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Epidemiology of Bloodstream *Candida* spp. Infections Observed During a Surveillance Study Conducted in Spain

R. Cisterna, G. Ezpeleta and O. Tellería
Clinical Microbiology and Infection Control Department
Basurto Hospital, Avenida Montevideo – Bilbao
Spain

1. Introduction

Candida bloodstream infections (BSI) have become a major healthcare problem, specially in tertiary- care hospitals worldwide (Al-Jasser & Elkhizzi, 2004, Almirante et al., 2005, Alonso-Valle et al., 2003, Atunes et al., 2004 Asmundsdottir et al., 2002, Costa et al., 2000, Fraser et al., 1992, Garbino et al., 2002, Luzzati et al. 2000, Marchetti et al., 2004, Pappas et al., 2003, Viudes et al., 2002). Several risk factor identified among patients hospitalized for long periods such as the exposition to broad spectrum antimicrobial and/or immunosuppressive chemotherapy, parenteral nutrition, and invasive medical procedures have contributed to this fact (Blumberg et al., 2001, Fraser et al., 1992). Despite some improvements in fungal BSI diagnosis during last years, candidemia diagnosis remains difficult. Besides, following the data appeared in the classical study from Berenguer and colleagues, only 50% of patients with disseminated candidiasis will have positive blood cultures and even fewer will have an antemortem diagnosis (15% to 40%) (Berenguer et al., 1993). Therefore, invasive candidemia is not easy to diagnose, has an expensive treatment and finally is a serious, often life-threatening infection (Girmenia et al., 1996, Messer et al., 2009).

Although the incidence of candidemia has increased steadily among hospitalized patients during the eighties and nineties, recent series suggest that This increase has stabilized, but with great variations between different geographical locations with similar socio-economical development even in the same continent. For instance, in The Netherlands an increasing incidence of candidemia has been reported during the period between eighties and nineties (Voss et al., 1996) but on the other hand, in a neighbouring country such as Switzerland the incidence of *Candida* BSI infections remained unchanged during the same period (Marchetti et al., 2004). Therefore, it seems that there are some differences in the epidemiology of candidemia between different countries.

Besides, in recent years, a trend towards increasing resistance to both traditional and more recently introduced antifungal agents has been observed amongst invasive *Candida* infections, underscoring the need for continuous surveillance to monitor trends in incidence, species distribution, and antifungal drug susceptibility profiles.

The epidemiology of candidemia has been extensively studied in many countries and there are some large series published in this field (Alonso-Valle et al., 2003, Atunes et al., 2004, Banerjee et al., 1991, Colombo et al., 2006, Diekema et al., 2002, Kao et al., 1999, Messer et al., 2009, San Miguel et al., 2005, Silva et al., 2004, Tortorano et al., 2004, Trick et al., 2002). But, most of the data on candidemia in Spain until recent days are limited to retrospective reviews of medical records or observational studies conducted in a limited geographical area (Almirante et al., 2005, Alonso-Valle et al., 2003, Pemán et al., 2002, Pemán et al., 2011). Regarding the Spanish data available on antifungal resistance is often assessed by occasional surveys or reported in summaries of sporadically occurring cases of treatment failures. The purpose of such investigations is to monitor levels of susceptibility to different agents. However, long-term prospective studies of antifungal susceptibility have the advantage of eliminating a number of variable factors which may affect these assessments. Some of these factors include temporary changes in patterns of Invasive Candida infections (as stated before) and transient alterations in antifungal resistance due to special conditions (e.g. candidemia outbreaks in ICUs). Consequently, the epidemiological data about candidemia and its impact in the healthcare system is unknown, and no reliable nationwide data are available. In order to make a realistic global perspective of invasive Candida BSI, we designed a prospective laboratory-based surveillance study comprising 40 tertiary care hospitals across the country, to assess the incidence, species distribution, frequency of antifungal resistance, and risk factors for candidemia.

2. Materials and methods

Study design

A prospective laboratory-based surveillance was established to monitor the predominant *Candida* species and antifungal resistance patterns of nosocomial and community-acquired invasive *Candida* infections via a network of sentinel hospitals distributed by geographic location across the country.

The participating institutions include 40 medical centers which provide medical care either to adults and children in several medical specialties. Each participant hospital contributed prospectively clinical and epidemiological results (organism identification, date of isolation, hospital location, intrinsic and extrinsic risk factors for candidemia) on clinically significant consecutive blood culture isolates of Candida spp. (one isolate per patient) detected during the 12-month period from June, 2008 through June, 2009. All isolates were saved on agar slants and were sent on a trimestral basis to the Mycology Laboratory at Basurto Hospital for storage, further characterization and reference susceptibility testing.

Clinical definitions

Clinical and case definitions were according the NHSN (formerly NNISS) methodology. Statements defining a case and other clinical conditions are summarized in Table 1.

Quality control measures of clinical data

The clinical case report list of each hospital was compared with the isolates received at Basurto Hospital to perform the antifungal susceptibility in order to verify that neither cases nor isolates were missed. Audits of medical records to verify accuracy of data and completeness were performed on 25% of cases.

Incident case of candidemia:	The incident isolation of *Candida spp*. from a blood culture.
New incident case of candidemia:	An episode of candidemia occurring more than 30 days after the initial incident isolation.
Breakthrough candidemia:	The incident isolation of *Candida* spp. from a blood culture from a patient receiving systemic antifungal therapy for any reason.
Fever:	Peripheral body temperature equal or higher than 37.8°C
Neutropenia:	An absolute neutrophil count of less than 500 cells / mm^3.
Adult patients:	All patients whose age was over 14 years old.

Table 1. Definitions according to NHSN (formerly NISS) used in this study

In vitro susceptibility testing

Antifungal susceptibility tests were performed by using the broth microdilution assay according to the methodology recommended by the CLSI (formerly known as NCCLS), document M27-A2 (NCCLS, 2002) using a microtiter plate. Each isolate was tested against different antifungal drugs at the indicated concentration range suggested in the CLSI document. Quality control (QC) was ensured by testing the CLSI recommended QC strains, *C. krusei* ATCC 6258, and *C. parapsilosis* ATCC 22019.The MIC endpoint for amphotericin B, azoles and echinocandins and interpretative MIC breakpoints for azoles and echinocandins were those suggested by the CLSI document M27-A2, but for the definition of the amphotericin B MIC breakpoints we used the values suggested from a previous study published by Nguyen *et al.* (Nguyen et al., 1998).

Statistical analysis

The numbers of admissions and patient-days were collected to calculate incidence rates. The incidence rate for each hospital was calculated as the number of candidemias per 1,000 admissions, whereas the overall incidence was determined using summed denominators of patient-days and admissions to calculate pooled mean rates. The data generated during the year of the surveillance on the different risk factors, underlying diseases, morbidity and mortality were recorded in a Microsoft Access 2003 (Microsoft Corporation, Redmond, WA) based case report database. Categorical data were analyzed using Chi-square or Fisher's exact tests as appropriate, and continuous variables were compared using the t-test or Wilcoxon test according to the significance of the normality test. Spearman rank-order correlation was used to measure the relationship between the MICs of fluconazole and voriconazole. We performed univariate and multivariate analysis of factors associated with candidemia caused by isolates with decreased susceptibility to fluconazole. Variables significant at *p*-values of less than 0.05 by univariate analysis were included in a multivariate model using a repeated measures logistic regression model (backward and forward). Data were analyzed using the SPSS 11.0.1 software (SPSS, Inc. Chicago, IL) and Stata 8.0 (Stata Corporation, Lenexa, TX).

3. Distribution of Candida blodostream infections

During the 12-month study period a total of 984 Candida BSIs were reported. The calculated overall incidence was 1.09 cases per 1,000 admissions, however the incidence rate changed a lot between the 40 centers enrolled in this study and ranged from 0.76 to 1.49 cases per 1,000 admissions.

Among the invasive Candida BSIs, 45.3 % occurred in patients in an medical service, 23.5% in patients hospitalized in an intensive care unit, 17.6% in patients in a surgical ward, 7.41% in a pediatric ward and finally 4.06% in other services. Most of the patients (98.7%) were hospitalized and only nine of them were outpatients at the time of diagnosis.

Candidemia incidence was slightly higher in males (64.02% of the case patients) and the global average age at the onset of the episode was 41 years with a median age was 53 years among adult patients and 7 months among children.

The frequency of BSIs due to the most frequently isolated species of *Candida* in the study sites are presented in Table 2.

Species	No. (%) of cases	Range (in %) between clinical settings
C. albicans	483 (49.08%)	27 – 54
C. parapsilosis	204 (20.73%)	7 – 40
C. glabrata	134 (13.61%)	2 – 14
C. tropicalis	106 (10.77%)	16 – 29
C. krusei	21 (2.13%)	0 – 9
Other species [a]	36 (3.65 %)	0 – 4

[a] Species with less than 10 isolates are included in this category. This category includes *C. famata*, *C. lusitaniae*, *C. pelliculosa* and *Candida spp*.

Table 2. Species distribution and incidence among 984 cases of candidemia detected during prospective sentinel surveillance in Spain from June 2008 to June 2009

Overall, the 49.08% of the cases were attributable to *C. albicans*, 20.73% were attributable to *C. parapsilosis*, 13.61% were attributable to *C. glabrata*, 10.77% were attributable to *C. tropicalis*, 2,13% to *C. krusei* and the rest of the cases (3.65%) were attributable to other species. The distribution of Candida species among adult population was similar to the one found in pediatric cases, however, the distribution of species varied considerably when analyzed between centers as it has been reflected in the ranges specified in Table 2. The species distribution among our study isolates is similar to that described by Pfaller et al. (Pfaller et al., 1998) in Latin America with data collected by the Sentry Antimicrobial Surveillance Program. As Pfaller and colleagues described previously, the proportion of species isolated varies considerably among medical centers beign unclear the reasons for such differences and they could be attributed to many different influences.

Table 3 summarizes the overall clinical characteristics and outcome of the 984 candidemia cases identified.

At the time of candidemia diagnosis, neoplasia was documented for 195 (19.84%) patients, 35 of which (17.94%) were affected with hematologic malignancies Prior surgery was recorded from 311 (31.6%) patients (311 of a total of 984), being most of them abdominal surgeries (64% of total surgical patients). Two third of the patients (66.97%) had a central venous catheter and one quarter (26.93%) of them were under mechanical ventilation. Neutropenia and dialysis were rare conditions which was only documented in only 35 case patients (3.55%) and 12 patients (1.21%) respectively. Invasive *Candida* spp. infection complications such as endocarditis or endophalmitis were infrequent and with 17 cases documented for the former complication (2%) and 3 patients for the later.

Variable	Value for all total cases	Value for species			
		C. albicans	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*
Average age (range)	41 (0–96)	46 (0–92)	48 (0–96)	33 (0–89)	52 (0–88)
No. of males	577 (58.64)	273 (56.52)	182 (89.21)	51 (48.11)	62 (46.27)
No. of outpatients	7 (0.71)	3 (0.62)	1 (0.49)	3 (2.83)	0 (0.00)
Median no. of days (range) until candidemia	20 (0–385)	20 (0–114)	19 (0–385)	19 (0–47)	19 (0–115)
No. of cases of underlying diseases					
Cancer	311 (31.61)	127 (26.29)	86 (42.16)	34 (32.07)	26 (19.40)
Hematological malignancy	20 (2.03)	5 (1.04)	6 (2.94)	1 (0.94)	1 (0.75)
Coronary artery disease	82 (8.33)	33 (6.83)	23 (11.27)	5 (4.72)	8 (5.97)
Chronic Obstructive Pulmonary disease (COPD)	71 (7.21)	40 (8.28)	11 (5.39)	5 (4.72)	9 (6.71)
Neurological disease	35 (3.55)	14 (2.80)	12 (5.88)	2 (1.89)	2 (1.49)
Diabetes	120 (12.20)	53 (10.97)	22 (10.78)	8 (7.55)	20 (14.93)
Organ transplantation	45 (4.57)	14 (2.90)	21 (10.29)	2 (1.89)	3 (2.23)
HIV infection	33 (3.35)	18 (3.73)	3 (1.47)	3 (2.83)	3 (2.24)
Parenteral drug abusers	22 (2.23)	10 (2.07)	4 (1.96)	2 (1.89)	1 (0.75)
No. of patients with characteristic					
Previous or actual corticosteroid therapy	180 (18.29)	80 (16.56)	50 (24.50)	13 (12.26)	21 (15.67)
Immunosuppressive therapy and/or neutropenia	265 (26.93)	102 (21.12)	75 (36.76)	28 (26.41)	28 (20.90)
In the ICU at diagnosis	252 (25.61)	120 (24.84)	68 (33.33)	21 (19.81)	25 (18.66)
Mechanical ventilation	265 (26.93)	133 (27.54)	71 (34.80)	15 (14.15)	26 (19.40)
Hemodialysis at diagnosis	12 (1.22)	2 (0.41)	4 (1.96)	1 (0.94)	3 (2.24)
Previous surgery	311 (31.61)	148 (30.64)	82 (40.20)	19 (17.92)	36 (26.87)
Central venous catheter	659 (66.79)	295 (61.07)	187 (91.66)	52 (49.06)	61 (45.52)
Urinary catheter	450 (45.73)	207 (42.86)	112 (54.90)	31 (29.25)	52 (38.81)
Prior antibiotic therapy	747 (75.91)	337 (69.77)	106 (51.96)	60 (56.60)	71 (52.98)
Prior fluconazole use	78 (7.93)	29 (6.00)	10 (4.90)	12 (11.32)	9 (6.71)
Death attributed to candidemia	134 (13.62)	60 (12.42)	20 (9.80)	13 (12.26)	15 (11.19)
Mortality due to other conditions	103 (10.47)	48 (9.94)	36 (17.65)	7 (6.60)	14 (10.45)
Overall mortality	237 (24.10)	108 (22.36)	56 (27.45)	20 (18.87)	29 (21.64)

Table 3. Demographics, clinical characteristics, and mortality for *Candida* spp. BSI episodes identified during prospective sentinel surveillance conducted in Spain from June 2008 to June 2009.

There were no statistically significant differences when the risk mentioned above were analyzed for the pediatric population of patients.

4. Antifungal susceptibility

In vitro susceptibility testing of the 984 BSI isolates of Candida species against amphotericin B, fluconazole, voriconazole, caspofungin and anidulafungin revealed that when globally analyzed *Candida* strains causing BSI are rarely resistant to a wide number of antifungal agents. However, the resistance rates among the different species vary a lot as it can be shown on Table 4.

Species	Antifungal agent	MIC (ug/ml)			No. of resistant or SDD isolates
		Range	50%	90%	
C. albicans (483)	Amphotericin B	0.125–1.0	0.5	1.0	0 (0.00)
	Fluconazole	0.125–64	0.5	2.0	10 (2.07)
	Voriconazole	< 0.03–4	0.03	0.03	5 (1.04)
	Caspofungin	<0.03–4	0.25	2.0	3 (0.62)
	Anidulafungin	<0.03–2	0.03	0.03	0 (0.00)
C. parapsilosis (204)	Amphotericin B	0.25–1.0	1.0	1.0	0 (0.00)
	Fluconazole	0.125–64	0.5	4.0	11 (5.39) [a]
	Voriconazole	0.03–2	0.03	0.125	3 (1.47) [b]
	Caspofungin	0.125–64	1.0	2.0	12 (5.88)
	Anidulafungin	0.03–64	1.0	2.0	7 (3.43)
C. glabrata (134)	Amphotericin B	0.25–1.0	0.5	1.0	0 (0.00)
	Fluconazole	1.0–64	4.0	32	25 (18.67)
	Voriconazole	0.03–4.0	0.125	0.5	3 (2.24)
	Caspofungin	0.03–0.5	0.06	0.125	0 (0.00)
	Anidulafungin	0.06–0.5	0.06	0.125	0 (0.00)
C. tropicalis (106)	Amphotericin B	0.125–1.0	0.5	1.0	0 (0.00)
	Fluconazole	0.25–128	1	2	2 (1.89)
	Voriconazole	0.03–8	0.06	0.125	2 (1.89)
	Caspofungin	0.03–0.5	0.06	0.125	0 (0.00)
	Anidulafungin	0.03–0.5	0.03	0.125	0 (0.00)
C. krusei (21)	Amphotericin B	0.125–2.0	0.25	0.75	0 (0.00)
	Fluconazole	16–128	4.0	8.0	21 (100.00)
	Voriconazole	0.03–4.0	0.25	1.0	2 (9.52)
	Caspofungin	0.125–8	0.125	1.0	1 (4.76)
	Anidulafungin	0.03–8	0.06	0.25	1 (4.76)

[a] All the isolates except one exhibit decreased susceptibility (SDD) to fluconazole. [b] All the isolates are SDD to voriconazole.

Table 4. Antifungal susceptibility test results for selected species of *Candida* isolated during prospective, sentinel surveillance in Spain from June 2008 to June 2009

When we considered the *C. glabrata* isolates obtained during the study only the 81.33% of them were were susceptible to fluconazole and 97.76% were susceptible to voriconazole, but on the contrary, 97.93% and 98.96% of the isolates of *C. albicans* were susceptible to fluconazole and voriconazole respectively. The proportion of isolates that was resistant to the studied azole drugs was comparable with that observed in the other recently published studies. (Messer et al., 2009, Pemán et al., 2011).

The antifungal activities of voriconazole, fluconazole, amphotericin B, caspofungin and anidulafungin against the 984 *Candida spp* isolated during the study period are summarized in Table 4. Among the azole compounds, voriconazole was the most active drug overall with an MIC90 of 0.25 μg/ml. Against *C. albicans*, *C. parapsilosis* and *C. tropicalis* isolates, voriconazole (MIC90 range 0.03-0.25 μg/ml) was much more active than fluconazole (MIC90, 2-4). Although these differences in the drug activity, both azole compounds showed lower MICs for fluconazole and voriconazole for the species mentioned before when compared to *C. glabrata* and *C. krusei* isolates. Despite this good susceptibility profile, we found five *Candida albicans* isolates with a MIC greater than 4 μg/ml to voriconazole and two *C. krusei* isolates that had a voriconazole MIC of 2 ug/ ml. All these isolates were also resistant to fluconazole.

We found that there was a statistically significant moderate linear correlation between fluconazole and voriconazole MICs (r = 0.574; $P \leq 0.01$). having higher voriconazole MICs those isolates from patients who received fluconazole before the candidemia episode when compared to those without previous exposure to fluconazole (MIC90s of 0.25 μg/ml and 0.06 μg/ml, respectively; $P \leq 0.05$

Table 5 summarize the risk factors we identified during the study with a candidemia episode due to an isolate with decreased susceptibility (SDD or resistant) to fluconazole using univariate statistical techniques.

	No of isolates (%) susceptible to fluconazole	No. of isolates (%) with decreased susceptibility or resistant to fluconazole	P - value
Neoplasia	27 (2.95)	6 (8.70)	≤ 0.01
Neutropenia	37 (4.04)	11(15.94)	≤ 0.01
Prior fluconazole use	27 (2.95)	10 (14.49)	≤ 0.01

[a] Only statistically significant variables are summarized in the table

Table 5. Summary of univariate statistical analysis between fluconazole susceptible isolates vs. resistant or SDD ones

We found that this condition was associated with neoplasia (9% versus 3%; $P \leq 0.01$), current neutropenia (16% versus 4%; $P \leq 0.01$), and prior fluconazole use (14% versus 3%; $P \leq 0.001$). These independent factors identified using the univariate statistical approach, were analyzed more deeply using a repeated measures logistic regression model. We obtained significant results for neoplasia (odds ratio, 2.9; 95% confidence interval, 1.4 to 5.9; $P \leq 0.05$) and prior use of fluconazole (odds ratio, 3.8; 95% confidence interval, 1.7 to 8.2; $P \leq 0.01$). (Table 6).

	Odds ratio	95 percent confidence Limits	P - value
Neoplasia	2.9	1.4 – 5.9	≤ 0.05
Prior fluconazole use	3.8	1.7 – 8.2	≤ 0.01

[a] Only statistically significant variables are summarized in the table.

Table 6. Summary of multivariate statistical analysis of risk factors for candidemia caused by fluconazole susceptible isolates vs. resistant or SDD ones [a]

Caspofungin and anidulafungin resistance was low (16 cases for caspofungin and 8 cases for anidulafungin) (Table 4). Despite this low rate of in vitro resistance to echinocandins of the isolates studied MICs from *C. parapsilosis* and *C. guilliermondi* were higher compared to the MIC obtained from other *Candida spp*. as it has been described in others studies.

5. Antifungal treatment

At the time of diagnosis and inclusion in this study, 122 case patients (12.3%) were receiving a systemic antifungal agent and were considered breakthrough infections (fluconazole, 78 patients, amphotericin B, 31 patients, itraconazole and voriconazole, 3 patient each; and echinocandins, 5 patients). Although the reason for this high rate of breakthrough infections is not clear, it is possible that other factors besides the antifungal resistance have got a role in the explanation of this phenomenon. A deep analysis of these 122 cases showed that either the antifungal therapy duration or the election of the antifungal drug was inadequate. A total of 536 case patients (54.5%) received antifungal therapy, started at a median of 3 days from the *Candida* isolation or onset of candidemia.

6. Mortality

The crude mortality rate was 24.10%, but the mortality rate among children was significantly lower. (see Table 3) As it has been described in different studies published in the medical literature candidemia due to *C. parapsilosis* had a lower mortality rate than the rate due other Candida species (Morgan et al., 2005, Pappas et al., 2004, Pemán et al., 2002), but no statistically significant result when analyzing the death rate of patients infected by a susceptible isolate (54%) or a less-susceptible isolate (SDD or resistant) (64%) among patients who received fluconazole as treatment. ($P \leq 0.44$).

7. Discussion and remarks

This prospective candidemia surveillance study represents one of the largest multicenter studies conducted in Spain and provide one of the most representative data on the epidemiology of candidemia to date. The first remarkable finding of our study was the higher incidence of candidemia than those reported from centers located in the Northern Hemisphere which ranged between 0.28 to 0.96 per 1,000 admissions (Banerjee et al., 1991, Doczi et al., 2002, Marchetti et al., 2002, Pfaller et al., 1998, Pfaller et al., 2004, Richet et al., 2002, Sandven et al., 1998, Tortorano et al., 2002, Tortorano et al., 2004) and including those published before in Spain (0.76 to 0.81 per 1,000 admissions) (Almirante et al., 2005, Alonso-Valle et al., 2003, Pemán et al., 2002, Pemán et al., 2011, San Miguel et al., 2005). Although the reasons for this high rate are not entirely clear, it is possible that this may be related to a

combination of multiple factors, including differences in medical care resources, transplantation programs, implementation of infection control measures in hospitals, empirical antifungal therapy and prophylaxis for high-risk patients. Another possibility is that our series may not reflect the current trends, but the data from a prospective study held in Spain and recently published by Peman *et al.* support our data (Pemán et al., 2011, Pfaller & Diekema, 2007) (see Table 7).

Country and period	Total number of isolates	% of total by most representative species				
		C. albicans	*C. parapsilosis*	*C. tropicalis*	*C. glabrata*	*C. krusei*
USA 1992-1993	837	52	21	10	12	4
USA 1993-1995	79	56	15	10	15	-
USA 1995-1997	1593	46	14	12	20	2
USA 1995-1998	934	53	10	12	20	3
USA 1998-2000	935	45	13	12	24	2
USA 2001-2004	2773	51	14	7	22	2
USA 2008-2009	1354	48	17	10	18	2
Canada 1992-94	415	69	10	7	8	1
Latin America 1995-1996	145	37	25	24	4	1
Latin America 2001-2004	1565	50	16	20	7	2
Asia-Pacific 2001-2004	1344	56	16	14	10	2
Taiwan 1994-2000	1095	50	14	21	12	<1
Europe 1992-94	249	49	11	11	10	9
Europe 1997-99	2089	56	13	7	14	2
Europe 2001-2004	2515	60	12	9	10	5
Norway 1991-2003	1415	70	6	7	13	2
Denmark 2003-2004	307	63	4	4	20	3
Spain 2002-2003	351	51	23	10	9	4
Spain 2001-2006	1997	47	19	10	12	5
Spain 2008-2009	984	49	21	13	11	2
Spain 2009-2010	1377	45	29	12	8	2

Table 7. Summary of geographical differences in species distribution in Candida BSI isolates. (Adapted and modified from Pemán et al., 2001 and Pfaller & Diekema, 2007).

Despite this fact, it seems that probably a combination of factors may have affected the overall rates of fungemia cases in Spain. The differences appeared in the average age of our patients when compared to other surveillance series from the United States (Ostrosky-Zeichner et al., 2003, Pappas et al., 2003), are probably due to the high proportion of children in our study, especially in the Spanish hospitals located in the Southern part of the country (32% of children in the Spanish hospitals from the Southern part of the country compared to 9% described in the study from the United States) (data not shown). Therefore, this condition reflects that there are great differences among patients of different geographical locations as it was mentioned in the introduction and the demographical composition and lastly the risk factors, could be very different from one population to another. (Table 7). In fact, the number of cases of invasive candidemia was not homogeneous across the country., the distribution of the clinical isolates obtained during the study period along four different

geographical areas in Spain,. (North, Center, East and South). *C. albicans* covers almost half (49.08%) of the global cases, remaining as the most frequently isolated specie, but the rates between the four different areas were not homogenous., for instance, in the Southern part of the country the rate of isolates of C. albicans (39.2%) was similar to the one of *C. parapsilosis* (37.4%).

Some studies have reported a shift in the etiology of candidemia reporting an increase of candidemia cases caused by non-*albicans Candida* species during the last decade (Colombo et al., 2006, Richet et al., 2002, Tortorano et al., 2004) (Table 7). Although C. albicans remain the most frequently isolated specie, reasons for the emergence of non-*albicans* species remain unclear, but some medical conditions may explain increasing incidence of candidemia due to non-*albicans* species. It has been noted in previous reports that infections due to *C. tropicalis* candidemia is associated with neoplasia and neutropenia (Komshian et al., 1989) and those attributable to *C. parapsilosis* are often associated with the presence of intravascular catheters and are not influenced by exposure to fluconazole or other antifungal agents (Clark et al., 2004, Girmenia et al., 1996, Levy et al., 1998, Sandven et al., 1998). The last situation, may us to consider *C. parapsilosis* as an exogenous pathogen and breaches of catheter care and of infection control practice should be investigated and revised within institutions where this species has become a common blood culture isolate.

The increasing incidence some of non-*albicans Candida* species with reduced susceptibility to azoles, such as *C. glabrata*, creates new therapeutic challenges and leads to another important question such as the influence of previous antifungal therapy in the development of non-*albicans* species candidemia. Recent studies, such as those published by Marr *et al.* and Tortorano *et al.* had addressed this question and showed interesting results about the association with previous exposure to azoles and the risk of development of fungemias due to *C. krusei* and / or *C. glabrata* (Marr et al., 2000, Tortorano et al., 2004). In fact, the differences in antifungal susceptibilities among isolates between different regions in Spain was almost entirely attributable to high-level resistance to azoles observed among *C. glabrata* and *C. krusei* isolates (Table 4).

We are not aware of these epidemiological changes mentioned in the paragraph above and our findings from our study are supportive of them. *C. parapsilosis* fungemia account for the large majority of non-*albicans* species (in the same manner that that been described for other European countries) and candidemia due to *C. krusei* is rare in Spain as it has been described in other series (Almirante et al., 2005, Alonso-Valle et al., 2003, Ostrosky-Zeichner et al., 2003, Pemán et al., 2002, Pemán et al., 2011, Tortorano et al., 2004). The explanation for this species distribution in Spain which is more or less similar to other Latin American countries is not clear and perhaps many factors are involved. However, this species distribution has got a great importance in the developing of therapeutic schemes and in the prevention of antifungal resistance.

While most non-albicans *Candida* species are associated with high fluconazole minimum inhibitory concentrations (MIC), *C. parapsilosis* is typically susceptible to most antifungals, although is associated with higher echinocandin MIC that vary between different agents. Moreover, the rate of persistently positive fungemia in patients treated with caspofungin was reported as almost double for *C. parapsilosis* versus other Candida species. Even more, if we consider clinical patients at risk of suffering a candidemia episode, such as

immunocompromised patients, where clinical responses are poorer the picture is not good. Summarizing, we are concerned about the use of echinocandins alone based on the identification of non-albicans *Candida* specie. Grouping these agents into one treatment scheme is difficult due to the variability not only in the susceptibility of the isolates, as well as the microbiological responses seen between different echinocandins.

Regarding the susceptibility of the studied isolates, antifungal resistance was an infrequent finding in our study and was restricted to a few isolates, and none of them were resistant to amphotericin B. This condition is similar to the findings published in three recent studies (Almirante et al., 2005, Messer et al., 2009, Pemán et al., 2011). Our proportion of fluconazole-resistant isolates (6.32%) was low, similarly to the rate observed with Spanish (Almirante et al., 2005, Pemán et al., 2011) European (5.2%) and North American isolates (6.6%) (Messer et al., 2009, Richardson & Lass-Flörl et al., 2008) (see Table 7). Mixing the ideas exposed above we can argue that the differences in the activity and susceptibility of the antifungal compounds studied suggest that azole drugs and echinocandins have got a complementary susceptibility profile. While azoles has got excellent in vitro activity to *C. albicans*, *C. parapsilosis* and *C. tropicalis* bloodstream isolates, echinocandins showed excellent activity against *C. glabrata* and *C. krusei* which are are associated with higher azole MICs. On the contrary, species with high MICs to echinocandins such as *C. parapsilosis* and *C. guilliermondii* showed excellent activity to azole agents. (Table 8).

Specie of *Candida*	Susceptibility to antifungal agent				
	Amphotericin B	Fluconazole	Voriconazole	Caspofungin	Anidulafungin
C. albicans	S	S	S	S	S
C. parapsilosis	S	S	S	S - I	S – I
C. glabrata	S – NS	S – SDD – R	S – NS	S	S
C. tropicalis	S	S	S – NS	S	S
C. krusei	S	R	S	S	S

[a] S, susceptible; NS, non-susceptible (intermediate for CLSI M27-A2 clinical breakpoints); SDD, sensitive dose-dependent; R, resistant.

[b] The clinical breakpoints adopted in this table are those reflected in the CLSI M27-A2 methodology. No new clinical breakpoints or epidemiological cut-offs were used, in order to make the data comparable to the one reflected in our work.

Table 8. Summary of commonly associated in-vitro susceptibility profiles for *Candida* spp. BSI isolates. (Adapted and modified from Richardson & Lass-Flörl, 2008) [a, b]

These ideas are of great importance because previous exposure to fluconazole was a strong and independent factor associated with candidemia caused by fluconazole non-susceptible isolates as it had been reported previously by Marr *et al.* and Lin and colleagues and higher voriconazole MICs tended to be associated with prior exposure to fluconazole. Although these obtained results are statistically significant, they must be taken with some caution because the low resistant proportion of isolates in our study. However, they depict a situation of concern and illustrate the potential problem of cross-resistance between azoles with a direct impact in treatment failure and the outcome of the patient. Moreover, the potential for voriconazole resistant *C. glabrata* to emerge as a threat in people receiving voriconazole therapy and or prophylaxis has been raised in reports of breakthrough infections (Imhof et al., 2004, Pfaller et al., 2004).

Despite these concerning matters exposed above, voriconazole was the azole which exhibited the best in vitro antifungal activity in our study, and only one of six fluconazole-resistant isolates was cross-resistant to voriconazole. The combination of a third generation azole such as voriconazole or posaconazole with an echinocandin could be of benefit for some patients, especially in those with persistent candidemia.

The crude mortality rate observed in our study was similar to that reported in other series (Almirante et al., 2005, Colombo et al., 2006, Gudlaugsson et al., 2003, Pappas et al., 2003, Pfaller et al., 2004). Adults had higher mortality rates than pediatric patients (24.10% to 16%). Similar to other reports, patients with *C. parapsilosis* candidemia had the lowest death rates (Nucci et al., 1998, Pappas et al., 2003).

Summarizing, the epidemiological and susceptible data described along the text, document important differences and similarities in the epidemiology of candidemia in Spain compared to updated reports from other countries. This report shows that candidemia is a source of significant morbidity and mortality with high associated healthcare costs. Although our high rates of candidemia may be related to many factors, reasons for them are not clear and further study is necessary. Determining them may lead to identify potential measures that can help in disease prevention. In addition, our data support that fluconazole non-susceptibility could be associated with prior fluconazole exposure and suggest that such exposure could lead to other new azoles cross-resistance and complicate the clinical outcome of some patients.

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Epidemiology Insights

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This book represents an overview on the diverse threads of epidemiological research, brings together the expertise and enthusiasm of an international panel of leading researchers to provide a state-of-the art overview of the field. Topics include the epidemiology of dermatomycoses and Candida spp. infections, the epidemiology molecular of methicillin-resistant Staphylococcus aureus (MRSA) isolated from humans and animals, the epidemiology of varied manifestations neuro-psychiatric, virology and epidemiology, epidemiology of wildlife tuberculosis, epidemiologic approaches to the study of microbial quality of milk and milk products, Cox proportional hazards model, epidemiology of lymphoid malignancy, epidemiology of primary immunodeficiency diseases and genetic epidemiology family-based. Written by experts from around the globe, this book is reading for clinicians, researchers and students, who intend to address these issues.

InTech Europe
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