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1 **Hospital outbreak of fluconazole-resistant *Candida parapsilosis*: arguments for clonal**
2 **transmission and long-term persistence**

3

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19

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25

26 **ABSTRACT**

27 The worldwide emergence of multidrug-resistant pathogenic fungi is a threat to human health.
28 At this very moment, an emergence of *Candida parapsilosis* isolates harbouring a resistance
29 to fluconazole, one of the most popular antifungal drugs, is being described in several
30 countries. We seek to better understanding the epidemiology, pathogenicity and transmission
31 of resistant *Candida parapsilosis*. Faced with an outbreak of invasive infections due to
32 resistant isolates of *C. parapsilosis*, we performed a 7-year retrospective and prospective
33 analysis of 283 *C. parapsilosis* isolates collected in 240 patients, among who 111 had
34 invasive candidiasis. Study included review of hospital records, genotyping analysis and
35 susceptibility testing that allow determining the type and outcome of infections, as well as the
36 spatial and temporal spread of clusters. Overall the incidence of azole resistance was 7.5%.
37 Genotyping analysis unveiled several previously undetected outbreaks and clonal spread of
38 susceptible and resistant isolates over a long period of time. In comparison with susceptible
39 isolates, resistant ones have a more restricted genetic diversity and seem to be more likely to
40 spread and more frequently associated with invasive infections. In intensive care units,
41 patients with invasive infections due to resistant isolates had poorer outcome (overall
42 mortality at day 30 of 40%; 4/10) than susceptible ones (overall mortality at day 30 of 26.5%;
43 9/34). Our results suggest that the propensity of *C. parapsilosis* to spread on an epidemic
44 fashion is underestimated, which warrants reinforced control and epidemiological survey of
45 this species.

46

47 **1. INTRODUCTION**

48 *Candida parapsilosis* is one of the most common *Candida* species responsible for human
49 infections, accounting for 15%-30% of candidemia (1-4). It is generally susceptible to azole
50 drugs, especially fluconazole and voriconazole (2, 5). Isolates of azoles-resistant *C.*

51 *parapsilosis* have occasionally been found in intensive care unit (ICU) (5) and resulted in
52 high mortality rates in immunocompromised patients (6).
53 Outbreaks of fluconazole-resistant *C. parapsilosis* infections have been described in recent
54 years (7-10). These isolates were responsible for infections occurring on an epidemic mode
55 with clonal spread and were associated with high morbidity and mortality. Our institution has
56 recently faced several cases of invasive infections due to fluconazole-resistant isolates among
57 patients in the same ICU. We have therefore initiated routine prospective screening of
58 fluconazole resistance for all *C. parapsilosis* isolates and started a retrospective investigation
59 of cases. Our study aimed at defining the antifungal susceptibility pattern of *C. parapsilosis*
60 isolates as well as the incidence and evolution of azole resistance. We also performed a
61 genotyping analysis using microsatellite markers to determine their genomic distribution and
62 evolutionary dynamics.

63

64 **2. MATERIALS AND METHODS**

65 **2.1. Study design.** The study covers the period from March 2012 to October 2019 (92
66 months) and includes a prospective part and a retrospective part. From March 2012 to
67 September 2018, *C. parapsilosis* isolates responsible for infections and/or for which Minimal
68 Inhibitory Concentrations (MIC) of antifungal drugs had been determined were kept and
69 therefore available for the study; others isolates, e.g. responsible for colonization without
70 MICs determination were not kept. From October 2018 to October 2019, any *C. parapsilosis*
71 isolate found in a hospitalized patient was considered and prospectively included in the study.
72 All isolates were identified by MALDI-TOF mass spectrometry using the MSI database.

73 **2.2. Minimal Inhibitory Concentrations of antifungal drugs.** Fluconazole MIC was
74 determined for all isolates included in the study by a gradient concentration strips method
75 (Etest, Biomérieux). For all fluconazole resistant isolates (MIC above 4 mg/L by Etest) and

76 for a random selection of susceptible isolates, fluconazole susceptibility was also assessed by
77 broth micro-dilution (EUCAST method). Antifungal susceptibility testing was extended to
78 other antifungal drugs. *C. parapsilosis* ATCC 22019 or *C. krusei* ATCC 6258 strains were
79 used as quality control. Isolates were classified as susceptible, intermediate or resistant
80 according to the EUCAST clinical breakpoints available at
81 <http://www.eucast.org/astoffungi/clinicalbreakpointsforantifungals/>

82 **2.3. Genotyping and Erg11 sequencing.** Microsatellite genotyping was performed as
83 previously described (11). Briefly, a panel of 6 short tandem repeat was used resulting in a
84 unique twelve-marker microsatellite profile for each isolate. Resulting microsatellite profiles
85 were then exported and submitted to unweighted-pair group method with arithmetic mean
86 (UPGMA) cluster analysis (Dendro-UPGMA, available at <http://genomes.urv.es/UPGMA/>) to
87 generate a dendrogram, considering data as categorical values. Isolates with 100% identical
88 genotypes by microsatellite typing were considered as clonal. The *erg11* gene sequencing was
89 performed using primers and conditions previously described by Grossman *et al* (12).

90 **2.4. Statistical analysis.** Statistical analyses were performed using Prism 5 (GraphPad
91 software). Continuous and categorical variables are presented as mean (and median;
92 interquartile range [IQR]) and number (percentage), respectively. Categorical variables were
93 compared using the Fisher exact test, and the Mann-Whitney U test was used for continuous
94 variables. Survival distributions were compared using the log-rank (Mantel-Cox) test.

95

96 **3. RESULTS**

97 **3.1. Origin of the isolates.** The study involved 283 *C. parapsilosis* isolates obtained from
98 240 patients between March 2012 and October 2019 at the La Pitié-Salpêtrière Hospital, a
99 1800-bed tertiary care center in Paris, France. It is a pavilion hospital that comprises 26
100 separate buildings destined for patient care. The origin of isolates and the clinical implications

101 are presented in Table 1. A total of 131 isolates (46.3 %) from 111 patients (46.3%) were
102 responsible for invasive infections. Colonization involved 104 isolates from 93 patients and
103 was predominantly superficial cutaneous colonization. Positive catheter cultures (29 isolates,
104 25 patients) that can be considered either colonization or infection-related were accounted for
105 separately.

106

107 **3.2. Antifungal susceptibility testing.** Of the 283 isolates tested, 26 (9.2%) from 18 patients
108 (7.5% of patients in whom *C. parapsilosis* was isolated) were classified as resistant to
109 fluconazole (MIC >4 mg/L) by Etest and confirmed by EUCAST (supplemental data Table
110 S1). A panel of 24 isolates classified as susceptible to fluconazole by E-test (MIC ≤ 2 mg/L)
111 was taken as a control group and was found susceptible by the EUCAST method. In addition,
112 these susceptible isolates did not show reduced susceptibility to any of the other antifungal
113 drugs tested. The 26 fluconazole-resistant isolates showed different susceptibility profiles to
114 others azoles drugs (Table S1). Voriconazole-resistant isolates were those with the highest
115 MICs for fluconazole (≥ 32 mg/L). No azole-resistant isolates showed resistance to either
116 echinocandins or amphotericin B.

117

118 **3.3. *Erg11* sequencing.** The 26 fluconazole-resistant isolates and a random selection of 21
119 fluconazole-susceptible isolates were subjected to *erg11* gene sequencing. Results highlighted
120 the A395T mutation that confers the Y132F amino-acid substitution in all fluconazole-
121 resistant isolates except one (identification number PSL0172) for which no specific alteration
122 was found. The latter showed a resistance profile to all azoles. The A395T mutation was
123 absent in all 21 fluconazole-susceptible isolates tested as controls.

124

125 **3.4. Microsatellite analysis.** The 26 fluconazole-resistant isolates and a random selection of
126 65 fluconazole-susceptible isolates were subjected to microsatellite genotyping. As shown by
127 the UPGMA-dendrogram in Figure 1, azole-susceptible *C. parapsilosis* isolates were
128 characterized by high genetic diversity. Of the 65 genotyped susceptible isolates, 58 isolates
129 showed separate and unique genetic patterns, while 7 isolates were distributed into 3 clusters
130 (referred to as S1 to S3). On the other hand, the 26 fluconazole-resistant isolates (18 patients)
131 fell into only 6 clusters, 4 of which were restricted to one single isolate, while 2 clusters
132 (referred to as R1 and R2) comprised 8 isolates (4 patients) and 14 isolates (10 patients),
133 respectively. So, compared to fluconazole-susceptible isolates (55 colonized/infected patients,
134 61 clusters), fluconazole-resistant isolates (18 colonized/infected patients, 6 clusters) showed
135 a reduced genetic diversity ($p=0.014$ by Chi-square test).

136 Interestingly, antifungal susceptibility profiles were different between clones R1 and R2, the
137 former having lower MICs to fluconazole (8-16 mg/L) and being intermediate to
138 voriconazole, the latter having higher MICs to fluconazole (≥ 32 mg/L) and being resistant to
139 voriconazole.

140

141 **3.5. Spatial and temporal circulation of clustered isolates.** Most of the susceptible and
142 resistant isolates from the clusters S1-S3 (7 patients) and R1-R2 (14 patients) came from
143 patients related to the same ward (cardiac surgery ICU; 14/21, 66.7%) or the same building
144 (16/21, 76.2%) that we will call for convenience “Building A” (Figure 2 and Table 2).

145 R1 cluster isolates (R1 clone) were detected in 4 patients between March 10, 2012 and
146 November 10, 2017. While the initial detection of a R1 clone occurred in another building
147 (Building C), this patient (Patient 1) was hospitalized in Building A a few weeks earlier
148 (Figure 2). Three of the 4 patients carrying R1 clone were linked to the same ICU.
149 Furthermore, a review of hospital records showed that one of these 3 patients (Patient 5) was

150 transferred from the cardiac surgery ICU to another unit (surgical critical care unit-2) where
151 the fourth patient (Patient 7) was cared for only a few days before he was detected with a R1
152 clone.

153 R2 cluster isolates (R2 clone) were detected in 10 patients between November 6, 2017 and
154 October 15, 2019. They were found in 7 patients hospitalized in Building A and in 2 patients
155 hospitalized in another building but who previously stayed in Building A.

156

157 **3.6. Comparison of patient profiles between susceptible and resistant isolates.**

158 We then analyzed whether there were differences between patients who developed invasive
159 infection due to a fluconazole-susceptible isolate and those who developed invasive infection
160 due to a fluconazole-resistant isolate. Relevant data were available for 78 patients (Table 3).
161 No differences were observed with respect to the clinical form (candidemia, mediastinitis, or
162 others) or the presence or absence of risk factors usually associated with invasive candidiasis
163 (surgical procedure, immunosuppression, broad-spectrum antibiotherapy therapy, presence of
164 external devices). Importantly, pre-exposure to an azole antifungal drug was also not a related
165 factor. On the other hand, ICU stay was statistically associated with infection due to a
166 fluconazole-resistant *Candida parapsilosis* isolate.

167 We then focused on patients in ICU, as these are the main source of invasive infections
168 related to fluconazole-resistant isolates. During the prospective part of the study (where the
169 analysis of *C. parapsilosis* isolates is exhaustive) and focusing on 36 ICU patients, we
170 observed that 5 out of 9 patients (55.5%) with a resistant isolate developed an invasive
171 candidiasis while only 6 out of 27 patients (22.2%) with a susceptible isolate developed
172 invasive candidiasis ($p=0.09$; Fisher's exact test). Considering the number of *Candida* isolates
173 rather than the number of patients, 6 out of 10 resistant isolates were responsible for invasive

174 infection while 8 out of 35 susceptible isolates were responsible for invasive infection
175 (p=0.049; Fischer's exact test).

176

177 **3.7. Ecological investigations.** The clustering of resistant isolates and their predominant
178 presence in a single unit along with some susceptible clonal isolates raised the question of an
179 existing environmental and/or human reservoir, and the possibility of patient-to-patient
180 transmission. Following discussion with the hospital's hygiene team, the local nosocomial
181 infection control committee and the unit's medical head, we conducted an epidemiological
182 investigation in the mentioned ICU in search of an environmental reservoir. We took 100
183 swab samples. Different rooms and corridors were examined. The floor, bed rails, ultrasound
184 scanners, washbasins, care trolleys, ECMO (extra-corporeal membrane oxygenation) devices
185 were swabbed. Only two colonies of *C. parapsilosis* were found in one sample (washbasin).
186 They were susceptible to azole drugs, had the same microsatellite profile and were not linked
187 to any of the clinical isolates (Figure 1).

188

189 **4. DISCUSSION**

190 Historically, the first described *C. parapsilosis* outbreaks had been related to environmental
191 reservoirs and medical devices, whit direct contamination of the patients. In 1977, Plouffe *et*
192 *al* reported an outbreak of *C. parapsilosis* fungemia related to the contamination of a vacuum
193 system in the preparation room for intravenous infusions (13). Years later, outbreaks due to
194 contaminated hospital environmental reservoirs (14) as well as epidemics related to hand
195 contamination by health care workers have also been reported (15). Thus, the epidemic
196 transmission of *C. parapsilosis* seems to be related both to a human and/or an environmental
197 reservoirs with direct or indirect contamination of the patients. But in most cases, it is very
198 difficult or even impossible to detect an irrefutable unique source of contamination (16).

199 Moreover, several works report outbreaks but few provide genetic analysis of the isolates to
200 confirm their clonal nature.

201 Recently, outbreaks of *C. parapsilosis* infection due to fluconazole-resistant isolates have
202 been described in several countries (7-10) with no explanation for this emergence. In our
203 study, resistance to fluconazole (and voriconazole) revealed two major *C. parapsilosis* clones
204 involved in two separate outbreaks. The A395T mutation that confers the Y132F amino-acid
205 substitution was found in 96.1% (25/26) of the fluconazole-resistant isolates and is
206 presumably the main mechanism that confers azole resistance. This mutation, which affects
207 an amino acid located close to the drug-target interaction area (17) is now widely reported
208 among resistant *C. parapsilosis* (9, 12, 18) and has also been shown to confer fluconazole
209 resistance to *Candida albicans* (19) or *Candida tropicalis* (20). However, the existence of
210 different susceptibility profiles between the different clusters raises the question of others
211 acquired resistance mechanisms such as those involving CDR1, MDR1, MRR1, TAC1 or
212 UPC2 (21). This aspect is worth considerations and requires investigation in further study. Of
213 note, apart from two patients, none had received or been pre-exposed to azole therapy when
214 the resistant isolates were identified and pre-exposure to an azole antifungal drug was not
215 related to the occurrence of invasive infection due to a resistant isolate.

216 The results of our study call for several comments. First and importantly, genotyping of *C.*
217 *parapsilosis* isolates may reveal undetected epidemic transmission, even over a very large
218 time scale. Indeed, the possibility of transmission leading to colonization without further
219 infection, the low number of cases and their sporadic nature, the significant delay that may
220 exist between two cases; all these points make it very difficult to detect epidemic
221 transmission.

222 Analysis of the R1 clone is particularly informative at this level. It was first detected in a
223 patient in March 2012 (Patient 1) and again a year and a half later (Patient 3), which suggests

224 the possibility of a persistent environmental or human reservoir among healthcare personnel.
225 Surprisingly, it was found again more than 4 years later (Patient 5). It should be noted that
226 some isolates were responsible for colonization only and therefore not tested for MICs nor
227 kept frozen and may have been missed during this period. One of the patients infected by an
228 R1 clone (Patient 5) and hospitalized in the cardiac surgery ICU was transferred to another
229 ICU (surgical critical care-2) where one more patient (Patient 7) was hospitalized just a few
230 days before he was detected positive with the R1 clone. This strongly suggests a transmission
231 from patient to patient mediated by health-care workers or medical devices. Also surprisingly,
232 R1 clone disappeared and has not been detected since.

233 The second point deals with genotyping analysis as well. It shows that genetic diversity is
234 reduced for resistant isolates compared to susceptible isolates, or that resistant isolates have
235 an increased ability to spread in a clonal mode. Moreover, they were more frequently related
236 to invasive infections than the azole-susceptible isolates. Whether the Y132F substitution that
237 confers azoles resistance might also be linked to a particular fitness of *C. parapsilosis*, that
238 would favour either its human/environmental persistence and/or its pathogenicity, requires
239 further investigation. Moreover, patients diagnosed with an invasive infection due to resistant
240 isolates had a poorer outcome than the patients infected by susceptible ones but this result has
241 to be confirmed on larger series, as it did not reach statistical significance. Thus, 30-day
242 mortality rate of invasive infections due to susceptible isolates was comparable to those
243 reported in others studies (1, 22). Intriguingly, Grossman *et al* also reported that *C.*
244 *parapsilosis* isolates harbouring the Y132F alteration tended to be closely related genetically
245 and limited to a small number of hospitals whereas others resistant isolates had no hospital
246 specificity (12). More recently, Choi *et al* have also provided arguments for a greater
247 propensity of Y132F isolates to cause clonal spread and to persist in hospitals (9). Our results
248 are in accordance with these observations and suggest that resistant *C. parapsilosis* isolates

249 with Y132F modification have a capacity to persist over a long period of time. As they are
250 thought to represent important mechanisms of pathogens spreading and virulence, it would be
251 interesting to evaluate the isolates harbouring the Y132F modification for their fitness, ability
252 of adherence, biofilms formations (23) or their survival to surface disinfection agents such as
253 quaternary ammonium.

254 Finally, it should be noted that the genotyping also revealed 3 grouped cases due to wild-type
255 azole susceptible isolates. It indicates that susceptible isolates might also be related to clonal
256 spreading although their susceptible phenotype makes them less intriguing and possibly more
257 difficult to detect.

258 As a consequence of our study, hygiene recommendations have been taken, notably
259 strengthening hand disinfection with hydro-alcoholic solutions and the cleaning of rooms. As
260 *C. parapsilosis* has been showed to accommodate with very various environment (24), alive
261 (human skin, body surface of insects, fruits, pine trees) or inert (tapwater, surface in
262 residential environment), we also performed hundred of environmental swabbing but failed to
263 find any source for the *C. parapsilosis* azoles-resistant clones. Investigations should be
264 extended to healthcare workers.

265

266 **5. CONCLUSION**

267 In conclusion, our study shed light on the propensity of *C. parapsilosis* to lead to an epidemic
268 over a long period of time. The particular fluconazole-resistant phenotype linked to the
269 Y132F substitution seems related to clonal spreading, invasive infection and high mortality.
270 Our results also underline the importance of genotyping *C. parapsilosis* isolates even for
271 susceptible isolates to unveil unsuspected clustered cases and thus apply hygiene measures to
272 limit nosocomial transmission.

273

274 **LEGENDS:**

275

276 Table 1: clinical origin and incidence of fluconazole resistance among 283 *Candida*
277 *parapsilosis* isolates collected in 240 patients from a single hospital between 2012 and 2019

278

279 Table 2: information for 26 fluconazole-resistant *Candida parapsilosis* isolates sampled in 18
280 patients

281

282 Table 3: data for 78 patients who developed an invasive infection due to *Candida*
283 *parapsilosis*. Comparison between susceptible and fluconazole-resistant isolates

284

285 Figure 1: UPGMA (unweighted pair group method with arithmetic mean)-dendrogram of 93
286 *Candida parapsilosis* isolates typed by microsatellite approach. Fluconazole-resistant isolates
287 (n=26) are highlighted in yellow. Isolates from a single intensive care unit are in red. R1 and
288 R2 represent 2 clusters of fluconazole-resistant isolates responsible for 2 outbreaks in 4 and
289 10 patients, respectively. S1-S3 represent clusters of susceptible isolates.

290

291 Figure 2: Spatial and temporal circulation of *Candida parapsilosis* fluconazole-resistant
292 isolates belonging to a single cluster (R1) among patients with invasive candidiasis hosted in
293 different building of a single hospital between 2012 and 2017

294

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300 **ETHICAL APPROVAL**

301 Not required.

302

303

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	Numbers of patients (%)	Number of isolates		
		Fluconazole-susceptible isolates (%)	Fluconazole-resistant isolates (%)	Total (%)
	240¹	257	26 (9.2)	283
Infections²	111 (46.3)	115 (44.7)	16 (61.5)	131 (46.3)
Candidemias	69 (62.1)	70 (60.9)	12 (75)	82 (62.6)
Osteo-articular infections	19 (17.1)	19 (16.5)	1 (6.3)	20 (15.3)
Mediastinitis	6 (5.4)	7 (6)	2 (12.5)	9 (6.9)
Other deep infections	19 (17.1)	19 (16.5)	1 (6.3)	20 (15.3)
Colonization³	93 (38.8)	98 (38.1)	6 (23.1)	104 (36.7)
Superficial / Mucocutaneous	57 (61.3)	57 (58.2)	3 (5)	60 (57.7)
Respiratory tract	23 (24.7)	26 (26.5)	1 (3.7)	27 (26)
Urinary tract	11 (11.8)	10 (10.2)	1 (9.1)	11 (10.6)
Other	6 (6.4)	6 (6.1)	0 (0)	6 (5.8)
Catheter	25 (10.4)	25 (9.7)	4 (15.4)	29 (10.2)
Data not available	18 (7.5)	19 (7.4)	0 (0)	19 (6.7)

Table 1: clinical origin and incidence of fluconazole resistance among 283 *Candida parapsilosis* isolates collected in 240 patients from a single hospital between 2012 and 2019

¹ : seven patients had isolates responsible for colonization, infection and/or found in catheter culture

² : one patient had mediastinitis, fungemia and other deep infection

³ : three patients had superficial and respiratory tract colonization, one patient had superficial and urinary tract colonization

Patients	Isolate identification number	Sample type	Date of sample	Genotype	Unit	Building	Previous stay in building A	Underlying condition	Day-30 evolution
1	PSL0010	Blood culture	March 10, 2012	R1	Surgical critical care - 1	C	yes (18 days ago)	Cardiothoracic surgery	Alive
2	PSL0020	Blood culture	August 5, 2013	Other	Neurological critical care	D	no	Guillain-Barré syndrome	Alive
3	PSL0014	Central catheter	September 5, 2013	R1	Cardiac surgery Intensive Care Unit	A	NA	Cardiothoracic surgery	Death
	PSL0011	Blood culture	September 10, 2013		Cardiac surgery Intensive Care Unit	A			
4	PSL1009	Blood culture	April 4, 2014	Other	Cardiac surgery Intensive Care Unit	A	NA	Cardiothoracic surgery	Death
5	PSL0007	Peritoneal fluid	August 27, 2017	R1	Cardiac surgery Intensive Care Unit	A	NA	Cardiothoracic surgery	Death
	PSL0015	Mediastinitis	September 26, 2017		Surgical critical care - 2	B			
	PSL0001	Pyelic urine	October 31, 2017		Vascular surgery	B			
	PSL0016	Blood culture	November 10, 2017		Surgical critical care - 2	B			
6	PSL0004	Central catheter	November 6, 2017	R2	Cardiac surgery Intensive Care Unit	A	NA	Cardiothoracic surgery	Death
	PSL0018	Mediastinitis	November 20, 2017		Cardiac surgery Intensive Care Unit	A			
7	PSL0003	Blood culture	November 9, 2017	R1	Surgical critical care - 2	B	no	Abdomial surgery	Alive
8	PSL0008	Blood culture	January 13, 2018	R2	Surgical critical care - 2	B	yes (14 days ago)	Cardiothoracic surgery	Alive
	PSL0005	Urine	January 27, 2018		Surgical critical care - 2	B			
9	PSL0013	Pacemaker pocket	August 16, 2018	R2	Cardiac surgery Intensive Care Unit	A	NA	Heart transplantation	Alive
10	PSL0019	Blood culture	September 29, 2018	R2	Medical critical care	A	NA	Heart transplantation	Alive
	PSL0106	Blood culture	October 18, 2018		Medical critical care	A			
11	PSL0102	Broncho-alveolar lavage	October 15, 2018	R2	Cardiac surgery Intensive Care Unit	A	NA	Heart transplantation	Death
	PSL0126	Blood culture	December 18, 2018		Cardiac surgery Intensive Care Unit	A			
12	PSL0107	Anal swab	October 28, 2018	R2	Cardiac surgery Intensive Care Unit	A	NA	Cardiothoracic surgery	Alive
13	PSL0119	Blood culture	November 29, 2018	Other	Medical critical care	A	NA	Cardiogenic shock / ECMO	Death
14	PSL0151	Catheter	March 25, 2019	R2	Cardiac surgery Intensive Care Unit	A	NA	Cardiogenic shock / ECMO	Alive
15	PSL0172	Diabetic foot	July 1, 2019	Other	Diabetology	E	no	Diabetes melitus	Alive
16	PSL0201	Nasal swab	July 31, 2019	R2	Surgical critical care - 2	B	no	Bariatric surgery	Alive
17	PSL0211	Nasal swab	September 3, 2019	R2	Cardiac surgery Intensive Care Unit	A	NA	Cardiothoracic surgery	Alive
18	PSL0225	Catheter	October 15, 2019	R2	Surgical critical care – 2	B	yes (7 months ago)	Abdomial surgery	Alive

Table 2: information for 26 fluconazole-resistant *Candida parapsilosis* isolates sampled in 18 patients

	Invasive infections due to fluconazole susceptible isolates	Invasive infections due to fluconazole resistant isolates	p value
Number of patients	67	11	
Mean age in year (median; interquartile)	59.4 (62; [51-72])	59.6 (65; [56-65.5])	0.78
Sex (male/female)	52/15	9/2	1
Clinical presentation			
Candidemia	55 (82.1%)	9/11 (81.8%)	1
Mediastinitis	3 (4.5%)	2/11 (18.2%)	0.14
Others	9 (13.4%)	0	0.34
Hospital ward			
ICU	34 (50.7%)	10 (90.9%)	0.019
non-ICU	22 (32.8%)	0	0.028
Chirurgical unit	11 (16.4%)	1 (9.1%)	1
Immunosuppression ¹	25/60 (41.7%)	3/11 (27.3%)	0.5
Presence of catheter and/or external devices	46/53 (86.8%)	11/11 (100%)	0.34
Recent chirurgical intervention (last 30 days)	42/57 (73.7%)	10/11 (90.9%)	0.44
Broad-spectrum antibiotic therapy	40/50 (80%)	10/10 (100%)	0.19
Pre-exposition to antifungal drugs (last 3 months)	8/47 (17%)	2/10 (20%)	1
Targeted antifungal therapy	36/39 (92.3%)	8/10 (80%)	0.27
Echinocandin-based	12/36 (33.3%)	5/8 (62.5%)	0.22
Fluconazole-based	21/36 (58.3%)	1/8 (12.5%)	0.046
Other	3/36 (8.3%)	2/8 (25%)	0.22
All cause mortality			
All patients			
Day 30	15/67 (22.4%)	5/11 (45.5%)	0.082
Day 90	19/64 (29.7%)	6/11 (54.5%)	0.12
ICU's patients			
Day 30	9/34 (26.5%)	4/10 (40%)	0.40
Day 90	11/31 (35.5%)	5/10 (50%)	0.46

Table 3: data for 78 patients who developed an invasive infection due to *Candida parapsilosis*. Comparison between susceptible and fluconazole-resistant isolates. ICU (intensive care unit). Statistically significant values are in bold.

¹ Immunocompromized conditions included solid organ transplantation, HIV infection, neutropenia (<500 cells/ μ L), long term corticosteroid therapy (> 3weeks), malignancies, allogenic hematopoietic stem cell transplantation

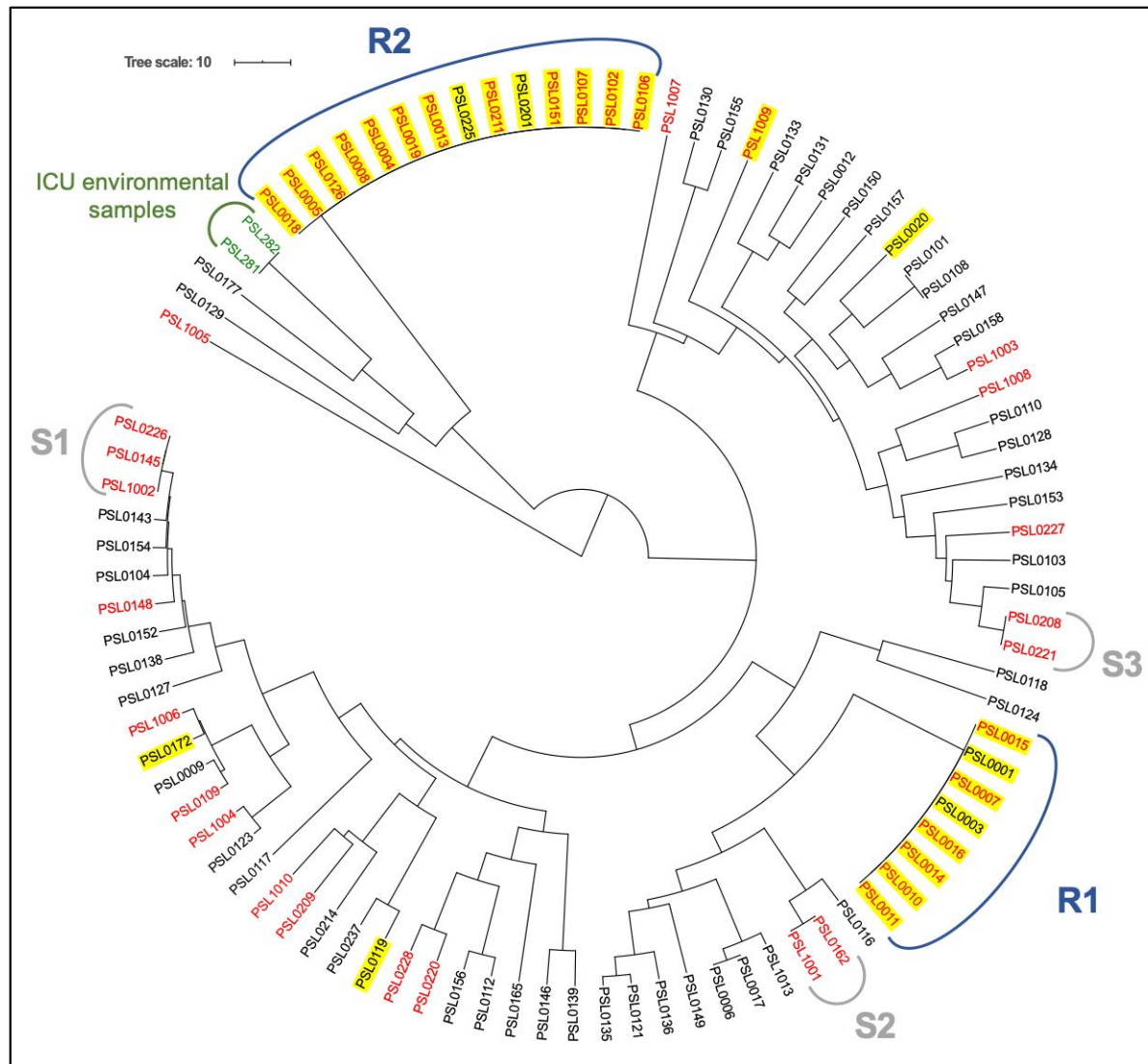


Figure 1: UPGMA (unweighted pair group method with arithmetic mean)-dendrogram of 93 *Candida parapsilosis* isolates typed by microsatellite approach. Fluconazole-resistant isolates (n=26) are highlighted in yellow. Isolates from a single intensive care unit (ICU) are in red. R1 and R2 represent 2 clusters of fluconazole-resistant isolates responsible for 2 outbreaks in 4 and 10 patients, respectively. S1-S3 represent clusters of susceptible isolates.

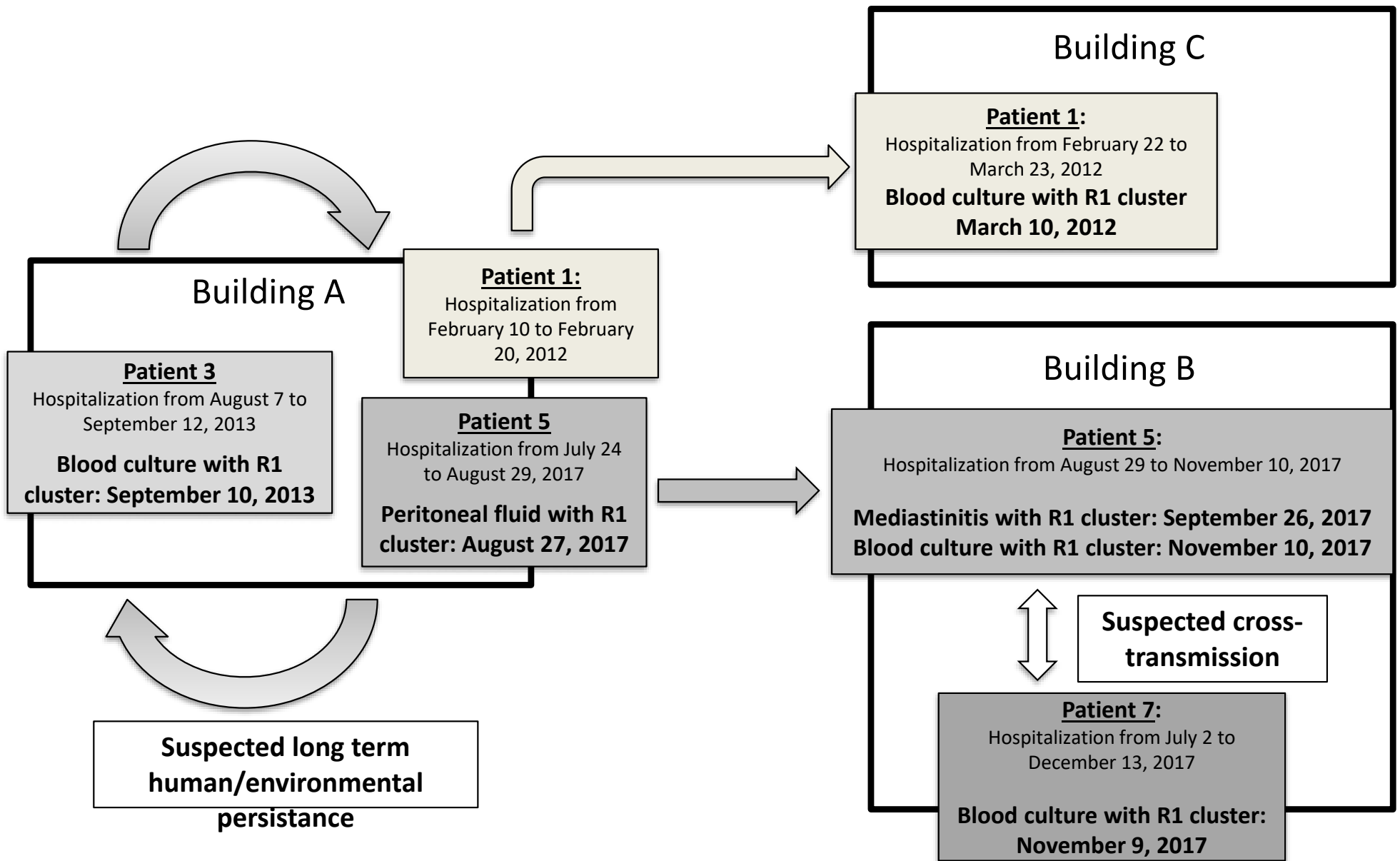


Figure 2: Spatial and temporal circulation of *Candida parapsilosis* fluconazole-resistant isolates belonging to a single cluster (R1) among patients with invasive candidiasis hospitalized in different building of a single hospital between 2012 and 2017.