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## Azoles susceptibility profiles of more than 9,000 clinical yeast isolates belonging to 40 common and rare species

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1 **Azoles susceptibility profiles of more than 9,000 clinical yeast isolates belonging to 40 common**  
2 **and rare species**

3 **Running title : Azoles distribution of 40 common & rare yeast species**

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20

21 **Abstract**

22 Invasive yeast infections represent a major global public health issue and only few antifungal  
23 agents are available. Azoles are one of the classes of antifungals used for treatment of  
24 invasive candidiasis. The determination of antifungal susceptibility profiles using

25 standardized methods is important to identify resistant isolates and to uncover the potential  
26 emergence of intrinsically-resistant species. We here report data on 9,319 clinical isolates  
27 belonging to 40 pathogenic yeast species recovered in France over 17 years. The antifungal  
28 susceptibility profiles were all determined at the National Reference Center for Invasive  
29 Mycoses and Antifungals based on the EUCAST broth microdilution method. The centralized  
30 collection and analysis allowed us to describe the trends of azoles susceptibility of isolates  
31 belonging to common species, confirming the high susceptibility for *C. albicans* (n=3,295),  
32 *C. tropicalis* (n=641), *C. parapsilosis* (n=820), and decreased susceptibility for *C. glabrata*  
33 (n=1,274), and *P. kudriavzevii* (n=343). They also provide interesting data concerning azole  
34 susceptibility of *Cr. neoformans* species complex: showing comparable MICs distribution for  
35 the three species but lower MIC50 and MIC90 for serotype D (n=208) compared to serotype  
36 A (n=949) and AD hybrids (n=177). Finally, these data provide useful information for rare  
37 and/or emerging species such as *C. lusitaniae* (n=221), *S. clavata* (n=184), *M. guilliermondii*  
38 complex (n=150), *C. haemulonii* complex (n=87), *R. mucilaginosa* (n=55), *W. anomalus*  
39 (n=36).

40

## 41 **Introduction**

42 Invasive fungal infections (IFIs) represent a major worldwide public health issue with an  
43 incidence of 5.9 to 20.3 cases/100 000 patients per year in France, up to 27.2 / 100 000 in  
44 USA and 14.1/100 000 in the United Kingdom (1-5). Despite treatment, the mortality remains  
45 high, ranging from 7.5 to 27.6%, with an estimation of 1.5 million deaths annually (6). Yeasts  
46 are the main causative agents of these infections and only few antifungals are effective against  
47 the most common Ascomycetous yeasts, and even less against Basidiomycetous yeasts. The  
48 azoles are the largest class of antifungal agents used for treatment of IFIs, especially  
49 fluconazole for treatment of candidemia (7, 8). Azoles inhibit the 14-alpha-lanosterol

50 demethylase (Cyp51), resulting in depletion of ergosterol synthesis, a major component of the  
51 fungal cell wall and in accumulation of toxic 14- $\alpha$ -demethylated sterols in the membrane  
52 (9). The determination of *in vitro* antifungal susceptibility allows determination of wild type  
53 population and those with acquired or mutational resistance to the drug. Therefore,  
54 epidemiological surveys and standardized methods of antifungal susceptibility testing are  
55 important to confirm or not that the antifungal susceptibility of a given species remains  
56 unchanged/stable. Among internationally recognized standards, two broth microdilution  
57 methods are well standardized by the EUCAST (European Committee on Antimicrobial  
58 Susceptibility Testing) and the CLSI (Clinical and Laboratory Standards Institute) for  
59 determining minimal inhibitory concentrations (MICs). Expert committees in both instances  
60 establish breakpoints (BPs) and epidemiological cut-off (abbreviated ECOFFs for EUCAST,  
61 and ECVs for CLSI) values in order to easily distinguish between susceptible and resistant  
62 and wild-type and non-wild-type isolates, respectively. BPs are defined for a limited number  
63 of species and antifungal agents based on MICs distributions, clinical data,  
64 pharmacodynamics and pharmacokinetics of the drugs while ECOFFs are determined based  
65 only on MICs distributions. An ECOFF corresponds to the MIC that separates a population of  
66 isolates into wild-type and non-wild type. Unlike BP, ECOFF does not necessarily predict  
67 clinical failure but it can be helpful for clinical decision (SOP10.1  
68 <https://eucast.org/documents/sops/> (10)). The EUCAST Antifungal Susceptibility Testing  
69 subcommittee (EUCAST-AFST) regularly updates data concerning MICs distributions, and  
70 ECOFFs for frequent species involved in human infections based on data generated by more  
71 than 15 European centers  
72 (<http://www.eucast.org/astoffungi/clinicalbreakpointsforantifungals/>, (11)). Furthermore, all  
73 over the world, mono- or multicentric studies provide reports adding to the knowledge on  
74 antifungal susceptibility profiles, mostly of common yeast species (12-20). For rare species

75 however, obtaining robust data is difficult given the low number of reported cases (12).  
76 Centralized data are therefore important for these species to determine their normal  
77 susceptibility profile (10).

78 The French National Reference Center for Invasive Fungal Infections and Antifungals  
79 (NRCMA) provides antifungal susceptibility testing results using the EUCAST method for all  
80 the isolates collected through its missions of expertise on pathogenic fungi and surveillance of  
81 IFIs in France. We here report the results obtained for azoles on more than 9,000 clinical  
82 isolates belonging to 40 different species, collected nationwide in France between 2003 and  
83 2019. These data provide an overview of the susceptibility to azoles of frequent and rare  
84 pathogenic yeast species in France.

85

## 86 **Results**

87 A total of 9,319 yeast isolates were included in the analysis. These isolates, received at the  
88 NRCMA between the 1<sup>st</sup> of September 2003 and the 31<sup>st</sup> December of 2019, were mainly  
89 involved in IFIs (80.8%; 6,853 recovered from blood cultures, and 679 from cerebrospinal  
90 fluids or brain abscesses cultures). The isolates belonged to 40 different species (32/40 were  
91 Ascomycetes corresponding to 17 genera, and 8/40 were Basidiomycetes belonging to 4  
92 genera). Eleven species were represented by more than 100 isolates: *Candida albicans*,  
93 *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis* and *Pichia kudriavzevii* (syn.  
94 *Candida krusei*) and *Cryptococcus neoformans* complex followed by *Clavispora lusitaniae*,  
95 *Kluyveromyces marxianus*, *Saprochaete clavata*, *Candida dubliniensis* and *Meyerozyma*  
96 *guilliermondii* (Table 1). Ranges of MICs, MIC50 and MIC90 for fluconazole, voriconazole  
97 and posaconazole are listed in Table 1 while MICs distributions are presented in Table S1. Of  
98 note, while the median MIC values for each batch of QC strains (ATCC22019 and  
99 ATCC6258) were always in the range of acceptable MIC defined by EUCAST-AFST for

100 fluconazole and voriconazole, they were one dilution higher for posaconazole between 2011  
101 and 2015. However, the proportion of posaconazole-resistant (MIC>0.06mg/L) isolates was  
102 not higher at that time (Figure 1).

103 We first compared the MIC distributions recorded at the NRCMA to those available in the  
104 EUCAST dataset. For fluconazole and voriconazole, ranges of MICs were similar or with a  
105 maximum variation of 2 dilutions. Medians of MICs were equal or with a maximum of one  
106 dilution difference for *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*,  
107 *P. kudriavzevii*, *W. anomalus*, *K. marxianus*, *C. lusitaniae* and *M. guilliermondii* (data not  
108 shown). For posaconazole, MIC distributions were also similar except for *C. parapsilosis*, *C.*  
109 *tropicalis* and *M. guilliermondii* (Supplemental Figure 1) with a median MIC value for the  
110 NRCMA dataset 2 dilutions higher than for the EUCAST dataset.

111 Among the *Candida parapsilosis* complex, the three species had similar MIC distribution for  
112 posaconazole while *C. metapsilosis* isolates had lower MICs for voriconazole and fluconazole  
113 but a higher MIC<sub>50</sub> for fluconazole than *C. parapsilosis* and *C. orthopsilosis*. Using  
114 EUCAST BP for fluconazole (R>4mg/L), *C. orthopsilosis* has the highest number of resistant  
115 isolates (12.2%) (Table 1, Figure 2).

116 For *Cr. neoformans*, range of MICs, MIC<sub>50</sub> and MIC<sub>90</sub> were determined for serotype A,  
117 serotype D and AD hybrids. The distribution of MICs was comparable for the three species  
118 but for the three azoles, serotype D isolates exhibited lower MIC<sub>50</sub> and MIC<sub>90</sub> than serotypes  
119 A and AD hybrids (Table 1 and Table S1).

120 Using the EUCAST breakpoints (BP) for fluconazole, 32.4% *C. glabrata* isolates were  
121 resistant (R > 16mg/L) compared to less than 7.5% of the isolates for all the other common  
122 species (R>4 mg/L) (Table 1). Using the non-species related BP defined by EUCAST for  
123 fluconazole (R>4mg/L), four species (*C. haemulonii*, *C. duobushaemulonii*, *C.*

124 *palmiophila*, *C. auris*) had more than 90% of resistant isolates while three others (*C.*  
125 *metapsilosis*, *C. orthopsilosis* and *C. nivariensis*) had 2.2%, 12.2% and 7.7% of fluconazole-  
126 resistant isolates, respectively. According to the BPs for posaconazole (R>0.25 mg/L) and  
127 voriconazole (R>0.06 mg/L), the percentage of resistant isolates was 23.9% and 2.2% for *C.*  
128 *parapsilosis* and 27.3% and 9.5% for *C. tropicalis*, respectively (Table 1).

129 According to the EUCAST ECOFF, 26.8% of *P. kudriavzevii* (ECOFF=128mg/L) and 20% of  
130 *M. guilliermondii* (ECOFF=16mg/L) isolates were considered as non-wild type for  
131 fluconazole. Likewise, 9.6% of *C. glabrata* (ECOFF=1mg/L), 4.5% of *C. lusitaniae*  
132 (ECOFF=0.06mg/L), 13.9% of *M. guilliermondii* (ECOFF=0.25mg/L) and 2.6% of *P.*  
133 *kudriavzevii* (ECOFF=1mg/L) isolates were considered as non-wild type for voriconazole  
134 (Table 1).

135 Among the population of *C. glabrata* isolates, 7.9 % (101/1274) were simultaneously  
136 resistant to fluconazole and considered non-wild-type for voriconazole. Among the 155 *C.*  
137 *albicans* isolates resistant to at least one azole, 49 (31.6%) were resistant to the three azoles.  
138 Cross-resistance to the three azoles concerned 0/8 *C. dubliniensis*, 29/192 (15.1%) *C.*  
139 *tropicalis*, and 7/236 (3.0%) *C. parapsilosis* (Supplemental Figure 2). The majority (>80%) of  
140 isolates resistant to at least one azole were resistant to posaconazole. The proportion of  
141 posaconazole-resistant isolates varied according to the year of isolation for *C. tropicalis* and  
142 *C. parapsilosis* (Figure 1). This variation was correlated with variation of voriconazole-  
143 resistance for *C. tropicalis*.

144

## 145 **Discussion**

146 In the present study, we report the azoles susceptibility profiles for 40 yeast species involved  
147 in IFIs based on the MIC distribution of 9,319 clinical isolates recovered in France between  
148 2003 and 2019. We provide azole profiles for common, emerging and rare species thanks to

149 our varied and important collection of isolates. Our results confirm that *C. albicans*, *C.*  
150 *dublinsiensis*, *C. tropicalis*, *C. parapsilosis* complex, *K. marxianus* and *C. lusitaniae* can be  
151 considered as susceptible *in vitro* to fluconazole, voriconazole and posaconazole (14, 16, 18,  
152 19, 21), while (i) *C. neoformans* complex has decreased *in vitro* susceptibility to fluconazole,  
153 (ii) *P. kudriavzevii* and uncommon species *C. haemulonii* complex, *M. guilliermondii*  
154 complex, *P. norvegensis*, *S. clavata*, *G. candidus* and *R. mucilaginosa* can be considered as  
155 intrinsically resistant to fluconazole (MIC<sub>90</sub> ≥ 64mg/L) (17, 19, 20, 22-24). Of note, *C.*  
156 *haemulonii* and *C. duobushaemulonii*, which belong to the same species complex and are  
157 closely related to the emerging *C. auris*, can also be considered intrinsically resistant to  
158 voriconazole and posaconazole (19, 25, 26). Other uncommon species such as *S. cerevisiae*,  
159 *K. ohmeri*, *C. palmiophila*, *P. cactophila*, *M. capitatus*, *Y. lipolytica*, *Cr. gattii* and *T.*  
160 *asahii* should be considered as less susceptible *in vitro* to fluconazole, with “intermediate”  
161 MIC value (MIC<sub>90</sub> ≥ 16mg/L) (27, 28) while voriconazole seems to be the most potent azole  
162 *in vitro*, which is already known for *Trichosporon* spp. (7, 23). When considering the *C.*  
163 *parapsilosis* complex, *C. metapsilosis* was the species the most sensitive *in vitro* to azoles  
164 with the lowest percentage of fluconazole-resistant isolates while *C. orthopsilosis* seemed to  
165 be more resistant than the other species of the complex with the highest percentage of  
166 fluconazole-resistant isolates and the highest MIC<sub>90</sub> for fluconazole, voriconazole and  
167 posaconazole (12, 19). There may be a link between the low frequency of *C. metapsilosis*  
168 recovered from IFIs in the literature (29) and this highest azole sensitivity. Another  
169 hypothesis proposed by Gago *et al.* is that *C. metapsilosis* has a reduced ability to produce  
170 some of the known virulence factors (30). Finally, we confirmed that very rare species *L.*  
171 *elongisporus* and *Cy. fabianii* were susceptible to the three azoles while *W. anomalus*, *K.*  
172 *ohmeri* and *Cy. jadinii* were less susceptible to fluconazole with “intermediate” MICs (12, 31-  
173 38).



174 Despite reports of azole resistance acquired after treatment, in common and rare yeast species,  
175 and description of an increasing percentage of resistant isolates (39), this phenomenon  
176 remains rare (1, 16, 40, 41) or has geographical specificity (42, 43), and seems to concern  
177 especially *C. tropicalis*, *C. glabrata* and *C. parapsilosis* (17). In the present study, we  
178 observed a proportion of fluconazole-resistant isolates among *C. albicans* and *C. parapsilosis*  
179 isolates similar to that reported in international surveys (17, 19, 20, 28, 44). We observed  
180 posaconazole-resistant isolates of *C. tropicalis* and *C. parapsilosis* complex (23.9 and 27.3%,  
181 respectively), with a variable proportion, according to the year of isolation. The increased  
182 proportion of posaconazole-resistant *C. tropicalis* isolates correlated with an increased  
183 proportion of voriconazole-resistance of those isolates. This heterogeneous percentage of  
184 resistant isolates in our collection remained unexplained. Indeed, we raised a few hypotheses:  
185 (i) Technical issues related to the batch of antifungal powder or RPMI medium that translated  
186 into a higher median posaconazole and not the other azoles MIC for the QC strains, but this  
187 was not associated with an increase in the proportion of posaconazole-resistant isolates in the  
188 related series; (ii) Geographic specificity and/or local outbreak could be responsible as  
189 described for instance in the multicentric CHIF-Net study (19) and in a national survey in  
190 Belgium (43), but none was reported to our knowledge; (iii) Bias related to the isolates  
191 analyzed knowing that 22% of the isolates were sent for expertise following therapeutic  
192 failure and not part of the epidemiological surveys (2, 45). However, posaconazole-resistance  
193 was not restricted to the isolates sent for expertise.

194 We also report an important percentage of *C. glabrata* isolates resistant to fluconazole  
195 (32.4%). The proportion of *C. glabrata* resistant to fluconazole seems to be very  
196 heterogeneous according to the country, the center and the year of isolation. In fact, Xiao *et*  
197 *al.* reported 10.3 to 19% of resistant isolates in China between 2009-2017 (19, 20, 23), based  
198 on the CHIF-Net study, while Pfaller *et al.* published 0 to 28.6%, according to the year of

199 isolation in the SENTRY study (44), Bassetti *et al.*, reported a percentage of 0 to 21.2% in the  
200 SENTRY and ARTEMIS studies depending on the continent (46), Borman *et al.* and Trouvé  
201 *et al.* reported 12.7% and 11.3% of resistance, respectively in national studies (28, 43). Of  
202 note, the BP values for *C. glabrata* was modified by EUCAST-AFST committee in 2020,  
203 from R>32mg/L to R>16mg/L (11). When using the previous BP (MIC>32mg/L), 18.8% of  
204 *C. glabrata* in our collection was identified as resistant to fluconazole, which is comparable to  
205 the studies published earlier before the change of the BP threshold.

206 Our data confirm azole susceptibility profiles for frequent species and, to our knowledge, it is  
207 one of the only studies to assemble MIC data for more than 40 pathogenic yeast species,  
208 mainly involved in invasive infection, including big samples of isolates, even for rare and  
209 emerging species such as *C. lusitaniae* (n=221), *S. clavata* (n=184), *M. guilliermondii*  
210 complex (n=150), *C. haemulonii* complex (n=87), *R. mucilaginosa* (n=55), *W. anomalus*  
211 (n=36) (12). This emphasizes the importance of centralization of isolates for collection and  
212 analysis by National Reference Centers. Our results confirm that an antifungal susceptibility  
213 profile is associated with each species, hence the importance of accurate yeast species  
214 identification as soon as possible to infer the susceptibility profile. Of course, it goes without  
215 saying that the determination of MICs, using standardized methods, remains important for  
216 rare species and the monitoring of potential emergence of resistance including in the context  
217 of therapeutic failure.

218

## 219 **Materials & Methods**

### 220 **Isolates**

221 Between the 1<sup>st</sup> January of 2003 and the 31<sup>st</sup> December of 2019, a total of 9,449 yeast were  
222 sent to the National Reference Center for Mycosis & Antifungal agents (NRCMA). We

223 analyzed MICs data for 9,319 isolates belonging to the species for which at least 5 isolates  
224 were available. Majority of the isolates were sent for expertise (7358/9,319; 78.8%; *i.e.*  
225 species identification, antifungal susceptibility testing) and/or as participation to  
226 epidemiological surveys. For rare species represented by 10 or less isolates, all isolates were  
227 recovered from different patients.

## 228 **Species identification**

229 For all isolates, purity was checked on chromogenic medium (BBL™ Chromagar™ Candida  
230 Medium, BD, GmbH) or Niger seed agar for *Cryptococcus* spp.. Phenotypic identification  
231 was performed using carbon assimilation profiles (ID32C, BioMérieux, Marcy-l'Etoile,  
232 France) before 2014, and matrix assisted laser desorption ionisation-time of flight mass  
233 spectrometry (MALDI-TOF, MALDI Biotyper, Bruker Daltonik, GmbH) since 2014. Duplex  
234 PCR was performed to differentiate *Candida dubliniensis* and *Candida albicans* (47). For all  
235 isolates, PCR and sequencing of ITS1-5.8S-ITS2 and D1D2 regions were performed, except  
236 for *C. glabrata*, *C. tropicalis*, *P.kudriavzevii* and *K. marxianus*, using V9D/LS266 (48, 49)  
237 and NL1/NL4 (50) primers, respectively. In addition, part of the actin gene (for *C. lusitaniae*  
238 (51) and *Debaryomyces* species (52)), part of the RPBI gene (for *M. guilliermondii*, *M.*  
239 *caribbica*, *C. carpophila* (53)) or the IGS1 region (for *Trichosporon* species (54)) were  
240 sequenced. Sequences were compared to sequences of the type strain of each species and of  
241 the closely related species. Serotype was determined for *Cr. neoformans* isolates by PCR  
242 amplification of part of GPA1 and PAK1 genes using a triplex PCR with primers specific of  
243 the serotype previously described (55).

## 244 **Antifungal susceptibility**

245 Minimal inhibitory concentrations (MICs) were determined for all isolates, for 3 antifungal  
246 agents (fluconazole provided by Pfizer Inc (NewYork, USA), Sigma-Aldrich (Merck KGaA,

247 Darmstadt, Germany) and Alsachim (Shimadzu Group Company, France); voriconazole  
248 provided by Pfizer and Alsachim and posaconazole provided by SheringPlough (Merck & Co.  
249 Inc., USA) and Alsachim) by using the standardized broth microdilution method EUCAST  
250 following the procedure E. DEF 7.3.2  
251 ([https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/AFST/Files/EUCAST\\_E](https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_E_Def_7.3.2_Yeast_testing_definitive_revised_2020.pdf)  
252 [Def 7.3.2 Yeast testing definitive revised 2020.pdf](https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Files/EUCAST_E_Def_7.3.2_Yeast_testing_definitive_revised_2020.pdf)), in 96 plate (sterile Tissue culture  
253 plates, 96 well flat bottom in clear polystyrene, TPP® Techno Plastic Products AG,  
254 Switzerland, Reference 92096). The concentrations tested ranged between 0.0015 mg/L to 8  
255 mg/L for posaconazole and voriconazole and between 0.125 mg/L to 64 mg/L for fluconazole.  
256 QC strains (ATCC22019, ATCC6258) were included in each set. The concentrations  
257 corresponding to the MIC that inhibited 50% (MIC50) and 90% (MIC90) of the isolates were  
258 determined for species having 10 or more isolates.

259 Our dataset (MIC distribution) was compared with that available on the EUCAST website  
260 (<https://mic.eucast.org/Eucast2/SearchController/search.jsp?action=init>, June 2020).

261 The BP or ECOFF values determined by EUCAST for some species and some antifungal  
262 agents  
263 ([https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/AFST/Clinical\\_breakpoint](https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Clinical_breakpoint_s/AFST_BP_v10.0_200204.pdf)  
264 [s/AFST\\_BP\\_v10.0\\_200204.pdf](https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Clinical_breakpoint_s/AFST_BP_v10.0_200204.pdf)) were used to calculate percentage of resistant (R) isolates  
265 and non-wild-type (NWT), respectively. Non-species related BP for fluconazole, defined by  
266 EUCAST, (MIC >16 mg/L) for *Candida* were also used to calculate percentage of resistant  
267 isolate for *C. orthopsilosis*, *C. metapsilosis*, *C. nivariensis*, *C. haemulonii*, *C.*  
268 *duobushaemulonii* and *C. palmiophila*. Since not all isolates were collected through  
269 unbiased epidemiological survey (7358/9,319; 78.8%), we did not determine local ECOFF,  
270 and reported only the percentage of resistant or non-wild-type isolates in our collection.

271

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### 318 **Figure Legends**

319 **Figure 1. Percentage of fluconazole, voriconazole and posaconazole -resistant isolates,**  
320 **according to the year of isolation, for (A) *Candida albicans*.** The proportion of resistant  
321 isolates being similar for posaconazole and fluconazole between 2016 and 2019, the lines are

322 overlaid; **(B) *Candida parapsilosis***, and **(C) *Candida tropicalis*** (voriconazole and  
323 fluconazole lines overlaid between 2015 and 2019).

324

325 **Figure 2. MIC distribution of *Candida parapsilosis* complex isolates for (A)**  
326 **Voriconazole, (B) Fluconazole and (C) Posaconazole.** EUCAST breakpoint values (R) for  
327 *C. parapsilosis sensu stricto* are indicated in the graphs.

328

329 **Supplemental Figure 1. Comparison of EUCAST and CNRMA data for posaconazole**  
330 **MIC distribution of *C. tropicalis* (A), *C. parapsilosis* (B) and *M. guilliermondii*.** EUCAST  
331 breakpoint value (R) or ECOFF value are indicated in the graphs.

332

333 **Supplemental Figure 2. Azoles cross-resistance.** Graphs represent the number of isolates  
334 resistant for each azoles, for two or three azoles among isolates resistant for at least one azoles  
335 for **(A) *Candida albicans*, (B) *Candida dubliniensis*, (C) *Candida tropicalis* and (D)**  
336 ***Candida parapsilosis***

337

## 338 **References**

- 339 1. Lortholary O, Renaudat C, Sitbon K, Madec Y, Denoeud-Ndam L, Wolff M, Fontanet A,  
340 Bretagne S, Dromer F, French Mycosis Study G. 2014. Worrisome trends in incidence and  
341 mortality of candidemia in intensive care units (Paris area, 2002-2010). *Intensive Care Med*  
342 40:1303-12.
- 343 2. Bitar D, Lortholary O, Le Strat Y, Nicolau J, Coignard B, Tattevin P, Che D, Dromer F. 2014.  
344 Population-based analysis of invasive fungal infections, France, 2001-2010. *Emerg Infect Dis*  
345 20:1149-55.

- 346 3. Gangneux JP, Bougnoux ME, Hennequin C, Godet C, Chandener J, Denning DW, Dupont B,  
347 Life program tSfdmmS-sg. 2016. An estimation of burden of serious fungal infections in  
348 France. *J Mycol Med* 26:385-390.
- 349 4. Pegorie M, Denning DW, Welfare W. 2017. Estimating the burden of invasive and serious  
350 fungal disease in the United Kingdom. *J Infect* 74:60-71.
- 351 5. Webb BJ, Ferraro JP, Rea S, Kaufusi S, Goodman BE, Spalding J. 2018. Epidemiology and  
352 Clinical Features of Invasive Fungal Infection in a US Health Care Network. *Open Forum*  
353 *Infect Dis* 5:ofy187.
- 354 6. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. 2012. Hidden killers:  
355 human fungal infections. *Sci Transl Med* 4:165rv13.
- 356 7. Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O, European Society  
357 of Clinical M, Infectious Diseases Fungal Infection Study G, European Confederation of  
358 Medical M. 2014. ESCMID and ECMM joint clinical guidelines for the diagnosis and  
359 management of rare invasive yeast infections. *Clin Microbiol Infect* 20 Suppl 3:76-98.
- 360 8. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, Reboli  
361 AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. 2016. Clinical Practice  
362 Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society  
363 of America. *Clin Infect Dis* 62:e1-50.
- 364 9. Bhattacharya S, Sae-Tia S, Fries BC. 2020. Candidiasis and Mechanisms of Antifungal  
365 Resistance. *Antibiotics (Basel)* 9.
- 366 10. Berkow EL, Lockhart SR, Ostrosky-Zeichner L. 2020. Antifungal Susceptibility Testing:  
367 Current Approaches. *Clin Microbiol Rev* 33.
- 368 11. Arendrup MC, Friberg N, Mares M, Kahlmeter G, Meletiadis J, Guinea J, Subcommittee on  
369 Antifungal Susceptibility Testing of the EECfAST. 2020. How to interpret MICs of antifungal  
370 compounds according to the revised clinical breakpoints v. 10.0 European committee on  
371 antimicrobial susceptibility testing (EUCAST). *Clin Microbiol Infect*  
372 doi:10.1016/j.cmi.2020.06.007.



- 373 12. Borman AM, Muller J, Walsh-Quantick J, Szekely A, Patterson Z, Palmer MD, Fraser M,  
374 Johnson EM. 2020. MIC distributions for amphotericin B, fluconazole, itraconazole,  
375 voriconazole, flucytosine and anidulafungin and 35 uncommon pathogenic yeast species from  
376 the UK determined using the CLSI broth microdilution method. *J Antimicrob Chemother*  
377 75:1194-1205.
- 378 13. Diaz-Garcia J, Alcala L, Martin-Rabadan P, Mesquida A, Sanchez-Carrillo C, Reigadas E,  
379 Munoz P, Escribano P, Guinea J. 2019. Susceptibility of uncommon *Candida* species to  
380 systemic antifungals by the EUCAST methodology. *Med Mycol* doi:10.1093/mmy/myz121.
- 381 14. Guinea J, Zaragoza O, Escribano P, Martin-Mazuelos E, Peman J, Sanchez-Reus F, Cuenca-  
382 Estrella M, Candipop Project G-G, Reipi. 2014. Molecular identification and antifungal  
383 susceptibility of yeast isolates causing fungemia collected in a population-based study in  
384 Spain in 2010 and 2011. *Antimicrob Agents Chemother* 58:1529-37.
- 385 15. Jung IY, Jeong SJ, Kim YK, Kim HY, Song YG, Kim JM, Choi JY. 2020. A multicenter  
386 retrospective analysis of the antifungal susceptibility patterns of *Candida* species and the  
387 predictive factors of mortality in South Korean patients with candidemia. *Medicine*  
388 (Baltimore) 99:e19494.
- 389 16. Pfaller MA, Castanheira M, Messer SA, Jones RN. 2015. In vitro antifungal susceptibilities of  
390 isolates of *Candida* spp. and *Aspergillus* spp. from China to nine systemically active  
391 antifungal agents: data from the SENTRY antifungal surveillance program, 2010 through  
392 2012. *Mycoses* 58:209-14.
- 393 17. Pfaller MA, Diekema DJ, Turnidge JD, Castanheira M, Jones RN. 2019. Twenty Years of the  
394 SENTRY Antifungal Surveillance Program: Results for *Candida* Species From 1997-2016.  
395 *Open Forum Infect Dis* 6:S79-S94.
- 396 18. Pfaller MA, Messer SA, Jones RN, Castanheira M. 2015. Antifungal susceptibilities of  
397 *Candida*, *Cryptococcus neoformans* and *Aspergillus fumigatus* from the Asia and Western  
398 Pacific region: data from the SENTRY antifungal surveillance program (2010-2012). *J*  
399 *Antibiot (Tokyo)* 68:556-61.

- 400 19. Xiao M, Chen SC, Kong F, Xu XL, Yan L, Kong HS, Fan X, Hou X, Cheng JW, Zhou ML, Li  
401 Y, Yu SY, Huang JJ, Zhang G, Yang Y, Zhang JJ, Duan SM, Kang W, Wang H, Xu YC.  
402 2020. Distribution and Antifungal Susceptibility of *Candida* Species Causing Candidemia in  
403 China: An Update From the CHIF-NET Study. *J Infect Dis* 221:S139-S147.
- 404 20. Xiao M, Sun ZY, Kang M, Guo DW, Liao K, Chen SC, Kong F, Fan X, Cheng JW, Hou X,  
405 Zhou ML, Li Y, Yu SY, Huang JJ, Wang H, Xu YC, China Hospital Invasive Fungal  
406 Surveillance Net Study G. 2018. Five-Year National Surveillance of Invasive Candidiasis:  
407 Species Distribution and Azole Susceptibility from the China Hospital Invasive Fungal  
408 Surveillance Net (CHIF-NET) Study. *J Clin Microbiol* 56.
- 409 21. Astvad KMT, Hare RK, Arendrup MC. 2017. Evaluation of the in vitro activity of  
410 isavuconazole and comparator voriconazole against 2635 contemporary clinical *Candida* and  
411 *Aspergillus* isolates. *Clin Microbiol Infect* 23:882-887.
- 412 22. Perez-Hansen A, Lass-Flörl C, Lackner M, Rare Yeast Study G. 2019. Antifungal  
413 susceptibility profiles of rare ascomycetous yeasts. *J Antimicrob Chemother* 74:2649-2656.
- 414 23. Xiao M, Chen SC, Kong F, Fan X, Cheng JW, Hou X, Zhou ML, Wang H, Xu YC, China  
415 Hospital Invasive Fungal Surveillance Net Study G. 2018. Five-year China Hospital Invasive  
416 Fungal Surveillance Net (CHIF-NET) study of invasive fungal infections caused by  
417 noncandidal yeasts: species distribution and azole susceptibility. *Infect Drug Resist* 11:1659-  
418 1667.
- 419 24. Jamiu AT, Albertyn J, Sebolai OM, Pohl CH. 2020. Update on *Candida krusei*, a potential  
420 multidrug-resistant pathogen. *Med Mycol* doi:10.1093/mmy/myaa031.
- 421 25. Bretagne S, Renaudat C, Desnos-Ollivier M, Sitbon K, Lortholary O, Dromer F, French  
422 Mycosis Study G. 2017. Predisposing factors and outcome of uncommon yeast species-related  
423 fungaemia based on an exhaustive surveillance programme (2002-14). *J Antimicrob*  
424 *Chemother* 72:1784-1793.
- 425 26. Desnos-Ollivier M, Robert V, Raoux-Barbot D, Groenewald M, Dromer F. 2012. Antifungal  
426 susceptibility profiles of 1698 yeast reference strains revealing potential emerging human  
427 pathogens. *PLoS One* 7:e32278.

- 428 27. Fernandez-Ruiz M, Guinea J, Puig-Asensio M, Zaragoza O, Almirante B, Cuenca-Estrella M,  
429 Aguado JM, Project C, Geih G, Reipi. 2017. Fungemia due to rare opportunistic yeasts: data  
430 from a population-based surveillance in Spain. *Med Mycol* 55:125-136.
- 431 28. Borman AM, Muller J, Walsh-Quantick J, Szekely A, Patterson Z, Palmer MD, Fraser M,  
432 Johnson EM. 2019. Fluconazole Resistance in Isolates of Uncommon Pathogenic Yeast  
433 Species from the United Kingdom. *Antimicrob Agents Chemother* 63.
- 434 29. Canton E, Peman J, Quindos G, Eraso E, Miranda-Zapico I, Alvarez M, Merino P, Campos-  
435 Herrero I, Marco F, de la Pedrosa EG, Yague G, Guna R, Rubio C, Miranda C, Pazos C,  
436 Velasco D, Group FS. 2011. Prospective multicenter study of the epidemiology, molecular  
437 identification, and antifungal susceptibility of *Candida parapsilosis*, *Candida orthopsilosis*,  
438 and *Candida metapsilosis* isolated from patients with candidemia. *Antimicrob Agents*  
439 *Chemother* 55:5590-6.
- 440 30. Gago S, Garcia-Rodas R, Cuesta I, Mellado E, Alastruey-Izquierdo A. 2014. *Candida*  
441 *parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* virulence in the non-  
442 conventional host *Galleria mellonella*. *Virulence* 5:278-85.
- 443 31. Al-Obaid K, Ahmad S, Joseph L, Khan Z. 2018. *Lodderomyces elongisporus*: a bloodstream  
444 pathogen of greater clinical significance. *New Microbes New Infect* 26:20-24.
- 445 32. Al-Sweih N, Ahmad S, Khan S, Joseph L, Asadzadeh M, Khan Z. 2019. *Cyberlindnera*  
446 *fabianii* fungaemia outbreak in preterm neonates in Kuwait and literature review. *Mycoses*  
447 62:51-61.
- 448 33. Bougnoux ME, Gueho E, Potocka AC. 1993. Resolutive *Candida utilis* fungemia in a  
449 nonneutropenic patient. *J Clin Microbiol* 31:1644-5.
- 450 34. Dutra VR, Silva LF, Oliveira ANM, Beirigo EF, Arthur VM, Bernardes da Silva R, Ferreira  
451 TB, Andrade-Silva L, Silva MV, Fonseca FM, Silva-Vergara ML, Ferreira-Paim K. 2020.  
452 Fatal Case of Fungemia by *Wickerhamomyces anomalus* in a Pediatric Patient Diagnosed in a  
453 Teaching Hospital from Brazil. *J Fungi (Basel)* 6.

- 454 35. Jung J, Moon YS, Yoo JA, Lim JH, Jeong J, Jun JB. 2018. Investigation of a nosocomial  
455 outbreak of fungemia caused by *Candida pelliculosa* (*Pichia anomala*) in a Korean tertiary  
456 care center. *J Microbiol Immunol Infect* 51:794-801.
- 457 36. Lin HC, Lin HY, Su BH, Ho MW, Ho CM, Lee CY, Lin MH, Hsieh HY, Lin HC, Li TC,  
458 Hwang KP, Lu JJ. 2013. Reporting an outbreak of *Candida pelliculosa* fungemia in a neonatal  
459 intensive care unit. *J Microbiol Immunol Infect* 46:456-62.
- 460 37. Park JH, Oh J, Sang H, Shrestha B, Lee H, Koo J, Cho SI, Choi JS, Lee MH, Kim J, Sung GH.  
461 2019. Identification and Antifungal Susceptibility Profiles of *Cyberlindnera fabianii* in Korea.  
462 *Mycobiology* 47:449-456.
- 463 38. Zhou M, Yu S, Kudinha T, Xiao M, Wang H, Xu Y, Zhao H. 2019. Identification and  
464 antifungal susceptibility profiles of *Kodamaea ohmeri* based on a seven-year multicenter  
465 surveillance study. *Infect Drug Resist* 12:1657-1664.
- 466 39. Arastehfar A, Lass-Flörl C, Garcia-Rubio R, Daneshnia F, Ilkit M, Boekhout T, Gabaldon T,  
467 Perlin DS. 2020. The Quiet and Underappreciated Rise of Drug-Resistant Invasive Fungal  
468 Pathogens. *J Fungi (Basel)* 6.
- 469 40. Castanheira M, Deshpande LM, Davis AP, Rhomberg PR, Pfaller MA. 2017. Monitoring  
470 Antifungal Resistance in a Global Collection of Invasive Yeasts and Molds: Application of  
471 CLSI Epidemiological Cutoff Values and Whole-Genome Sequencing Analysis for Detection  
472 of Azole Resistance in *Candida albicans*. *Antimicrob Agents Chemother* 61.
- 473 41. Lass-Flörl C, Mayr A, Aigner M, Lackner M, Orth-Holler D. 2018. A nationwide passive  
474 surveillance on fungal infections shows a low burden of azole resistance in molds and yeasts  
475 in Tyrol, Austria. *Infection* 46:701-704.
- 476 42. Tacconelli E, Sifakis F, Harbarth S, Schrijver R, van Mourik M, Voss A, Sharland M,  
477 Rajendran NB, Rodriguez-Bano J, Group EP-NC-M. 2018. Surveillance for control of  
478 antimicrobial resistance. *Lancet Infect Dis* 18:e99-e106.
- 479 43. Trouve C, Blot S, Hayette MP, Jonckheere S, Patteet S, Rodriguez-Villalobos H, Symoens F,  
480 Van Wijngaerden E, Lagrou K. 2017. Epidemiology and reporting of candidaemia in Belgium:  
481 a multi-centre study. *Eur J Clin Microbiol Infect Dis* 36:649-655.

- 482 44. Pfaller MA, Carvalhaes CG, Smith CJ, Diekema DJ, Castanheira M. 2020. Bacterial and  
483 fungal pathogens isolated from patients with bloodstream infection: frequency of occurrence  
484 and antimicrobial susceptibility patterns from the SENTRY Antimicrobial Surveillance  
485 Program (2012-2017). *Diagn Microbiol Infect Dis* 97:115016.
- 486 45. Lortholary O, Renaudat C, Sitbon K, Desnos-Ollivier M, Bretagne S, Dromer F, French  
487 Mycoses Study G. 2017. The risk and clinical outcome of candidemia depending on  
488 underlying malignancy. *Intensive Care Med* 43:652-662.
- 489 46. Bassetti M, Vena A, Bouza E, Peghin M, Munoz P, Righi E, Pea F, Lackner M, Lass-Flörl C.  
490 2020. Antifungal susceptibility testing in *Candida*, *Aspergillus* and *Cryptococcus* infections:  
491 are the MICs useful for clinicians? *Clin Microbiol Infect* 26:1024-1033.
- 492 47. Donnelly SM, Sullivan DJ, Shanley DB, Coleman DC. 1999. Phylogenetic analysis and rapid  
493 identification of *Candida dubliniensis* based on analysis of ACT1 intron and exon sequences.  
494 *Microbiology* 145 1871-82.
- 495 48. de Hoog GS, van den Ende GAH. 1998. Molecular diagnostics of clinical strains of  
496 filamentous Basidiomycetes. *Mycoses* 41:183-9.
- 497 49. Masclaux F, Gueho E, de Hoog GS, Christen R. 1995. Phylogenetic relationships of human-  
498 pathogenic *Cladosporium (Xylohypha)* species inferred from partial LS rRNA sequences. *J*  
499 *Med Vet Mycol* 33:327-38.
- 500 50. O'Donnell K. 1993. *Fusarium* and its near relatives, p 225-233. In Taylor DRRaJW (ed), The  
501 fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics, CAB  
502 International, Wallingford, United kingdom.
- 503 51. Guzman B, Lachance MA, Herrera CM. 2013. Phylogenetic analysis of the angiosperm-  
504 floricolous insect-yeast association: have yeast and angiosperm lineages co-diversified? *Mol*  
505 *Phylogenet Evol* 68:161-75.
- 506 52. Martorell P, Fernandez-Espinar MT, Querol A. 2005. Sequence-based identification of species  
507 belonging to the genus *Debaryomyces*. *FEMS Yeast Res* 5:1157-65.

- 508 53. Desnos-Ollivier M, Bretagne S, Boullie A, Gautier C, Dromer F, Lortholary O, French  
509 Mycoses Study G. 2019. Isavuconazole MIC distribution of 29 yeast species responsible for  
510 invasive infections (2015-2017). *Clin Microbiol Infect* 25:634 e1-634 e4.
- 511 54. Sugita T, Nakajima M, Ikeda R, Matsushima T, Shinoda T. 2002. Sequence analysis of the  
512 ribosomal DNA intergenic spacer 1 regions of *Trichosporon* species. *J Clin Microbiol*  
513 40:1826-30.
- 514 55. Lengeler KB, Cox GM, Heitman J. 2001. Serotype AD strains of *Cryptococcus neoformans*  
515 are diploid or aneuploid and are heterozygous at the mating-type locus. *Infect Immun* 69:115-  
516 22.
- 517