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

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Arnaud Fekkar[1,2,#], Marion Blaize[1], Adrien Bouglé[3], Anne-Cécile Normand[1], Audrey Raoelina[1], Dimitri Kornblum[1], Laure Kamus[1], Renaud Piarroux[1,4], Sébastien Imbert[1,2]

[1] AP-HP, Groupe Hospitalier La Pitié-Salpêtrière, Service de Parasitologie Mycologie, Paris, France

[2] Sorbonne Université, Inserm, CNRS, Centre d'Immunologie et des Maladies Infectieuses, Cimi-Paris, Paris, France

[3] AP-HP, Groupe Hospitalier La Pitié-Salpêtrière, Département d'Anesthésie et Réanimation, Paris, France

[4] Sorbonne Université, Inserm, Institut Pierre Louis d'Epidemiologie et de Santé Publique, Paris, France

[#] Corresponding author

Corresponding author:

A. Fekkar, Service de Parasitologie-Mycologie, Pavillon Laveran, Hôpital de La Pitié-Salpêtrière, Boulevard de l'Hôpital, 75013 Paris, France

E-mail: arnaud.fekkar@aphp.fr Tel: +33 1 42 16 01 84 Fax: +33 1 42 16 01 15

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ABSTRACT

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

1. INTRODUCTION

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

parapsilosis have occasionally been found in intensive care unit (ICU) (5) and resulted in high mortality rates in immunocompromised patients (6).

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

2. MATERIALS AND METHODS

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

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

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

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

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

3. RESULTS

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

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

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

3.3. E*rg11* sequencing. The 26 fluconazole-resistant isolates and a random selection of 21 fluconazole-susceptible isolates were subjected to *erg11* gene sequencing. Results highlighted the A395T mutation that confers the Y132F amino-acid substitution in all fluconazole-resistant isolates except one (identification number PSL0172) for which no specific alteration was found. The latter showed a resistance profile to all azoles. The A395T mutation was absent in all 21 fluconazole-susceptible isolates tested as controls.

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

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

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

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

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

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

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

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

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

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

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

4. DISCUSSION

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

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

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

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

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

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

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

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

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

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

5. CONCLUSION

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

LEGENDS:

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

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

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

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 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 hosted in different building of a single hospital between 2012 and 2017

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ETHICAL APPROVAL

Not required.

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	Numbers of patients (%)	Number of isolates		
		Fluconazole-susceptible isolates (%)	Fluconazole-resistant isolates (%)	Total (%)
	240 [1]	**257**	**26 (9.2)**	**283**
Infections[2]	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)
Colonization[3]	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 mediastinis, 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
Immunosuppresion [1]	25/60 (41.7%)	3/11 (27.3%)	0.5
Presence of cathether and/or external devices	46/53 (86.8%)	11/11 (100%)	0.34
Recent chirugical 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 antifgunal 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 acre unit). Statistically significant values are in bold.

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

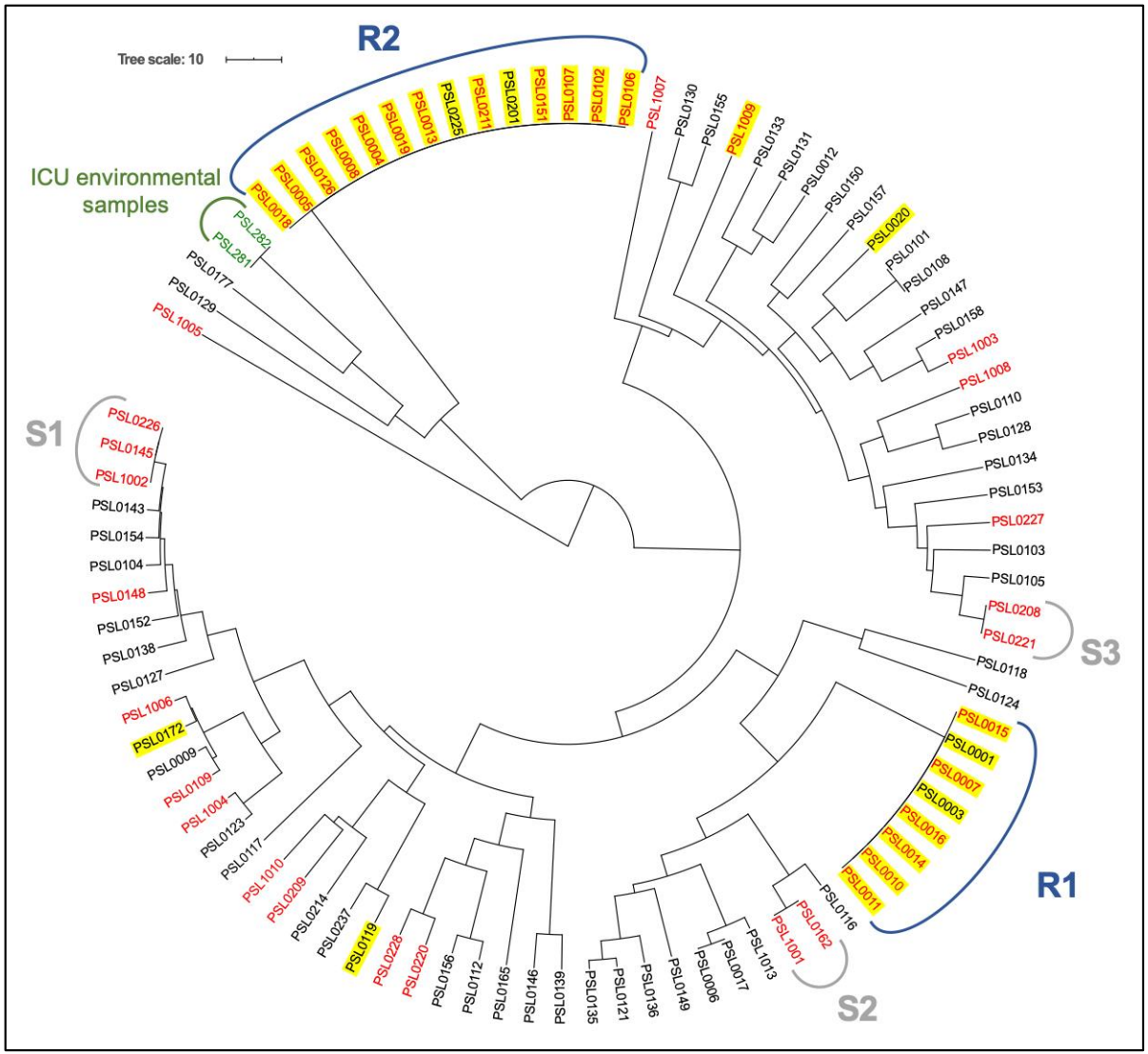

Figure 1: UPGMA (unweighted pair group method with arithmetic mean)-dendrogram of 93 *Candida parapsilosis* isolates typed by microsatellite approach. Fluconazole-resistant isolates (n=26) are highlighted in yellow. Isolates from a single intensive care unit (ICU) are in red. R1 and R2 represent 2 clusters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

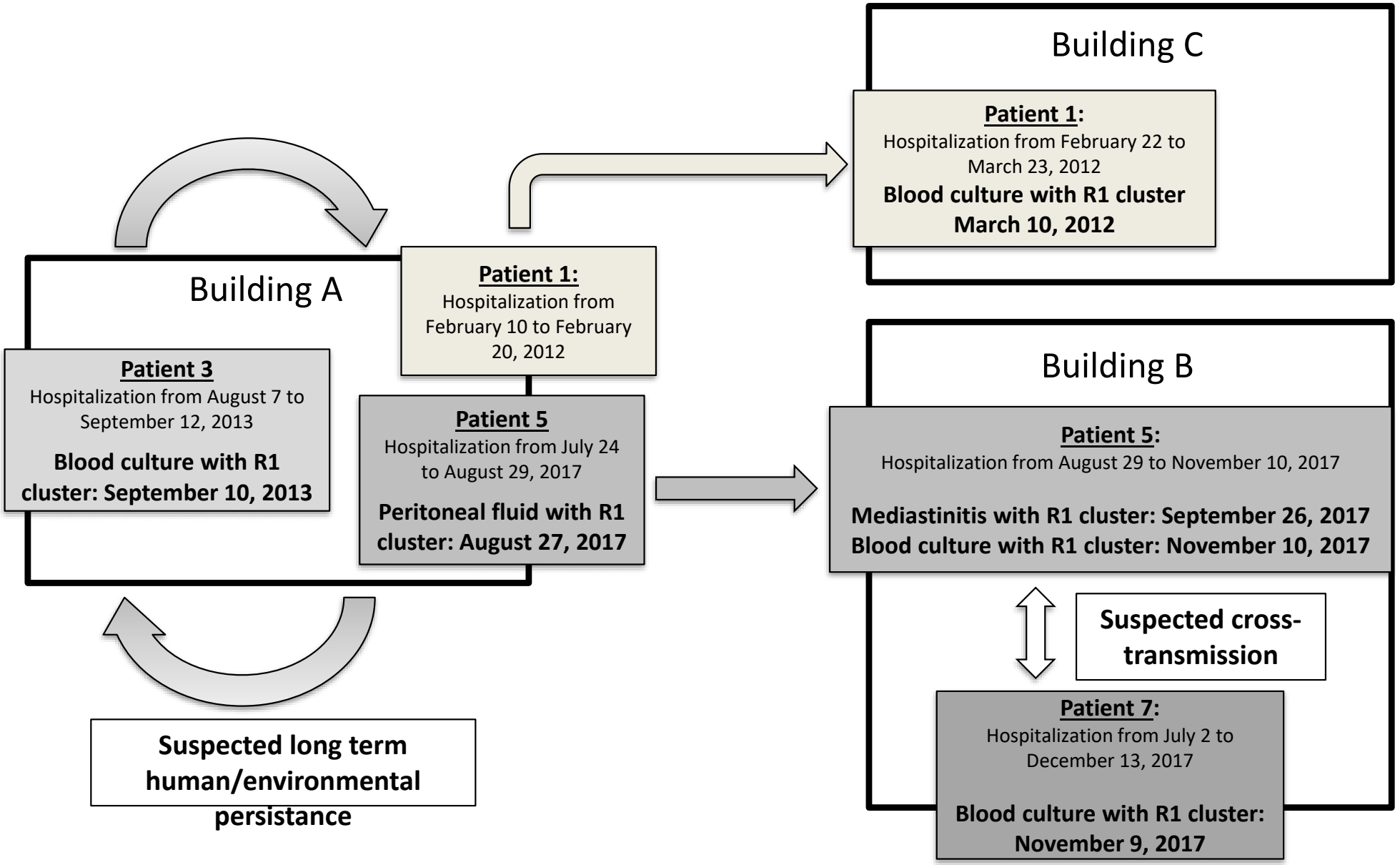

Figure 2: Spatial and temporal circulation of *Candida parapsilosis* fluconazole-resistant isolates belonging to a single cluster (R1) among patients with invasive candidiasis hospitalized in different building of a single hospital between 2012 and 2017.