



BRIEF REPORT

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



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### KEYWORDS

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**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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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<sup>2,11</sup>. 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 worldwide<sup>2</sup>. However, the urinary tract infection caused by *Trichosporon asahii* is the most prevalent, regardless of the geography<sup>6,7,16</sup>.

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 >10<sup>5</sup> 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 characteristics<sup>9</sup>.

DNA sequencing was performed to confirm identification: *Trichosporon* DNA was extracted using the method proposed by Chong *et al.*<sup>3</sup>. 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'-ATCCTTTGCGACGACTTGA-3') and 5SR (5'-AGCTTGACTTCGCAGATCGG-3'), and sequencing was performed according to Sugita *et al.*'s method<sup>14</sup>.

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 linkages<sup>2</sup>. 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 Institute<sup>4</sup>, 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.*<sup>12</sup>.

*T. asahii* seems to be the most common species isolated from the urine of hospitalized patients in Brazil<sup>5,13</sup>, followed by *Trichosporon inkin* and *Trichosporon asteroides*. In addition, *T. asahii* is the most common species identified in disseminated trichosporonosis<sup>5</sup>.

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 earlier<sup>16</sup>.

Currently, there are 12 known genotypes of *T. asahii*<sup>17</sup>, however, in Brazil, the vast majority (>80%) of the isolates genotyped so far belongs to genotype 1, followed by genotypes 3, 4, and 6<sup>2,5</sup>. 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

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