intermediate and resistant isolates conducted separately showed no statistically significant differences.

The significance of the differences in MICs was determined by Student's *t*-test (unpaired, unequal variance). In order to approximate a normal distribution, the MICs were transformed to $\log_2$ values to establish susceptibility differences between species. A P value of <0.01 was considered significant. Both on-scale and off-scale results were included in the analysis. The off-scale MIC values were converted to the next concentration up. Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS, version 12.0) (SPSS S.L., Madrid, Spain).

Results

Table 1 summarizes the species distribution and *in vitro* susceptibilities of the 351 incident bloodstream isolates of *Candida* species to amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and caspofungin. The results are reported as MIC ranges, geometric mean (GM) MIC values and the MICs at which 90% of the isolates were inhibited (MIC_{90}s). *C. albicans* was the most common isolate (51%), followed by *C. parapsilosis* (23%), *C. tropicalis* (10%), *C. glabrata* (9%) and *C. krusei* (4%).

Amphotericin B MICs were in the range 0.03–0.5 mg/L, with isolates of *C. krusei* demonstrating the highest GM MIC and MIC_{90} values (0.3 and 0.5 mg/L, respectively). Flucytosine MICs were in the range 0.125–4 mg/L, with isolates of *C. krusei* again showing the highest GM and MIC_{90} values (2.43 and 4 mg/L, respectively). With regard to the three azole agents, fluconazole, itraconazole and voriconazole showed good activity against the majority of isolates, with GM MICs of 0.39, 0.03 and 0.02 mg/L, respectively. Caspofungin MICs were in the range 0.015–16 mg/L. Overall, a caspofungin MIC of ≥1 mg/L was demonstrated for 19 isolates (5.4%).

A total of 24 isolates (6.8%) showed decreased susceptibility to fluconazole (MIC ≥ 16 mg/L) (Table 2). Six isolates were classified as resistant to this compound (MIC ≥ 64 mg/L) and 18 were SDD (MIC, 16–32 mg/L). Although 25 of 31 incident *C. glabrata* isolates were classified as fluconazole-susceptible, MICs of 4–8 mg/L were demonstrated for these isolates. Overall, itraconazole MICs of ≥0.25 mg/L were demonstrated for 43 of 351 isolates (12.3%); 34 were classified as SDD (MIC 0.25–0.5 mg/L) and nine as resistant (MIC ≥ 1 mg/L). Notably, most of the isolates for which fluconazole MICs were ≥16 mg/L showed decreased susceptibility to itraconazole ($P<0.01$). Interpretative breakpoints for susceptibility testing of voriconazole have not been established, but voriconazole MICs of ≥1 mg/L were demonstrated for only three isolates (0.85%) (one each of *Candida tropicalis*, *C. glabrata* and *C. krusei*).

A total of 20 serial isolates were obtained from 16 cases of *Candida* BSI: seven cases were due to *C. albicans*, six to *C. parapsilosis*, two to *C. tropicalis* and one to *C. krusei*. Most of these patients were treated with fluconazole for several weeks, but the MICs of fluconazole for the first and successive isolates were comparable. No significant differences in susceptibility were demonstrated for any of the antifungal agents tested between the initial and subsequent isolates ($P>0.01$).

Discussion

This report provides the first population-based description of the species distribution and *in vitro* drug susceptibilities of bloodstream isolates of *Candida* species from Spain. It provides more representative information than previous reports, which were based only on selected hospitals or particular groups of hospitalized patients. Our findings indicate that the distribution of *Candida* species causing BSI in Spain is similar to that documented in recent reports from several other European countries.

Table 2. *In vitro* susceptibilities to other antifungal agents of *Candida* bloodstream isolates with decreased susceptibility to fluconazole (MIC ≥ 16 mg/L)

Species	*n*/%[a]	Amphotericin B	Flucytosine	Itraconazole	Voriconazole	Caspofungin
C. parapsilosis	1/1.2%	0.25	1	0.06	0.5	0.5
C. tropicalis	1/2.8%	0.5	0.25	8	8	0.03
C. glabrata	6/19.4%	0.06–0.125	0.12–0.25	0.5–2	0.5–2	0.06–0.25
C. krusei	14/100%	0.25–0.5	2–4	0.125–0.25	0.25–1	0.5–2
Others	2/18.2%	0.06–0.25	1	0.125–0.25	0.01–0.25	0.125
Total	24/6.8%	0.06–0.5	0.125–4	0.06–8	0.01–8	0.03–2

Table displays ranges of MICs in mg/L.
[a]Number of isolates and percentage of cases per species.

C. albicans was the predominant organism, accounting for 51% of isolates, followed by *C. parapsilosis* (23%), *C. tropicalis* (10%), *C. glabrata* (9%) and *C. krusei* (4%). Although several previous reports from Spain have indicated that almost 40% of cases of *Candida* BSI were due to *C. parapsilosis*,[23–27] the lower proportion reported here (23%) is more consistent with recently reported rates of 15%–16% from France and Italy.[28,29] It is, however, higher than the rates of 1%–10% reported for *C. parapsilosis* from England and Wales, Finland, Iceland and Switzerland.[12,30–32] These differences in rates might be attributable to differences in the representativeness of the populations studied, but variations in healthcare practices could also be an important factor. It has been suggested that the higher prevalence of *C. parapsilosis* in some institutions might be related to poor catheter care or infection control practices, but ecological and climatological factors could also be involved.[26,33]

In this study, *C. glabrata* and *C. krusei* accounted for 9% and 4%, respectively, of bloodstream isolates. *C. glabrata*, a species that easily acquires azole drug resistance, is represented in European surveillance data of the 1990s at proportions in the range 9%–16%, depending on the geographic location.[12,28–32] The low proportion of *Candida* BSI due to *C. krusei*, a species that is intrinsically resistant to fluconazole, is also consistent with reports from other European countries[12,28–31] and the USA.[10,11,34,35]

Our findings confirm the negligible proportions of fluconazole resistance among *C. albicans* bloodstream isolates that have been reported elsewhere.[8,10–13,30,36] Our susceptibility results for *Candida* species other than *C. albicans* are also consistent with those of other studies[8,10–13,30,36] in showing a low level of fluconazole resistance among *C. tropicalis* and *C. parapsilosis*, and the expected high level of resistance in *C. krusei*. A total of 43 isolates (12.3%) demonstrated decreased susceptibility to itraconazole (MIC > 0.25 mg/L), a similar proportion to that reported elsewhere.[8,23,36] Of these less-susceptible strains, 28 were identified as *C. glabrata*, eight as *C. krusei* and two as *C. tropicalis*.

In a population-based surveillance programme in the USA, conducted during 1998–2000, Hajjeh *et al.*[11] documented amphotericin B Etest MICs in the range 0.002–12 mg/L, with MICs of ≥0.38 mg/L for 10% of isolates, ≥1 mg/L for 1.7% and ≥2 mg/L for 0.4%. In this study, we documented amphotericin B MICs in the range 0.03–0.5 mg/L, with MICs of >0.25 mg/L for 10% of isolates. These results are consistent with those of reports from other European countries.[12,30] The decreased susceptibility of *C. krusei* to amphotericin B (MIC_{90}, 0.5 mg/L) is consistent with previous reports for this organism.[11,34,35]

Pfaller *et al.*[37] reported the *in vitro* activities of flucytosine against >8000 incident isolates of *Candida* spp. obtained from blood and other deep sites at more than 200 hospitals worldwide. Only 3% of *C. albicans* and 1% of *C. glabrata* were resistant to this agent *in vitro* (MIC ≥ 32 mg/L). More recently, Hajjeh *et al.*[11] reported that 4.3% of North American *C. albicans* bloodstream isolates were resistant to flucytosine, compared with <1% of *C. parapsilosis* and *C. tropicalis* isolates and no *C. glabrata* isolates. In this study, we documented flucytosine MICs in the range 0.125–4 mg/L, with MICs of >0.5 mg/L for 10% of isolates. Again, our results are consistent with those of reports from other European countries.[12,30,38]

Although no established interpretative breakpoints are available for the new triazole agents and echinocandins, both voriconazole and caspofungin were active *in vitro* against the majority of bloodstream isolates of *Candida* species tested in this study, even those strains resistant to other agents. However, as has been noted elsewhere,[14] the activity of voriconazole was reduced among isolates that showed decreased susceptibility to fluconazole and/or itraconazole. Our findings confirm those of Pfaller *et al.*[15] who tested 351 clinical isolates of *Candida* species resistant to fluconazole against caspofungin and observed that 99% were inhibited by caspofungin at an MIC of ≤2 mg/L.

In conclusion, the results of this population-based surveillance study indicate that the majority of strains causing *Candida* BSI are still susceptible to fluconazole (93%) and itraconazole (88%). Our results indicate that fluconazole is a reasonable alternative for empirical treatment of candidaemia. However, 13% of cases were due to organisms that were either intrinsically resistant to fluconazole (*C. krusei*) or possessed the ability to develop fluconazole resistance rapidly (*C. glabrata*). Prompt identification of isolates causing *Candida* BSI is important in selecting the most appropriate antifungal therapy.

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References

1. Edmond, M. B., Wallace, S. E., McClish, D. K. *et al.* (1999). Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clinical Infectious Diseases* **29**, 239–44.

2. Pfaller, M. A., Messer, S. A., Hollis, R. J. *et al.* (1999). Trends in species distribution and susceptibility to fluconazole among blood stream isolates of *Candida* species in the United States. *Diagnostic Microbiology and Infectious Diseases* **33**, 217–22.

3. Pfaller, M. A. & Diekema, D. J. (2004). Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates of *Candida*. *Clinical Microbiology and Infection* **10**, Suppl. 1, 11–23.

4. Sandven, P., Bevanger, L., Digranes, A. *et al.* (1998). Constant low rate of fungemia in Norway, 1991 to 1996. The Norwegian Yeast Study Group. *Journal of Clinical Microbiology* **36**, 3455–9.

5. Trick, W. E., Fridkin, S. K., Edwards, J. R. *et al.* (2002). Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1989-1999. *Clinical Infectious Diseases* **35**, 627–30.

6. Voss, A., Kluytmans, J. A., Koeleman, J. G. *et al.* (1996). Occurrence of yeast bloodstream infections between 1987 and 1995 in five Dutch university hospitals. *European Journal of Clinical Microbiology and Infectious Diseases* **15**, 909–12.

7. Pfaller, M. A., Jones, R. N., Doern, G. V. *et al.* (2000). Bloodstream infections due to *Candida* species: SENTRY antimicrobial surveillance program in North America and Latin America, 1997-1998. *Antimicrobial Agents and Chemotherapy* **44**, 747–51.

8. Pfaller, M. A., Diekema, D. J., Jones, R. N. *et al.* (2001). International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and *in vitro* susceptibilities to fluconazole, ravuconazole, and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. *Journal of Clinical Microbiology* **39**, 3254–9.

9. Sanglard, D. & Odds, F. C. (2002). Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. *Lancet Infectious Diseases* **2**, 73–85.

10. Diekema, D. J., Messer, S. A., Brueggemann, A. B. *et al.* (2002). Epidemiology of candidemia: 3-year results from the emerging infections and the epidemiology of Iowa organisms study. *Journal of Clinical Microbiology* **40**, 1298–302.

11. Hajjeh, R. A., Sofair, A. N., Harrison, L. H. *et al.* (2004). 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. *Journal of Clinical Microbiology* **42**, 1519–27.

12. Kibbler, C. C., Seaton, S., Barnes, R. A. *et al.* (2003). Management and outcome of bloodstream infections due to *Candida* species in England and Wales. *Clinical Microbiology and Infection* **54**, 18–24.

13. Pfaller, M. A., Diekema, D. J., Jones, R. N. *et al.* (2002). Trends in antifungal susceptibility of *Candida* spp. isolated from paediatric and adult patients with bloodstream infections: SENTRY Antimicrobial Surveillance Program, 1997 to 2000. *Journal of Clinical Microbiology* **40**, 852–6.

14. Pfaller, M. A., Messer, S. A., Hollis, R. J. *et al.* (2002). *In vitro* activities of ravuconazole and voriconazole compared with those of four approved systemic antifungal agents against 6,970 clinical isolates of *Candida* spp. *Antimicrobial Agents and Chemotherapy* **46**, 1723–7.

15. Pfaller, M. A., Messer, S. A., Boyken, L. *et al.* (2003). Caspofungin activity against clinical isolates of fluconazole-resistant *Candida*. *Journal of Clinical Microbiology* **41**, 5729–31.

16. Pfaller, M. A., Diekema, D. J., Messer, S. A. *et al.* (2003). *In vitro* activities of caspofungin compared with those of fluconazole and itraconazole against 3,959 clinical isolates of *Candida* spp., including 157 fluconazole-resistant isolates. *Antimicrobial Agents and Chemotherapy* **47**, 1068–71.

17. Kurtzman, C. P. & Fell, J. W. (1998). *The Yeasts. A taxonomic study*, 4th edn. Elsevier, Amsterdam, The Netherlands.

18. Rodriguez-Tudela, J. L., Barchiesi, F., Bille, J. *et al.* (2003). Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts. *Clinical Microbiology and Infection* **9**, I–VIII.

19. National Committee for Clinical Laboratory Standards. (2002). *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast—Second Edition: Approved Standard M27-A2*. NCCLS, Wayne, PA, USA.

20. Cuenca-Estrella, M., Lee-Yang, W., Ciblak, M. A. *et al.* (2002). Comparative evaluation of NCCLS M27-A and EUCAST broth microdilution procedures for antifungal susceptibility testing of *Candida* species. *Antimicrobial Agents and Chemotherapy* **46**, 3644–7.

21. Odds, F. C., Motyl, M., Andrade, R. *et al.* (2004). Interlaboratory comparison of results of susceptibility testing with caspofungin against *Candida* and *Aspergillus* species. *Journal of Clinical Microbiology* **42**, 3475–82.

22. Bartizal, C. & Odds, F. C. (2003). Influences of methodological variables on susceptibility testing of caspofungin against *Candida* species and *Aspergillus* fumigatus. *Antimicrobial Agents and Chemotherapy* **47**, 2100–7.

23. Cuenca-Estrella, M., Rodero, L., Garcia-Effron, G. *et al.* (2002). Antifungal susceptibilities of *Candida* spp. isolated from blood in Spain and Argentina, 1996-1999. *Journal of Antimicrobial Chemotherapy* **49**, 981–7.

24. Duran, M. T., Velasco, D., Canle, D. *et al.* (2003). Antifungal susceptibility of *Candida* spp. isolates from blood cultures in a five-year period (1997-2001). *Enfermedades Infecciosas y Microbiología Clínica* **21**, 488–92.

25. Peman, J., Canton, E., Orero, A. *et al.* (2002). Epidemiology of candidemia in Spain—Multicenter study. *Revista Iberoamericana de Micología* **19**, 30–5.

26. Rodriguez-Tudela, J. L. & Cuenca-Estrella, M. (1999). A multicenter study on fungemia caused by yeasts in Spain (April-June, 1997). A Work Group to Study Fungemia. *Revista Clínica Española* **199**, 356–61.

27. Marco, F., Danes, C., Almela, M. *et al.* (2003). Trends in frequency and *in vitro* susceptibilities to antifungal agents, including voriconazole and anidulafungin, of *Candida* bloodstream isolates. Results from a six-year study (1996-2001). *Diagnostic Microbiology and Infectious Diseases* **46**, 259–64.

28. Richet, H., Roux, P., Des, C. C. *et al.* (2002). Candidemia in French hospitals: incidence rates and characteristics. *Clinical Microbiology and Infection* **8**, 405–12.

29. Tortorano, A. M., Biraghi, E., Astolfi, A. *et al.* (2002). European Confederation of Medical Mycology (ECMM) prospective survey of candidaemia: report from one Italian region. *Clinical Microbiology and Infection* **51**, 297–304.

30. Asmundsdottir, L. R., Erlendsdottir, H. & Gottfredsson, M. (2002). Increasing incidence of candidemia: results from a 20-year nationwide study in Iceland. *Journal of Clinical Microbiology* **40**, 3489–92.

31. Marchetti, O., Bille, J., Fluckiger, U. *et al.* (2004). Epidemiology of candidemia in Swiss tertiary care hospitals: secular trends, 1991–2000. *Clinical Infectious Diseases* **38**, 311–20.

32. Poikonen, E., Lyytikainen, O., Anttila, V. J. *et al.* (2003). Candidemia in Finland, 1995-1999. *Emerging Infectious Diseases* **9**, 985–90.

33. Colombo, A. L., Nucci, M., Salomao, R. *et al.* (1999). High rate of non-albicans candidemia in Brazilian tertiary care hospitals. *Diagnostic Microbiology and Infectious Diseases* **34**, 281–6.

34. Kao, A. S., Brandt, M. E., Pruitt, W. R. *et al.* (1999). The epidemiology of candidemia in two United States cities: results of a population-based active surveillance. *Clinical Infectious Diseases* **29**, 1164–70.

35. Pfaller, M. A., Jones, R. N., Messer, S. A. *et al.* (1998). 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. *Diagnostic Microbiology and Infectious Diseases* **30**, 121–9.

36. Ostrosky-Zeichner, L., Rex, J. H., Pappas, P. G. *et al.* (2003). Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates

in the United States. *Antimicrobial Agents and Chemotherapy* **47**, 3149–54.

37. Pfaller, M. A., Messer, S. A., Boyken, L. *et al.* (2002). In vitro activities of 5-fluorocytosine against 8,803 clinical isolates of *Candida* spp.: global assessment of primary resistance using National Committee for Clinical Laboratory Standards susceptibility testing methods. *Antimicrobial Agents and Chemotherapy* **46**, 3518–21.

38. Cuenca-Estrella, M., Diaz-Guerra, T. M., Mellado, E. *et al.* (2001). Flucytosine primary resistance in *Candida* species and *Cryptococcus neoformans*. *European Journal of Clinical Microbiology and Infectious Diseases* **20**, 276–9.