

Molecular Genotyping of *Candida parapsilosis* Species Complex

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Abstract

Background The *Candida parapsilosis* complex species has emerged as an important cause of human disease. The molecular identification of *C. parapsilosis* isolates at the species level can be helpful for epidemiological studies and then for the establishment of appropriate therapies and prophylactic measures.

Methods The present study was undertaken to analyze 13 short tandem repeat (STR) markers (7 minisatellites and 6 microsatellites) in a global set of 182 *C. parapsilosis* complex isolates from different origins including invasive and superficial clinical sites.

Results Upon the analysis of 182 strains of *C. parapsilosis* complex species, 10–17 haplotypes were detected for each minisatellite marker. The combination of 7 minisatellite markers yielded 121 different genotypes with a 0.995 *D* value. Upon the analysis of 114 isolates (68 from invasive infections and 46 from superficial infections), 21–32 genotypes were detected for each microsatellite marker. The combination of all 13 markers yielded 96 different genotypes among 114 isolates with a high degree of discrimination (0.997 *D* value). The same multilocus genotype was shared by isolates recovered from some patients and from the hand of their correspondent healthcare worker. For another patient, the same multilocus genotype of *C. metapsilosis* was detected in blood and skin confirming that candidemia usually arises as an endogenous infection following prior colonization.

Conclusions These STR markers are a valuable tool for the differentiation of *C. parapsilosis* complex strains, to support epidemiological investigations especially studies of strain relatedness and pathways of transmission.

Keywords *Candida parapsilosis* · *Candida orthopsilosis* · *Candida metapsilosis* · STR · Genetic diversity · Genotyping

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Introduction

Clinically, *Candida parapsilosis* is an important pathogen among the non *C. albicans* emerging species, and its significance and prevalence have dramatically increased over the past two decades [1, 2].

Candida parapsilosis strains, though, are more heterogeneous than other *Candida* species. Tavanti et al. suggested a division of the *C. parapsilosis* group into three species named *C. parapsilosis sensu stricto*, *C. orthopsilosis*, and *C. metapsilosis* [3]. Species of *C. parapsilosis* complex are important agents of nosocomial infections, because they can easily colonize hospital environments, essentially medical devices and hands of health care workers [1, 2]. The rapid identification of the strains involved in the infection can contribute to the development of new strategies not only for treatment but also for the prevention of the infections [4]. Then, molecular identification of *C. parapsilosis* isolates at the species level is very important for optimal therapeutic options and studies of nosocomial cross-transmission [5, 6]. But, the vast majority of clinical isolates belonging to *C. parapsilosis sensu stricto* is characterized by low levels of nucleotide sequence variation, which constitutes a challenge for the development of genotyping techniques [5–7]. Instead, the trend in *C. parapsilosis* genotyping is toward reliance on length variations associated with microsatellites [4, 8, 9]. But, there are several studies from different investigation groups that have already described microsatellite markers for *Candida parapsilosis* genotyping with different degrees of specificity regarding *C. orthopsilosis* and *C. metapsilosis* [7, 8], which emphasize the need of a new set of markers for better genotyping of these two species.

Accordingly, the present study was undertaken to analyze 13 short tandem repeat (STR) markers (7 minisatellites and 6 microsatellites) in a global set of *C. parapsilosis* complex isolates from different origins including invasive and superficial clinical sites. We also aimed to analyze the genetic structure to distinguish epidemiologically related isolates, and to determine possible routes of acquisition of a strain in the hospital environment or identify possible reservoirs.

Materials and Methods

Fungal Strain

A total number of 182 *C. parapsilosis* complex species isolates analyzed in this study were recovered from clinical samples received by Department of Parasitology—Mycology-University Hospital—Sfax, Tunisia, during a 14-year period (from January 2002 to January 2016). They were 172 strains of *C. parapsilosis sensu stricto*, 6 strains of *C. metapsilosis* and 4 strains of *C. orthopsilosis*. The isolates of *C. parapsilosis sensu stricto* were obtained from various clinical specimens including blood (36.05%), urine (16.86%), auricular samples (22.68%), respiratory tract (2.33%), catheters (5.23%), and other sites such as skin (4.07%), nails (1.16%), oral cavity (1.74%), vagina (0.58%), peritoneal fluid (0.58%), and hand carriage (8.72%). *C. metapsilosis* was isolated from blood, urine, skin, and auricular samples. *C. orthopsilosis* were isolated from blood, urine, auricular sample, and hand carriage. Isolates were identified to the species level by standard methods [morphological characteristics and biochemical tests using ID 32C (BioMerieux, France)]. Molecular identification of the *C. parapsilosis* species complex was performed according to Tavanti et al. [3], and supplemented, as needed by internal transcribed spacer 1 (ITS1), 5.8S, and ITS2 region rRNA sequence analysis [10]. Eleven of these isolates were as reference strains, identified by ITS sequence analysis of the ribosomal DNA, and deposited in GenBank: *C. parapsilosis sensu stricto* (KT948326), *C. metapsilosis* (KU665248, KU665249, KU665250, KU665251, KU665252, and KX421284), and *C. orthopsilosis* (KU665253, KU665254, KU665255, and KU665256).

Four reference strains were included: *C. parapsilosis* (ATCC 22019), *C. orthopsilosis* (1219482, 1343124), and *C. metapsilosis* (1240011).

One *Candida albicans* (ATCC 3153), one *Candida glabrata* (CBS 138), one *Candida tropicalis* (ATCC 66029), and two *Aspergillus flavus* (CBS 12685.7 and JX 852615) strains were used in the specificity tests.

STR Design

To collect the published sequences of *C. parapsilosis* genome, the following websites were consulted: NCBI (<http://ncbi.nlm.nih.gov>), DDBJ (www.ddbj.nig.ac)

and EMBL (<http://www.embl.fr/>) databases. The Tandem Repeats Finder software (<http://tandem.bu.edu/trf/trf.html>) was used to identify the short tandem repeats in *C. parapsilosis* genome. Selection criteria for the STR markers were: to have perfect repeat sequences with 100% identity between repeat units and to be highly repetitive sequences (> 20 repeats and ≥ 2 repeats if consensus size is more than 50 bp). Six microsatellites and seven minisatellites loci were then selected. The Primer (version 3) software (<http://frodo.wi.mit.edu>) was used to design primers. BLASTn search (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was consulted to verify the specificity of primers to *C. parapsilosis*.

DNA Extraction

DNA was extracted using a QIAamp DNA Mini Kit (QIAGEN), as indicated by the manufacturer's instructions.

PCR Amplification of STR Markers

Amplification reactions were performed in final volumes of 25 μ l containing 1 ng of genomic DNA, 5 μ l of 5X reaction buffer (pH 8.5), 3 mM MgCl₂, 0.2 mM (each) dATP, dCTP, dGTP, and dTTP (Promega), 0.5 μ M of each primer, and 2.5 U of GOTaq[®] DNA polymerase (Promega). PCR amplification was performed in a thermocycler (Bio-Rad). It consisted of initial denaturation for 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 56 °C, and 1 min at 72 °C, and a final extension for 10 min at 72 °C. Different markers were amplified using different fluorescent labels (FAM, HEX, TET). One microliter of the tenfold diluted amplification products (with formamide) was added to 15 μ l of formamide and 0.5 μ l of internal size marker LIZ 500 (Applied Biosystems Inc.). The PCR products were then denatured and injected with polymer POP-7 for run onto an ABI 310 genetic analyzer (Applied Biosystems Inc.).

For minisatellite analysis, the amplification products were then migrated on 3% agarose gel. The capture and analysis of the gels were performed by the Quantity One software package (Bio-Rad) coupled to the imaging system Gel Doc XR⁺™.

Data Analysis

Different DNA preparations of the same isolate with repeated analyses of the same DNA preparation were tested to evaluate the repeatability of the STR markers. The GENEPOP software version 4.2 was used to calculate genotype frequencies for each marker [11]. For each marker and for each possible marker combination, the Simpson index of diversity (*D*) [12] was calculated. A combination is considered as parsimonious if *D* value ≥ 0.95 . The NTSYS-PC numerical taxonomy and multivariate analysis system (version 2.1; Exeter Software, Setauket, NY) was used to calculate the degree of similarity by applying the Dice coefficient test. The genetic distance between strains of *C. parapsilosis* complex species was determined by the dice index. An UPGMA dendrogram was generated. The ARLEQUIN software package calculated the fixation index (*FST*) on all loci [13]. The minimum spanning tree (MStree) method as implemented by Network V4.6 estimated the phylogenetic relationships [14].

Results

A total of 26041 STR were found among 13.0501 Mb of *C. parapsilosis* genomic sequences available in online databases (February 2015); 0.68.6% consisted of repeat units greater than 7 nucleotides (minisatellites) and 31.4% contained repeat units ranging from 1 to 6 nucleotides in length. For the microsatellites, they were: mononucleotide (12.2%), dinucleotide (14.4%), trinucleotide (37%), tetranucleotide (4.9%), pentanucleotide (4.6%), and hexanucleotide (26.9%). For the minisatellites, they ranged from 7 to 50 bp (94.1%), 51 to 100 bp (4%), 101 to 150 bp (1.2%), and ≥ 151 bp (0.7%). Thirteen markers were selected for further assays since they matched the selection criteria (Table 1): four dinucleotides, two trinucleotides, and seven minisatellites (with the following repeat unit sizes: 88, 12, 24, 81, 82, 134, and 138 bp).

Specificity of STR Markers

The amplification of the 13 STR markers, using the genomic DNA of 3 *Candida* species [1 *C. albicans* (ATCC 3153), 1 *C. glabrata* (CBS 138), 1 *C. tropicalis* (ATCC 66029)] and 2 *Aspergillus flavus*

Table 1 Features of the 13 polymorphic STR sequences of *Candida parapsilosis* complex species upon analysis of 182 isolates

Marker names	Primer sequences (5'–3')	Repeat units
STR-AC	FAM-TCGCCTTTAGTTGCATTGTGT AAATAAGCACGGGTGTTGGT	AC
STR-ACA	HEX-GGAGCAGCTACCACTACCAA GACTCTCCTGCTTTGTTGCC	ACA
STR-GA	TET-TGCTACTGACTTGACCGCTA GCCCAATCGCACTTTGAAT	GA
STR-TC	FAM-GCATTGCATCCCATCGTGTA AGGAGTTTGGGCAATGGTA	TC
STR-TG	HEX-GTTGCTTCAAGGATCACGGT AAATCCGCATGTGAAGGTCA	TG
STR-TTG	TET-TGCTATGAGCTGTGAGTA AGTTCGGTCACGTCAAT	TTG
STR88	GCAATCTTGTGTGGGAAT CTACTCTTGACGAAAACGAG	TACTAACAACATGTACAGTCACGTGGTT TTGTAAGGTTATAGACAGCTAG GCTTATGATGATTACTCGCCCCCTCTAAACAAAGTGCT
STR12	CAATTGAGCCCACCATGTGC TGATTCCAGTTTGTGCGCAA	AAATGTCTGTCA
STR24	AGAATGGCTGACGAGGAAGA GGGCTTGAAGAATCTTTTGACG	GCTGAAGAAGAGGCTAAGAGAAAA
STR81	GGACTTGGTAGGACTCAGGG TATACCTCAACGCCGTGAGA	TTGGGGGCCAAACGAATCACAAATGG CGTGTCTAAGAATGACG ACGAAAACCAAAGTTCGAATCCAAATCGGTTGGATGCT
STR82	ATGCGTGCCACATTTGTGTTG TCAAACCTACAAGTGGACGGGA	CGCAAAAAATGCCCGTAAACAAAC AAGTTTGCAGCAGAATA AAATGGAGGGCCAATTTCTGATACTT TTCGTTATCAAAC
STR134	TGCATCACTCTCGGAAAGGT TGTGCCAGTTTATAGACACCA	TACACATTGTAAGTGCCTAAGCTGAT GTATCTCTGTTATACGTCAC TCTGCCACTAATGTAGTACCGTAGCCATTGATCCT TGCTGTTACGAAATCTATCCTCTTCATTC ATTTTCATGATACTCCAGCTTGA
STR138	CTGTCAATCAGGACATTGC AGCCATGTGGTGGTAATC	ACCACAACCTGTCACTGACATGACAACAAG TACCCACACCACTGTGG TTACCACTACTTGGCTCGTGACCAACTCGGAT GGGTCTGTTGAAACTGAAACAGGTATTGCAACTCCATC GGACACCAACTCGTACGAATCT

(CBS 12685.7 and JX 852615), revealed that the 13 new loci were specific for *C. parapsilosis* complex (no PCR product was generated).

All microsatellite–minisatellite markers reacted with DNA from *C. orthopsilosis* and *C. metapsilosis* isolates.

Genetic Diversity

Upon the analysis of 182 strains of *Candida parapsilosis* complex species isolated from 2002 to 2016,

5–8 distinct alleles and 10–17 genotypes were detected for each minisatellite marker (Table 2). The highest discriminatory power for a single minisatellite locus was obtained with the STR12 marker, which had 7 distinct alleles, 17 genotypes, and a *D* value of 0.858 (Table 2). The combination of 7 minisatellites markers yielded 121 different genotypes with a 0.995 *D* value (Table 3).

Upon the analysis of 114 isolates (68 from invasive infections and 46 from superficial infections were part of the 182 strains), 8–17 distinct alleles and 21–32

Table 2 Characteristics of the STR loci

Marker names	No. of alleles	Size range (bp)	No. of genotypes/strains	Genotype frequency	<i>D</i>	Heterozygosity	
						Observed	Expected
STR-AC	12	217–263	30/114	0.009–0.088	0.960	0.765	0.873
STR-ACA	8	240–264	19/114	0.009–0.202	0.881	0.296	0.815
STR-GA	17	160–226	21/114	0.009–0.500	0.730	0.304	0.622
STR-TC	12	211–249	18/114	0.009–0.421	0.792	0.696	0.736
STR-TG	17	208–254	32/114	0.009–0.132	0.956	0.426	0.910
STR-TTG	11	206–241	21/114	0.009–0.219	0.874	0.678	0.740
STR88	7	200–600	10/182	0.005–0.286	0.788	0.472	0.618
STR12	7	200–500	17/182	0.011–0.269	0.858	0.363	0.738
STR24	8	200–550	17/182	0.005–0.467	0.757	0.841	0.686
STR81	5	200–400	7/182	0.044–0.407	0.772	0.423	0.629
STR82	7	200–500	10/182	0.011–0.522	0.688	0.302	0.575
STR134	5	200–600	11/182	0.005–0.313	0.782	0.374	0.680
STR138	6	200–700	12/182	0.005–0.401	0.785	0.753	0.743

Table 3 Discriminatory indices (*D*) of different STR marker combinations

Locus combination	No. profiles/isolates	<i>D</i>
STR-AC + STR-TG	78/114	0.992
STR-AC + STR-TG + STR-ACA	85/114	0.994
STR-AC + STR-TG + STR-TTG	85/114	0.994
STR-AC + STR-TG + STR-ACA + STR-TTG	86/114	0.995
STR-AC + STR-TG + STR-ACA + STR-TTG + STR-TC	86/114	0.995
Six microsatellites	87/114	0.995
STR12 + STR138	54/182	0.959
STR12 + STR88	52/182	0.967
STR12 + STR134	45/182	0.949
STR12 + STR138 + STR88	84/182	0.985
STR12 + STR134 + STR88	78/182	0.982
STR12 + STR138 + STR134	71/182	0.972
STR12 + STR138 + STR134 + STR88	98/182	0.989
STR12 + STR138 + STR134 + STR88 + STR81	108/182	0.992
STR12 + STR138 + STR134 + STR88 + STR81 + STR24	117/182	0.994
Seven minisatellites	121/182	0.995
STR-AC + STR-TG + STR-ACA + STR12 + STR138 + STR88	96/114	0.997
Six microsatellites + seven minisatellites	96/114	0.997

genotypes were detected for each microsatellite marker (Table 2). The highest discriminatory power for a single microsatellite locus was obtained with the STR-AC marker, which had 12 distinct alleles, 30 genotypes, and a *D* value of 0.960 (Table 2). The combination of 6 microsatellites markers yielded 87 different genotypes with a 0.995 *D* value (Table 3).

The combination of all 13 markers yielded 96 different genotypes among 114 isolates with a 0.997 *D* value. A 6-marker combination (STR-AC, STR-TG, STR-ACA, STR12, STR138, and STR88) yielded also 96 different genotypes with a 0.997 *D* value (Table 3). This six-marker combination was selected as the most parsimonious panel achieving *D* > 0.95.

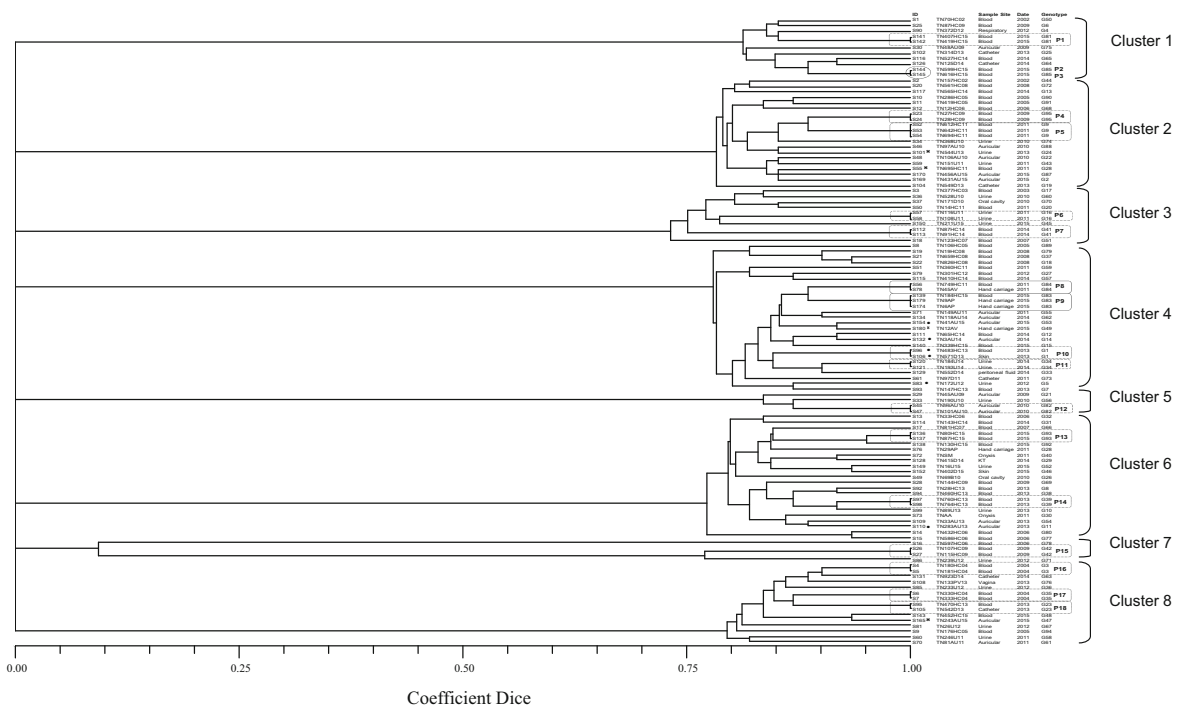


Fig. 1 UPGMA dendrogram based on the Dice similarity coefficient upon analysis of 13 new STR markers in 114 *Candida parapsilosis* species complex isolates. The isolates are identified by the culture collection number. Six strains of *C. metapsilosis* (dots) and four strains of *C. orthopsilosis* (times) were included. The bordered strains represent the cases of

multiple strains for 11 patients with invasive infections (dotted rectangle P1, P4, P5, P7, P10, P13, P14, P15, P16, P17, and P18) and 3 patients with superficial infections (dashed rectangle P6, P11, and P12). For two patients (solid rectangle P8 and P9), they were strains isolated from blood culture and from hand carriage of hers health care workers

The observed percent of heterozygosity was similar to estimated percent for four STR (STR-AC, STR-TC, STR-TTG, and STR138), superior for one STR (STR24) and inferior for all the others STR (Table 2).

Cluster Analysis

The dendrogram based on the Dice similarity coefficient upon analysis of 13 STR markers in 114 *Candida parapsilosis* species complex isolates revealed 8 clusters (Fig. 1). The majority of the strains belonging to cluster 1 (11 strains) were isolated from blood or catheter. Each of clusters 2 (20 strains) and 4 (27 strains) was subdivided in 2 subclusters: 1 for isolates from blood and the other for isolates from superficial sites. The cluster 3 (10 strains), 6 (23 strains), 7 (4 strains), and 8 (15 strains) contained strains from different clinical sites. Cluster 5 (four strains) contained strains isolated only from urine and auricular samples.

For *C. orthopsilosis* and *C. metapsilosis*, they were not grouped in homogeneous groups or independent clusters. But, two strains of *C. orthopsilosis* belonged to cluster 2 (subcluster of superficial sites) and five strains of *C. metapsilosis* and one *C. orthopsilosis* belonged to cluster 4 (subcluster of superficial sites). The genetic diversity per locus (F_{ST}) values were calculated for the three species of *C. parapsilosis* complex and were noted to range between 0.039 and 0.041, indicating a moderate differentiation between the three species (Table 4).

Similarity Dice Coefficient

Pairwise Dice coefficient of similarity values between strains ranged from 0.09 to 1 (Fig. 1). The dice value was 1 for cases with multiple strains.

They were eleven patients with invasive infections (P1, P4, P5, P7, P10, P13, P14, P15, P16, P17, and P18). For patient, 18 identical multilocus genotypes

Table 4 Pairwise F_{st} values for the three species of *Candida parapsilosis* complex

	<i>C. parapsilosis</i>	<i>C. orthopsilosis</i>	<i>C. metapsilosis</i>
<i>C. parapsilosis</i>	–		
<i>C. orthopsilosis</i>	0.039 $p = 0.11712^*$	–	
<i>C. metapsilosis</i>	0.041 $p = 0.09009^*$	0.039 $p = 0.27027^*$	–

* p values calculated from 10,000 permutations

for both strains isolated from blood culture and catheter were obtained. For patient 10, the same genotype of *C. metapsilosis* was detected in blood and skin. It was noted also that patient 2 and patient 3 (hospitalized in two different departments) presented bloodstream infection with strains which had identical genotypes.

The same multilocus genotypes were obtained for each of the three patients (P6, P11, and P12) with multiple strains in superficial infections (urinary infection and otomycosis).

For each patient P8 and P9, the strains isolated from blood culture and from hand carriage of health care workers displayed the same multilocus genotypes. The strains causing these two infections were closely related in cluster analysis (Fig. 1).

Distance Interpopulation

A minimum spanning tree (MSTree) was generated from the analysis of 182 *Candida parapsilosis* species complex isolates (74 strains from invasive infections and 108 from superficial infections) using Network V4.6 (Fig. 2). The findings revealed that cluster A (131 strains) was dominated by strains from the of the invasive infection population; cluster B (51 strains) was dominated by strains from the superficial infection population.

A factorial analysis correspondence graph was generated to illustrate if there is population structure associated with the period of isolation (Fig. 3). In this two-dimensional graphical representation of genetic similarity between individuals, the information for all loci is condensed into a smaller dimension (two factors, axis I and axis II) with a minimum loss of information. A graphical proximity between two individuals indicates a genetic similarity. This graph illustrated that the majority of isolates were grouped with the exception of some strains isolated in 2011 and 2015. The most distant strains were isolated in 2013

and 2016. In fact, the pairwise F_{st} values of 2016 compared to others years were the most important and ranged from 0.191 (with 2011) to 0.644 (with 2008).

Discussion

Opportunistic infections are an increasingly common problem in hospitals worldwide. The *Candida parapsilosis* complex species has emerged as an important cause of human disease with the ability to infect different body sites.

For a better understanding of the epidemiology of this complex, it is essential to discriminate exactly *C. parapsilosis* strains, and to identify rapidly the strains involved in the infections [2, 15]. So, a variety of strain typing methods have been used including electrophoretic karyotype analysis [16], DNA fingerprinting with probe Cp3-13 [17], RAPD analysis [18], RFLP analysis [19], multilocus sequence typing (MLST) [3], and pyrosequencing [20].

But, given the clonal nature of *C. parapsilosis*, most of the genotyping methods became not discriminant enough to distinguish closely related isolates and to establish routes of transmission [21, 22]. However, microsatellite analysis may be more able than others genotyping methods to distinguish between strains with low degrees of sequence variation, because microsatellite loci behave as codominant markers and evolve rapidly in a genome [8].

In fact, the STR markers proposed in the present study yielded promising results in terms of identifying intraspecies polymorphisms in *C. parapsilosis* complex strains. In a multilocus analysis, 96 genotypes were found among 114 isolates, resulting in a D of 0.997, similar than that published by Reiss et al. [7]. In 2006, Lasker et al. described a set of seven dinucleotide microsatellite markers, able to discriminate *C. parapsilosis* sensu stricto strains, with a discriminatory power of 0.97 [8]. In 2010, Sabino et al. reported a

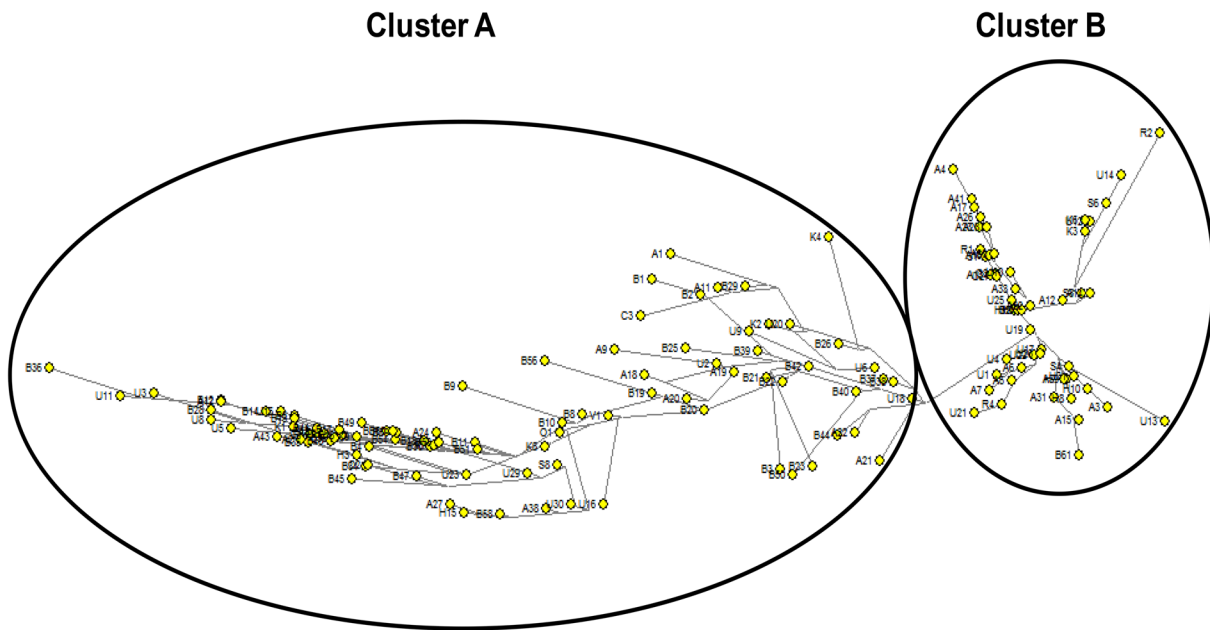
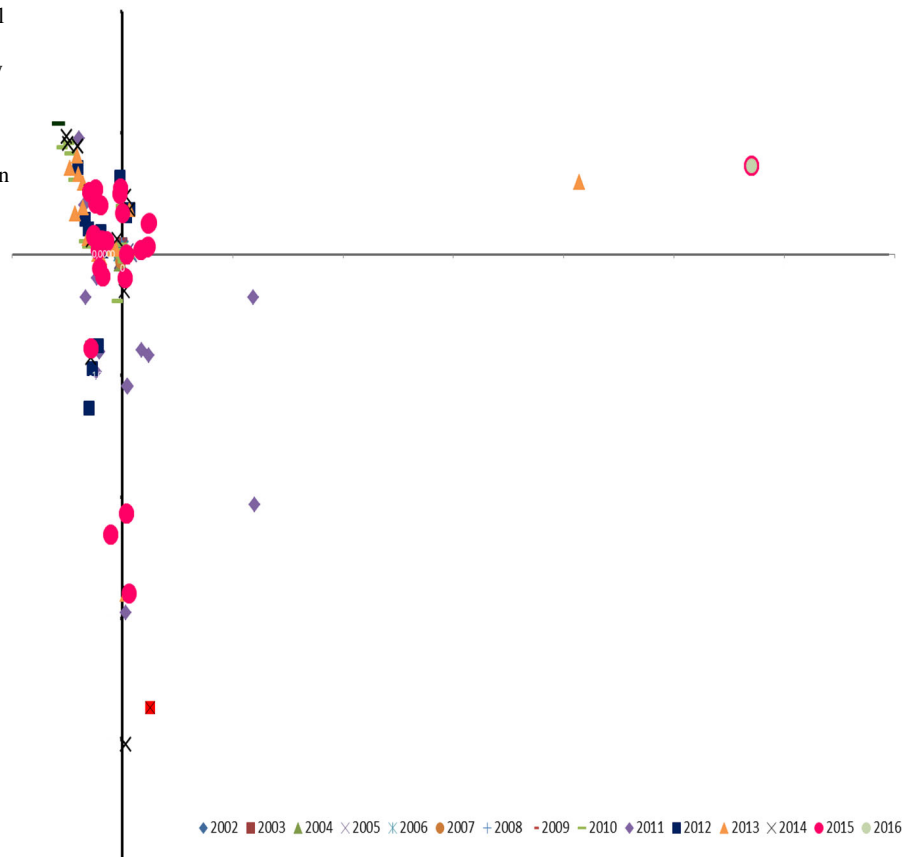


Fig. 2 Minimum spanning tree (MSTree) generated from the analysis of 182 *Candida parapsilosis* species complex isolates (74 strains from invasive infections and 108 from superficial infections) using Network V4.6

Fig. 3 Two-dimensional factorial analysis correspondence graph representing the genetic similarity between *Candida parapsilosis* species complex strains isolated during the period from 2002 to 2016. Graphical proximity between two isolates indicates genetic similarity



high degree of polymorphisms by microsatellite analysis in *C. parapsilosis*, with 192 different genotypes found among 233 isolates, based on 4 hyper variable loci (D of 0.99) [4]. In the present work, the combination of seven minisatellite markers yielded a 0.995 D value. This is very interesting because these markers showed a high discriminatory power using gel electrophoresis, which is less expensive and easy to implement in laboratories with basic molecular biology equipment.

The 13 STR markers presented in this work showed high levels of discrimination and specificity for the inter-strain differentiation of *C. parapsilosis* complex species. Although the strains studied are from the same geographical origin, a genetic heterogeneity was detected among the *C. parapsilosis* complex strains. In fact, microsatellite typing analysis was a very powerful technique to clarify epidemiologic associations, to detect micro-evolutionary variations, and to confirm nosocomial infection caused by *C. parapsilosis* [9, 15, 21].

Our study is consistent with other previous reports implicating the hands of healthcare workers as the source of fungemia [15, 22]. In fact, the same multilocus genotype was shared by isolates recovered from patients and from the hand of their correspondent healthcare workers, suggesting that these could be possible routes of transmission and that infections due to *C. parapsilosis* may be mainly related with exogenous horizontal transmission to the patient.

This study confirms that candidemia in intensive care unit patients is caused by strain colonizing hands of healthcare workers. So, it is necessary to implement surveillance program for better dissemination of information to health care workers which must be careful to the correct use of gloves and hand washing [23, 24].

For the same patient, the same multilocus genotype of *C. metapsilosis* was detected in blood and skin. This observation is in accordance with what is generally agreed stating that candidemia usually arises as an endogenous infection, following prior colonization of the gastrointestinal tract, skin, or vagina [22].

This property of *C. parapsilosis* complex species as a skin colonizer facilitates transmission from an exogenous source, the hands of healthcare workers, to patient during installation and maintenance of intravascular catheters [2, 7]. In fact, identical multilocus genotypes for both strains isolated from blood

culture and catheter were obtained for another of our patients. Then, appropriate control measures must be implemented against both exogenous and endogenous infection reservoirs for prevention strategies aimed at *C. parapsilosis* [6].

In our work, all the 13 STR amplified DNA from *C. orthopsilosis* and *C. metapsilosis*. In a previous study realized by Lasker et al., DNA obtained from single representatives of these two species was not amplified by all seven primer pairs [8]. Reiss et al. tested also cross-hybridization of five STR markers with the species *C. metapsilosis* and *C. orthopsilosis*. Some markers were completely unreactive for some isolates [7].

In cluster analysis, we noted that *C. orthopsilosis* and *C. metapsilosis* were not grouped in homogeneous groups or independent clusters. This can be explained by the limited number of tested isolates which hinders a statistical analysis to prove/disprove the existence of cluster structure within these species. Moreover, the use of microsatellite markers for strain identification among *C. parapsilosis* complex species may be limited by the high degree of polymorphism observed with this molecular method which could be correlated with a high mutation rate [4]. Furthermore, it may be due to the homoplasmy which is explained by constraints on the microsatellite size distribution leading to alleles with an identical size, but not necessarily with a common ancestor [8].

Distance interpopulation study revealed that cluster A was dominated by strains from that of the invasive infection population and cluster B was dominated by strains from the superficial infection population. So, it seems that not all *C. parapsilosis* isolates are equally as likely to cause human infections and some of them appear to be clinically most relevant and cause invasive infections significantly more often than others [25].

A factorial analysis correspondence graph illustrated that the majority of isolates were grouped with the exception of some strains isolated in 2011 and 2015. So it is clear that “some genotypes may have evolved over time by spontaneous changes”, and clonal complexes of closely related genotypes were than generated [6, 7, 24]. It was also suggested that strains have insufficient time to diversify completely, but show adaptation to the environment [26].

In our study, we found that the most distant strains were isolated in 2013 and 2016. A micro-evolutionary process might be involved between these strains,

differentiating them from a common ancestry [24, 26]. But, microsatellites loci are inherently unstable, so they would not be useful for genetic relatedness studies on larger time scale or on population structure [4, 24].

Conclusion

STR analysis presents a high discriminatory power and may be very useful for studies of kinetics of the colonization-to-infection process and for epidemiological studies of *C. parapsilosis* complex species. Then, we emphasize the importance of appropriate control measures for prevention of these infections. The identification of genotypes and population structure of *C. parapsilosis* complex species could be also a significant marker for further investigations of virulence factors and drug resistance.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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