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Blood and intra-abdominal *Candida* spp. from a multicentre study conducted in Madrid using EUCAST: emergence of fluconazole resistance in *Candida parapsilosis*, low echinocandin resistance and absence of *Candida auris*

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Objectives: We prospectively monitored the epidemiology and antifungal susceptibility of *Candida* spp. from blood cultures and intra-abdominal samples in patients admitted to hospitals in the Madrid area.

Methods: Between 2019 and 2021, we prospectively collected incident isolates [one per species, patient and compartment (blood cultures versus intra-abdominal samples)] from patients admitted to any of 16 hospitals located in Madrid. We studied the antifungal susceptibilities to amphotericin B, triazoles, micafungin, anidulafungin and ibrexafungerp following the EUCAST E.Def 7.3.2 procedure.

Results: A total of 2107 *Candida* spp. isolates (1895 patients) from blood cultures (51.7%) and intra-abdominal samples were collected. *Candida albicans*, the *Candida glabrata* complex, the *Candida parapsilosis* complex, *Candida tropicalis* and *Candida krusei* accounted for 96.9% of the isolates; in contrast, *Candida auris* was undetected. Fluconazole resistance in *Candida* spp. was higher in blood cultures than in intra-abdominal samples (9.1% versus 8.2%; $P > 0.05$), especially for the *C. parapsilosis* complex (16.6% versus 3.6%, $P < 0.05$), whereas echinocandin resistance tended to be lower in blood cultures (0.5% versus 1.0%; $P > 0.05$). Resistance rates have risen, particularly for fluconazole in blood culture isolates, which increased sharply in 2021. Ibrexafungerp showed *in vitro* activity against most isolates. Species distributions and resistance rates varied among hospitals.

Conclusions: Whereas no *C. auris* isolates were detected, fluconazole-resistant *C. parapsilosis* isolates have been spreading across the region and this has pulled up the rate of fluconazole resistance. In contrast, the rate of echinocandin resistance continues to be low.

Introduction

Candidaemia and intra-abdominal infections are the typical clinical presentations of invasive candidiasis.[1,2] Antifungal resistance in *Candida* spp. is a worldwide concern. Although most candidaemia cases are attributable to *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*, their epidemiology may vary among hospitals and regions.[3] Diminished susceptibility to antifungal agents is a trait of infrequent species such as *Candida auris*.[4]

Multicentre surveillance studies provide solid and representative data for large regions, whereas single-centre ones are particularly useful for local decision-making.[5] Prior multicentre studies conducted on blood culture isolates reported fluconazole and echinocandin resistance rates up to 11% and 8.5%, respectively.[6–8] The epidemiology and antifungal susceptibility of yeasts collected from blood cultures were assessed in two multicentre studies conducted in Spain 10 years ago and showed low fluconazole (6.9%) and echinocandin (3.1%) resistance rates.[9,10] Since then, *C. auris* has been reported in two Spanish cities. Moreover, the incidence of candidaemia has increased during the COVID-19 pandemic, which may have had an impact on its epidemiology.[5,11–14] In contrast, the limited number of multicentre studies with intra-abdominal isolates reported rates of fluconazole and echinocandin resistance of 26.5% and 2%, respectively.[2,15] Recently, our group pointed to the abdominal cavity being a reservoir of antifungal resistance in a single-centre study, this being one of the few Spanish studies in which antifungal susceptibility of intra-abdominal isolates was assessed.[16]

There is a paucity of Spanish multicentre studies to assess the epidemiology and antifungal resistance of yeast isolates collected simultaneously from blood and abdominal samples. In light of this, we prospectively monitored and compared the epidemiology and antifungal susceptibility—including to ibrexafungerp—of *Candida* spp. collected from blood cultures and intra-abdominal samples from 16 hospitals in Madrid.

Materials and methods

Study period, participating hospitals and isolate selection

From 1 January 2019 to 31 December 2021, *Candida* spp. isolates from two different compartments (bloodstream and the intra-abdominal cavity) were prospectively collected from patients cared for at 16 hospitals in Madrid (Spain) covering an area with around 6 750 000 inhabitants. The selected hospitals, all located in highly populated areas, cover around 70% of this population (Figure 1). One incident isolate per species, patient and compartment (blood culture and/or any intra-abdominal samples) was studied.

Isolate identification, antifungal susceptibility testing and characterization of resistant isolates

We used MALDI-TOF to determine the identity of isolates and molecular identification for further confirmation.[16,17]

Antifungal susceptibilities to amphotericin B, fluconazole, voriconazole and posaconazole (Sigma–Aldrich, Madrid, Spain), isavuconazole (Basilea Pharmaceutica, Basel, Switzerland), micafungin and anidulafungin (Sigma–Aldrich, Madrid, Spain) and ibrexafungerp (Scynexis, Inc., Jersey City, NJ, USA) were assessed by the EUCAST E.Def 7.3.2 broth dilution method with tissue-treated plates (CELLSTAR® Ref. 655180, Greiner Bio-One, Frickenhausen, Germany).[18] We used *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 as quality controls.

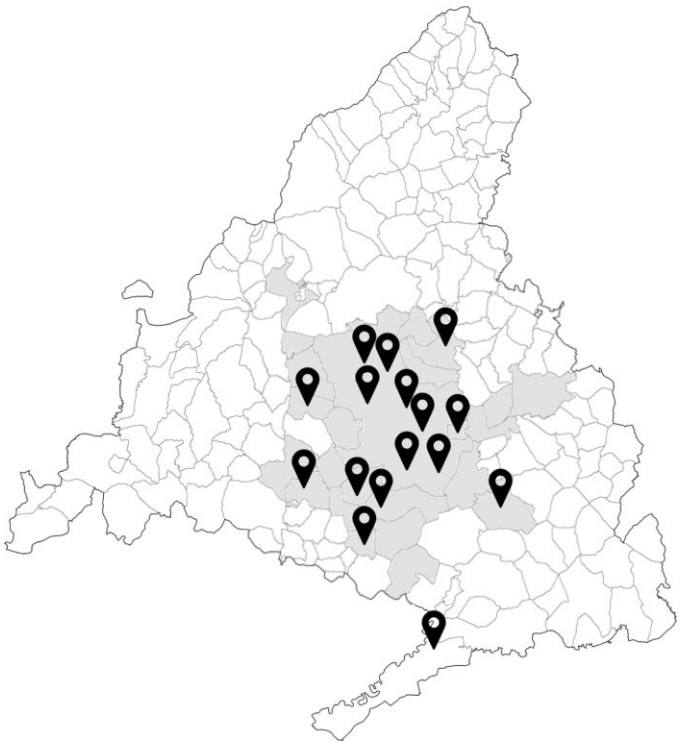

Figure 1. Location of the 16 participating hospitals in the Madrid area: Hospital General Universitario Gregorio Marañón, (Madrid); Hospital Universitario 12 de Octubre (Madrid); Hospital Universitario Ramón y Cajal (Madrid); Hospital Universitario Clínico San Carlos (Madrid); Hospital Universitario Severo Ochoa (Leganés); Hospital Universitario de Getafe (Getafe); Hospital Universitario La Paz (Madrid); Hospital Universitario de La Princesa (Madrid); Hospital Universitario Puerta de Hierro (Majadahonda); Hospital Universitario de Móstoles (Móstoles); Hospital Universitario Infanta Sofía (San Sebastián de los Reyes); Hospital Universitario Infanta Cristina (Parla); Hospital Universitario Infanta Leonor (Madrid); Hospital Universitario del Henares (Coslada); Hospital Universitario del Sureste (Arganda del Rey); and Hospital Universitario del Tajo (Aranjuez).

Isolates were categorized as resistant (or non-WT in the absence of species-specific breakpoints) according to EUCAST clinical breakpoints, epidemiological cut-off values (ECOFFs) or WT upper limits (the latter for isavuconazole and ibrexafungerp).[16,18,19] Given that *C. krusei* is intrinsically fluconazole resistant, fluconazole resistance rates were assessed overall and excluding *C. krusei* isolates. The *FKS* genes were sequenced in either echinocandin-non-WT or ibrexafungerp-non-WT isolates.[16,20] We sequenced the *ERG11* gene in fluconazole-resistant *C. albicans*, *C. parapsilosis* and *C. tropicalis* isolates.[21–23]

We compared proportions using a standard binomial method for the calculation of 95% CIs (Epidat v.4.2 Consellería de Sanidade, Xunta de Galicia, Spain).

Ethics

This study was approved by the Ethics Committee of Gregorio Marañón Hospital (CEim; study no. MICRO.HGUGM.2019-001).

Table 1. Distribution of *Candida* species and percentages of resistant or non-WT isolates from blood cultures and intra-abdominal samples

		Resistant/non-WT isolates, *n* (%)							
Species	*n*	Amphotericin B	Fluconazole	Voriconazole	Posaconazole	Isavuconazole	Micafungin	Anidulafungin	Ibrexafungerp
Blood cultures									
C. albicans	490	0 (0)	3 (0.6)	2 (0.4)	2 (0.4)	2 (0.4)	2 (0.4)	2 (0.4)	0 (0)
C. glabrata	202	0 (0)	11 (5.4)	5 (2.5)	6 (3.0)	11 (5.4)	3 (1.5)	3 (1.5)	0 (0)
C. parapsilosis complex	277	0 (0)	**46 (16.6)**	**40 (14.4)**	4 (1.4)	11 (4.0)	0 (0)	0 (0)	0 (0)
C. tropicalis	57	0 (0)	1 (1.8)	1 (1.8)	1 (1.8)	1 (1.8)	0 (0)	0 (0)	1 (1.8)
C. krusei	28	0 (0)	28 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other *Candida* spp.	35	ND	10 (28.6)	ND	ND	ND	ND	ND	ND
Overall	1089	0 (0)	99 (9.1)	**48 (4.4)**	13 (1.2)	25 (2.3)	5 (0.5)	5 (0.5)	1 (0.1)
Intra-abdominal samples									
C. albicans	554	0 (0)	2 (0.4)	1 (0.2)	1 (0.2)	2 (0.4)	3 (0.5)	3 (0.5)	0 (0)
C. glabrata complex	234	0 (0)	23 (9.8)	10 (4.3)	8 (3.4)	18 (7.7)	3 (1.3)	5 (2.1)	2 (0.9)
C. parapsilosis complex	84	0 (0)	**3 (3.6)**	**3 (3.6)**	1 (1.2)	1 (1.2)	1 (1.2)	0 (0)	0 (0)
C. tropicalis	68	0 (0)	1 (1.5)	1 (1.5)	1 (1.5)	0 (0)	1 (1.5)	1 (1.5)	1 (1.5)
C. krusei	47	0 (0)	47 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other *Candida* spp.	31	ND	7 (22.6)	ND	ND	ND	ND	ND	ND
Overall	1018	0 (0)	83 (8.2)	**15 (1.5)**	11 (1.1)	21 (2.1)	8 (0.8)	9 (0.9)	3 (0.3)

Numbers in bold indicate comparisons between blood cultures and intra-abdominal samples reaching statistically significant differences ($P<0.05$). Underlined figures indicate non-WT isolates according to ECOFFs or WT upper limits in the absence of clinical breakpoints. ND, not done.

Results

Isolates and patients

We collected 2107 *Candida* spp. isolates (1895 patients) from blood cultures ($n=1089$, 51.7%) and intra-abdominal samples [$n=1018$, 48.3%; peritoneal fluid ($n=548$, 53.8%), liver samples, including bile fluid and abscess ($n=206$, 20.2%), peritoneal abscess ($n=177$, 17.4%), abdominal drainage ($n=42$, 4.1%), abdominal wound exudate ($n=26$, 2.6%), spleen ($n=13$, 1.3%), peritoneal biopsy ($n=4$, 0.4%) and other ($n=2$, 0.2%)]. Although most patients yielded one isolate each ($n=1711$), 130 patients (6.9%) yielded ≥2 different species simultaneously isolated either from intra-abdominal samples ($n=104$, 5.5%) or blood cultures ($n=26$, 1.4%); for the remaining patients ($n=54$, 2.8%) we performed simultaneous isolations (either different species or the same species) in both compartments.

Epidemiology of the species

We identified 22 species, some exclusively found in blood cultures ($n=7$) or in intra-abdominal samples ($n=4$) (Figure 2). *C. albicans*, *C. glabrata* complex, *C. parapsilosis* complex, *C. tropicalis* and *C. krusei* accounted for 96.9% of the isolates from both compartments. Other *Candida* spp. represented 3.2% and 3.0% of the isolates in blood cultures and intra-abdominal samples, respectively (Table 1 and Figure 2); *C. auris* was not identified among the analysed samples. Percentages were lower in blood cultures than in intra-abdominal samples for *C. albicans* (45.0% versus 54.4%), *C. glabrata* (18.6% versus 23.0%) and *C. krusei* (2.6% versus 4.6%), while the opposite was found for the *C. parapsilosis* complex (25.4% versus 8.3%) ($P<0.05$). No statistically significant percentage differences between blood isolates versus intra-abdominal isolates were found for *C. tropicalis* (5.2% versus 6.7%) and other *Candida* spp. (3.2% versus 3.0%).

Antifungal susceptibility testing and characterization of resistant isolates

Table S1 and Table S2, available as Supplementary data at *JAC* Online, show the MIC distributions against the studied isolates. No resistance to amphotericin B was found. Overall, fluconazole and echinocandin resistance rates were 8.6% and 0.7%, respectively.

A total of 9.1% ($n=99/1089$) isolates from blood cultures were fluconazole resistant (*C. parapsilosis*, $n=45$; *C. krusei*, $n=28$; *C. glabrata*, $n=11$; *Candida guilliermondii*, $n=5$; *C. albicans*, $n=3$; *Candida pararugosa*, $n=3$; *Candida blankii*, $n=1$; *Candida lusitaniae*, $n=1$; *Candida orthopsilosis*, $n=1$; and *C. tropicalis*, $n=1$). Resistance rates per species ranged from 0.6% (*C. albicans*) to 16.6% (*C. parapsilosis* complex) (Table 1 and Figure 2a and c). A total of 8.2% of ($n=83/1018$) *Candida* spp. isolates from intra-abdominal samples were fluconazole resistant (*C. krusei*, $n=47$; *C. glabrata*, $n=23$; *C. guilliermondii*, $n=5$; *C. parapsilosis*, $n=3$; *C. albicans*, $n=2$; *C. tropicalis*, $n=1$; *Candida inconspicua*, $n=1$; and *Candida kefyr*, $n=1$). Resistance rates per species ranged from 0.4% (*C. albicans*) to 9.8% (*C. glabrata*) (Table 1 and Figure 2b and c). Exclusion of *C. krusei* from the analysis pulled down the rates of fluconazole resistance overall (5.3%), in blood isolates (6.7%) and in intra-abdominal isolates (3.7%) ($P<0.05$; Figure 2 and Figure S1). No statistically significant differences

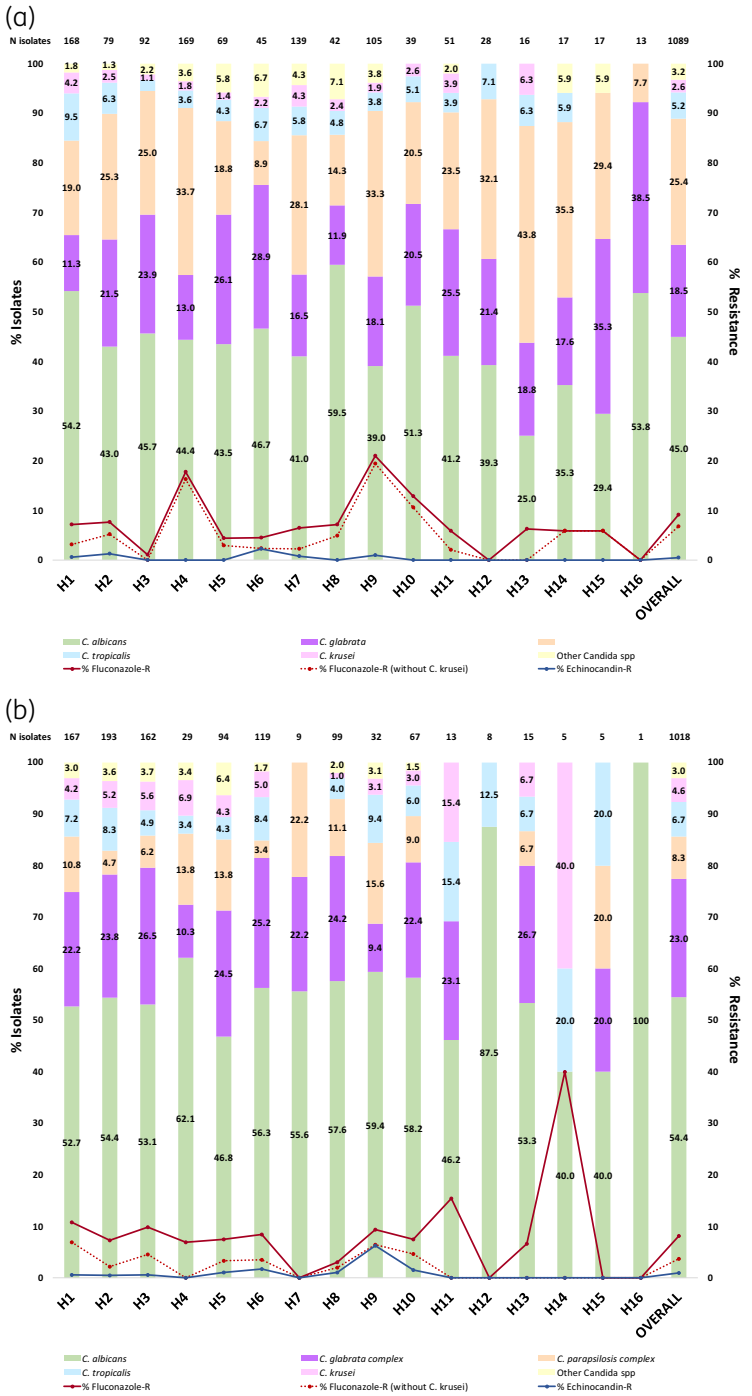

Figure 2. Overall and per hospital species distribution and antifungal resistance to fluconazole and echinocandins in blood cultures (a) and intra-abdominal samples (b). Species from fluconazole-resistant isolates and percentage of fluconazole resistance from blood and intra-abdominal samples (c). (a) Species distribution in blood cultures was as follows: *C. albicans* ($n=490$, 45.0%); *C. parapsilosis* complex {$n=277$, 25.4%; [*C. parapsilosis* sensu stricto ($n=271$, 97.8%), *C. orthopsilosis* ($n=3$, 1.1%), *C. metapsilosis* ($n=2$, 0.7%) and *Lodderomyces elongisporus* ($n=1$, 0.4%)]}; *C. glabrata* ($n=202$, 18.6%); *C. tropicalis* ($n=57$, 5.2%); *C. krusei* ($n=28$, 2.6%); and other *Candida* spp. [$n=35$, 3.2%; *C. lusitaniae* ($n=10$, 28.5%), *C. guilliermondii* ($n=8$, 22.9%), *C. dubliniensis* ($n=7$, 20%), *C. pararugosa* ($n=3$, 8.5%), *C. kefyr* ($n=2$, 5.7%), *C. blankii* ($n=1$, 2.9%), *C. fermentati* ($n=1$, 2.9%), *C. lipolytica* ($n=1$, 2.9%), *C. pelliculosa* ($n=1$, 2.9%) and *C. rugosa* ($n=1$, 2.9%)]. (b) Species distribution from intra-abdominal samples was as follows: *C. albicans* ($n=554$, 54.4%); *C. glabrata* complex {$n=234$, 23.0%; [*C. glabrata* ($n=232$, 99.2%), *C. bracarensis* ($n=1$, 0.4%) and *C. nivariensis* ($n=1$, 0.4%)]}; *C. parapsilosis* complex {$n=84$, 8.3%; [*C. parapsilosis* sensu stricto ($n=83$, 98.8%), *C. orthopsilosis* ($n=1$, 1.2%)]; *C. tropicalis* ($n=68$, 6.7%); *C. krusei* ($n=47$, 4.6%); and other *Candida* spp. [$n=31$, 3.0%; *C. dubliniensis* ($n=9$, 29.0%), *C. lusitaniae* ($n=7$, 22.6%), *C. guilliermondii* ($n=6$, 19.4%), *C. kefyr* ($n=6$, 19.4%), *C. bovina* ($n=1$, 3.2%), *C. fermentati* ($n=1$, 3.2%) and *C. inconspicua* ($n=1$, 3.2%)].

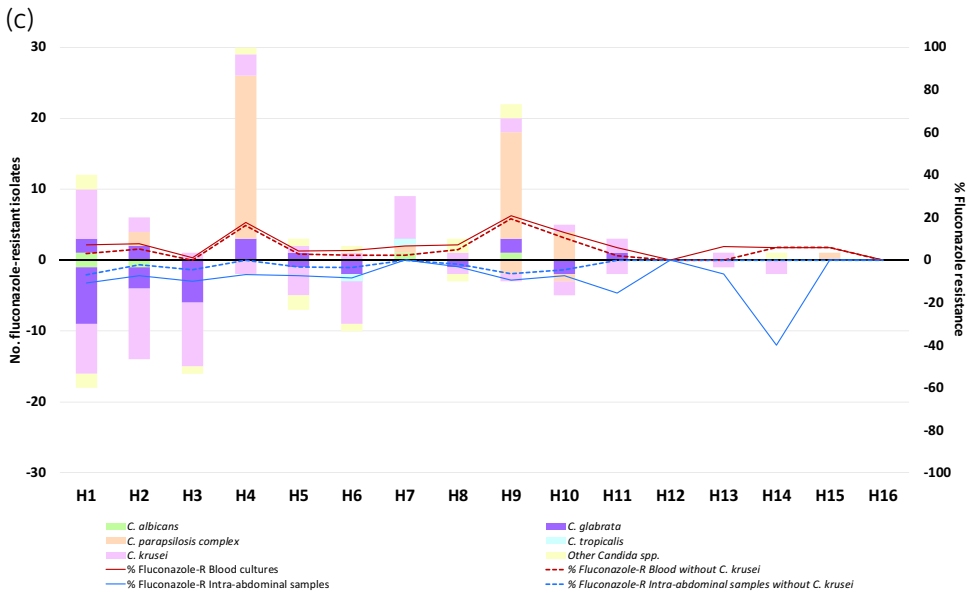

Figure 2. Continued

were found in fluconazole resistance rates between isolates from blood cultures and the intra-abdominal cavity, except for the *C. parapsilosis* complex, for which the resistance rate was higher in blood (16.6% versus 3.6%, $P<0.05$; Table 1). Seven blood isolates (*C. albicans*, $n=6$; *C. parapsilosis*, $n=1$) and 11 from the abdominal cavity (*C. albicans*, $n=9$; *C. parapsilosis*, $n=1$; *C. tropicalis*, $n=1$) were fluconazole intermediate. With the exception of fluconazole-resistant *C. glabrata* isolates, the remaining isolates from the species complex were considered intermediate to that drug, regardless of the clinical source. Overall, *Candida* isolates from blood versus intra-abdominal samples that were found to be non-WT to voriconazole (5.1% versus 1.8%; $P<0.05$), posaconazole (1.2% versus 1.1%) or isavuconazole (2.3% versus 2.1%) were also fluconazole non-WT (Table S1 and Table S2). Substitutions in the *ERG11* gene were found in 52/55 isolates: *C. parapsilosis* (Y132F-R398I, $n=43$; G458S, $n=5$), *C. albicans* (A114S-Y257H, $n=1$; D115E-K128T-F145L-I471L/I, $n=1$; D116E/D-D153E/D, $n=1$) and *C. tropicalis* ($n=1$; F449V). Analysis of fluconazole resistance rates per patient showed identical results in blood cultures and intra-abdominal samples (9.3%).

A total of 0.5% ($n=5$) of blood *Candida* spp. isolates were resistant to micafungin and anidulafungin (*C. glabrata*, $n=3$; *C. albicans*, $n=2$). In intra-abdominal samples, 1.0% ($n=10$) of *Candida* spp. isolates were resistant (or non-WT for micafungin and *C. tropicalis*) to micafungin, anidulafungin or both (*C. glabrata*, $n=5$; *C. albicans*, $n=3$; *C. parapsilosis*, $n=1$; and *C. tropicalis*, $n=1$) (Table 1, Table 2 and Figure 2). No statistically significant differences were found between blood cultures and intra-abdominal sample isolates ($P>0.05$; Table 1). *FKS* gene mutations were found in 14/15 echinocandin-resistant/non-WT isolates (Table 2). Cross-resistance between fluconazole and echinocandins was found in four *C. glabrata* isolates. The rate of echinocandin resistance per patient tended to be higher in isolates from intra-abdominal samples than in blood cultures (0.5% versus 1.1%; $P>0.05$).

Ibrexafungerp showed *in vitro* activity against the isolates tested (Table S1 and Table S2), except for four isolates (two *C. tropicalis* and two *C. glabrata* isolates) from different hospitals. Ibrexafungerp-non-WT *C. glabrata* isolates were anidulafungin resistant (± micafungin resistant) and harboured the F659S *FKS* substitution, whereas the ibrexafungerp-non-WT *C. tropicalis* isolates were echinocandin susceptible with a WT *FKS* gene sequence (Table 2).

Epidemiology and antifungal resistance analysis per hospital

The number of isolates collected each year were comparable: $n=697$ (33.1%) in 2019, $n=723$ (34.3%) in 2020 and $n=687$ (32.6%) in 2021 ($P>0.05$). Hospitals 1, 4, 7 and 9 accounted for 53% of the isolates from blood cultures, whereas hospitals 1, 2, 3 and 6 accounted for 63% of the isolates from intra-abdominal samples. The number of isolates and species distributions varied among hospitals and, with a few exceptions, *C. albicans* was the most frequent species, regardless of the hospital and studied sample (Figure 2).

Antifungal resistance rates of *Candida* spp. varied over the 3 years of the study (Table 3). For 2021, we observed a remarkable increase in the rate of fluconazole resistance in blood culture isolates, mostly attributable to the emergence of fluconazole-resistant *C. parapsilosis*. In fact, differences reaching statistical significance were found when comparing overall fluconazole resistance rates in blood isolates collected in 2019 and 2020 with those in 2021 overall (6.8% and 5.9% versus 14.4%, $P<0.05$) and excluding *C. krusei* (4.3% and 3.8% versus 11.8%; $P<0.05$). Such an effect was not observed in intra-abdominal samples. We also observed that fluconazole resistance from blood cultures and intra-abdominal samples was comparable in 2019 and 2020, but higher in blood cultures in 2021, overall (14.4% versus 8.7%; $P<0.05$) and excluding *C. krusei* (11.8% versus 4.0%;

Table 2. Characteristics of isolates showing phenotypic echinocandin and/or ibrexafungerp non-WT phenotypes

Isolate	Sample	MIC (mg/L)			*FKS* mutations	Hospital	Ward	Date of isolation
		Micafungin	Anidulafungin	Ibrexafungerp				
	C. albicans							
1	Peritoneal abscess	**1**	**0.25**	0.125	S645P *FKS1* HS1	6	Urology	14/10/2019
2	Blood culture	**0.5**	**0.25**	0.125	S645P *FKS1* HS1	6	ICU	10/01/2020
3	Peritoneal abscess	**0.5**	**0.125**	0.125	S645P *FKS1* HS1	6	ICU	29/01/2020
4	Blood culture	**0.03**	**0.06**	0.125	R1361H *FKS1* HS2	2	Surgery	25/03/2021
5	Peritoneal fluid	**0.06**	**0.06**	0.125	F641L *FKS1*HS1	9	Hepatic transplants	16/11/2021
	C. glabrata							
6	Abdominal exudate	0.03	**0.125**	**2**	F659S *FKS2* HS1	8	Reanimation	22/06/2019
7[a]	Peritoneal abscess	0.03	**0.125**	1	F708S *FKS2* outside the HS1	1	Reanimation	07/04/2020
8[a]	Blood culture	**0.25**	**2**	0.5	F708S *FKS2* outside the HS1	1	Surgery	26/04/2020
9[a]	Blood culture	**0.25**	**1**	0.5	S663P *FKS2* HS1	9	Reanimation	12/03/2021
10[a]	Peritoneal fluid	**>8**	**4**	0.25	S663P *FKS2* HS1	2	Nephrology	30/07/2021
11	Peritoneal fluid	**0.06**	**0.25**	**4**	F659S *FKS2* HS1	5	Reanimation	04/09/2021
12	Blood culture	**4**	**2**	1	S663P *FKS2* HS1	7	Internal medicine	07/10/2021
13	Liver	**0.125**	**0.5**	0.25	S663F *FKS2* HS1	3	ICU	02/10/2021
	C. parapsilosis							
14	Peritoneal fluid	**4**	4	0.5	*FKS* WT	9	Nephrology	31/05/2020
	C. tropicalis							
15	Peritoneal fluid	**0.5**	**0.25**	0.5	S654P/S *FKS1* HS1 + V1352I/V, V1404I/V *FKS1* outside the HS2	10	Reanimation	24/07/2020
16	Peritoneal fluid	0.016	0.03	**4**	*FKS* WT	2	Surgery	19/08/2021
17	Blood culture	0.03	0.03	**4**	*FKS* WT	1	Reanimation	18/12/2021

Numbers in bold indicate resistance or non-WT isolates. Each isolate was obtained from a single patient, except for isolates 1 + 2 and 7 + 8, each pair obtained from a single and the same patient.
[a]Isolates showing fluconazole and echinocandin cross-resistance.

Table 3. Fluconazole and echinocandin resistance in blood cultures and intra-abdominal samples per year

Year	Sample type	No. of isolates	Overall resistance (%)		Fluconazole resistance without *C. krusei*	
			Fluconazole	Echinocandins	No. of isolates	Resistance (%)
2019	Blood cultures	307	6.8	0.0	299	4.3
	Intra-abdominal samples	390	6.9	0.5	379	4.2
2020	Blood cultures	407	5.9	0.5	398	3.8
	Intra-abdominal samples	316	9.2	1.3	295	2.7
2021	Blood cultures	375	**14.4**	0.8	364	**11.8**
	Intra-abdominal samples	312	**8.7**	1.3	297	**4.0**

Numbers in bold indicate statistically significant differences ($P < 0.05$).

$P < 0.05$). Rates of echinocandin resistance in *Candida* spp. isolates from blood cultures and intra-abdominal samples tended to increase slightly during the study period ($P > 0.05$).

We observed variable resistance rates per hospital (Figure 2). Fluconazole resistance was found in isolates from 14 hospitals in blood cultures (range: 0% to 21%) and intra-abdominal samples (range: 0% to 40%), notably driven by *C. krusei*, *C. glabrata* and *C. parapsilosis*, the latter species in some hospitals (Figure 2c). Echinocandin resistance in *Candida* spp. was found in isolates from nine hospitals in blood cultures (range: 0% to 2.2%) and in intra-abdominal samples (range: 0% to 6.3%), mostly driven by *C. glabrata*.

Discussion

With some exceptions, our study shows that *C. albicans* is the dominant species, regardless of the type of sample and hospital. We were unable to detect *C. auris*, but in 2021 the fluconazole resistance rate increased due to the emergence of fluconazole-resistant *C. parapsilosis* isolates causing candidaemia in different hospitals. The low rate of echinocandin resistance was mainly associated with *C. glabrata* and *C. albicans*.

In this study, species epidemiology in blood culture isolates coincides with prior multicentre studies, including the CANDIPOP study conducted in Spain 10 years ago.[9,24] Moreover, this is the first multicentre study conducted in Spain with intra-abdominal isolates—supported by prior studies—that points to *C. glabrata* being the second most frequently detected species.[2,15] *C. auris* is an emergent species able to cause outbreaks in different geographical regions, including some regions in Spain, although it remains undetected in Madrid.[11–13]

The CANDIPOP study showed low rates of fluconazole and echinocandin resistance (6.9% and 3.1%, respectively) in Spain; the resistance rate in the Madrid region at that time was 5%.[9] Our study shows the emergence of fluconazole resistance (8.6%), mostly attributable to the presence of fluconazole-resistant *C. parapsilosis* isolates, which accounts for 26.4% of all fluconazole-resistant *Candida* isolates (45.5% of fluconazole-resistant isolates if only those from blood cultures are considered). The presence of fluconazole-resistant *C. parapsilosis* isolates harbouring the Y132F *ERG11* gene substitution was recently reported for the first time in Spain in a hospital in the Balearic Islands.[25] In our study we identify and describe fluconazole-resistant *C. parapsilosis* genotypes harbouring the Y132F *ERG11* gene substitution and its spreading for the first time in Madrid; moreover, to the best of our knowledge this is the first report of *C. parapsilosis* harbouring the G458S *ERG11* gene substitution in Spain. An in-depth analysis of these fluconazole-resistant *C. parapsilosis* isolates is reported elsewhere.[5,26] In contrast, the echinocandin resistance rate is low for the time being.

We compare the antifungal resistance rates in blood culture isolates and intra-abdominal samples. In a previous report, fluconazole and echinocandin resistance tended to be higher in the abdominal cavity compared with blood cultures,[16] confirmed in the present study for echinocandins (0.5% versus 1.0%); however, fluconazole resistance is higher in blood culture isolates versus abdominal sample isolates (9.1% versus 8.2%), probably due to the emergence of fluconazole-resistant *C. parapsilosis* clones that cause candidaemia. The slight increase in the resistance rate over the 3 year study period needs further monitoring in the region.

Ibrexafungerp is a novel oral glucan synthase inhibitor with activity against *Candida* spp. that might be a good alternative to echinocandins. We previously reported the WT upper limits of ibrexafungerp against *Candida* spp. and the activity of the drug against some *C. glabrata FKS2* gene mutant isolates.[19,27] Our study includes a large number of isolates and proves the high activity of ibrexafungerp against *Candida*, with only four isolates resulting in a non-WT phenotype. *C. glabrata* isolates are echinocandin resistant and harbour the F659S *FKS2* gene substitution, confirming previous observations;[27,28] in contrast, *C. tropicalis* isolates are echinocandin susceptible with a WT *FKS1* gene sequence; this suggests an alternative resistance mechanism to the presence of *FKS1* gene hot-spot mutations.

A limitation of this study is the analysis of a single incident isolate from intra-abdominal samples, which might have prevented us from identifying emerging antifungal-resistant isolates. In a previous study from our group, we detected a number of resistant isolates in the abdominal cavity by studying sequential isolates per patient.[16]

In conclusion, *Candida* spp. epidemiology in blood cultures and abdominal samples from patients recently treated at hospitals located in Madrid coincide with prior studies conducted in Spain and elsewhere; the region seems to be free from *C. auris*. In contrast, the rates of fluconazole resistance have increased, mainly due to the emergence of fluconazole-resistant *C. parapsilosis* isolates spreading across the region.

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Supplementary data

Figure S1 and Tables S1 and S2 are available as Supplementary data at *JAC* Online.

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