Global Emergence of Resistance to Fluconazole and Voriconazole in Candida parapsilosis in Tertiary Hospitals in Spain During the COVID-19 Pandemic

Nuria Trevijano-Contador,1 Alba Torres-Canó,1 Cristina Carballo-González,1 Mireia Puig-Agussenso,1,2,* María Teresa Martín-Gómez,1 Emilio Jiménez-Martínez,3,4 Daniel Romero,5 Francesc Xavier Nuvials,6 Roberto Olmos-Arenas,7 María Clara Moretó-Castellsagüe,9 Lucia Fernández-Delgado,8 Graciela Rodriguez-Sevilla,9 María-Mercedes Aguilar-Sánchez,8 Josefina Ayats-Ardite,8 Carmen Ardanuy-Tisaisa,15,16 Isabel Sánchez-Romero,8 María Muñoz-Algarra,8 Paloma Merino-Amador,8,9,1011 Fernando González-Romo,8,9,10,11 Gregoria Megías-Lobón,12 Jose Angel García-Campos,1 María Ángeles Mantecon-Vallejo,1 Eva Alcoceba,1 Pilar Escrivano,14,15,16 Jesús Guinea,14,16 María Teresa Durán-Valle,17 Arturo Manuel Fraile-Torres,17 María Pia Roiz-Mesones,18 Isabel Lara-Plaza,18 Ana Pérez de Ayala,19 María Simón-Sarcristán,19 Ana Collazos-Blanco,19 Teresa Nebreda-Mayoral,20 Gabriel March-Roselló,21 Laura Alcázar-Fuoli,1,2;22 and Oscar Zaragoza1,22

1Mycology Reference Laboratory, National Centre for Microbiology, Instituto de Salud Carlos III, Carretera Majadahonda-Pozuelo, Madrid, Spain, 2Department of Infectious Diseases, Hospital Universitari de Bellvitge-Institut d’Investigació Biomèdica de Bellvitge (IDIBELL), Barcelona, Spain, 3Center for Biomedical Research in Network in Infectious Diseases (CIBERINFE, CB21/13/00006), Instituto de Salud Carlos III, Madrid, Spain, 4Department of Microbiology, Vall d’Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain, 5Intensive Care Unit, Vall d’Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain, 6Microbiology Department, Hospital Universitari de Bellvitge, IDIBELL, Barcelona, Spain, 7Center for Biomedical Research Network in Respiratory Diseases (CIBERS-CB06/00037), Instituto de Salud Carlos III, Madrid, Spain, 8Microbiology Department, Hospital Universitario Puerta de Hierro, Majadahonda, Madrid, Spain, 9Microbiology Department, Hospital Universitario Clínico San Carlos, Madrid, Spain, 10Institute of Investigation Sanitary Hospital Clínic San Carlos (IdiISSC), Madrid, Spain, 11Department of Medicine, Universidad Complutense School of Medicine, Madrid, Spain, 12Department of Clinical Microbiology, Hospital Universitario de Burgos, Burgos, Castilla y León, Spain, 13Clinical Microbiology Department, Hospital Universitat Son Espases, Mallorca, Spain, 14Clinical Microbiology and Infectious Diseases, Hospital General Universitario Gregorio Marañón, Madrid, Spain, 15Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain, 16Institute for Biomedical Research Network in Respiratory Diseases (CIBERS-CB06/00098), Madrid, Spain, 17Microbiology and Parasitology Department, Hospital Universitario de Móstoles, Madrid, Spain, 18Microbiology Unit, Universitary Hospital 12 de Octubre, Madrid, Spain, 19Microbiology and Parasitology Department, Hospital Central de la Defensa Gómez Ulla, Madrid, Spain, 20Microbiology and Immunology Unit, Universitary Hospital of Valladolid, Valladolid, Spain, and 21Center for Biomedical Research in Network in Infectious Diseases (CIBERINFE-CB21/13/00105), Instituto de Salud Carlos III, Madrid, Spain

Background. Candida parapsilosis is a frequent cause of candidemia worldwide. Its incidence is associated with the use of medical implants, such as central venous catheters or parenteral nutrition. This species has reduced susceptibility to echinocandins, and it is susceptible to polyenes and azoles. Multiple outbreaks caused by fluconazole-nonsusceptible strains have been reported recently. A similar trend has been observed among the C. parapsilosis isolates received in the last 2 years at the Spanish Mycology Reference Laboratory.

Methods. Yeast were identified by molecular biology, and antifungal susceptibility testing was performed using the European Committee on Antimicrobial Susceptibility Testing protocol. The ERG11 gene was sequenced to identify resistance mechanisms, and strain typing was carried out by microsatellite analysis.

Results. We examined the susceptibility profile of 1315 C. parapsilosis isolates available at our reference laboratory between 2000 and 2021, noticing an increase in the number of isolates with acquired resistance to fluconazole, and voriconazole has increased in at least 8 different Spanish hospitals in 2020–2021. From 121 recorded clones, 3 were identified as the most prevalent in Spain (clone 10 in Catalonia and clone 96 in Castilla-Leon and Madrid, whereas clone 67 was found in 2 geographically unrelated regions, Cantabria and the Balearic Islands).

Conclusions. Our data suggest that concurrently with the coronavirus disease 2019 pandemic, a selection of fluconazole-resistant C. parapsilosis isolates has occurred in Spain, and the expansion of specific clones has been noted across centers. Further research is needed to determine the factors that underlie the successful expansion of these clones and their potential genetic relatedness.

Keywords. Candida parapsilosis; antifungal resistance; fluconazole; outbreaks; voriconazole.
with a higher risk of infection [5, 6]. Besides sporadic infections, C. parapsilosis is well known to cause nosocomial outbreaks through direct and indirect contact via the hands of health care workers and through contaminated patient care equipment.

Candida parapsilosis exhibits a reduced natural in vitro susceptibility to echinocandins [7], so the main therapeutic options for invasive infections due to this species are the triazoles, mainly fluconazole or, alternatively, polyenes. Acquired resistance to fluconazole in C. parapsilosis is a rare phenomenon, <5% of isolates in different epidemiological studies [2, 7–10]. In recent years, however, a steady increase in resistance has been observed worldwide, mostly in the context of nosocomial outbreaks [11–20]. In many cases, these outbreaks are monoclonal and are associated with mutations in ERG11 (mainly with the Y132F mutation), overexpression of efflux pumps (as Mdr1 and Cdr1), and mutations in MRR1, which encodes a transcription factor that regulates the expression of some efflux pumps [11–13, 16, 18, 21, 22].

The National Centre for Microbiology from Instituto de Salud Carlos III (CNM-ISCHII, Madrid, Spain) acts as a national reference center for clinically isolated fungi, providing services such as genotyping and confirmation of antifungal susceptibility profiles by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standardized methodology. Since 2020, a significant increase in the number of fluconazole-nonsusceptible (FNS) C. parapsilosis isolates received was noted, most of them coming from tertiary hospitals across the country reporting strong epidemiological suspicion of ongoing outbreaks.

The aim of this work was to describe the antifungal susceptibility profile of all the C. parapsilosis isolates received in the Spanish Mycology Reference Laboratory (SMRL) to gain insights about the susceptibility profile and appearance of resistance in this species. Typing analysis confirmed genetic relatedness between isolates and their distribution across centers not always in the geographical vicinity.

This nationwide study adds new data to the worrisome phenomenon of worldwide emergingazole resistance in C. parapsilosis and points toward a temporal relationship between the coronavirus disease 2019 (COVID-19) pandemic and the expansion of clonal outbreaks in several Spanish tertiary hospitals.

**METHODS**

**Media and Strains Identification**

The isolates were primarily isolated, identified, and screened for fluconazole non-susceptibility at local laboratories following the routine methodologies of each center. Isolates sent to the CNM-ISCHII from 2000 to 2021 and identified as C. parapsilosis sensu stricto were subcultivated onto Sabouraud solid or liquid medium. Identification was confirmed by sequencing the internal transcribed spacer 1 (ITS1) and ITS2 regions from the ribosomal DNA as previously described [23].

**Antifungal Susceptibility**

Antifungal susceptibility testing was performed following the EUCAST protocol [24] (RPMI 1640 medium; Merck, Sigma-Aldrich). The medium was buffered with MOPS (Merck, Sigma-Aldrich) at pH 7 and supplemented with 2% glucose (Merck, Sigma-Aldrich). The following antifungals were tested in the concentration range indicated in brackets: amphotericin B (AmB; Merck, Sigma-Aldrich, 16–0.03 mg/L), fluconazole (FLC; Merck, Sigma-Aldrich, 64–0.125 mg/L), itraconazole (ITZ; Janssen Pharmaceutical Research and Development, 8–0.016 mg/L), voriconazole (VOR; Pfizer Pharmaceutical Group, 8–0.016 mg/L), posaconazole (POS; Merck, Sigma-Aldrich, 8–0.016 mg/L), isavuconazole (ISV; Pfizer Pharmaceutical Group, 8–0.016 mg/L), caspofungin (CSP; Merck, Sigma-Aldrich, 16–0.016 mg/L), micafungin (MICA; Astellas Pharma Inc, 2–0.004 mg/L), and anidulafungin (ANID; Pfizer Pharmaceutical Group, 4–0.008 mg/L). The minimal inhibitory concentration (MIC) was defined as the concentration that caused 50% of growth inhibition compared with the control well without an antifungal, except for amphotericin B (90%). Strains were categorized as susceptible (S), resistant (R), or intermediate or susceptible increased exposure (I) following the breakpoints established by EUCAST (see https://www.eucast.org/astoffungi/clinicalbreakpointsforantifungals, document from February 4, 2020) [24]. Control strains C. parapsilosis ATCC 22019 and C. krusei ATCC 6258 were included in all the assays.

**Sequencing of the ERG11 Gene**

To identify mutations at the ERG11 gene, we designed different primers (Table 1). The whole gene was amplified using oligonucleotides 01_F and 02_R using the following polymerase chain reaction (PCR) conditions: 94 °C for 2 minutes and 35 cycles of amplification (94 °C for 30 seconds, 56 °C for

### Table 1. Oligonucleotides Designed to Sequence the Candida parapsilosis ERG11 Gene

| Oligonucleotide Name | Sequence (5’ - 3’) |
|----------------------|--------------------|
| 01_F_CpERG11         | CGTCAAATGTACAGCTGTC |
| 02_R_CpERG11         | TCATTGGAAGTTGAATC |
| 03_F_CpERG11         | TGGGTGCTGAGGGTATC |
| 05_F_CpERG11         | ACCATCTCCACGCTGAC |
| 07_F_CpERG11         | GTGCAATTGGGAGGAAGC |
| 09_F_CpERG11         | CCAAGGTTGTTAATCC |
| 10_R_CpERG11         | GACATAGGCAAACTTTCAC |
| 08_R_CpERG11         | CCACCTTACCAGATAAGG |
| 06_R_CpERG11         | GCATACTTGGACAAATGAG |
| 04_R_CpERG11         | CCAAGTACACGGTCATTA |

Abbreviations: F, forward; R, reverse.
RESULTS AND DISCUSSION

We collected all the isolates available at our laboratory (SMRL) and analyzed the evolution of the antifungal susceptibility pattern from 2000 to 2021. A total of 1315 isolates were studied. As shown in Table 1, resistance to fluconazole remained low (3%–7%) among the isolates from our collection until 2016. However, a dramatic change in this resistance rate among the isolates received at the Reference Laboratory was noted thereafter, being particularly notable from 2019 onwards. Throughout the latter period, the percentage of fluconazole resistance significantly increased (27% in 2019, around 60% in 2020 and 2021) (Table 2) as compared with previous years. This trend was also observed for voriconazole (Table 3). Before 2019, the voriconazole resistance rate was below 2%, but since 2020, the percentage of susceptible increased exposure (I, MIC = 0.25 mg/L) and resistant strains (MIC > 0.25 mg/L) increased up to around 60% among the strains received at the laboratory.

Regarding itraconazole and posaconazole, there was a slight trend toward higher MICs, but they were still categorized as susceptible (Tables 4 and 5). Only 3 isolates were fully resistant to fluconazole: voriconazole, itraconazole, and posaconazole. For isavuconazole, although there are no breakpoints to define resistant strains, an increase in the MICs among the isolates received since 2020 was found. The isavuconazole modal MIC rose from 0.016 mg/L before 2020 to 0.06 mg/L later on (Table 6), similar to what was observed for itraconazole and posaconazole. All the isolates were considered wild-type to the rest of the antifungals tested (AmB, flucytosine, caspofungin, micafungin, anidulafungin).

The presence of ERG11 mutations in fluconazole non-susceptible isolates was investigated. The ERG11 gene of 244 strains from 2019, 2020, and 2021, including S (n = 34), I (susceptible, increased exposure, n = 7), and R (n = 203) strains to FLC, was sequenced. The ERG11 gene was found to be wild-type in all the susceptible strains, and in 4.8% of the FNS isolates. Among the latter (n = 11), 1 strain was also susceptible increased exposure (I), and 6 were resistant to voriconazole. The remaining fluconazole-resistant isolates (n = 192, 94.6%) harbored the Y132F mutation, which has already been associated with FLC resistance in C. parapsilosis (Table 7). In addition, we found that 1 of the resistant isolates harbored the K143R mutation in the ERG11 gene, which has been detected in azole-nonsusceptible strains causing monoclonal outbreaks in India [26], as well as in combination with the Y132F mutation [11]. This mutation has also been associated with pan-azole resistance in C. tropicalis [27]. Another strain harbored the G458S mutation, which has been related to azole resistance in Candida parapsilosis [4, 28]. Finally, many isolates harbored R398I (data not shown), but this mutation was also found in several susceptible isolates, which suggest that it is not related to FLC resistance.

MIC analysis was performed with SPSS software. For each year, the distribution of MICs was reported. We also calculated the geometric mean of the MIC values, the median, and the minimal and maximal values of the distributions.
Interestingly, in up to 35% of FNS strains, the Y132F substitution was found in heterozygosis. Thus, we analyzed if the triazole MIC distribution differed among homozygous or heterozygous strains. We observed that strains that harbored the Y132F mutation in homozygosis had higher MICs to fluconazole (geometric mean = 26.1 mg/L) compared with strains carrying the mutation in heterozygosis (geometric mean = 12.5). A similar situation was found for voriconazole (GM of heterozygous strains = 0.39 mg/L vs GM for homozygous strains = 0.5 mg/L). The Y132F substitution did not have a significant influence on susceptibility to isavuconazole, posaconazole, and itraconazole. Moreover, for these 3 antifungals, the Y132F mutation in homozygosis tended to result in lower GM than in heterozygous strains (Table 8).

To investigate if there was any genetic correlation between the FNS strains, we performed a microsatellite-based genotyping of 270 C. parapsilosis (from 2019, 2020, and 2021) isolates from 234 different patients and 8 environmental strains, including 81 susceptible, 6 susceptible increased exposure (I), and 183 resistant isolates (fluconazole categorization). The majority of the strains were isolated from blood (38.3% among FLC-S strains and 44.4% among FLC-R/I strains), skin (13.6% among FLC-S strains and 20.1% among FLC-R/I strains), and respiratory samples (9.9% among FLC-S strains and 11.64% among FLC-R/I strains). Among the susceptible isolates, we included strains from the same hospitals that had resistant strains, but also others not related to these outbreaks. Microsatellite genotyping identified 121 different genotypes. The relationship between the obtained genotypes is illustrated in Figures 1 and 2 and Supplementary Table 1.

As compared with the FNS isolates, genotypic variability was greater among fluconazole-susceptible strains, which could be attributed, in part, to the fact that most of the susceptible strains were recovered from unrelated cases.

Remarkably, in the case of contemporary resistant isolates, there was a markedly well-defined geographical distribution of genotypes. Genotype 10 was found to be the dominant cluster among strains of 2 hospitals from the area of Barcelona (Bellvitge Universitary Hospital and Vall d’Hebron University Hospital). This cluster was noted for the first time in 3 isolates from 1 of these 2 centers by 2019 (Bellvitge University Hospital), and was also detected in a third center of the metropolitan area of Barcelona that contributed with a single isolate sent to the reference laboratory the same year. These 2 former hospitals also shared the closely related genotype 12. Neither genotype 10 nor genotype 12 was found in
centers from other regions in Spain. Genotype 96 was found to be highly prevalent among isolates obtained from centers located in Madrid and Burgos (Castilla León Region). Genotype 67 was found in a hospital in the north of Spain (Hospital Marqués de Valdecilla, Santander, Cantabria), geographically distant from Madrid and Barcelona. Interestingly, genotype 67 was found in outbreaks from 2 distant hospitals, Son Espases Hospital (Balearic Islands) and Marqués de Valdecilla Hospital (Santander, Cantabria). The outbreak in the Balearic Islands has been previously described [29]. Additionally, fluconazole-susceptible strains isolated in the context of another nosocomial outbreak (Universitary Clinic Hospital from Valladolid) were received, displaying genotypes clearly different from the abovementioned and closely related to each other (genotypes 45–50). The geographical distribution of the genotypes of the resistant strains is shown in Figure 1.

A minimum spanning tree was built, showing that some genotypes have evolved by spontaneous changes in one of the microsatellite markers. The microsatellite analysis showed a distribution of clades that grouped by geographic origin, with resistant strains clustering together (Figure 2).

Our work shows a significant increase in the number of C. parapsilosis samples resistant to fluconazole and voriconazole received at the SMRL from several Spanish hospitals and arising in a relatively short period. These isolates seem to be part of outbreaks that have emerged almost simultaneously in distant cities and that can be attributed to clones that are shared almost exclusively among geographically close related centers. From these data, a generalized increment in fluconazole resistance among Spanish isolates of C. parapsilosis cannot be inferred as it is not mandatory to report all the infections caused by these species. All together, our data are in sharp contrast to what has being described in several former epidemiological studies carried out in Spain [6, 7, 30, 31], suggesting a new and worrisome change in the epidemiological incidence of FNS C. parapsilosis strains. Furthermore, our work also suggests that there has been a dispersion of several genotypes between different hospitals from the same and different regions. In particular, genotype 10 has disseminated through different hospitals and Cataluña, genotype 96 was first found in Madrid and Burgos, and genotype 67 was found in the Balearic Islands and Cantabria. Similar findings have recently been described from hospitals in Madrid [32]. When analyzing the sample isolation dates, our data suggest that the major clone that circulated in hospitals in Madrid disseminated to Burgos Hospital. Of interest is the case of the hospitals in the Balearic Islands and

Table 3. Distribution of the Percentage of MIC to Voriconazole of C. parapsilosis Strains Received at the SMRL Since 2000

| Year | 0.016 | 0.031 | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | >8 | No. | % I | % S | % R |
|------|--------|--------|------|-------|------|-----|---|---|---|---|---|-----|-----|-----|-----|
| 2000 | 79     | 14     | 7    | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 42 | 50  | 0  | 0  |
| 2001 | 73     | 23     | 1    | 3     | 0    | 0   | 0 | 0 | 0 | 0 | 74 | 100 | 0  | 0  |
| 2002 | 85     | 14     | 0    | 0     | 0    | 1   | 0 | 0 | 0 | 0 | 80 | 99  | 0  | 1  |
| 2003 | 85     | 11     | 0    | 1     | 0    | 0   | 0 | 0 | 0 | 0 | 94 | 100 | 0  | 0  |
| 2004 | 96     | 4      | 0    | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 75 | 100 | 0  | 0  |
| 2005 | 93     | 6      | 0    | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 82 | 99  | 0  | 1  |
| 2006 | 94     | 4      | 1    | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 71 | 100 | 0  | 0  |
| 2007 | 85     | 6      | 4    | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 73 | 99  | 1  | 0  |
| 2008 | 90     | 4      | 2    | 0     | 1    | 1   | 1 | 0 | 0 | 0 | 92 | 97  | 3  | 0  |
| 2009 | 88     | 5      | 1    | 3     | 1    | 0   | 0 | 0 | 0 | 0 | 55 | 98  | 2  | 0  |
| 2010 | 85     | 4      | 5    | 2     | 0    | 0   | 0 | 0 | 0 | 0 | 22 | 100 | 0  | 0  |
| 2011 | 77     | 18     | 5    | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 22 | 100 | 0  | 0  |
| 2012 | 73     | 14     | 0    | 7     | 0    | 0   | 0 | 0 | 0 | 0 | 14 | 100 | 0  | 0  |
| 2013 | 69     | 23     | 4    | 0     | 0    | 0   | 4 | 0 | 0 | 0 | 26 | 96  | 0  | 4  |
| 2014 | 88     | 9      | 0    | 2     | 0    | 0   | 0 | 0 | 0 | 0 | 46 | 100 | 0  | 0  |
| 2015 | 70     | 13     | 13   | 4     | 0    | 0   | 0 | 0 | 0 | 0 | 23 | 100 | 0  | 0  |
| 2016 | 57     | 26     | 0    | 9     | 0    | 4   | 4 | 0 | 0 | 0 | 23 | 91  | 0  | 9  |
| 2017 | 87     | 0      | 0    | 0     | 0    | 13  | 0 | 0 | 0 | 0 | 15 | 87  | 0  | 13 |
| 2018 | 27     | 26     | 0    | 7     | 20   | 7   | 13 | 0 | 0 | 0 | 15 | 53  | 7  | 40 |
| 2019 | 24     | 7      | 1    | 13    | 22   | 32  | 1 | 0 | 0 | 0 | 76 | 32  | 13 | 55 |
| 2020 | 28     | 2      | 3    | 5     | 25   | 27  | 7 | 1 | 1 | 0 | 204 | 38  | 25 | 37 |

The table includes the number of strains analyzed each year and the % of susceptible, susceptible increased exposure, and resistant isolates. Cells in orange: mode (most frequent value). In yellow: left and right values to the mode.

Abbreviations: I, susceptible increased exposure; MIC, minimum inhibitory concentration; R, resistant; S, susceptible; SMRL, Spanish Mycology Reference Laboratory.
Santander (Cantabria), which are more distant than the rest of the hospitals described in this work. In this case, since the outbreak in Son Espases Hospital occurred in 2015 [29], we hypothesized that this clone was disseminated to Cantabria.

Recent emergence of FNS isolates in C. parapsilosis has been described in other countries in the literature [11–20], so our data support that the increase of azole resistance in C. parapsilosis might be a global problem. In this study, the majority of resistant isolates harbored the Y132F mutation, which has been largely associated in the literature with the appearance of clonal outbreaks. However, we also detected a few isolates that did not have this mutation. For this reason, further studies should be performed to describe all the resistance mechanisms circulating among Spanish hospitals.

At the moment, the reasons for the increase in the incidence of azole-resistant C. parapsilosis strains in Spain are unknown, but we hypothesized that this phenomenon may be related to the negative impact that the COVID-19 pandemic has had in Spanish hospitals for several reasons. There seems to be a clear temporal correlation between the increase in the number of resistant isolates received at the reference laboratory and the clinical impact of the pandemic. The COVID-19 pandemic has resulted in a severe overcrowding of hospitals and, in particular, of intensive care units, along with the necessity of recruiting large numbers of health care professionals that were not properly trained in infection control measures. Moreover, during the pandemic, there were changes in personal protective equipment protocols, and the same gloves could have been used between patients [33, 34]. This might have increased the risk of cross-transmission between patients and caused hospital outbreaks. Furthermore, during the pandemic, there has been a significant transfer of patients and health care professionals between different hospitals, which might have contributed to the dispersion of resistant clones between clinical tertiary centers. A similar situation has been described in multicenter studies in India [26], which highlights the ability of FNS isolates to spread and colonize hospital environments. Interestingly, some of the analyzed strains in this work in 1 of the hospitals (Bellvitge Universitary Hospital) were isolated from an environmental origin in the hospital, and we found that the most prevalent genotype obtained from patients in this hospital (genotype 10) was also present in different hospital locations. This correlation suggests that C. parapsilosis clones mis hospit-  

| Year | 0.016 | 0.031 | 0.061 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | >8 | No. | % S | % R |
|------|-------|-------|-------|-------|------|-----|---|---|---|---|----|-----|-----|-----|
| 2000 | 58    | 30    | 9     | 2     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 43  | 100 | 0   |
| 2001 | 30    | 55    | 11    | 4     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 74  | 100 | 0   |
| 2002 | 25    | 58    | 18    | 0     | 1    | 0   | 0 | 0 | 0 | 0 | 0 | 80  | 99  | 1   |
| 2003 | 94    | 56    | 12    | 1     | 0    | 1   | 0 | 0 | 0 | 0 | 0 | 115 | 99  | 1   |
| 2004 | 61    | 31    | 7     | 1     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 75  | 100 | 0   |
| 2005 | 32    | 41    | 6     | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 82  | 100 | 0   |
| 2006 | 73    | 20    | 6     | 1     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 71  | 100 | 0   |
| 2007 | 93    | 25    | 8     | 1     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 17  | 99  | 1   |
| 2008 | 92    | 40    | 4     | 0     | 2    | 1   | 0 | 0 | 0 | 0 | 0 | 92  | 97  | 3   |
| 2009 | 78    | 21    | 0     | 1     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 73  | 100 | 0   |
| 2010 | 95    | 0     | 2     | 2     | 2    | 0   | 0 | 0 | 0 | 0 | 0 | 55  | 98  | 2   |
| 2011 | 86    | 9     | 5     | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 22  | 100 | 0   |
| 2012 | 95    | 5     | 0     | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 22  | 100 | 0   |
| 2013 | 71    | 29    | 0     | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 14  | 100 | 0   |
| 2014 | 85    | 23    | 4     | 0     | 4    | 4   | 0 | 0 | 0 | 0 | 0 | 26  | 92  | 8   |
| 2015 | 72    | 28    | 0     | 0     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 46  | 100 | 0   |
| 2016 | 35    | 22    | 30    | 13    | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 23  | 100 | 0   |
| 2017 | 26    | 35    | 22    | 9     | 9    | 0   | 0 | 0 | 0 | 0 | 0 | 23  | 91  | 9   |
| 2018 | 7     | 20    | 67    | 7     | 0    | 0   | 0 | 0 | 0 | 0 | 0 | 23  | 100 | 0   |
| 2019 | 7     | 13    | 93    | 40    | 7    | 0   | 0 | 0 | 0 | 0 | 0 | 15  | 93  | 7   |
| 2020 | 15    | 20    | 40    | 23    | 2    | 0   | 0 | 0 | 0 | 0 | 0 | 36  | 98  | 2   |
| 2021 | 23    | 28    | 22    | 20    | 6    | 1   | 1 | 0 | 0 | 0 | 0 | 204 | 89  | 11  |

Cells in orange: mode (most frequent value). In yellow: left and right values to the mode.

Abbreviations: MIC, minimum inhibitory concentration; SMRL, Spanish Mycology Reference Laboratory.
**Table 5. Distribution of the Percentage of MIC to Posaconazole of *C. parapsilosis* Strains Received at the SMRL Since 2000**

| Mutation          | Susceptible | Resistant |
|-------------------|-------------|-----------|
|                   | MIC, mg/L   |           |
|                   | 0.016       | 0.031     | 0.06     | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | >8 | No. | % S | % R |
| ERG11לש | 2001 | 55 | 45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 100 | 0 |
|                   | 2002 | 65 | 31 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 80 | 100 | 0 |
|                   | 2003 | 66 | 19 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 94 | 100 | 0 |
|                   | 2004 | 83 | 17 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 75 | 100 | 0 |
|                   | 2005 | 85 | 33 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 82 | 100 | 0 |
|                   | 2006 | 87 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 70 | 100 | 0 |
|                   | 2007 | 87 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 71 | 99 | 1 |
|                   | 2008 | 70 | 26 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 92 | 97 | 3 |
|                   | 2009 | 81 | 19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 73 | 100 | 0 |
|                   | 2010 | 74 | 20 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 54 | 96 | 4 |
|                   | 2011 | 95 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 100 | 0 |
|                   | 2012 | 91 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 100 | 0 |
|                   | 2013 | 93 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 14 | 100 | 0 |
|                   | 2014 | 92 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 26 | 100 | 0 |
|                   | 2015 | 78 | 20 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 46 | 100 | 0 |
|                   | 2016 | 22 | 52 | 22 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 96 | 4 |
|                   | 2017 | 26 | 57 | 13 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 23 | 96 | 4 |
|                   | 2018 | 13 | 47 | 33 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 93 | 7 |
|                   | 2019 | 13 | 41 | 33 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 87 | 13 |
|                   | 2020 | 33 | 45 | 20 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 76 | 98 | 2 |
|                   | 2021 | 23 | 38 | 20 | 12 | 4 | 2 | 1 | 0 | 0 | 0 | 0 | 204 | 81 | 19 |

Cells in orange: mode (most frequent value). In yellow: left and right values to the mode.

Abbreviations: MIC, minimum inhibitory concentration; SMRL, Spanish Mycology Reference Laboratory.

**Table 6. Distribution of the Percentage of MIC to Isavuconazole of *C. parapsilosis* Strains Received at the SMRL Since 2016**

| Mutation          | MIC, mg/L |
|-------------------|-----------|
|                   | Year       | 0.016 | 0.031 | 0.06 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | >8 | No. |
|                   | 2016       | 88 | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 |
|                   | 2017       | 75 | 15 | 5 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20 |
|                   | 2018       | 87 | 7 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
|                   | 2019       | 53 | 13 | 7 | 13 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 15 |
|                   | 2020       | 39 | 14 | 39 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 72 |
|                   | 2021       | 38 | 23 | 29 | 7 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 196 |

Cells in orange: mode (most frequent value). In yellow: left and right values to the mode.

Abbreviations: MIC, minimum inhibitory concentration; SMRL, Spanish Mycology Reference Laboratory.

**Table 7. Mutations in the *ERG11* Gene Found in Susceptible, Susceptible Increased Exposure, and Resistant Strains to Fluconazole**

| ERG11 Mutation | FLC Susceptible | FLC Susceptible Increased Exposure | FLC Resistant |
|----------------|-----------------|-----------------------------------|--------------|
|                | VOR_S           | VOR_I                             | VOR_R        |
| WT             | 34              | 1                                 | 1            |
| Y132F_HET      | 0               | 3                                 | 1            |
| Y132F_HOMO     | 0               | 0                                 | 1            |

For each category, we also include the susceptibility profile (S/I/R) for voriconazole.

Abbreviations: HET, heterozygous; HOMO, homozygous; I, susceptible increased exposure; MIC, minimum inhibitory concentration; R, resistant; S, susceptible; SMRL, Spanish Mycology Reference Laboratory.
Table 8. Susceptibility Profile of WT or Mutant Strains Harboring the Y132F in Homozygosity or Heterozygosity

| Antifungal | ERG11 Mutation | No. | Median | Geometric Mean | Minimal | Maximal |
|------------|----------------|-----|--------|----------------|---------|---------|
| Fluconazole | WT             | 43  | 0.5    | 0.78           | 0.125   | >64     |
|             | Y132F_HET      | 60  | 16     | 12.7           | 4       | 32      |
|             | Y132F_HOM      | 120 | 16     | 25             | 8       | >64     |
| Voriconazole | WT             | 43  | 0.031  | 0.032          | 0.016   | 4       |
|             | Y132F_HET      | 60  | 0.5    | 0.4            | 0.125   | 1       |
|             | Y132F_HOM      | 120 | 0.5    | 0.5            | 0.06    | 2       |
| Itraconazole | WT             | 43  | 0.03   | 0.033          | 0.016   | 1       |
|             | Y132F_HET      | 60  | 0.125  | 0.10           | 0.031   | 0.25    |
|             | Y132F_HOM      | 120 | 0.06   | 0.05           | 0.016   | 0.25    |
| Posaconazole | WT             | 43  | 0.03   | 0.029          | 0.016   | 0.25    |
|             | Y132F_HET      | 60  | 0.06   | 0.065          | 0.016   | 0.5     |
|             | Y132F_HOM      | 120 | 0.031  | 0.035          | 0.016   | 0.25    |
| Isavuconazole | WT             | 43  | 0.016  | 0.021          | 0.016   | 1       |
|             | Y132F_HET      | 60  | 0.06   | 0.07           | 0.031   | 0.125   |
|             | Y132F_HOM      | 120 | 0.031  | 0.037          | 0.016   | 0.5     |

Abbreviation: WT, wild-type.

Figure 1. Geographical distribution of the different genotypes of FLC-resistant isolates. The pie charts denote the distribution of the different genotypes in different tertiary hospitals from different metropolitan areas in Spain. Abbreviation: FLC, fluconazole. Template of the map of Spain was obtained from a free repository (https://es.m.wikipedia.org/wiki/Archivo:Provinces_of_Spain_%28Blank_map%29.png) and its use and modification is allowed according to the GNU Free Documentation License, version 1.2.
diseases, such as COVID-associated pulmonary aspergillosis (CAPA) [36–38], mucormycosis [39–41], and Candida infections [42, 43] (see reviews in [44, 45]).

The impact of the COVID-19 pandemic and clinical management of COVID patients does not fully explain why there has been a selection of azole-resistant strains and why these genetically different resistant strains have emerged almost simultaneously in distant places across Spain. An increase in the use of antimicrobials has been reported since the appearance of the COVID-19 pandemic in some geographical regions [46]. Among azoles, an increase in the use of echinocandins and voriconazole has been reported [46], which might have favored the selection of fluconazole and voriconazole-resistant C. parapsilosis. To validate this hypothesis, we were able to obtain data on fluconazole and voriconazole use from some of the hospitals, and, as shown in Table 9, there was not a significant increase in the use of fluconazole. A similar trend was found for voriconazole use, although 2 hospitals (Burgos University Hospital and Bellvitge University Hospital) reported an increase of around 2-fold in the use of this last antifungal. These data suggest that the increase in the incidence of FNS strains from C. parapsilosis has not been mainly driven by the selective pressure of the antifungal use. In agreement, no correlation between previous azole treatment and infection by FNS C. parapsilosis strains has been found in the outbreaks from Son Espases University Hospital (Balearic Islands) and Puerta de Hierro University Hospital (Madrid) [29, 47].

Another possibility is that resistance to azoles affects virulence traits. In this sense, it has been described that C. parapsilosis strains harboring the Y132F mutation in ERG11 have reduced ability to form biofilms [11], which raises the hypothesis that these strains have a greater ability to spread and disseminate. Furthermore, several studies have associated the incidence of resistant strains with higher mortality of the patients [11, 28], which warrants further studies on the virulence of FLC-nonsusceptible C. parapsilosis strains. In our case, the

| Hospital                      | Fluconazole 2018 | Fluconazole 2019 | Fluconazole 2020 | Fluconazole 2021 |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| Vall d’Hebron University Hospital | 3.94            | 2.68            | 1.98            | 2.43            |
| 12 de Octubre Hospital         | 2.21            | 2.31            | 3.05            | 2.56            |
| Marqués de Valdecilla University Hospital | 2.16          | 2.11            | 2.24            | 1.94            |

**Table 9. General Fluconazole and Voriconazole Use (Expressed and Defined Daily Doses per 100 Patients Days) in Several Hospitals From Different Geographical Regions From 2018 to 2021**

*Data from use in the intensive care unit.*
clinical management of the patients might have contributed to the selection of preexisting resistant clones circulating in the hospitals before the COVID-19 pandemic [47]. In our case, this idea is supported by the fact that we identified that some of the resistant clones were already detected in samples from 2019 (ie, Bellvitge Hospital) and also in some strains present in our collection from 2019 from the same geographical region. For this reason, future research to investigate the genetic proximity of the resistant isolates is needed, and to compare them not only between different hospitals but also with isolates described in different countries.

Despite the epidemiological limitations and interpretations of our work, we believe that the data presented herein are an indicator of an emerging clinical problem, that is, the selection of azole resistance in *C. parapsilosis* during the COVID-19 pandemic. In particular, we report the appearance of a significant increase in the resistance rate to fluconazole and voriconazole simultaneously in multiple hospitals in Spain. This increase has a temporal correlation with the COVID-19 pandemic, suggesting that the increased incidence of FNS *C. parapsilosis* strains is a consequence of the impact of the pandemic. We also present data that indicate that there has been a dissemination of some genotypes between hospitals, not only from the same cities but from different geographical regions, and despite the clonal diversity documented, only a few of them dominated across centers. We would like to note that the increase in FLC-resistant isolates in tertiary hospitals in Spain is agreement with the worldwide context, where an increasing number of outbreaks caused by FNS *C. parapsilosis* strains are being reported. Our work highlights the importance of national surveillance programs carried out by reference laboratories to detect epidemiological changes and to characterize outbreaks, especially those that involve the selection of microbiol-resistant isolates. We encourage the clinical community to investigate the presence of these clones in the hospital environment, to make an effort to perform susceptibility testing in strains of noninvasive origins (colonization, isolated from hospital surfaces, etc.), and to design specific measures to prevent the expansion of the associated resistance mechanisms.

**Supplementary Data**

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

**Acknowledgments**

**Financial support.** O.Z. was funded by grants SAF2017–86912-R and PID2020–114546RB–100 from the Spanish Ministry for Science and Innovation. This work was also funded by the National Centre for Microbiology (Instituto de Salud Carlos III) through the Surveillance Program of Antifungal Resistance and the Center for Biomedical Research in Network of Infectious Diseases CIBERINFECTRBC21/13/00105 (O.Z. and L.A.F.), CIBERINFECC–CB21/13/00009 (M.P.-A.), CIBERES-CB06/06/0037 (C.A.-T.), and CIBERES-CB06/06/0058 (J.G.). L.A.-F. was supported by Fondo de Investigación Sanitaria (MPY 117/18 and MICYT 15/05/20). We thank Dr. David Campany Herrero (Vall d’Hebron Hospital), Noelia Garrido Peño (Móstoles Hospital), David Gómez Gómez y Aitziber Illarre Uranga (Marques de Valdecilla Hospital), María Ángeles Machín Morón (Burgos Hospital), Jose Manuel Caro Teller (Doce de Octubre Hospital), Marina Calvo (Puerta de Hierro Hospital), and Ariadna Padulles (Bellvitge Hospital) for providing the data on antifungal consumption from their hospitals. We also thank Ángel Zaballos and Pilar Jiménez from the Genomics Core Facility from Instituto de Salud Carlos III for their technical help with the microsatellite analysis technique.

**Potential conflicts of interest.** All authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**Patient consent.** This study does not include factors necessitating patient consent.

**References**

1. Toth R, Nosek J, Mora-Montes HM, et al. *Candida parapsilosis* from genes to the bedside. *Clin Microbiol Rev* 2019; 32:e00111–18.
2. Pfaffer MA, Diekema DJ, Turndidge JD, Castanheira M, Jones RN. Twenty years of the SENTRY antifungal surveillance program: results for Candida species from 1997–2016. *Open Forum Infect Dis* 2019; 6:579–94.
3. Lamothe F, Lockhart SR, Berkow EL, Calandra T. Changes in the epidemiological landscape of invasive candidiasis. *J Antimicrob Chemother* 2018; 73:14–13.
4. Arastehfar A, Lass-Flores C, García-Rubio R, et al. The quiet and underappreciated rise of drug-resistant invasive fungal pathogens. *J Fungi* (Basel) 2020; 6:138.
5. Yamin DH, Husin A, Harun A. Risk factors of Candida parapsilosis catheter-related bloodstream infection. *Front Public Health* 2021; 9:631865.
6. Puig-ASENSIO M, Padilla B, Garnacho-Montero J, et al. Epidemiology and predictive factors for early and late mortality in Candida bloodstream infections: a population-based surveillance in Spain. *Clin Microbiol Infect* 2014; 20:0245–54.
7. Guinea J, Zaragoza O, Escribano P, et al. Molecular identification and antifungal susceptibility of yeast isolates causing fungemia collected in a population-based study in Spain in 2010 and 2011. *Antimicrob Agents Chemother* 2014; 58: 1529–37.
8. Jatta R, Caggiano G, Cuna T, Montagna MT. Antifungal susceptibility testing of a 10-year collection of Candida spp. isolated from patients with candidemia. *J Chemother* 2011; 23:92–6.
9. Ziccardi M, Souza LO, Gandra RM, et al. *Candida parapsilosis* (sensu latu) isolated from hospitals located in the southeast of Brazil: species distribution, antifungal susceptibility and virulence attributes. *Int J Med Microbiol* 2015; 305:848–59.
10. Battistolo L, Glampakdis E, Damonti L, et al. Increasing morbidity and mortality of candidemia over one decade in a Swiss university hospital. *Mycoses* 2021; 64: 1512–20.
11. Arastehfar A, Daneshnia F, Hâlimoglu-Polar S, et al. First report of candidemia clonal outbreak caused by emerging fluconazole-resistant *Candida parapsilosis* isolates harboring Y132F and/or Y132F + K143R in Turkey. *Antimicrob Agents Chemother* 2020; 64:e01001–20.
12. Choi YJ, Kim YJ, Yong D, et al. Fluconazole-resistant *Candida parapsilosis* bloodstream isolates with Y132F mutation in ERG11 gene, South Korea. *Emerg Infect Dis* 2018; 24:1768–70.
13. Corzo-Leon DE, Peacock M, Rodriguez-Zulueta P, Salazar-Tamayo GJ, MacCallum DM. General hospital outbreak of invasive candidiasis due to azole-resistant Candida parapsilosis associated with an ErG11 Y132F mutation. *Med Mycol* 2021; 59:664–71.
14. Fekkar A, Blaize M, Rougue A, et al. Hospital outbreak of fluconazole-resistant Candida parapsilosis: arguments for clonal transmission and long-term persistence. *Antimicrob Agents Chemother* 2021; 65:e02036–20.
15. Govender NP, Patel I, Magobo RE, et al. Emergence of azole-resistant Candida parapsilosis causing bloodstream infection: results from laboratory-based sentinel surveillance in South Africa. *J Antimicrob Chemother* 2016; 71:1994–2004.
16. Martini C, Torelli R, de Groot T, et al. Prevalence and clonal distribution of azole-resistant Candida parapsilosis isolates causing bloodstream infections in a large Italian hospital. *Front Cell Infect Microbiol* 2020; 10:232.
17. Mesini A, Mikułska M, Giacobbe DR, et al. Changing epidemiology of candidaeae: increase in fluconazole-resistant Candida parapsilosis. *Mycoses* 2020; 63: 361–8.
18. Demirci-Duarte S, Arakan-Akdagli S, Gulmez D. Species distribution, azole resistance and related molecular mechanisms in invasive Candida parapsilosis
complex isolates: increase in fluconazole resistance in 21 years. Mycoses 2021;64:823–30.

19. Thomaz DY, de Almeida JN Jr, Lima GME, et al. An azole-resistant Candida parapsilosis outbreak: clonal persistence in the intensive care unit of a Brazilian teaching hospital. Front Microbiol 2018; 9:2997.

20. Thomaz DY, Del Negro GMB, Ribeiro LB, et al. A Brazilian inter-hospital candidemia outbreak caused by fluconazole-resistant Candida parapsilosis in the COVID-19 era. J Fungi (Basel) 2022; 8:100.

21. Grossman NT, Pham CD, Cleveland AA, Lockhart SR. Molecular mechanisms of fluconazole resistance in Candida parapsilosis isolates from a U. S. surveillance system. Antimicrob Agents Chemother 2015; 59:1030–7.

22. Thomaz DY, de Almeida JN Jr, Sejas ONE, et al. Environmental clonal spread of azole-resistant Candida parapsilosis with Erg11-Y132F mutation causing a large candidemia outbreak in a Brazilian cancer referral center. J Fungi (Basel) 2021; 7:259.

23. White T, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA Gefland DH Sninsky JJ, and White TJ, eds. PCR Protocols: A Guide to Methods and Applications. Academic Press; 1990:315–322.

24. Arendrup MC, Meletiadis J, Mouton JW, et al. EUCAST technical note on isavuconazole breakpoints for Aspergillus,itraconazole breakpoints for Candida and updates for the antifungal susceptibility testing method documents. Clin Microbiol Infect 2016; 22:571 e1–4.

25. Diab-Elschahawi M, Forstner C, Hagen F, et al. Microsatellite genotyping clarified conspicuous accumulation of Candida parapsilosis at a cardiothoracic surgery intensive care unit. J Clin Microbiol 2012; 50:3422–6.

26. Singh A, Singh PK, de Groot T, et al. Emergence of clonal fluconazole-resistant Candida parapsilosis clinical isolates in a multicentre laboratory-based surveillance study in India. J Antimicrob Chemother 2019; 74:1260–8.

27. Kisto MI, Caramballo RD, Rocha DA, et al. Pan-azole-resistant Candida tropicalis carrying homoyzgous erg1 mutations at position K143R: a new emerging superbug? J Antimicrob Chemother 2017; 72:988–92.

28. Arastehfar A, Hilmioglu-Polat S, Daneshfar F, et al. A clonal candidemia outbreak by Candida parapsilosis carrying Y132F in Turkey: evolution of a persisting challenge. Front Cell Infect Microbiol 2021; 11:676177.

29. Alcoceda E, Gomez A, Lara-Escol P, et al. Fluconazole-resistant Candida parapsilosis clonally related genotypes: first report proving the presence of endemic isolates harbouring the Y132F ERG11 gene substitution in Spain. Clin Microbiol Infect 2022; 28:1113–9.

30. Peman J, Canton E, Quindos G, et al. Epidemiology, species distribution and in vitro antifungal susceptibility of fungaemia in a Spanish multicentre prospective survey. J Antimicrob Chemother 2012; 67:1181–7.

31. Canton E, Peman J, Quindos G, et al. Prospective multicenter study of the epidemiology, molecular identification, and antifungal susceptibility of Candida parapsilosis, Candida orthopsilosis, and Candida metapsilosis isolated from patients with candidemia. Antimicrob Agents Chemother 2011; 55:5590–6.

32. Diaz-Garcia J, Gomez A, Alcala I, et al. Evidence of fluconazole-resistant Candida parapsilosis genotypes spreading across hospitals located in Madrid, Spain and harboring the Y132F ERG11p substitution. Antimicrob Agents Chemother 2022; 66:e0071022.

33. Sturdy A, Barsab M, Cotter M, et al. Severe COVID-19 and healthcare-associated infections on the ICU: time to remember the basics? J Hosp Infect 2020; 105:593–5.

34. Abelenda-Arons G, Puig-Asensio M, Jimenez-Martinez E, et al. Impact of the COVID-19 pandemic on infection control practices in a university hospital. Infect Control Hosp Epidemiol 2022; 26:1–3.

35. Cultrera R, Barozzi A, Libanore M, et al. Co-infections in critically ill patients with or without COVID-19: a comparison of clinical microbial culture findings. Int J Environ Res Public Health 2021; 18:4358.

36. Chong WH, Neu KP. Incidence, diagnosis and outcomes of COVID-19-associated pulmonary aspergillosis (CAPA): a systematic review, J Hosp Infect 2021; 113:115–29.

37. Thompson Iii GR, Cornely OA, Pappas PG, et al. Invasive aspergillosis as an under-recognized superinfection in COVID-19. Open Forum Infect Dis 2020; 7:ofoa242.

38. Bartoletti M, Pascale R, Cricca M, et al. Epidemiology of invasive pulmonary aspergillosis among COVID-19 intubated patients: a prospective study. Clin Infect Dis 2021; 73:e1606–14.

39. Singh K, Kumar S, Shastri S, Sudershah A, Mansotra V. Black fungus immunosuppressive epidemic with COVID-19 associated mucormycosis (zygomycosis): a clinical and diagnostic perspective from India. Immunogenetics 2022; 74:197–206.

40. Ravindra K, Ablawat A. Five probable factors responsible for COVID-associated mucormycosis outbreak in India. Int J Infect Dis 2021; 112:278–80.

41. Sahu RK, Salem-Bekhit MM, Bhattacharjee B, et al. Mucormycosis in Indian COVID-19 patients: insight into its patho-genesis. Clinical manifestation, and management strategies. Antibiotics (Basel) 2021; 10:1079.

42. Segrelles-Calvo G, de S Araújo GR, Llopis-Pastor E, et al. Candida spp. co-infection in COVID-19 patients with severe pneumonia: prevalence study and associated risk factors. Respir Med 2021; 188:106619.

43. Arastehfar A, Carvalho A, Nguyen MH, et al. COVID-19-associated candidiasis (CAC): an underestimated complication in the absence of immunological predispositions? J Fungi (Basel) 2020; 6:211.

44. Roudsbary M, Kumar S, Kumar A, Cernaková L, Nikoomanesh F, Rodrigues CF. Overview on the prevalence of fungal infections, immune response, and microbiome role in COVID-19 patients. J Fungi (Basel) 2021; 7:720.

45. Abdoli A, Falahi S, Kenarzooohi A. COVID-19-associated opportunistic infections: a snapshot on the current reports. Clin Exp Med 2022; 22:327–46.

46. Grau S, Hernandez S, Escherrya Ensal D, et al. Antimicrobial consumption among 66 acute care hospitals in Catalonia: impact of the COVID-19 pandemic. Antibiotics (Basel) 2021; 10:943.

47. Ramos-Martinez A, Pintos-Pascual I, Guinée J, et al. Impact of the COVID-19 pandemic on the clinical profile of candidemia and the incidence of fungemia due to fluconazole-resistant Candida parapsilosis. J Fungi (Basel) 2022; 8:451.