Monitoring the Epidemiology and Antifungal Resistance of Yeasts Causing Fungemia in a Tertiary Care Hospital in Madrid, Spain: Any Relevant Changes in the Last 13 Years?

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ABSTRACT We conducted an updated analysis on yeast isolates causing fungemia in patients admitted to a tertiary hospital in Madrid, Spain, over a 13-year period. We studied 896 isolates associated with 872 episodes of fungemia in 857 hospitalized patients between January 2007 and December 2019. Antifungal susceptibility was assessed by EUCAST EDef 7.3.2. Mutations conferring azole and echinocandin resistance were further studied, and genotyping of resistant clones was performed with species-specific microsatellite markers. *Candida albicans* (45.8%) was the most frequently identified species, followed by the *Candida parapsilosis* complex (26.4%), *Candida glabrata* (12.3%), *Candida tropicalis* (7.3%), *Candida krusei* (2.3%), other *Candida* spp. (3.1%), and non-*Candida* yeasts (2.8%). The rate of fluconazole resistance in *Candida* spp. was 4.7%, ranging from 0% (*C. parapsilosis*) to 9.1% (*C. glabrata*). The overall rate of echinocandin resistance was 3.1%. Resistance was highly influenced by the presence of intrinsically resistant species. Although the number of isolates between 2007 and 2013 was almost 2-fold higher than that in the period from 2014 to 2019 (566 versus 330), fluconazole resistance in *Candida* spp. was greater in the second period (3.5% versus 6.8%; $P < 0.05$), while overall resistance to echinocandins remained stable (3.5% versus 2.4%; $P > 0.05$). Resistant clones were collected from different wards and/or time points, suggesting that there were no epidemiological links. The number of fungemia episodes has been decreasing over the last 13 years, with a slight increase in the rate of fluconazole resistance and stable echinocandin resistance. Antifungal resistance is not the cause of the spread of resistant clones.

KEYWORDS fungemia, yeast, *Candida*, EUCAST, microsatellite genotyping, antifungal resistance, fluconazole, echinocandins

Candidemia is the most common fungal bloodstream infection in hospitalized patients. The overall pooled incidence rate in Europe is 3.88 episodes per 100,000 inhabitants and is associated with a crude mortality rate that ranges from 10% to 47% (1). *Candida albicans* remains the most commonly isolated species, although recent reports alert on the emergence of other *Candida* spp., non-*Candida* yeasts, echinocandin-resistant *Candida glabrata* isolates, or the multiresistant *Candida auris* (2).

Species-specific patterns may be used to try to predict antifungal susceptibility by identifying the species involved in the infection, which is generally sufficient due to intrinsically resistant isolates (3). However, detecting secondary resistance requires further antifungal susceptibility testing to monitor the resistance and guide empirical

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TABLE 1 Species distribution of 896 isolates causing fungemia 857 patients

Species	No. of isolates	%
<i>C. albicans</i> ^a	410	45.8
<i>C. parapsilosis</i> complex ^b	237	26.4
<i>C. glabrata</i> ^c	110	12.3
<i>C. tropicalis</i>	65	7.3
<i>C. krusei</i>	21	2.3
<i>C. guilliermondii</i> complex ^d	11	1.2
<i>C. dubliniensis</i>	8	0.9
<i>C. lusitaniae</i>	5	0.6
<i>C. kefyr</i>	2	0.2
<i>C. inconspicua</i>	1	0.1
<i>C. pelliculosa</i>	1	0.1
<i>Rhodotorula mucilaginosa</i>	7	0.8
<i>Trichosporon</i> spp. ^e	7	0.8
<i>Cryptococcus</i> spp. ^f	7	0.8
<i>Magnusiomyces capitatus</i>	2	0.2
<i>Arxula adeninivorans</i>	1	0.1
<i>Kodamaea ohmeri</i>	1	0.1
Overall	896 ^g	

^aOne isolate proved to be *C. africana* (0.11%).

^bThree isolates proved to be *C. metapsilosis* (0.33%); seven isolates proved to be *C. orthopsilosis* (0.78%).

^cOne isolate proved to be *C. nivariensis* (0.11%).

^dTwo isolates proved to be *C. fermentati* (0.22%).

^e*T. inkin* ($n = 3$ [0.33%]), *T. asahii* ($n = 2$ [0.22%]), *T. dermatis* ($n = 1$ [0.11%]), and *T. asteroides* ($n = 1$ [0.11%]).

^f*C. neoformans* ($n = 5$ [0.56%]) and *C. deneoformans* ($n = 2$ [0.22%]).

^gForty-eight isolates caused 24 polyfungal episodes; species were distributed as follows: *C. albicans* ($n = 17$), *C. glabrata* ($n = 15$), *C. parapsilosis* ($n = 8$), *C. tropicalis* ($n = 3$), *C. krusei* ($n = 2$), *C. dubliniensis* ($n = 1$), *C. guilliermondii* ($n = 1$), and *C. metapsilosis* ($n = 1$).

antifungal treatment. Therefore, periodic surveillance of isolates causing fungemia may provide further insight on the epidemiology and antifungal resistance. Nationwide fungemia studies have reported resistance rates ranging from 2.7% (Iceland) to 9.4% (Denmark) for fluconazole and 0% (Brazil and Iceland) to 15% (Portugal) for echinocandins (4–7). In Spain, resistance rates for fluconazole and echinocandins are 7% and 2%, respectively (8). Population-based studies are useful, although they may overlook inter-hospital differences (9).

In a previous study, we found low azole and echinocandin resistance rates in isolates causing fungemia obtained from patients admitted to our hospital (Hospital General Universitario Gregorio Marañón, Madrid, Spain) between January 2007 and December 2013 (10). In this study, we assessed the etiology and antifungal resistance of isolates collected between January 2014 and December 2019. We made an overall reassessment of the agents causing fungemia in patients admitted to a tertiary care hospital in Madrid (Spain) over a 13-year period, as well as their amphotericin B, azole, and echinocandin antifungal susceptibility using updated EUCAST clinical breakpoints, and the potential spread of resistant clones.

RESULTS

Epidemiology of species causing fungemia. We studied 896 isolates causing 872 episodes of fungemia—single-species episodes ($n = 848$) and polyfungal episodes ($n = 24$)—in 857 patients. Isolates were from patients admitted to medical wards (30.2%), intensive care units (ICUs) (20.1%), surgical wards (15.7%), oncology-hematology (14.5%), neonatology (12.5%), and other wards (7%). Table 1 shows the distribution of species. *C. albicans* (45.8%) was the most frequently identified species, followed by the *C. parapsilosis* complex (26.4%), *C. glabrata* (12.3%), *C. tropicalis* (7.3%), *C. krusei* (2.3%), other *Candida* spp. (3.1%), and non-*Candida* yeasts (2.8%). In cases with polyfungal episodes, the following were isolated: *C. albicans*-*C. glabrata* ($n = 11$), *C. albicans*-*C. parapsilosis* ($n = 4$), *C. glabrata*-*C. tropicalis* ($n = 2$), and 1 case for each of the

FIG 1 Species distribution of isolates causing fungemia and rate of resistance to fluconazole and echinocandins during the study period.

following combinations: *C. albicans*-*C. dubliniensis*, *C. albicans*-*C. krusei*, *C. glabrata*-*C. parapsilosis*, *C. glabrata*-*C. metapsilosis*, *C. parapsilosis*-*C. guilliermondii*, *C. parapsilosis*-*C. metapsilosis*, and *C. tropicalis*-*C. krusei*.

The number of episodes varied by year, ranging from 115 (2007) to 39 (2017). Figure 1 and Supplemental Table S1 show the percentage of isolates by individual species and year. *C. albicans* accounted for 40% to 48% of the isolates per year, except for 2010 (59%), whereas *C. parapsilosis* accounted for 17% to 36% of the isolates per year, although with a decreasing trend from initial years of the study period. In contrast, the proportion of other *Candida* spp. and non-*Candida* yeast isolates showed a spiked pattern. The distribution by species in different hospital locations is shown in Fig. S1 and Supplemental Table S2. With the exception of oncology-hematology, *C. albicans* was the dominant species, particularly in neonatology and other wards. In contrast, *C. parapsilosis* was more frequently detected in neonatology and oncology-hematology. The highest percentages of *C. glabrata* and *C. tropicalis* were found in medical wards and the ICU, respectively. *C. krusei*, *Candida* spp., and non-*Candida* yeasts were anecdotal in neonatology but notoriously frequent in oncology-hematology.

The number of isolates detected in the first period was almost twice that in the second period ($n = 566$ [63.2%] versus $n = 330$ [36.8%]). The proportions of *C. albicans* and

FIG 2 Species distribution of yeasts causing fungemia and fluconazole- and/or echinocandin-resistant isolates found in both subperiods.

C. parapsilosis isolates were similar in both subperiods, while those of *C. glabrata* and *C. krusei* significantly increased in the second period (10.2% versus 15.8% and 1.6% versus 3.6%, respectively; $P < 0.05$). Finally, the proportions of infections by *C. tropicalis* (8.3% versus 5.5%; $P > 0.05$), other *Candida* spp. (3.4% versus 2.7%; $P > 0.05$), and non-*Candida* yeasts (3.4% versus 1.8%; $P > 0.05$) decreased in the second period (Fig. 2).

Antifungal susceptibility testing and characterization of resistance. Table 2 lists MIC distributions of the drugs tested. No resistance to amphotericin B was detected.

Table 3 shows the characteristics of fluconazole-resistant *Candida* spp. isolates. Overall, 4.7% ($n = 41$) of isolates were fluconazole resistant (*C. krusei* [$n = 21$], *C. glabrata* [$n = 10$], *C. albicans* [$n = 4$], *C. guilliermondii* [$n = 3$], *C. lusitanae* [$n = 2$], and *C. inconspicua* [$n = 1$]). Resistance rates in individual species ranged from 0% (*C. parapsilosis*) to 9.1% (*C. glabrata*). For the remaining triazoles, the overall rate of non-wild-type *Candida* species isolates was 1.3% for voriconazole ($n = 11$; *C. albicans* [$n = 6$] and *C. glabrata* [$n = 5$]), 1.1% for posaconazole ($n = 10$; *C. albicans* [$n = 4$] and *C. glabrata* [$n = 6$]), and 1.6% for isavuconazole ($n = 14$; *C. albicans* [$n = 4$] and *C. glabrata* [$n = 10$]). All isolates showing a non-wild-type phenotype to voriconazole, posaconazole, or isavuconazole were fluconazole non-wild type. The four fluconazole-resistant *C. albicans* isolates harbored mutations in *ERG3* (V351A [$n = 3$], T293I V351A [$n = 1$]) and/or *ERG11* (D116E/D E266D V488I/V [$n = 1$] and A114S Y257H G464S/G [$n = 1$]) and were non-wild type to the other azoles. The remaining two fluconazole non-wild-type *C. albicans* isolates also harbored mutations in *ERG3* (V351A [$n = 2$]) and *ERG11* (A114S Y257H [$n = 2$]). All fluconazole-resistant *C. glabrata* isolates exhibited cross-resistance to the four azoles ($n = 5$), posaconazole and isavuconazole ($n = 1$), or isavuconazole ($n = 4$). The updated fluconazole EUCAST breakpoints resulted in up to a 9% increase of the resistance rate in *C. glabrata* (compared to the 7% detected using the former 2018 breakpoints).

Table 4 presents the characteristics of echinocandin-resistant isolates. Only a few

TABLE 2 MIC distributions of studied antifungals versus isolates and rates of resistant/non-wild-type isolates^a

Organism(s) and drug	No. of isolates at each MIC, in mg/liter															No. of isolates (%)			
	≤0.001	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64	Resistant	Non-wild type
<i>C. albicans</i> (n = 410)																			
Amphotericin B	—	—	0	0	0	0	12	98	217	78	5	0	0	0	—	—	—	0 (0)	0 (0)
Fluconazole	—	—	—	—	—	—	28	239	125	12	2	0	0	0	0	0	3	4 (1.0)	6 (1.5)
Voriconazole	4	46	280	61	11	2	2	0	0	1	0	0	0	0	—	—	—	4 (1.0)	6 (1.5)
Posaconazole	1	4	83	205	81	27	5	1	0	0	0	0	0	0	—	—	—	4 (1.0)	4 (1.0)
Isavuconazole	25	239	120	16	6	0	0	0	0	1	0	0	0	0	—	—	—	ND	4 (1.0)
Micafungin	10	104	244	42	10	0	0	0	0	0	0	0	0	0	—	—	—	0 (0)	0 (0)
Anidulafungin	13	178	190	19	9	1	0	0	0	0	0	0	0	0	—	—	—	0 (0)	0 (0)
<i>C. parapsilosis</i> complex (n = 237)																			
Amphotericin B	—	—	0	0	0	0	4	20	105	101	7	0	0	0	—	—	—	0 (0)	0 (0)
Fluconazole	—	—	—	—	—	—	0	3	116	93	22	3	0	0	0	0	0	0 (0)	0 (0)
Voriconazole	0	0	16	125	78	15	3	0	0	0	0	0	0	0	—	—	—	0 (0)	0 (0)
Posaconazole	0	0	0	10	76	106	45	0	0	0	0	0	0	0	—	—	—	0 (0)	0 (0)
Isavuconazole	0	33	104	83	16	1	0	0	0	0	0	0	0	0	—	—	—	ND	0 (0)
Micafungin	0	0	0	0	0	0	0	0	10	36	140	50	0	0	—	—	—	0 (0)	0 (0)
Anidulafungin	0	0	0	0	0	0	1	0	3	14	53	153	13	0	—	—	—	0 (0)	0 (0)
<i>C. glabrata</i> (n = 110)																			
Amphotericin B	—	—	0	0	0	0	3	14	65	27	1	0	0	0	—	—	—	0 (0)	0 (0)
Fluconazole	—	—	—	—	—	—	0	0	0	0	0	5	37	49	9	2	8	10 (9.1)	10 (9.1)
Voriconazole	0	0	0	0	0	2	15	35	47	4	2	4	1	0	—	—	—	ND	5 (4.5)
Posaconazole	0	0	0	0	0	1	1	8	32	53	9	4	2	0	—	—	—	ND	6 (5.5)
Isavuconazole	0	1	0	1	1	14	35	32	16	1	5	3	1	0	—	—	—	ND	10 (9.1)
Micafungin	0	0	0	93	15	2	0	0	0	0	0	0	0	0	—	—	—	0 (0)	0 (0)
Anidulafungin	0	0	0	0	41	64	4	1	0	0	0	0	0	0	—	—	—	1 (0.9)	1 (0.9)
<i>C. tropicalis</i> (n = 65)																			
Amphotericin B	—	—	0	0	0	0	1	4	36	22	2	0	0	0	—	—	—	0 (0)	0 (0)
Fluconazole	—	—	—	—	—	—	0	10	37	14	4	0	0	0	0	0	0	0 (0)	0 (0)
Voriconazole	0	0	0	6	34	17	7	1	0	0	0	0	0	0	—	—	—	0 (0)	0 (0)
Posaconazole	0	0	1	16	21	25	2	0	0	0	0	0	0	0	—	—	—	0 (0)	0 (0)
Isavuconazole	0	3	28	31	2	1	0	0	0	0	0	0	0	0	—	—	—	ND	0 (0)
Micafungin	0	0	0	3	44	15	1	1	0	0	0	1	0	0	—	—	—	ND	2 (3.1)
Anidulafungin	0	0	0	15	38	8	3	0	0	0	0	1	0	0	—	—	—	1 (1.5)	1 (1.5)
<i>C. krusei</i> (n = 21)																			
Amphotericin B	—	—	0	0	0	0	0	0	1	17	3	0	0	0	—	—	—	0 (0)	0 (0)
Fluconazole	—	—	—	—	—	—	0	0	0	0	0	0	0	0	6	8	7	21 (100)	0 (0)
Voriconazole	0	0	0	0	0	0	0	4	9	8	0	0	0	0	—	—	—	ND	0 (0)
Posaconazole	0	0	0	0	1	2	4	8	6	0	0	0	0	0	—	—	—	ND	0 (0)
Isavuconazole	0	0	0	0	0	0	2	12	6	1	0	0	0	0	—	—	—	ND	0 (0)
Micafungin	0	0	0	0	0	0	15	6	0	0	0	0	0	0	—	—	—	ND	0 (0)
Anidulafungin	0	0	0	0	0	14	7	0	0	0	0	0	0	0	—	—	—	0 (0)	0 (0)

(Continued on next page)

TABLE 2 (Continued)

Organism(s) and drug	No. of isolates at each MIC, in mg/liter																No. of isolates (%)		
	≤0.001	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	≥64	Resistant	Non-wild type
Other <i>Candida</i> spp. (n = 28)																			
Amphotericin B	—	—	0	0	0	2	6	10	6	4	0	0	0	0	—	—	—	ND	ND
Fluconazole	—	—	—	—	—	—	2	5	5	0	0	5	5	1	2	2	1	6 (21.4)	ND
Voriconazole	0	0	3	8	2	0	6	4	3	1	0	0	1	0	—	—	—	ND	ND
Posaconazole	0	0	2	2	8	2	5	3	6	0	0	0	0	0	—	—	—	ND	ND
Isavuconazole	0	5	5	3	0	2	3	5	1	3	0	1	0	0	—	—	—	ND	ND
Micafungin	0	1	0	1	6	4	4	5	2	3	0	1	1	0	—	—	—	ND	ND
Anidulafungin	0	0	1	7	2	3	3	0	2	2	6	2	0	0	—	—	—	ND	ND
Non- <i>Candida</i> yeasts (n = 25)																			
Amphotericin B	—	—	0	0	0	0	0	1	1	5	9	5	0	4 ^b	—	—	—	ND	ND
Fluconazole	—	—	—	—	—	—	0	0	0	2	5	1	5	1	1	2	8	ND	ND
Voriconazole	0	1	1	1	3	2	4	1	2	4	3	2	0	1 ^b	—	—	—	ND	ND
Posaconazole	0	0	0	2	3	5	5	3	4	3	0	0	0	0	—	—	—	ND	ND
Isavuconazole	0	1	1	1	5	2	6	1	2	3	1	2	0	0	—	—	—	ND	ND
Micafungin	0	0	0	0	0	0	0	1	0	1	0	1	1	21 ^b	—	—	—	ND	ND
Anidulafungin	0	0	0	0	0	0	0	0	0	2	0	1	2	20 ^b	—	—	—	ND	ND

^aUnderlined values indicate non-wild-type isolates according to ECOFFs, and values in bold indicate resistant isolates (EUCAST breakpoint table v 10.0), with the exception of *C. krusei*, which was considered intrinsically resistant to fluconazole (30, 31). —, nontested antifungal concentration. ND, not done, as either breakpoints or ECOFFs were not available.

^bIsolates with MICs of ≥8 mg/liter.

TABLE 3 Characteristics of fluconazole-resistant isolates from patients with candidemia

Isolate no.	Species	Fluconazole MIC (mg/liter)	ERG3-ERG 11 sequence	Ward	Date of isolation (day/mo/yr)	Genotype
1	<i>C. albicans</i> ^a	>64	V351A-D116E/D E266D V488I/V	Digestive	23/08/2010	CA-170
2	<i>C. albicans</i> ^a	8	V351A-A114S Y257H G464S/G	Neonatology ICU	27/07/2011	CA-276
3	<i>C. albicans</i> ^a	>64	T291I V351A-wild type	Internal medicine	13/11/2019	CA-313
4	<i>C. albicans</i> ^a	>64	V351A-wild type	Neurosurgery	15/03/2015	CA-413
5	<i>C. glabrata</i> ^a	64	ND ^d	Oncology	25/05/2018	CG-01
6	<i>C. glabrata</i> ^a	>64	ND	Oncology	08/11/2011	CG-06
7	<i>C. glabrata</i> ^b	64	ND	Urology	22/12/2008	CG-09
8	<i>C. glabrata</i> ^a	>64	ND	ICU	09/10/2007	CG-13
9	<i>C. glabrata</i> ^b	32	ND	ICU	12/01/2014	CG-15
10	<i>C. glabrata</i> ^b	64	ND	Digestive	13/08/2015	CG-15
11	<i>C. glabrata</i> ^a	64	ND	Emergency	21/10/2007	CG-23
12	<i>C. glabrata</i> ^b	32	ND	ICU	26/01/2010	CG-24
13	<i>C. glabrata</i> ^a	>64	ND	Surgery	11/09/2015	CG-24
14	<i>C. glabrata</i> ^c	64	ND	Surgery	12/07/2019	CG-27
15	<i>C. krusei</i>	32	ND	Geriatrics	02/01/2007	ND
16	<i>C. krusei</i>	16	ND	Oncology	27/11/2007	ND
17	<i>C. krusei</i>	32	ND	ICU	28/09/2010	ND
18	<i>C. krusei</i>	64	ND	ICU	27/12/2010	ND
19	<i>C. krusei</i>	32	ND	ICU	08/03/2011	ND
20	<i>C. krusei</i>	32	ND	Oncology	02/01/2012	ND
21	<i>C. krusei</i>	32	ND	Digestive	05/03/2012	ND
22	<i>C. krusei</i>	64	ND	Nephrology	29/05/2012	ND
23	<i>C. krusei</i>	64	ND	Infectious diseases	05/04/2013	ND
24	<i>C. krusei</i>	16	ND	Digestive	14/11/2014	ND
25	<i>C. krusei</i>	64	ND	Onco/Hemato ^e	24/11/2014	ND
26	<i>C. krusei</i>	32	ND	Onco/Hemato	24/04/2015	ND
27	<i>C. krusei</i>	16	ND	Onco/Hemato	16/07/2015	ND
28	<i>C. krusei</i>	32	ND	ICU	06/04/2016	ND
29	<i>C. krusei</i>	16	ND	Oncology	15/06/2016	ND
30	<i>C. krusei</i>	16	ND	Onco/Hemato	29/11/2016	ND
31	<i>C. krusei</i>	16	ND	Oncology	07/03/2017	ND
32	<i>C. krusei</i>	32	ND	Internal medicine	22/09/2018	ND
33	<i>C. krusei</i>	64	ND	Onco/Hemato	29/09/2018	ND
34	<i>C. krusei</i>	64	ND	Intensive medicine	17/03/2019	ND
35	<i>C. krusei</i>	64	ND	Intensive medicine	09/04/2019	ND
36	<i>C. guilliermondii</i>	16	ND	Surgery	10/10/2008	ND
37	<i>C. guilliermondii</i>	16	ND	Digestive	23/04/2013	ND
38	<i>C. guilliermondii</i>	>64	ND	Internal medicine	31/01/2018	ND
39	<i>C. lusitanae</i>	16	ND	Onco/Hemato	30/05/2010	ND
40	<i>C. lusitanae</i>	32	ND	Neurosurgery	28/12/2014	ND
41	<i>C. inconspicua</i>	8	ND	Traumatology	22/01/2015	ND

^aPan-azole-resistant isolate.^bCross-resistance among fluconazole and isavuconazole.^cCross-resistance among fluconazole, posaconazole, and isavuconazole.^dND, not done.^eOnco/Hemato, Oncology and Hematology.

Candida species isolates were resistant to echinocandins ($n=3$ [0.3%]; *C. tropicalis* [$n=2$] and *C. glabrata* [$n=1$]). *FKS* mutations were found in the three isolates, two of *C. tropicalis* (R656G/R *FKS1* HS1 and S654F *FKS1* HS1) and one of *C. glabrata* (F659S *FKS2* HS1). No cross-resistance between azoles and echinocandins in *Candida* spp. was

TABLE 4 Characteristics of echinocandin-resistant isolates from patients with candidemia

Isolate no.	Species	MIC (mg/liter) ^a			Ward	Date on which species was isolated (day/mo/yr)	Genotype
		Micafungin	Anidulafungin	FKS mutation			
1	<i>C. tropicalis</i>	0.125	0.03	R656G/R <i>FKS1</i>	ICU	31/10/2011	CT-033
2	<i>C. tropicalis</i>	2	2	S654F <i>FKS1</i>	Oncology and Hematology	23/03/2018	CT-165
3	<i>C. glabrata</i>	0.03	0.125	F659S <i>FKS2</i>	Digestive	04/08/2016	CG-024

^aNumbers in bold indicate resistance or non-wild-type isolates.

found. Non-*Candida* yeasts showed decreased fluconazole susceptibility and intrinsic echinocandin resistance. Pooled MICs against *Candida* spp. and non-*Candida* yeasts showed 3.1% ($n = 28$) echinocandin resistance.

Rates of resistance to fluconazole (against *Candida* spp.) and echinocandins (in *Candida* spp. and non-*Candida* yeasts) fluctuated throughout the study period (Fig. 1). Fluconazole resistance rates in *Candida* spp. ranged from 0% (2009) to 9.8% (2015) and were highly influenced by the presence of species with intrinsic lower fluconazole susceptibility (*C. glabrata* and *C. krusei*), which accounted for 75.6% of all fluconazole-resistant *Candida* species isolates. Likewise, overall echinocandin resistance rates ranged between 0% (2015 and 2017) and 6.0% (2009) and were highly influenced by the presence of intrinsically echinocandin-resistant non-*Candida* species (89.3% of all echinocandin-resistant isolates).

Percentages of fluconazole (3.5% versus 6.8%; $P < 0.05$) and echinocandin (0.2% versus 0.6%; $P > 0.05$) resistance in *Candida* spp. were higher in the second period. However, pooled MICs against *Candida* spp. and non-*Candida* yeasts showed stable echinocandin resistance rates (3.5% versus 2.4%; $P > 0.05$) in the second period (Fig. 2).

Admission wards of patients with resistant isolates. There was no relationship between patients infected with resistant *Candida* species isolates and specific hospital wards (Tables 3 and 4). The four fluconazole-resistant *C. albicans* isolates had different genotypes; this was also observed for the two echinocandin-resistant *C. tropicalis* isolates (Tables 3 and 4). In contrast, the 10 fluconazole-resistant *C. glabrata* isolates had eight genotypes; genotype CG-24 was detected in 3 isolates, 2 fluconazole-resistant isolates, and 1 echinocandin-resistant isolate. No epidemiological association was found between patients harboring *C. glabrata* clones since they were not admitted to the same wards or during the same period of candidemia onset.

DISCUSSION

Slight changes in the etiology and antifungal susceptibility in yeast species causing fungemia have been observed over time in our hospital. Despite the drop in the number of episodes since the beginning of the study, the rate of fluconazole resistance has increased, whereas resistance to echinocandin has remained stable. Moreover, resistance to fluconazole is not caused by the spread of resistant clones but is due to the presence of species showing intrinsic reduced susceptibility.

Population-based studies, aimed to assess the epidemiology of fungemia in large geographic areas, have proven *C. albicans* to be the main etiological agent. The second species in terms of frequency may be either *C. parapsilosis* (southern Europe), *C. glabrata* (northern Europe and North America), or *C. tropicalis* (Asia and Latin America) (2, 5–8, 11, 12). However, these types of studies may overlook interhospital nuances and differences that may be relevant to initiating empirical treatment while identifying the pathogen. Furthermore, periodic surveillance is also needed to follow epidemiological and antifungal resistance trends. We have been actively conducting monitoring studies with isolates causing fungemia at our hospital since 2007 (10). The current study is an update in which only incident isolates were included. We reassessed the data, dividing the study period into two subperiods, and applied the EUCAST 2020 updated fluconazole clinical breakpoints and epidemiological cutoffs (ECOFFs). The epidemiology of the examined species is in line with that reported in southern European hospitals (8, 11). The number of episodes decreased considerably (566 versus 330) between the two subperiods. This may explain why the implementation of educational campaigns, focused on catheter care and management, initiated in our hospital in 2010 led to a decrease in the number of fungemia infections (13). Health care workers' knowledge on the guidelines for the prevention of catheter-related bloodstream infections was assessed before and after the program, and care bundles were implemented in adult and neonatal units, the pediatric ICU, and the internal medicine department (13–16). In the present study, no statistically significant epidemiological differences were observed, except for *C. glabrata* and *C. krusei*, for which increases were seen in the

second period, in line with what has been reported elsewhere (5). *C. glabrata* is commonly found in elderly patients and among transplant recipients, whereas *C. krusei* is often isolated from patients with underlying hematological malignancies under fluconazole prophylaxis (6). *C. albicans* and *C. parapsilosis* are frequently associated with catheter-related candidemia. This may explain why the infection control campaign helped decrease the number of *C. parapsilosis* and *C. albicans* cases, while *C. glabrata* and *C. krusei* cases remained the same. On the other hand, rare *Candida* species and non-*Candida* yeasts showed a trend toward decrease over time, and *C. auris* was not detected in this study.

Antifungal resistance has been associated with poor outcome in patients with fungemia, as well as with higher hospital care costs (17). Echinocandin resistance is related to poor clinical response (18), whereas in cases of azole resistance, this is less clear (17). International guidelines recommend antifungal susceptibility testing in invasive *Candida* isolates, ideally using reference methods (3, 19). Given antifungal susceptibility species-specific patterns, correctly identifying the species is an invaluable tool to predict antifungal susceptibility and guide antifungal treatment (3). Nevertheless, antifungal susceptibility testing is necessary to detect either acquired fluconazole resistance in purportedly susceptible species such as *C. albicans*, *C. tropicalis*, and *C. parapsilosis* (9) or echinocandin resistance in species such as *C. albicans*, *C. tropicalis*, and *C. glabrata* (18, 20).

Fluconazole resistance is driven by species epidemiology, as shown in countries with a large number of *C. glabrata*- and *C. krusei*-related candidemia cases, which show high resistance rates (2). Globally, echinocandin resistance is generally low in most common *Candida* species, except for *C. glabrata*, for which increasing resistance rates have been reported in North America and northern Europe (2, 5, 18). In the multicenter CANDIPOP study carried out in Spain, a 6.9% rate of fluconazole resistance and low rates of echinocandin resistance were found (8). A later study, also conducted in Spain, showed lower rates of fluconazole resistance (3.4%) and full susceptibility to echinocandins (12). The rates of fluconazole resistance in *Candida* spp. (4.7%) and overall echinocandin resistance (3.1%) reported in this study are in line with those reported in the CANDIPOP study.

We observed an increase of fluconazole resistance over time, particularly due to the presence of *C. glabrata* and *C. krusei*. EUCAST antifungal susceptibility clinical breakpoint update testing affected only *C. glabrata* and fluconazole; the new breakpoint was lowered from ≥ 64 mg/liter to ≥ 32 mg/liter, implying that the rate of resistance in this species has increased (from 7% to 9%). Acquired resistance to azoles in *C. albicans* is due to efflux pumps or alterations in the *ERG3* and *ERG11* genes. We found polymorphisms in the *ERG3* genes of the four azole-resistant *C. albicans* strains. However, the effects mutations in the *ERG3* gene have on the acquisition of high-level azole resistance remain unclear (21). We further sequenced the *ERG11* gene and detected previously reported mutations (D116E/D E266D V488I/V and A114S Y257H G464S/G) (22).

The echinocandin resistance communicated here is low and stable. We detected previously reported *FKS* gene mutations in our *C. glabrata* and *C. tropicalis* echinocandin-resistant isolates (23). The F659S substitution in *C. glabrata* isolates confers low-level resistance exclusively to anidulafungin (23–25). Amino acid substitutions in resistant *C. albicans* isolates at the S645 and R647 positions lead, respectively, to high MICs of echinocandins and slight MIC elevations but resistance exclusively to micafungin (26). The above-mentioned phenotypes in *C. albicans* were confirmed in this study for both echinocandin-resistant *C. tropicalis* isolates; furthermore, both S654F and R656G/R substitutions have been previously reported (27).

Monitoring resistant *Candida* clones may be helpful to avoid dissemination of resistance and prevent outbreaks (9). In our hospital, there is no spread of resistant *C. albicans* and *C. tropicalis* clones. Two *C. glabrata* clones infected 2 and 3, respectively, epidemiologically unrelated patients, suggesting the clones may have a limited capacity to cause outbreaks. Moreover, no association was found between resistant isolates and

specific hospital locations. It is not uncommon to find clones involving patients who were either admitted to the same hospital but without a clear epidemiological link or admitted to different hospitals that in some cases were located in different countries (13, 28). The haploid species *C. glabrata* shows a relatively low genetic diversity compared to diploid *Candida* species such as *C. albicans*, although with sufficient typing discrimination; the two molecular methods mainly used for *C. glabrata* genotyping—multilocus sequence typing and microsatellite markers—correlate well (29). Clones without a clear epidemiological link may indicate the presence of common widespread clones or low typing resolution of the microsatellites (e.g., homoplasy, an intrinsic limitation of microsatellite typing analysis, leading to alleles with identical sizes but different sequences), or they may simply be a consequence of the lower diversity of a haploid species. Future studies using whole-genome sequencing will help decipher the role of epidemiologically unrelated clones.

We conclude that the etiology and antifungal susceptibility of the pathogens that cause fungemia have not significantly changed over time at our hospital. Antifungal resistance should be carefully monitored in order to check future trends.

MATERIALS AND METHODS

Definition of fungemia episode and study period. We studied the incident isolates causing fungemia in patients admitted to Hospital General Universitario Gregorio Marañón (Madrid, Spain) between January 2007 and December 2019. A fungemia episode was defined as the presence of yeasts in blood cultures. The presence of two different species in a single patient was considered a polyfungal episode (both species found simultaneously in the same set of blood cultures) or different episodes (both species found in different sets of blood cultures).

Isolate identification and antifungal susceptibility testing. Isolates were confirmed by amplification of the ITS1-5.8S-ITS2 region (10). Their *in vitro* antifungal susceptibilities to amphotericin B, fluconazole, voriconazole, and posaconazole (Sigma-Aldrich, Madrid, Spain), isavuconazole (Basilea Pharmaceutica, Basel, Switzerland), micafungin (Astellas Pharma, Inc., Tokyo, Japan), and anidulafungin (Pfizer Pharmaceutical Group, New York, NY) was assessed by the EUCAST EDef 7.3.2 broth dilution method using tissue-treated plates (CELLSTAR, 655180; Greiner bio-one, Frickenhausen, Germany) (30). Ranges of concentrations tested for each antifungal are shown in Table 2. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as quality controls.

Isolates were categorized as resistant, susceptible, wild type, or non-wild type according to clinical breakpoints or tentative epidemiological cutoff values (ECOFFs) provided by EUCAST (31). Since there are no available clinical breakpoints for isavuconazole, we used tentative ECOFFs or wild-type upper limits previously proposed for *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis*, and *C. krusei* isolates (32). Phenotypically resistant isolates were retested. The *FKS1* and *FKS2* genes were sequenced in anidulafungin- and/or micafungin-non-wild-type *C. glabrata* and *C. tropicalis* isolates (27). The *ERG3* and *ERG11* genes were sequenced in fluconazole non-wild-type *C. albicans* (33). Resistance/non-wild-type rates were calculated per isolate exclusively. In the absence of available clinical breakpoints/ECOFFs against non-*Candida* yeasts, resistance/non-wild-type rates were calculated either exclusively (azoles) in *Candida* species isolates or overall (echinocandins) in *Candida* spp. plus non-*Candida* yeasts given that echinocandins show systematically high MICs against non-*Candida* yeasts.

Proportions of species isolates and percentages of resistance found in the subperiods from 2007 to 2013 and 2014 to 2019 were compared (Epidat v.4.2; Consellería de Sanidade, Xunta de Galicia, Spain).

Genotyping. Species-specific microsatellite markers were used to genotype *C. albicans* (CDC3, EF3, HIS3 CAI, CAIII, and CAVI), *C. parapsilosis* (CP1, CP4a, CP6, and B), *C. glabrata* (MTI, ERG3, GLM4, Cg4, Cg7, and Cg10), and *C. tropicalis* (Ctrm1, Ctrm10, Ctrm12, Ctrm21, Ctrm24, and Ctrm28) isolates showing resistance to any of the drugs tested (28, 34). We sized and scored the electrophoretic bands with GeneMapper version 4.0 (Applied Biosystems-Life Technologies Corporation, CA). The allelic composition was studied for each locus and converted to binary data by scoring the presence or absence of each allele. Genetic relationships between genotypes were clarified by constructing a minimum spanning tree using BioNumerics version 7.6 (Applied Maths, Sint-Martens-Latem, Belgium). Singletons were genotypes found only once. Isolates were considered genotypically identical when they showed the same alleles with all markers; identical genotypes were further defined as a match (found in serial samples of a given patient) or as a cluster (found in ≥ 2 patients). Different genotypes were codified as follows: CA-X (*C. albicans*), CP-X (*C. parapsilosis*), and CT-X (*C. tropicalis*), where X represents the internal code of the genotype in our collection.

Ethical considerations. This study was approved by the Ethics Committee of Hospital Gregorio Marañón (CEIm; study no. MICRO.HGUGM.2020-011).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.23 MB.

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