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Isolation and Drug Susceptibility of *Candida parapsilosis* Sensu Lato and other Species of *C. parapsilosis* Complex from Patients with Blood Stream Infections and Proposal of a Novel LAMP Identification Method for the Species

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Abstract *Candida parapsilosis* complex (CPC) is the third *Candida* species isolated in blood cultures of patients from our Hospital, following *C. albicans* and *C. tropicalis*. From 2006 to 2010, the median annual distribution of CPC was 8 cases/year. Records of 36 patients were reviewed. CPC were 31 (86.1 %) *C. parapsilosis*; 4 (11.1 %) *C. orthopsilosis*; and 1 (2.8 %) *C. metapsilosis*. Clinical characteristics were central venous catheter, 34 (94.4 %); parental nutrition, 25 (70 %); surgery, 27 (57.9 %); prior bacteremia, 20 (51.3 %); malignancy, 18 (50 %). General mortality was 47.2 %. Death was higher in immunosuppressed patients (17 vs. 11; $p = 0.003$). Three out four (75 %) patients with *C. orthopsilosis* and 14 out 31 (45.2 %) with *C. parapsilosis* died ($p = 0.558$). Thirty-nine individual isolates were tested for susceptibility to seven antifungal drugs, with MICs values showing susceptibility to all of them. Two isolates, one *C. orthopsilosis* and one *C. parapsilosis*, had fluconazole MIC = 4 μg/mL. Differentiation among CPC has implication in caring for patients with invasive candidiasis since there are differences in virulence, pathogenicity and drug susceptibility. A method targeting the topoisomerase II gene based on loop-mediated isothermal amplification (LAMP) was developed. LAMP emerges as a promising tool for the identification of fungal species due to the high sensitivity and specificity. LAMP can be performed at the point-of-care, being no necessary the use of expensive equipment. In our study, the method was successful comparing to the DNA sequencing and proved to be a reliable and fast assay to distinguish the three species of CPC.

Keywords *Candida parapsilosis* · Candidemia · Mycoses · Antifungal susceptibility · Molecular diagnostic techniques · LAMP

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Introduction

Invasive candidiasis is the most frequent, as well as life-threatening fungal infection in severely ill

patients, either in medical, surgical or burn intensive care units (ICU) [1]. The prompt introduction of specific and highly effective antifungal therapy is of a major importance. However, significant epidemiological changes were witnessed during the past decades, either regarding the increasing prevalence of non-*albicans Candida* species, as well as the emergence of resistance to fluconazole [2, 3].

Despite the increase in the incidence of *C. glabrata* reported recently [2, 3], *C. parapsilosis* remains as the most frequent non-*albicans Candida* species in South American and Brazilian hospitals [3–6].

Several publications reported *C. parapsilosis* as a frequent causative agent of blood stream infection in the last decade [7–10] and as a major pathogen causing candidemia in neonates [11, 12], children [13] and onco-hematologic patients [14]. The ability of *C. parapsilosis* to adhere onto vascular catheters, prosthetics devices and the hands of health care workers [15, 16] represents a potential risk of the development of candidemia and a challenge for their control.

Since 2005, *C. parapsilosis* sensu lato has been recognized as a complex of three closely related species: *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis* [17]. The three species of the *Candida parapsilosis* complex (CPC) are closely related even in their biochemical characteristics, which makes extremely difficult, if not impossible, differentiate the CPC members by phenotypic methods. Nevertheless, differentiation among CPC members has a potential implication in caring for patients with invasive candidiasis, since *C. parapsilosis* sensu stricto appears to be more resistant than *C. orthopsilosis* and *C. metapsilosis* for a variety of antifungal agents [7]. Based on in vitro experiments, there are also evidences that the three components of CPC have different pathogenicity, being *C. parapsilosis* sensu stricto more pathogenic than *C. orthopsilosis* which is, in turn, more pathogenic than *C. metapsilosis* [18]. Therefore, in order to determine and identify each species correctly, sequencing of ITS regions or other molecular techniques has been necessary. Molecular techniques have been applied for the identification of fungi, including DNA microarray [19, 20], real-time polymerase chain reaction (real-time PCR) [21] and DNA sequencing [22]. DNA microarray has been demonstrated as highly specific tool to simultaneously detect multiple pathogens on a 4-h runtime assay, which can be performed at the point-of care [19, 23]. Real-time PCR can quantify the DNA amount, showing a higher level of accuracy when compared to the standard PCR, though requires expensive equipment and skilled personnel to be performed [21, 24, 25]. Despite the high specificity and discrimination power that made DNA sequencing be chosen as the gold standard for fungi identification, this technique is a 12-h time-consuming assay, it is not widely available at the point-of-care and also requires skilled personnel and expensive supplies to be performed [22]. Since such works are tedious and time-consuming, more simple and rapid procedures are desired [26]. Recently, a DNA amplification technique, loop-mediated isothermal amplification (LAMP), was developed and has been applied to the detection of a variety of microorganisms. LAMP method consist of a PCR that uses a set of six primers, being two outer-inserted (forward and backward) in the 3′ position (F3; B3), two inner-inserted (forward and backward) in the ITS region (FIP; BIP), and two loop-inserted (loop-F; loop-B). Amplification starts with the pairing of the six primers with their counterpart, which allows a more specific identification of pathogen when comparing with conventional real-time PCR. Although LAMP can also quantify DNA amount in real time, the amplification can be visually checked by turbidity, making not necessary a dye labeling [27]. The simplicity and rapidness of LAMP have been confirmed [28–30]. In this paper, the usefulness of newly developed LAMP identification primers for the CPC was reported, and the performance of this method was compared with others [26, 31]. We also describe the clinical and epidemiological features of blood stream infections caused by CPC and the antifungal susceptibility testing.

Materials and Methods

This retrospective study was performed comprising 39 strains of CPC isolated from blood cultures of individual patients hospitalized in a Brazilian tertiary care university hospital. From January 2006 to December 2010, the epidemiological and clinical data from charts of 36 adult patients were reviewed. Blood cultures and yeast identification were performed by automated microbiological system BacT/ALERT 3D FA and PF bottles and Vitek 2YST card (bioMérieux Inc. Durham, NC, USA). The isolates were kept in sterile distilled water and, for the purpose of this study, the fungal isolates were cultured in CHROMagar

(CHROMagar, Paris, France) and Sabouraud dextrose agar (SDA, Difco, Detroit, MI, USA) plate and kept on potato dextrose agar (PDA, Difco) slants before use.

Drug Susceptibility Tests

Drug susceptibility tests of 39 strains of CPC were performed by broth microdilution method according to Clinical Laboratory Standards Institute (CLSI) document M27-A2 [32] for flucytosine, itraconazole, fluconazole, voriconazole, micafungin and miconazole. Minimum inhibitory concentration (MIC) for amphotericin B was determined by E test [33]. *C. parapsilosis* ATCC 22019 was used as a reference strain. MICs at which 50 % (MIC_{50}) and 90 % (MIC_{90}) of strains were inhibited were reported. Interpretative criteria for fluconazole MICs were: ≤8 μg/mL: susceptible; 16–32 μg/mL: susceptible dose dependent and ≥64 μg/mL: resistant. MICs for micafungin were considered susceptible if ≤2 μg/mL and; however, an interpretative criterion for amphotericin B has not yet been established by CLSI, isolates with amphotericin B MIC ≤1 μg/mL were considered susceptible [34]. CLSI endpoints interpretation was compared to the recently suggested clinical endpoints for antifungal agents [35–38].

DNA Sequencing

DNA sequencing PCR were performed with universal fungus-specific primer pair ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) (Sigma-Aldrich, Saint Louis, MO, USA) [39, 40]. The PCR products were purified with ExoSAP-IT (Affymetrix |USB, Cleveland, OH, USA) and then sequenced using Big Dye® terminator reagent kit (Applied Biosystem, Foster City, CA, USA) according to the manufacturer's instructions in an ABI PRISM® 3100 Genetic analyzer (Applied Biosystems, Foster City, CA, USA). The sequence data were assembled by ATSQ software version 6.0.1 (Genetix Corporation, Tokyo, Japan) and then submitted to GenBank.

Molecular Identification by LAMP Method

For the molecular identification by LAMP method, following cultures were used as reference strains: *Candida albicans* ATCC 90028 (IFM 40213), *Candida dubliniensis* CBS 7987^T (IFM 48313), *Candida glabrata* ATCC 2001^T (IFM 46843), *Candida guilliermondii* ATCC 6260^T (IFM 46823), *Candida kefyr* ATCC 4135^T (IFM 5773), *Candida krusei* ATCC 62587^T (IFM 46834), *Candida parapsilosis* ATCC22019^T (IFM 46829) and *Candida tropicalis* ATCC 750^T (IFM 5777). All fungal strains were grown on SDA slant at 27 or 37 °C for 4–7 days. Rapid preparation of DNA from fungi was performed by a modification of our previous report [41]. Small samples of fungal cells on SDA plate were suspended in a saline, and DNA was extracted with a DNA extraction kit (Dr. GenTLE, Takara Bio Inc., Shiga, Japan). Sets of LAMP primers were developed specifically for differentiating CPC, using sequence information of the topoisomerase II gene; known sequences of targeted genes were searched in the GenBank (http://www.ncbi.nlm.nih.gov/genbank/), Centraalbureau voor Schimmelcultures Fungal Biodiversity Centre (CBS; http://www.cbs.knaw.nl), and DNA Data Bank of Japan (DDBJ; http://www.ddbj.nig.ac.jp) databases. Then, six to ten sequences, each one having more than 600 base pairs (bp) and less than 800 bp, were aligned using the software Clustal-W (http://www.clustal.org). The final alignment sequence was used to design the specific primers for LAMP (PrimerExplorer V4, Eiken Chemical Co., Japan, http://primerexplorer.jp/e/). Primers were manufactured by Sigma-Aldrich Japan (http://www.sigmaaldrich.com/japan.html/). The LAMP reaction was performed with a Loopamp DNA amplification kit (Eiken Chemical Co., Tokyo, Japan) in reaction mixtures composed of 40 pmol each of primers FIP and BIP, 5 pmol each of primers F3 and B3, 20 pmol each primers LF and LB, 12.5 μL of 2× reaction mixture, 1 μL of *Bst* DNA polymerase, 2 μL DNA samples and distilled water up to a final volume of 25 μl. The mixtures were incubated at 63 °C for 60 min and then heated at 80 °C for 2 min to terminate the reaction. The turbidity was measured every 6 s using a real-time turbidity meter (LoopAmp EXIA; Eiken Chemical Co., Japan). Start of amplification of LAMP products at around 30 min suggested the positive reaction due to the presence of corresponding fungal DNA by specific LAMP primer sets [28, 41].

Results

During the study period, 313 candidemia episodes were identified, and the CPC was the third *Candida*

Table 1 Characteristics of 36 patients with candidemia due to *Candida parapsilosis* complex according to the outcome

Characteristics	Survived ($n = 19$)	Died ($n = 17$)	p value
Gender (female)	9	7	0.70
Mean age (years)	48	56.9	0.15
Underlying conditions			
Autoimmune disorder	1	1	
Infection	1	3	
Hematological diseases	2	5	
Pancreatitis	3	0	
Chronic diseases (hypertension, cardiac insufficiency, kidney insufficiency)	0	3	
Trauma	5	0	
Gastrointestinal tumor	5	5	
Other tumor	1	0	
Liver transplant	1	0	
Median number of days of hospitalization	39	26	0.98
Mean number of days of mechanical ventilation	5.2	17.5	0.11
Mean number of days of central venous catheter	21	11	0.51
Mean number of days of parenteral nutrition	10.6	17.7	0.52
Mean number of days of urinary catheter	10	20	0.42
Mean number of days from 1st day of hospitalization to positive blood culture	18	30	0.13
Mean number of days from positive blood culture to outcome (death or alive)	25	13	0.04*
Mean number of days from blood culture to antifungal therapy	2	2	
ICU hospitalization >48 h (yes)	8	9	0.51
Surgical procedure (yes)	16	11	0.25
Immunosuppressive condition (yes)	11	17	0.003*
Use of parenteral nutrition (yes)	13	10	0.73
Use of mechanical ventilation (yes)	7	11	0.09
Use of urinary catheter (yes)	15	14	0.79
Use of antifungal for treatment (yes)	15	11	0.46
Use of steroids (yes)	4	4	1

ICU intensive care unit

* $p < 0.05$

species isolated after *C. albicans* and *C. tropicalis*. Charts of 36 patients were reviewed. CPC were distributed as follows: *C. parapsilosis* sensu stricto: 31 (86.1 %), *C. orthopsilosis*: 4 (11.1 %) and *C. metapsilosis*: 1 (2.8 %). Annual distribution was similar during the period with a median of 8 (range 3–13) cases per year. Main characteristics of the patients are in Table 1. Fifty percent of the patients had cancer (onco-hematological: 7 or gastrointestinal: 10) as the main underlying disease. General mortality rate was 47.2 % (17 patients), and main risk factors were: ICU admission <48 h: 17 (47.2 %); surgery: 27 (57.9 %); parental nutrition: 25 (70 %); prior bacteremia: 20 (51.3 %); CVC: 34 (94.4 %); mean time from blood culture to antifungal treatment: 2.6 days. Antifungal treatment was administered to 28 (75 %) patients. The univariate analysis comparing patients' outcomes (survival or death) showed a mean number of days from blood culture to outcome significantly higher in patients that survived (25 vs. 13 days; $p = 0.04$). Death was higher in patients with immunosuppressive conditions (17 vs. 11 patients; $p = 0.003$); three out of four patients with *C. orthopsilosis* died (75 %), while 14 of 31 patients with *C. parapsilosis* sensu stricto died ($p = 0.558$).

Drug susceptibilities of 39 isolates of CPC (*C. parapsilosis*: 33; C. *orthopsilosis*: 5; *C. metapsilosis*: 1) were compared with those of the reference *C. parapsilosis* ATCC 22019. MICs values are displayed in Table 2. Fluconazole MIC_{50}/MIC_{90} were 0.5/1 μg/mL ranging from 0.5 to 4 μg/mL. One isolate of *C. orthopsilosis* and one isolate of *C. parapsilosis* showed MIC = 4 μg/mL for fluconazole. Most of 39 isolates showed lower MIC values against itraconazole (MIC_{50}/MIC_{90}: 0.06/0.125 μg/mL) and voriconazole (MIC_{50}/MIC_{90}: ≤ 0.015/0.06 μg/mL) when compared with those of reference *C. parapsilosis* ATCC 22019. MICs of amphotericin B (MIC_{50}/MIC_{90}: 0.5/1 μg/mL), flucytosine (MIC_{50}/MIC_{90}: 0.25/0.25 μg/mL) and itraconazole (MIC_{50}/MIC_{90}: 0.06/0.125 μg/mL) were very similar among the isolates. Micafungin MIC_{50}/MIC_{90} was 0.5/0.5 μg/mL ranging from 0.125 to 1 μg/mL. These data suggest that our strains showed good susceptibility against all tested antifungal agents. No correlation was seen comparing mortality and the susceptibility to antifungal drugs. Fluconazole was administered to 23 patients and amphotericin B to 3 patients. In the group of patients that received fluconazole, 12 patients survived and 11 died.

The targets of the LAMP primers developed in this investigation were conserved region sequences inside the topoisomerase II. Our first screening works, which

Table 2 Distribution of MIC_{50}, MIC_{90}, geometric mean and MIC range of 39 isolates of *Candida* psilosis complex from bloodstream infections to antifungal drugs

In vitro antifungal activity (μg/mL) ($N = 39$ isolates)	VRC	FCZ	ITR	MFG	AMB	5FC	MCZ
MIC_{50}	≤0.015	0.5	0.06	0.5	0.5	0.25	0.25
MIC_{90}	0.06	1	0.125	0.5	1	0.25	1
Range	≤0.015–1	0.5–4	≤0.015–0.25	0.125–1	0.125–2	0.25–0.5	0.06–1
Geometric mean	0.022	0.5743	0.0508	0.3242	0.7071	0.1363	0.3339
C. parapsilosis ATCC[a] 22019	0.03	0.5	0.06	0.125	0.5	0.25	0.5
C. orthopsilosis (LIF[b] 12)	0.06	1	0.25	1	1	0.125	1
C. orthopsilosis (LIF 77)	0.06	1	0.06	1	1	0.125	1
C. orthopsilosis (LIF 193)	0.06	4	0.125	1	1	0.25	1
C. orthopsilosis (LIF 246)	0.03	1	0.25	1	1	0.125	1
C. orthopsilosis (LIF 265)	0.015	0.5	0.06	1	0.25	0.125	0.5
C. metapsilosis (LIF 48)	0.03	1	0.125	0.5	1	0.125	

VRC voriconazole, *FCZ* fluconazole, *ITR* itraconazole, *MFG* micafungin, *AMB* amphotericin B, *5FC* 5-flucytosine, *MCZ* miconazole

[a] American Type Culture Collection

[b] Research Laboratory in Fungi

lead to specific LAMP primers, were repeated, and many possibly useful specific LAMP candidate primer sets for each species of the psilosis complex were prepared. Among their LAMP primer sets, the primers that showed a rising of the turbidity curves at around 30 min were selected. The primers' sequence information, which showed a sharp DNA amplification in each species of the psilosis complex, was listed in Table 3. Usefulness of each of the three primer sets, thus prepared for the identification of *C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis*, was confirmed in the experiments as shown in Fig. 1a–c, respectively. Primer sets, which amplify the DNA of *C. parapsilosis* sensu stricto only, did not amplify the DNA of other eight *Candida* species including *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. kefyr*, *C. krusei* and *C. tropicalis*. Also, the LAMP primer sets amplified DNA of three clinical isolates of *C. parapsilosis* sensu stricto. This primer set did not amplify the DNA of two isolates of *C. orthopsilosis* and one isolate of *C. metapsilosis*.

LAMP primer sets designed for *C. orthopsilosis* and *C. metapsilosis*, respectively, could amplify DNA for respective species, but no cross-reactivity against *C. parapsilosis* was observed. Either LAMP primer sets for the two species did not show cross-reactivity with *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. glabrata*, *C. guilliermondii*, *C. krusei* and *C. kefyr*. Furthermore, it was confirmed that newly developed LAMP primer sets did not amplify the any DNA samples from *Cryptococcus neoformans*, which is frequently isolated from clinical samples of yeast-like fungal infection patients.

Discussion

The current epidemiology of candidemia in our hospital stressed CPC as agents that led to high mortality rate. In our hospital, the prevalence of *C. orthopsilosis* (11 %) and *C. metapsilosis* (2 %) among the CPC was similar to studies previously reported of Brazilian and South America data [34] and conversely higher than many other countries [34, 42]. In a recent study [43], Wisplinghoff et al. reported that CPC were the second most frequent agent of candidemia, accounting for 210 (17.4 %) cases among 1,218 *Candida* isolates obtained between 1998 and 2006 from 52 geographically dispersed hospitals in the USA. In this study, *C. albicans* (50.7 %) and *C. glabrata* (16.7 %) were the first and third most common *Candida* species, respectively [43]. Mortality associated with CPC candidemia in our patients (47.2 %) was higher than the current literature [1, 8] but similar to other Brazilian publications [6, 44]. In addition, *C. orthopsilosis* in our hospital exhibited higher mortality rate (75 %) than *C. parapsilosis* sensu stricto. Mortality rate due to *C. metapsilosis*

Table 3 Sequences of the LAMP primers used in this study

Specific LAMP primer for	Primer name	Sequence (5′–3′)
C. parapsilosis	F3	TGATCTTTGTGGTACGGTAA
	B3	CATCACTGACAGCAAACG
	FIP	ACCTCTCCTTGATTGCTTTAACATACATCTATCTCAATGACAAGCGA
	BIP	CCAGAGCCAGAAGGAGAAGGCCACTTCCCATCTTTCGT
	LF	CATAGCTCTTGAAGCTAGTGACGGG
	LB	CCCAAGAATTACACCACAATTGTAC
C. orthopsilosis	F3	TTGTCGCTTCTGACAACT
	B3	TTGTTCCATCTTTTGTACCTG
	FIP	TGGCATTGGCAATATCACGGTAATCAGCCGTGTATTAAGGAC
	BIP	ACGAGGATAAAGCATTGCAAAAAGCTTTATTTGCGTCTACTAAATTGAC
	LF	ATCTTTTCAGCTATCCCA
	LB	GAAACTGCGTATCAAAGGTC
C. metapsilosis	F3	CATTGAAAATCCCGCATTCA
	B3	CTTCCGTCAACTTTTTGCA
	FIP	GGCTACGAATTTTTCTTTACCTCCACATCCCAAACAAAGGAGCA
	BIP	ACCTTATTGCCCGTGTATTGAAAGCCTTATCTTCATTAGCGTTG
	LF	GAGACACCTTTGTTGTTAG
	LB	CCGGAATAGCTGAAAAGATTCG

LAMP loop-mediated isothermal amplification

could not be calculated because only one strain was isolated from 2006 to 2010. Since the number of patients in the present study was not high, we need to continue monitoring the mortality rate for further comparative studies on the recently isolated CPC.

Candidemia due to CPC, in our hospital, was mainly in patients with malignancies (50 %), long term of hospitalization and relatively old age. Surgery was performed in 27 (75 %) patients, of whom 70 % were related to the gastrointestinal tract. Most of our patients had several risk factors and invasive procedures known as associated with candidemia. The worse outcome was associated with patients with immunosuppressive conditions ($p = 0.003$) and short-time survival days ($p = 0.04$). The high mortality rate of our patients might be secondarily to multiple variables.

Although there were some fluctuations in MIC values in each species, the CPC isolates were very susceptible, and their MIC values were within those of the reference strain or proposed MIC values (CLSI). No resistant strains were confirmed. CPC is known for its decreased susceptibility to echinocandins [45]; however, our strains were very susceptible to micafungin (MIC_{50}/MIC_{90}: 0.5/0.5 μg/mL; range 0.125–1 μg/mL) and that could be explained because during the present study, echinocandins were sporadic prescribed. Two isolates, one *C. orthopsilosis* and one *C. parapsilosis,* had MIC = 4 μg/mL, which is considered susceptible dose dependent to fluconazole, according to the proposed species-specific epidemiologic cut-off value for *C. parapsilosis* [32, 46].

Though closely related, the species of the CPC differ from each other not only in prevalence [34] but also in virulence [47] and pathogenicity [18]. Recently, Kasahara et al. [48] reported the use of LAMP to detect *Candida* spp. in dairy products. The authors started from 4 *C. parapsilosis*, 1 *C. parapsilosis* and 1 *C. orthopsilosis* belonging to one private Culture Collection, in order to design a set of common primers, which were able to identify the CPC. Conversely, in our study, we design three sets of primers that are able to distinctly identify each one of *C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis*. This differentiation among the components of the *C. parapsilosis* complex has significance in the medical field, since each species can present differences in virulence [47], pathogenicity [18] and drug susceptibility, as we demonstrated at least for fluconazole. Therefore, the search for a fast, cost-effective and

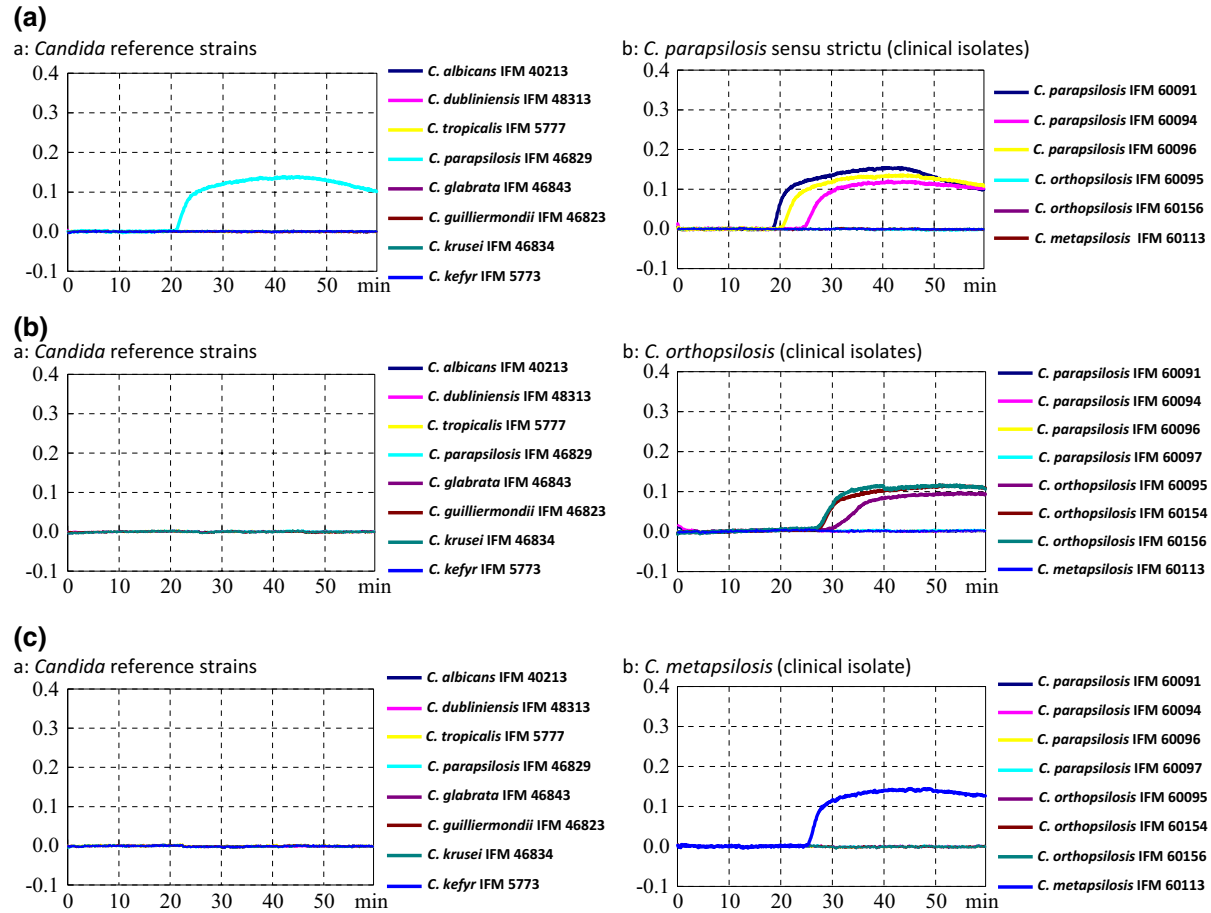

Fig. 1 Accurate identification profiles for three respective *Candida* species (*C. parapsilosis* sensu stricto, *C. orthopsilosis* and *C. metapsilosis*) by LAMP assay using specific primers for each *Candida* species. Following *Candida* strains were used as reference *Candida* species: *C. albicans* IFM 40213, *C. dubliniensis* IFM 48313, *C. tropicalis* IFM 577, *C. parapsilosis* IFM 46829, *C. glabrata* IFM 46843, *C. guilliermondii* IFM 46823, *C. krusei* IFM 46834, and *C. kefyr* IFM 5773. Amplified products based on rising curves of turbidity indicate specific DNA amplification for identifying corresponding *Candida* species. **a–c** indicate identification of (**a**) *C. parapsilosis* sensu stricto, **b** *C. orthopsilosis* and **c** *C. metapsilosis*, respectively

reliable method that discriminated among the species would be an important aid for the choice of optimal antifungal treatment in patients with life-threatening diseases. Many scientists had investigated molecular techniques for rapid diagnosis of fungal infection. Still identification or classification by sequencing of pathogenic fungi so far has been considered to be a "gold standard" for *Candida* yeast identification [26, 49]. However, sequencing of fungi is time-consuming and labor-intensive and may have reproducibility limitations. Therefore, the need of the application of fast and appropriate identification to species level is still increasing. The high potential of LAMP for the development of improved DNA-based diagnostic kits was reported [50]. In general, LAMP was found to be either similar or superior to PCR and more specific [51]. Actually, LAMP based approaches have been applied to a wide range of samples, such as paraffin-embedded tissues, whole blood and swabs [50]. Since there is concern over the increasing rates of the CPC infections worldwide [7, 49, 52], our present experiments confirmed the usefulness of LAMP primers for correct identification of each the three species of the complex.

The use of PCR assay for detection or discrimination of the *Candida* psilosis complex has been reported [7, 53]. Recently, a new identification of *C. parapsilosis* (sensu lato) by use of mitochondrial DNA and

real-time PCR was proposed [7]. Actually, the method can distinguish *C. parapsilosis* (sensu stricto), *C. metapsilosis* and *C. orthopsilosis.* However, LAMP has some advantages over PCR and real-time PCR, including isothermal conditions for amplification, and in addition LAMP does not need special thermo-cycler equipment [41].

In conclusion, we analyzed the clinical and epidemiological data of a 5 years surveillance of patients with candidemia due to CPC in our hospital and the antifungal susceptibility of the isolates. We proposed a novel molecular method, LAMP, for the identification of species of CPC. Since we identified two isolates with fluconazole MICs = 4 μg/mL and a high mortality rate among the patients there is a need for a fast, cost-effective and reliable molecular method that can distinguished the members of the CPC and to monitor the trends in antifungal susceptibility and clinical outcomes. In addition, LAMP method might be very useful method in discriminating the CPC in studies such as the clinical trials monitoring the changes in the species distribution and antifungal susceptibility.

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Conflict of interest All authors declare no conflict of interests.

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