Araújo de Almeida, Adriana; do Amaral Crispim, Bruno; Barufatti Grisolia, Alexéia; Estivalet Svidzinski, Terezinha Inez; Gonçalvez Ortolani, Lais; Pires de Oliveira, Kelly Mari

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BRIEF REPORT

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

Adriana Araújo de Almeida a, Bruno do Amaral Crispim b, Alexéia Barufatti Grisolía c, Terezinha Inez Estivalet Svidzinski d, Lais Gonçalvez Ortolani a, Kelly Mari Pires de Oliveira a, *

a Faculty of Health Sciences, Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil
b Faculty of Exact Sciences and Technology, Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil
c Faculty of Biological and Environmental Sciences, Federal University of Grande Dourados, Dourados, Mato Grosso do Sul, Brazil
d Department of Clinical Analyses, State University of Maringá, Maringá, Paraná, Brazil

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**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...
**Trichosporon asahii** isolated from urine and biofilm

Identificación molecular; Máxima parsimonia

*Trichosporon* causa infecciones superficiales y sistémicas nosocomiales.\(^2\)\(^-\)\(^1\(^1\)\) Estos levaduras han sido encontradas como agentes causales de infecciones en pacientes hospitalizados en todo el mundo.\(^2\) Sin embargo, las especies de *Trichosporon* con enzimas responsables de infecciones en pacientes con inmunodepresión son las más prevalentes, a pesar de la geografía.\(^6\)\(^-\)\(^7\)\(^-\)\(^1\(^6\)\)

El objetivo de este estudio fue investigar el genotipo, la susceptibilidad antifúngica y la identificación de *Trichosporon asahii* y de otras especies.\(^3\)Seis de 18 pacientes admitidos en el Hospital Universidad Federal de Grande Dourados, Dourados, Central-West del Brasil estuvieron infectados con *Trichosporon*.

Entre octubre de 2010 y enero de 2011, 18 pacientes admitidos en este hospital fueron diagnosticados con infecciones urinarias (infecciones urinarias). Nine (9) de los 18 pacientes estaban infectados por *Trichosporon asahii* con más de 10\(^5\) colonies forming units/ml. Se obtuvieron de 6 de estos 18 pacientes (33%). Estos isolados fueron analizados fenotípicamente y confirmados usando técnicas moleculares. Se encontraron una serie de infecciones urinarias que identificaron durante el periodo de cuatro meses.

La pureza de las culturas se confirmó creciendo a partir de CHROM agar *Candida* (Difco\(^a\), Sparks, MD, USA) y levaduras fueron identificadas de acuerdo con el método clásico de macroscópico, microscópico y bioquímico características.\(^6\)

La secuenciación de DNA fue determinada usando la técnica propuesta por Choong et al.\(^1\)\(^1\) \(^1\) para *Trichosporon* identificar la secuencia de *Trichosporon* a nivel de especie, la región IGS1 región amplificada por PCR (ABI 3730 XL: Applied Biosystems, Foster City, CA). Se identificó que el *Trichosporon* se consideran genotipos de *Trichosporon asahii* y *Trichosporon asteroides*.

Molecular epidemiology markers were assessed by *Trichosporon asahii* genotyping and phylogenetic analysis. The IGS1 sequences of the *Trichosporon 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.\(^6\) 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,\(^8\) determining 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.\(^1\)\(^2\)

*Trichosporon asahii* seems to be the most common species isolated from the urine of hospitalized patients in Brazil.\(^5\)\(^-\)\(^1\(^3\)\) isolated by *Trichosporon asahii* and *Trichosporon asteroides*. In addition, *Trichosporon asahii* is the most common species identified in disseminated trichosporonosis.\(^3\)

The nine isolates were phylogenetically identified as *Trichosporon asahii* and the maximum parsimony analysis of the IGS1 sequence revealed three novel genotypes that have not been yet described in Brazil and worldwide.\(^1\)\(^7\)\(^-\)\(^1\(^1\)\) 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 *Trichosporon 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.\(^1\)\(^6\)

Currently, there are 12 known genotypes of *Trichosporon asahii*\(^1\)\(^7\), however, in Brazil, the vast majority (~80%) of the isolates genotyped so far belongs to genotype 1, followed by genotypes 3, 4, and 6.\(^2\)\(^-\)\(^3\) 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 asahii* 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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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*

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Patientsa</th>
<th>Antifungal susceptibility (µg/ml)</th>
<th>Biofilmb</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AMB</td>
<td>FLU</td>
<td>ITZ</td>
</tr>
<tr>
<td>1</td>
<td>MLC</td>
<td>0.25</td>
<td>2</td>
<td>0.03</td>
</tr>
<tr>
<td>2</td>
<td>MNS</td>
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<td>2</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>LC</td>
<td>1</td>
<td>0.5</td>
<td>0.03</td>
</tr>
<tr>
<td>4</td>
<td>MNS</td>
<td>1</td>
<td>0.5</td>
<td>0.03</td>
</tr>
<tr>
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<td>MNS</td>
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<td>0.5</td>
<td>0.03</td>
</tr>
<tr>
<td>6</td>
<td>PSL</td>
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<td>0.25</td>
<td>0.03</td>
</tr>
<tr>
<td>7</td>
<td>ASP</td>
<td>0.25</td>
<td>4</td>
<td>0.03</td>
</tr>
<tr>
<td>8</td>
<td>SMS</td>
<td>0.25</td>
<td>4</td>
<td>0.03</td>
</tr>
<tr>
<td>9</td>
<td>PSL</td>
<td>0.25</td>
<td>4</td>
<td>0.03</td>
</tr>
</tbody>
</table>

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+) positive.

B, 0.25–4 µg/ml for fluconazole, and 0.03–0.06 µg/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.

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.

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.

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