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Prospective Multicenter Study of the Epidemiology, Molecular Identification, and Antifungal Susceptibility of *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* Isolated from Patients with Candidemia▽

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A 13-month prospective multicenter study including 44 hospitals was carried out to evaluate the epidemiology of *Candida parapsilosis* complex candidemia in Spain. Susceptibility to amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, caspofungin, and micafungin was tested by the microdilution colorimetric method. A total of 364 *C. parapsilosis* complex isolates were identified by molecular methods: *C. parapsilosis* (90.7%), *Candida orthopsilosis* (8.2%), and *Candida metapsilosis* (1.1%). Most candidemias (*C. parapsilosis*, 76.4%; *C. orthopsilosis*, 70.0%; *C. metapsilosis*, 100%) were observed in adults. No *C. orthopsilosis* or *C. metapsilosis* candidemias occurred in neonates. *C. parapsilosis* was most frequent in adult intensive care unit (28.8%), surgery (20.9%), and internal medicine (19.7%) departments; and *C. orthopsilosis* was most frequent in hematology (28.6%), pediatrics (12.0%), and neonatology (11.5%) departments. The geographic distribution of *C. orthopsilosis* and *C. metapsilosis* was not uniform. According to CLSI clinical breakpoints, all *C. orthopsilosis* and *C. metapsilosis* isolates were susceptible to the nine agents tested. Resistance (MICs > 1 mg/liter) was observed only in *C. parapsilosis*: amphotericin B, posaconazole, itraconazole, and caspofungin (0.3% each), anidulafungin (1.9%), and micafungin (2.5%). Applying the new species-specific fluconazole and echinocandin breakpoints, the rates of resistance to fluconazole for *C. parapsilosis* and *C. orthopsilosis* increased to 4.8% and 0.3%, respectively; conversely, for *C. parapsilosis* they shifted from 1.9 to 0.6% (anidulafungin) and from 2.5 to 0.6% (micafungin). Our study confirms the different prevalence of *C. parapsilosis* complex candidemia among age groups: neither *C. orthopsilosis* nor *C. metapsilosis* was isolated from neonates; interestingly, *C. metapsilosis* was isolated only from adults and the elderly. The disparity in antifungal susceptibility among species could be important for therapy.

The incidence of invasive candidiasis has been increasing throughout the world in recent years, and invasive candidiasis is associated with a high mortality rate (41). At present, a large proportion of bloodstream infections are due to non-*Candida albicans Candida* spp., with *Candida parapsilosis* being the second most commonly isolated *Candida* spp. from blood cultures in Asia, Latin America, and some European countries (2, 16, 35, 36, 40). Furthermore, *C. parapsilosis* is the second most common yeast causing candidemia in children (3, 5, 57), and it has even become the predominant species in some pediatric hospitals (29, 33).

C. parapsilosis is a common saprophyte of the skin, and many infections caused by this species are associated with its carriage on the hands of health care workers (6). This species is commonly related to catheter- and intravenous hyperalimentation-associated candidemia due to its capability to adhere to and develop biofilms on the surfaces of intravascular devices (7, 27,

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45). In fact, *C. parapsilosis* has emerged as a significant nosocomial pathogen, with clinical manifestations that include endophthalmitis, endocarditis, septic arthritis, peritonitis, and fungemia usually being associated with invasive procedures or prosthetic devices (54).

On account of its heterogeneity, *C. parapsilosis* was divided into three groups on the basis of differences of randomly amplified polymorphic DNA (RAPD), DNA sequencing of different genes, and morphotyping (10, 25, 28, 30, 44). In 2005, Tavanti et al. (48) suggested that the *C. parapsilosis* complex could be replaced by 3 different closely related species named *C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis*. These species may show differences in antifungal susceptibilities and virulence, and an increased interest in the study of their epidemiology has arisen in the last years (8, 19, 23, 31, 34, 46, 47, 51, 52). Interestingly, the incidence of *C. orthopsilosis* and *C. metapsilosis* infections may have increased since 2004 (31), with prevalence rates ranging from 2.3 to 9% and 0.9 to 6.9%, respectively, depending on the geographical area and clinical specimens analyzed. However, there are few national reports, including reports with extensive demographic information, such as age, hospitalization unit, or the patient's underlying condition. The object of this study was to describe the epidemiological characteristics, clinical significance, and *in vitro* susceptibilities to nine systemic antifungal agents of *C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis* isolates yielded in the FUNGEMYCA multicenter study (35).

MATERIALS AND METHODS

Study design. The FUNGEMYCA survey was a prospective, sequential, hospital population-based study. Forty-four Spanish institutions, widely distributed throughout the country, including the Canary and Balearic Islands, participated in this study. Participating hospitals were required to collect and identify the isolates from blood cultures and to complete, for each fungemia episode, a questionnaire with demographic information, clinical signs of sepsis, and risk factors or predisposing diseases within the preceding 30 days. Approval for the study was obtained from the ethic committees of all participating institutions.

Period of study. The study was carried out over a 13-month period, from January 2009 to February 2010.

Definitions. An episode of fungemia was defined as the isolation of a yeast or mold species from a culture of blood from a patient with temporally related clinical signs and symptoms. In patients with more than one episode of fungemia, an episode was defined as a new case if it occurred more than 30 days after the previous episode. Outpatient-acquired fungemia was considered when the fungal etiologic agent was isolated in blood in the first 48 h after hospital admission. Neonates were defined to be less than 30 days of age; children were between 1 month and 15 years old, adults were between 16 and 64 years old, and the elderly were more than 64 years old.

Identification of organisms and antifungal susceptibility testing. All yeast species isolated from blood cultures were identified at the participating institutions by the routine methods in use at each laboratory: AUXACOLOR (Bio-Rad, Madrid, Spain) or API 20C AUX, ID 32C, or Vitek-2 (bioMérieux, Madrid, Spain). Isolates were stored as suspensions in sterile water at ambient temperature for ulterior studies. Antifungal susceptibility testing was performed in the first isolate from each fungemia episode at the participating hospitals by the microdilution colorimetric Sensititre YeastOne SYO-09 panel (TREK Diagnostic Systems, Cleveland, OH). This commercial method determines the MICs of nine antifungal agents: amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, caspofungin, and micafungin. To categorize the isolates, CLSI breakpoints have been applied (13), on the basis of the good correlation between the two methods. Since no breakpoints have been published for posaconazole and amphotericin B, isolates inhibited by >1 mg/liter were considered resistant to these drugs. The recently published species-specific clinical breakpoints for fluconazole and echinocandins were also applied (14, 38, 42). Isolates for which fluconazole MICs were ≤2 mg/liter were categorized susceptible and were categorized resistant if MICs were >4 mg/liter. Furthermore, for the three echinocandins, isolates inhibited by ≤2 mg/liter and >4 mg/liter were classified susceptible and resistant, respectively. As a control, *C. parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were assayed in each center before the start of the study. All MIC results for control strains were within the ranges for susceptible and resistant.

Molecular identification of cryptospecies from *C. parapsilosis*. The identities of *C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis* isolates were confirmed as previously described by Tavanti et al. (48, 49).

Isolates were grown on fresh Sabouraud agar (Difco, St. Louis, MO) and incubated at 37°C for 24 h. A 3-μl equivalent of yeast was scraped from the plate and resuspended in 20 μl of sterile water. The yeast suspensions were treated by heating to 95°C for 8 min and then placed in a −80°C freezer for 1 to 2 h.

For identification of *C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis*, the amplification of the *SADH* gene was performed by PCR with the primers S1F (5′-TTGATGCTGTTGGATTGT-3′) and S1R (5′-CAATGCCAA ATCTCCCAA-3′), which amplify a fragment of 716 bp (48, 49). PCR mixtures were prepared as suggested by the manufacturer (Bioline, London, United Kingdom). Each 25-μl reaction mix contained 2 μl of the prepared yeast supernatant. The amplification conditions were as follows: a first cycle of 5 min at 95°C, followed by 40 cycles at 92°C for 1 min, at 45°C for 1 min, and at 68°C for 1 min, with a final extension step of 10 min at 68°C. The PCR product was then digested with BanI enzyme (New England BioLabs, Ipswich, MA) in a 40-μl volume containing 20 μl of the PCR product and 40 U of BanI and incubated at 37°C for 2 h. The digestion products were separated on a 1% agarose gel, stained with GelRed, and visualized with UV light. Isolates of *C. parapsilosis* sensu stricto, *C. orthopsilosis*, and *C. metapsilosis* were identified by differences in *SADH* amplicons containing the restriction sites that were one, zero (no restriction site), and three BanI restriction sites, respectively. All isolates identified as *C. orthopsilosis* or *C. metapsilosis* were digested a second time for confirmation. Furthermore, the identities of these species were confirmed by DNA sequencing of *ITS1* and *ITS4* regions of the 28S rRNA gene to avoid the possibility of misidentification due to a possible point mutation in *C. parapsilosis* sensu stricto. The PCR was carried out with panfungal *ITS1* and *ITS4* primers (55, 56) under the following conditions: a first cycle of 5 min at 95°C, followed by 30 cycles at 95°C for 1 min, at 60°C for 30 s, and at 72°C for 1 min, with a final extension step of 10 min at 72°C. The amplicons were sequenced and BLAST searches were performed for species identification.

Statistical analyses. Data were analyzed with SPSS software (version 10.0.7; SPSS Inc., Chicago, IL). Continuous variables were compared by Student's *t* test, and categorical variables were compared by the chi-square test or Fisher's exact test. Comparison of antifungal susceptibility was carried out with $\log_2$ MIC. Differences in antifungal susceptibility patterns among species and age groups were evaluated using one-way analysis of variance with Bonferroni adjustment for multiple comparisons. A P value of <0.05 was considered significant.

RESULTS

During the study period (January 2009 to February 2010), 1,356 cases of fungemia were included in the FUNGEMYCA project. *C. parapsilosis* sensu lato was isolated from 400 episodes, representing an incidence of 29.1%, in 400 patients, comprising 231 males (57.7%) and 169 females 50.6 ± 29.1 and 47.4 ± 29.7 years of age (mean ± standard deviation), respectively. No statistically significant differences between sexes were found ($P = 0.436$).

Of these 400 episodes, there were 364 *C. parapsilosis* sensu lato isolates, identified by molecular methods: 330 (90.7%) *C. parapsilosis* sensu stricto isolates, 30 (8.2%) *C. orthopsilosis* isolates, and 4 (1.1%) *C. metapsilosis* isolates. PCR identification was not available for 36 isolates. Table 1 depicts the species distribution by age group. The mean age of patients with *C. parapsilosis* fungemia was 48.4 ± 29.3 years (range, 3 days to 97 years); that for patients with *C. orthopsilosis* fungemia was 46.7 ± 31.2 (range, 36 days to 87 years), and that for patients with *C. metapsilosis* fungemia was 62.2 ± 14.8 (range, 48 to 74 years). *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* fungemias were mainly observed in patients older than 15 years of age: 76.4%, 70%, and 100% of episodes, respec-

TABLE 1. Species distribution by age group

Age group[a]	No. (%) of isolates			
	C. parapsilosis	*C. orthopsilosis*	*C. metapsilosis*	Total
Neonates	20 (6.1)	0	0	20 (5.5)
Children	58 (17.6)	8 (26.7)	0	66 (18.1)
Adults	129 (39.1)	10 (33.3)	2 (50)	141 (38.7)
Elderly	123 (37.3)	11 (36.7)	2 (50)	137 (37.6)
Total	330 (90.7)	30 (8.2)	4 (1.1)	364 (100)

[a] Neonates, <1 month old; children, 1 month to 15 years old; adults, 16 to 64 years old; elderly, >64 years old.

tively. Interestingly, there were no *C. orthopsilosis* or *C. metapsilosis* fungemias in neonates.

Moreover, nine patients showed mixed fungemia episodes, all of them caused by *C. parapsilosis* sensu stricto species and other species of *Candida*: *Candida albicans* (in six episodes), *Candida tropicalis* (in two episodes), and *Candida lusitaniae* (in one episode). No outpatient-acquired fungemia by *C. parapsilosis* sensu lato species was identified during the study period.

Overall, 34.6% of the *C. parapsilosis* sensu lato fungemia episodes were observed in intensive care units (ICUs), but there were no *C. orthopsilosis* or *C. metapsilosis* fungemias recorded in either pediatrics or ICU-neonatal departments (Table 2). *C. parapsilosis* sensu stricto was isolated most frequently from patients with fungemia in the following departments: ICU-adults (28.8%), surgery (20.9%), internal medicine (19.7%), neonatology (7%), and pediatrics (6.7%). It was the most commonly isolated species in all the departments included in the current study. Of interest, hematology (6/21, 28.6%), pediatrics (3/25, 12.0%), and neonatology (3/26, 11.5%) were the departments with the highest proportion of *C. orthopsilosis* fungemias.

Table 3 shows the underlying conditions of patients with candidemia by *C. parapsilosis* sensu lato. The presence of an indwelling catheter was the most frequent underlying condition for all species: *C. metapsilosis* (4 out of 4 episodes, 100%), *C. orthopsilosis* (22/30, 73.3%), and *C. parapsilosis* (215/330, 65.2%). Interestingly, 46.7% (14/30) of *C. orthopsilosis* fungemias occurred in surgical patients. The geographic distribution of *C. orthopsilosis* and *C. metapsilosis* fungemias was not uniform throughout the country; most isolates of *C. orthopsilosis* (54%) were observed in only two Spanish regions (Andalusia and Valencia), situated in the south and east of Spain, respectively. In relation to all *C. parapsilosis* sensu lato isolates, the geographic prevalence rate of *C. orthopsilosis* candidemia ranged from 0% to 30.8%, with the Balearic Islands (30.8%), Asturias (20%), Valencia (12.3%), and Galicia (11.8%) being the regions where this species was more frequently isolated. Conversely, 2 out of 4 cases of candidemia by *C. metapsilosis* were observed in Asturias, where it represented 40% of *C. parapsilosis* sensu lato isolated in this geographic area.

TABLE 2. Clinical hospitalization units and species distribution

Hospitalization unit	No. (%) of isolates			
	C. parapsilosis	*C. orthopsilosis*	*C. metapsilosis*	Total
Surgery	69 (20.9)	5 (16.7)	1 (25)	75 (20.6)
Hematology	14 (4.2)	6 (20.0)	1 (25)	21 (5.8)
Internal medicine	65 (19.7)	4 (13.3)	1 (25)	70 (19.2)
Neonatology	23 (7.0)	3 (10.0)	0	26 (7.1)
Oncology	19 (5.8)	2 (6.7)	0	21 (5.8)
Pediatrics	22 (6.7)	3 (10.0)	0	25 (6.9)
ICU				
Adult	95 (28.8)	7 (23.3)	1 (25)	103 (28.3)
Neonatal	9 (2.7)	0	0	9 (2.5)
Pediatrics	14 (4.2)	0	0	14 (3.8)
Total	330 (100)	30 (100)	4 (100)	364 (100)

TABLE 3. Patients' underlying conditions and species distribution

Underlying condition[a]	No. (%) of isolates			
	C. parapsilosis	*C. orthopsilosis*	*C. metapsilosis*	Total
Catheterization	215 (65.2)	22 (73.3)	4 (100)	241 (66.2)
Major surgery	133 (40.3)	14 (46.7)	1 (25)	148 (40.6)
HIV infection	5 (1.5)	0	0	5 (1.4)
Burns	19 (5.8)	0	0	19 (5.2)
Neutropenia	17 (5.2)	4 (16.7)	1 (25)	23 (6.3)
SOT	7 (2.1)	2 (6.7)	0	9 (2.5)
HSCT	23 (7.0)	2 (6.7)	0	25 (6.9)
Premature birth	18 (5.5)	2 (6.7)	0	20 (5.5)

[a] SOT, solid organ transplant; HSCT, hematopoietic stem cell transplant; neutropenia, <500 cells/ml.

Table 4 summarizes the *in vitro* susceptibilities of *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* isolates. When comparing the susceptibility of isolates in the four age groups, for the three species, the geometric mean (GM) MICs of all antifungal agents were very similar, although, in general, GM MICs were slightly higher in the adult group. The upper limits of the MIC ranges were higher for *C. parapsilosis* sensu stricto isolates, and no statistically significant interspecies differences were found when the GM MICs were analyzed, except for micafungin and *C. parapsilosis* sensu stricto and *C. orthopsilosis* (0.79 and 0.40 mg/liter, respectively; $P = 0.004$).

All isolates of *C. orthopsilosis* and *C. metapsilosis* were susceptible to the nine antifungal agents tested according to CLSI clinical breakpoints. Resistance was observed only in *C. parapsilosis* sensu stricto isolates. The rate of resistance to amphotericin B, posaconazole, itraconazole, and caspofungin was very low: only one isolate each (0.3%). In addition, six isolates (1.8%) were resistant to anidulafungin and eight (2.4%) to micafungin. Interestingly, all isolates of this species were susceptible to fluconazole, voriconazole, and flucytosine.

Applying the new species-specific clinical breakpoints for fluconazole and echinocandins (14, 38, 42), the rates of fluconazole resistance increased for *C. parapsilosis* sensu stricto and *C. orthopsilosis* to 5.5% and 0.3%, respectively. Conversely, the rates of anidulafungin and micafungin resistance for *C. parapsilosis* sensu stricto shifted from 1.8 to 0.6% for anidulafungin and from 2.4 to 1.2% for micafungin. Results for 5 (1.5%) and 10 (3.0%) isolates of *C. parapsilosis* sensu stricto were above epidemiological cutoff values (ECVs) for posaconazole and voriconazole (>0.25 and >0.12 mg/liter, respectively) (37), and the result for only 1 isolate (0.3%) of *C. orthopsilosis* was above the voriconazole ECV (Table 4).

DISCUSSION

The importance of *C. parapsilosis* as a cause of candidemia and invasive candidiasis has risen in the last years. In some European and South American countries, *C. parapsilosis* sensu

TABLE 4. Antifungal *in vitro* susceptibility of *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* isolates

Species (no. of isolates tested)	Drug	GM MIC (mg/liter) for indicated group					MIC (mg/liter)			No. (%) of resistant isolates	
		Neonates	Children	Adults	Elderly	Overall	Range	50%	90%	CLSI	CBP/ECV[a]
C. parapsilosis (330)	Anidulafungin	1.1	0.85	1.00	0.82	0.90	0.016–8	1	2	6 (1.8)	2 (0.6)
	Caspofungin	0.35	0.32	0.37	0.31	0.34	0.008–8	0.5	1	1 (0.3)	1 (0.3)
	Micafungin	0.86	0.82	0.89	0.72	0.81	0.008–16	1	2	8 (2.4)	4 (1.2)
	Fluconazole	0.77	0.76	0.98	0.85	0.88	0.06–32	1	2	0	15 (5.5)
	Itraconazole	0.06	0.06	0.07	0.08	0.07	0.016–1	0.06	0.12	1 (0.3)	ND
	Posaconazole	0.04	0.04	0.04	0.05	0.04	0.008–2	0.03	0.12	1 (0.3)	5 (1.5)
	Voriconazole	0.01	0.01	0.02	0.02	0.02	0.008–1	0.016	0.06	0	10 (3.0)
	Amphotericin B	0.24	0.28	0.32	0.28	0.29	0.12–2	0.25	0.5	1 (0.3)	ND
	Flucytosine	0.08	0.10	0.10	0.10	0.10	0.06–8	0.06	0.25	0	ND
C. orthopsilosis (30)	Anidulafungin		0.50	0.76	0.47	0.56	0.12–2	0.5	1	0	0
	Caspofungin		0.28	0.35	0.26	0.30	0.12–1	0.25	0.5	0	0
	Micafungin		0.37	0.47	0.28	0.37	0.12–2	0.5	1	0	0
	Fluconazole		0.61	0.93	0.84	0.85	0.25–16	0.5	2	0	1 (0.3)
	Itraconazole		0.08	0.09	0.09	0.09	0.016–0.25	0.12	0.25	0	ND
	Posaconazole		0.08	0.06	0.05	0.06	0.016–0.25	0.06	0.12	0	0
	Voriconazole		0.01	0.02	0.02	0.02	0.008–0.25	0.016	0.03	0	1 (0.3)
	Amphotericin B		0.23	0.34	0.19	0.24	0.12–0.5	0.25	0.5	0	ND
	Flucytosine		0.07	0.07	0.11	0.08	0.06–4	0.06	0.12	0	ND
C. metapsilosis (4)	Anidulafungin			0.25	0.71	0.42	0.25–1	0.25	1	0	0
	Caspofungin			0.50	0.50	0.50	0.50	0.5	0.5	0	0
	Micafungin			0.71	0.50	0.59	0.5–1	0.5	1	0	0
	Fluconazole			1.41	1.00	1.19	1–2	1	2	0	0
	Itraconazole			0.06	0.35	0.15	0.03–0.5	0.12	0.5	0	ND
	Posaconazole			0.04	0.03	0.04	0.06–0.12	0.12	0.12	0	0
	Voriconazole			0.06	0.13	0.09	0.03–0.06	0.03	0.06	0	0
	Amphotericin B			0.35	1.00	0.50	0.25–1	0.5	1	0	ND
	Flucytosine			0.09	0.18	0.13	0.06–0.25	0.12	0.25	0	ND

[a] CBP, new clinical breakpoint (12, 39) for fluconazole and echinocandins; ECV, epidemiological cutoff value for posaconazole and voriconazole; ND, not determined.

lato is the second or even the first most common etiological agent of candidemia (2, 15, 21, 22, 53). This species is more important in neonates in neonatal ICUs (4, 12). The current report corroborates the rising importance of members of the *C. parapsilosis* complex as bloodstream pathogens and shows recent data from a nationwide study on the epidemiology, clinical relevance, and antifungal *in vitro* susceptibilities of members of this species complex isolated during 2009 in 44 Spanish hospitals. At present, there are few national reports analyzing the epidemiology or the *in vitro* susceptibility of these species. The existing studies are mostly multinational or local and include only partial demographic information, such as age, hospitalization unit, or the patient's underlying condition, with the consequent difficulty for comparison to previous studies (Table 5).

TABLE 5. Previous studies of invasive infections caused by the *Candida parapsilosis* complex[a]

Authors (reference)	Origin(s) of isolates	Kind of multicenter study	No. of participating centers	Total no. of isolates		No. of indicated species (% with respect to *Candida* spp., % with respect to *C. parapsilosis* sensu lato)		
				Candida spp.	*C. parapsilosis* sensu lato	*C. parapsilosis*	*C. orthopsilosis*	*C. metapsilosis*
Diekema et al. (18)	Blood and SS	Worldwide	100	14,007	2,027	1,895 (13.5, 93.5)	102 (0.7, 5.0)	30 (0.2, 1.5)
Lockhart et al. (31)	Blood and SS	Worldwide	89		1,929	1,778 (UD, 92.2)	117 (UD, 6.1)	34 (UD, 1.8)
García-Effron et al. (20)	Blood		1	756	293	218 (28.8, 74.4)	69 (9.1, 23.5)	6 (0.8, 2.1)
Blyth et al. (5)	Blood	National	52	1,005	191	180 (17.9, 94.2)	7 (0.7, 3.7)	4 (0.4, 2.1)
Gonçalves et al. (24)	Blood	National	11		141	124 (UD, 88)	13 (UD, 9)	4 (UD, 3)
Gómez-López et al. (23)	Blood	Local	14	345	87	76 (22.0, 87.3)	5 (1.4, 5.7)	6 (1.7, 6.9)
Kocsubé et al. (26)	Blood	National	2	125	12	11 (8.8, 91.6)	0	1 (0.8, 8.4)
Silva et al. (46)	Blood		1		60	60 (UD, 100)	0	0
Chen et al. (11)	Blood		1		59	54 (UD, 91.5)	2 (UD, 3.4)	3 (UD, 5.0)
de Toro et al. (17)	Blood		1		62	56 (UD, 90.3)	6 (UD, 9.6)	0
Miranda-Zapico et al. (32)	Blood		1		128	125 (UD, 97.7)	2 (UD, 1.6)	1 (UD, 0.8)
Our study	Blood	National	44	1,356	364	330 (24.3, 90.7)	30 (2.2, 8.2)	4 (0.3, 1.1)

[a] SS, sterile sites; UD, unknown data.

In the FUNGEMYCA study, 400 out of 1,356 isolates were identified as *C. parapsilosis* sensu lato (29.5%), with this organism being the species isolated the second most frequently from blood, after *C. albicans*, in Spain (35). Of these 400 isolates, 364 were identified by molecular methods, with *C. parapsilosis* sensu stricto representing 90.7% of isolates, *C. orthopsilosis* 8.2%, and C. *metapsilosis* 1.1%. This distribution of species agrees with the distributions previously published in other multicenter candidemia studies (Table 5), where *C. parapsilosis* sensu stricto has been the most frequently isolated species (87.3 to 94.2%) among the *C. parapsilosis* sensu lato organisms, followed by *C. orthopsilosis* (0 to 9%) and *C. metapsilosis* (1.5 to 8.4%) (18, 23, 24, 26, 31). Although the species distribution rates vary depending on continent, country, or institution, *C. parapsilosis* and *C. metapsilosis* have been the most and least frequently isolated species, respectively, in many studies. However, some authors have reported that *C. metapsilosis* was more common than *C. orthopsilosis* (11, 23, 26, 43, 46).

C. parapsilosis sensu stricto was the most frequently isolated species in all age groups analyzed. *C. orthopsilosis* was not isolated from neonates, and in agreement with other reports (5, 20, 31), the highest percentage of this species was observed in the elderly. Moreover, *C. metapsilosis* was not isolated from patients younger than 15 years of age, as has also been previously described (20, 31). Conversely, Tay et al. (50) did not observe *C. metapsilosis* isolates in blood from adult patients. The reported frequency of isolation of *C. metapsilosis* in blood is very low. It has also been isolated from other body sites or fluids, such as the respiratory tract, mucosal surface, urine, and even the hands of health care workers (11, 11, 46, 51). This lower frequency of *C. metapsilosis* infection could be in line with the recent report indicating that this species is the least virulent of the *C. parapsilosis* complex in cellular infection models (34). In fact, the absence among pediatric patients in this study may simply be related to the overall rarity of this species. In our study, *C. parapsilosis* is also the species most frequently isolated from all hospitalization units. The highest percentages of *C. parapsilosis* sensu stricto (28.8%) and *C. orthopsilosis* (23.3%) isolates are observed in the ICU-adult setting. On the other hand, the most common underlying condition contributing to fungemia for the three species is the presence of indwelling catheters (65.3 to 100%). These findings confirm and extend those reported in previous studies (17, 20, 32, 46).

The prevalence of candidemia caused by *C. orthopsilosis* and *C. metapsilosis* is highly variable in Spain. Most isolates of *C. orthopsilosis* (56.6%) were isolated in only two Mediterranean Spanish regions (Andalusia and Valencian). However, the Balearic Islands (30.8%) and Asturias (20%) are the Spanish regions where *C. orthopsilosis* is isolated more frequently in blood cultures. Conversely, the four isolates of *C. metapsilosis* were cultured in three hospitals from Asturias (two isolates), Madrid, and Valencia. To our knowledge, there are two Spanish studies published on the prevalence of the *C. parapsilosis* complex in Seville (17) and Barcelona (23), and two more, from the Bilbao area and a Valencian institution, have recently been published (20, 32). The prevalence of *C. metapsilosis* ranges from 0 to 6.9% and that for *C. orthopsilosis* ranges from 1.6 to 23.5% among blood isolates previously identified as *C. parapsilosis* sensu lato. Lockhart et al. (31), in their global surveillance study, also included 49 *C. parapsilosis* sensu lato isolates from Spanish hospitals in Madrid, with *C. metapsilosis* and *C. orthopsilosis* comprising 2.0 and 4.1% of isolates, respectively. Our results are similar to those previously published in different Spanish studies, but the climatic, socioeconomic, and sanitary conditions and recruitment characteristics of each study could explain this variation in the frequency of these species.

There is an increasing concern about the antifungal resistance or tolerance of *C. parapsilosis* to current and new antifungal agents. Different *in vitro* antifungal susceptibility patterns have been reported, with *C. parapsilosis* sensu stricto being less susceptible to amphotericin B, echinocandins, and fluconazole than *C. metapsilosis* or *C. orthopsilosis* (26, 31). In our study, MICs of antifungal agents were within the MIC ranges reported by other authors (18, 23, 24, 31, 46, 49–51). All *C. orthopsilosis* and *C. metapsilosis* isolates were very susceptible to the nine antifungal agents tested. In contrast, in the study of Diekema et al. (18), which included the highest number of isolates tested to date, 30.4% of *C. orthopsilosis* isolates and 10% of *C. metapsilosis* isolates were inhibited by >1 mg/liter of amphotericin B. Moreover, they 1 observed fluconazole-resistant isolate out of 102 *C. orthopsilosis* isolates (MIC = 64 mg/liter).

However, in the current study, which applied the new species-specific clinical breakpoints or the ECV, one *C. orthopsilosis* isolate was considered resistant to fluconazole and in another the voriconazole MIC was above the ECV. For *C. parapsilosis* sensu stricto, the rates of fluconazole resistance increased from 0% to 5.5%, and for anidulafungin and micafungin, they shifted from 1.8 to 0.6% and from 2.4 to 0.6%, respectively. Moreover, 1.5 and 1.2% of isolates of *C. parapsilosis* sensu stricto had values above the ECVs for posaconazole and voriconazole, respectively. These variations in antifungal susceptibilities have also been reported by other authors (17, 23, 24) and could be of great importance to the therapeutic approach to these invasive infections that is taken. It must be commented that all MIC values reported by these authors have been determined using the CLSI M27-A3 methodology; although breakpoints are method specific, we have used them in this study for the SYO-09 panel since this method has been approved and has shown results that correlate with those of the CLSI methodology. Discrepant results are possible but rare, particularly for isolates with borderline MIC values, mainly for azole agents (1, 9, 39).

The current multicenter study confirms the increasing importance of the species in the *C. parapsilosis* complex as etiological agents in bloodstream *Candida* infections. There are differences in the presence of *C. metapsilosis* and *C. orthopsilosis* infections in the different age groups. Interestingly, at the present, neither *C. orthopsilosis* nor *C. metapsilosis* has been isolated in neonates. Moreover, *C. metapsilosis* has been recovered only in adult patients. Finally, the disparity in antifungal susceptibility, with *C. metapsilosis* and *C. orthopsilosis* being more susceptible to antifungal drugs than *C. parapsilosis* sensu stricto, could have importance in the treatment of candidemia. These data emphasize the necessity for further studies monitoring the epidemiology and antifungal susceptibility of *C. metapsilosis*, *C. orthopsilosis*, and *C. parapsilosis*.

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