Rev Iberoam Micol. 2015;32(3):153–158

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Original article

Genotypic variability and antifungal susceptibility of *Candida tropicalis* isolated from patients with candiduria

Adriana Araújo de Almeida[a], Sandra Sayuri Nakamura[b], Adriana Fiorini[b], Alexéia Barufatti Grisolia[c], Terezinha Inez Estivalet Svidzinski[b], Kelly Mari Pires de Oliveira[c,*]

[a] *Department of Health Sciences, Grande Dourados Federal University, Dourados, MS, Brazil*
[b] *Center for Health Sciences, State University of Maringa, Maringa, PR, Brazil*
[c] *Department of Biological and Environmental Sciences, Grande Dourados Federal University, Dourados, MS, Brazil*

ARTICLE INFO

Article history:
Received 5 August 2013
Accepted 2 June 2014
Available online 14 October 2014

Keywords:
Candiduria
Candida tropicalis
RAPD-PCR
Microsatellite
Antifungal susceptibility

ABSTRACT

Background: *Candida tropicalis* is an emerging major human pathogen in nosocomial infections, and it is considered the second or third species of *Candida* most isolated from urine cultures.
Aims: The study aimed at characterizing genotypically *C. tropicalis* strains from patients with candiduria in a university hospital, and assessed the antifungal susceptibility profile.
Methods: The study was conducted with hospitalized patients who developed urinary tract infection from *C. tropicalis* from June 2010 to June 2011 at the Grande Dourados University Hospital of the Federal University, Dourados, MS, Brazil. Susceptibility to the antifungal agents amphotericin B and fluconazole was determined by broth microdilution. The genotypic variability of isolates of *C. tropicalis* was analyzed by microsatellite markers and RAPD-PCR.
Results: Only one isolate was resistant to amphotericin B (MIC → 16 μg/ml); the others were susceptible to fluconazole and amphotericin B. The genotypic variability by RAPD-PCR resulted in distinct profiles for RAPD markers. A total of 10 alleles were observed for the microsatellite loci, *URA3* and *CT14*, which were grouped differently, and four associations were observed for locus *URA3* and eight for locus *CT14*.
Conclusions: *C. tropicalis* isolates from urine were susceptible to the antifungal agents tested. The genotyping techniques make possible proving the similarity and genetic diversity among isolates of *C. tropicalis* involved in nosocomial infections. This knowledge is important for the control and prevention of nosocomial infections caused by this yeast species.

Variabilidad genotípica y sensibilidad antifúngica de aislamientos de *Candida tropicalis* procedentes de pacientes con candiduria

Palabras clave:
Candiduria
Candida tropicalis
RAPD-PCR
Microsatélite
Sensibilidad antifúngica

RESUMEN

Antecedentes: *Candida tropicalis* es un patógeno humano emergente en las infecciones nosocomiales y es considerado la segunda o tercera especie de *Candida* más aislada en cultivos de orina.
Objetivos: El objetivo del estudio fue caracterizar genotípicamente aislamientos de *C. tropicalis* procedentes de pacientes con candiduria de un hospital universitario, y evaluar su perfil de sensibilidad a los antifúngicos.
Métodos: La investigación fue realizada con pacientes hospitalizados que desarrollaron una infección urinaria por *C. tropicalis* desde junio de 2010 hasta junio de 2011 en el Hospital Universitario de la Universidad Federal de Grande Dourados, Dourados, MS, Brasil. La sensibilidad a los agentes antifúngicos anfotericina B y fluconazol fue determinada mediante el método de microdilución en caldo. La variabilidad genotípica de los aislamientos de *C. tropicalis* se analizó mediante marcadores microsatélites y RAPD-PCR.

* Corresponding author.
E-mail address: kellyoliveira@ufgd.edu.br (K.M.P. Oliveira).

http://dx.doi.org/10.1016/j.riam.2014.06.003
1130-1406/

Resultados: Sólo un aislamiento fue resistente a la anfotericina B (MIC → 16 mg/ml); los restantes aislamientos fueron sensibles al fluconazol y la anfotericina B. La variabilidad genotípica por RAPD-PCR dio como resultado perfiles distintos para los marcadores utilizados. Se observó un total de 10 alelos de los loci microsatélites, *URA3* y *CT14* fueron agrupados de manera diferente y se observaron cuatro asociaciones para el locus *URA3* y ocho para el locus *CT14*.
Conclusiones: Los aislamientos de *C. tropicalis* obtenidos de orina fueron sensibles a los antifúngicos probados. Las técnicas de genotipificación permiten demostrar la similitud y la diversidad genética de los aislamientos de *C. tropicalis* implicados en las infecciones nosocomiales. Este conocimiento es importante para el control y la prevención de las infecciones hospitalarias causadas por esta especie de levadura.

Nosocomial infections caused by *Candida* yeasts have increased significantly worldwide in recent years and have been the growing cause of morbidity and mortality in hospitalized patients, especially those severely ill and immunocompromised.[5,13,27,33,35]

Nosocomial urinary tract infections caused by *Candida* spp. frequently occur in hospitalized patients, and account for 10–15% of all urinary tract infections.[4] Episodes of candiduria in hospitalized patients increase morbidity and offer the risk of mortality.[14,34] Furthermore, candiduria can be considered a risk factor for candidemia in adult patients.[16]

Candida tropicalis is an emerging major human pathogen in nosocomial infections[5,21,22,31] and it is considered the second or third *Candida* species most frequently isolated in urine cultures.[15,18,24] This species has some important virulence characteristics such as increased resistance to azole antifungal drugs, significant adherence to the epithelium, more so than to the silicone, biofilm formation and expression of total hemolytic activity.[23,27,32]

Currently several molecular typing methods have been used for molecular characterization and correlation of *Candida* species in hospital infections. Among them, the Randomly Amplified Polymorphic DNA (RAPD) and microsatellite markers stand out.[3,10,30] Genotyping of clinical isolates by RAPD technique involves the amplification of DNA fragments by polymerase chain reaction using short primers of random sequence.[11] However, microsatellite markers also amplify DNA fragments by polymerase chain reaction and are co-dominant markers which allow the heterozygous loci to be differentiated from the homozygous ones.[12] The application of these molecular tools in infections within a hospital environment is to characterize genetic variations and demonstrate the degree of similarity between the isolates. Genotyping of different *Candida* species is important due to its prognostic and therapeutic significance, thus generating information for clinical epidemiology, allowing the correct treatment and control of infections in hospitals.

Studies aimed at assessing the genetic diversity of species of *Candida* non-*Candida albicans* (CNCA) isolated from hospitalized patients, as well as the evaluation of antifungal susceptibility profile, are relevant for improving therapeutic approaches and control hospital fungal infections. Therefore, the present study aimed at evaluating the molecular genetic diversity of *C. tropicalis* and studying its susceptibility to antifungal agents in patients with candiduria hospitalized in a university hospital.

Materials and methods

Samples

Urine samples from patients with urinary infection hospitalized at the University Hospital of the Grande Dourados Federal University (Dourados, State of Mato Grosso do Sul, Brazil) from June 2010 to June 2011, whose results were positive for *C. tropicalis*, were processed in this study.

In order to characterize a urinary tract infection caused by *C. tropicalis*, the presence of more than 10^5 colony forming units per milliliter (CFU/mL) in urine was considered.

Isolation and identification of isolates

The yeasts were screened by cultivation in CHROMagar *Candida*® (Difco, BD, Franklin Lakes, NJ, USA) through routine laboratorial analysis and stored in Sabouraud Dextrose Broth (Difco, BD, Franklin Lakes, NJ, USA) with 20% glycerol in a freezer at −70 °C. Isolates of *C. tropicalis* were identified phenotypically by way of macroscopic, microscopic and biochemical features described in the classical method, including colony morphology, micromorphological analyses, and carbohydrate assimilation and fermentation tests.[37]

Antifungal susceptibility testing

The antifungal susceptibility was determined by broth microdilution method, performed according to the document M27-A3 of the Clinical and Laboratory Standards Institute.[7]

The antifungal agents used were amphotericin B and fluconazole, and the susceptibility cutoffs were in accordance to the parameters established by Yang et al.[36] with MIC values ≤1 μg/ml considered susceptible and ≥2 μg/ml resistant to amphotericin, and by the supplement document M27-A3–M27-S3[8] to fluconazole.

Genomic DNA extraction

Yeasts were cultured in Sabouraud Dextrose Broth (Difco, BD, Franklin Lakes, NJ, USA) and maintained overnight at 25 °C. Genomic DNA was extracted as described by Chong et al.[6] The amount and purity of genomic DNA were determined by optical density in a spectrophotometer (Genesys 10, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Molecular identification by species-specific PCR primer

The identification of the species *C. tropicalis* was confirmed by the amplification of the internal transcribed regions 1 and 2 of the rRNA gene of *Candida* species.[20] The PCR reaction, adapted from Alves et al.,[2] was performed with genomic DNA (10–20 ng), forward and reverse primers (10 pmol each), PCR Master Mix (Axygen Scientific, Union City, CA, USA) (12.5 μl), and water for a final volume of 25 μl. The primers used for the amplification reaction of the internal transcribed spacer 1 region (ITS1)-5.8S-ITS2 of the rRNA gene were: forward (*C. tropicalis*, 5-AAGAATTTAACGTGGAAACTTA-3) and reverse (5-TCCTCCGCTTATTGATATGC-3) (GenBank Accession

No.: EU2888196.1). All PCR reactions were performed in Mastercycler Gradient Thermal Cycler (Eppendorf, Hamburg, Germany). Amplification conditions were initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 63 °C for 1 min, extension at 72 °C for 1 min, and final extension at 72 °C for 5 min.

The amplified products were subjected to electrophoresis in 1.5% agarose gel (110 V, 40 min), stained with ethidium bromide (0.5 mg ml^{-1}) and visualized under ultraviolet light. The size of the amplified product (149 bp), specific to *C. tropicalis*, was determined by using the molecular weight marker 50 bp.

RAPD-PCR assay

The molecular characterization of *C. tropicalis* isolates from urine was performed by RAPD-PCR based on the methodology of Bautista-Muñoz et al.[3] The reaction contained 10 ng of genomic DNA, 0.4 μM of the primer, 2 mM of $MgCl_2$, 1.2 U of Taq polymerase and 0.8 mM dNTP. The primers used for the reaction were OPA-18 (5-AGCTGACCGT-3), OPE-18 (5-GGACTGCAGA-3) and P4 (5-AAGAGCCCGT-3). The reaction conditions for RAPD-PCR were initial denaturation at 94 °C for 5 min, 45 cycles of denaturation at 94 °C for 1 min, annealing at 36 °C for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 10 min.

The amplified products were subjected to electrophoresis in agarose gel at 1.2% in 0.5× TBE buffer (110 V, 60 min), stained with ethidium bromide (0.5 mg ml^{-1}), and visualized under ultraviolet light. The determination of the sizes of the fragments was performed using the molecular weight marker 100 bp.

Genetic variability analyses were performed with the Bionumerics® software, version 4.6 (Applied Maths, Sint-Martens-Latem, Belgium). The similarity was verified by the coefficient (SAB) between the patterns for each pair of isolates A and B and was calculated using the formula $SAB = 2E/(2E + a + b)$, where E is the number of common bands in the patterns of A and B, *a* being the number of bands in the pattern of *a* with no correlates in B pattern, and *b* is the number of bands in pattern B with no correlation in pattern A.

From the similarity matrix, units were grouped by the method UPGMA (Unweighted Pair-Group Method with arithmetical Average). SAB value of 1.00 indicates that the band pattern for lineage A is identical to B; values between 0.80 and 0.99 means very similar clinical isolates, but not identical, and may suggest a microevolution of a single strain; SAB values lower than 0.80 represent independent strains.[3,29]

Microsatellite assay

The reaction was adapted from the method described by Desnos-Ollivier et al.[10] Two microsatellite markers were used, one upstream of the *URA3* genes (*URA3*, GenBank Accession No. EU288195.1) and one non-annotated sequence *CT14*. Each reaction contained 50 ng of genomic DNA, 0.1 μM of each primer pair, 5 mM of $MgCl_2$, 1.25 U of Taq polymerase and 0.8 mM of dNTP. The forward and reverse primers used for the reactions were: URAF (5-ATTGGATAGTCCCTCTAAACTCACTACTA-3)/CTU2R (5-GTTGGAACATCAATTGATGCACATAAAT-3) and CT14a (5-GTAAATCTTGTATACCGTGGA-3)/CT14b (5-TAGCCCATTTTCTAGTTTTGC-3). The conditions for amplification were: 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 7 s and final extension at 72 °C for 5 min.

The amplification products were subjected to electrophoresis in 8% polyacrylamide gel in 1× TBE buffer (140 V, 5 h and 30 min), stained with ethidium bromide (0.5 mg ml^{-1}), and visualized under ultraviolet light. The determination of the sizes of the fragments was performed using the molecular weight marker 100 bp.

The characteristics of co-dominance of microsatellite markers allow the identification of homozygotes and heterozygous genotypes, enabling the estimation of the discriminating power of each marker.[12] The latter was calculated according to the Simpson index[17] for each of the markers (*URA3* and *CT14*).

Table 1
Susceptibility test to amphotericin B and fluconazole for 15 isolates of *C. tropicalis* obtained from 12 patients with candiduria.

Variables	MIC values (μg/ml)	
	Amphotericin B	Fluconazole
MIC range	0.125 → 16	0.25–4
Geometric mean MIC	0.91	0.74
MIC_{50}[a]	1	1
MIC_{90}[b]	1	2

[a] MIC_{50}: lowest concentration able to inhibit the growth of 50% of the isolates.
[b] MIC_{90}: lowest concentration able to inhibit the growth of 90% of the isolates.

Results

The phenotypic analysis for the identification of the *Candida* species from the 12 patients was able to identify 15 isolates of *C. tropicalis* obtained from cases of candiduria. The method of species-specific PCR of the regions ITS1 and ITS2 of the rRNA gene confirmed the species *C. tropicalis* in all the 15 isolates.

Antifungal susceptibility test

The results for the susceptibility test are shown in Table 1. The range of the Minimal Inhibitory Concentration (MIC) value was 0.125-16 μg/ml for amphotericin B and 0.25–4 μg/ml for fluconazole. Only one isolate was resistant to amphotericin B and the others were susceptible to fluconazole and amphotericin B (Table 2).

RAPD-PCR profiles

The isolates showed six (A–F), two (A and B) and four (A–D) different molecular profiles for the markers OPA-18, OPE-18 and P4 respectively (Table 2 and Fig. 1).

The amplification using the primer OPA-18 allowed the formation of four clusters named I, II, III and IV, which grouped 13.3%, 26.7%, 20% and 26.7% of the samples respectively. Clusters I and II were more related to each other with a similarity coefficient of approximately 90%. Cluster IV correlated to the others in approximately 70%. Samples 14 and 15 had a correlation to the other with a similarity coefficient of 65 and 87.6%, respectively. The values found were: SAB 0.73 ± 0.11, mean 73.86 and standard deviation of 11.08 (Fig. 1a).

The results of amplified products by the primer OPE-18 led to the formation of two clusters (I and II), which grouped 33.3% and 66.6% of the samples, respectively. The similarity coefficient between the two clusters was 93.3%. The values found were: SAB 0.96 ± 0.33, mean 96.82 and standard deviation of 3.33 (Fig. 1b).

Analyzing the clusters constructed by means of amplified products of the primer P4, the formation of clusters I and II, which grouped 13.3% and 73.33% of the samples, respectively, was observed. The similarity coefficient between the two clusters was 80%. Samples 14 and 1 correlated to the others with a coefficient of similarity of 78% and 63.1%, respectively. The values found were: SAB 0.74 ± 0.08, mean 74.63 and standard deviation 8.66 (Fig. 1c).

Table 2
Data from isolates of *C. tropicalis*, RAPD profiles and microsatellite genotypes.

Patients	Isolated	Date of isolation	MIC		RAPD			Microsatellites	
			AMB	FLU	OPA-18	OPE-18	P4	*URA3*	*CT14*
P1	1	21/06/2010	0.5	0.5	B	B	D	178:199	151:157
P2	2	20/07/2010	1	1	D	B	B	176:176	148:157
P3	3	22/09/2010	>16	0.5	B	B	A	172:172	151:154
P4	4	19/12/2010	1	1	B	A	B	172:172	148:148
P5	5	24/01/2011	0.5	1	B	A	B	172:172	148:148
P6	6	03/02/2011	1	0.25	A	A	B	172:172	145:151
P6	7	07/02/2011	0.5	4	A	B	A	172:172	145:151
P7	8	04/03/2011	0.5	2	C	A	B	172:172	145:145
P8	9	19/03/2011	1	0.5	D	B	B	172:172	145:151
P8	10	23/03/2011	0.5	2	D	B	B	172:172	148:154
P8	11	30/03/2011	1	1	C	A	B	172:172	145:151
P9	12	05/04/2011	0.5	1	C	B	B	172:172	154:154
P10	13	25/04/2011	1	1	D	B	B	172:172	154:154
P11	14	18/05/2011	1	0.5	F	B	C	172:191	148:148
P12	15	19/05/2011	1	1	E	B	B	172:172	145:151

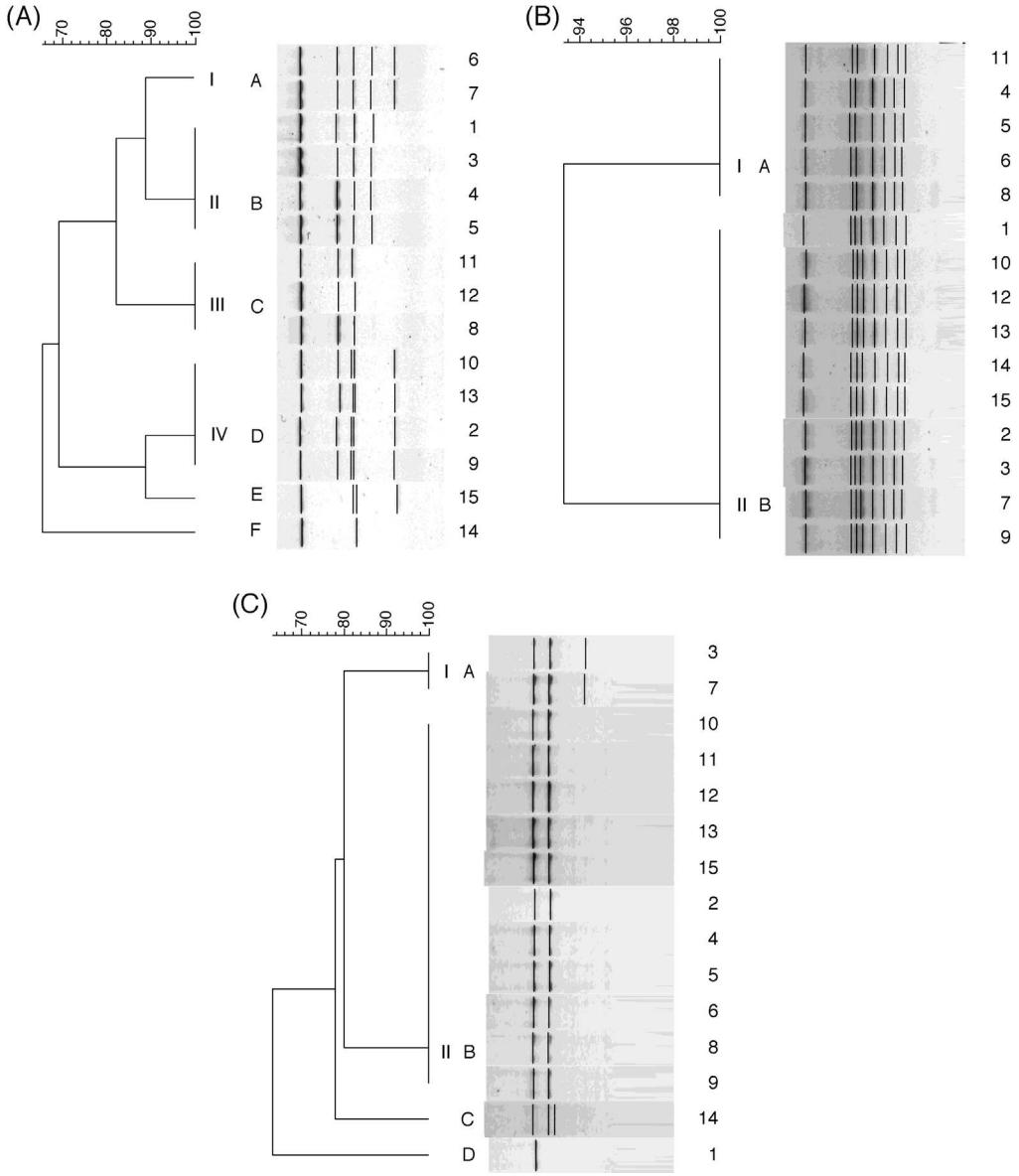

Fig. 1. Dendrogram of cluster isolates from candiduria by UPGMA determined by RAPD-PCR using *primers* (A) OPA-18, (B) OPE-18, and (C) P4. The similarity among genotypes was determined with the Dice coefficient and calculated by the BioNumerics software version 4.6. The roman algorithms (I–IV) represent the cluster, and the letters (A–F) represent profiles.

Microsatellite analysis

Amplified products from microsatellites were observed for all *C. tropicalis* isolates. The differences in size of the amplified fragments between the two markers are related to the different number of repeats of microsatellites. Isolates that showed two fragments were heterozygous and those that showed one fragment were homozygous.

A total of 10 alleles were observed for the 15 isolates, five for each of the *URA3* locus and *CT14*. Four allelic combinations were observed for the locus *URA3* and 8 for the locus *CT14* (Table 2).

Discriminatory power based on the Simpson index for the *URA3* marker was 0.372, and 0.86 for *CT14*, while combining the two markers the discriminatory power provided was 0.88.

Discussion

The occurrence of candiduria may be due to factors such as anatomical abnormalities in the urinary tract, comorbidities, urinary drainage devices, abdominal surgery, admission to the Intensive Care Unit (ICU), use of broad spectrum antibiotics, diabetes mellitus, increasing age and belonging to female gender.[1,25]

C. tropicalis has been featured among nosocomial infections as a global emerging pathogen among species of CNCA.[13] It is a commensal microorganism in the human gastrointestinal tract with the potential to cause invasive infections due to virulence factors, which have greater potential to spread and cause mortality in ICU patients than *C. albicans* or other CNCA species.[15,19,23]

Studies have demonstrated the need to perform antifungal susceptibility testing for *C. tropicalis*, because some isolates may present a resistance to antifungal agents.[15,26,32] However, the results of our study indicate that the isolates of *C. tropicalis* from urine were susceptible to the antifungal agents tested, except for one isolate which was resistant to amphotericin B. This profile may be related to microbiota established in this hospital or to the clinical and pathological profile of patients.

The analysis of RAPD-PCR allowed us to evaluate the genotypic profile of each isolate, making it possible to differentiate them genotypically. This method has been used for the study of clinical isolates of *Candida* spp., with the purpose of identification, correlation and phylogenetic analysis.[28,30] The primer OPA-18 was more discriminatory than OPE-18 and P4 for genotypic analysis by RAPD-PCR; however, all indicated similarity among isolates.

With regard to microsatellites, the marker *CT14* showed greater genetic difference between the isolates, as eight allelic combinations were observed. The combination of the two markers revealed nine different genotypes among the isolates, with the predominance of genotypes 172:172 and 145:151 for *URA3* and *CT14*, respectively. However, the discriminatory power (0.88) obtained from the combination of markers is not favorable to explain the typing results with confidence.[17]

The occurrence of two isolates in one patient with different genotypic profiles by RAPD-PCR and microsatellite techniques was observed. Genotypic variation of these isolates may be associated to the reproduction of microorganisms during the in vivo or in vitro growth. A considerable alteration in the MIC of the isolates from patient 6 (isolates 6 and 7) to fluconazole points out the importance of antifungal susceptibility testing and warns about the tendency of decreased sensitivity of CNCA species.

Nosocomial infections caused by *Candida* may be due to the presence of yeasts in the patient's own microflora (endogenous) or the transmission to the patient from the microbiota in health professionals, on inanimate surfaces, catheters and probes (exogenous).[9] The combination of the two techniques for genotyping revealed that isolates 4 and 5, from different patients and with different periods of hospitalization, had genotypic similarities, confirming the possibility of a spread, exogenously, of the infectious agent of a single strain from patient to patient. Thus, knowledge of the source of infection can help to prevent the spreading of resistant microorganisms and, therefore, help with the appropriate prophylactic treatment.

The results show the possibility of assessing the genetic similarity among isolates of *Candida* species involved in nosocomial infections, and comparing the genotypes among isolates from different sites, as already reported by other authors[3,10] which aids in the investigation of outbreaks. The RAPD-PCR and microsatellite techniques allowed us to visualize the genetic diversity and molecularly define the isolates, as it was observed by other authors who used these techniques of molecular genotyping to assess the genetic diversity of species of *Candida*.[3,10–12] This information regarding genetic diversity is important for the control and prevention of nosocomial infections of endogenous or exogenous origin caused by yeasts, especially those isolates of *C. tropicalis*, which have emerged as an isolated species of CNCA in hospital infections.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

Our thanks to Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) for their financial aid for the execution of this research, to Grande Dourados Federal University for its support, and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship.

References

1. Achkar JM, Fries BC. *Candida* infections of the genitourinary tract. Clin Microbiol Rev. 2010;23:253–7.
2. Alves IA, Camargo FP, Goulart LS. Identificação por PCR e sensibilidade a antifúngicos de isolados clínicos vaginais de *Candida* sp. Rev Soc Bras Med Trop. 2010;43:575–9.
3. Bautista-Muñoz C, Boldo XM, Villa-Tanaca L, Hernández-Rodríguez C. Identification of *Candida* spp. by randomly amplified polymorphic DNA analysis and differentiation between *Candida albicans* and *Candida dublinienses* by direct PCR methods. J Clin Microbiol. 2003;41:414–20.
4. Bukhary ZA. Candiduria: a review of clinical significance and management. Saudi J Kidney Dis Transpl. 2008;19:350–60.
5. Chi HW, Yang YS, Shang ST, Chen KH, Yeh KM, Chang FY, et al. *Candida albicans* versus non-*albicans* bloodstream infections: the comparison of risk factors and outcome. J Microbiol Immunol Infect. 2011;44:369–75.
6. Chong PP, Lee YL, Tan BC, Ng KP. Genetic relatedness of *Candida* strains isolated from women with vaginal candididasis in Malaysia. J Med Microbiol. 2003;52:657–66.
7. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard M27-A3. 3rd ed. Wayne: Clinical and Laboratory Standards Institute; 2008.
8. Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution Antifungal Susceptibility testing of yeasts; 3rd Informational Supplement, M27-S3. Wayne: Clinical and Laboratory Standards Institute; 2008.
9. Colombo AL, Guimarães T. Epidemiologia das infecções hematogênicas por *Candida* spp. Rev Soc Bras Med Trop. 2003;36:599–607.
10. Desnos-Ollivier M, Bretagne S, Bernède C, Robert V, Raoux D, Chachaty E, et al. Clonal population of flucytosine-resistant *Candida tropicalis* from blood cultures, Paris, France. Emerg Infect Dis. 2008;14:557–65.
11. Durán EL, Mujica MT, Jewtuchowicz VM, Finquelievich JL, Pinoni MV, Iovannitti CA. Estudio de la variabilidad genetic entre aislamientos clínicos de *Candida albicans* formadores de biopelículas. Rev Iberoam Micol. 2007;24:268–71.
12. Escribano P, Rodríguez-Créixems M, Sánchez-Carrillo C, Muñoz P, Bouza E, Guinea J. Endemic genotypes of *Candida albicans* causing fungemia are frequent in the hospital. J Clin Microbiol. 2013;51:2118–23.
13. Falagas ME, Roussos N, Vardakas KZ. Relative frequency of *albicans* and the various non-*albicans Candida* spp. among candidemia isolates from inpatients in various parts of the world: a systematic review. Int J Infect Dis. 2010;14:954–66.

14. Fraisse T, Crouzet J, Lachaud L, Durand A, Charachon S, Lavigne JP, et al. Candiduria in those over 85 years old: a retrospective study of 73 patients. Intern Med. 2011;50:1935–40.
15. Freitas AR, Baeza LC, Faria MGI, Dota KFD, Martínez PG, Svidzinski TIE. Yeasts isolated from nosocomial urinary infections: antifungal susceptibility and biofilm production. Rev Iberoam Micol. 2014;31:104–8.
16. Gürcüoğlu E, Akalın H, Ener B, Ocakoğlu G, Sınırtaş M, Akçağlar S, et al. Nosocomial candidemia in adults: risk and prognostic factors. J Med Mycol. 2010;20:269–78.
17. Hunter PR, Gaston MA. Numerical index of the discriminatory of typing systems: an application of Simpson's index of diversity. J Clin Microbiol. 1988;26:2465–6.
18. Jain M, Dogra V, Mishra B, Thakur A, Loomba PS, Bhargava A. Candiduria in catheterized intensive care unit patients: emerging microbiological trends. Indian J Pathol Microbiol. 2011;54:552–5.
19. Kothavade RJ, Kura MM, Valand AG, Panthaki MH. *Candida tropicalis*: its prevalence, pathogenicity and increasing resistance to fluconazole. J Med Microbiol. 2010;59:873–80.
20. Li YL, Leaw JH, Chen H, Chang HC, Chang TC. Rapid identification of yeasts commonly found in positive blood cultures by amplification of the internal transcribed spacer regions 1 and 2. Eur J Clin Microbiol Infect Dis. 2003;22:693–6.
21. Marra AR, Camargo LFA, Pignatari ACC, Sukiennik T, Behar PRP, Medeiros EAS, et al., Brazilian scope study group. Nosocomial bloodstream infections in Brazilian hospitals: analysis of 2563 cases from a prospective nationwide surveillance study. J Clin Microbiol. 2011;49:1866–71.
22. Negri M, Martins M, Henriques M, Svidzinski TIE, Azeredo J, Oliveira R. Examination of potencial virulence factors of *Candida tropicalis* clinical isolates from hospitalized patients. Mycopathologia. 2010;169:175–82.
23. Negri M, Silva S, Breda D, Henriques M, Azeredo J, Oliveira R. *Candida tropicalis* biofilms: effect on urinary epithelial cells. Microb Pathog. 2012;53:95–9.
24. Ozhak-Baysan B, Ogunc D, Colak D, Ongut G, Donmez L, Vural T, et al. Distribution and antifungal susceptibility of *Candida* species causing nosocomial candiduria. Med Mycol. 2012;50:529–32.
25. Paul N, Mathai E, Abraham OC, Michael JS, Mathai D. Factors associated with candiduria and related mortality. J Infect. 2007;55:450–5.
26. Pfaller MA, Castanheira M, Messer SA, Moet GJ, Jones RN. Echinocandin and triazole antifungal susceptibility profiles for *Candida* spp., *Cryptococcus neoformans*, and *Aspergillus fumigatus*: application of new CLSI clinical breakpoints and epidemiologic cutoff values to characterize resistance in the SENTRY Antimicrobial Surveillance Program (2009). Diag Microbiol Infect Dis. 2011;69:45–50.
27. Pfaller MA, Diekema DJ, Jones RN, Sader HS, Fluit AC, Hollis RJ, et al., SENTRY participant group. International surveillance of bloodstream infections due to *Candida* species: frequency of occurrence and *in vitro* susceptibilities to fluconazole, ravuconazole and voriconazole of isolates collected from 1997 through 1999 in the SENTRY antimicrobial surveillance program. J Clin Microbiol. 2001;39:3254–9.
28. Rocha BA, Negro GM, Yamamoto L, Souza MV, Precioso AR, Okay TS. Identification and differentiation of *Candida* species from pediatric patients by random amplified polymorphic DNA. Rev Soc Bras Med Trop. 2008;41:1–5.
29. Soll DR. The ins and outs of DNA fingerprinting the infectious fungi. Clin Microbiol Rev. 2000;13:332–70.
30. Tay ST, Na SL, Chong J. Molecular differentiation and antifungal susceptibilities of *Candida parapsilosis* isolated from patients with bloodstream infections. J Med Microbiol. 2009;58:185–91.
31. Tortorano AM, Pen J, Bernhardt H, Klingspor L, Kibbler CC, Faure O, et al, ECMM Working Group on Candidaemia Epidemiology of candidaemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. Eur J Clin Microbiol Infect Dis. 2004;23:317–22.
32. Tortorano AM, Rigoni AL, Biraghi E, Prigitano A, Viviani MA, FIMUA-ECMM Candidemia Study Group. The European Confederation of Medical Mycology (ECMM) survey of candidemia in Italy: antifungal susceptibility patterns of 261 non-*albicans Candida* isolates from blood. J Antimicrob Chemother. 2003;52:679–82.
33. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis. 2004;39:309–17.
34. Wynn JL, Tan S, Gantz MG, Das A, Goldberg RN, Adams-Chapman I, et al., NICHD Neonatal Research Network. Outcomes following candiduria in extremely low birth weight infants. Clin Infect Dis. 2012;54:331–9.
35. Yamamoto ACA, Paula CR, Dias LB, Tadano T, Martins ERM, Amadio JVRS, et al. Epidemiological and clinical characteristics of nosocomial candidiasis in university hospitals in Cuiabá – Mato Grosso, Brazil. Rev Iberoam Micol. 2012;29:164–8.
36. Yang YL, Wang AH, Wang CW, Cheng WT, Li SY, Lo HJ. Suscetibilities to amphotericin B and fluconazole of *Candida* species in Taiwan surveillance of antimicrobial resistance of yeasts 2006. Diag Microbiol Infect Dis. 2008;61:175–80.
37. Yarrow D. Methods for the isolation, maintenance and identification of yeasts. In: Kurtzman CP, Fell JW, editors. The yeast, a taxonomic study. New York: Elsevier; 1998. p. 77–99.