Medical Mycology March 2010, **48**, 346–354

informa
healthcare

Epidemiology of candidemia in oncology patients: a 6-year survey in a Portuguese central hospital

R. SABINO*§, C. VERÍSSIMO*, J. BRANDÃO*, C. ALVES*, H. PARADA*, L. ROSADO*, E. PAIXÃO†, Z. VIDEIRA‡, T. TENDEIRO‡, P. SAMPAIO§ & C. PAIS§

**Laboratório de Micologia, Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, †ONSA – Observatório Nacional de Saúde do Instituto Nacional de Saúde Dr. Ricardo Jorge, Lisboa, ‡Laboratório de Bacteriologia, Instituto Português de Oncologia Prof. Francisco Gentil, Lisboa, and §CBMA – Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Braga, Portugal*

This study presents data on the incidence of candidemia in a Portuguese oncology hospital during a 6-year period. The species distribution and their antifungal susceptibility, as well as the clinical outcomes associated with candidemia were evaluated. A total of 119 episodes were reported, with the majority occurring among patients older than 56 years. The most common underlying medical conditions were solid tumors (64.5%) and hematological disease (28.2%). The most frequent species found was *Candida albicans* (48.7%), followed by *C. parapsilosis* (20.2%), *C. tropicalis* (8.4%), *C. krusei* (6.7%) and *C. glabrata* (5.0%), but *Saccharomyces cerevisiae* and *Rhodotorula mucilaginosa* were also isolated. *Candida albicans* was more frequently associated with solid tumors of the gastrointestinal and genitourinary tracts and breast ($P = 0.005$), while non-*C. albicans Candida* species were most frequently recovered from hematological patients ($P = 0.007$). The mortality rate associated with candidemia was 31.9% ($P = 0.016$). All *C. albicans* and *C. parapsilosis* isolates were susceptible to fluconazole, voriconazole and itraconazole. Resistance to caspofungin was only observed in *C. albicans* and in the *R. mucilaginosa* isolates. Posaconazole was active against all *C. parapsilosis* isolates tested but resistant strains were found among *C. albicans* (4.9%), *C. tropicalis* (12.5%), *C. krusei* (25%) and *C. glabrata* (50%). This study provides useful information regarding the local epidemiology of candidemia in cancer patients.

Keywords Candidemia, blood cultures, cancer patients

Introduction

Candidemia is one of the most frequent fungal infections in hospitalized patients, often associated with a prolonged hospital stay and significant mortality rate. In fact, over the past two decades the incidence of *Candida* bloodstream infections among immunocompromised patients has increased 15 to 20-fold [1] and is presently the third most common nosocomial infection in the United States [1–3]. Despite the widespread use of antifungals for prophylaxis and treatment of invasive fungal infections, candidemia has been associated with the highest crude mortality rate of all bloodstream infections and even in non–neutropenic patients, the crude mortality of candidemia may exceed 50% [4]. Cancer chemotherapy, neutropenia, organ transplantation, indwelling catheters and devices, autoimmune diseases, burns, antimicrobial therapy, abdominal surgery, radiotherapy, and intensive care are among the main risk factors predisposing for *Candida* infections [5–7]. *Candida albicans* is the most frequently isolated species from deeper tissue, blood and organs. According to recent literature, although *C. albicans* remains the most common fungal isolate from blood, several studies have detected a trend toward an increased prevalence of other *Candida* species [8–10]. Compared to 20 years ago, a larger proportion of *Candida* bloodstream infections is presently caused by

Received 10 February 2009; Received in final revised form 8 June 2009; Accepted 4 July 2009

Correspondence: Célia Pais, CBMA-Centre of Molecular and Environmental Biology, Department of Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. Tel: +351 253604312; Fax: +351 253678980. E-mail: cpais@bio.uminho.pt

DOI: 10.3109/13693780903161216

C. glabrata in the United States [7] and by *C. parapsilosis* and *C. tropicalis* in European, Canadian and Latin American hospitals [8,11–13] An increasing incidence of non-*C.albicans* candidemia has been reported mainly in cancer patients with hematologic malignancies [14–16]. In Portugal, the candidemia rate found in acute leukemia patients (6.3%) was similar to rates published in the literature and *C. parapsilosis* was present in one third of the cases studied [17]. However, data related to the epidemiology of candidemia in oncological patients in our country are scarce. The present study was performed in an oncology hospital in Lisbon, during a 6 year period, and its main objective was to evaluate the local epidemiology of candidemia in oncological patients, namely the incidence of *Candida* species and distribution, their *in vitro* antifungal susceptibility, and the clinical outcomes associated with this infection.

Materials and methods

Study population and definitions

The survey was performed from 2002 to 2007 in a 280-bed central oncology hospital in Lisbon, Portugal during the course of a surveillance program. This hospital has an average occupation rate of 80% and during the study period, a mean number of 9828 patients/year were hospitalized. The average hospitalization period was 80,214 days/year or 8.1 days/patient. A mean of 164,826 patients/year were treated in ambulatory care. This hospital provides care to patients from the central and southern areas of the country, as well as patients from the Azores and Madeira Islands. The incidence of candidemia was calculated per 1000 hospital admissions and per 1000 patient-days, the last calculated as the difference between the date of admission and the date of collection of a positive blood culture [5]. An episode of candidemia was defined as the first isolation of any *Candida* species from the blood and did not include positive cultures obtained only from catheters, with no associated blood culture. Candidemias that occurred >30 days after the initial case were counted as new cases. Cases occurring either prior to or within 2 days of hospital admission were considered outpatient acquired [5,18]. Demographic and clinical data were recorded and included ward, age, gender, day of yeast isolation, underlying disease, clinical outcome and the presence of a central venous catheter.

Yeast culture and identification

Yeasts were isolated in blood cultures incubated on BACTEC 9240 System (Becton Dickinson, Sparks, MD), or from catheters by seeding it in Sabouraud agar with chloramphenicol (Difco, Detroit, MI) and blood agar (bioMérieux SA, Marcy-l'Etoile, France). Yeasts were identified by using ID 32 C strips or VITEK YBC identification cards (bioMérieux SA) and typed by molecular fingerprinting with primer T3B [19]. When conclusive identification could not be obtained with these methods, isolates were identified by sequencing the D1/D2 region of 26S rDNA gene as described by Sampaio *et al*. [20]. Isolates were stored at −70°C in 10% (v/v) sterile glycerol.

Antifungal testing

Susceptibility of yeast isolates to caspofungin, posaconazole, itraconazole, voriconazole, fluconazole and amphotericin B was determined. Minimal inhibitory concentrations (MICs) were calculated by using the E-test diffusion method according to the manufacturer's instructions (AB BioDisk, Solna, Sweden). The MIC interpretative breakpoints (μg/ml) used for itraconazole, voriconazole and fluconazole were those suggested by CLSI [21]. Interpretative breakpoints for amphotericin B and posaconazole have not been established yet and *Candida* strains with MICs <2 μg/ml for amphotericin B and <1μg/ml for posaconazole were considered as susceptible [22,23]. Regarding caspofungin, strains with MICs >2 μg/ml were considered to have reduced susceptibility [24,25]. MIC_{50} and MIC_{90} were defined as the concentrations of each antifungal agent necessary to inhibit 50% and 90% of the isolates.

Statistical analysis

The Pearson chi-square test or the Fisher Exact Test for 22 tables were used to compare proportions and analyze differences in species distribution. Two-sided *P*-values from tests were used to summarize the comparability and a 5% significance level was set. The SPSS v15.0 program for Windows was used to perform the statistical analysis.

Results

Clinical and demographic data

During the period of study, 421 cancer patients showing clinical signs and symptoms of infection were screened for the presence of yeast in different body fluids and secretions, as needed for diagnostic purposes. From these, 110 (26.1%) patients had positive yeast blood cultures and were diagnosed as having candidemia. A total of 119 episodes of candidemia were reported during the six years. In the first two years of the study (2002 and 2003), the number of candidemias was high (27 and 26 cases, respectively), decreasing markedly to 2005 and keeping approximately the same values until 2007 (Table 1).

Table 1 Yeast species and number of isolates collected during the period 2002–2007

Yeast species (Number of isolates)	Years					
	2002	2003	2004	2005	2006	2007
C. albicans (*n* = 58)	10	6	14	8	9	11
C. famata (*n* = 2)	1	1	0	0	0	0
C. glabrata (*n* = 6)	1	2	0	1	2	0
C. guilliermondii (*n* = 2)	0	2	0	0	0	0
C. krusei (*n* = 8)	1	3	2	1	0	1
C. lusitaniae (*n* = 1)	1	0	0	0	0	0
C. parapsilosis (*n* = 23)	8	6	1	1	4	3
C. tropicalis (*n* = 10)	3	2	1	1	2	1
Candida sp. (*n* = 4)	2	1	0	1	0	0
Rhodotorula mucilaginosa (n = 2)	0	1	0	0	1	0
Saccharomyces cerevisiae (n = 3)	0	2	1	0	0	0
Total	27	26	19	13	18	16

Of the 110 patients, 90 (81.8%) were adults with an average age of 56 years and 20 (18.2%) were children s 15 years, with an average age of 8 years. Sixty-two (56.4%) patients were females, ranging in age from 1 to 90 with a median age of 53 years and 48 (43.6%) were males, ranging in age from 2 to 93 years with also a median age of 53 years. The majority of candidemia cases (*n* = 74; 62.2%) occurred among patients aged 45 years and older. Thirty four (28.6%) cases occurred in patients older than 65 (*n* = 31; 28.4%). Fifty two isolates were recovered from males and 67 from women (Fig. 1).

Twenty-six percent of the candidemia cases were diagnosed in non-hospitalized individuals or within 48 h of the hospitalization of the patients, indicating outpatient-acquired candidemia. However, candidemias in cancer patients were mainly nosocomial infections (74%). The median time between admission and date of a positive blood culture for yeasts among the 88 hospitalized patients was 13 days (range 3–133 days). The median duration of hospitalization in the period of occurrence of candidemia was 41 days (range 3–351 days), with an average of 42 days. According to these values, the incidence of candidemia and nosocomial candidemia were respectively, 2.02 and 1.49 per 1,000 hospital admissions.

Forty-two percent (*n* = 50) of the patients had a previous hospitalization within a two month period preceding candidemia. In five cases (4.5%), previous colonization with *Candida* spp. was detected in other body sites of the patients. Sixty seven (56.3%) of the 119 candidemia cases occurred in patients with a central venous catheter.

Medical conditions of the patients

The most common underlying medical conditions of the patients with candidemia were solid organ tumors noted in 64.5% (*n* = 71) of the cases, hematological/immunological disorders in 28.2% (*n* = 31) and unspecified disease in 7.3% (*n* = 8) of the cases.

Solid tumors were mainly located in the gastrointestinal tract (*n* = 26; 36.6%), breast and genitourinary tract (*n* = 20; 28.2%), head/neck and central nervous system (*n* = 11; 15.5%) and other different locations (*n* = 14; 19.7%). The incidence of candidemia in this group of patients was 1.3 per 1,000 hospital admissions. The median time between admission and date of a positive yeast blood culture was 13 days, and the median duration of the hospital stay was 33 days. All these patients belonged to a risk group for candidemia, i.e., in 42% related had a previous hospital stay and in 51.9% of the cases there was the presence of central venous catheters, but no positive associations were found. In 31 (40.3%) cases, patients died in a period shorter than 30 days after the first positive blood culture (*P* = 0.016).

Among the patients with hematological malignancies, the majority had leukemia (*n* = 21; 67.7%) and 32.3%

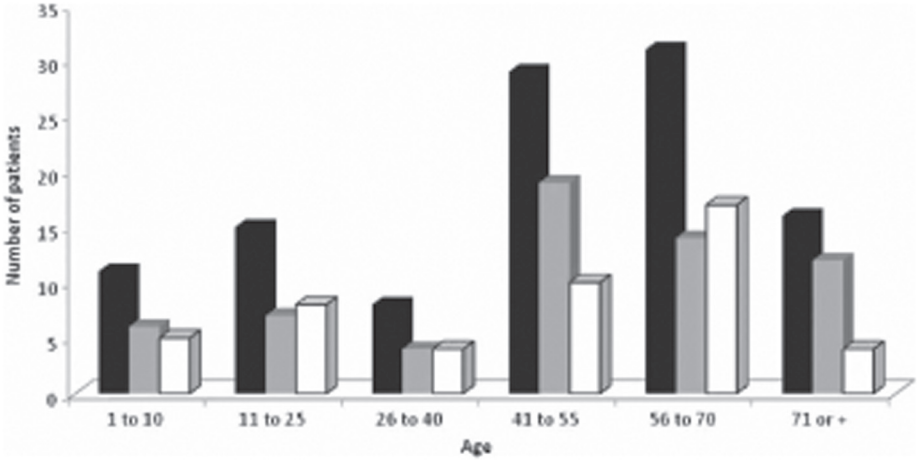

Fig. 1 Age and gender of the patients with positive blood cultures (■ Number of patients in each age group; ▩ Females; □ Males).

(n = 10) suffered from lymphoma. The incidence of candidemia in this group of patients was 0.56 per 1,000 hospital admissions. The median time between hospital admission and date of positive blood culture was 17 days and the median duration of hospitalization during the period of the occurrence of candidemia was 30 days. Most of these candidemia cases (n = 23; 69.7%) occurred among patients who had central venous catheters and approximately half of them had a previous hospitalization period less than two months prior to the current admission. As above, no positive associations were found (Table 2). In five cases (15.1%), patients died in a period shorter than 30 days after yeast isolation.

The median age was 56 years (range 2–93) among patients with solid tumors and 39 years (range 1–75) among patients with hematological malignancies. In the cases of unspecified disease, the median age was 55 years (range 5–85). When comparing adults vs. children and the type of tumor, hematological malignancies were more frequently in children (P = 0.002). Patients aged 45 years and older presented more incidence of solid tumors (P = 0.001) (Table 2).

Etiology

A total of 119 yeast isolates were recovered, with 77 from patients presenting solid tumors, 33 from patients with hematological malignancies and 9 from patients with other pathologies. *Candida albicans* was the most frequently isolated species (n = 58; 48.7%), followed by *C. parapsilosis* (n = 23; 19.3%), *C. tropicalis* (n = 10; 8.4%), *C. krusei* (n = 8; 6.7%), *C. glabrata* (n = 6; 5.0%), *Candida* spp. (n = 4; 3.4%), *C. famata* (n = 2; 1.7%), *C. guilliermondii* (n = 2; 1.7%) and *C. lusitaniae* (n = 1; 0.8%). Besides *Candida* species, three isolates (2.5%) were identified as *Saccharomyces cerevisiae* and two (1.7%) as *Rhodotorula mucilaginosa* (Table 1).

The distribution of the yeast species relative to the medical conditions of the patients is shown in Fig. 2. In patients with solid tumors, *C. albicans* represented 55.8% (n = 43) of the yeast isolates, followed by *C. parapsilosis* at 16.9% (n = 13), *C. glabrata* and *C. tropicalis* each being recovered 7.8% (n = 6) and the other yeasts were less represented. It is noteworthy that in patients with hematological/immunological disorders the relative percentages of the species found was quite different, with *C. albicans* and *C. parapsilosis* representing 27.3% (n = 9) and 24.2% (n = 8), respectively, *C. krusei* 15.1% (n = 5) and the other yeasts were also significantly represented (33.4%). Eleven of the patients with hematological/immunological disorders were children and the three isolates of *Saccharomyces cerevisiae* were recovered from this group.

Table 2 Patients characterization and association with different risk factors

Variables	Solid tumors (%)	Hematological malignancies (%)	P-value
Demographic factors			
Age			
Adult/children s 15 years	76.9/36.8	23.1/63.2	[0.002]
$<$ 45 years old/ $\geq$ 45 years old	51.2/81.2	48.8/18.8	0.001
$<$ 65 years old/ $\geq$ 65 years old	67.5/76.7	32.5/23.3	NS
Gender			
Male	66.7	33.3	NS
Female	72.3	27.7	
Predisposing conditions			
Central venous catheters	62.3	37.7	NS
Gastrointestinal surgery	36.6	0.0	$<$0.001
Previous hospital stay ($<$2 months)	64.7	35.3	NS
Time period until first candidemia case			
$\leq$ 48 hours	66.7	33.3	NS
$>$ 48 hours	70.9	29.1	
Length of hospitalization period			
$\leq$ 48 hours	56.3	43.8	NS
3–20 days	83.3	16.7	
21–40 days	68.8	31.3	
41–60 days	66.7	33.3	
61–80 days	84.6	15.4	
81–100 days	50.0	50.0	
$\geq$ 101 days	100.0	0.0	
Death in a period $<$ 30 days after yeast isolation	84.6	15.4	[0.016]
Overall mortality	78.1	21.9	[0.036]
Aetiological agent *C. albicans*	82.7	58.6	[0.007]
Aetiological agent non-*C. albicansspecies*	17.3	41.4	

NS, Not significant.

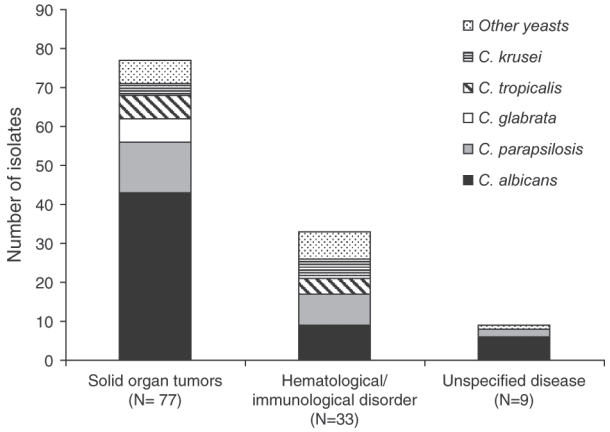

Fig. 2 Distribution of yeast species by medical conditions of the patients with positive blood cultures.

The number of isolates from patients with unspecified disease was much smaller (9) and in this group *C. albicans* was present in six (66.7%) of the cases, *C. parapsilosis* in two (22.2%), and *Rhodotorula mucilaginosa* in one (11.1%).

From the analysis of the species distribution over the different years of this study, we observed that the proportion *Candida albicans*/ non-*C. albicansCandida* species and other yeasts changed. In the first two years, there was a higher number of non-*C. albicansCandida* species and other yeasts than *C. albicans* (10/17 in 2002 and 6/20 in 2003). In 2004 and 2005, this ratio was the reverse, i.e., 14/5 and 8/5, respectively. In 2006, the number was the same (9/9) and in 2007 again the number of cases of candidemia caused by *C. albicans* was higher than the number of candidemias caused by non-*C. albicans Candida* species (Table 1). We noted that *C. albicans* was more frequently isolated from patients with solid tumors of the gastrointestinal tract, breast and genitourinary tract ($P = 0.005$), while non-*C. albicansCandida* species were more frequently found in hematological patients ($P = 0.007$) and in those with head/neck and central nervous system tumors (Table 2, Fig. 2).

Six patients had more than one positive blood culture separated by at least one month. Four of these cases represented reinfection, two with *C. parapsilosis* and two with *C. albicans*. In other patients, *C. parapsilosis* was isolated from the first blood culture but *C. tropicalis* was present in the second. In another patient, *C. albicans* was isolated in the first collection date and *C. krusei* was recovered in the second culture. Mixed infections with two different species also occurred, *C. albicans* and *C. parapsilosis* in two cases and *C. albicans* and *C. krusei* in another case.

Outcome

The crude mortality rate of cancer patients with candidemia in the period of study was 58.2% (64 patients died). Patients presenting solid tumors had a worse outcome than those with hematological malignancies ($P = 0.036$). Of the 119 candidemia cases, 38 (31.9%) resulted in the mortality of the patient in a period ≤30 days after the first yeast isolation in the blood culture ($P = 0.016$) (Table 2). The overall mortality rate in a period ≤30 days after the recovery of a yeast in blood cultures was 58.3% for patients infected with *C. albicans*, 8.3% for patients infected with *C. glabrata*, 11.1% for patients infected with *C. parapsilosis, C. tropicalis*, 5.6% for patients infected with *Candida* spp. and 2.8% for patients infected with *C. krusei* and with *S. cerevisiae*. Overall, a higher mortality rate was observed in patients infected with *C. albicans* than in patients infected with non-*C. albicansCandida* species and other yeasts, however no positive association was found when comparing these two groups.

Antifungal susceptibility of the isolates

The isolates were evaluated for their susceptibility to fluconazole, voriconazole, itraconazole, amphotericin B, caspofungin and posaconazole (Table 3). All *C. albicans* and *C. parapsilosis* isolates were susceptible to fluconazole, voriconazole, itraconazole and amphotericin B. Resistance to fluconazole, voriconazole and itraconazole was observed with *C. krusei* and *R. mucilaginosa* isolates. With respect to caspofungin, all the tested isolates were susceptible, except three *C. albicans* strains and two *R. mucilaginosa* isolates which presented reduced susceptibility to that antifungal. Posaconazole was active against 100% of *C. parapsilosis,* 95.1% of *C. albicans,* 87.5% of *C. tropicalis*, 75% of *C. krusei*, and 50% of *C. glabrata* strains. The susceptibility pattern of isolates from sequential infections to all the antifungals tested did not change.

The isolates of *S. cerevisiae* recovered in this study were not tested for antifungal susceptibility but it is important to emphasize the resistance of the two *R. mucilaginosa* isolates to all antifungals tested.

Discussion

Candidemia is one of the most frequent life-threatening fungal diseases, contributing to the morbidity and mortality of seriously ill patients. During a six-year survey, we found that the incidence of candidemia in an oncology hospital was 2.0 cases per 1,000 hospital admissions a number much lower than the values reported by other authors in patients with the same underlying diseases [14] and lower than the 2.7 previously reported in an Portuguese epidemiological survey of fungemia in a general hospital [26]. In several studies the presence of a hematologic malignancy, long duration neutropenia and the use of a central venous catheter were described as potential risk factors for candidemia and

Table 3 *In vitro* activity of six antifungal agents against the different *Candida* species bloodstream isolates tested

Candida species (no. strains tested)	MIC50/MIC90 and no. of susceptible and resistant/with reduced susceptibility strains																					
	Posaconazole				Caspofungin				Amphotericin B				Itraconazole				Fluconazole				Voriconazole			
	MIC50	MIC90	S	RS	MIC50	MIC90	S	RS	MIC50	MIC90	S	RS	MIC50	MIC90	S	R	MIC50	MIC90	S	R	MIC50	MIC90	S	R
C. albicans ($n = 41$)	0.023	0.094	39	2	0.016	1	38	3	<0.002	0.125	4	0	0.016	0.125	4	0	0.19	0.5	4	0	0.008	0.023	4	0
C. parapsilosis ($n = 19$)	0.016	0.125	19	0	0.25	0.25	19	0	0.094	0.19	8	0	0.032	0.047	8	0	0.125	0.25	8	0	0.004	0.008	8	0
C. tropicalis ($n = 8$)	0.012	0.047	7	1	0.016	0.023	8	0	n.d	n.d			n.d	n.d			n.d	n.d			n.d	n.d		
C. glabrata ($n = 4$)	0.016	>32	2	2	0.032	0.25	4	0	n.d	n.d			n.d	n.d			n.d	n.d			n.d	n.d		
C. krusei ($n = 7$)	0.38	8	4	3	0.016	0.25	7	0	0.25	0.25	1	0	2	2	0	1	>256	>256	0	1	0.19	0.19	0	1
Candida spp. ($n = 5$)	0.125	4	4	1	0.032	0.25	5	0	n.d	n.d			n.d	n.d			n.d	n.d			n.d	n.d		
Rhodotorula mucilaginosa ($n = 2$)	0.25	>32	1	1	>32	>32	0	2	n.d	n.d			n.d	n.d			>256	>256	0	2	>32	>32	0	2
Total no.			76	10			81	5			13	0			12	1			12	3			12	3

n.d, Not determined; RS, Reduced susceptibility.

breakthrough candidemia [27,28]. Our results demonstrate that a significant number of yeast isolates were recovered from catheterized patients and the rate of catheter-associated candidemia was very similar to the data reported by Almirante *et al*. (57.4% vs. 56.3%) [18]. However, in contrast to previously published studies the presence of central venous catheters was not significantly associated with candidemia in our study studies [18,26–30]. We found that candidemia was mainly a nosocomial infection. Nevertheless, about 26% of the cases were outpatient-acquired, a relatively high percentage when compared with the value reported in a similar study that 10% of candidemias in cancer patients occurred outside of the hospital [15]. The higher percentage observed in the present study may be related to the fact that most of the outpatients had short stays at the hospital in the 60 days preceding candidemia. The length of hospitalization period though was not significantly correlated with the development of candidemia. The majority of candidemia cases studied occurred in patients with solid tumors (64.5%). These findings agree with a recent report from Pasqualotto *et al*. [16] in which 77.1% of the cancer patients with candidemia had solid tumors, primarily of the alimentary tract. Our findings indicate that, similarly to previous studies, gastrointestinal surgery might be a predisposing factor to the development of candidemia in patients with solid tumors [15,31–32]. In agreement with Pasqualotto *et al*. [32], patients with hematological malignancies were younger (median age 39 years) in comparison with patients with solid tumors (median age 56 years). Interestingly, the median age of hematological patients in this study was similar to that found in a previous report in acute leukemia patients from a Portuguese hospital [17]. No national data from patients with solid tumors are yet available. Since most of the previous studies [14,15,33] have found a higher incidence of candidemia in patients with hematological malignancies, further investigations will be needed to determine whether the present results reflect only the characteristics of patients treated in the study hospital. The mortality rate associated with candidemia was 31.9%, slightly lower than reported in other studies [32,34].

Candida albicans was the main cause of candidemia, accounting for 48.7% of the cases in the hospital surveyed, while *C. parapsilosis* (20.2%) was the second most common species causing bloodstream infections among the patients. *C. tropicalis*, *C. krusei* and *C. glabrata* together accounted for 20% of all the bloodstream infections recorded. Eight cases of *C. krusei* bloodstream infections were detected over the six years of the survey, which is a very high number when compared to other reports, considering that the population in the present study was considerably smaller [18,35]. According to previous reports, *C. parapsilosis* was also found to be the second most common isolate from blood in countries like Spain and Italy [13,35]. Although as

previously noted, *C. albicans* accounted for about half of the episodes of candidemia in cancer patients [15,16], when we compared the yeasts isolated from patients with different types of cancer we observed a predominance (72.7%) of non-*C. albicans Candida* species and other yeasts causing bloodstream infections in patients with hematological malignancies as compared to those with solid tumors (55.8%). Infections in patients with solid tumors are mainly endogenous in origin, while those in patients with hematological malignancies are more likely to have been caused by yeasts that are not part of the normal human flora.

Multiple-species candidemia was detected in several patients, with the most common combination being *C. albicans* plus *C. parapsilosis*. Candidemia by multiple *Candida* species has also been reported by Boktour *et al*. [36] who noted the most frequent combination was C. *albicans* and *C. glabrata,* followed by *C. albicans* and *C. tropicalis. Candida albicans* and *C. parapsilosis* dual infections were found primarily in neutropenic patients with underlying hematologic malignancies. In contrast, the majority of cases in our study were observed in patients with solid tumors.

Several surveillance programs have produced data documenting different trends in the distribution of species recovered in positive blood cultures and in antifungal susceptibility patterns [26,29,35,37]. Some variations have been shown to occur among institutions, localities or countries which may be due to differences in antifungal prescription and infection control practices [12]. *In vitro* antifungal resistance was rarely detected in European multi-institutional surveys [29]. Our study also suggests that antifungal resistance has not emerged among bloodstream isolates of *C. albicans*. The results in the present investigation with respect to fluconazole and voriconazole showed that these antifungals were active against 92.3% of the tested *Candida* strains. For posaconazole, 10.7% of the tested isolates presented reduced susceptibility and only *C. parapsilosis* strains were 100% susceptible to this antifungal. As expected, the MIC range was higher for *C. krusei* (<0.002–16 μg/ml) and *C. glabrata* (0.016 –>32 μg/ml), which is in accord with the MIC range obtained by Sims *et al*. [38]. Regarding caspofungin, 96.4% of all tested strains were susceptible, including all isolates of *C. parapsilosis*. These results agree with those presented by Pfaller *et al*. [39] but different from those described by Costa-de-Oliveira *et al*. [26]. The latter found 100% reduced susceptibility of *C. parapsilosis* isolates from a Portuguese hospital. Overall, these results reinforce the importance of local epidemiological studies of *Candida* prevalence and susceptibility patterns. Ongoing surveillance of serious infections caused by *Candida* species will be important for tracking changes in the epidemiology and in antifungal susceptibility among these important healthcare associated pathogens. It is also important to observe that usually nonpathogenic yeast species like *Saccharomyces cerevisiae* and *Rhodotorulamucilaginosa* were detected in three and in two blood cultures, respectively. Other authors have reported systemic infections due to these species [40–43] and the extreme antifungal resistance of *Rhodotorula* spp. to azoles and echinocandins was well documented by Lunardi *et al*. [44].

It is important to bear in mind some limitations of this study. First, the true incidence of candidemia in the group of patients considered might have been underestimated since we only considered positive blood cultures. It is known from recent clinical investigations that 30–50% of the patients with disseminated candidiasis may have negative blood cultures [1]. Second, the differences found between solid tumors and hematological patients may be related mainly to differences in the underlying disease as we did not have a control group without candidemia. Third, the small study population might have prevented some associations to reach statistical significance. Nevertheless, our data confirm the important role of *Candida* species as a cause of bloodstream infection in cancer patients. Although *C. albicans* was the most frequent species found, following a worldwide tendency, non-*C. albicansCandida* species represented the main etiological agents of candidemia in this study and were significantly associated with patients with hematological disease. The results here presented are an important contribution to the knowledge of the epidemiological situation regarding candidemia in cancer patients in our country and reinforce the need for monitoring species distribution and antifungal response.

Acknowledgements

This research was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal through a multi-year contract with Centre of Molecular and Environmental Biology (CBMA), University of Minho. Raquel Sabino was financially supported by a fellowship from FCT, Portugal (contract BD/22100/2005).

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1 Perlroth J, Choi B, Spellberg B. Nosocomial fungal infections: epidemiology, diagnosis and treatment. *Med Mycol* 2007; **45**: 321–346.

2 Wisplinghoff H, Seifert H, Tallent SM, *et al*. Nosocomial bloodstream infections in pediatric patients in United States hospitals: epidemiology, clinical features and susceptibilities. *Pediatr Infect Dis J* 2003; **22**: 686–691.

3 Wisplinghoff H, Seifert H, Wenzel RP, Edmond MB. Current trends in the epidemiology of nosocomial bloodstream infections in patients with hematological malignancies and solid neoplasms in hospitals in the United States. *Clin Infect Dis* 2003;36:1103–1110.
4 Kulberg BJ, Lashof AML. Epidemiology of opportunistic invasive mycoses. *Eur J Med Res* 2002; **7**: 183–191.
5 Hajjeh RA, Sofair AN, Harrison LH, *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**: 1519–1527.
6 Pemán J, Cantón E, Gobernado M, and the Spanish ECMM Working Group on Candidemia. 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* 2004; **23**: 1267–1275.
7 Trick WE, Fridkin SK, Edwards JR, Hajjeh RA, Gaynes RP, National Nosocomial Infections Surveillance System Hospitals. Secular trend of hospital-acquired candidemia among intensive care unit patients in the United States during 1998–1999. *Clin Infect Dis* 2002; **35**: 627–630.
8 Wingard JR. Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. *Clin Infect Dis* 1995; **20**: 115–125.
9 Pfaller MA. Nosocomial candidiais: emerging species, reservoirs, and modes of transmission. *Clin Infect Dis* 1996; **22**: S89–94.
10 Chai YAL, Wang Y, Khoo AL, *et al*. Predominance of *Candida tropicalis* bloodstream infections in a Singapore teaching hospital. *Med Mycol* 2007; **45**: 435–439.
11 Lupetti A, Tavanti A, Davini P, *et al*. Horizontal transmission of *Candida parapsilosis* candidemia in a neonatal intensive care unit. *J Clin Microbiol* 2002; **40**: 2363–2369.
12 St-Germain G, Laverdière M, Pelletier R, *et al*. Prevalence and antifungal susceptibility of 442 *Candida* isolates from blood and other normally sterile sites: results of a 2-year (1996 to 1998) multicenter surveillance study in Quebec, Canada. *J Clin Microbiol* 2001; **39**: 949–953.
13 Almirante B, Rodriguez D, Cuenca-Estrella M, *et al*. Epidemiology, risk factors and prognosis of *Candida parapsilosis* bloodstream infections: case-control population-based surveillance study of patients in Barcelona, Spain, from 2002 to 2003. *J Clin Microbiol* 2006; **44**: 1681–1685.
14 Abi-Said D, Anaissie E, Omrum U, *et al*. The epidemiology of hematogenous candidiasis caused by different *Candida* species. *Clin Infect Dis* 1997; **24**: 1122–1128.
15 Viscoli C, Girmenia C, Marinus A, *et al*. Candidemia in cancer patients: a prospective, multicenter surveillance study by the Invasive Fungal Infection Group (IFIG) of the European Organization for Research and Treatment of Cancer (EORTC). *Clin Infect Dis* 1999; **28**: 1071–1079.
16 Pasqualotto AC, Rosa DD, Medeiros LR, Severo LC. Candidaemia and cancer: patients are not all the same. *BMC Infect Dis* 2006; **6**: 50–56.
17 Ribeiro P, Sousa AB, Nunes O, *et al*. Candidemia in acute leukemia patients. *Supp Care Canc* 1997; **5**: 249–251.
18 Almirante B, Rodriguez D, Park BJ, *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**: 1829–1835.
19 Correia A, Sampaio P, Almeida J, Pais C. Study of molecular epidemiology of candidiasis in Portugal by PCR fingerprinting of *Candida* clinical isolates. *J Clin Microbiol* 2004; **42**: 5899–5903.
20 Sampaio JP, Gadanho M, Santos S, *et al*. Polyphasic taxonomy of the basidiomycetous yeast genus *Rhodosporidium: Rhodosporidium kratochvilovae* and related anamorphic species. *Int J Syst Evol Microbiol* 2001; **51**: 687–697.
21 National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts – 2nd edn: Approved Standard M27-A2 2nd edn: Wayne, PA: NCCLS, 2002.
22 Bedini A, Venturelli C, Mussini C, *et al*. Epidemiology of candidemia and antifungal susceptibility patterns in an Italian tertiary-care hospital. *Clin Microbiol Infect* 2006; **12**: 75–80.
23 Diekema DJ, Messer SA, Hollis RJ, *et al*. Evaluation of Etest and disk diffusion methods compared with broth microdilution antifungal susceptibility testing of clinical isolates of *Candida* spp. against posaconazole. *J Clin Microbiol* 2007; **45**: 1974–1977.
24 Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Reviews. 2007; **20**: 133–163.
25 Baixench MT, Aoun N, Desnos-Ollivier M, *et al*. Acquired resistance to echinocandins in *Candida albicans* case report and review. *J Antimicrob Chemother* 2007; **59**: 1076–1083.
26 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**: 365–374.
27 Nucci M, Colombo AL. Risk factors for breakthrough candidemia. *Eur J Clin Microbiol Infect Dis* 2002; **21**: 209–211.
28 Raad I, Hanna H, Boktour M, *et al*. Management of central venous catheters in patients with cancer and candidemia. *Clin Infect Dis* 2004; **38**: 1119–1127.
29 Tortorano AM, Kibbler C, Peman J, *et al*. Candidemia in Europe: epidemiology and resistance. *Int J Antimicrob Agents* 2006; **27**: 359–366.
30 Pasqualotto AC, Severo LC. The importance of central venous catheter removal in patients with candidemia: time to rethink our practice? *Clin Microbiol Infect* 2008; **14**: 2–4.
31 DiNubile MJ, Hille D, Sable CA, Kartsonis NA. Invasive candidiasis in cancer patients: observations from a randomized clinical trial. *J Infect* 2005; **50**: 443–449.
32 Pasqualotto AC, Nedel WL, Machado TS, Severo LC. Comparative study of risk factors and outcome among outpatient-acquired and nosocomial candidemia. *J Hospital Infect* 2005; **60**: 129–134.
33 Chung JW, Lee SO, Choi SH, *et al*. Risk factors and outcome for breakthrough candidemia in patients with cancer. *Cancer* 2004; **101**: 1860–1865.
34 Kovacicová G, Spánik S, Kunová A, *et al*. Prospective study of fungaemia in a single cancer institution over a 10-y period: etiology, risk factors, consumption of antifungals and outcome in 140 patients. *Scand J Infect Dis* 2001; **33**: 367–374.
35 Tortorano AM, Prigitano A, Biraghi E, Viviani MA on behalf of the FIMUA-ECMM Candidemia Study Group. The European Confederation of Medical Mycology (ECMM) survey of candidemia in Italy: *in vitro* susceptibility of 375 *Candida albicans* isolates and biofilm production. *J Antimicrob Chemother* 2005; **56**: 777–779.
36 Boktour MR, Kontoyiannis DP, Hanna HA, *et al*. Multiple-species candidemia in patients with cancer. *Cancer* 2004; **101**: 1860–1865.
37 Cuenca-Estrella M, Rodriguez D, Almirante B, *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* 2002; **55**: 194–199.
38 Sims CR, Paetznick VL, Rodriguez JR, Chen E, Ostrosky-Zeichner L. Correlation between microdilution, e-test, and disk diffusion methods for antifungal susceptibility testing of posaconazole against *Candida* spp. *J Clin Microbiol* 2006; **44**: 2105–2108.

39 Pfaller MA, Diekema DJ, Gibbs DL, *et al*. Geographic and temporal trends in the isolation and antifungal susceptibility of *Candida parapsilosis*: a global assessment from the ARTEMIS disk antifungal surveillance program 2001 to 2005. *J Clin Microbiol* 2008; **46**: 842–849.
40 Wenzel R, Edmond M. The impact of hospital-acquired bloodstream infections. *Emerg Infect Dis* 2001; **7**: 174–177.
41 Tuon FF, Almeida G, Costa SF. Central venous catheter-associated fungemia due to *Rhodotorula* spp. – A systematic review. *Med Mycol* 2007; **45**: 441–447.
42 Bassetti S, Frei R, Zimmerli W. Fungemia with *Saccharomyces cerevisiae* after treatment with *Saccharomyces boulardii*. *Am J Med* 1998; **105**: 71–72.
43 Llanos R, Querol A, Pemán J, Gobernado M, Fernández-Espinar MT. Food and probiotic strains from the *Saccharomyces cerevisiae* species as a possible origin of human systemic infections. *Int J Food Microbiol* 2006; **1**: 286–290.
44 Lunardi LW, Aquino VR, Zimerman RA, Goldani LZ. Epidemiology and outcome of *Rhodotorula* fungemia in a tertiary care hospital. *Mycoses* 2006; **49**: 114–118.

This paper was first published online on Early Online on 01 February 2010.