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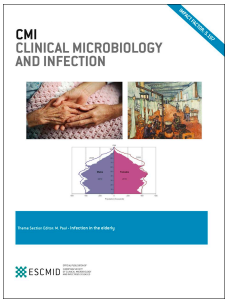

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Analysis of clinical and environmental *Candida parapsilosis* isolates by microsatellite genotyping – a tool for hospital infections surveillance

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

Abstract

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

Keywords:

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

Introduction

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

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

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

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

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

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

Materials and methods

Yeast strains and DNA extraction

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

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

PCR amplification conditions fragment size determination.

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

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

Statistical analysis.

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

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

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

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

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

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

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

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

Global analysis and most common genotypes

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

The most common multilocus genotypes in isolates from bloodcultures are 222/243 300/300 282/336 127/127 and 240/252 300/300 285/285 147/149, shared by 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 multiloccus 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

Discussion

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

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

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

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

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

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

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

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

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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

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

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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

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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

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

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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

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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)

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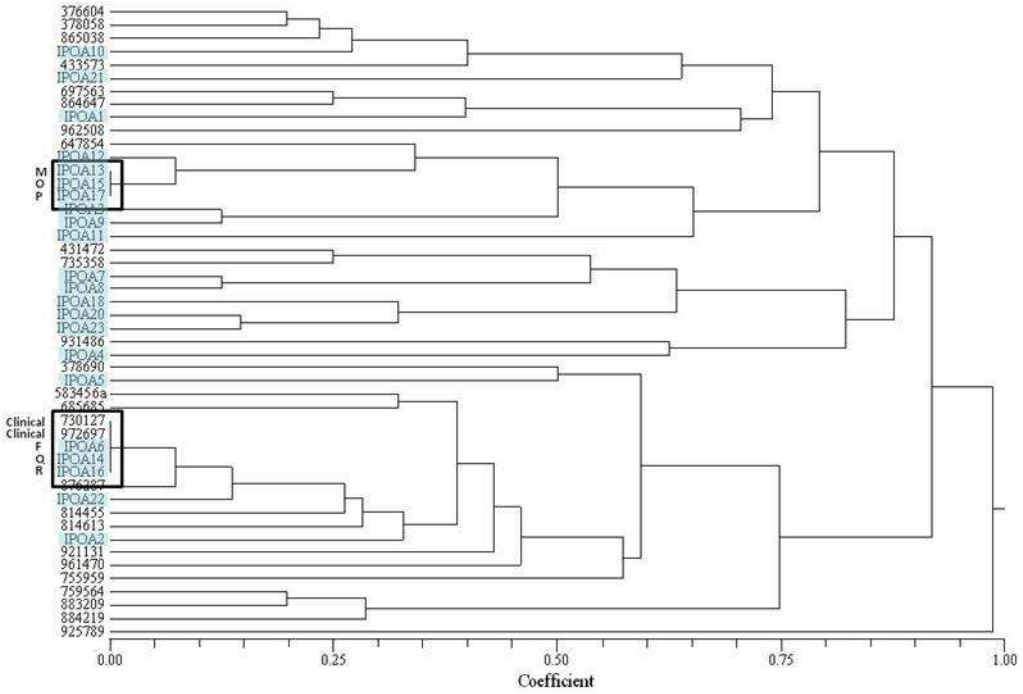

Figure 1. Dendrogram showing the clustering of clinical and environmental *Candida parapsilosis* isolates from the same hospital, based on microsatellite multilocus genotyping. Genetic distances were calculated by using Populations 1.2.30 software program and clustering performed by using UPGMA method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