Rev Iberoam Micol. 2015;32(4):221–228

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Original article

In vitro susceptibility and molecular characterization of *Candida* spp. from candidemic patients

Patricia Fernanda Herkert[a], Renata Rodrigues Gomes[a], Marisol Dominguez Muro[b], Rosangela Lameira Pinheiro[b], Gheniffer Fornari[a], Vânia Aparecida Vicente[a,d], Flávio Queiroz-Telles[a,c,*]

[a] *Postgraduate Program in Microbiology, Parasitology and Pathology, Biological Sciences, Department of Basic Pathology, LabMicro – Laboratory of Microbiology and Molecular Biology, Federal University of Paraná, Curitiba, Paraná, Brazil*
[b] *Support and Diagnosis Unit, Mycology Laboratory, Hospital of Clinics, Federal University of Paraná, Brazil*
[c] *Hospital de Clínicas, Federal University of Paraná, Brazil*
[d] *Fellowship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil*

ARTICLE INFO

Article history:
Received 25 March 2014
Accepted 15 July 2014
Available online 20 December 2014

Keywords:
Candida
In vitro susceptibility
Molecular sequencing
Antifungals

ABSTRACT

Background: *Candida* species are the main cause of hospital acquired fungal bloodstream infections. The main risk factors for candidemia include parenteral nutrition, long-term intensive care, neutropenia, diabetes, abdominal surgery and the use of central venous catheters. The antifungal drugs used to treat candidemia are mainly the echinocandins, however some isolates may be resistant to these drugs.
Aims: This work aims to evaluate the in vitro susceptibility patterns of various *Candida* species isolated from blood samples and provide their identification by molecular characterization.
Methods: Antifungal susceptibility testing was performed using the broth microdilution method. The sequencing of the ITS and D1/D2 regions of rDNA was used for molecular characterization.
Results: Seventy-four of the 80 isolates were susceptible to anidulafungin, 5 were intermediate, and 1 was resistant. For micafungin 67 were susceptible, 8 were intermediate and 5 were resistant. All isolates were susceptible to amphotericin B. Lastly, 65 isolates were susceptible to fluconazole, 8 were dose-dependent and 4 were resistant. The molecular identification corroborated the phenotypic data in 91.3% of the isolates.
Conclusions: Antifungal susceptibility data has an important role in the treatment of candidemia episodes. It was also concluded that the molecular analysis of isolates provides an accurate identification and identifies genetic variability within *Candida* species isolated from patients with candidemia.

Sensibilidad in vitro y caracterización molecular de aislamientos de *Candida* procedentes de pacientes con candidemia

Palabras clave:
Candida
Sensibilidad in vitro
Secuenciación molecular
Antifúngicos

RESUMEN

Antecedentes: Los hongos del género *Candida* son la causa principal de infección micótica del torrente sanguíneo adquirida en el hospital. Entre los factores de riesgo asociados a la candidemia destacan la nutrición parenteral, la estancia prolongada en una unidad de cuidados intensivos, la neutropenia, la diabetes, la cirugía abdominal y la utilización de catéter venoso central. Los agentes antifúngicos más utilizados para tratarla son las equinocandinas, pero determinados aislamientos son resistentes a dichos componentes, por lo que algunos pacientes no responden al tratamiento.
Objetivos: Este trabajo tiene como objetivo evaluar la sensibilidad in vitro de varios aislamientos de *Candida* procedentes de muestras de sangre y realizar su caracterización molecular.
Métodos: Se hicieron pruebas de sensibilidad a los antifúngicos mediante el método de microdilución en caldo. Para la caracterización molecular se utilizó la secuenciación de las regiones ITS y D1/D2 del DNAr.

* Corresponding author.
E-mail address: queiroz.telles@uol.com.br (F. Queiroz-Telles).

http://dx.doi.org/10.1016/j.riam.2014.07.007
1130-1406/

Resultados: De los 80 aislamientos evaluados, 74 fueron sensibles a la anidulafungina, 5 mostraron sensibilidad intermedia y solo uno era resistente. Cuando se utilizó la micafungina, 67 aislamientos resultaron sensibles, 8 presentaron sensibilidad intermedia y 5 fueron resistentes. Los 80 aislamientos fueron sensibles a la anfotericina B. Al menos 65 aislamientos eran sensibles al fluconazol, 8 presentaron sensibilidad dependiente de la dosis y 4 se mostraron resistentes. La identificación molecular confirmó la identificación fenotípica en un 91,3% de los aislamientos.
Conclusiones: Teniendo en cuenta los resultados obtenidos con las pruebas de sensibilidad a los antifúngicos, estas resultan indispensables para el tratamiento adecuado de la candidemia. Se concluye además que la identificación molecular proporciona una identificación precisa y consigue identificar la variabilidad genética de las especies del género *Candida* aisladas en pacientes con candidemia.

Among the medically important fungi, *Candida* species are of great importance due to the high frequency of colonization and infection in human hosts.[8] Under normal conditions, most do not cause damage to their hosts, and only cause tissue invasions and systemic infections when host defense mechanisms are weakened.[26] *Candida* infections account for about 80% of the total fungal infections of the bloodstream, urinary tract and surgical site infections.[8] The bloodstream infections caused by *Candida* have a high prevalence, morbidity and mortality,[19] and have a profound economic impact due to the long hospitalization periods, intensive care and treatment.[23]

The incidence of candidemia in tertiary care hospitals in Brazil is 1.38 cases per 1.000 hospital admissions[21] with a 54% mortality rate.[6] The underlying conditions are cancer, neutropenia, surgery (mainly abdominal), mechanical ventilation, dialysis, parenteral nutrition and central venous catheter.[6] In Brazil, *Candida albicans* is the leading agent, followed by *Candida parapsilosis*, *Candida tropicalis*, *Candida guilliermondii*, *Candida glabrata* and *Candida krusei*. Species as *Candida intermedia*, *Candida haemulonii*, *Candida lusitaniae*, *Candida famata* and *Candida norvegensis* are less frequent.[21]

The differences in the epidemiology and therapeutic approach for the various *Candida* species justify identification of the species responsible for the disease. This information is essential not only for appropriate patient management, but also for the control of nosocomial infections. Additionally, this information provides hospital-specific data, as antifungal species and susceptibility patterns often vary between institutions.[8] Therefore, this study was aimed to evaluate both the in vitro susceptibility patterns and the molecular characterization of those *Candida* species isolated from patients with candidemia.

Materials and methods

Isolates

This study assessed the in vitro susceptibility and molecular characterization of 80 *Candida* species obtained from blood samples that had been deposited into the mycology collection of the Laboratory of Mycology, UFPR Hospital, between January 2005 and June 2012 (Table 1).

Molecular characterization

For the DNA extraction, physical maceration performance with silica:celite (2:1) in CTAB (cetyl trimethylammonium bromide) and CIA (acidic solution of chloroform isoamyl alcohol)[32] was used. The sequencing was performed on an ABI3500 sequencer. For ITS sequencing the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) were used.[33] For the amplification of D1/D2 region primers NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′) were used,[22] with the same conditions used as for the ITS sequencing. For the *C. albicans* ABC genotyping, primers CA-int-L (5′-ATAAGGGAAGTCGGCAAAATAGATCCG TAA-3′) and CA-int-R (5′-CCTTGGCTGTGGTTTCGCTAGATAGTAGAT-3′) were used.[18]

Alignment and phylogenetic construction

Sequences were edited with the BioEdit program,[14] and compared with reference sequences for detection of similarity with the BLAST program.[1] The alignment was performed with the MAFFT,[16] and visual inspection by the MEGA 5.1 version.[29] The clinical isolates included in the study and 36 reference sequences were used for the phylogenetic analysis (Table 1). An isolate of *Neurospora crassa* was included as an outgroup.[9,10,30] The MEGA version 5.1 program[29] was used to estimate the best-fitting evolutionary models for each data and the Maximum Likelihood analysis. Bootstrap support was estimated by 1000 replicates.

Antifungal susceptibility testing

The susceptibility tests against amphotericin B (Sigma–Aldrich Quimica, Madrid, Spain), fluconazole (Sigma–Aldrich Quimica, Madrid, Spain), micafungin (Mycamine®; Astellas Pharma Inc., Toyama, Japan) and anidulafungin (Ecalta-Pfizer, Kent, United Kingdom) were performed with the broth microdilution technique in accordance with the guidelines in CLSI document M27-A3.[4,5] A reference strain *C. albicans* ATCC 10231 was included with each set of experiments for quality control. The MIC values for the echinocandins were verified by LEMI reference laboratory (UNIFESP – São Paulo – Brazil).

Results

The 80 isolates were identified as *C. albicans* (27 isolates), *C. parapsilosis* complex (24 isolates), *C. glabrata* (8 isolates), *C. tropicalis* (7 isolates), *C. guilliermondii* (5 isolates), *C. krusei* (3 isolates), *C. pelliculosa* (3 isolates), *C. lusitaniae* (2 isolates), and *C. dubliniensis* (1 isolate). Molecular identification was consistent with the phenotypic data in 91.3% isolates. Isolate LMICRO112 identified only as a *Candida* sp., was later confirmed as *C. tropicalis* with the molecular characterization. LMICRO133 thought to be *C. famata* was identified as *C. lusitaniae* through molecular markers; and LMICRO134, also thought to be *C. famata*, was reidentified as *C. tropicalis*. A single *C. albicans* isolate (LMICRO158) was molecularly characterized as *C. dubliniensis*, and *C. guilliermondii* LMICRO180 was identified as *C. parapsilosis* through the molecular analysis. Two isolates in the *C. parapsilosis* complex, LMICRO168 and LMICRO175, were subsequently reidentified as *C. metapsilosis* and *C. orthopsilosis*, respectively.

According to the Maximum Likelihood analysis based on ITS sequencing regions, the isolates were clustered into nine different

Table 1
Species, identification and GenBank access numbers of strains included in the study.

SPECIES	ID	GENBANK ITS/D1D2	SPECIES	ID	GENBANK ITS/D1D2	SPECIES	ID	GENBANK ITS/D1D2
C. lusitaniae	LMICRO110	KJ451634/–	*C. albicans*	LMICRO148	KJ451672/–	*C. glabrata*	LMICRO186	KJ451710/–
C. tropicalis	LMICRO111	KJ451635/KJ451714	*C. albicans*	LMICRO149	KJ451673/–	*C. pelliculosa*	LMICRO187	KJ451711/–
C. glabrata	LMICRO112	KJ451636/–	*C. pelliculosa*	LMICRO150	KJ451674/–	*C. parapsilosis*	LMICRO188	KJ451712/–
C. glabrata	LMICRO113	KJ451637/–	*C. albicans*	LMICRO151	KJ451675/–	*C. pelliculosa*	LMICRO189	KJ451713/–
C. glabrata	LMICRO114	KJ451638/–	*C. albicans*	LMICRO152	KJ451676/–	*C. albicans*	CBS 562	AB018037/AY497682
C. glabrata	LMICRO115	KJ451639/–	*C. albicans*	LMICRO153	KJ451677/–	*C. albicans*	ATCC 10231	FJ159643/–
C. glabrata	LMICRO116	KJ451640/–	*C. albicans*	LMICRO154	KJ451678/–	*C. albicans*[a]	CBS 1905	–/AY497673
C. glabrata	LMICRO117	KJ451641/–	*C. albicans*	LMICRO155	KJ451679/–	*C. dubliniensis*	CBS 7988	AB035590/–
C. tropicalis	LMICRO118	KJ451642/–	*C. albicans*	LMICRO156	KJ451680/–	*C. dubliniensis*[a]	CBS 7987	NR103562/U57685
C. tropicalis	LMICRO119	KJ451643/–	*C. albicans*	LMICRO157	KJ451681/–	*C. dubliniensis*	IFM 5422	–/AB828136
C. guilliermondii	LMICRO120	KJ451644/–	*C. dubliniensis*	LMICRO158	KJ451682/KJ451717	*C. parapsilosis*	WM 0295	EF568035/–
C. krusei	LMICRO121	KJ451645/–	*C. albicans*	LMICRO159	KJ451683/–	*C. parapsilosis*[a]	CBS 604T	AJ635316/CPU45754
C. albicans	LMICRO122	KJ451646/–	*C. albicans*	LMICRO160	KJ451684/–	*C. parapsilosis*[a]	ATCC 96138	–/AY497665
C. tropicalis	LMICRO123	KJ451647/–	*C. albicans*	LMICRO161	KJ451685/–	*C. metapsilosis*	CBS 2916	AY391844/–
C. tropicalis	LMICRO124	KJ451648/–	*C. albicans*	LMICRO162	KJ451686/–	*C. metapsilosis*[a]	ATCC 96144T	AJ698049/FJ746055
C. parapsilosis	LMICRO125	KJ451649/–	*C. albicans*	LMICRO163	KJ451687/–	*C. metapsilosis*	ATCC 14054	–/KC881060
C. parapsilosis	LMICRO126	KJ451650/–	*C. albicans*	LMICRO164	KJ451688/–	*C. orthopsilosis*	CBS 10906	FJ872018/–
C. parapsilosis	LMICRO127	KJ451651/–	*C. parapsilosis*	LMICRO165	KJ451689/–	*C. orthopsilosis*[a]	ATCC 96139T	AJ698048/FJ746056
C. parapsilosis	LMICRO128	KJ451652/–	*C. parapsilosis*	LMICRO166	KJ451690/–	*C. orthopsilosis*	CBS 8825	–/AJ508575
C. parapsilosis	LMICRO129	KJ451653/–	*C. parapsilosis*	LMICRO167	KJ451691/–	*C. tropicalis*	WM 233	EF568042/–
C. parapsilosis	LMICRO130	KJ451654/–	*C. metapsilosis*	LMICRO168	KJ451692/KJ451718	*C. tropicalis*[a]	IFM 5446	AB437068/–
C. parapsilosis	LMICRO131	KJ451655/–	*C. parapsilosis*	LMICRO169	KJ451693/–	*C. tropicalis*[a]	CBS 94	–/U45749
C. parapsilosis	LMICRO132	KJ451656/–	*C. parapsilosis*	LMICRO170	KJ451694/–	*C. tropicalis*	DMKUXE318	–/AB847528
C. lusitaniae	LMICRO133	KJ451657/KJ451715	*C. parapsilosis*	LMICRO171	KJ451695/–	*C. glabrata*[a]	CBS 138	AY198398/–
C. tropicalis	LMICRO134	KJ451658/KJ451716	*C. parapsilosis*	LMICRO172	KJ451696/–	*C. glabrata*	WM 02.57	EF568002/–
C. guilliermondii	LMICRO135	KJ451659/–	*C. parapsilosis*	LMICRO173	KJ451697/–	*C. famata*[a]	CBS 1795T	AM992910/AJ508559
C. krusei	LMICRO136	KJ451660/–	*C. parapsilosis*	LMICRO174	KJ451698/–	*C. famata*[a]	CBS 767	GU246256/AY497693
C. albicans	LMICRO137	KJ451661/–	*C. orthopsilosis*	LMICRO175	KJ451699/KJ451719	*M. guilliermondii*[a]	WM 02374	EF568007/–
C. albicans	LMICRO138	KJ451662/–	*C. parapsilosis*	LMICRO176	KJ451700/–	*M. guilliermondii*	CBS 2030	EF568003/AY497675
C. albicans	LMICRO139	KJ451663/–	*C. parapsilosis*	LMICRO177	KJ451701/–	*M. guilliermondii*	HB 31–2	–/AB568329
C. albicans	LMICRO140	KJ451664/–	*C. parapsilosis*	LMICRO178	KJ451702/–	*P. anomala*[a]	PY1	AB331898/–
C. albicans	LMICRO141	KJ451665/–	*C. krusei*	LMICRO179	KJ451703/–	*P. anomala*[a]	CBS 5759	DQ249196/–
C. albicans	LMICRO142	KJ451666/–	*C. parapsilosis*	LMICRO180	KJ451704/KJ451720	*C. lusitaniae*[a]	CBS 4413	EF568024/AJ508571
C. albicans	LMICRO143	KJ451667/–	*C. guilliermondii*	LMICRO181	KJ451705/–	*C. lusitaniae*[a]	CBS 6936	AY321464/U44817
C. albicans	LMICRO144	KJ451668/–	*C. guilliermondii*	LMICRO182	KJ451706/–	*P. kudriavzevii*	WM 03.204	EF568016/–
C. albicans	LMICRO145	KJ451669/–	*C. guilliermondii*	LMICRO183	KJ451707/–	*P. kudriavzevii*[a]	CBS 573	EF568018/–
C. albicans	LMICRO146	KJ451670/–	*C. tropicalis*	LMICRO184	KJ451708/–	*S. cerevisiae*	CBS 1171	AB018043/–
C. albicans	LMICRO147	KJ451671/–	*C. glabrata*	LMICRO185	KJ451709/–	*Neurospora crassa*	MYA-4619 or FGSC8771	GU327635/FR774249

[a] Type.
–: data not provided.

clades: Albicans, Dubliniensis, Tropicalis, Parapsilosis Complex, Glabrata, Pelliculosa, Guilliermondii, Lusitaniae and Krusei, supported by bootstrap values (Fig. 1). Sequencing analysis of the variable D1/D2 region was performed to confirm the identity of the isolates (LMICRO112, 133, 134, 158, 168, 175, and 180) that exhibited a discordance between molecular and phenotypic identification. The D1/D2 sequencing analysis confirmed the results from ITS sequencing, demonstrating that the isolates belonged

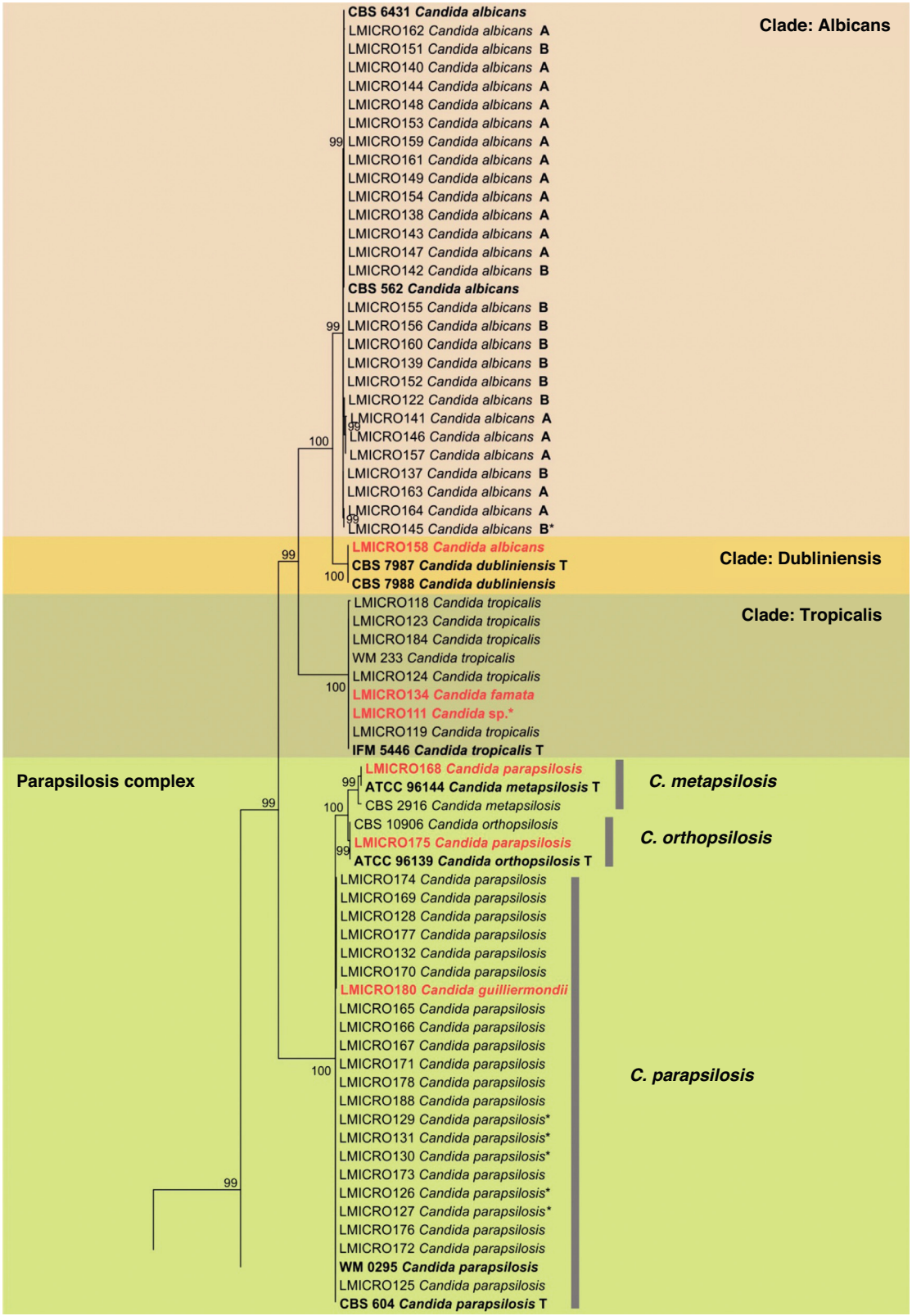

Fig. 1. Phylogenetic tree of Maximum likelihood based on the alignment of ITS regions and 5.8S rDNA built with 1000 bootstrap using the evolutionary model Tamura 3-parameters with gamma distribution, using mega version 5.1 program. *Neurospora crassa* (mya-4619) was used as an outgroup. A/B: genotype of *C. albicans*. *Isolates with altered susceptibility profile. T: type strain. Red: isolates with discordant molecular and phenotypic identification.

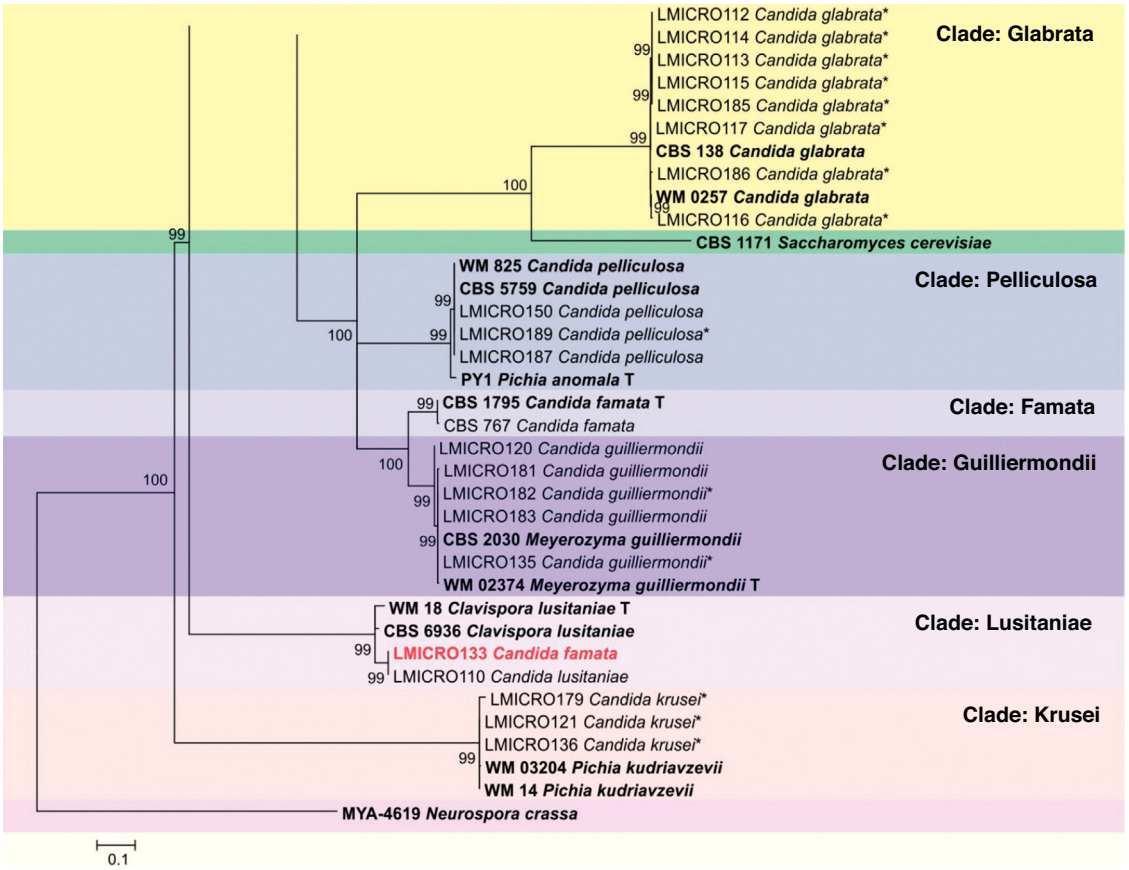

Fig. 1. (*Continued*)

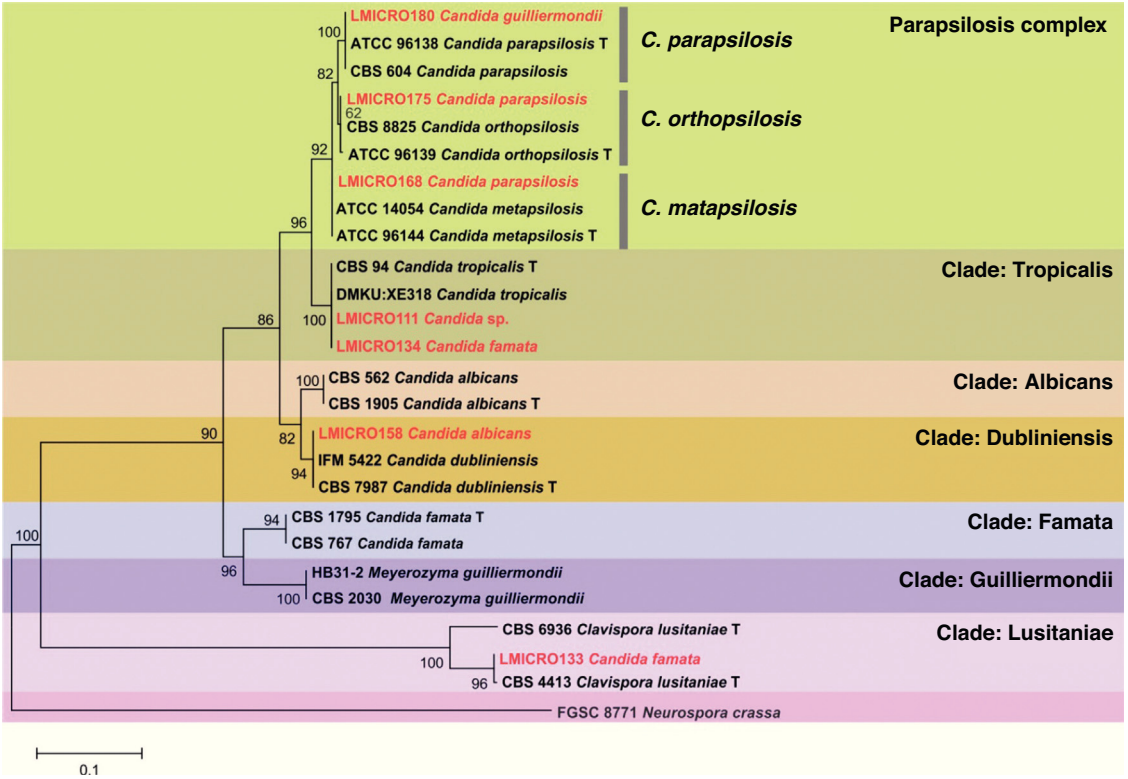

Fig. 2. Phylogenetic tree of Maximum likelihood based on the alignment of D1/D2 region of rDNA built with 1000 bootstrap using the evolutionary model Tamura-Nei with gamma distribution, using mega version 5.1 program. *Neurospora crassa* (FGSC 8771) was used as an outgroup. T: type strain. Red: isolates with discordant molecular and phenotypic identification.

Table 2
Variation of minimum inhibitory concentrations (μg/ml) obtained according to the tested *Candida* species.

Species	Antifungal agent (no. tested)	No. of isolates with MIC (μg/ml)											
		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
C. albicans	Anidulafungin (27)	–	2	–	24	1	–	–	–	–	–	–	–
	Micafungin (27)	6	17	3	–	–	–	1	–	–	–	–	–
	Amphotericin B (27)	–	2	3	14	8	–	–	–	–	–	–	–
	Fluconazole (27)	–	–	4	11	6	1	4	–	1	–	–	–
C. parapsilosis	Anidulafungin (22)	–	1	1	1	11	6	2	–	–	–	–	–
	Micafungin (22)	–	–	–	–	3	6	8	5	–	–	–	–
	Amphotericin B (22)	–	1	–	14	7	–	–	–	–	–	–	–
	Fluconazole (22)	–	–	2	5	8	6	1	–	–	–	–	–
C. metapsilosis	Anidulafungin (1)	–	–	–	–	1	–	–	–	–	–	–	–
	Micafungin (1)	–	–	–	–	–	1	–	–	–	–	–	–
	Amphotericin B (1)	–	–	–	–	1	–	–	–	–	–	–	–
	Fluconazole (1)	–	–	–	–	–	1	–	–	–	–	–	–
C. orthopsilosis	Anidulafungin (1)	–	–	–	–	1	–	–	–	–	–	–	–
	Micafungin (1)	–	–	–	–	–	–	1	–	–	–	–	–
	Amphotericin B (1)	–	–	–	–	1	–	–	–	–	–	–	–
	Fluconazole (1)	–	–	–	–	–	–	1	–	–	–	–	–
C. glabrata	Anidulafungin (8)	–	–	–	7	–	–	1	–	–	–	–	–
	Micafungin (8)	2	1	–	4	–	1	–	–	–	–	–	–
	Amphotericin B (8)	–	–	–	3	5	–	–	–	–	–	–	–
	Fluconazole (8)	–	–	–	–	–	–	–	–	1	6	–	1
C. tropicalis	Anidulafungin (7)	–	3	1	3	–	–	–	–	–	–	–	–
	Micafungin (7)	–	–	–	7	–	–	–	–	–	–	–	–
	Amphotericin B (7)	–	1	1	3	2	–	–	–	–	–	–	–
	Fluconazole (7)	–	–	–	2	2	1	1	–	–	–	1	–
C. guilliermondii	Anidulafungin (5)	–	1	–	–	–	–	4	–	–	–	–	–
	Micafungin (5)	–	–	–	–	–	1	2	2	–	–	–	–
	Amphotericin B (5)	–	–	–	1	4	–	–	–	–	–	–	–
	Fluconazole (5)	–	–	–	–	–	2	1	–	–	–	–	2
C. krusei	Anidulafungin (3)	–	–	–	3	–	–	–	–	–	–	–	–
	Micafungin (3)	–	–	–	1	2	–	–	–	–	–	–	–
	Amphotericin B (3)	–	–	–	–	2	1	–	–	–	–	–	–
	Fluconazole (3)	–	–	–	–	–	–	–	–	–	1	2	–
C. pelliculosa	Anidulafungin (3)	1	–	–	2	–	–	–	–	–	–	–	–
	Micafungin (3)	–	–	2	–	1	–	–	–	–	–	–	–
	Amphotericin B (3)	1	–	–	–	1	1	–	–	–	–	–	–
	Fluconazole (3)	–	–	–	–	–	–	3	–	–	–	–	–
C. lusitaniae	Anidulafungin (2)	–	–	–	2	–	–	–	–	–	–	–	–
	Micafungin (2)	–	–	–	1	1	–	–	–	–	–	–	–
	Amphotericin B (2)	–	–	1	1	–	–	–	–	–	–	–	–
	Fluconazole (2)	–	–	1	–	–	–	1	–	–	–	–	–
C. dubliniensis	Anidulafungin (1)	–	1	–	–	–	–	–	–	–	–	–	–
	Micafungin (1)	–	–	1	–	–	–	–	–	–	–	–	–
	Amphotericin B (1)	–	–	–	–	1	–	–	–	–	–	–	–
	Fluconazole (1)	–	–	–	1	–	–	–	–	–	–	–	–

to the clades Tropicalis, Lusitaniae, Dubliniensis, and Parapsilosis complex (Fig. 2). A low genetic variability rate among the *C. albicans* isolates was observed, and considerable interspecific differences were noted for the Parapsilosis complex, dividing it in three species: *C. parapsilosis*, *C. metapsilosis* and *C. orthopsilosis*.

The ABC genotyping of the *C. albicans* isolates distinguished between genotypes A and B, with a wider prevalence of genotype A (62%). It was noticed that both were susceptible to the tested antifungals, with the exception of isolate LMICRO145 of genotype B that was resistant to fluconazole. The mortality rate was similar between both genotypes, with seven deaths in both groups.

Antifungal susceptibility data showed several resistant isolates of *C. glabrata*, with 5 of them being resistant to micafungin and intermediate to anidulafungin, and 7 susceptible-dose dependent to fluconazole. The *C. albicans* species were the most susceptible to the antifungals tested, with only one isolate resistant to fluconazole. Among the C. *parapsilosis* complex, 5 isolates of *C. parapsilosis* had intermediate susceptibility to micafungin while the remaining isolates studied were susceptible to all the antifungals tested (Table 2).

Those isolates resistant to at least one antifungal (LMICRO112, 113, 115, 116, 117, 135 and 145) were further studied analyzing the relationship between the clinical data and the MIC values (Table 3). With regard to clinical response to treatment and the in vitro susceptibility tests results, it was observed that the LMICRO116 isolate (*C. glabrata*) was obtained from a patient whose blood cultures remained positive during micafungin (MCF) therapy, but was successfully treated after the addition of amphotericin B. The susceptibility tests revealed resistance of the isolate to MCF and anidulafungin (AND), but susceptibility to amphotericin B.

Discussion

The frequency of invasive mycosis by opportunistic fungal pathogens has increased significantly along the last years. Besides, more than 17 different *Candida* species have been identified as etiological agents of bloodstream infections.[27]

C. albicans is still considered the most common cause of candidemia in tertiary Brazilian hospitals, with a rate of about 40% of the episodes.[11,13,21,25] The results obtained in this study corroborate this evidence. However, a rising incidence of infections caused by the non-*C. albicans Candida* species, especially *C. tropicalis*, *C. parapsilosis*, *C. glabrata* and *C. krusei*[6,7,13,21,28,35] has been noticed. These species were found in the present study as well. It is also difficult to phenotypically identify cryptic species within species complexes without the addition of molecular sequencing[8,20,34]

Table 3
Correlation between the clinical data of the patients and the in vitro susceptibility profile of the tested isolates.

Isolate	Disease associated	Treatment	Response	Susceptibility profile
LMICRO112 *C. glabrata*	Intestinal sub-occlusion, abdominal surgery	FLU	Undetermined	AND (I) MCF (R) AMB (S) FLU (SDD)
LMICRO113 *C. glabrata*	Metabolic disease, abdominal surgery	FLU	Favorable	AND (I) MCF (R) AMB (S) FLU (SDD)
LMICRO115 *C. glabrata*	Crohn disease, adenocarcinoma of the pancreas	Untreated[a]	–	AND (I) MCF (R) AMB (S) FLU (SDD)
LMICRO116 *C. glabrata*	Burkitt lymphoma, dialysis	AMB	Favorable	AND (R) MCF (R) AMB (S) FLU (R)
LMICRO117 *C. glabrata*	Gastric ulcer, diverticular disease, abdominal surgery	Untreated[b]	–	AND (I) MCF (R) AMB (S) FLU (SDD)
LMICRO135 *C. guilliermondii*	ALL, bone marrow transplantation	FLU, voriconazole	Undetermined	AND (S) MCF (I) AMB (S) FLU (R)
LMICRO145 *C. albicans*	Anorectal malformation, abdominal surgery	FLU, AMB	Favorable	AND (S) MCF (S) AMB (S) FLU (R)

ALL: acute lymphoblastic leukemia; AND: anidulafungin; MCF: micafungin; AMB: amphotericin B; FLU: fluconazole; S: susceptible; SDD: susceptible-dose dependent; I: intermediate; R: resistant.

[a] Died before the beginning of the treatment.
[b] Transient candidemia; undetermined: died.

methods. This was observed with the isolates of the Parapsilosis complex and those of *C. dubliniensis* and *C. lusitaniae* (Fig. 2), confirming the necessity to complement the presumptive identification based on morphology and biochemistry with molecular data. The identification of the Parapsilosis complex is necessary as these isolates vary in their antifungal susceptibility profiles.[17,31] According to this study *C. parapsilosis* isolates showed intermediate susceptibility to micafungin, corroborating previous findings that suggest a decreased susceptibility in *C. parapsilosis*.[3,12,17] The results of the present study show that the MIC values for amphotericin B among the three species of the Parapsilosis complex were similar. However, Lockhart et al.[17] observed higher MIC values for *C. parapsilosis*. This supports the relevance of the in vitro susceptibility tests for appropriate patient management.

The ABC genotyping of the *C. albicans* isolates was also included in this study in order to provide additional discriminatory data regarding this species. According to McCullough et al.,[18] genotype A is more frequent among the isolates of this species, and it has being correlated with lower susceptibility to flucytosine. In our study, genotype A was predominant (62%) among the analyzed isolates, and most of the isolates were susceptible to all of the antifungals tested regardless of the genotype, with the exception of one genotype B isolate which showed resistance to fluconazole. It was also observed that the mortality rates of the patients were similar, regardless of genotype involved. It remains unclear if genotype A is more virulent.[36] Fluconazole has a good therapeutic activity against *C. albicans*, and has been used to prevent the systemic candidiasis for many years; in these cases, the susceptibility to fluconazole can reach 95%, being effective against most infections by this species.[37] However, the repetitive and long-term use of fluconazole for chronic infections and its prophylactic use has favored the appearance of resistant isolates.

Infections due to *C. glabrata* have risen in the last few years and this appears to be related to the high usage of fluconazole in hospitals and the occurrence of resistant isolates to this antifungal.[24] As a result, the echinocandins have been recently added as a first-line indication for the candidemia,[8] but it has been noticed that some *C. glabrata* isolates are resistant to this agent as well.[15] In our study, 62% of the *C. glabrata* isolates (5/8) exhibited resistance to micafungin and 87% (7/8) showed resistance to fluconazole. One of these highly resistant isolates (*C. glabrata* LMICRO116) was used by Bizerra et al.[2] to investigate the mutations associated with resistance. The authors confirmed the presence of a S663F mutation in the FKS2 gene, resulting in the production of a 1,3-β-glucan synthase enzyme with reduced susceptibility to the echinocandins, and a strong potential for clinical failure. Furthermore, species such as *C. albicans*, *C. parapsilosis*, *C. guilliermondii*, and *C. tropicalis* showed high MIC values in this study, a finding that is not consistent with a previous study conducted at the same hospital.[11] This increased resistance to antifungals appears related to the rise of prophylactic and empirical therapies using fluconazole and echinocandins in this hospital.

Most of the *Candida* isolates showed susceptibility to the antifungal agents tested. However, *C. glabrata* presented the largest number of isolates resistant to the echinocandins and fluconazole. Accordingly, antifungal susceptibility testing has an important role in the treatment of candidemia, and the molecular analysis of isolates provides accurate identification of cryptic species in the *C. parapsilosis* complex and demonstrates the genetic variability between *Candida* species.

Acknowledgements

We are grateful to CAPES and CNPq, Brasília, Brazil, for the financial support.

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