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Trends in antifungal resistance in *Candida* from a multicenter study conducted in Madrid (CANDIMAD study): fluconazole-resistant *C. parapsilosis* spreading has gained traction in 2022

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ABSTRACT We previously conducted a multicenter surveillance study on *Candida* epidemiology and antifungal resistance in Madrid (CANDIMAD study; 2019–2021), detecting an increase in fluconazole-resistant *Candida parapsilosis*. We here present data on isolates collected in 2022. Furthermore, we report the epidemiology and antifungal resistance trends during the entire period, including an analysis per ward of admission. *Candida* spp. incident isolates from blood cultures and intra-abdominal samples from patients cared for at 16 hospitals in Madrid, Spain, were tested with the EUCAST E.Def 7.3.2 method against amphotericin B, azoles, micafungin, anidulafungin, and ibrexafungerp and were molecularly characterized. In 2022, we collected 766 *Candida* sp. isolates (686 patients; blood cultures, 48.8%). *Candida albicans* was the most common species found, and *Candida auris* was undetected. No resistance to amphotericin B was found. Overall, resistance to echinocandins was low (0.7%), whereas fluconazole resistance was 12.0%, being higher in blood cultures (16.0%) mainly due to fluconazole-resistant *C. parapsilosis* clones harboring the Y132F-R398I ERG11p substitutions. Ibrexafungerp showed *in vitro* activity against the isolates tested. Whereas *C. albicans* was the dominant species in most hospital wards, we observed increasing *C. parapsilosis* proportions in blood. During the entire period, echinocandin resistance rates remained steadily low, while fluconazole resistance increased in blood from 6.8% (2019) to 16% (2022), mainly due to fluconazole-resistant *C. parapsilosis* (2.6% in 2019 to 36.6% in 2022). Up to 7 out of 16 hospitals were affected by fluconazole-resistant *C. parapsilosis*. In conclusion, rampant clonal spreading of *C. parapsilosis* fluconazole-resistant genotypes is taking place in Madrid.

KEYWORDS *Candida*, antifungal resistance, *C. parapsilosis*, Y132F, Madrid

Some institutions have reported changes in *Candida* species distribution alongside an increase in antifungal resistance rates in recent years, mainly because of the emergence of *Candida auris* or fluconazole-resistant *Candida parapsilosis*, which partially intensified during the COVID-19 pandemic (1–3). To date, Spain, France, Italy, Greece, Austria, Switzerland, Czech Republic, Germany, Poland, Belgium, the Netherlands, the UK, Ireland, Denmark, Norway, Sweden, Finland, Russia, and Turkey are the European countries that have reported *C. auris* infections (4, 5). Moreover, fluconazole-resistant *C. parapsilosis* isolates harboring the Y132F ERG11p substitution have also been recently reported in Turkey, France, Italy, Slovakia, and Spain (1, 3, 6).

Prospective surveillance studies on invasive *Candida* isolates are key to monitoring resistance rates, studying local epidemiology, and detecting clones or species of

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particular interest. Unfortunately, such studies are rarely conducted in Spain (7–10). Therefore, we conducted a multicenter surveillance study (named CANDIMAD, CANDIdaemia in MADrid) on *Candida* isolates collected from patients admitted to the main hospitals located in Madrid, Spain, which cover an area of around seven million people. The main observation from isolates collected between 2019 and 2021 was the emergence of fluconazole-resistant *C. parapsilosis* causing candidemia in patients admitted to five of the participating hospitals. All resistant isolates harbored the Y132F (in four hospitals) or G458S (in a fifth hospital) ERG11p substitutions (11–13). The rate of fluconazole resistance in *C. parapsilosis* at that time showed an upward trend suggesting that the problem in Madrid was increasing in magnitude (13).

Most surveillance studies assessed antifungal resistance during a limited period of time, thus lacking a long-term perspective, which is particularly required after the outbreak of the COVID-19 pandemic (14, 15). Here, we report data from isolates collected in the CANDIMAD study in 2022, the year in which the COVID-19 pandemic started to recede, and assessed the trend of antifungal resistance since the beginning of the project in 2019. Furthermore, we provide additional detailed information concerning *Candida* spp. epidemiology and rates of resistance per type of ward of admission of patients.

RESULTS

Species epidemiology and antifungal resistance rates found in isolates collected in 2022

We collected 766 *Candida* sp. isolates (n = 686 patients) from blood cultures (n = 374, 48.8%) and intra-abdominal samples [n = 392, 51.2%; peritoneal fluid (n = 175, 44.6%), liver samples, (n = 98, 25.0%), peritoneal abscess (n = 88, 22.5%), abdominal drainage (n = 22, 5.6%), spleen (n = 6, 1.5%), abdominal wound exudate (n = 2, 0.5%), and peritoneal biopsy (n = 1, 0.3%)]. Most patients yielded one isolate each (n = 622), but 9.3% of patients (n = 64) yielded ≥2 isolates. Species distribution is shown in Fig. 1. We found that proportions of *C. albicans*, *C. krusei*, and other *Candida* spp. were lower in blood cultures than in intra-abdominal samples (39.8% versus 51.4%, 1.1% versus 3.6%, and 1.3% versus 5.1%, respectively; $P < 0.05$), whereas *C. parapsilosis* complex proportions were higher in blood cultures than in intra-abdominal samples (36.1% versus 10.2%; $P < 0.05$). We did not detect *C. auris*.

No resistance to amphotericin B was found. Overall, echinocandin and fluconazole resistance rates in *Candida* were 0.7% (n = 5/766) and 12.0% (n = 92/766) of isolates and 0.7% (n = 5/686) and 13.1% (n = 90/686) of patients, respectively. Resistant isolates came from 12/16 hospitals, and no cross-resistance between azoles and echinocandins was found.

A total of 0.3% (n = 1/374; *C. glabrata*, P633T *FKS1* HS1) of blood culture isolates and 1.0% [n = 4/392; *C. glabrata* (*FKS* sequence wild type, F659S *FKS2* HS1, D666E *FKS2* HS1, and D666G *FKS2* HS1)] of intra-abdominal isolates were echinocandin resistant (Fig. 1A and B). Per-patient echinocandin resistance rates were 0.3% and 1.5% in blood cultures and intra-abdominal samples ($P > 0.05$), respectively. Ibrexafungerp showed *in vitro* activity against the isolates tested (Tables S1 and S2), as we only found three ibrexafungerp non-wild-type isolates from intra-abdominal samples: two *C. albicans* isolates (echinocandin susceptible and harboring *FKS* wild-type sequence) and one *C. glabrata* isolate (echinocandin resistant and harboring an F659S *FKS2* HS1 substitution).

A total of 16.0% (n = 60/374) of blood culture isolates were fluconazole resistant (*C. parapsilosis*, n = 49; *C. glabrata*, n = 6; *C. krusei*, n = 4; and *C. pararugosa*, n = 1). Excluding *C. krusei* isolates from the analysis, fluconazole resistance rate was 15.1% (Fig. 1A). Likewise, a total of 8.2% (n = 32/392) of isolates from intra-abdominal samples were fluconazole resistant (*C. krusei*, n = 14; *C. parapsilosis*, n = 7; *C. glabrata*, n = 5; *C. albicans*, n = 1; *C. lusitaniae*, n = 2; *C. guilliermondii*, n = 1; *C. inconspicua*, n = 1; *C. tropicalis*, n = 1); excluding *C. krusei* isolates from the analysis, fluconazole resistance rate decreased to 4.8% (Fig. 1B). Per-patient fluconazole resistance rates were 16.5% and 9.5% in blood cultures and intra-abdominal samples ($P < 0.05$) and 15.6% and 5.5% excluding *C. krusei*

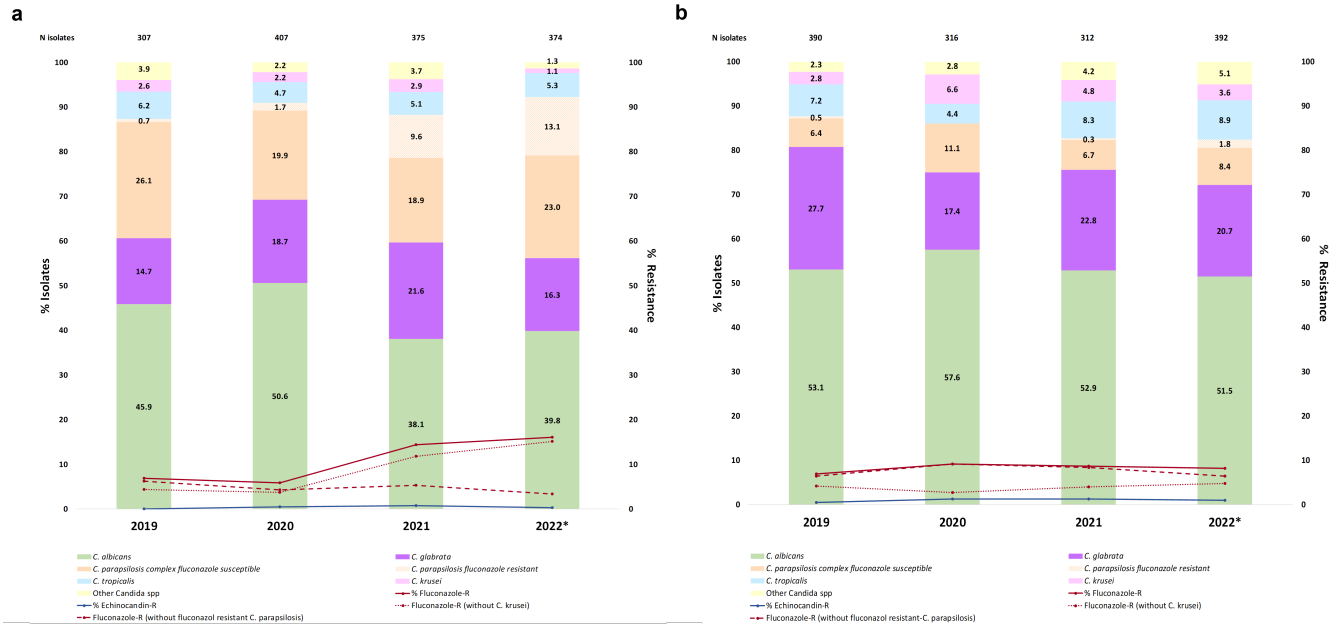

FIG 1 Species distributions and resistance rates per year in *Candida* sp. isolates from (a) blood cultures and (b) intra-abdominal samples. *Isolates collected in 2022. Blood cultures: *C. parapsilosis* complex (*C. parapsilosis sensu stricto*, *n* = 134; and *C. orthopsilosis*, *n* = 1); *Candida* spp. (*C. lusitaniae*, *n* = 3; *C. guilliermondii*, *n* = 1; and *C. pararugosa*, *n* = 1); non-*Candida* (*Saccharomyces cerevisiae*, *n* = 2; *Rhodotorula mucilaginosa*, *n* = 2; and *Cryptococcus neoformans*, *n* = 1), which represented 1.3% of blood isolates. Intra-abdominal samples: *C. parapsilosis* complex (*C. parapsilosis sensu stricto*, *n* = 39; and *C. orthopsilosis*, *n* = 1); *Candida* spp. (*C. lusitaniae*, *n* = 11; *C. dubliniensis*, *n* = 4; *C. guilliermondii*, *n* = 3; *C. pararugosa*, *n* = 1; and *C. inconspicua*, *n* = 1); non-*Candida* (*Pichia manshurica*, *n* = 1; *Wickerhamomyces onychis*, *n* = 1), which represented 0.5% of intra-abdominal isolates. Non-*Candida* isolates were excluded from the analysis. Adapted from a previously reported publication (12); reprinted with the journal's permission (license number: 5623520933072); data from 2022 are here newly reported.

isolates ($P < 0.05$), respectively. Isolates from blood/intra-abdominal samples that were non-wild type to voriconazole (15.0%/2.8%), posaconazole (2.4%/1.5%), or isavuconazole (5.6%/1.8%) were also fluconazole non-wild type (Tables S1 and S2), except for an isavuconazole-non-wild-type but fluconazole-susceptible *C. glabrata* isolate from blood.

The fluconazole-resistant *C. parapsilosis* isolates (*n* = 56) were sourced from 54 patients (two patients simultaneously harbored isolates from blood cultures and intra-abdominal samples) cared for at six hospitals. Isolates were detected in four of the five hospitals previously reported and were newly reported in Hospital 3 and Hospital 7. Most resistant isolates harbored the Y132F-R398I ERG11p substitutions (*n* = 53) and were grouped into four clonally related genotypes [CP-451 (*n* = 46 isolates from *n* = 44 patients cared for at six hospitals), CP-674 (*n* = 4 isolates from one patient each, all of them cared for at a single hospital), CP-707 (*n* = 2 isolates from one patient each from two hospitals), and CP-795 (*n* = 1)]. The remaining resistant isolates harbored the G458S ERG11p substitution (*n* = 3 isolates from one patient each) and the CP-675 genotype and were exclusively found in one hospital (Fig. 2). We found the V437I *ERG11* gene substitution in a *C. albicans* isolate.

Trends in species epidemiology over time

Since 2020, in blood cultures, we observed increasing proportions of *C. parapsilosis* (2020 = 21.6% < 2021 = 28.5% < 2022 = 36.1%; $P < 0.05$) and decreasing proportions of *C. albicans* (2020 = 50.6% > 2021 = 38.1%; $P < 0.05$). We detected an increase in *C. glabrata* from 2019 to 2021 (2019 = 14.7% > 2021 = 21.6%; $P < 0.05$) and a decrease in other *Candida* spp. in 2022 (1.3%) compared with 2019 (3.9%) and 2021 (3.7%) ($P < 0.05$) (Fig. 1). Few differences were found in species distributions in intra-abdominal samples over time; we found decreasing proportions of *C. glabrata* (2019 = 27.7% > 2020 = 17.4%; $P <$ 0.05) and increasing proportions of *C. krusei* (2019 = 2.8% > 2020 = 6.6%; $P < 0.05$) from

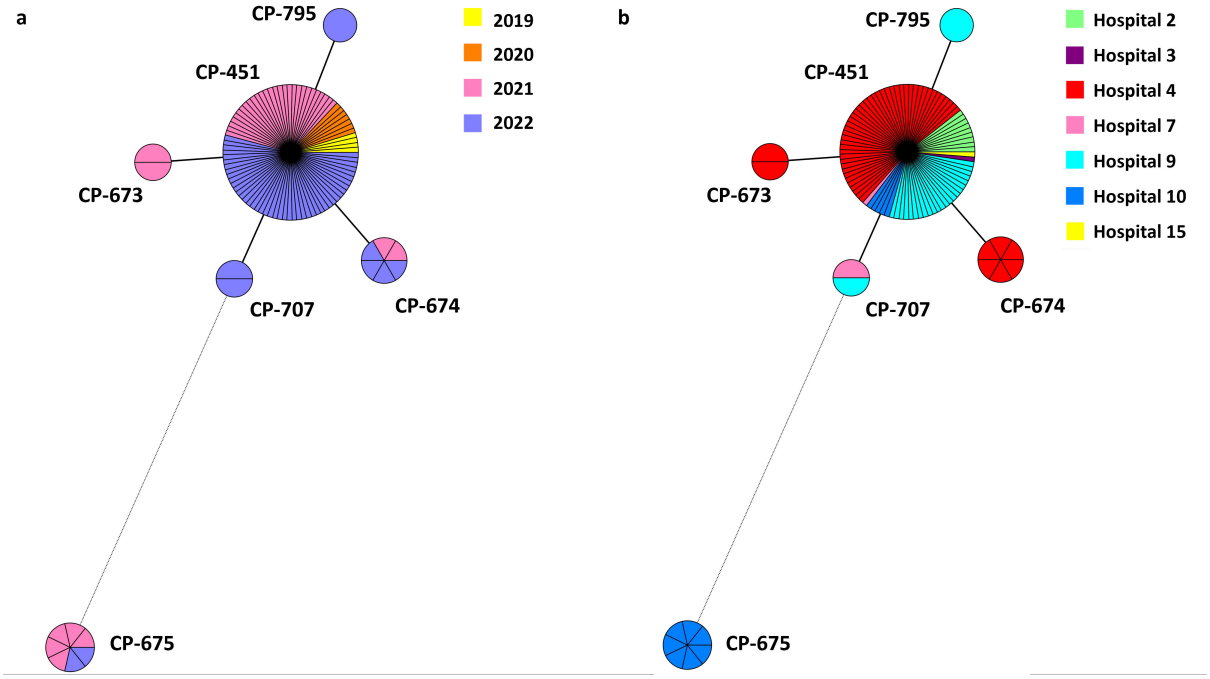

FIG 2 Minimum spanning tree showing fluconazole-resistant *C. parapsilosis* genotypes found during the study period (2019–2022) per year of detection (A) or per hospital (B) Circles represent different genotypes, and circle size, the number of isolates belonging to the same genotype. Connecting lines between the circles show profile similarities. The solid bold line indicates differences in only one marker, and the dotted line indicates differences in four or more markers. Genotypes CP-673, CP-674, CP-707, and CP-795 differ from CP-451 at microsatellite markers B, CP6, CP4a, and CP6, respectively.

2019 to 2020. We also observed an increase in other *Candida* spp. between 2019 and 2022 (2019 = 2.3% > 2022 = 5.1%; $P < 0.05$).

Species distributions are hospital dependent (Fig. S1). We studied the epidemiology of *Candida* spp. from blood cultures per hospital ward. *C. albicans* accounted for 22.9% to 60.9% of isolates, being the dominant species in most wards, except for the following wards: pediatrics, oncology-hematology, and other wards. *C. parapsilosis* complex accounted for 20.9% to 42.9% of cases, being particularly frequent in pediatric wards. *C. glabrata* was frequent in medical (22.6%) and surgical wards (20.2%) but undetected in pediatrics and neonatology. *C. tropicalis* proportions were particularly high in pediatrics (14.3%) and accounted for 4.0%–7.2% in other areas. Finally, *C. krusei* and other *Candida* spp. were especially frequent in pediatrics (8.6% and 11.4%, respectively) (Fig. 3). Species distribution in intra-abdominal samples was more homogeneous, and isolates mainly came from surgical and medical wards. *C. albicans* was the dominant species in all hospital wards followed by *C. glabrata*, except for pediatrics and neonatology (data not shown).

Trends in antifungal resistance rates over time

Overall, echinocandin resistance rates were low and steady, regardless of the sample type (Fig. 1). Fluconazole resistance rates in blood cultures are shown in Fig. 1A. Resistance to fluconazole increased from 6.8% in 2019 to 16% in 2022 and was mainly impacted by fluconazole-resistant *C. parapsilosis*, whose rates of fluconazole resistance have been on the rise over the years (2019 = 2.6% < 2020 = 8.0% < 2021 = 34.0% < 2022 = 36.6%; $P < 0.05$). In fact, the exclusion of fluconazole-resistant *C. parapsilosis* resulted in significantly lower resistance rates (2019 = 6.2%; 2020 = 4.3%; 2021 = 5.3%; 2022 = 3.4%; $P < 0.05$). Such an impact was not observed when excluding *C. krusei* (2019 = 4.3%; 2020 = 3.8%; 2021 = 11.8%; 2022 = 15.1%; $P > 0.05$) (Fig. 1A). Resistance rates are hospital dependent, as shown by the increasing rate of *C. parapsilosis* from 2020 to

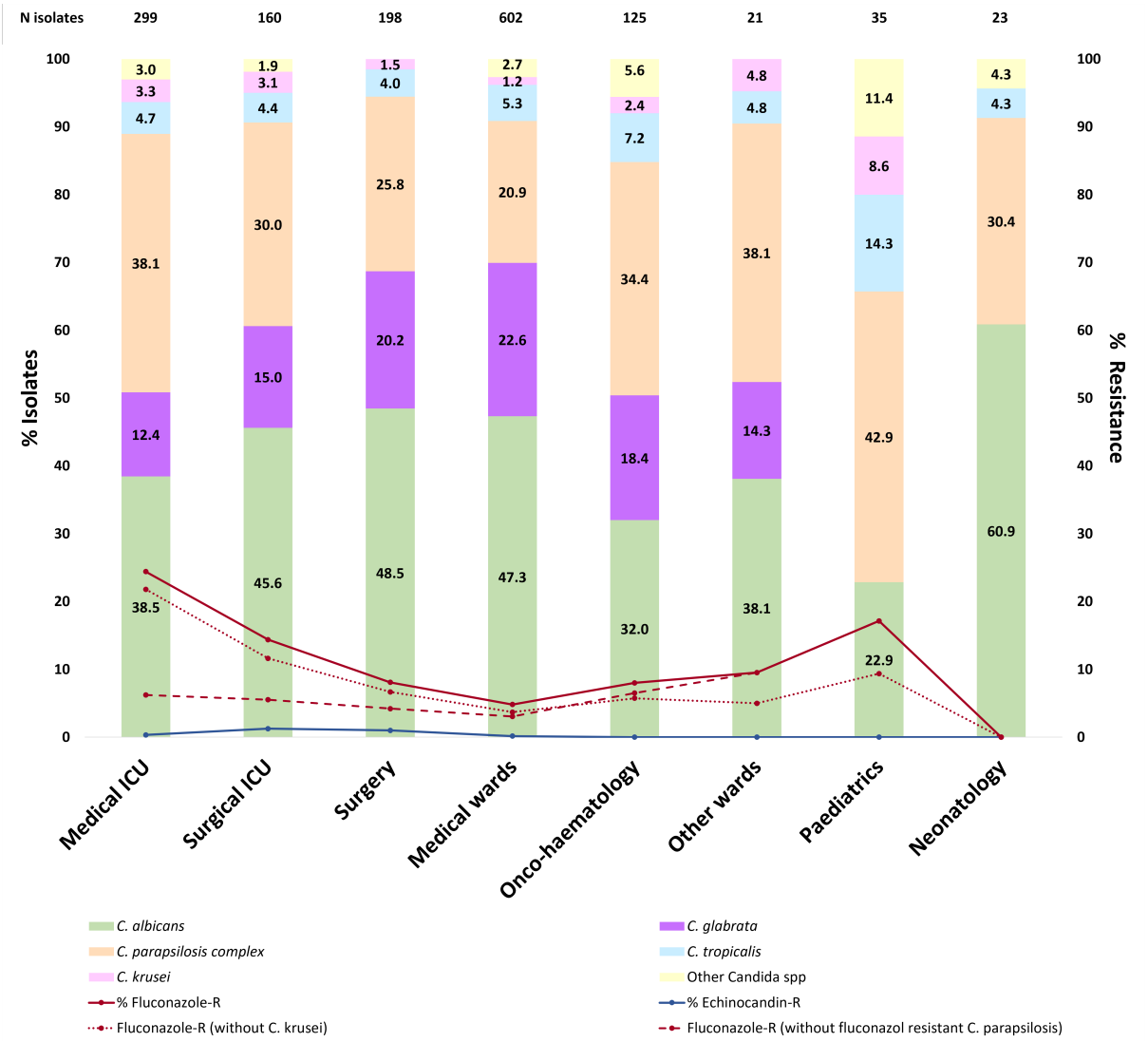

FIG 3 Species distributions and resistance rates of *Candida* spp. isolates from blood cultures per ward of admission during the 2019–2022 period.

2021 in Hospitals 2, 4, 9, and 10 (Table 1 and Fig. S1). In addition to the presence of fluconazole-resistant *C. parapsilosis* isolates in two newly affected hospitals (Hospitals 3 and 7) in 2022, the most relevant observation was the dramatic increase in the number of such isolates in Hospital 4, where resistance rates in blood isolates significantly increased

TABLE 1 Per-year *C. parapsilosis* fluconazole resistance rates at the seven hospitals affected[c]

Fluconazole-resistant *C. parapsilosis, N* (%)				
Hospital	**2019**	**2020**	**2021**	**2022**
2	0 (0)	1 (16.7)	1 (7.7)	7 (33.3)
3	0 (0)	0 (0)	0 (0)	1 (4.3)
4	0 (0)	**3 (17.6)**	**20 (66.7)**	**30 (76.9)**
7	0 (0)	0 (0)	0 (0)	2 (9.5)
9	4 (36.4)	3 (27.3)	10 (55.6)	8 (36.4)
10	0 (0)	0 (0)	5 (71.4)[a]	8 (88.9)[b]
15	0 (0)	0 (0)	1 (33.3)	0 (0)

[a]All resistant isolates harbored the G458S ERG11p substitution.
[b]A total of 55.6% and 33.3% of resistant isolates harbored the Y132F and G458S ERG11p substitutions, respectively.
[c]Numbers in bold indicate differences reaching statistical significance (2020 versus 2021 and 2020 versus 2022; $P < 0.05$).

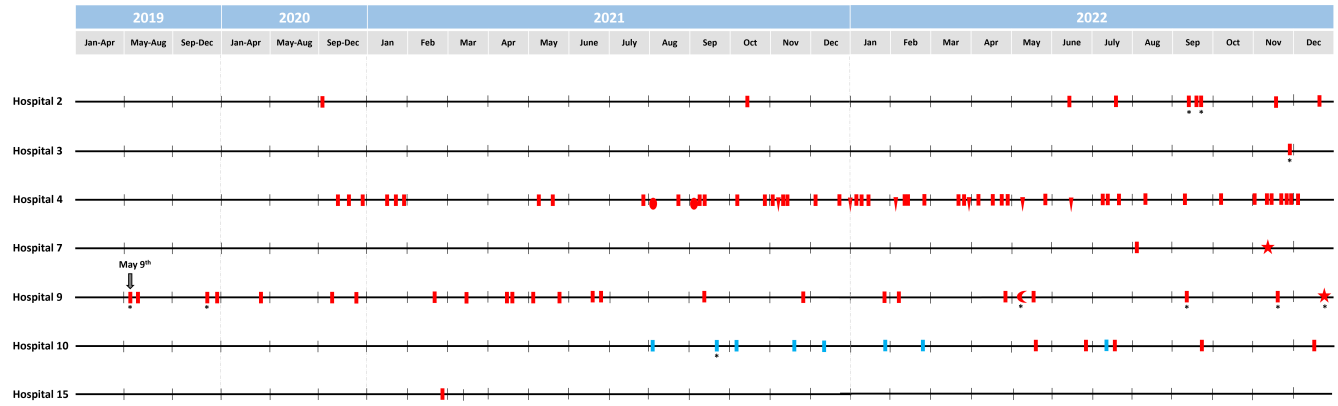

FIG 4 Timeline of the detection of fluconazole-resistant *C. parapsilosis* isolates at each affected hospital over the 4-year study period. Colored symbols refer to fluconazole-resistant *C. parapsilosis* isolates from blood cultures, an asterisk represents isolates from intra-abdominal samples. Red indicates isolates within the clonal complex (CP-451, bar; CP-673, oval; CP-674, triangle; CP-707, star; and CP-795, moon) of genotypes harboring the Y132F-R398I ERG11p substitution. Blue indicates isolates within the CP-675 genotype harboring the G458S substitution. Arrow indicates the first time resistant isolates were detected. Adapted from a previously reported figure (11); reprinted with the journal's permission (order license ID: 1397047–1); data from 2022 are here newly reported.

between 2020 and 2022 (Table 1; $P < 0.05$). In intra-abdominal samples, fluconazole resistance rates did not change over the years and were hugely affected by *C. krusei*, since its exclusion lowered rates of resistance especially in 2020 and 2021 ($P < 0.05$) (2019 = 4.2%; 2020 = 2.7%; 2021 = 4.0%; 2022 = 4.8%) (Fig. 1B). In contrast, an increase in fluconazole-resistant *C. parapsilosis* isolates was detected in intra-abdominal samples in 2022 in Hospital 9 (Fig. S2).

The timeline of the fluconazole-resistant *C. parapsilosis* isolates detected over the 4-year period is shown in Fig. 4. Resistant *C. parapsilosis* isolates were found in seven hospitals (Table 1) and were mainly driven by the presence of genotype CP-451, which has been constantly spreading across hospitals over the years; from time to time and to a lesser extent, clonally related fluconazole-resistant genotypes were also detected (Fig. 2 and 4).

DISCUSSION

Our study demonstrates rampant clonal spreading of *C. parapsilosis* fluconazole-resistant genotypes in Madrid, a spread that gained traction at some hospitals in 2022. *C. auris* remains undetected in blood and intra-abdominal samples in Madrid's hospitals.

The species distribution in blood isolates per ward of admission was as expected. Whereas *C. albicans* was usually the most common species, *C. parapsilosis* was frequent in intensive care unit (ICU) wards and neonatology (16), *C. glabrata* was associated with abdominal surgery and elderly patients (17), and *C. tropicalis* was frequent in patients with hematological malignancies (18). Species distribution in pediatrics was dominated by non-albicans *Candida* spp.; neither *C. glabrata* nor *C. krusei* isolates were found in neonatology (19, 20).

The distribution of *Candida* species in isolates collected during the study period, in both blood cultures and intra-abdominal samples, was comparable to previous studies (9, 21). *C. auris* has been detected in some hospitals in the Mediterranean area of Spain (22–24); however, it remains undetected in invasive isolates in Madrid. In contrast, since 2019, we have detected an increase in fluconazole-resistant *C. parapsilosis* isolates from blood cultures along with a decrease in *C. albicans* proportions, as reported elsewhere (25, 26). This fluconazole-resistant *C. parapsilosis* increase overtook overall fluconazole resistance rates in blood cultures, mainly in patients cared for in ICU wards. In fact, fluconazole-resistant *C. parapsilosis* in blood cultures increased 14-fold between 2019 and 2022; by excluding such isolates, fluconazole resistance rates would have been low and steady over the study period in Madrid (27). In a recent study conducted in European

countries, Arendrup and collaborators detected a high fluconazole resistance rate (17%) in *C. parapsilosis* blood isolates. Those isolates sourced from Greece, Turkey, and Italy, countries where the fluconazole resistance rates in *C. parapsilosis* was up to 37%, a figure that is line with the fluconazole resistance rates reported in Madrid between 2021 and 2022 (26).

The increasing detection of fluconazole-resistant *C. parapsilosis* in Madrid can be attributed to the presence of genotypes harboring the Y132F ERG11p substitution, which could be more associated with patient-to-patient transmission rather than prior azole exposure (1). The fluconazole-resistant *C. parapsilosis* isolates detected in Madrid mostly belonged to the dominant CP-451 genotype, accounting for 53.5% of all fluconazole-resistant isolates, which has become endemic in many of the hospitals affected. The CP-451 genotype was detected for the first time in 2019; since then, its presence has gained traction and two hospitals became newly affected in 2022. The fact that fluconazole-resistant *C. parapsilosis* increased in blood cultures and emerged in intra-abdominal samples in one hospital in 2022 is a matter of concern. Furthermore, the presence of three *C. parapsilosis* fluconazole-resistant isolates from Hospital 3 (two rectal swabs and one skin catheter) and two isolates from Hospital 10 (a wound exudate and a catheter tip) suggests that it might be the tip of the iceberg (data not shown). In contrast, echinocandin resistance was steadily low throughout the entire study period and was mainly due to *C. glabrata* isolates from the abdominal cavity.

C. parapsilosis shows intrinsic low susceptibility to echinocandins, thus making the emergence of fluconazole resistance a matter of concern. Ibrexafungerp, a new inhibitor of (1, 3)-β-D-glucan synthase, has partial activity against *FKS*-mutant *C. glabrata* isolates (28) and here exhibited potent activity against all isolates tested, including fluconazole-resistant *C. parapsilosis*. Our *in vitro* observations open the door to future clinical evaluations of the efficacy of ibrexafungerp for the treatment of invasive infections caused by fluconazole-resistant *C. parapsilosis*.

This study is subject to limitations. We did not collect colonizing samples from patients or environmental samples. Since we were unaware of the nosocomial infection control policies at each hospital, we could not explain the different patterns of fluconazole-resistant *C. parapsilosis* spreading. Finally, we did not collect the clinical data of infected patients; such analysis will form part of a future study.

In conclusion, our study demonstrates rampant clonal spreading of *C. parapsilosis* fluconazole-resistant genotypes in Madrid; this spreading gained traction at some hospitals in 2022. Hospital control measures should urgently be taken to bar further spreading and prevent new hospitals from becoming affected. *C. auris* remains undetected in blood and intra-abdominal samples in Madrid's hospitals.

MATERIALS AND METHODS

Study period and isolate selection

We studied *Candida* spp. isolates from blood cultures and intra-abdominal samples sourcing from patients cared for at 16 hospitals in Madrid, Spain (CANDIMAD study) collected from 1 January 2019 to 31 December 2022. As previously reported, one available incident isolate per species, patient, and compartment (blood culture and/or any intra-abdominal samples) was studied (12).

Species identification and antifungal susceptibility testing

Isolates were molecularly identified (12), and we assessed antifungal susceptibilities to amphotericin B, fluconazole, voriconazole, posaconazole, micafungin, and anidulafungin (Sigma-Aldrich, Madrid, Spain), isavuconazole (Basilea Pharmaceutica, Basel, Switzerland), and ibrexafungerp (Scynexis, Inc., Durham, NC, USA) using the EUCAST E.Def 7.3.2 broth dilution method (12, 29). Isolates were categorized as resistant/non-wild type as previously reported (12). Resistant isolates were re-tested.

Molecular characterization of resistant isolates

The *FKS1* and *FKS2* genes were sequenced in either echinocandin-resistant or ibrexafungerp-non-wild-type isolates (12). We sequenced the *ERG11* gene in fluconazole-resistant *C. albicans*, *C. tropicalis*, and *C. parapsilosis* isolates (12). Fluconazole-resistant *C. parapsilosis* isolates were genotyped by means of species-specific microsatellite markers (CP1, CP4a, CP6, and B) (11, 13) that were highly discriminatory, since the probability of identity for *C. parapsilosis* was 1.2×10^6 (30). Two or more isolates were considered genotypically identical when they presented the same alleles with all markers. Clonally related genotypes were those that differed in one microsatellite marker (13).

Data analysis

We here report new data regarding the epidemiology and antifungal resistance rates found in isolates collected in 2022. Comparisons of species epidemiology and antifungal resistance rates over time (from 2019 to 2022) were assessed per isolate, sourcing blood cultures or intra-abdominal samples separately, per patient, and also broken down per ward of patient admission. We calculated fluconazole resistance rates per year and compartment considering overall isolates or excluding fluconazole-resistant *C. parapsilosis* or *C. krusei*. We also calculated fluconazole-resistant *C. parapsilosis* proportions exclusively in affected hospitals. Proportions were compared using Epidat v.4.2 (Consellería de Sanidade, Xunta de Galicia, Spain).

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Judith Díaz-García, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review and editing | Marina Machado, Resources, Writing – review and editing | Luis Alcalá, Resources, Writing – review and editing | Elena Reigadas, Resources, Writing – review and editing | Ana Pérez-Ayala, Resources, Writing – review and editing | Elia Gómez-García de la Pedrosa, Resources, Writing – review and editing | Fernando Gónzalez-Romo, Resources, Writing – review and editing | María Soledad Cuétara, Resources, Writing – review and editing | Coral García-Esteban, Resources, Writing – review and editing | Inmaculada Quiles-Melero, Resources, Writing – review and editing | Nelly Daniela Zurita, Resources, Writing – review and editing | María Muñoz-Algarra, Resources, Writing – review and editing | María Teresa Durán-Valle, Resources, Writing – review and editing | Aida Sánchez-García, Resources, Writing – review and editing | Patricia Muñoz, Resources, Writing – review and editing | Pilar Escribano, Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing | Jesus Guinea, Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

ETHICS APPROVAL

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

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Tables S1 and S2; Figure S1 and S2 (AAC00986-23-S0001.docx). Additional tables and figures complementary to the main document.

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