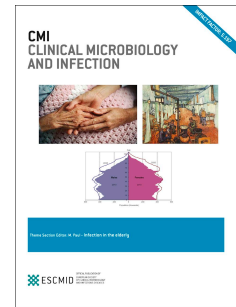


# Accepted Manuscript

Analysis of clinical and environmental *Candida parapsilosis* isolates by microsatellite genotyping – a tool for hospital infections surveillance

Raquel Sabino, Paula Sampaio, Laura Rosado, Zélia Videira, Frederic Grenouillet, Célia Pais



PII: S1198-743X(15)00605-9

DOI: [10.1016/j.cmi.2015.06.001](https://doi.org/10.1016/j.cmi.2015.06.001)

Reference: CMI 301

To appear in: *Clinical Microbiology and Infection*

Received Date: 24 February 2015

Revised Date: 18 May 2015

Accepted Date: 1 June 2015

Please cite this article as: Sabino R, Sampaio P, Rosado L, Videira Z, Grenouillet F, Pais C, Analysis of clinical and environmental *Candida parapsilosis* isolates by microsatellite genotyping – a tool for hospital infections surveillance, *Clinical Microbiology and Infection* (2015), doi: 10.1016/j.cmi.2015.06.001.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **CLM-15-8307**

2  
3 **Analysis of clinical and environmental *Candida parapsilosis* isolates by**  
4 **microsatellite genotyping – a tool for hospital infections surveillance**

5  
6 **Raquel Sabino<sup>a,b\*</sup>, Paula Sampaio<sup>a</sup>, Laura Rosado<sup>b</sup>, Zélia Videira<sup>c</sup>, Frederic**  
7 **Grenouillet<sup>d</sup> and Célia Pais<sup>a</sup>**

8  
9 (a) Centre of Molecular and Environmental Biology (CBMA), Department of Biology,  
10 University of Minho, Braga, Portugal

11 (b) URSZ- Mycology, Department of Infectious Diseases, National Institute of Health  
12 Dr. Ricardo Jorge, Lisbon, Portugal

13 (c) Bacteriology Laboratory, Portuguese Institute of Oncology Prof. Francisco Gentil,  
14 Lisbon, Portugal

15 (d) Mycology Department, University Hospital, Besançon, France

16  
17 \* Corresponding author:

18 Raquel Sabino

19 National Institute of Health Dr. Ricardo Jorge – Mycology Laboratory, Infectious  
20 Diseases Department, Lisbon, Portugal, Av. Padre Cruz, 1649-016 Lisboa

21 Tel: (+351)217519247

22 Fax: (+351)217526400

23 raquelsabino@hotmail.com

24  
25 **Running Title:** Microsatellite genotyping of clinical and environmental *Candida*  
26 *parapsilosis* isolates

**27 Abstract**

28 *Candida parapsilosis* emerged as an important opportunistic pathogen, causing  
29 candidemia worldwide. Nosocomial outbreaks triggered by this species have been  
30 frequently described, particularly in cancer patients. For a better understanding of its  
31 epidemiology, several typing methods are used and microsatellite analysis has been  
32 reported as highly discriminant. The main objective of this work was to study *C.*  
33 *parapsilosis* isolates by application of microsatellite genotyping to distinguish  
34 epidemiologically related strains, compare clinical and environmental isolates and  
35 determine possible routes of dispersion of the isolates in the hospital setting. A total  
36 of 129 *C. parapsilosis* isolates from different origins, including hospital environment  
37 and hands of health care workers, were genotyped using four microsatellite markers.  
38 The isolates were recovered from different health institutions. Analysis of *C.*  
39 *parapsilosis* isolates from hospital environment showed great genotypic diversity,  
40 however the same or very similar genotypes were also found. The same multilocus  
41 genotype was shared by isolates recovered from the hand of a healthcare worker,  
42 from the hospital environment, and from patients of the same health care institution,  
43 suggesting that these could be possible routes of transmission and that infections  
44 due to *C. parapsilosis* may be mainly related with exogenous transmission to the  
45 patient. Examination of sequential isolates from the same patients showed that  
46 colonizing and bloodstream isolates had the same multilocus genotype in the  
47 majority of cases. We demonstrate that this typing method is able to distinguish  
48 clonal clusters from genetically unrelated genotypes and can be a valuable tool to  
49 support epidemiological investigations in the hospital setting.

50

51 **Keywords:**

52 *Candida parapsilosis*, microsatellite genotyping, nosocomial infections, health care  
53 workers, hospital air and surfaces.

54

55

56 **Introduction**

57 Infections caused by *Candida* species have been rising in the last decades mainly  
58 due to the multiple medical resources used currently, such as chemotherapy,  
59 antibiotics, catheters and parenteral nutrition [1-5]. Although *C. albicans* continues to  
60 be the most common cause of bloodstream infections, longitudinal studies have  
61 detected a shift towards non-*albicans* *Candida* infections, specifically *C. glabrata*, *C.*  
62 *tropicalis* and *C. parapsilosis* [1, 6, 7].

63 *Candida parapsilosis* is associated with approximately 25% of *Candida* infections  
64 in European hospitals and it is now the second most commonly isolated *Candida*  
65 species from bloodcultures in Europe, Canada and Latin America, and in some  
66 European hospitals even outranks *C. albicans* [5, 7]. Invasive disease caused by *C.*  
67 *albicans* and *C. tropicalis* is normally preceded by prior colonization while, in  
68 contrast, invasive disease produced by *C. parapsilosis* can occur without prior  
69 colonization and is frequently transmitted horizontally via contaminated external  
70 sources such as infusates [8], the hands of health care workers [9, 10], prosthetic  
71 devices [11], catheters [12-14], parenteral hyperalimentation [15, 16]. It is possible  
72 that inanimate environmental surfaces and the colonized patients themselves  
73 constitute a reservoir from which other patients may acquire the fungus and in some  
74 cases develop candidemia [17, 18]. *Candida parapsilosis* is a particular problem as it

75 tends to grow as biofilms on implanted medical devices, conferring almost total  
76 resistance to antifungal drugs, and this ability to grow as a biofilm seems to be  
77 directly related with the capacity to cause clinically significant disease [19].

78 The laboratory identification and typing of pathogens is frequently used by  
79 epidemiologists for providing evidence for the biological and genetic relatedness of  
80 these organisms as an aid in the epidemiological investigation of nosocomial  
81 infections. The rationale for such subspecies or strain delineation is that repeated  
82 isolation of the same strain from one or more patients suggests that the organism  
83 may have originated from a single clone and thus is more likely to represent infection  
84 in the case of a single patient or transmission from patient to patient from a common  
85 source or by a common mechanism [20]. The genetic relationship between clinical  
86 isolates, route of acquisition, nosocomial transmission, or the emergence of  
87 antifungal resistant strains can be studied by using DNA-based typing methods and  
88 the need to discriminate among *C. parapsilosis* strains has been reported in several  
89 studies for a better understanding of the epidemiology of this species [10, 21, 22].  
90 However, most of the genotyping methods are not discriminant enough to distinguish  
91 closely related isolates and to establish routes of transmission.

92 A microsatellite-based typing method to genotype *C. parapsilosis* sensu stricto  
93 isolates, using four loci composed by tandemly repetitive stretches of three  
94 nucleotides, has been recently described, achieving a discriminatory power of 99.9%  
95 [23]. Our aim was the study of *C. parapsilosis* isolates with different origins by  
96 application of these microsatellite markers in order to distinguish epidemiologically  
97 related isolates, identify prevalent strains in the hospital setting, compare between  
98 environmental and clinical isolates and determine possible routes of acquisition of a

99 strain in the hospital environment or identify possible reservoirs. We demonstrate that  
100 this typing method is able to distinguish clonal clusters from genetically unrelated  
101 genotypes and can be a valuable tool to support epidemiological investigations.

102

103

## 104 **Materials and methods**

105

### 106 *Yeast strains and DNA extraction*

107 A total of 129 *C. parapsilosis* strains with different origins were analysed in this  
108 study (Table 1). Several isolates were collected from the same patient in different  
109 time periods or in the same period but from different biological products. In these  
110 cases, clinical presentation of the patients and dates of collection are presented in  
111 Tables 4 and 5. The environmental *C. parapsilosis* isolates analysed were actively  
112 collected from the Haematology ward of an oncological hospital during four sampling  
113 periods (January, April, September and February), in the same time period and at the  
114 same time that isolates from the patients were collected as well. Isolates from the air  
115 were collected using an air sampler (Millipore) with a velocity air rate of 140 L/min.  
116 The sampled air (500 m<sup>3</sup>) was directly impacted onto 2% malt extract agar (MEA)  
117 with chloramphenicol (0.05 g/L). Samples from hard surfaces were collected by  
118 swabbing them with a cotton swab pre-moistened with sterile saline solution,  
119 according to the International Standard ISO 18593 [24]. The sample swabs were  
120 then streaked onto MEA. Collection of the Isolates from the hands of health care  
121 workers (14 nurses, 4 physicians, 6 medical auxiliaries or hospital technicians, 4  
122 members of the administrative staff) was previously approved by the Ethical

123 committee of the selected hospital). Those isolates were collected using contact  
124 plates at the palm of the hand and swabs in nails and inter-digital spaces of the  
125 hands. *Candida parapsilosis* isolates were identified using the methodology  
126 previously described [23, 25]. Stock cultures were maintained on Sabouraud glucose  
127 agar medium at 4°C. Prior to DNA isolation, yeast cells were grown for 24 hours on  
128 Sabouraud glucose agar medium at 37°C. DNA extraction was performed by using  
129 the High Pure PCR Template kit (Roche Diagnostics Corp., Indianapolis, Ind.),  
130 according to the manufacturer's instructions.

131

132 *PCR amplification conditions fragment size determination.*

133 Four *C. parapsilosis* specific microsatellite markers were used, designated by  
134 CP1, CP4, CP6 and B and PCR amplification was performed following the  
135 methodology developed and described previously [23].

136 Following PCR, denaturated samples were run in an ABI 310 Genetic Analyser  
137 (AB Applied Biosystems) and the PCR products size was determined by using the  
138 GeneScan 3.7 Analysis software and alleles were designated by their sizes in base  
139 pairs (bp) [23].

140

141 *Statistical analysis.*

142 Allelic and genotypic frequencies were determined by using ARLEQUIN ver.2.000  
143 software. Genetic distance between *C. parapsilosis* isolates was calculated by using  
144 shared allele distance (DAS) in the Populations 1.2.30 software program. The  
145 clustering of the isolates was performed with NTSys software, using UPGMA  
146 method.

## 147 Results

148

149 *Analysis of Candida parapsilosis isolates from the hospital environment and*  
150 *healthcare workers*

151 Air from the Haematology unit of an oncological hospital, as well as from several  
152 hard surfaces, such as doors knobs, bedside tables, water taps and medical trolleys  
153 of the same unit were sampled and screened for *C. parapsilosis*. The hands of health  
154 care workers of that same ward were also analysed and several *C. parapsilosis*  
155 isolates were collected. Great genotype diversity was observed amongst hospital  
156 environmental isolates (18 different multilocus genotypes), however some of them  
157 were found to share the same multilocus genotype (M, O, P and F, Q, R) (Table 2).

158 Isolates M, O and P were all collected in the same day, one of them from a  
159 medical trolley and the other two from doors knobs. The places from where these  
160 isolates were collected suggest a possible strain transmission due to manual  
161 handling (Table 3). Interestingly, none of the patients of the studied hospital unit  
162 presented infections due to *C. parapsilosis* with a similar multilocus genotype. Isolate  
163 F was collected from a health care worker hand in January/07 and presented the  
164 same multilocus genotype as an isolate recovered from a water tap and another one  
165 collected from a medical trolley in April/07 (Table 3).

166 In opposition to what was observed in the previous case, two patients presented  
167 infections caused by isolates with the same multilocus genotype. The first one was  
168 hospitalized in the same hospital ward in May/07 and the strain was isolated from a  
169 bloodculture. The other clinical isolate presenting the same genotype was collected  
170 in August/05 from the pus of a patient hospitalized in the Gastroenterology Unit. A



171 dendrogram showing the degree of genetic similarity based on microsatellite  
172 genotyping, among the clinical and environmental *Candida parapsilosis* isolates  
173 obtained in that hospital is presented in Fig. 1.

174

175 *Analysis of isolates from the same patients and possible occurrence of an*  
176 *outbreak*

177 Sequential *C parapsilosis* isolates obtained from the same patients in different  
178 time periods, as well as multiple isolates from the same patient recovered from  
179 different biological products were analysed in six hospitalized patients. Table 4  
180 shows the clinical data associated with each patient and the microsatellite multilocus  
181 genotypes obtained in each case.

182 In four of the studied patients, the sequential isolates presented similar genotypes:  
183 from patient #1 three isolates were recovered from bloodcultures separated by about  
184 one month from each other and patient #3 presented one isolate from the catheter  
185 and another one from a bloodculture. The same multilocus genotype was observed in  
186 these isolates indicating that they were the same or very similar strains. . Patient #4  
187 presented a positive bloodculture preceded by a positive urine culture two days  
188 before, and the infecting strains displayed the same genotype. From patient #6,  
189 isolates were recovered from different places and dates but all the strains shared the  
190 same multilocus genotype.

191 The scenario was different regarding the remaining cases whose isolates collected  
192 from the same patient presented different genotypes: in patient #2 it was observed  
193 that the multilocus genotypes of the two isolates recovered were not the same.  
194 However, the difference was only observed in one of the locus (CP6), indicating that

195 the strains causing these two infections were very closely related. From patient #5,  
196 the isolate collected from the skin and the one recovered from the blood were  
197 different strains since they presented distinct genotypes. Therefore, most probably  
198 these two strains were acquired from different sources and the strain colonizing the  
199 skin was not the cause of the bloodstream infection.

200 It was observed that three *C. parapsilosis* isolates collected from blood and urine  
201 of three different patients hospitalized in the Paediatrics Unit at the same time, within  
202 a period of 20 days, shared the same multilocus genotype indicating that they were  
203 the same or very closely related strains (Table 5). The presence of the same strain in  
204 three different patients at the same period of time suggests the possible occurrence  
205 of an outbreak in that hospital unit. Interestingly, no other *C. parapsilosis* strain was  
206 isolated during that period in the same unit and, as no further sampling was  
207 performed, it was not possible to trace the origin of the outbreak.

208

#### 209 *Global analysis and most common genotypes*

210 The 129 *C. parapsilosis* isolates analysed in this study included 45 from  
211 bloodcultures and catheter tips, 31 from skin and nails, 29 from other body sources  
212 and 24 from air and surfaces of the hospital environment. A great genotypic diversity  
213 was found among the isolates, corresponding to 108 different multilocus genotypes.  
214 The most frequent genotypes found in the different biological products are presented  
215 in Table 6.

216 The most common multilocus genotypes in isolates from bloodcultures are  
217 222/243 300/300 282/336 127/127 and 240/252 300/300 285/285 147/149, shared by  
218 three (6.6%) isolates each. The first corresponds to the strains isolated from the

219 described possible outbreak in the Paediatric Unit (Table 5). Interestingly, the second  
220 was only observed in five strains from the French hospital suggesting the possibility  
221 of a local or resident strain.

222 In the case of the isolates from skin and nails, the most common genotype was  
223 222/243 354/354 282/336 129/129 and shared by seven (22.5%) isolates. One of  
224 these isolates was collected from a HCW hand of the Haematology Unit studied and  
225 the other six were recovered from six different non-hospitalized patients and in  
226 different dates, suggesting that this clone could be particularly adapted to skin and  
227 nails. Regarding other clinical products, the multilocus genotype 222/243 354/354  
228 282/282 129/129 is the only one that is shared by three (10.3%) isolates. From the  
229 environmental strains, two multilocus genotypes are shared by three (12.5%) isolates  
230 each: multilocus genotypes 240/243 342/342 285/285 103/103 and 240/240 342/342  
231 285/285 103/103. In this case, three of the isolates were collected from the air of the  
232 same health institution and the other three correspond to the isolates M, O and P  
233 (Table 3).

234 Globally, the multilocus genotype 222/243 354/354 282/336 129/129 was the  
235 most frequently found, shared by 11 isolates. These isolates were collected from the  
236 hospital environment (two of them), from the hands of a health care worker (one  
237 isolate), from hospitalized patients (pus and blood) and from non-hospitalized  
238 patients mainly originated from skin and nails (7). These isolates were collected in  
239 two different and independent Portuguese health institutions and were not observed  
240 in isolates from other geographical origins.

241

242

## 243 Discussion

244 In order to determine the possibility of infection or transmission it is necessary that  
245 the isolates of *Candida parapsilosis* can be exactly discriminated at the strain level  
246 since the results of genotyping methods with low discriminatory potential may lead to  
247 misleading ideas concerning the surveillance of candidiasis [26]. Thus, distinguishing  
248 among strains of *C. parapsilosis* is crucial to understand the epidemiology of this  
249 pathogen [27]. The amount of genetic variation between isolates gives a measure of  
250 their relatedness and molecular typing is performed to determine whether different  
251 isolates give the same or different results for one or more tests. Epidemiologically  
252 related isolates share the same DNA profile or fingerprint, whereas sporadic or  
253 epidemiologically unrelated isolates have distinctly different patterns [28]. According  
254 to several studies that have been performed to distinguish *C. parapsilosis* strains,  
255 ITS group I /RFLP subtype VII-1/ *C. parapsilosis sensu stricto* isolates have high  
256 genome homogeneity [21, 22, 27, 29, 30], which was considered a difficulty to  
257 perform epidemiological studies with this organism. Microsatellite markers have been  
258 applied in the study of clinical yeast species and have shown a high discriminatory  
259 power in discriminating *C. albicans* strains [18, 22, 31-36]. In 2007, Lasker *et al.* [22]  
260 described a set of seven dinucleotidic microsatellites markers, able to discriminate *C.*  
261 *parapsilosis sensu stricto* strains, with a discriminatory power of 0.97. Brillowska-  
262 Dabrowska and colleagues [37] used a combined methodological approach with  
263 pyrosequencing, multilocus sequence typing, random amplified polymorphism and  
264 microsatellite genotyping to study a *C. parapsilosis* nosocomial outbreak in a  
265 haematology ward and they concluded that the last one appears to be the highest  
266 resolution method.

267 The polymorphic microsatellite markers described by Sabino *et al.* [23] showed a  
268 higher discriminatory power than other methodologies and were applied in the study  
269 of several possible outbreaks [38-41]. In the present work they were used to  
270 distinguish among epidemiologically related isolates and to study several *C.*  
271 *parapsilosis* populations.

272 When applied to clinical isolates and when sequential isolates from patients were  
273 genotyped, in four of six cases, the series of isolates displayed the same genotype.  
274 The maintenance of multilocus genotypes was also observed by other authors in  
275 infections due to *Candida albicans* [33, 34, 42]. We detected at a given time, in the  
276 same patient, strains presenting identical multilocus genotype isolated from multiple  
277 anatomical sites, and also a colonizing strain and a bloodstream isolate, from the  
278 same patient, sharing the same multilocus genotype. These observations are in  
279 accordance to what is generally agreed stating that candidaemia usually arises as an  
280 endogenous infection following prior colonization of the gastrointestinal tract, skin, or  
281 vagina [43, 44]. Colonizing strains, however, are not always the source of infection.  
282 In contrast to what happens with *C. albicans*, infections by *C. parapsilosis* may occur  
283 without prior colonization of the patients [22]. That fact was observed in patient #5,  
284 from whom *C. parapsilosis* skin isolate did not share the same multilocus genotype  
285 than the blood isolate, suggesting that the bloodstream infection might have been of  
286 exogenous source. In fact, it has been reported that clustering of *Candida* strains in  
287 time and space may result from cross-infection from an exogenous source, usually  
288 transmitted by contaminated healthcare workers [8].

289 Molecular typing methods have illustrated the link between hand carriage of *C.*  
290 *parapsilosis* and the horizontal transmission and outbreak of infections of *C.*

291 *parapsilosis* in hospital environments by showing the genetic similarities among  
292 isolates from health care workers and clinical isolates [9, 10]. High degree of genetic  
293 similarity was found among isolates collected from the hospital environment and  
294 hands of healthcare workers. This could be observed particularly in the case of  
295 isolates F, Q and R, where one of them was collected from the hand of a healthcare  
296 worker, two with similar genotype from the hospital ward environment, and other two  
297 from patients of the same health care institution. All these isolates shared the same  
298 multilocus genotype which was also the most common genotype found. It is a  
299 genotype well established in this hospital and the hypothesis that its route of  
300 transmission is through the hands of healthcare workers and the contaminated  
301 medical trolleys and doors knobs cannot be ruled out. Moreover, this genotype was  
302 found in 2005 and 2007 and according with Shimdit *et al.* [45], a long-term success of  
303 a given strain is enhanced if it is broadly adaptable. The other environmental isolates  
304 collected in the same hospital ward displayed multilocus genotypes that were not  
305 found in the patients isolates, indicating that in these cases the infections might not  
306 have been hospital acquired.

307 The presence of a prevalent genotype could be due to the lack of a sexual cycle in  
308 *C. parapsilosis* and to the expansion of some clones [22, 46], and may also suggest  
309 a development of a global dominance of a single *C. parapsilosis* genotype.

310 Although microsatellite multiplex genotyping is highly discriminatory, the possible  
311 convergence of ancestral alleles to the same length by different mutational events,  
312 an effect known as homoplasy, can be a limiting factor for strain identification.  
313 Nevertheless, as several loci are considered, the high microsatellite variability often  
314 largely compensates for their eventual homoplastic evolution. Thus, the application of

315 microsatellite loci in studies such as molecular epidemiology and population studies  
316 is highly recommended since the accurate discrimination of genetically divergent  
317 groups within pathogenic species is critical to the appropriate development and use  
318 of treatment strategies [47].

319 In conclusion, the four *C. parapsilosis* microsatellite markers used in this study are  
320 sufficiently polymorphic to allow a high discriminatory power thus permitting their  
321 application in epidemiological studies for recurrent infections and nosocomial  
322 outbreaks. The results obtained in the present study have proven to be very valuable  
323 in studying the genetic relatedness among *C. parapsilosis* isolates, which can be  
324 easily differentiated with high discriminatory power, allowing the detection of  
325 outbreaks.

326

### 327 **Acknowledgments**

328 This research was supported by FCT/MEC, Portugal through Portuguese funds  
329 (PIDDAC) - Pest-OE/BIA/UI4050/2014 (CBMA), University of Minho. Raquel Sabino  
330 was financially supported by a fellowship from FCT, Portugal (contract  
331 BD/22100/2005).

332

### 333 **References**

334 [1] Safdar A, van Rhee F, Henslee-Downey JP, Singhal S, Mehta J. *Candida*  
335 *glabrata* and *Candida krusei* fungemia after high-risk allogeneic marrow  
336 transplantation: no adverse effect of low-dose fluconazole prophylaxis on incidence  
337 and outcome. Bone Marrow Transplant 2001;28:873–878.

338

339 [2] Pemán J, Cantón E, Gobernado M, The Spanish ECMM Working Group on  
340 Candidemia. Epidemiology and antifungal susceptibility of *Candida* species isolated  
341 from blood: results of a 2-year multicentre study in Spain. Eur J Clin Microbiol Infect  
342 Dis 2004;23:1267-1275.

343

344 [3] Bedini, A, Venturelli C, Mussini C, *et al.* Epidemiology of candidaemia and  
345 antifungal susceptibility patterns in an Italian tertiary-care hospital. Clin Microbiol  
346 Infect 2006;12:75-80.

347

348 [4] Perlroth J, Choi B, Spellberg B. Nosocomial fungal infections: epidemiology,  
349 diagnosis and treatment. Med Mycol 2007;45:321-346.

350

351 [5] Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public  
352 health problem. Clin Microbiol Rev 2007;20:33-163.

353

354 [6] Capoor MR, Nair D, Deb M, Verma PK, Srivastava L, Aggarwal P. Emergence of  
355 non-*albicans* *Candida* species and antifungal resistance in a tertiary care hospital.  
356 Jpn J Infect Dis 2005;58:344-348.

357

358 [7] Almirante B, Rodriguez D, Park BJ, *et al.* Epidemiology and predictors of mortality  
359 in cases of *Candida* bloodstream infection: results from population-based  
360 surveillance, Barcelona, Spain, from 2002 to 2003. J Clin Microbiol 2005;43:1829-  
361 1835.

362



- 363 [8] San-Miguel LG, Pla J, Cobo J, Navarro F, Sánchez-Sousa A, Alvarez ME, *et al.*  
364 Morphotypic and genotypic characterization of sequential *Candida parapsilosis*  
365 isolates from an outbreak in a pediatric intensive care unit. *Diagn Microbiol Infect Dis*  
366 2004;49:189-196.
- 367
- 368 [9] Lupetti A, Tavanti A, Davini P, Ghelardi E, Corsini V, Merusi I, *et al.* Horizontal  
369 transmission of *Candida parapsilosis* candidemia in a neonatal intensive care unit. *J*  
370 *Clin Microbiol* 2002;40:2363-2369.
- 371
- 372 [10] van Asbeck, EC, Huang YC, Markham AN, Clemons KV, Stevens DA. *Candida*  
373 *parapsilosis* fungemia in neonates: genotyping results suggest healthcare workers  
374 hands as source, and review of published studies. *Mycopathologia* 2007;164:287–  
375 293.
- 376
- 377 [11] Kan C-D, Luo C-Y, Lin P-Y, Yang Y-J. Native-valve endocarditis due to *Candida*  
378 *parapsilosis*. *Interact Cardiovasc Thorac Surg* 2002;1:66–68.
- 379
- 380 [12] Raad II, Bodey GP. Infectious complications of indwelling vascular catheters.  
381 *Clin Infect Dis* 1992;15:197-210.
- 382
- 383 [13] Levin AS, Costa SF, Mussi NS, Levin AS1, Costa SF, Mussi NS, Basso M, Sinto  
384 SI, *et al.* *Candida parapsilosis* fungemia associated with implantable and semi-  
385 implantable central venous catheters and the hands of healthcare workers. *Diagn*  
386 *Microbiol Infect Dis* 1998;30:243-249.

387 [14] Deshpande K. *Candida parapsilosis* fungaemia treated unsuccessfully with  
388 amphotericin b and fluconazole but eliminated with caspofungin: a case report. Crit  
389 Care Res 2003;5:20-23.

390

391 [15] Weems JJ Jr, Chamberland ME, Ward J, Willy M, Padhye AA., and Solomon  
392 SL. *Candida parapsilosis* fungemia associated with parenteral nutrition and  
393 contaminated blood pressure transducers. J Clin Microbiol 1987;25:1029-1032.

394

395 [16] Trofa D, Gácsér A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal  
396 pathogen. Clin Microbiol Rev 2008;21:606–625.

397

398 [17] Hota B. Contamination, disinfection, and cross-colonization: are hospital surfaces  
399 reservoirs for nosocomial infection? Clin Infect Dis 2004;39:1182–1189.

400

401 [18] Stephan F, Bah MS, Desterke C, Rézaiguia-Delclaux S, Foulet F, Philippe  
402 Duvaldestin P, *et al.* Molecular diversity and routes of colonization of *Candida*  
403 *albicans* in a surgical intensive care unit, studied using microsatellite markers. Clin  
404 Infect Dis 2002; 35:1477-1483.

405

406 [19] Laffey SF, Butler G. Phenotype switching affects biofilm formation by *Candida*  
407 *parapsilosis*. Microbiology 2005;151:1073-1081.

408

409 [20] Pfaller MA. Molecular approaches to diagnosing and managing infectious  
410 diseases: practicality and costs. Emerg Infect Dis 2001;7:312-318.

- 411
- 412 [21] Tavanti A, Davidson AD, Gow NA, Maiden MC, Odds FC. *Candida orthopsilosis*  
413 and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis* groups II and III.  
414 J Clin Microbiol 2005;43:284–292.
- 415
- 416 [22] Lasker BA, Butler G, Lott TJ. Molecular genotyping of *Candida parapsilosis*  
417 Group I clinical isolates by analysis of polymorphic microsatellite markers. J Clin  
418 Microbiol 2006;44:750-759.
- 419
- 420 [23] Sabino R, Rosado L, Sampaio P, Stevens DA, Clemons KV, Pais C. New  
421 polymorphic microsatellite markers able to distinguish among *Candida parapsilosis*  
422 sensu stricto isolates. J Clin Microbiol 2010;48:1677-1682.
- 423
- 424 [24] ISO 18593:2004: Microbiology of food and animal feeding stuffs -- Horizontal  
425 methods for sampling techniques from surfaces using contact plates and swabs.  
426 International Organization for Standardization (ISO). Geneva, International  
427 Organization for Standardization ISO, and Brussels, Euro-pean Committee for  
428 Standardization CEN, September 2003.
- 429
- 430 [25] Correia A, Sampaio P, Almeida J, Pais C. Study of molecular epidemiology of  
431 candidiasis in Portugal by PCR fingerprinting of *Candida* clinical isolates. *J Clin*  
432 *Microbiol* 2004;42:5899-5903.
- 433

434 [26] Dalle F, Lafon I, L'Ollivier C, Ferrant E, Sicard P, Labruère C, et al. A  
435 prospective analysis of the genotypic diversity and dynamics of the *Candida albicans*  
436 colonizing flora in neutropenic patients with de novo acute leukemia. *Haematologia*  
437 2008; 93:581-587.

438

439 [27] van Asbeck EC, Clemons KV, Stevens DA. *Candida parapsilosis*: a review of its  
440 epidemiology, pathogenesis, clinical aspects, typing and antimicrobial susceptibility.  
441 *Crit Rev Microbiol* 2009;35:283-309.

442

443 [28] Pfaller MA. Epidemiological typing methods for mycoses. *Clin Infect Dis* 1992;  
444 14:S4-10.

445

446 [29] Shin JH, Shin DH, Song JW, Kee SJ, Suh SP, Ryang DW. Electrophoretic  
447 karyotype analysis of sequential *Candida parapsilosis* isolates from patients with  
448 persistent or recurrent fungemia. *J Clin Microbiol* 2001;39:1258-1263.

449

450 [30] van Asbeck EC, Clemons KV, Markham AN, Stevens DA. Correlation of  
451 restriction fragment length polymorphism genotyping with internal transcribed spacer  
452 sequence, randomly amplified polymorphic DNA and multilocus sequence groupings  
453 for *Candida parapsilosis*. *Mycoses* 2009;52:493-498.

454

455 [31] Dalle F, Franco N, Lopez J, Vagner O, Caillot D, Chavanet P, Cuisenier B, et al.  
456 Comparative genotyping of *Candida albicans* bloodstream and non bloodstream  
457 isolates at a polymorphic microsatellite locus. *J Clin Microbiol* 2000;38:4554-4559.

458

459 [32] Dalle F, Dumont L, Franco N, Mesmacque D, Caillot D, Bonnin P, et al.  
460 Genotyping of *Candida albicans* oral strains from healthy individuals by polymorphic  
461 microsatellite locus analysis. J Clin Microbiol 2003;41:2203–2205.

462

463 [33] Eloy O, Marque S, Botterel F, Stephan F, Costa J-M, Lasserre V, et al. Uniform  
464 distribution of three *Candida albicans* microsatellite markers in two French ICU  
465 populations supports a lack of nosocomial cross-contamination. BMC Infect Dis  
466 2006;6:162-169.

467

468 [34] Sampaio P, Gusmão L, Correia A, Alves C, Rodrigues AG, Pina-Vaz C, et al.  
469 New microsatellite multiplex PCR for *Candida albicans* strain typing reveals  
470 microevolutionary changes. J Clin Microbiol 2005;43:3869-76.

471

472 [35] Foulet F, Nicolas N, Eloy O, Botterel F, Gantier JC, Costa JM, et al.  
473 Microsatellite marker analysis as a typing system for *Candida glabrata*. J Clin  
474 Microbiol 2005; 43:4574-4579.

475

476 [36] Garcia-Hermoso D, Maccallum DM, Lott TJ, Sampaio P, Buitrago Serna MJ,  
477 Grenouillet F, et al. A multicenter collaborative study for the standardization of  
478 *Candida albicans* genotyping using a polymorphic microsatellite marker. J Clin  
479 Microbiol 2010;8:2578-2581

480

481 [37] Brillowska-Dabrowska A, Pannanusorn ST, Lönnbro N, et al. A nosocomial  
482 outbreak of *Candida parapsilosis* in southern Sweden verified by genotyping. Scand  
483 J Infect Dis 2009;41:135-142.

484

485 [38] Vaz C, Sampaio P, Clemons KV, Huang YC, Stevens DA, Pais C. Microsatellite  
486 multilocus genotyping clarifies the relationship of *Candida parapsilosis* strains  
487 involved in a neonatal intensive care unit outbreak. Diagn Microbiol Infect Dis 2011;  
488 71:159-62.

489

490 [39] Romeo O, Delfino D, Cascio A, Lo Passo C, Amorini M, Romeo D, et al.  
491 Microsatellite-based genotyping of *Candida parapsilosis sensu stricto* isolates  
492 reveals dominance and persistence of a particular epidemiological clone among  
493 neonatal intensive care unit patients. Infect Genet Evol 2013;13:105–108.

494

495 [40] Diab-Elschahawi M, Forstner C, Hagen F, Meis JF, Lassnig AM, Presterl E, et al.  
496 Microsatellite genotyping clarified conspicuous accumulation of *Candida parapsilosis*  
497 at a cardiothoracic surgery intensive care unit. J Clin Microbiol 2012; 50:3422-3426.

498

499 [41] Delfino D, Scordino F, Pernice I, Lo Passo C, Galbo R, David A, et al. Potential  
500 association of specific *Candida parapsilosis* genotypes, bloodstream infections and  
501 colonization of health workers' hands. Clin Microbiol Infect 2014;20:O946-O951.

502

503 [42] Sampaio P, Gusmão L, Alves C, Pina-Vaz C, Amorim A, Pais C. Highly  
504 polymorphic microsatellite for identification of *Candida albicans* strains. J Clin  
505 Microbiol 2003;41:552-557.

506

507 [43] Traoré O, Springthorpe V, Sattar S. A quantitative study of the survival of two  
508 species of *Candida* on porous and non-porous environmental surfaces and hands. J  
509 Appl Microbiol 2002;92:549-555.

510

511 [44] Miranda LN, van der Heijden IM, Costa SF, Sousa AP, Sienna RA, Gobara S, et  
512 al. *Candida* colonisation as a source for candidaemia. J Hosp Infect 2009;72:9-16.

513

514 [45] Schmid J, Herd S, Hunter PR, et al. Evidence for a general-purpose genotype in  
515 *Candida albicans*, highly prevalent in multiple geographical regions, patient types and  
516 types of infection. Microbiology 1999;145:2405–2413.

517

518 [46] van Asbeck EC, Clemons KV, Markham AN, Stevens DA and The *Candida*  
519 *parapsilosis* Global Epidemiology Group. Molecular epidemiology of the global and  
520 temporal diversity of *Candida parapsilosis*. Scand J Infect Dis 2008;40:827-834.

521

522 [47] Sampaio P, Correia A, Gusmão L, Amorim A, Pais C. Sequence analysis reveals  
523 complex mutational processes for allele length variation at two polymorphic  
524 microsatellite loci in *Candida albicans*. In: Méndez-Vilas A, ed. Communicating  
525 Current Research and Educational Topics and Trends in Applied Microbiology.  
526 Badajoz: Formatex, 2007:926-935.

527 **Table 1.** Origin of the *Candida parapsilosis* isolates

528

---

Origin of the isolates	Portuguese institutions	French institutions	Total
Bloodcultures and catheter tips	21	24	45
Skin and nails	28	3	31
Other biological products	21	8	29
Hospital environment	22	2	24
Total	92	37	129

---

529

530

531

532

533

534

535

536

537

538

539

540

541

542

543

544



545 Table 2. Microsatellite multilocus analysis of *Candida parapsilosis* isolates obtained from the  
 546 environment of a hospital ward and their health care workers

547

Isolate identification	Source	Date	Multilocus Genotypes			
			CP1	CP4	CP6	B
A	Nursery 3 water tap	Jan-2007	234 / 240	318 / 318	267 / 267	145 / 147
B	Nursery 3 Bedside table 4	Jan-2007	222 / 246	354 / 357	336 / 336	129 / 129
C	Nursery 2 water tap	Jan-2007	240 / 243	327 / 342	285 / 285	143 / 145
D	HCW hands	Jan-2007	240 / 246	375 / 375	333 / 333	127 / 127
E	HCW hands	Jan-2007	240 / 243	360 / 360	273 / 321	129 / 129
F	HCW hands	Jan-2007	222 / 243	354 / 354	282 / 336	129 / 129
G	Nursery 2 Bedside table	Jan-2007	240 / 243	294 / 294	273 / 291	127 / 133
H	Nursery 3 Bedside table1	Jan-2007	240 / 243	294 / 294	273 / 294	127 / 133
I	Patient W.C. - shower	Jan-2007	240 / 243	327 / 339	285 / 285	143 / 145
J	Nursery 2 door knob	Jan-2007	237 / 240	309 / 309	261 / 279	145 / 147
K	Patient's W.C.	Jan-2007	237 / 240	399 / 399	285 / 306	129 / 129
L	Nursery 1 Bedside table	Jan-2007	237 / 240	342 / 342	285 / 285	105 / 105
M	Patient individual room's door knob	Apr-2007	240 / 240	342 / 342	285 / 285	103 / 103
O	Patient W.C door knob	Apr-2007	240 / 240	342 / 342	285 / 285	103 / 103
P	Nursery Medical trolley	Apr-2007	240 / 240	342 / 342	285 / 285	103 / 103
Q	Water tap (treatment room)	Apr-2007	222 / 243	354 / 354	282 / 336	129 / 129
R	Nursery medical trolley	Apr-2007	222 / 243	354 / 354	282 / 336	129 / 129
S	HCW hands	Sep-2007	237 / 246	297 / 297	276 / 276	127 / 133
T	Air from individual room no.5	Feb-2008	237 / 243	297 / 297	276 / 294	127 / 133
U	Water tap (inside) treatment room	Feb-2008	243 / 243	309 / 309	264 / 279	145 / 145
V	Shower Patients' WC	Feb-2008	222 / 243	354 / 354	282 / 282	129 / 129
X	Air from nursery 24	Feb-2008	237 / 243	297 / 297	276 / 276	133 / 133

548

549

550

551 **Table 3.** Common multilocus microsatellite genotypes of *Candida parapsilosis* isolates obtained from the environment of a hospital ward and  
 552 from their health care workers.

Isolate Identification	Source	Hospital ward	Date	Multilocus Genotypes			
				CP1	CP4	CP6	B
M	Patient individual room door knob	Haematology	11/04/07	240 / 240	342 / 342	285 / 285	103 / 103
O	Patients W.C. door knob	Haematology	11/04/07	240 / 240	342 / 342	285 / 285	103 / 103
P	Nursery medical trolley	Haematology	11/04/07	240 / 240	342 / 342	285 / 285	103 / 103
F	HCW hands	Haematology	20/01/07	222 / 243	354 / 354	282 / 336	129 / 129
Q	Water tap	Haematology	11/04/07	222 / 243	354 / 354	282 / 336	129 / 129
R	Nursery medical trolley	Haematology	11/04/07	222 / 243	354 / 354	282 / 336	129 / 129
Patient isolate H972697	Bloodculture	Haematology	04/05/07	222 / 243	354 / 354	282 / 336	129 / 129
Patient isolate G730127	Pus	Gastroenterology	05/08/05	222 / 243	354 / 354	282 / 336	129 / 129

553 **Table 4.** Microsatellite multilocus analysis of several sequential isolates obtained from the same patients.

Patient #	Origin	Patient's data	Clinical data	Biological Product	Date	Multilocus Genotypes			
						CP1	CP4	CP6	B
1	Portuguese hospital	Male, 17 years old	Acute lymphoblastic leukemia	Bloodculture	24/11/03	240 / 240	354 / 354	282 / 282	129 / 129
				Bloodculture	30/12/03	240 / 240	354 / 354	282 / 282	129 / 129
				Bloodculture	12/01/04	240 / 240	354 / 354	282 / 282	129 / 129
2	Portuguese hospital	Female 55 years old	Thyroid carcinoma	Bloodculture	04/04/06	222 / 243	354 / 354	279 / 279	129 / 129
				Bloodculture	04/05/07	222 / 243	354 / 354	282 / 336	129 / 129
3	French Hospital	Female 56 years old	Liver carcinoma	Bloodculture	27/06/03	240 / 252	300 / 300	285 / 285	147 / 149
				Catheter	30/06/03	240 / 252	300 / 300	285 / 285	147 / 149
4	French Hospital	Male 46 years old	Peritonitis, laparotomy	Urine	21/03/06	243 / 243	264 / 297	267 / 267	131 / 131
				Bloodculture	23/03/06	243 / 243	264 / 297	267 / 267	131 / 131
5	French Hospital	Male 73 years old	Lung carcinoma	Skin	23/09/05	240 / 252	240 / 252	177 / 195	147 / 149
				Bloodculture	24/09/05	240 / 252	300 / 300	279 / 285	147 / 149
6	French Hospital	Male 75 years old	Aorto-femoral bypass	Abdominal drain effluent	20/12/06	243 / 258	318 / 357	297 / 297	141 / 141
				Tracheal aspirate	27/12/06	243 / 258	318 / 357	297 / 297	141 / 141
				Oropharyngeal swab	09/01/07	243 / 258	318 / 357	297 / 297	141 / 141

555 **Table 5.** Clinical data and multilocus genotype of the *Candida parapsilosis* isolates regarding a possible outbreak in a hospital unit.

556

Patient #	Sex	Age	Clinical data	Hospital unit	Outcome	Biological Product	Collection Date	Genotypes			
								CP1	CP4	CP6	B
1	Female	12	Ewing sarcoma	Pediatry	Died	Urine	29/08/03	222 / 243	354 / 354	282 / 336	127 / 127
2	Male	3	Lymphocytosis	Pediatry	Survived	Bloodculture	08/09/03	222 / 243	354 / 354	282 / 336	127 / 127
3	Male	13	Liver Carcinoma	Pediatry	Survived	Bloodculture	17/09/03	222 / 243	354 / 354	282 / 336	127 / 127

557

558

559

560

561

562

563

564

565

566

567 **Table 6.** Multilocus genotypes most frequently found in the different biological products.

Origin of the isolates	Multilocus genotypes				No. Isolates (%)
	CP1	CP4	CP6	B	
Bloodcultures and catheter tips (n=45)	222 / 243	354 / 354	282 / 336	127 / 127	3 (6.6)
	240 / 252	300 / 300	285 / 285	147 / 149	3 (6.6)
Skin and nails (n=31)	222 / 243	354 / 354	282 / 336	129 / 129	7 (22.5)
Other biological products (n=29)	222 / 243	354 / 354	282 / 282	129 / 129	3 (10.3)
Hospital environment (n=24)	240 / 240	342 / 342	285 / 285	105 / 105	3 (12.5)
	240 / 243	342 / 342	285 / 285	103 / 103	3 (12.5)

568

569

570

571

572

573

574

575

576

577

578

579

580

581

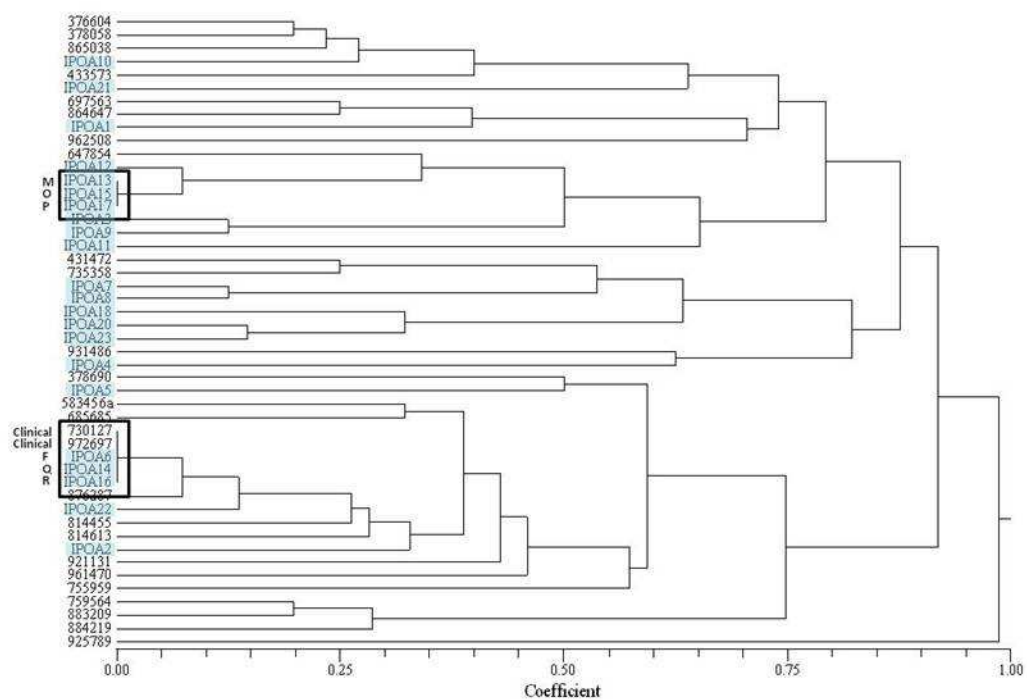
582

583

584

585

586



587

588 **Figure 1.** Dendrogram showing the clustering of clinical and environmental *Candida*  
 589 *parapsilosis* isolates from the same hospital, based on microsatellite multilocus genotyping.  
 590 Genetic distances were calculated by using Populations 1.2.30 software program and  
 591 clustering performed by using UPGMA method ( $r=0.91357$ ). The isolates highlighted are  
 592 environmental strains.