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Molecular identification, genotyping, and antifungal susceptibility testing of clinically relevant *Trichosporon* species from Argentina

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Abstract

Trichosporon species are emerging causative agents of mycoses; most are documented in immunocompromised patients. Species identification is important for epidemiological purposes in order to better define species clinical associations and to improve antifungal treatment. Here, we studied a collection of 41 *Trichosporon* strains recovered from hospitalized patients in Argentina. All strains were identified by sequencing the D1/D2 domain of 26S, internal transcribed spacer (ITS) regions, and intergenic spacer 1 (IGS1) region. In addition, we determined the IGS1 region genotypes of the suspected *T. asahii* strains. Antifungal susceptibility of all strains was investigated. Thirty-eight of the 41 strains in this study were identified as follows: 29 *T. asahii*, 3 *T. inkin*, 3 *T. montevideense*, 2 *T. faecale,* and 1 *T. dermatis*. The identity of the three remaining strains could not be confirmed. Strain DMic 114126 (Culture collection of the Mycology Department (DMic), National Institute of Infectious Diseases "Dr. Carlos G. Malbrán".) may represent a *T. asahii* subspecies or a new *Trichosporon* species, strain DMic 94750 was identified as *T.* cf. *guehoae* and strain DMic 114132 as *T.* cf. *akiyoshidainum*. The distribution of *T. asahii* genotypes was as follows: 12 genotype 3, 9 genotype 1, 4 genotype 4, 2 genotype 5, and 2 genotype 7. Amphotericin B minimal inhibitory concentrations (MICs) were ≤1 mg/l for 78% (32/41) of the strains. Fluconazole MICs were ≥2 mg/l for 90% of the strains. However, itraconazole, voriconazole, ketoconazole, and posaconazole MICs were ≤1 mg/l for 100% of the strains. Terbinafine MICs were ≤1 mg/l for 98% 40/41 of the strains.

Key words: IGS1, genotyping, *Trichosporon*, identification, sequencing.

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Introduction

Trichosporon spp. are basidiomycetous, yeast-like anamorphic organisms that are widely distributed in nature. These fungal species can colonize different parts of the human body, including the gastrointestinal tract, oral cavity, respiratory tract, skin, and the vagina and they can cause deep-seated, mucosa-associated infections or superficial infections [1]. Deep-seated infections due to *Trichosporon* spp. are emerging mycoses that are usually documented in patients with hematological malignancies or other medical conditions associated with immunosuppression. Also, these infections are commonly associated with central venous catheters, urinary catheters, and peritoneal catheter-related devices [1]. Infection outcome has very poor prognosis and, usually, high mortality rates [2–8].

Because the susceptibility profile can vary according to species, *Trichosporon* spp. identification is important to aid in epidemiological purposes, to better define a species clinical association, and to improve antifungal treatment [1,3,9,10]. Several molecular studies have shown that identification of *Trichosporon* spp. by conventional methods is often difficult and frequently inconclusive [10–12]. The use of molecular techniques to clarify and identify *Trichosporon* spp. modified the taxonomy of the genus [13–19]. Thereafter, numerous new species have been described [12,20–26]. More recently, the matrix-assisted, laser desorption, ionization time-of-flight mass spectrometry (MALDI-TOF/MS) technique has demonstrated good potential for identification and classification of medically relevant species of arthroconidial yeasts, including *Trichosporon* species [27]. The latest edition of *The Yeasts, A Taxonomic Study* listed 37 *Trichosporon* species [28]. Furthermore, a review of the genus informed the existence of 50 species in the *Trichosporon* genus [1], including 16 that are clinically relevant. Recently, *T. vanderwaltii* sp. nov. was added to the long list of *Trichosporon* species and raised to 51 the number of members of the genus [29].

Today, ribosomal DNA sequence analysis is the molecular technique that is most frequently used to identify and classify fungal pathogens [30,31]. The analyses of the internal transcribed spacer (ITS) regions and D1/D2 domain have shown that conspecific strains have a difference of $<1\%$ in their sequences and that the similarity between different species is usually $<99\%$. However, some phylogenetically closely related species have shown similarity of $\geq 99\%$, making it difficult to differentiate between them [32,33]. On the other hand, intergenic spacer 1 (IGS1) region sequence analysis has been demonstrated to be superior compared with ITS regions or D1/D2 domain sequence analyses in differentiating phylogenetically closely related species [15]. Moreover, IGS1 region sequence analysis also has shown good potential as an epidemiological tool. A correlation between the IGS1 genotype and the geographical substructure of *T. asahii* isolates has been suggested [3,10,11,34–36]. Currently, nine *T. asahii* genotypes have been reported and/or submitted to GenBank [15,34,35,37].

The correct characterization of *Trichosporon* species can be significant to the improvement of antifungal treatment because *T. asahii* seems to be more resistant *in vitro* to amphotericin B (AMB) than triazole compounds. In addition, the non–*T. asahii* species seem to be more resistant *in vitro* to triazole agents compared with AMB [10,38,39].

Trichosporon species are uncommon pathogens, and not much is known about their distribution worldwide. In Argentina, almost no data about species distribution, genotypic and phylogenetic diversity, and antifungal susceptibilities of *Trichosporon* pathogens exist.

In this work, we studied a collection of 41 Argentinean *Trichosporon* strains. Species were identified using sequence analyses of the D1/D2 domain of 26S, the ITS regions, and the IGS1 region. This last region was also used for genotyping of the *T. asahii* strains. In addition, we performed a phylogenetic analysis of the ITS regions plus D1/D2 domain sequences of all strains in order to study their relationships. An additional phylogenetic analysis of the IGS1 region sequences of all *T. asahii* and *T. faecale* strains was performed in order to study the relationships between their genotypes. We also performed antifungal susceptibility tests of seven antifungal drugs commonly used in clinical treatment.

Materials and methods

Strains and clinical source

Forty-one *Trichosporon* strains, which belong to the culture collection of the Mycology Department (DMic) of the National Institute of Infectious Diseases "Dr Carlos G. Malbrán," were analyzed. Strains were recovered from 39 patients hospitalized in 22 hospitals and health institutions at 11 localities in Argentina from 1988 to 2011. Isolates were recovered from the following: 12, blood; 10, urine; 3, skin; 3, bronchial lavage; 2, nail of hand; 1, blood catheter; 1, open fracture; 1, bile; 1, throat; 1, vitreous humor; 1, subxiphoid puncture; 1, biopsy of skin lesion; and 4, unknown clinical sources (Table 1). Strains were checked for purity and viability, and genus identity was confirmed using standard phenotypic methods [28].

DNA extraction

DNA extraction was performed according to the method reported by Möller et al. [40], modified as previously described [41]. The DNA was stored at −20°C.

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Table 1. *Trichosporon* strain characteristics.

Strain no.	Clinical source	Molecular identification (IGS1 genotype)	D1/D2 domain GenBank accession no.[a]	ITS regions GenBank accession no.[a]	IGS1 region GenBank accession no.[a]
DMic 88201	Unknown	*T. asahii* (1)	JX476272	JX476281	JX476292
DMic 951503	Skin	*T. asahii* (1)	JX476272	JX476281	JX476292
DMic 114122[b]	Blood	*T. asahii* (1)	JX476272	JX476281	JX476292
DMic 114124	Blood	*T. asahii* (1)	JX476272	JX476281	JX476292
DMic 114125	Blood	*T. asahii* (1)	JX476272	JX476281	JX476292
DMic 114129[c]	Catheter	*T. asahii* (1)	JX476272	JX476281	JX476292
DMic 114133	Urine	*T. asahii* (1)	JX476272	JX476282	JX476292
DMic 114135	Open fracture	*T. asahii* (1)	JX476272	JX476281	JX476292
DMic 114136	Urine	*T. asahii* (1)	JX476272	JX476282	JX476292
DMic 021058[d]	Urine	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 021060[d]	Urine	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 021261[d]	Urine	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 021262[d]	Urine	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 031403[d]	Urine	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 031707[d]	Urine	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 114120	Unknown	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 114123	Blood	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 114134	Blood	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 114137	Blood	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 114138	Blood	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 124142	Blood	*T. asahii* (3)	JX476272	JX476281	JX476293
DMic 114119	Blood	*T. asahii* (4)	JX476272	JX476281	JX476294
DMic 114121[b]	Blood	*T. asahii* (4)	JX476272	JX476281	JX476294
DMic 114127	Urine	*T. asahii* (4)	JX476272	JX476281	JX476294
DMic 114128[e]	Skin	*T. asahii* (4)	JX476272	JX476281	JX476294
DMic 052616	Blood	*T. asahii* (5)	JX476272	JX476281	JX476295
DMic 114130	Blood	*T. asahii* (5)	JX476272	JX476281	JX476295
DMic 90437	Unknown	*T. asahii* (7)	JX476272	JX476281	JX476296
DMic 114118	Bile	*T. asahii* (7)	JX476272	JX476281	JX476296
DMic 88177	Nail of hand	*T. faecale*	JX476273	JX476283	JX476297
DMic 993262	Skin	*T. faecale*	JX476274	JX476284	JX476298
DMic 114131	Fauces	*T. dermatis*	JX476275	JX476285	JX476299
DMic 85105	Unknown	*T. inkin*	JX476276	JX476286	JX476300
DMic 01534	Vitreous humor	*T. inkin*	JX476276	JX476287	JX476301
DMic 993198	Subxiphoid puncture	*T. inkin*	JX476276	JX476286	JX476300
DMic 052617[e]	Bronchial lavage	*T. montevideense*	JX476277	JX476288	JX476302
DMic 052618[e]	Bronchial lavage	*T. montevideense*	JX476277	JX476288	JX476302
DMic 052619[e]	Bronchial lavage	*T. montevideense*	JX476277	JX476288	JX476302
DMic 94750	Urine	*Trichosporon sp.*	JX476278	JX476289	JX476303
DMic 114126	Biopsy of skin lesion	*Trichosporon sp.*	JX476279	JX476290	JX476304
DMic 114132	Nail of hand	*Trichosporon sp.*	JX476280	JX476291	JX476305

[a]Identical sequences have the same GenBank accession number.
[b, c]Strains isolated from the same patient.
[d, e]Strains isolated from different patients in the same clinical institution.

Polymerase chain reactions, gel electrophoresis, and purification

ITS regions (ITS1–5.8s-ITS2), the D1/D2 domain of 26S, and the IGS1 region were amplified using previously described primers ITS1/ITS4, NL1/NL4, and 26SF/5SR, respectively [15,42]. Reactions were performed in 100 µl containing Tris-HCl (pH, 8.4) 20 mM; KCl 50 mM; Mg_2Cl 2 mM for ITS regions and the IGS1 region or 2.5 mM for 26S; dimethyl sulfoxide 5.2% for ITS regions or 5.0% for the D1/D2 domain of 26S; dTAP, dCTP, dGTP, and dTTP 0.2 mM each (Fermentas International Inc.) for the D1/D2 domain of 26S and the IGS1 region or 0.25 mM each for ITS regions; primers 0.1 µM each; Taq DNA polymerase 1U (Invitrogen-Life Technologies, Brazil); and 30 ng of DNA.

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Amplifications were performed in an iCycler (Bio-Rad Laboratories Inc., CA) with the following parameters: 95°C for 7 min, followed by 40 cycles at 95°C for 1 min, 54°C for 2 min, and 72°C for 1 min; followed by one final extension at 72°C for 10 min. Polymerase chain reaction (PCR) products were purified using the PureLink purification kit (Invitrogen-Life Technologies, Brazil).

DNA sequencing and editing

PCR products were sequenced on both strands using an ABI Genetic Analyzer 3500 (Applied Biosystems, CA). Sequences were edited, and the consensus sequences were obtained using BioloMICS software (Bioaware SA, NV). All sequences were deposited in the GenBank database, and their accession numbers are listed in Table 1.

Molecular identification and genotype

Molecular identification was performed based on the sequence analyses of the D1/D2 domain, the ITS regions, and the IGS1 region using the BLASTN tool of the National Center for Biotechnology Information website, Library of Medicine, Bethesda, MD, USA (http://www.ncbi.nlm.nih.gov/BLAST/). Genotyping was based on the analysis of the IGS1 region sequences, comparing them with references sequences retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Phylogenetic analyses

Reference strain sequences retrieved from GenBank were included in the analyses. Two phylogenetic analyses were performed: the first included all strains and the sequences of the D1/D2 domain, and the ITS regions were analyzed (the sequences of each strain were concatenated manually); the second included only the *T. asahii* strains and those closely related, and the sequences of the IGS1 region were analyzed. Alignments were performed using Clustal W software [43]. Phylogenetic trees of the concatenated D1/D2 domain plus ITS regions and the IGS1 region were obtained using MEGA, version 5.05 [44]. The neighbor-joining algorithm and the number-of-differences model were chosen. All gaps and missing data were excluded from the analyses, and branch support was ascertained using 2000 bootstrap replicates.

Antifungal susceptibility testing

The minimal inhibitory concentration (MIC) was determined according to the European Committee on Antimicrobial Susceptibility Testing definitive document revision [45]. The following minor modification was included to improve growth: the plates were agitated at 250 rpm according to the technique proposed by Rodríguez-Tudela et al. [46]. All strains were tested against AMB, fluconazole (FCZ), itraconazole (ITZ), voriconazole (VCZ), ketoconazole (KTZ), posaconazole (POS), and terbinafine (TBF). For AMB and TBF, the MIC endpoints were defined as the lowest drug concentration that caused a prominent reduction (≥90%) of growth compared with that of the control. For all triazole drugs, the MIC endpoints were defined as the lowest drug concentration that caused a reduction of ≥50% in growth compared with that of the control. *Candida parapsilosis*, American Type Culture Collection (ATCC) 22019, and *Candida krusei*, ATCC 6258, were used as quality-control strains.

Results

Molecular identification

Of the 41 strains, 38 were identified as belonging to the following species: *T. asahii* ($N = 29$), *T. inkin* ($N = 3$), *T. montevideense* ($N = 3$), *T. faecale* ($N = 2$), and *T. dermatis* ($N = 1$). Three strains were not unambiguously identified to the species level (Table 1). The three target sequence analyses separately allowed us to determine the species level of the strains in most cases. However, the similarity percentages between closely related species varied depending on the target analyzed. For example, all *T. asahii* strains presented D1/D2 domain sequences with 100% similarity to *T. asahii* CBS2479, yet they also had 99% similarity to *T. japonicum* CBS8641, *T. asteroides* CBS2481, *T. insectorum* ATCC20506, *T. faecale* CBS4828, and *T. coremiiforme* CBS2482. Similarly, almost all *T. asahii* strains had ITS regions sequences with 100% similarity to *T. asahii* CBS2479; however, two strains had 99% similarity to *T. asahii* CBS2479 and all *T. asahii* strains also had 99% similarity to the other closely related species named above. However, all *T. asahii* strains had IGS1 region sequences with 100% similarity to any of the *T. asahii* genotypes and <81% similarity to other *Trichosporon* species. Another example was strain DMic 993262, which had a D1/D2 domain sequence with 100% similarity to *T. insectorum* ATCC20506 and 99% similarity to *T. faecale* CBS4828, *T. asahii* CBS2479, *T. japonicum* CBS8641, *T. asteroides* CBS2481, and *T. coremiiforme* CBS2482. Similarly, the ITS regions sequence had 99% similarity to *T. faecale* CBS4828 and the other closely related species named above. However, the IGS1 region sequences had 100% similarity to *T. faecale* G01 and <81% similarity to other *Trichosporon* species.

The three strains that could not be unambiguously identified to the species level showed IGS1 region sequences with

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Table 2. Distribution of *Trichosporon asahii* genotypes reported in the literature.

Country	Reference	*Trichosporon asahii* genotype (G)								
		G 1	G 2	G 3	G 4	G 5	G 6	G 7	G 8	G 9
Argentina	Rodríguez-Tudela et al. 2007 [34]							1[*]		
	Present study	9		12	4	2		2		
Brazil	Sugita et al. 2002 [15]	1		2						
	Rodríguez-Tudela et al. 2007 [34]							1[a]		
	Araujo-Ribeiro et al. 2008 [47]	5		1		2		1[a]		
	Chagas-Neto et al. 2008 [9]	13		1	1					
China	Guo et al. 2011 [11]	9		7	18		1			
Spain	Rodríguez-Tudela et al. 2007 [34]	7		1	2	3				
Japan	Sugita et al. 2002 [15]	26	1		2	1				
Thailand	Mekha et al. 2010 [36]	45	2	35		1		18		
Turkey	Kalkansi et al. 2010 [35]	69		6	1	7	3			1
	Sugita 2008 (published only in GenBank)								1	
United States	Sugita et al. 2002 [15]			7		4				
	Total	184	3	72	28	20	6	21	1	1

*Genotype 6 for Rodríguez-Tudela et al. 2007 [34] and Araujo-Ribeiro et al. 2008 [47].

no similar sequences in the BLASTN search; strain DMic 114126 had D1/D2 domain and ITS regions sequences with 100% similarity to *T. asahii* CBS2479. However, its IGS1 region sequence had 85% similarity to *T. faecale* CNM-CL6515 (97% coverage) and 83% similarity to *T. asahii* CBS2479 (100% coverage). The strain DMic 114126 was identified as *Trichosporon* sp.; strain DMic 94750 had D1/D2 domain and ITS regions sequences with 99% similarity to *T. guehoae* CBS8521. However, its IGS1 region sequence (566 pb) had 98% and 90% similarity to both ends of the *T. guehoae* CBS8521 IGS1 region sequence (268 pb); however, only 41% of the sequence was analyzed. No other *T. guehoae* IGS1 region sequence was available and no full similar sequence was found for the complete DMic 94750 IGS1 region sequence. This strain was identified as *Trichosporon* cf. *guehoae*; strain DMic 114132 had a D1/D2 domain sequence with 100% similarity to *T. akiyoshidainum* JCM12595 and 99% similarity to *T. laibachii* CBS5790. It also had an ITS regions sequence with 100% similarity to *T. laibachii* CBS5790, *T. multisporum* CBS2495, and *T. akiyoshidainum* ATCC-MYA4129 and 99% similarity to *T. akiyoshidainum* JCM12595, *T. cacaoliposimilis* ATCC20505, and *T. gracile* CBS8189. Its IGS1 region sequence (488 pb) had 100% and 91% similarity to both ends of the *T. laibachii* CBS5790 IGS1 region sequence (411 pb); however, only 75% of the sequence was analyzed. No IGS1 region sequences were available for any *T. akiyoshidainum* and *T. multisporum* strains and no full similar sequence was found for the complete DMic 114132 IGS1 region sequence. The strain also showed 100% D1/D2 domain sequence similarity to *Trichosporon* sp. IFM 56913, *Trichosporon* sp. HP-2023, and *Trichosporon* sp. SC4505, as well as 100% ITS regions sequence similarity to *Trichosporon* sp. IFM 56913 and *Trichosporon* sp. HP-2023. No IGS1 region sequences were available for the HP-2023, SC4505, and IFM 56913 strains. The strain DMic 114132 was identified as *Trichosporon* cf. *akiyoshidainum*.

Genotyping

Of the 29 *T. asahii* strains, 9 were genotype 1, 12 genotype 3, 4 genotype 4, 2 genotype 5, and 2 genotype 7 (Table 1). Table 2 shows the distribution of *T. asahii* genotypes reported both in the bibliography as well as in this study. Genotype 1 is the most frequently reported genotype worldwide and is predominant in all countries studied, except the United States. Genotype 3 is predominant in the United States and is the second most frequently reported worldwide. Genotype 4 is predominant in China. Genotypes 4 and 5 are less frequently reported worldwide than genotypes 1 and 3; however, they have been recovered from several countries.

Phylogenetic analyses

The neighbor-joining tree based on ITS regions plus the D1/D2 domain (Fig. 1) formed well-supported groups (bootstrap >88) that correspond to the clades Ovoides, Cutaneum, Porosum, and Gracile/Brassicae, previously

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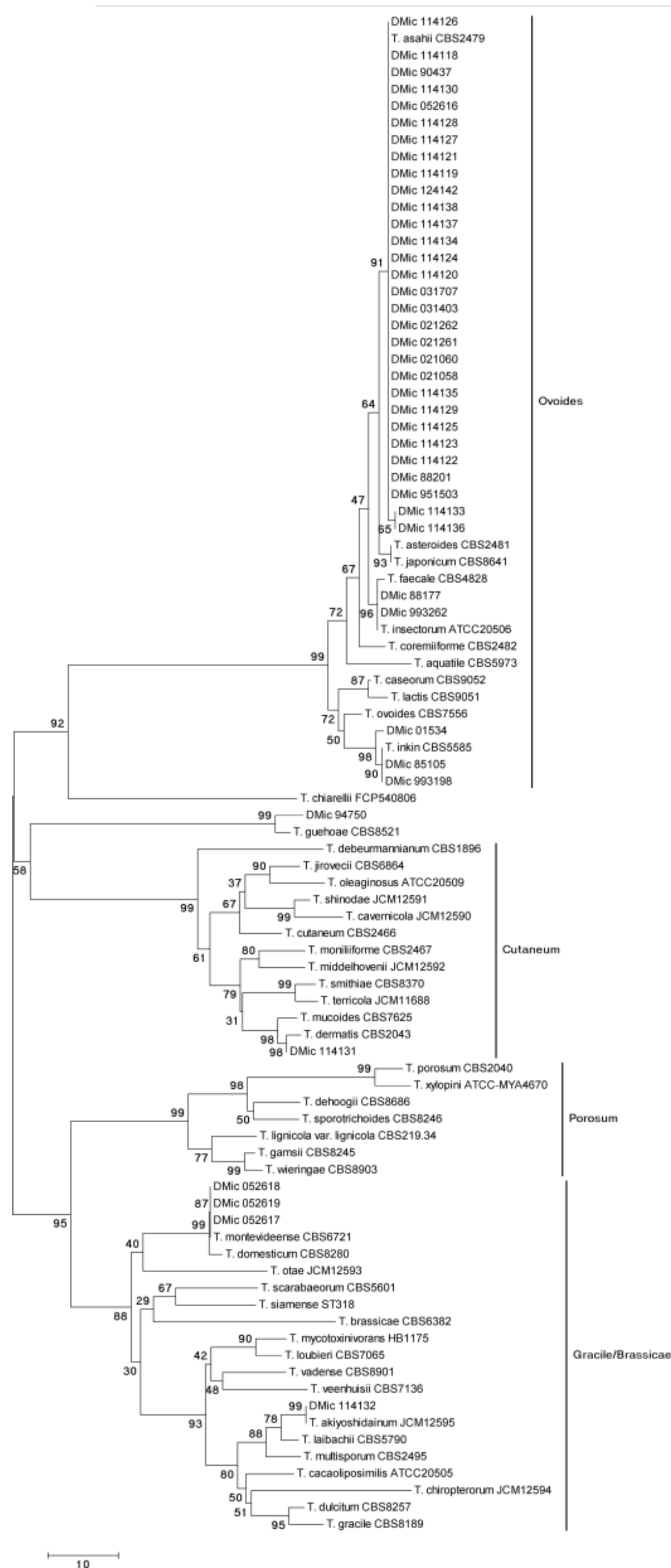

Figure 1. Neighbor-joining tree based on ITS regions plus D1/D2 domain sequences showing the phylogenetic relationship among *Trichosporon* species and strains. Bootstrap percentages from 2000 replicates are shown in each node. Scale bar indicates number of differences.

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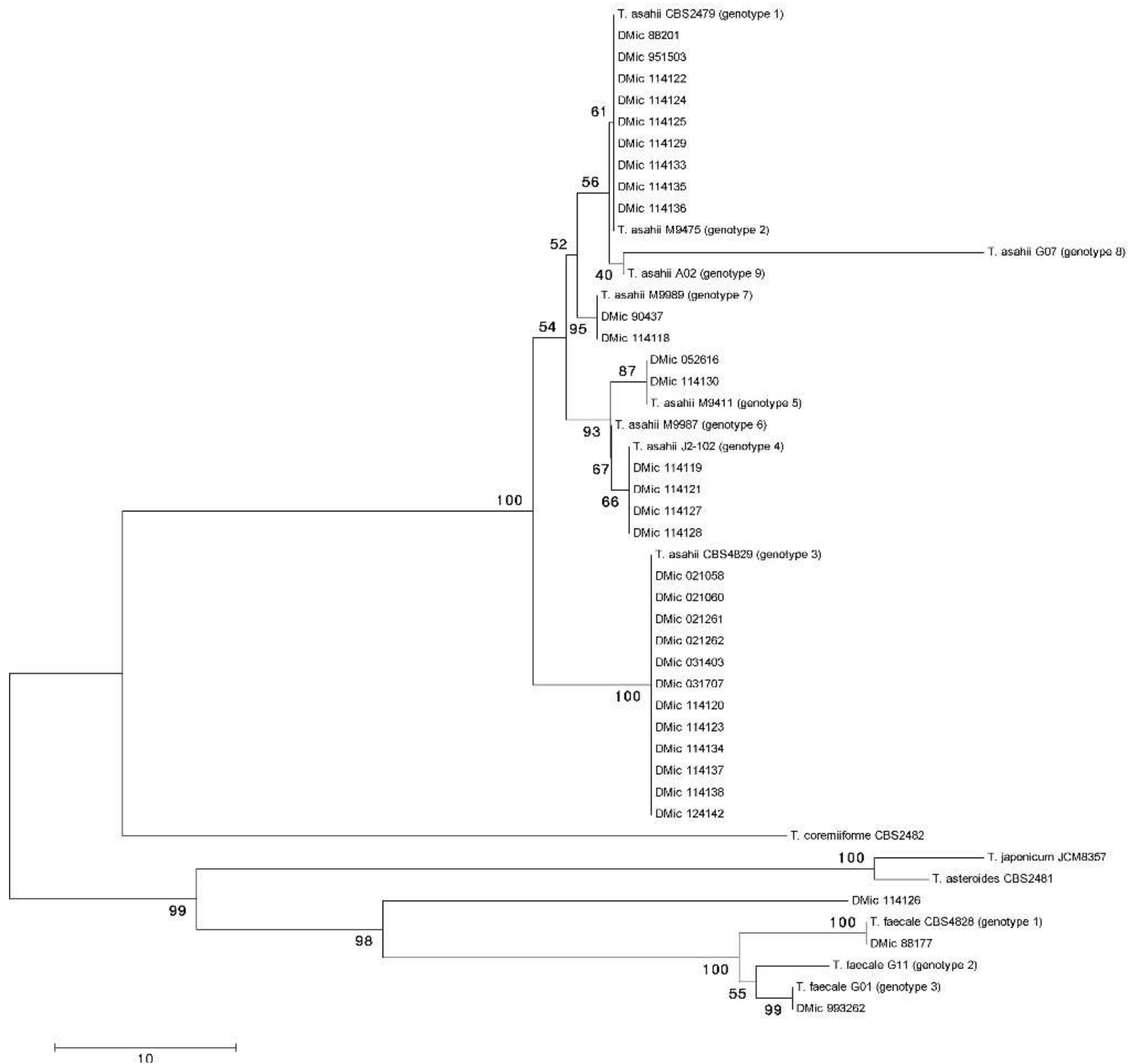

Figure 2. Neighbor-joining tree based on IGS1 region sequences showing the phylogenetic relationship among *Trichosporon asahii* and *T. faecale* genotypes. Closely related *Trichosporon* species and strain DMic 114126 are included. Bootstrap percentages from 2000 replicates are shown in each node. Scale bar indicates number of differences.

described by Sugita et al. [37] and Middelhoven et al. [18]. However, the Brassicae clade formed a single group with the Gracile clade. *Trichosporon chiarelli* formed a separated branch as a sister group of the Ovoides clade. Similarly, *T. guehoae* formed a separated branch as a sister group of the Cutaneum clade. The tree shows isolates DMic 114126, DMic 94750, and DMic 114132 to be closely related to *T. asahii*, *T. guehoae*, and *T. akyoshidainum*, respectively.

The neighbor-joining tree based on IGS1 region sequences focused on *T. asahii*, and related taxa (Fig. 2) formed well-supported groups (bootstrap >40) corresponding to the different *T. asahii* genotypes, except for genotype 2, which formed a single group with genotype 1 (both differ by only one nucleotide base). The tree shows the isolate DMic 114126 to be closely related to *T. faecale*.

Antifungal susceptibility tests

The MIC values of the seven antifungal drugs tested against the *Trichosporon* strains are summarized in Table 3. AMB MICs were ≤1 mg/l against 76% (22/29) of the *T. asahii* strains and 83% (10/12) of the non–*T. asahii* strains. FCZ MICs were ≥2 mg/l against 90% (26/29) of the *T. asahii* strains and 92% (11/12) of the non–*T. asahii*. ITZ, VCZ,

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Table 3. Minimal inhibitory concentration values against the seven antifungal drugs tested summarized by species.

	Minimal inhibitory concentration (mg/l)													
	Amphotericin B		Fluconazole		Itraconazole		Voriconazole		Ketoconazole		Posaconazole		Terbinafine	
Trichosporon spp. (no. of isolates)	GM	Range	GM	Range	GM	Range	GM	Range	GM	Range	GM	Range	GM	Range
T. asahii (29)	0.89	0.25–4	7.63	1–64	0.17	0.03–0.5	0.12	0.03–0.5	0.33	0.03–1	0.14	0.015–1	0.23	0.03–2
non-*T. asahii* (12)	0.50	0.25–2	2.12	1–4	0.09	0.03–0.25	0.08	0.03–0.13	0.11	0.06–0.5	0.06	0.015–0.25	0.25	0.06–1
T. faecale (2)		1–2		4–4		0.13–0.25		0.13–0.13		0.13–0.5		0.13–0.25		0.13–0.13
T. dermatis (1)		0.13		2		0.06		0.06		0.06		0.015		0.25
T. inkin (3)	0.50	0.25–1	2.00	2–2	0.06	0.03–0.13	0.06	0.03–0.13	0.08	0.06–0.13	0.06	0.06–0.06	0.32	0.13–1
T. montevideense (3)	0.25	0.25–0.25	1.59	1–2	0.08	0.06–0.13	0.10	0.06–0.13	0.10	0.06–0.13	0.03	0.03–0.06	0.25	0.06–1
Trichosporon cf. *guehoae* DMic 94750		0.5		2		0.13		0.06		0.06		0.06		0.13
Trichosporon sp. DMic 114126		2		2		0.25		0.06		0.25		0.13		0.5
Trichosporon cf. *akiyoshidainum* DMic 114132		0.5		2		0.06		0.13		0.13		0.06		0.5

GM, geometric mean.

KTZ, and POS MICs were ≤1mg/l against all strains, including *T. asahii* strains. TBF MICs were ≤1 mg/l against all strains, except for one *T. asahii* strain (TBF MIC = 2 mg/l).

Discussion

The three molecular targets separately allowed us to accurately identify most of the strains at the species level. However, a few strains had D1/D2 domain and ITS regions sequences that showed high similarity to any of the groups of closely related species, making it difficult to gain an accurate identification. In this sense, IGS1 region sequence analysis allowed us to identify accurately those strains because IGS1 region sequence analysis showed more discriminatory power between closely related species than D1/D2 domain and ITS regions sequences analyses, as reported by others [10,11,15,47]. Despite this, IGS1 region sequence analysis is not widely performed and is used for genotyping rather than species identification. The IGS1 region sequences of all *Trichosporon* species are still not available or only one IGS1 region sequence of some species is available.

In this study, we were not able to accurately identify the following three strains at the species level: strain DMic 114126, which may represent a subspecies or a new *Trichosporon* species; strain DMic 94750, its identity could not be confirmed and it was finally identified as *Trichosporon* cf. *guehoae*; and strain DMic 114132, its identity could not be confirmed and it was finally identified as *Trichosporon* cf. *akiyoshidainum*. It is worth noting that strain DMic 114132 had 100% D1/D2 domain and ITS regions sequence similarity to the strain *Trichosporon* sp. HP-2023, which was isolated from the Las Yungas rain forest in Tucumán, Argentina [48].

Trichosporon asahii seems to be the most common *Trichosporon* species that is a causative agent of deep-seated infections in Argentina (see Table 1); this is in agreement with what has been reported by others around the world [3,11,35,47,49,50]. Furthermore, *T. asahii* was the only species causative of trichosporonemia in our study.

Among the *T. asahii* strains, genotype 3 accounted for most of the strains (12/29). However, six of those were part of a possible outbreak (described below) and, consequently, we consider genotype 1 (9/29) to be the most common *T. asahii* genotype in Argentina. We also report the first isolation of *T. asahii* genotypes 4 and 5 in Argentina. Genotypes 1 and 7 have been previously described by Rodríguez-Tudela et al. [34] in Argentina; however, only four isolates were included in that study.

The usefulness of IGS1 region genotyping as a new epidemiological tool is still under study. It appears that *T. asahii* genotypes 1 and 3 are the most common isolated

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genotypes with a worldwide distribution, except for genotype 1 in the United States (Table 2). The other genotypes are less common and may involve local epidemiological trends. Interestingly, genotyping has allowed us to report, for the first time here, two mixed infections by two *T. asahii* genotypes: strains DMic 114121 (genotype 4) and DMic 114122 (genotype 1) were recovered from a catheter sample and a blood sample of one patient and, similarly, strains DMic 114128 (genotype 4) and DMic 114129 (genotype 1) were recovered from a skin sample and a catheter sample of another patient.

In this study, we observed one possible outbreak due to six *T. asahii* genotype 3 strains that were isolated from urine samples of intensive care unit patients hospitalized in the same clinical institution. Apparently, the same container for the discharge and measurement of urine was used for several patients with bladder catheterization and that container could have been the source of the infection. We also observed one pseudo-outbreak that was due to *T. montevideense* strains isolated from bronchial lavage of three patients hospitalized in the same clinical institution. In that case, a bronchoscope that was presumed to be contaminated was used with those patients and that instrument assumed to be the source of the pseudo-outbreak. In this respect, several authors have reported on the recovery of isolated clusters or the description of outbreaks due to a *Trichosporon* species [51–55]. Although *Trichosporon* spp. are uncommon infection agents, it seems that these species are likely to contaminate and persist in clinical devices, in turn, causing outbreak or pseudo-outbreak infections in hospitalized patients.

The phylogenetic tree of ITS regions plus the D1/D2 domain is consistent with previous phylogenetic analyses [18,19,22,24,37]. However, in our study, the Brassicae clade proposed by Sugita et al. [37] forms a single group with the Gracile clade, supporting the finding of Middelhoven et al. [18]. *Trichosporon guehoae* and the closely related strain DMic 94750 form a separate branch as a sister group of the Cutaneum clade; this is consistent with the results of others that are based on the D1/D2 domain [22,24] but differs from the results that were based on ITS regions [18], where *T. guehoae* is part of the Porosum clade. *Trichosporon chiarelli* forms a separated branch as a sister group of the Ovoides clade, which is consistent with the results of Pagnocca et al. that were based on the D1/D2 domain [24]. Strain DMic 114126 seems to belong to the Ovoides clade and to be closely related to *T. asahii* and *T. faecale*. Strain DMic 114132 seems to belong to the Gracile/Brassicae clade and to be closely related to *T. akiyoshidainum*.

Antifungal susceptibility profiles of *Trichosporon* species are still under study. Most *T. asahii* strains in our study showed AMB MICs ≤1 mg/l and FCZ MICs ≥2 mg/l. However, the AMB and FCZ geometric mean (GM) MIC values for *T. asahii* strains (0.89 mg/l and 7.63 mg/l) were higher than for non–*T.asahii* strains (0.5 mg/l and 2.12 mg/l). On the other hand, there were nonsignificant differences in GM MIC values of the other azole drugs when tested against *T. asahii* and non–*T. asahii* strains; those drugs had MIC values ≤1 mg/l compared with all strains tested. The results discussed above are in agreement with the findings of others [3,11,12]. Interestingly, our strains showed lower TBF MIC values than previously reported [56,57]. In agreement with other authors, we suggest that antifungal susceptibility testing be performed on all clinical isolates not only to guide therapy but also to document local epidemiological trends [11,12,34–36].

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper. The findings and conclusions of this article are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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