ORIGINAL ARTICLE 10.1111/1469-0691.12380

Epidemiology and predictive factors for early and late mortality in *Candida* bloodstream infections: a population-based surveillance in Spain

M. Puig-Asensio[1], B. Padilla[2,3], J. Garnacho-Montero[4], O. Zaragoza[5], J. M. Aguado[6], R. Zaragoza[7], M. Montejo[8], P. Muñoz[2,3], I. Ruiz-Camps[1], M. Cuenca-Estrella[5], B. Almirante[1] and on behalf of the CANDIPOP Project and GEIH-GEMICOMED (SEIMC) and REIPI

1) *Infectious Diseases Department, Medicine Department, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona,* 2) *Department of Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid,* 3) *Department of Medicine, Universidad Complutense de Madrid, CIBER de Enfermedades Respiratorias (CIBER RES), CD06/06/0058, Palma de Mallorca,* 4) *Critical Care and Emergency Department, Hospital Universitario Virgen del Rocío, Seville,* 5) *Department of Mycology, Spanish National Centre for Microbiology, Instituto de Salud Carlos III, Madrid, Spain,* 6) *Infectious Diseases Unit, Medicine Department, University Hospital 12 de Octubre, Instituto de Investigación I+12, Universidad Complutense de Madrid, Madrid,* 7) *Intensive Care Medicine Department, Hospital Universitario Dr Peset, Valencia, and* 8) *Infectious Diseases Unit, Hospital Universitario de Cruces, Bilbao, Spain*

Abstract

A prospective, multicentre, population-based surveillance programme for *Candida* bloodstream infections was implemented in five metropolitan areas of Spain to determine its incidence and the prevalence of antifungal resistance, and to identify predictors of death. Between May 2010 and April 2011, *Candida* isolates were centralized to a reference laboratory for species identification by DNA sequencing and for susceptibility testing by EUCAST reference procedure. Prognostic factors associated with early (0–7 days) and late (8–30 days) death were analysed using logistic regression modelling. We detected 773 episodes: annual incidence of 8.1 cases/100 000 inhabitants, 0.89/1000 admissions and 1.36/10 000 patient-days. Highest incidence was found in infants younger than 1 year (96.4/100 000 inhabitants). *Candida albicans* was the predominant species (45.4%), followed by *Candida parapsilosis* (24.9%), *Candida glabrata* (13.4%) and *Candida tropicalis* (7.7%). Overall, 79% of *Candida* isolates were susceptible to fluconazole. Cumulative mortality at 7 and 30 days after the first episode of candidaemia was 12.8% and 30.6%, respectively. Multivariate analysis showed that therapeutic measures within the first 48 h may improve early mortality: antifungal treatment (OR 0.51, 95% CI 0.27–0.95) and central venous catheter removal (OR 0.43, 95% CI 0.21–0.87). Predictors of late death included host factors (e.g. patients' comorbid status and signs of organ dysfunction), primary source (OR 1.63, 95% CI 1.03–2.61), and severe sepsis or septic shock (OR 1.77, 95% CI 1.05–3.00). In Spain, the proportion of *Candida* isolates non-susceptible to fluconazole is higher than in previous reports. Early mortality may be improved with strict adherence to guidelines.

Keywords: antifungal resistance, *Candida* bloodstream infections, early mortality, epidemiology, prognostic factors, surveillance
Original Submission: 27 June 2013; **Revised Submission:** 21 August 2013; **Accepted:** 24 August 2013
Editor: M. Paul

Clin Microbiol Infect

Corresponding author: Dr Benito Almirante, Infectious Diseases Department, Hospital Universitari Vall d'Hebron, Pg Vall d'Hebron 119-129, 08035 Barcelona, Spain
E-mail: balmirante@vhebron.net

*Members of the CANDIPOP Project are listed in Appendix 1.

Introduction

European surveillance studies show that the incidence of *Candida* bloodstream infections (BSI) ranges from nearly 3 to 8.6 per 100 000 population per year [1–6].

Despite the introduction of new antifungal agents, this infection remains a severe disease associated with significant mortality [7]. Hence, changes in clinical practices have already occurred, with prophylactic and empirical antifungal therapies in high-risk patients. However, these strategies may be linked to a shift towards non-*albicans* species and the emergence of isolates with decreased fluconazole susceptibility [8].

The epidemiology of candidaemia has been extensively studied in the USA [9–12] and northern and central Europe. In Spain, however, data are limited to surveys conducted in specific areas [6,13] or tertiary centres [14]. Furthermore, we are lacking information about the reasons for the poor current outcome of candidaemia. Studies that have reported determinants of mortality are based on retrospective data or have focused on the impact of therapeutic measures from a restricted viewpoint [15–20].

We conducted a population-based surveillance for *Candida* BSI in Spain to determine its incidence and the distribution and susceptibility pattern of *Candida* species, and to examine prognostic risk factors for mortality.

Materials and Methods

Setting, patients and study design

The CANDIPOP study is a prospective, population-based surveillance programme on *Candida* BSI, conducted from May 2010 to April 2011 in 29 hospitals located in five of the largest municipal areas of Spain: Barcelona, Bilbao, Madrid, Seville and Valencia (population 9 498 980, or 20% of the Spanish population). Patients were identified by local laboratories and reported to study coordinators, who collected data using a standardized case report form. Demographic characteristics, underlying conditions, predisposing risk factors within the preceding month, and 30-day follow-up outcome were recorded in a dedicated database created for the study. Given the observational nature of this research, patients were managed according to routine clinical care.

Audits were carried out to ensure that all cases were reported. The study was approved by the local institutional review boards, and written consent was obtained from patients.

Definitions

Definitions have been described in a previous publication [6]. In brief, an incident case was the first positive *Candida* spp. blood culture. Candidaemias occurring >30 days after the incident episode or isolation of a different *Candida* species after the initial case were considered new episodes. Outpatient-acquired cases were candidaemias detected ≤2 days after hospitalization. The Charlson index was used to represent comorbidity in adults [21]. Sepsis, severe sepsis or septic shock were recorded on the day of candidaemia [22]. Proven catheter-related candidaemia has been described elsewhere [23]. Timing to central venous catheter (CVC) removal and to antifungal administration was the interval between incident blood culture and implementation of these measures. Adequate antifungal treatment was the use of the correct dose of antifungal agent for a susceptible *Candida* isolate (see Supplementary material, Table S1). Patients receiving >3 days of systemic antifungal drug before the first positive blood culture were considered to have breakthrough candidaemias.

Incidence

Population and age-specific incidence rates were expressed as number of cases per 100 000 population, using the 2011 Spanish national census data. Overall incidence of hospitals was calculated using as denominators the summed number of admissions and patient-days of each hospital during the study period.

Microbiological studies

Candida isolates were forwarded to a reference laboratory, the Spanish National Centre for Microbiology in Madrid, for species confirmation and antifungal susceptibility testing. Species identification was performed by sequencing the internal transcribed spacer (ITS) regions from ribosomal DNA. ITS1 and ITS2 regions were directly amplified by PCR from yeast suspensions and sequenced using universal primers [24,25]. Susceptibility to antifungal drugs and interpretation of resistance rates were investigated according to the protocols [26,27] and clinical breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [http://www.eucast.org/clinical_breakpoints/]. Of note, *Candida glabrata* or *Candida guilliermondii* are considered intermediate or resistant to fluconazole, as there is insufficient evidence on whether the wild-type population of these pathogens can be considered fluconazole-susceptible.

Data analysis

Quantitative variables are reported as median and interquartile range (IQR) and qualitative variables as number (%). Categorical data were analysed using the chi-squared or Fisher exact test. Significance was set at a p-value of <0.05. Prognostic factors associated with early (0–7 days) and late (8–30 days) death were assessed using logistic regression analysis. To preserve the assumption of independence of observations, only the first episode of candidaemia recorded for an individual patient was included in this analysis. Neonates and infants younger than 1 year were excluded from the

predictor analysis because epidemiology and risk factors for candidaemia might differ from those described in adults and older children. Episodes caused simultaneously by different *Candida* species were also excluded. Variables with p <0.1 in the univariate analysis and considered clinically relevant were entered in a multivariate model. The best model was selected according to Mallows' Cp statistic. Model adequacy was assessed by the Hosmer–Lemeshow goodness-of-fit test and the area under the receiver operating characteristic curve. For early mortality, it was decided a priori that antifungal treatment and CVC removal would remain in the final multivariate analysis given the belief that these factors might be associated with outcome. Potential confounders of therapy were maintained in the multivariate model. No interactions between variables were found. Statistical analyses were performed with Microsoft SPSS-PC+, version 15.0 (SPSS, Chicago, IL, USA).

Results

Incidence rates

We identified 773 episodes of *Candida* BSI, yielding an annual incidence of 8.1 cases/100 000 inhabitants, 0.89/1000 admissions and 1.36/10 000 patient-days. Differences in incidence rates between geographical areas are shown in the Supplementary material (Table S2). Highest age-specific incidence was observed in infants younger than 1 year (96.4/100 000 inhabitants), and a later peak occurred in persons aged 71–80 years (26.5/100 000 inhabitants) (Fig. 1).

Study population

Twenty-one patients declined to participate. Hence, this report is based on 752 episodes of candidaemia detected in 729 patients. Baseline characteristics of the study population are outlined in Table 1. Ninety (12%) cases were outpatient-acquired, and the remaining occurred among hospitalized patients, including 264 (35.1%) admitted to the intensive care unit. Median length of hospitalization before *Candida* BSI was 22 days (IQR 13–39), and 356 (47.3%) cases had recent healthcare exposure (i.e hospitalization within the previous 3 months).

Clinical data

Sepsis was the clinical presentation of candidaemia in 512 (68.1%) cases. Regarding haematogenous dissemination, ocular candidiasis was reported in 20 cases (2.7%), endocarditis in 14 (1.9%), and metastatic renal infection in three (0.4%). Central nervous system involvement occurred in seven cases (0.9%), all but one in infants. Excluding the 45 patients who survived ≤48 h, follow-up blood samples were obtained in 477 (67.5%) cases at a median of 3 days (IQR 4–8). Of these, 144 (30.1%) cases had persistent candidaemia.

Species distribution and antifungal susceptibility testing

In 159 cases, incident blood culture was polymicrobial: a bacterial strain was identified in 145 (19.3%) and two different *Candida* species were simultaneously isolated in 14 (1.9%). Hence, 766 *Candida* strains were obtained from 752 episodes. *Candida albicans* was the predominant species (348, 45.4%), followed by *Candida parapsilosis* (191, 24.9%), *C. glabrata* (103, 13.4%), *Candida tropicalis* (59, 7.7%), *Candida krusei* (15, 2%), and other rarer species (50, 6.5%). Differences in distribution of *Candida* spp. between metropolitan areas are outlined in the Supplementary material (Figure S1).

Compared with other *Candida* species, *C. parapsilosis* was more likely to occur in children younger than 1 year (18.9% versus 9.9%, p 0.001) and in catheter-related candidaemia (48.1% versus 29.3%, p <0.001). *Candida glabrata* cases were more frequent in persons older than 65 years (62.9% versus 43.4%, p <0.001) and where there was an abdominal source of infection (8.2% versus 2.7%, p 0.011). *Candida albicans* was more often related to previous colonization by the same

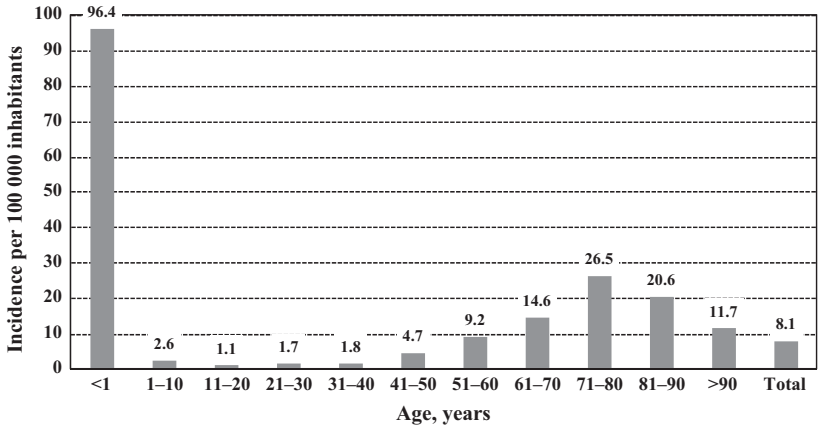

FIG. 1. Annual incidence of candidaemia by age in Spain from 2010 to 2011.

TABLE 1. Baseline characteristics of study population and clinical data of candidaemia episodes according to *Candida* species, Spain (2010–2011)

Characteristic	All cases (*N* = 752)	*Candida* species[a]				
		C. albicans (*n* = 337)	*C. parapsilosis* (*n* = 185)	*C. glabrata* (*n* = 97)	*C. tropicalis* (*n* = 57)	*C. krusei* (*n* = 14)
Demographics						
Median age, years	63 (43–75)	65 (45–75)	54 (6–69.5)	71 (56.5–78.5)	68 (54.5–78.5)	54.5 (47.5–66.8)
Age <1 year	92 (12.2)	43 (12.8)	35 (18.9)	1 (1)	4 (7)	–
Male sex	442 (58.8)	185 (54.9)	122 (65.9)	57 (58.8)	30 (52.6)	10 (71.4)
Outpatient	90 (12)	31 (9.2)	24 (13)	21 (21.6)	4 (7.1)	1 (7.1)
Days in hospital until *Candida* BSI[b]	22 (13–39)	20 (12–34)	28 (17–55)	23 (12.3–36.8)	20 (11–36.5)	24 (13–42.5)
Comorbidities						
Diabetes mellitus	161 (21.4)	76 (22.6)	29 (15.7)	29 (29.9)	16 (28.1)	2 (14.3)
Malignancy (≤1 year)	257/749 (34.3)	101/334 (33.2)	54 (29.2)	42 (43.3)	25 (43.9)	7 (50)
Previous renal failure	194 (25.8)	106 (31.5)	32 (17.3)	26 (26.8)	14 (24.6)	3 (21.4)
Transplant recipient	48 (6.4)	11 (3.3)	15 (8.1)	10 (10.3)	5 (8.8)	3 (21.4)
Liver cirrhosis	32 (4.3)	13 (3.9)	4 (2.2)	5 (5.2)	2 (3.5)	2 (14.3)
HIV infection	16 (2.1)	6 (1.8)	1 (0.5)	3 (3.1)	1 (1.8)	–
Risk factors for candidaemia						
Central venous catheter	581/750 (77.5)	258 (76.6)	156/183 (85.2)	66 (68)	38 (66.7)	13 (92.9)
Total parenteral nutrition	365 (48.5)	176 (52.2)	95 (51.4)	41 (42.3)	20 (35.1)	8 (57.1)
Immunosuppressive therapy[c]	168 (22.3)	68 (20.2)	40 (21.6)	18 (18.6)	14 (24.6)	6 (42.9)
Neutropenia (<500 cell/mm^3)	35 (4.7)	10 (3)	6 (3.2)	3 (3.1)	5 (8.8)	2 (14.3)
Intubation	188/751 (25)	98/336 (29.1)	51 (27.6)	16 (16.5)	7 (12.3)	4 (28.6)
Prior surgery (3 months)	382 (50.8)	181 (53.7)	98 (53)	47 (48.5)	27 (47.4)	5 (35.7)
Abdominal surgery	211 (28.1)	95 (28.2)	54 (29.2)	34 (35.1)	15 (26.3)	4 (28.6)
Prior antibiotic therapy[c]	699/748 (93.5)	324/334 (97)	165/184 (89.7)	87 (89.7)	54 (94.7)	11 (78.6)
Prior fungal therapy[c]	160/751 (21.3)	46 (13.6)	57 (30.8)	23/96 (24)	12 (21.1)	8 (57.1)
Azole exposure	117/750 (15.6)	37 (11)	34 (18.4)	20/96 (20.8)	7/56 (12.5)	7 (50)
Echinocandin exposure	45/751 (6)	9 (2.7)	23 (12.4)	5/96 (5.2)	4 (7)	2 (14.3)
Prior *Candida* colonization	284/750 (37.9)	157/336 (46.7)	50 (27)	42 (43.3)	18 (31.6)	4/13 (30.8)
Source of infection						
Primary	423 (56.3)	202 (59.9)	89 (48.1)	54 (55.7)	33 (57.9)	8 (57.1)
Catheter-related	258 (34.3)	101 (30)	89 (48.1)	24 (24.7)	16 (28.1)	5 (35.7)
Urological	40 (5.3)	22 (6.5)	2 (1.1)	9 (9.3)	5 (8.8)	–
Abdominal	25 (3.3)	9 (2.7)	5 (2.7)	8 (8.2)	3 (5.3)	–
Others	6 (0.8)	3 (0.9)	–	2 (2.1)	–	1 (7.1)
Severity of infection						
Bacteria in incident culture	145 (19.3)	63 (18.7)	38 (20.5)	20 (20.6)	10 (17.5)	5 (35.7)
Septic shock or severe sepsis	240 (31.9)	120 (35.6)	46 (24.9)	30 (30.9)	21 (36.8)	7 (50.7)
Therapeutic measures (≤48 h)						
Adequate antifungal therapy[d]	428/749 (57.1)	203/337 (60.2)	121/184 (65.8)	23/96 (24)[e]	36/56 (64.3)	8/14 (57.1)
CVC removal[f]	275/575 (47.8)	127/253 (50.2)	70/156 (44.9)	38/66 (57.6)	15/38 (39.5)	7/13 (53.8)

Values are reported as no./total no (%) or median (interquartile range) unless otherwise indicated.
BSI, bloodstream infection; CVC, central venous catheter; HIV, human immunodeficiency virus.
[a]Cases in which two *Candida* species were isolated on incident blood culture are not included.
[b]Only includes nosocomial candidaemias, cases with positive blood culture after 2 days of hospitalization.
[c]Within the preceding month. Immunosuppressive therapy includes corticoids, chemotherapy and other immunosuppressive drugs.
[d]Appropriateness of antifungal treatment in the first 48 h was not available for three cases.
[e]We considered that azole use in the first 48 h was unsuitable for treating *C. glabrata* infections in 21 episodes. Among them, one patient died within 7 days.
[f]Data regarding CVC removal in the first 48 h were missing in six of 581 cases.

Candida spp. (46.7% versus 30.3, p <0.001), and *C. tropicalis* to haematological malignancies (17.5% versus 6.3%, p 0.005). Regarding the impact of antifungal exposure within the previous month, *C. parapsilosis* was more frequent in cases with previous use of echinocandins (12.4% versus 4%, p <0.001) and *C. krusei* with previous azole exposure (50% versus 15%, p 0.003).

Table 2 shows antifungal susceptibility results. For fluconazole, 79% (604/766) of *Candida* isolates were susceptible. The resistance rate of *C. tropicalis* was 22% (13/59). However, fluconazole non-susceptible isolates (MIC ≥4 mg/L) were uncommon in both *C. albicans* and *C. parapsilosis* (1% and 5%, respectively). A single case of *Candida kefyr* was resistant to amphotericin B (0.1%). Rates of resistance to echinocandins were 0.3% (1/348) for *C. albicans*, 1% (1/103) *C. glabrata*, 3.4% (2/59) *C. tropicalis*, and no resistance was found among *C. krusei*.

Therapeutic measures

Of 749 cases in 726 patients with available data, 137 (18.3%) were receiving antifungal drugs at blood culture collection; 101 of these episodes were considered breakthrough candidaemias that occurred while receiving azoles (62, 61.4%), echinocandins (26, 25.7%) or amphotericin B (13, 12.9%). Overall, 673 (89.5%) episodes received targeted antifungal therapy for candidaemia. Excluding the 137 cases receiving antifungal drugs at candidaemia onset, treatment was started at a median time of 2 days (IQR 1–3) after the incident blood culture. A detailed description of therapies is provided in the Supplementary material (Table S3).

Outcome and predictors of mortality

Nine patients were lost to follow up before day 30 (three before day 7). Overall, cumulative mortality at 7 and 30 days

TABLE 2. ***In vitro*** **susceptibilities of *Candida* bloodstream isolates to different antifungals**[a]

		MIC (mg/L)								
Species	Value	Amphotericin B	Flucytosine	Fluconazole	Itraconazole	Voriconazole	Posaconazole	Caspofungin	Micafungin	Anidulafungin
C. albicans	GM	0.051	0.16	0.21	0.016	0.016	0.015	0.31	0.03	0.03
	MIC_{90}	0.12	0.5	0.25	0.015	0.015	0.015	0.5	0.03	0.03
	Range	0.03–0.25	0.12–32	0.12–>64	0.015–>8	0.015–>8	0.015–8	0.12–2	0.03–1	0.03–0.25
C. parapsilosis	GM	0.14	0.49	0.48	0.018	0.017	0.016	1.36	0.84	0.93
	MIC_{90}	0.25	0.25	1	0.03	0.03	0.015	2	2	2
	Range	0.03–1	0.12–0.5	0.12–64	0.015–0.25	0.015–0.5	0.015–0.12	0.5–4	0.25–4	0.12–4
C. glabrata	GM	0.106	0.133	3.074	0.127	0.12	0.124	0.44	0.031	0.031
	MIC_{90}	0.25	0.12	16	1	0.5	0.5	1	0.03	0.03
	Range	0.03–0.5	0.12–>64	0.5–>64	0.015–>8	0.015–8	0.015–8	0.25–1	0.03–1	0.03–0.5
C. tropicalis	GM	0.079	0.154	1.83	0.057	0.13	0.047	0.41	0.034	0.034
	MIC_{90}	0.12	0.12	>64	8	>8	8	0.5	0.03	0.03
	Range	0.03–0.5	0.12–32	0.12–>64	0.015–>8	0.015–>8	0.015–>8	0.12–2	0.03–2	0.03–1
C. krusei	GM	0.236	3.48	33.5	0.066	0.3	0.048	0.87	0.08	0.034
	MIC_{90}	0.5	4	64	0.25	0.5	0.12	1	0.12	0.03
	Range	0.12–0.5	2–4	16–64	0.015–0.25	0.015–1	0.015–0.12	0.5–2	0.06–0.12	0.03–0.06
C. guilliermondii	GM	0.067	0.127	3.22	0.22	0.12	0.075	0.9	0·33	0.5
	MIC_{90}	0.12	0.25	16	1	0.25	0.25	2	0·5	1
	Range	0.03–0.12	0.12–0.25	0.12–32	0.06–2	0.06–0.25	0.015–0.25	0.5–2	0.25–0.5	0.25–1
C. lusitaniae	GM	0.06	0.287	0.45	0.02	0.023	0.018	0.92	0.05	0.036
	MIC_{90}	0.25	>64	32	0.12	0.5	0.06	1	0.06	0.12
	Range	0.03–0.25	0.12–>64	0.12–64	0.015–0.25	0.015–0.5	0.015—0.06	0.5–1	0.03–0.06	0.03–0.12

GM, geometric mean; MIC, minimum inhibitory concentration.
[a]The table describes susceptibility of species with higher prevalence ($n \geq 10$) in the CANDIPOP study.

after the first episode of candidaemia was 12.8% (93/726) and 30.6% (220/720), respectively.

On univariate analysis, numerous factors were associated with early mortality in patients older than 1 year (Table 3). When adjusted by primary source of candidaemia and severity of infection (severe sepsis or septic shock) in the multivariate regression analysis, appropriate antifungal treatment within the first 48 h was the only factor independently associated with lower mortality. To explore whether antifungal therapy was affected by CVC removal, a secondary analysis was performed in patients with CVCs. On multivariate analysis, adequate antifungal treatment (OR 0.51, 95% CI 0.27–0.95) and having the CVC removed (OR 0.43, 95% CI 0.21–0.87) within the first 48 h remained associated with decreased early mortality.

Independent risk factors for late mortality were related to host characteristics (age, immunosuppression), clinical presentation of candidaemia (septic shock or severe sepsis and primary infection), and signs of organ dysfunction (intubation and previous renal replacement therapy) (Table 4). To further assess the influence of CVC removal on late mortality, separate logistic regression analyses were performed. The possible benefit of CVC removal on univariate analysis disappeared after including host factors and clinical data in the multivariate analysis (OR 0.72, 95% CI 0.43–1.22). Similar results were obtained in patients with catheter-related candidaemia: none of the treatment-related factors were significantly associated with late mortality.

Because no paediatric severity of illness score was measured during the study and comorbidities may have an effect on outcome, similar multivariate models for early and late mortality were explored in the subgroup of adults (≥18 years). After entering the Charlson index as an independent variable, the benefit of therapeutic measures on early mortality remained stable. Conversely, the Charlson index was added as a prognostic factor for late mortality (OR 1.13, 95% CI 1.00–1.28) (data not shown).

Discussion

Candidaemia remains a life-threatening infection, especially in patients with severe underlying conditions. As our results show, overall incidence of *Candida* BSI in Spain is 8.1/100 000 population. Although we lack comparative studies, our incidence rate is similar to those of most population-based studies conducted in the USA between 1992 and 2001 [9–12] (6–8/100 000 population/year) and is comparable to a recent national surveillance performed in Denmark [4] (8.6/100 000 population). However, it differs from most northern and central European countries [2,3,5], which have described a much lower disease burden (nearly 3–5.7/100 000 population) and contrasts with the high rates in a contemporary US survey conducted in Atlanta and Baltimore (13.3 and 26.2 cases/100 000 population, respectively) [12]. In general, these geographic variations probably reflect demographic differences or variations in patient management.

In accordance with previous surveillance reports, the highest incidence rates are at the extremes of age. Of note, however, we found an unexpectedly high peak in children younger than 1 year (96.4 cases/100 000 population) in comparison with other European surveys [2–5] (range, 9.4–20.7/100 000 population).This result diverges from the

TABLE 3. Univariate and multivariate logistic regression analyses of prognostic factors for early mortality (0–7 days) in 629[a] patients

Variable	Alive (*n* = 547)	Died (*n* = 82)	Univariate analysis		Multivariate analysis[b]	
			OR (95% CI)	p value	OR (95% CI)	p value
Host factors						
Age, years	65.6 (51.3–76.0)	72.2 (55.4–80.2)	1.02 (1.01–1.04)	0.001	1.02 (1.01–1.04)	0.011
Male sex	317 (58)	57 (69.5)	1.65 (1.00–2.73)	0.048		
Charlson index[c]	2 (1–3)	3 (2–5)	1.23 (1.10–1.37)	<0.001		
Malignancy (≤1 year)	214/546 (39.2)	28/80 (35)	0.84 (0.52–1.36)	0.472		
Transplant recipient	42 (7.7)	3 (3.7)	0.46 (0.14–1.51)	0.199		
HIV infection	13 (2.4)	1 (1.2)	0.51 (0.07–3.93)	0.516		
Immunosuppressive therapy	137 (25)	16 (19.5)	0.73 (0.41–1.30)	0.278		
Neutropenia (<500 cell/mm^3)	29 (5.3)	5 (6.1)	1.16 (0.44–3.01)	0.766		
Abdominal surgery	166 (30.3)	20 (24.4)	0.74 (0.43–1.27)	0.272		
Previous RRT	40 (7.3)	5 (6.1)	0.82 (0.32–2.15)	0.691		
Intubation	111 (20.3)	21 (25.6)	1.35 (0.79–2.32)	0.272		
Clinical data						
Primary source	278 (50.8)	64 (78)	3.44 (1.99–5.96)	<0.001	3.43 (1.90–6.19)	<0.001
Catheter-related	208 (38)	11 (13.4)	0.25 (0.13–0.49)	<0.010		
Antifungal agent at time of blood culture collection	91/545 (16.7)	16 (19.5)	1.21 (0.67–2.18)	0.528		
Severe sepsis or septic shock	143 (26.1)	53 (64.6)	5.16(3.16–8.44)	<0.001	6.56 (3.85–11.17)	<0.001
Bacteria in incident culture	101 (18.5)	15 (18.3)	0.99 (0.54–1.80)	0.970		
Candida species						
C. albicans	249 (45.5)	38 (46.3)	1.03 (0.65–1.65)	0.889		
C. parapsilosis	132 (24.1)	12 (14.6)	0.54 (0.28–1.03)	0.060		
C. glabrata	77 (14.1)	15 (18.3)	1.37 (0.74–2.51)	0.315		
C. tropicalis	43 (7.9)	8 (9.8)	1.27 (0.57–2.80)	0.559		
C. krusei	10 (1.8)	4 (4.9)	2.75 (0.84–8.99)	0.093	3.10 (0.83–11.60)	0.092
Therapeutic measures (≤48 h)						
Adequate antifungal treatment	306/544 (56.3)	25 (30.5)	0.34 (0.21–0.56)	<0.001	0.35 (0.20–0.61)	
CVC removal[d]	206/416 (49.5)	13/55 (23.6)	0.32 (0.17–0.61)	0.001		

Values are reported as no./total no (%) or median (interquartile range).
CI, confidence interval; CVC, central venous catheter; HIV, human immunodeficiency virus; OR, odds ratio; RRT, renal replacement therapy.
[a]Only the first episode of candidaemia was included for patients with multiple episodes. Neonates and infants younger than 1 year, cases of candidaemia caused simultaneously by different species of *Candida*, and two adult patients who were lost to follow up in this period were excluded from the analysis.
[b]The best multivariate analysis model according to Mallows' Cp statistic included the following variables: age, primary source, severe sepsis or septic shock, *Candida krusei* and adequate antifungal treatment (Hosmer–Lemeshow p 0.90; area under the curve = 0.81).
[c]Recorded in adults (*n* = 600).
[d]Subset of patients with available information regarding CVC removal (*n* = 471 out of 474). Multivariate analysis in patients with CVC was as follows: increasing age (OR 1.03, 95% CI 1.00–1.05, p 0.017), primary source (OR 2.25, 95% CI 1.17–4.33, p 0.015), severe sepsis or septic shock (OR 4.50, 95% CI 2.40–8.44, p <0.001), *C. krusei* (OR 4.16, 95% CI 1.07–16.12, p 0.039), adequate antifungal treatment (OR 0.51, 95% CI 0.27–0.95, p 0.033), and CVC removal within 48 h (OR 0.43, 95% CI 0.21–0.87, p 0.019) (Hosmer–Lemeshow p 0.11; AUC = 0.79).

epidemiological trend towards the increasing relevance of the elderly population as the most important age-specific group affected by candidaemia [2,4,12]. We believe the high incidence rate in our infants is influenced by at least two factors. First, all the study regions included referral paediatric and neonatal units for community hospitals from other areas, which could have contributed to an overestimate of the incidence rates. Second, there was a high percentage of *C. parapsilosis* in children younger than 1 year in comparison with adults. Although the relevance of *C. parapsilosis* in neonatal candidaemia is well-recognized [28,29] and an endemic situation cannot be ruled out [30] these results may reflect the presence of nosocomial outbreaks and the need to improve infection control practices. Therefore, further molecular studies of *C. parapsilosis* strains are required, and particular consideration should be given to fluconazole prophylactic therapy in low-birthweight neonates, according to guidelines [31,32].

Over the last decade, there have been no substantial changes in *Candida* species distribution in Spain. The only exception is a possible increase in the percentage of *C. glabrata*, particularly in the elderly. In the present study, *C. glabrata* was the third most common species (13.4%), whereas in reports from the early 2000s its proportion was <9% [6,33]. Although these studies involved different surveillance regions and are not entirely comparable, the same trends have been reported in the USA [10,11], Denmark and Finland [2,4]. It is suggested that the rise in *C. glabrata* may be due to widespread azole use. However, this association remains unclear, and other host factors and medical practices may have contributed [34,35].

Our findings confirm that fluconazole susceptibility has decreased in Spain. Earlier studies in Barcelona [6,36] showed that >90% of *Candida* isolates were fluconazole-susceptible, whereas the present report documents fluconazole susceptibility at <80%. Although this decrease is mainly due to rises in *C. glabrata* infection, we also found significant fluconazole resistance in *C. tropicalis* strains (22%), never before reported in Spain. Resistance to echinocandins was very low, except for *C. parapsilosis*, which exhibited higher MICs than those of other *Candida* species. The clinical relevance of these findings warrants analysis in further studies because the correlation between MIC and clinical response to echinocandins remains uncertain.

TABLE 4. Univariate and multivariate logistic regression analyses of prognostic factors for late mortality (8–30 days) in 542[a] patients

Variable	Alive (*n* = 431)	Died (*n* = 111)	Univariate analysis		Multivariate analysis[b]	
			OR (95% CI)	p value	OR (95% CI)	p value
Host factors						
Age, years	64.7 (50.4–75.6)	68.7 (59.2–78.9)	1.02 (1.00–1.03)	0.001	1.03 (1.02–1.05)	<0.001
Male sex	256 (59.4)	57 (51.4)	0.72 (0.48–1.10)	0.127		
Charlson index[c]	2 (1–3)	3 (2–4)	1.18 (1.06–1.31)	0.002		
Malignancy (≤1 year)	168/430 (39.1)	44 (39.6)	1.02 (0.67–1.57)	0.913		
Transplant recipient	31 (7.2)	11 (9.9)	1.42 (0.69–2.92)	0.342		
HIV infection	9 (2.1)	4 (3.6)	1.75 (0.53–5.80)	0.358		
Immunosuppressive therapy[d]	100 (23.2)	36 (32.4)	1.59 (1.00–2.51)	0.047	2.50 (1.48–4.21)	0.001
Abdominal surgery	141 (32.7)	24 (21.6)	0.57(0.35–0.93)	0.025		
Neutropenia (<500 cell/mm^3)	20 (4.6)	9 (8.1)	1.81 (0.80–4.10)	0.153		
Previous RRT	22 (5.1)	18 (16.2)	3.60 (1.86–6.98)	<0.001	2.87 (1.34–6.15)	0.007
Intubation	65 (15.1)	46 (41.4)	3.99 (2.51–6.32)	<0.001	4.24 (2.42–7.42)	<0.001
Clinical data						
Primary source	208 (48.3)	68 (61.3)	1.70 (1.11–2.60)	0.015	1.63 (1.03–2.61)	0.039
Catheter-related	172 (39.9)	34 (30.6)	0.67 (0.43–1.04)	0.074		
Severe sepsis or septic shock	95 (22)	48 (43.2)	2.70 (1.74–4.18)	<0.001	1.77 (1.05–3.00)	0.034
Antifungal agent at time of blood culture collection	70/429 (16.3)	21 (18.9)	1.20 (0.70–2.05)	0.514		
Bacteria in incident culture	75 (17.4)	25 (22.5)	1.38 (0.83–2.30)	0.216		
Persistent candidaemia[e]	86/308 (27.9)	26/74 (35.1)	1.40 (0.82–2.40)	0.222		
Candida species						
C. albicans	186 (43.2)	60 (54.1)	1.55 (1.02–2.36)	0.041		
C. parapsilosis	110 (25.5)	21 (18.9)	0.68 (0.40–1.15)	0.149		
C. glabrata	64 (14.8)	13 (11.7)	0.76 (0.40–1.44)	0.400		
C. tropicalis	35 (8.1)	7 (6.3)	0.76 (0.33–1.76)	0.525		
C. krusei	7 (1.6)	3 (2.7)	1.68 (0.43–6.61)	0.456		
Therapeutic measures (≤48 h)						
Adequate antifungal treatment	245/429 (57.1)	60/110 (54.5)	0.90 (0.59–1.37)	0.628		
CVC removal[f]	167/321 (52)	38/93 (40.9)	0.64 (0.40–1.02)	0.059		

Values are reported as no./total no (%) or median (interquartile range).
CI, confidence interval; CVC, central venous catheter; HIV, human immunodeficiency virus; OR, odds ratio; RRT, renal replacement therapy.
[a]Only the first episode of candidaemia was included for patients with multiple episodes. Neonates and infants younger than 1 year, cases of candidaemia caused simultaneously by different species of *Candida*, deaths that occurred at days 0–7, and five adult patients who were lost to follow up in this period were excluded from the analysis.
[b]The best multivariate analysis model according to Mallows' Cp statistic included the following variables: age, immunosuppressive therapy, previous RRT, intubation, primary source and severe sepsis or septic shock (Hosmer–Lemeshow p 0.75; area under the curve = 0.76).
[c]Recorded in adults (*n* = 513).
[d]Includes corticoids, chemotherapy and other immunosuppressive drugs within the preceding month.
[e]Persistent candidaemia was defined as persistently positive blood cultures for ≥3 days after the incident blood sample. Analysis performed in the subset of patients with follow-up blood cultures (*n* = 382).
[f]Subset of patients with available information regarding CVC removal (*n* = 414 out of 417). Multivariate analysis in patients with CVC was as follows: age (OR 1.04, 95% CI 1.02–1.05, p <0.001), previous RRT (OR 2.84, 95% CI 1.28–6.31, p 0.011), intubation (OR 3.57, 95% CI 1.98–6.45, p <0.001), primary source (OR 1.90, 95% CI 1.13–3.18, p 0.015), severe sepsis or septic shock (OR 1.42, 95% CI 0.79–2.57, p 0.243), and CVC removal (OR 0.72, 95% CI 0.43–1.22, p 0.222) (Hosmer–Lemeshow p 0.32; area under curve = 0.77).

Overall 30-day mortality in our study was high (30.6%), but similar to recent data [2,5]. Multivariate analysis suggested the benefit of prompt therapeutic measures for decreasing early mortality, in keeping with previous reports. Furthermore, and for the first time, it was clearly seen that host factors and severity of infection were the main variables influencing mortality in the later period. These results support current guidelines, which consider appropriate antifungal therapy and catheter removal as the cornerstones of treatment for candidaemia. Nonetheless, prompt CVC removal as a prognostic factor of mortality remains controversial. Current data are provided by observational studies [18,19], and some reports have failed to demonstrate the benefit of CVC removal on outcome [16,20]. We believe CVC management should be carefully evaluated in each patient and removal performed whenever possible, especially if the catheter is the suspected source of infection [19].

In light of the low percentage of adequate antifungal treatment for *C. glabrata* found in this study, we considered that azoles at any dose were inappropriate for non-susceptible isolates [http://www.eucast.org/clinical_breakpoints/]. This interpretation could be controversial and might have biased the benefit of antifungal therapy towards the reduction of its effect. However, even with this definition, antifungal treatment was associated with better early survival. Further studies are needed to elucidate whether fluconazole is a good option for *C. glabrata* in terms of clinical and microbiological responses.

This study has some limitations. First, the epidemiology described is influenced by local medical practices, which limits the ability to generalize the results to other geographical areas. Second, it was difficult to control the analysis of prognostic risk factors of mortality for the variable severity of illness, since APACHE II score was only available in adult intensive care unit patients. Nevertheless, we used other markers related with concurrent illnesses (Charlson index) and we explored a wide range of clinical variables to adjust for confounding factors, which lends strength to the results. Third, the precise number of metastatic candidiasis cases could not

be determined because diagnostic procedures were performed at the physicians' discretion.

In conclusion, candidaemia is a severe infection that remains associated with high morbidity and mortality. Although our results confirm that the poor prognosis may be strongly associated with the fragile status of affected patients in whom the risk of death is inherently high, we should focus on the control of modifiable risk factors for mortality and improve adherence to guidelines. Prompt initiation of appropriate antifungal treatment and CVC removal could decrease early mortality in these patients.

Acknowledgements

We thank Celine Cavallo for English language support and Núria Fernández-Hidalgo and Santiago Pérez-Hoyos for statistical assistance.

Funding

This work was supported by research grants from Gilead, MSD, Astellas and Pfizer and by funding from Fundación SEIMC-GESIDA and Ministerio de Economía y Competitividad, Instituto de Salud Carlos III, co-financed by the European Development Regional Fund "A way to achieve Europe" ERDF, Spanish Network for the Research in Infectious Diseases (REIPI RD12/0015). These sources of funding had no involvement in the preparation of the manuscript.

Authors Contributions

BP, JGM, IRC, BA and MCE conceived, designed and coordinated the study. BP, JGM, JMA, RZ, MM and BA were local study coordinators ensuring correct data collection. OZ and MCE were responsible for *Candida* species confirmation and antifungal susceptibility testing. MPA and BA were responsible for data analysis and interpretation, and prepared the final version of the article. JGM, JMA, RZ, MM, PM and IRC contributed to the original intellectual content, reviewing and adding a critique of the report. All authors read the manuscript and approved the final version.

Transparency Declaration

BP has received honoraria for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas and Novartis. JGM has received honoraria for talks on behalf of Gilead Sciences, Merck Sharp and Dohme and Astellas. JMA has received grant support from Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer, The Instituto de Salud Carlos III and The Mutua Madrileña Foundation. He has been an advisor/consultant to Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme and Pfizer. He has received honoraria for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer and Astellas Pharma. RZ has received honoraria for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer and Astellas and a grant support from Pfizer. MM has received grant support from Pfizer, Astellas Pharma, Novartis, Merck Sharp and Dohme and Gilead Sciences. PM has received honoraria for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas and Novartis. IRC has received honoraria for talks on behalf of Pfizer, Merck Sharp and Dohme, Gilead, Astellas and Novartis. MCE has received grant support from Astellas Pharma, bioMérieux, Gilead Sciences, Merck Sharp and Dohme, Pfizer, Schering Plough, Soria Melguizo SA, Ferrer International, the European Union, the ALBAN programme, the Spanish Agency for International Cooperation, the Spanish Ministry of Culture and Education, the Spanish Health Research Fund, the Instituto de Salud Carlos III, the Ramon Areces Foundation and the Mutua Madrileña Foundation. He has been an advisor/consultant to the Pan-American Health Organization, Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering Plough. He has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering Plough. BA has received grant support from Gilead Sciences, Pfizer and the Instituto de Salud Carlos III, and he has received honoraria for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas and Novartis. MPA and OZ declare that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Percentages of *Candida* species among the 766 isolates according to each metropolitan area of Spain.

Table S1. Definitions used to classify the appropriateness of antifungal treatment.

Table S2. Incidence of candidaemia and characteristics of participating centres.

Table S3. Initial therapeutic measures for *Candida* bloodstream episodes in paediatric and adult patients.

Appendix 1

Other members of the CANDIPOP project

Jesús Guinea (Hospital General Universitario Gregorio Marañón), José Ramón Paño, Julio García and Carlos García (Hospital La Paz, Madrid), Jesús Fortún, Pilar Martín and Elia Gómez (Hospital Ramón y Cajal, Madrid), Pablo Ryan and Carolina Campelo (Hospital Infanta Leonor, Madrid), Ignacio de los Santos and Buenaventura Buendía (Hospital Universitario La Princesa, Madrid), Beatriz Perez and Mercedes Alonso (Hospital Niño Jesús, Madrid), Francisca Sanz (Hospital 12 de Octubre, Madrid), Paloma Merino and Fernando González (Hospital Clínico, Madrid), Miguel Gorgolas and Ignacio Gadea (Fundación Jiménez Díaz, Madrid), Juan Emilio Losa and Alberto Delgado-Iribarren (Hospital de Alcorcón, Madrid), Antonio Ramos, Yolanda Romero and Isabel Sánchez (Hospital Majadahonda, Madrid), Jesús Rodriguez-Baño and Ana Isabel Suarez (Hospital Universitario Virgen Macarena, Sevilla), Ana Loza, Estrella Martín-Mazuelos and Ana Isabel Aller (Hospital Universitario Virgen de Valme, Sevilla), Maite Ruiz (Hospital Universitario Virgen del Rocío, Sevilla), Carlos Ortiz (Hospital Sagrado Corazón, Sevilla), Mónica Chávez and Fernando L. Maroto (Hospital San Juan de Dios de Aljarafe, Sevilla), Miguel Salavert and Javier Pemán (Hospital Universitario la Fe, Valencia), José Blanquer and David Navarro (Hospital Clínico Universitario Valencia), Vicente Abril and Concepción Gimeno (Consorcio Hospital General Universitario de Valencia), Juan José Camarena (Hospital Universitario Dr. Peset, Valencia), Silvia Hernáez and Guillermo Ezpeleta (Hospital de Basurto, Bilbao), Elena Bereciartua, José L. Hernández (Hospital Universitario de Cruces, Bilbao), Rosa Ana Rivas and Rafael Ayarza (Hospital de Galdakano, Bilbao), Ana Mª Planes (Hospital Universitari Vall d'Hebron, Barcelona), José Mensa and Manel Almela (Hospital Clínic-IDIBAPS Barcelona), Mercè Gurgui and Ferran Sánchez-Reus (Hospital Universitari de Sant Pau i Santa Creu, Barcelona), Joaquin Martinez-Montauti and Montserrat Sierra (Hospital de Barcelona, Barcelona), Juan Pablo Horcajada, Luisa Sorli and Julià Gómez (Hospital del Mar, Barcelona), Amadeu Gené and Mireia Urrea (Hospital Sant Joan de Déu, Esplugues de Llobregat-Barcelona). *Study collaborators of CANDIPOP Project*: Maricela Valerio, Mario Fernández-Ruiz, Ana Díaz-Martín, Francesc Puchades, and Alessandra Mularoni.

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Change bold to non-bold type	(As above)	⌐
Insert 'superior' character	/ through character or ⌐ where required	Y or Y under character e.g. Y^{3} or Y^{3}
Insert 'inferior' character	(As above)	⌐ over character e.g. Y_{2}
Insert full stop	(As above)	⊙
Insert comma	(As above)	,
Insert single quotation marks	(As above)	Y or Y and/or Y or Y
Insert double quotation marks	(As above)	Y or Y and/or Y or Y
Insert hyphen	(As above)	⊢
Start new paragraph	⌐	⌐
No new paragraph	⌐	⌐
Transpose	⌐	⌐
Close up	linking ⁀ characters	⁀
Insert or substitute space between characters or words	/ through character or ⌐ where required	Y
Reduce space between characters or words	| between characters or words affected	⊤