Invasive fungal infections in Chile: a multicenter study of fungal prevalence and susceptibility during a 1-year period

VICTOR SILVA*, MARÍA CRISTINA DÍAZ*, NALDY FEBRÉ† & the CHILEAN INVASIVE FUNGAL INFECTIONS GROUP‡

**Microbiology and Mycology Program, Biomedical Sciences Institute, School of Medicine, University of Chile and †Mayor University, School of Nursing, Alameda, Santiago, Chile*

During the first year of an ongoing surveillance program of invasive fungal infections (IFI) a total of 130 patients (56% male) with fungal strains isolated from blood and other sterile sites were reported from 13 hospitals in Chile. Significant yeast isolates were obtained from 118 patients, and molds affected 12 patients. The main patient groups affected were neonates, children less than 1 year old and adults aged 50–79 years. All fungal bloodstream infections (BSI) were due to yeasts; 79 patients (61%) were affected. The main risk factors recorded were antibiotic therapy (76%), stay in the intensive care unit (ICU) (70%) and presence of a central venous catheter (65%). Nosocomial infections were represented in 83.5% of BSI. Overall, *Candida albicans* (40.8%), *C. parapsilosis* (13.1%), *C. tropicalis* (10%) and *Cryptococcus neoformans* (10%) were the most common species. *Aspergillus fumigatus* (3.1%) was the most frequent mold. *C. albicans* (48.1%) and *C. parapsilosis* (17.7%), were the most frequent agents recovered from blood. *Saccharomyces cerevisiae* and *Trichosporon mucoides*, two emerging pathogens, were also isolated. All yeasts tested were susceptible to amphotericin B with minimal inhibitory concentration (MIC) ≤ 1 μg/ml. Resistance to itraconazole (MIC ≥ 1 μg/ml) and fluconazole (MIC ≥ 64 μg/ml) was observed in 4 and 6% of cases, respectively. *C. glabrata* was the least susceptible species, with 50% of isolates resistant to itraconazole and 33% resistant to fluconazole, with one strain showing combined resistance. Reduction of BSI requires greater adherence to hand-washing and related infection control guidelines.

Keywords invasive fungal infections, bloodstream infections, susceptibility, Chilean surveillance program

Introduction

The incidence of invasive fungal infections (IFI), mainly candidemia, has increased significantly during the past 20 years; immunocompromised and intensive care unit (ICU) patients are the main groups involved in this increase [1,2]. *Candida* species are the most

Correspondence: Victor Silva, Microbiology and Mycology Program, ICBM, School of Medicine, University of Chile, Independencia 1027, Santiago, Chile. Tel: +56 2 678 6145; Fax: +56 2 735 5855; E-mail: vsilva@med.uchile.cl

‡ The Chilean Invasive Fungal Infections Group consists of one or more representatives from 13 clinical microbiology laboratories. The group includes E. Reyes, Asociación Chilena de Seguridad; Marcela San Martín, Carolina Allende, Angélica Aranda, Dina Concha, Sara Cisternas, Herta Barria, and Liliana Paredes, Hospital Barros Luco Trudeau; María Emilia Arce, Hospital Base de Valdivia; Ema Gaete, and Gerardo Peralta, Hospial Carlos Van Buren; Ana M. Guzmán, and Eugenia Pilar León, Centro de Diagnóstico Universidad Católica de Chile; Maggie Vecchiola, and Carmen Villalobos, Centro de Diagnóstico y Tratamiento Eloiza Díaz, Hospital San José; Mailing Chang, and Marcela Castillo, Hospital Clínico Universidad de Chile; Alejandro Joyas, Berta Osandón and Patricia Tapia, Hospital Gustavo Fricke; Ricardo Zepeda, and Pablo Herrera, Hospital Juan Noé de Arica; Teresa Siri, and Mónica Córdova; Hospital Luis Calvo Mackenna; Gloria Zenteno, and Beatriz Borquez; Hospital Militar del General Luis Felipe Brieva Arán; Mónica Lafourcade, and Angel Geraldo, Hospital San Juan de Dios; Patricia Gonzalez, and Marcela Gaete, Hospital Sótero del Río.

common opportunistic fungi causing IFI. Nosocomial fungemia rates in the United States were shown in recent surveys to have increased almost fourfold, with candidemia accounting for approximately 10–12% of nosocomial infections among ICU patients, and 8% of all hospital-acquired bloodstream infections (BSI) [3–5]. The proportion of opportunistic infections formed by nosocomial candidemia also increased in pediatric hospitals from 11% in 1989 to 23% in 1993 [6]. In Chile, *Candida albicans* ranks as the fourth most common agent of pediatric nosocomial BSI and as the most common agent of bloodstream infections related to parenteral nutrition [7]. Pneumonia caused by species of *Aspergillus* was one of several deep mold infections that occurred with increased frequency [5,8]. In the face of such an emergent problem, development of an organized surveillance program appeared essential to allow the collection and analysis of reliable information on prevalences, antifungal resistance trends, and emerging pathogens [5,9]. Several studies have documented trends in species distribution and antifungal susceptibility, and both these factors varied markedly among different countries and time periods [9–17]. In general, though, these studies show a mild increase in the proportion of candidemia due to non-*C. albicans* species, as well as the emergence of *Candida glabrata* and *C. parapsilosis* as important agents of BSI in recent years. The present report documents the findings of a Chilean multicenter surveillance program for all fungi isolated from blood and other normally sterile sites. Here we present the species prevalences of yeasts and molds isolated during the first year of surveillance, as well as the antifungal susceptibility profiles of the isolates.

Materials and methods

Study design

The Chilean surveillance program for IFI was established in March 2000. Its aim was to obtain up-to-date information on the prevalence of fungal opportunists and on antifungal susceptibility patterns of strains isolated from blood and other normally sterile sites. It involved 13 hospital laboratories (nine from Santiago and four from other Chilean provinces). Epidemiological and relevant clinical data were recorded on a standard form that included space for recording age, sex, site of infection, concomitant diseases, predisposing factors, the hospital and service area involved, occurrence of a positive clinical sample and identification of a nosocomial infection. All isolates were stored on Sabouraud glucose agar and sent to the Medical Mycology Laboratory of the Medical School, University of Chile, Santiago, for characterization and reference-level susceptibility testing. Data reported here were collected from March 2000 to March 2001 and results represent the species identification of the first etiologically significant strain obtained from a given patient.

Organism identification

Mold strains were identified by culture and microscopic morphology on potato dextrose agar and *Candida* species were characterized by germ tube analysis, morphological evaluation on cornmeal-Tween 80 agar and carbohydrate assimilation tests, supplemented, where necessary, with tests for growth at several temperatures, ascospore production and urease activity [18,19]. Yeast isolates were stored in drying gelatin disk at 4°C, according to Silva *et al.* [20]. In brief, 1 ml of inoculum at a density equal to the No. 3 McFarland standard was transferred to 1 ml 20% dissolved gelatin (Difco Laboratories, Detroit, MI) at pH 7.2. Immediately, drops (~ 50 µl) of the inoculum-impregnated gelatin were spotted onto a base of solid paraffin in a Petri dish and were incubated in the presence of silica gel at room temperature for 48 h. The dehydrated gelatin discs so produced were stored in tubes containing silica gel and cotton at 4°C.

Susceptibility testing

Antifungal susceptibility testing of *Candida* species and *Cryptococcus neoformans* strains was performed by a broth microdilution method following NCCLS recommendations [21], using RPMI 1640 (Sigma Aldrich, Milwaukee, WI) broth supplemented with 2% glucose. Visually read minimal inhibitory concentrations (MIC) were confirmed spectrophotometrically at 540 nm after 48 h incubation at 35°C, using broth inocula without antifungal agents as controls. For amphotericin B, the MIC was defined as the lowest drug concentration that gave an optical density (OD)-based reduction in turbidity of $\geq 90\%$ compared to a control. For azoles, the MIC was defined as a reduction of $\geq 50\%$ [22,23]. Amphotericin B was obtained from Bristol-Myers Squibb (Wallingford, CN) fluconazole from Pfizer (New York, NY) and itraconazole from Janssen Pharma (Beerse, Belgium). Quality control was performed by testing *C. parapsilosis* ATCC 22019 (American Type Culture Collection, Manassas, VA) and *Candida krusei* ATCC 6258 [24]. Interpretative susceptibility criteria for fluconazole and itraconazole were published by NCCLS [21] and Rex *et al.* [25].

Statistical analyses

The Epi Info 6.0 (Centers for Disease Control and Prevention and World Health Organization) statistical program was used. Relationships between proportions were analyzed by chi-squared tests and a P-value of less than 0.05 was used to determine statistical significance.

Results

During the 12-month study period, 130 patients (56% male and 44% female) with IFI were reported by the 13 participating hospitals (Table 1). Fungi were recovered from blood (79 patients), bronchoalveolar lavage (18 patients), cerebrospinal fluid (11 patients), brain, kidney, liver or eye tissues (eight patients), abdominal fluid (six patients), and other sterile sites (eight patients). Questionnaires were completed for 114 patients with ages ranging from 10 days to 91 years. Of these 114 patients, 41 were children, of which 13 were newborns and 18 were non-neonates less than 1 year old. The mean and median ages of the 73 adult patients were 56 and 62 years, respectively, and the most commonly affected age class was patients between 50 and 79 years old. HIV (19%), lung disease (18%), surgery (15%) and prematurity (11%) were the most common underlying diseases. Of 79 patients with BSI, 51% were male (data not shown) and the most frequent predisposing factors were antibacterial therapy (76%), stay in the ICU (70%), central venous catheters (65%), mechanical ventilation (42%), urinary catheters (35%) and parenteral nutrition (24%). A total of 72 (63%) of these 114 infections were considered nosocomial, and 66 (85.5%) of 79 BSI were nosocomial (data not shown).

The distribution of species in 130 strains analyzed is shown in Table 2. Overall, 118 cases were due to yeasts and 12 were due to mold. The main species involved were *C. albicans* 53 (40.8%), followed by *C. parapsilosis* 17 (13.1%), *C. tropicalis* 13 (10%) and *C. neoformans* 13 (10%). Among 12 strains of filamentous fungi, four were identified as *Aspergillus fumigatus*, two as *A. flavus*, two as *Fusarium solani* and the remaining four as *A. niger*, *Fusarium semitectum* (current name: *Fusarium incarnatum*), *Exophiala jeanselmei* and *Rhizopus oryzae*. The *Fusarium* strains were isolated from eye tissues and all other molds were isolated from broncheoalveolar lavage, except one *A. fumigatus* strain that was recovered from a brain biopsy.

The species most frequently isolated from blood were *C. albicans* with 38 isolates (48.1%), *C. parapsilosis* with 14 (17.7%), and *C. tropicalis* with 11 (13.9%). Apart from *Candida* species, other yeasts isolated from blood were two isolates of *C. neoformans,* two of

Table 1 Clinical data for patients with invasive mycosis in 13 Chilean centres surveyed from the year 2000–2001

Characteristic	No. (%) patients
Sex ($n = 130$)	
Male	73 (56)
Female	57 (44)
Sites of isolation ($n = 130$)	
Blood	79 (61)
Bronchoalveolar lavage fluid	18 (14)
Cerebrospinal fluid	11 (8)
Tissue (brain, kidney, liver, eye)	8 (6)
Abdominal fluid	6 (5)
Other sterile sites	8 (6)
Ages of pediatric patients ($n = 41/114$) (years)	
Newborn	13 (32)
< 1	18 (44)
2–5	3 (7)
6–10	2 (5)
11–15	5 (12)
Ages of adult patients ($n = 73/114$) (years)	
20–29	6 (8)
30–39	8 (11)
40–49	8 (11)
50–59	13 (18)
60–69	16 (22)
70–79	13 (18)
80–89	8 (11)
> 90	1 (1)
Underlying condition ($n = 114$)	
HIV	22 (19)
Lung disease	20 (18)
Surgery	17 (15)
Prematurity	13 (11)
Malignant disease	10 (9)
Splenic disease	9 (8)
Diabetes mellitus	7 (6)
Renal insufficiency	7 (6)
Leukemia	6 (5)
Organ transplant	3 (3)
Predisposing factors for BSI ($n = 79$)	
Antibacterial therapy	60 (76)
Stay in ICU	55 (70)
Central venous catheter	51 (65)
Mechanical ventilation	33 (42)
Urinary catheter	28 (35)
Parenteral nutrition	19 (24)
Chemotherapy	16 (20)
Neutropenia	10 (13)
Dialysis	7 (9)
Nosocomial infections ($n = 114$)	
Yes	72 (63)
No	32 (28)
Not determined	10 (9)

BSI, bloodstream infections; ICU, intensive care unit.

Table 2 Species distribution of 130 fungal isolates causing invasive mycosis in Chilean centres

Species	Blood isolates ($n = 79$) No. (%)	All isolates ($n = 130$) No. (%)	Number of cases positive for fungal filaments in direct microscopy (molds only)
Candida albicans	38 (48.1)	53 (40.8)	
Candida parapsilosis	14 (17.7)	17 (13.1)	
Candida tropicalis	11 (13.9)	13 (10.0)	
Cryptococcus neoformans	2 (2.5)	13 (10.0)*	
Candida glabrata	3 (3.8)	8 (6.1)	
Candida famata	5 (6.3)	5 (3.8)	
Aspergillus fumigatus		4 (3.1)	3‡
Aspergillus flavus		2 (1.5)	1‡
Saccharomyces cerevisiae	2 (2.5)	2 (1.5)	
Fusarium solani		2 (1.5)	2
Candida kefyr	1 (1.3)	1 (0.8)	
Candida krusei	1 (1.3)	1 (0.8)	
Trichosporon mucoides	1 (1.3)	1 (0.8)	
Aspergillus niger		1 (0.8)	1
Fusarium incarnatum		1 (0.8)	1
Exophiala jeanselmei		1 (0.8)	1
Rhizopus oryzae		1 (0.8)	1
Candida guilliermondii		1 (0.8)	
Candida lusitaniae		1 (0.8)	
Other yeasts†	1 (1.3)	2 (1.5)	

*Eleven were isolated from CSF. †One isolate was identified only as *Candida* sp. and another as *Cryptococcus* sp. ‡Accepted cases lacking positive direct microscopy results were from BAL; in these cases, direct microscopy was not done but the strains recorded were the only molds recovered in four tubes from each sample. Other clinical factors were also consistent with infection.

Saccharomyces cerevisiae and one of *Trichosporon mucoides*.

In-vitro susceptibility results for 125 isolates (the second strain from some patients were included) tested against amphotericin B, fluconazole and itraconazole are shown in Table 3. All isolates were susceptible to amphotericin B with $MIC_{90} \leq 1$ μg/ml. Azoles were highly active: 89% of isolates were susceptible to fluconazole and only 6% resistant, while 84% were susceptible to itraconazole and only 4% resistant. For fluconazole the MIC range was 0.25 to 128 μg/ml and the MIC_{90} was 16 μg/ml. The itraconazole MIC range was 0.03 to 16 μg/ml and the MIC_{90} was 0.5 μg/ml. The pattern of susceptibility shown in relation to the two azoles varied among the species tested. *C. albicans, C. parapsilosis* and *C. neoformans* showed an MIC_{90} of ≤ 8 μg/ml to fluconazole, and 97, 96 and 91% of isolates, respectively, were susceptible. No resistance was found. *C. tropicalis*, by contrast had an MIC_{90} of 128 μg/ml to fluconazole; 87% of isolates were susceptible and 7% resistant. *C. glabrata* was the least susceptible species to fluconazole with a MIC_{90} of 128 μg/ml and with 50% susceptible isolates and 33% resistant. The sole *C. krusei* isolate was resistant to fluconazole. No *C. albicans*, *C. parapsilosis, C. tropicalis* or *C. neoformans* isolates were resistant to itraconazole, and 98, 96, 80 and 73%, respectively, were susceptible. *C. famata* had an itraconazole MIC_{90} of 1 μg/ml with 33% of isolates resistant. *C. glabrata* exposed to itraconazole showed an MIC_{90} of 16 μg/ml with 50% of isolates resistant. One isolate of *C. glabrata* was resistant to both azoles.

Discussion

The majority of serious fungal infections affecting our 130 patients were BSI (61%), a situation similar to that reported by other studies on IFI [4,5,16]. The finding that the most common underlying diseases were HIV infection, lung disease and surgery is in agreement with the results of Rees *et al.* in the San Francisco area [5]. The predisposing factors summarized above are comparable to those observed by others [4,6,7,12,13,16,17]. Among these, antibacterial therapy, ICU stay, and presence of a central venous catheter were the factors most commonly signaled in our patients. The relatively small number of patients with filamentous fungal infection and the predominance among these of *A. fumigatus* infections is also in agreement with other findings [5,8].

Our finding that *C. albicans* made up 48.1% of BSI is in accordance with several reports that document a prevalence near 50% for this species in BSI

Table 3 *In-vitro* susceptibility patterns to amphotericin B, fluconazole and itraconazole for yeasts from invasive infections

Species (*n*)	Antifungal agents MIC (μg/ml)			%S–%R
	Range	50%	90%	
Candida albicans (59)				
Amphotericin B	0.03–1	0.06	1	100–0
Fluconazole	0.25–16	0.25	1	97–0
Itraconazole	0.03–0.5	0.03	0.06	98–0
C. famata (6)				
Amphotericin B	0.03–0.13	0.03	0.13	100–0
Fluconazole	8–16	8	16	50–0
Itraconazole	0.5–1	0.5	1	0–33
C. glabrata (6)				
Amphotericin B	0.06–1	0.5	1	100–0
Fluconazole	0.25–128	8	128	50–33
Itraconazole	0.03–16	0.5	16	33–50
C. parapsilosis (23)				
Amphotericin B	0.03–1	0.13	0.25	100–0
Fluconazole	0.25–128	1	4	96–4
Itraconazole	0.03–0.25	0.03	0.13	96–0
C. tropicalis (15)				
Amphotericin B	0.03–1	0.06	1	100–0
Fluconazole	0.25–128	0.5	128	87–7
Itraconazole	0.03–0.5	0.06	0.5	80–0
Cryptococcus neoformans (11)				
Amphotericin B	0.13–0.5	0.25	0.5	100–0
Fluconazole	0.5–16	2	8	91–0
Itraconazole	0.03–0.5	0.13	0.25	73–0
All yeasts* (125)				
Amphotericin B	0.03–1	0.13	1	100–0
Fluconazole	0.25–128	0.5	16	89–6
Itraconazole	0.03–16	0.03	0.5	84–4

S, susceptible; R, resistant. *Some second strains isolated from the same patients were included; data were thus incorporated for two *C. lusitaniae*, one *C. guilliermondii*, and one *C. kefyr*, as well as one *C. krusei* that was resistant to fluconazole with an MIC of ≥ 64 μg/ml.

[6,9,14,16,22,26], but differs from other reports showing frequencies over 60% [13,15] and less than 35% [10]. That the most common non-*C. albicans* species from blood was *C. parapsilosis* also agrees with other studies [6,9,11,13]. In Brazil and Argentina, however, *C. parapsilosis* was reported to occur with a frequency equal to that of *C. tropicalis* [10,14]. Between 1997 and 1998 an increased incidence of *C. parapsilosis* was reported in the US, concomitant with a decline in Canada and Latin America [26]. The authors did not provide an explanation for this. Candidemia caused by *C. parapsilosis* may be influenced by several factors: this is a pathogen associated with intravascular devices, and it is therefore a common cause of nosocomial infection clusters related to poor catheter care, hyperalimentation, contaminated solutions and biomedical devices, and poor adherence to infection control practices, especially hand-washing [11,12,17,26]. In a recent study, we documented *C. parapsilosis* colonization of the hands of medical students and showed that the prevalence, diversity and quantity of yeasts isolated increased according to the number of years these students had studied [27]. The high frequency of *C. parapsilosis* on hands suggests that exogenous transmission is important. To reduce the BSI, it appears necessary to remind practitioners about basic medical hygiene and to improve infection control practices.

Our study showed that candidemia caused by *C. glabrata* and *C. krusei* was uncommon, as has previously been observed in Spain [13]. These results differ from those reported from Norway, the US and Canada, where *C. glabrata* was found to be the most common non-*C. albicans* species causing candidemia [15,16,26]. This difference in incidence can be explained in part by the widespread use of azoles in the US, a practice that may exert selective pressure towards an increase in incidence of the less susceptible species, as has been documented for *C. glabrata* [16]. More

epidemiological and clinical research is required in order to identify local risk factors for candidemia. Such information is required for the design of specific preventive measures.

We identified *S. cerevisiae* and *T. mucoides* from blood. Both species have been reported as emerging agents of fungemia [28–30]. All *C. neoformans* strains isolated were from HIV patients. These strains mainly caused meningitis, though two cases of cryptococcal sepsis were also seen. This population clearly remains the group most susceptible to *C. neoformans* infection [5].

The susceptibility of all isolates to amphotericin B is similar results documented in many other areas [9,15,22,26], but differs from what was observed in Québec, Canada, by St-Germain *et al.* [16]. For all isolates tested against fluconazole and itraconazole, the MIC_{90} was susceptible-dose-dependent; however, our collection of yeast BSI strains was highly susceptible to both drugs. These results again are similar to those obtained elsewhere [9,10,14,16,26]. For the first time in Chile, one *C. glabrata* strain was identified that demonstrated co-resistance to both antifungals.

Six strains of *C. famata* were isolated from five patients over a short time in just one of the centers, suggesting a possible outbreak. These strains had relatively low levels of susceptibility to azoles, especially to itraconazole.

Other than *C. krusei* and *C. glabrata*, isolates showing *in-vitro* resistance to fluconazole made up low proportions, 7 and 4% respectively, of the *C. tropicalis* and *C. parapsilosis* isolates tested. Some previous authors also found no resistance in these species [26], but others obtained *C. parapsilosis* or *C. tropicalis* isolates with MICs ≥ 16 μg/ml to fluconazole [15,16]. A recent Sentry survey from 1997 to 1999 documented a trend in decreased fluconazole susceptibility, where resistant strains of *C. tropicalis* increased from 0 to 3%. However, *C. glabrata* isolates susceptible to fluconazole increased from 48 to 84% [9].

At present, it appears that resistance to antifungal drugs is rare in Chile and restricted mainly to *C. glabrata*. This preliminary information on prevalence and susceptibility of fungal strains isolated from IFI in Chile raises concerns regarding sources of infections that can be only answered by continued surveillance.

Acknowledgements

We acknowledge the advice of Eduardo Piontelli, Luis Thompson and Luis Zaror, and the excellent technical assistance of R. Contreras and our graduate students C. Abarca, M. Cabrera and D. Alvarado. We thank Dr M. O'Ryan for his critical review of the manuscript.

References

1 Banerjee SN, Emori TG, Culver DH, *et al*. Secular trends in nosocomial primary bloodstream infections in the United States, 1980–1989. *Am J Med* 1980; **91**(Suppl. 3B): 86–89.
2 Edmond MB, Wallace SE, McClish DK, *et al*. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clin Infect Dis* 1999; **29**: 239–244.
3 Beck-Sagué CM, Jarvis WR and the National Nosocomial Infections Surveillance System. Secular trends in the epidemiology of nosocomial infections in the United States, 1980–1990. *J Infect Dis* 1993; **167**: 1247–1251.
4 Jarvis WR. Epidemiology of nosocomial fungal infections, with emphasis on *Candida* species. *Clin Infect Dis* 1995; **20**: 1526–1530.
5 Rees JR, Pinner RW, Hajjeh RA, Brandt ME, Reingold AL. The epidemiological features of invasive mycotic infections in the San Francisco Bay area, 1992–1993: results of a population based laboratory active surveillance. *Clin Infect Dis* 1998; **27**: 1138–1147.
6 MacDonald L, Baker C, Chenoeth C. Risk factors for candidemia in a children's hospital. *Clin Infect Dis* 1998; **26**: 642–645.
7 Otaíza F, Brenner P. *Informe De Vigilancia Epidemiológica De Las Infecciones Intrahospitalarias, Chile – 1997–1998*. Santiago: Gobierno de Chile, Ministerio de Salud, 1999.
8 Pound MW, Drew RH, Perfect JR. Recent advances in the epidemiology prevention, diagnosis and treatment of fungal pneumonia. *Curr Opin Infect Dis* 2002; **15**: 183–194.
9 Pfaller AM, Diekema DJ, Jones RN, *et al*. 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. *J Clin Microbiol* 1997; **39**: 3254–3259.
10 Colombo AL. Epidemiology and treatment of hematogenous candidiasis: a Brazilian perspective. *Braz J Infect Dis* 2000; **4**: 113–118.
11 Girmenia C, Martino P, De Bernardis F, *et al*. Rising incidence of *Candida parapsilosis* fungemia in patients with hematologic malignancies: clinical aspects, predisposing factors, and differential pathogenicity of the causative strains. *Clin Infect Dis* 1996; **23**: 506–514.
12 Levy L, Rubin LG, Vasishtha S, Tucci V, Sood SK. Emergence of *Candida parapsilosis* as the predominant species causing candidemia in children. *Clin Infect Dis* 1998; **26**: 1086–1088.
13 Nolla-Salas J, Sitges-Serra JA, Leon-Gil C, *et al*. Candidemia in non-neutropenic critically ill patients: analysis of prognostic factors and assessment of systemic antifungal therapy. Study group of fungal infections in ICU. *Intensive Care Med* 1997; **23**: 23–30.
14 Rodero L, Davel G, Córdoba S, Soria M, Cantero C, Hochenfeller F. Multicenter study on nosocomial candidiasis in the Republic of Argentina. *Rev Argent Microbiol* 1999; **31**: 114–119.
15 Sandven P, Bevanger L, Digranes A, *et al*. Constant low rate of fungemia in Norway, 1991 to 1996. *J Clin Microbiol* 1998; **36**: 3455–3459.
16 St-Germain G, Lavendière M, Pelletier R, *et al*. Prevalence and antifungal susceptibility of 442 *Candida* isolates from blood and normally sterile sites: results of a 2-year (1996 to 1998) multicenter

surveillance study in Quebec, Canada. *J Clin Microbiol* 2001; **39**: 949–953.
17 Welbel SF, McNeil MM, Kuykendall RJ, *et al*. *Candida parapsilosis* bloodstream infections in neonatal intensive care unit patients; epidemiological laboratory confirmation of a common source outbreak. *Pediatr Infect Dis J* 1996; **15**: 998–1002.
18 Hoog GS, Guarro J, eds. *Atlas of Clinical Fungi*. Baar and Delft – Reus: Centraalbureau voor Schimmelcultures–Universitat Rovira i Virgili, 1996.
19 Yarrow D. Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP, Fell JW (eds). *The Yeasts: A Taxonomic Study*, 6th edn. Amsterdam: Elsevier, 1998: 75–107.
20 Silva V, Pires MF, Fischman O. In vitro maintenance of dermatophytes in gelatin disc. *Program and Abstracts of the 13th Congress of the International Society for Human and Animal Mycology (ISHAM)*. Salsomaggiore-Terme, Parma, Italy. 1997; Abstr. P399, University of Parma, Italy.
21 National Committee for Clinical Laboratory Standards. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. Approved Standard M 27-A*. Wayne, PA: National Committee for Clinical Laboratory Standards, 1997.
22 Alvarado D, Díaz MC, Silva V. Identification and antifungal susceptibility of *Candida* spp. isolated from invasive mycosis. Influence of growth inhibition percentage to determine minimal inhibitory concentration. *Rev Med Chile* 2002; **130**: 416–423.
23 Pfaller AM, Messer SA, Coffmann S. Comparison of visual and spectrophotometric methods of MIC endpoint determinations by using broth microdilution methods to test five antifungal agents, including the new triazole D 0870. *J Clin Microbiol* 1995; **33**: 1094–1097.
24 Rex JH, Pfaller MA, Lancaster M, *et al*. Quality control guidelines for national committee for clinical laboratory standards-recommended broth macrodilution testing of ketoconazole and itraconazole. *J Clin Microbiol* 1996; **34**: 816–817.
25 Rex JH, Pfaller MA, Galgiani JN, *et al*. Development of interpretative 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* 1997; **24**: 235–247.
26 Pfaller AM, Jones RN, Doern GV, *et al*. Bloodstream infections due to *Candida* species: SENTRY antimicrobial surveillance program in North America and Latin America, 1997–1998. *Antimicrob Agents Chemother* 2000; **44**: 747–751.
27 Silva V, Zepeda G, Rybac ME, Febré N. Yeast carriage on the hands of Medicine's students. *Rev Iberoam Micol* 2003; **20**: 41–45.
28 Parapoch J, Planes AM, Querol A, *et al*. Fungemia with *Saccharomyces cerevisiae* in two newborns, only one of whom had been treated with ultra-levura. *Eur J Clin Microbiol Infect Dis* 2000; **19**: 468–470.
29 Ruiz-Esquide F, Díaz MC, Wu E, Silva V. *Saccharomyces cerevisiae* endocarditis in a preterm infant. Report of one case. *Rev Med Chile* 2002; **130**: 1165–1169.
30 Wolf DG, Folk R, Hacham M, *et al*. Multidrug resistance in *Trichosporon asahii* from nongranulocytopenic patients in three intensive care units. *J Clin Microbiol* 2000; **139**: 4420–4425.