



BRIEF REPORT

Genotype, antifungal susceptibility, and biofilm formation of *Trichosporon asahii* isolated from the urine of hospitalized patients



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Received 2 July 2015; accepted 11 November 2015

Available online 22 February 2016

KEYWORDS

Urinary tract
infection;
Trichosporon asahii;
Molecular
identification;
Maximum parsimony

Abstract In this study, the culture analysis of urine samples from patients hospitalized in the Central-West region of Brazil was performed, and the isolated microorganisms were phylogenetically identified as *Trichosporon asahii*. Maximum parsimony analysis of the IGS1 sequences revealed three novel genotypes that have not been described. The minimum inhibitory concentrations of the nine isolates identified were in the range of 0.06–1 µg/ml for amphotericin B, 0.25–4 µg/ml for fluconazole, and 0.03–0.06 µg/ml for itraconazole. Approximately 6/9 of the *T. asahii* isolates could form biofilms on the surface of polystyrene microplates. This study reports that the microorganisms isolated here as *T. asahii* are agents of nosocomial urinary tract infections. Furthermore, the IGS1 region can be considered a new epidemiological tool for genotyping *T. asahii* isolates. The least common genotypes reported in this study can be related to local epidemiological trends.

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PALABRAS CLAVE

Infección del tracto
urinario;
Trichosporon asahii;

Genotipo, sensibilidad a los antifúngicos y formación de *biofilm* de aislados de *Trichosporon asahii* recuperados de la orina de pacientes hospitalizados

Resumen En este estudio fueron analizadas mediante el cultivo muestras de orina de pacientes hospitalizados en la región centro-oeste de Brasil; los microorganismos aislados fueron

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Identificación molecular;
Máxima parsimonia

identificados filogenéticamente como *Trichosporon asahii*. A través del análisis de máxima parsimonia de las secuencias de IGS1, fueron encontrados 3 genotipos que no habían sido descritos anteriormente. Las concentraciones inhibitorias mínimas frente a los 9 aislados identificados presentaron un rango de 0,06-1 µg/ml en el caso de la anfotericina B, de 0,25-4 µg/ml en el del fluconazol, y de 0,03-0,06 µg/ml en el del itraconazol. Aproximadamente 6/9 de los aislados de *T. asahii* formaron biopelículas en la superficie de microplacas de poliestireno. Este trabajo documenta el aislamiento de *T. asahii* como agente causal de infecciones urinarias nosocomiales. Además, demuestra que la región IGS1 puede ser considerada una nueva herramienta epidemiológica para la genotipificación de los aislados de *T. asahii*. Los genotipos menos comunes encontrados en este estudio pueden estar relacionados con las características epidemiológicas locales.

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Trichosporon causes superficial and systemic nosocomial infections^{2,11}. These yeasts have been found to be the causative pathogens of white piedra, and opportunistic pathogens in invasive infections in immunocompromised patients in tertiary hospitals worldwide². However, the urinary tract infection caused by *Trichosporon asahii* is the most prevalent, regardless of the geography^{6,7,16}.

The objective of this study was to investigate the genotypes, antifungal susceptibility, and biofilm formation of *T. asahii* isolates obtained from urine of patients admitted to the University Hospital, Federal University of Grande Dourados, Dourados, Central-West Brazil.

Between October 2010 and January 2011, 18 patients admitted to this hospital were diagnosed with urinary tract infection (mainly fungal on the basis of clinical and laboratory findings). Nine (9) pure *T. asahii* isolates with >10⁵ colony-forming units per millilitre were obtained from 6 of these 18 patients (33%). These isolates were analyzed phenotypically and confirmed by using molecular techniques. A remarkable observation is the large number of urinary tract infection cases that were identified over the span of four months.

The purity of the cultures was confirmed by growth on CHROM agar *Candida*[®] (Difco[®], Sparks, MD, USA) and yeasts were identified in accordance with the classical methodology based on macroscopic, microscopic, and biochemical characteristics⁹.

DNA sequencing was performed to confirm identification: *Trichosporon* DNA was extracted using the method proposed by Chong *et al.*³. To accurately identify the *Trichosporon* strains to species level, the IGS1 region was amplified by PCR (ABI 3730 XL; Applied Biosystems, Foster City, CA) and purified using the ABI PRISM Big Dye Terminator Cycle Sequencing Kit, according to the manufacturer's instructions. The primers used were 26SF (5'-ATCCTTTGCAGACGACTTGA-3') and 5SR (5'-AGCTTGACTTCGCAGATCGG-3'), and sequencing was performed according to Sugita *et al.*'s method¹⁴.

Molecular epidemiology markers were assessed by *T. asahii* genotyping and phylogenetic analysis. The IGS1 sequences of the *T. asahii* strains determined in this study were compared with DNA sequences deposited in GenBank. All phylogenetic analyses were performed using the Mega 6 software. The phylogenetic tree was constructed using the unweighted pair group method with average linkages². The

phylogram stability was assessed by parsimony bootstrapping with 1000 simulations.

The antifungal susceptibility profile was determined by broth microdilution, in accordance with the M27-A3 document of the Clinical and Laboratory Standards Institute⁴, testing the following antifungals: amphotericin B (Bristol – Myers Squibb), itraconazole (Janssen), and fluconazole (Pfizer).

Biofilm production was evaluated on the surfaces of polystyrene microplates using the method described by Shin *et al.*¹².

T. asahii seems to be the most common species isolated from the urine of hospitalized patients in Brazil^{5,13}, followed by *Trichosporon inkin* and *Trichosporon asteroides*. In addition, *T. asahii* is the most common species identified in disseminated trichosporonosis⁵.

The nine isolates were phylogenetically identified as *T. asahii* and the maximum parsimony analysis of the IGS1 sequence revealed three novel genotypes that have not been yet described in Brazil and worldwide (Fig. 1), which were named as genotype 13 (GenBank accession numbers: KJ176987, KJ176988, KJ176993, KJ176994), 14 (accession number: KJ176992), and 15 (accession number: KJ176995). The other three were identified as genotype 10 (accession numbers: KJ176991 and KJ176990) and 11 (accession number: KJ176989).

Interestingly, genotyping showed mixed infections by two *T. asahii* genotypes: isolates 2 (genotype 13) and 4 and 5 (genotype 10) were recovered from the same patient and, similarly, isolates 6 (genotype 14) and 9 (genotype 15) were recovered from another patient. However, this phenomenon was reported earlier¹⁶.

Currently, there are 12 known genotypes of *T. asahii*¹⁷, however, in Brazil, the vast majority (>80%) of the isolates genotyped so far belongs to genotype 1, followed by genotypes 3, 4, and 6^{2,5}. Our findings may indicate the identification of novel genotypes that are not so common in Brazilian hospitals. In fact, further studies need to be conducted considering the characteristics of each hospital and the geography of Brazil. To the best of our knowledge, this is the first documentation of the molecular characterization of *Trichosporon* isolates identified in the Brazilian countryside.

The minimal inhibitory concentration (MIC) of the nine isolates was in the range of 0.06–1 µg/ml for amphotericin

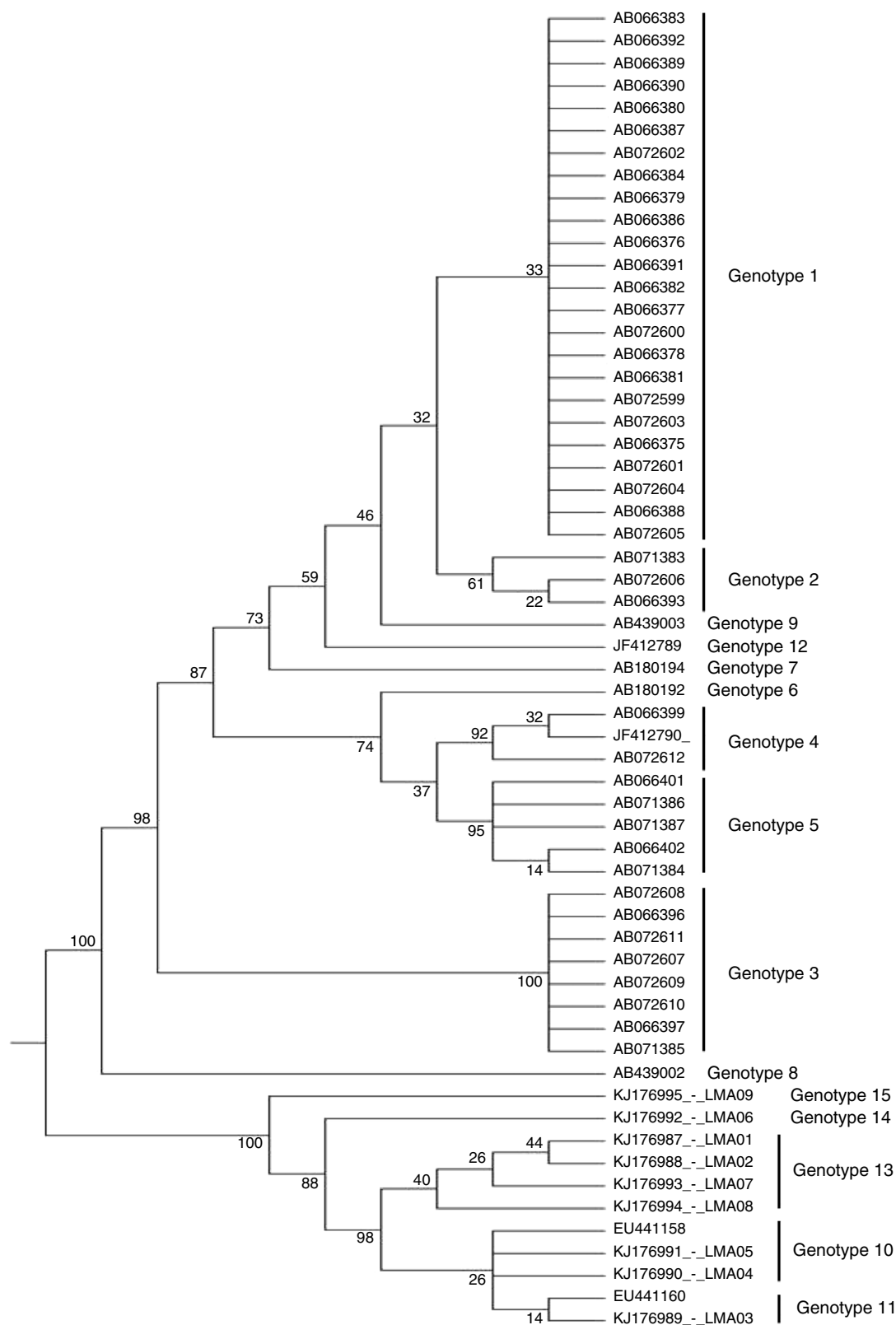


Figure 1 Molecular phylogenetic tree of *T. asahii* strains based on confidently aligned IGS1 sequences, including those from reference strains and the novel genotype strains found in Brazil. The code numbers correspond to the GenBank accession numbers. The values were obtained using maximum parsimony cluster analysis and 1000 bootstrap simulations.

Table 1 Results from the nine isolates of *T. asahii*

Isolates	Patients ^a	Antifungal susceptibility ($\mu\text{g/ml}$)			Biofilm ^b	Genotype
		AMB	FLU	ITZ		
1	MLC	0.25	2	0.03	1+	13
2	MNS	0.5	2	0.03	1+	13
3	LC	1	0.5	0.03	1+	11
4	MNS	1	0.5	0.03	Negative	10
5	MNS	0.06	0.5	0.03	1+	10
6	PSL	1	0.25	0.03	Negative	14
7	ASP	0.25	4	0.03	1+	13
8	SMS	0.25	4	0.03	2+	13
9	PSL	0.25	4	0.03	Negative	15

AMB, Amphotericin B; FLU, Fluconazole; ITZ, Itraconazole.

^a Initials of the patients' names.

^b The adherent biofilm layer was scored as either negative or weakly (1+), moderately (2+ or 3+), or strongly (4+) positive¹².

B, 0.25–4 $\mu\text{g/ml}$ for fluconazole, and 0.03–0.06 $\mu\text{g/ml}$ for itraconazole (Table 1). There are no defined criteria to determine the cutoff point in order to establish the susceptibility profile of *Trichosporon*. Interestingly, the MIC was low for all of our isolates for the antifungal agents tested, contradicting other studies on *T. asahii* susceptibility to triazoles and amphotericin B^{8,10}.

The ability to form biofilm on the surfaces of polystyrene microplates was observed in 6/9 of the isolates of *T. asahii* (Table 1), though the degree of biofilm formation was small: 1+ (55.5%) and 2+ (11.1%). *Trichosporon* spp. are known to form biofilms^{1,15}.

In conclusion, this study reports that the *T. asahii* microorganisms isolated here are agents of nosocomial urinary tract infections. This is the first study in the Central-West region of Brazil that identified novel genotypes not been previously described. Moreover, different genotypes were isolated from the same patient. The IGS1 region may be an epidemiological tool for genotyping *T. asahii* isolates. The least common genotypes reported in our study can be related to local epidemiological trends.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the responsible Clinical Research Ethics Committee and in accordance with those of the World Medical Association and the Helsinki Declaration.

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgements

This work was supported by Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

- Bonaventura GD, Pompilio A, Picciani C, Iezzi M, D'Antonio D, Piccolomini R. Biofilm formation by the emerging fungal pathogen *Trichosporon asahii*: development, architecture, and antifungal resistance. *Antimicrob Agents Chemother*. 2006;50:3269–76.
- Chagas-Neto TC, Chaves GM, Melo ASA, Colombo AL. Bloodstream infections due to *Trichosporon* spp.: species distribution, *Trichosporon asahii* genotypes determined on the basis of ribosomal DNA intergenic spacer 1 sequencing, and antifungal susceptibility testing. *J Clin Microbiol*. 2009;47:1074–81.
- Chong PP, Lee YL, Tan BC, Ng KP. Genetic relatedness of *Candida* strains isolated from women with vaginal candidiasis in Malaysia. *J Med Microbiol*. 2003;2:657–66.
- Clinical and Laboratory Standards Institute, editor. Reference method for broth dilution antifungal susceptibility testing of yeasts, vol. M27-A3, 3rd ed. Wayne: CLSI; 2008.
- Colombo AL, Padovan AC, Chaves GM. Current knowledge of *Trichosporon* spp. and Trichosporonosis. *Clin Microbiol Rev*. 2011;24:682–700.
- Gómez-Camarasa C, Liébana-Martos C, Navarro-Marí JM, Gutiérrez-Fernández J. Detection of unusual uropathogens during a period of three years in a regional hospital. *Rev Esp Quimioter*. 2015;28:86–91.
- Iturrieta-González IA, Padovan ACB, Bizerra FC, Hahn RC, Colombo AL. Multiple species of *Trichosporon* produce biofilms highly resistant to triazoles and amphotericin B. *PLOS ONE*. 2014;9:e109553.
- Kalkanci A1, Sugita T, Arikan S, Yucesoy M, Ener B, Otag F, Kiraz N, Kustimur S, Sancak B, Evcı C, Emektas G. Molecular identification, genotyping, and drug susceptibility of the basidiomycetous yeast pathogen *Trichosporon* isolated from Turkish patients. *Med Mycol*. 2010;48:141–6.
- Larone DH, editor. *Medically important fungi*. 5th ed. Washington: American Society for Microbiology; 2011.
- Mekha N, Sugita T, Ikeda R, Nishikawa A, Autthateinchai R, Poonwan N, Sawanpanyalert P. Genotyping and antifungal drug

- susceptibility of the pathogenic yeast *Trichosporon asahii* isolated from Thai patients. *Mycopathologia*. 2010;169:67–70.
11. Otero V, Galán-Sánchez F, García-Agudo L, García-Tapia AM, Guerrero-Lozano I, Rodríguez-Iglesias MA. Fungemia due to *Trichosporon asahii* in a patient with hematological malignancy. *Rev Iberoam Micol*. 2015;32:59–61.
 12. Shin JH, Kee1 SJ, Shin MG, Kim SH, Shin DH, Lee1 SK, Suh SP, Ryang DW. Biofilm production by isolates of *Candida* species recovered from nonneutropenic patients: comparison of bloodstream isolates with isolates from other sources. *J Clin Microbiol*. 2002;40:1244–8.
 13. Silvestre-Junior AM, Miranda MABR, Camargo ZP. *Trichosporon* species isolated from the perigenital region, urine and catheters of a Brazilian population. *Braz J Microbiol*. 2010;41: 628–34.
 14. Sugita T, Nakajima M, Ikeda R, Matsushima T, Shinoda T. Sequence analysis of the ribosomal DNA intergenic spacer 1 regions of *Trichosporon* species. *J Clin Microbiol*. 2002;40: 1826–30.
 15. Sun W, Su J, Xu S, Yan D. *Trichosporon asahii* causing nosocomial urinary tract infections in intensive care unit patients: genotypes, virulence factors and antifungal susceptibility testing. *J Med Microbiol*. 2012;61:1750–7.
 16. Taverna CG, Córdoba S, Murisengo OA, Vivot W, Davel G, Bosco-Borgeat ME. Molecular identification, genotyping, and antifungal susceptibility testing of clinically relevant *Trichosporon* species from Argentina. *Med Mycol*. 2014;52:356–66.
 17. Xia Z, Yang R, Wang W, Cong L. Genotyping and antifungal drug susceptibility of *Trichosporon asahii* isolated from Chinese patients. *Mycopathologia*. 2012;173:127–33.