






Correlation of *Trichosporon asahii* Genotypes with Anatomical Sites and Antifungal Susceptibility Profiles: Data Analyses from 284 Isolates Collected in the Last 22 Years across 24 Medical Centers

Elaine Cristina Francisco,^a João N. de Almeida Junior,^b  Flávio Queiroz-Telles,^c Valério Rodrigues Aquino,^d Ana Verena A. Mendes,^e Marcio de Oliveira Silva,^e Paulo de Tarso O. e Castro,^f  Thais Guimarães,^g Vinicius Ponzio,^h Rosane C. Hahn,ⁱ Guilherme M. Chaves,^j  Arnaldo L. Colombo^a

^aLaboratório Especial de Micologia, Division of Infectious Diseases, Universidade Federal de São Paulo, São Paulo, Brazil

^bHospital das Clínicas da FMUSP, São Paulo, Brazil

^cUniversidade Federal do Paraná, Curitiba, Paraná, Brazil

^dHospital das Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil

^eHospital São Rafael de Salvador, Salvador, Bahia, Brazil

^fHospital de Câncer de Barretos, Barretos, São Paulo, Brazil

^gHospital do Servidor Público Estadual de São Paulo, São Paulo, Brazil

^hHospital Nove de Julho, São Paulo, Brazil

ⁱUniversidade Federal de Mato Grosso, Cuiabá, Brazil

^jUniversidade Federal do Rio Grande do Norte, Natal, Brazil

ABSTRACT *Trichosporon asahii* is an opportunistic fungal pathogen that can cause severe infections with high mortality rates. Azole derivatives are the best-targeted therapy for *T. asahii* invasive infections, but azole-resistant isolates have been reported. To investigate peculiarities in the antifungal susceptibility profile (ASP) of *T. asahii* clinical isolates, we analyzed the genotype distribution, isolation sources, and ASP of 284 strains collected from 1997 to 2019 in different Brazilian medical centers. Species identification and genotype characterization were performed by analysis of the intergenic spacer (IGS1) region of the ribosomal DNA (rDNA). Antifungal susceptibility testing (AST) for amphotericin B and azoles was with the CLSI M27, 4th edition, microdilution broth method. Trends in the ASP of Brazilian *T. asahii* isolates were investigated using epidemiological cutoff values. Five different genotypes were found among the 284 isolates tested (G1, 76%; G3, 10%; G4, 3%; G5, 7%; and G7, 4%). The isolates were collected mainly from urine (55%) and blood/catheter tip samples (25%) where G1 was the most frequent genotype found ($P < 0.05$). The G7 isolates exhibited the highest MIC₉₀ values for azoles compared to those for the other genotypes ($P < 0.05$). Genotype 7 isolates also contributed to the increasing rates of voriconazole non-wild-type isolates found in recent years ($P = 0.02$). No significant differences were found among the AST results generated by isolates cultured from different anatomical sites. Monitoring *T. asahii* genotype distributions and antifungal susceptibility profiles is warranted to prevent the spread of azole-resistant isolates.

KEYWORDS *Trichosporon asahii*, genotypes, epidemiology, antifungal resistance, opportunistic infections

First described by Akagi in 1929 (1), *Trichosporon asahii* is a basidiomycete yeast-like fungi that is considered an emergent opportunistic fungal pathogen capable of causing life-threatening invasive infections, mostly in neutropenic cancer patients, patients submitted for organ transplantation, or critically ill patients exposed to antibiotics, corticosteroids, or invasive medical procedures (2). The prognosis of patients with

Citation Francisco EC, de Almeida Junior JN, Queiroz-Telles F, Aquino VR, Mendes AVA, de Oliveira Silva M, Castro PDTOE, Guimarães T, Ponzio V, Hahn RC, Chaves GM, Colombo AL. 2021. Correlation of *Trichosporon asahii* genotypes with anatomical sites and antifungal susceptibility profiles: data analyses from 284 isolates collected in the last 22 years across 24 medical centers. Antimicrob Agents Chemother 65:e01104-20. <https://doi.org/10.1128/AAC.01104-20>.

Copyright © 2021 American Society for Microbiology. All Rights Reserved.

Address correspondence to Arnaldo L. Colombo, arnaldolcolombo@gmail.com.

Received 29 May 2020

Returned for modification 22 August 2020

Accepted 3 December 2020

Accepted manuscript posted online 14 December 2020

Published 17 February 2021

invasive trichosporonosis is a concern given that crude mortality rates as high as 42% to 87% have been reported by different series (2, 3).

Due to the lack of clinical trials, recommendations for the clinical management of invasive trichosporonosis rely mostly on data generated from animal models, *in vitro* susceptibility tests, and reports of small clinical series (3–6). In this scenario, most authors suggest that triazoles are the best therapy for invasive infections caused by *Trichosporon* spp., despite some reports illustrating that some species and strain variability may occur in their susceptibilities to fluconazole and voriconazole (3, 7, 8).

Nowadays, a total of 15 *T. asahii* genotypes has been characterized by different research groups based on the genetic diversity described in their intergenic spacer 1 (IGS1) ribosomal DNA (rDNA) sequences (9). Notably, there is a lack of robust data illustrating their distribution in different anatomical sites of patients colonized or infected by *T. asahii*. In addition, it remains controversial whether the genetic diversity described for *T. asahii* isolates has any impact in terms of virulence or antifungal susceptibility to different antifungal drugs (5, 6, 9).

The present study describes the prevalence rates of *T. asahii* genotypes found among 284 clinical isolates cultured from different patients as well as their *in vitro* susceptibility to four antifungal drugs.

RESULTS

Prevalence rates of *T. asahii* genotypes cultured from different anatomical sites. Only five clinical centers were able to provide data on the annual incidence of *T. asahii*-positive cultures collected between 2010 and 2019. The pooled data showed that the incidence of cultures positive for *T. asahii* remained stable over the years, ranging from 0.5 to 2 isolates per 10,000 admissions each year. Clinical isolates were cultured from urine ($n = 157$ [55%]), blood samples/central venous catheter tips ($n = 71$ [25%]), respiratory tract samples ($n = 16$ [6%]), integumentary samples, including hair, nail, and skin samples ($n = 11$ [4%]), other sterile anatomical sites ($n = 11$ [4%]), and other sources ($n = 18$ [6%]).

The 284 clinical isolates were successfully identified as *T. asahii* by sequencing of the IGS1 rDNA region, with 100% of query coverage and percent identity and E value of 0.0. As illustrated in Fig. 1 and Fig. S1 in the supplemental material, the median-joining network analysis showed five different genotypes (G1, $n = 216$ [76%]; G3, $n = 28$ [10%]; G4, $n = 8$ [3%]; G5, $n = 21$ [7%]; G7, $n = 11$ [4%]), including representatives of G7 that were never previously reported among Brazilian isolates.

Three to five distinct genotypes of *T. asahii* were found in each anatomic site. Genotype 1 was present in all evaluated sites and represented 76% of all typed isolates. In urine cultures, *T. asahii* G1 was responsible for 138 of 157 (88%) isolates studied ($P < 0.001$). Remarkably, all five genotypes were detected among the 71 isolates cultured from blood samples, including 48 isolates of G1 (68%) and 11 isolates of G3 (16%). The median-joining genotype network of *T. asahii* clinical isolates and the prevalence in each anatomical site are shown in Fig. 1.

We found a higher percentage of *T. asahii* G7 in blood/catheter tip samples than other anatomical sites (9.8% versus 1.8%, $P = 0.005$). Notably, the 11 *T. asahii* G7 isolates were cultured from patients admitted in 3 medical centers from different regions, instead of representing a cluster of infections documented in a single hospital.

Antifungal susceptibility tests. Table 1 summarizes the MIC ranges ($\mu\text{g/ml}$), MIC₅₀, MIC₉₀, and geometric mean (GM) values obtained with the 284 *T. asahii* clinical isolates tested. At the 48-h reading, the MIC range values were 0.25 to ≥ 64 $\mu\text{g/ml}$ for fluconazole (FLC), 0.03 to 2 $\mu\text{g/ml}$ for both voriconazole (VRC) and posaconazole (POS), and 0.025 to ≥ 16 $\mu\text{g/ml}$ for amphotericin B (AMB). The highest MIC₉₀ values were found for FLC, followed by those for AMB, POS, and VRC.

We found substantial variation in MIC₉₀ values when comparing certain combinations of drugs and genotypes tested. In this scenario, the isolates representative of *T. asahii* G7, which emerged after 2013 in 3 medical centers only, exhibited higher MIC₉₀ values for FLC (MIC₉₀, 16 $\mu\text{g/ml}$; $P = 0.03$), VRC (MIC₉₀, 0.5 $\mu\text{g/ml}$; $P < 0.001$), and POS

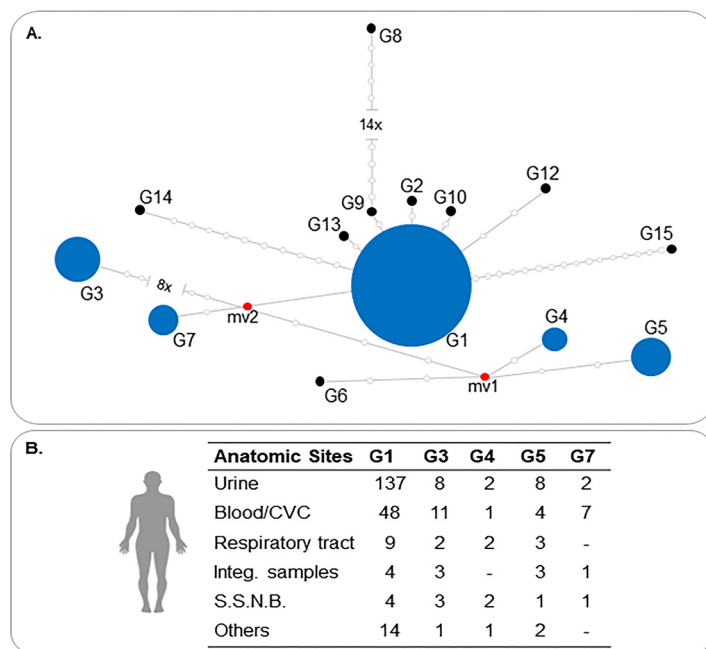


FIG 1 Median-joining genotypes network of *T. asahii* based on IGS1 rDNA sequences (A) and their distribution in anatomic sites (B). Circumference sizes are proportional to the frequencies of genotypes detected. Blue circles represent genotypes found in our study (G1, G3, G4, G5, and G7). Black circles represent genotypes that could not be grouped with any isolate from our collection. Red dots (median vectors [mv]) are hypothetical missing intermediates. CVC, catheter tips; Integ. samples, integumentary samples; S.S.N.B., sterile sites, nonblood.

(MIC₉₀, 0.1 µg/ml; $P < 0.02$) than all other genotypes. No significant differences were found in AMB MIC values for the different *T. asahii* genotypes ($P = 0.1$). MIC values tended to be similar when checking isolates cultured from different anatomic sites ($P > 0.05$). Blood/catheter tips and sterile site isolates showed FLC, VRC, POS, and AMB MIC₉₀ values of 8 µg/ml, 0.125 µg/ml, 0.5 µg/ml, and 4 µg/ml, respectively. Otherwise, isolates from all other anatomic sites showed FLC, VRC, POS, and AMB MIC₉₀ values of 4 µg/ml ($P = 0.7$), 0.125 µg/ml ($P = 0.2$), 0.5 µg/ml ($P = 0.7$), and 4 µg/ml ($P = 0.5$), respectively.

As shown in Fig. 2, by using the ECV values for *T. asahii* recently described by Francisco et al. (4), we found a trend for increasing rates of non-wild-type isolates of VRC in the second period of study (P1, 5/72 [7%] versus P2, 21/212 [10%]; $P = 0.02$). When isolates belonging to the G7 genotype were excluded from the analyses, the rates of non-wild-type isolates of VRC did not differ between the two periods (P1, 0/72 [0%] versus P2, 11/212 [5.2%]).

DISCUSSION

Trichosporon asahii has recently attracted worldwide medical attention due to its capability to cause a broad spectrum of infections in humans, including life-threatening invasive infections in immunocompromised hosts and critically ill patients. Additionally, in contrast with *Candida* spp., *Trichosporon* is a poor target for echinocandins and amphotericin B (2–7).

Trichosporon asahii usually comprises the majority of all *Trichosporon* spp. found in urine and blood cultures (3–6). Data on the incidence of this pathogen in clinical samples are scarce, and our results show an approximate incidence of 0.5 to 2 cultures per 10,000 hospital admissions. While a higher incidence of positive cultures was reported in Taiwan, >12 per 100,000 admissions, much lower rates are reported in US or European centers (10, 11).

Despite the large number of *T. asahii* genotypes described so far, there is a lack of

TABLE 1 *In vitro* antifungal susceptibility of 284 *Trichosporon asahii* clinical isolates tested against triazoles and amphotericin B

Anatomic site or genotype ^a	No. of isolates	MIC (μ g/ml)															
		Fluconazole			Voriconazole			Posaconazole			Amphotericin B						
		Range	50%	90%	GM	Range	50%	90%	GM	Range	50%	90%	GM				
Anatomic sites																	
Urine	157	0.25 to \geq 64	2	4	2.47	0.03–2	0.06	0.125	0.07	0.03–2	0.25	0.5	0.21	0.25 to \geq 16	2	4	1.65
Blood/CVC	71	0.25 to 32	2	8	2.08	0.03–1	0.06	0.125	0.07	0.03–1	0.25	0.5	0.18	0.25 to \geq 16	1	4	1.42
Respiratory	16	0.5 to 8	1	4	1.54	0.03–0.125	0.06	0.06	0.06	0.06–0.25	0.125	0.25	0.16	1 to 8	1	2	1.35
Integumentary	11	1 to 6	2	4	2.13	0.03–0.25	0.06	0.06	0.06	0.03–1	0.125	0.25	0.17	1 to 4	1	2	1.29
S.S.N.B.	11	1 to 16	2	4	2.57	0.03–1	0.125	0.125	0.12	0.06–1	0.25	0.25	0.21	0.5 to 4	1	4	1.55
Others	18	1 to 8	2	4	2.16	0.03–0.5	0.06	0.125	0.06	0.03–0.5	0.125	0.25	0.14	0.25 to \geq 16	1	4	1.71
Genotypes																	
G1	216	0.25 to \geq 64	2	8	2.30	0.03–2	0.06	0.25	0.06	0.03–2	0.25	0.5	0.18	0.25 to \geq 16	2	4	1.57
G3	28	0.5 to 16	2	2	1.95	0.03–1	0.06	0.125	0.08	0.06–1	0.25	0.25	0.22	0.5 to \geq 16	1	1	1.19
G4	8	1 to 4	1	2	1.54	0.03–0.125	0.06	0.06	0.05	0.125–0.25	0.125	0.125	0.13	0.5 to 2	1	2	1.09
G5	21	0.5 to 4	2	2	1.61	0.03–0.125	0.06	0.125	0.04	0.03–0.5	0.25	0.5	0.16	0.25 to 4	2	4	1.14
G7	11	1 to 32	2	16	4.83	0.06–1	0.125	0.5	0.19	0.06–1	0.5	1	0.36	1 to 4	2	4	2.13

^aCVC, catheter tips; SSNB, sterile sites, nonblood.

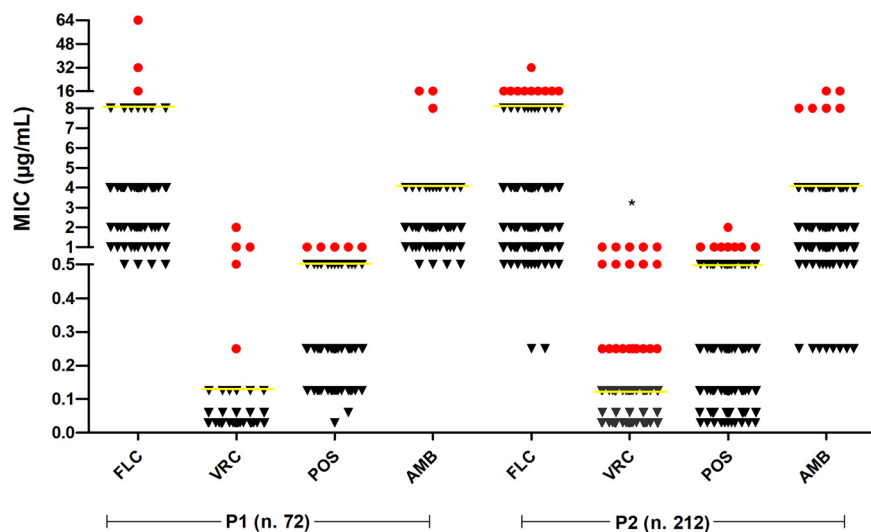


FIG 2 Historical trends in the antifungal susceptibility of Brazilian clinical isolates of *T. asahii* collected over the last 20 years. The isolates that exhibited MICs higher than the epidemiologic cutoff values for each antifungal tested (yellow lines) were identified by red circles. FLC, fluconazole; VRC, voriconazole; POS, posaconazole; AMB, amphotericin B; P1, 1997 to 2008; P2, 2009 to 2019; *, $P < 0.05$.

robust data on the real prevalence in different anatomical sites and potential peculiarities in terms of antifungal susceptibility.

Concerning the intraspecific diversity of *T. asahii*, our results showed the presence of five circulating genotypes in Brazilian patients: G1, G3, G4, G5, and G7. Consistent with previous studies, including Brazilian series, *T. asahii* G1 (76%) was the most common genotype, followed by G3 (9.8%), G5 (7.5%), G7 (3.9%), and G4 (2.8%) (5, 6, 12–22).

Interestingly, a high proportion of G7 isolates were found in blood/catheter tip samples. The G7 genotype was described in 2004 from blood samples of Japanese patients (21) and later found in Thailand, Taiwan, Greece, Argentina, Mexico, India, Tunisia, and China (5, 15–18, 23–25). These results suggest a more virulent trait of G7, but *in vitro* and *in vivo* studies comparing the virulence of all different described genotypes are warranted to confirm this issue.

We were not able to find any representative of genotypes 2, 6, or 8 to 15 in our *T. asahii* collection. These genotypes have been reported in different geographic regions, with variable prevalences and distributions. The first seven genotypes (G1 to G7) of *T. asahii* have been extensively recovered from a large diversity of clinical samples and patients living in different countries (5, 6, 12–22).

Otherwise, G8 was only mentioned by a single report published in 2010, and G9 has only been reported from Turkish studies (6, 19). Two different research groups, one from China and another from Greece, reported the isolation of genotypes 10 and 11 in their collections (23, 26). There are additional reports of isolation of G12 in patients from China, and *T. asahii* isolates representative of G13, G14, and G15 were reported in a single publication from Brazil (9, 26).

Although 15 different genotypes for *T. asahii* have been described around the world, some of them have only been found in single reports (9, 19) and require confirmation to determine if they represent the genetic diversity of this pathogen or if their description was related to some artifacts faced during their sequence analysis.

Despite the lack of statistical differences in the AST results obtained with isolates cultured from several anatomical sites, attention is drawn to the high MIC₉₀ values for FLC (8 µg/ml) in *T. asahii* isolates obtained from blood samples/central venous catheters. These data suggest that MIC determination may have a role in defining the best strategy to treat patients with *T. asahii* fungemia.

Voriconazole exhibits the best *in vitro* activity against *T. asahii* clinical isolates and is considered first-line therapy, especially in hematological patients (3). High MIC values

in *T. asahii* isolates are not commonly reported for this compound. In our study, the increase of rates of non-wild-type (NWT) *T. asahii* clinical isolates for VRC over the last 10 years was certainly influenced by the emergence of G7 isolates. Similar results were found in G7 clinical isolates tested against triazoles in a center from Greece (23). As reported in other yeast genera (*Candida* and *Cryptococcus*), we believe that the low susceptibility of G7 for azoles can be influenced by the selective pressure of their widespread use prophylactically or empirically (8). Otherwise, until now, these organisms have represented only a small proportion of the *T. asahii* clinical isolates found in Brazilian medical centers, and less than 15% in series published in other countries (5, 15–18, 23–25). A previous systematic review on *Trichosporon* fungemia shows a notable increase of azole-based treatments in the late 2000s, and the emergence of resistant isolates in recent years is a concern (27).

In conclusion, the present paper represents the largest series of *T. asahii* isolates characterized by sequencing IGS1 of rDNA region and antifungal susceptibility testing. We were able to demonstrate four main aspects: (i) despite the fact that 15 different *T. asahii* genotypes have been described by different authors, only 5 of them appeared in this large Brazilian isolate series; (ii) the five *T. asahii* genotypes found were cultivated from different clinical samples, illustrating their ability to colonize and infect several anatomic sites; (iii) isolates recovered from different anatomical sites exhibited similar MIC values to all tested drugs; (iv) isolates of G7 emerged throughout the last 10 years and exhibit limited susceptibility to all triazoles investigated as well as to amphotericin B.

Based on our findings, we may suggest that monitoring of *T. asahii* genotype distributions and antifungal susceptibility profiles is warranted to prevent the spread of azole-resistant isolates.

MATERIALS AND METHODS

Selection of *T. asahii* clinical isolates. We selected a total of 284 *T. asahii* clinical isolates obtained from different patients that were admitted in 24 Brazilian medical centers at different times in the period between January 1997 and May 2019. The isolates were obtained from different anatomical sites and sent to Laboratório Especial de Micologia, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil, for further identification and antifungal susceptibility testing.

Only one isolate per patient was selected for further molecular identification, genotyping investigation, and antifungal susceptibility testing. The medical centers were encouraged to provide the annual incidence of *T. asahii* cultures per 10,000 admissions/year. The study protocol was approved by UNIFESP's ethics committee (CEP-UNIFESP number 401.893/2013).

Molecular species identification of *T. asahii* isolates. Molecular identification was performed by sequencing the IGS1 region of rDNA using the 26SF and 5SR primer pair as previously described (12). Contigs were assembled based on two reads per isolates and edited using Phred-Phrap-Consed, targeting a Phred score of >30 (28, 29). The consensus was compared with the sequences deposited in the NCBI database (<http://ncbi.nlm.nih.gov>) using the BLASTn tool, looking for the percentage of identity and sequence coverage of $\geq 98\%$ and an E value of $< 10^{-5}$ (4). Additionally, the nucleotide sequences were aligned with sequences of *T. asahii* type strains deposited in the GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>). The phylogenetic tree was created by using the neighbor-joining method based on the Kimura two-parameter model with 1,000 bootstrap pseudoreplicates in MEGA7 (30–33).

Genotyping analysis of *T. asahii*. Sequences of the IGS1 of rDNA region from 284 clinical isolates identified at the species level were aligned with sequences deposited in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) representative of the 15 identified genotypes of *T. asahii*. Alignments were performed using the muscle algorithm implemented by SeaView 4.2.12 and adjusted by eye before performing haplotype analyses (33, 34). Genotype analysis was implemented in DNAsp 5.0, excluding gap positions, and the median-joining network was constructed and visualized using the software Network 5.0.1.1 (35, 36). To check the consistency of the network results, the *T. asahii* genotypes were also investigated by the neighbor-joining analysis performed by SeaView 4.2.12 (34), according to previous studies (5, 6, 13, 19, 21, 23–25).

Antifungal susceptibility testing of *T. asahii*. Antifungal susceptibility testing (AST) for fluconazole (FLC), voriconazole (VRC), posaconazole (POS), and amphotericin B (AMB) (Sigma, St. Louis, MO, USA) was performed by using the CLSI broth microdilution method according to the document M27, 4th edition (37). The MIC values were determined after 48 h of incubation (4, 37). The following drug concentration ranges were tested: 0.125 to 64 $\mu\text{g/ml}$ for FLC and 0.03 to 16 $\mu\text{g/ml}$ for VRC, POS, and AMB. Quality control organisms were included in all experiments and were represented by the ATCC 6258 *Candida krusei* and ATCC 22019 *Candida parapsilosis* strains. Antifungal MIC distributions obtained with 284 *T. asahii* isolates cultured from different anatomical sites and representatives of different genotypes were compared using analysis of variance (ANOVA) performed with the aid of SPSS program, and *P* values of < 0.05 were considered significant.

To check for historical trends in the prevalence rates of non-wild-type (NWT) clinical isolates regarding their susceptibility to antifungals, the total collection of isolates was divided into two different periods: (i) P1, including 72 clinical isolates collected between 1997 and 2008, and (ii) P2, including 212 clinical isolates collected between 2009 and 2019. The definition of wild-type and non-wild-type *T. asahii* isolates was based on the epidemiological cutoff values (ECVs) recently proposed by Francisco et al. (4): 8 µg/ml for FLC, 0.125 µg/ml for VRC, 0.5 µg/ml for POS, and 4 µg/m for AMB. The distributions of MICs obtained with all isolates collected during both periods (P1 versus P2) were compared by Student's *t* test ($P < 0.05$).

Data availability. The rDNA IGS sequences of the 284 *Trichosporon asahii* clinical isolates were deposited in the GenBank database under accession numbers [MT460677](#) to [MT460907](#), [FJ169359](#) to [FJ169365](#), [FJ172160](#) to [FJ172173](#), [FJ187679](#) to [FJ187697](#), [KM488282](#) to [KM488284](#), [EU934808](#) to [EU938058](#), and [EU980326](#). For complete information, see Table S1 in the supplemental material.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.1 MB.

ACKNOWLEDGMENTS

We thank Ângela Tavares Paes from Pró-Reitoria de Pós-Graduação e Pesquisa da UNIFESP/EPM for helping with the statistical analysis.

The study was partially supported by a grant received from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 484020-2013-7 and CNPq 307510/2015-8). E.C.F. received a scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq 138258/2013-9 and 165230/2015-0).

A.L.C. received research grants from Astellas and Pfizer. A.L.C. has also received educational grants from Astellas, Biotoscana, United Medical, Gilead, MSD, and Pfizer. The other authors report no conflicts of interest that may be relevant to this article.

REFERENCES

- Akagi S. 1929. Über eine neue mykotische Hautkrankheit. *Jpn J Dermatol Urol* 29:53–55.
- Colombo AL, Padovan ACB, Chaves GM. 2011. Current knowledge of *Trichosporon* spp. and trichosporonosis. *Clin Microbiol Rev* 24:682–700. <https://doi.org/10.1128/CMR.00003-11>.
- de Almeida Júnior JN, Hennequin C. 2016. Invasive *Trichosporon* infection: a systematic review on a re-emerging fungal pathogen. *Front Microbiol* 7:1629. <https://doi.org/10.3389/fmicb.2016.01629>.
- Francisco EC, de Almeida Junior JN, de Queiroz Telles F, Aquino VR, Mendes AVA, de Andrade Barberino MGM, de Tarso O Castro P, Guimaraes T, Hahn RC, Padovan ACB, Chaves GM, Colombo AL. 2019. Species distribution and antifungal susceptibility of 358 *Trichosporon* clinical isolates collected in 24 medical centres. *Clin Microbiol Infect* 25:909.e1–909.e5. <https://doi.org/10.1016/j.cmi.2019.03.026>.
- Guo L-N, Yu S-Y, Hsueh P-R, Al-Hatmi AMS, Meis JF, Hagen F, Xiao M, Wang H, Barresi C, Zhou M-L, de Hoog GS, Xu Y-C. 2019. Invasive infections due to *Trichosporon*: species distribution, genotyping, and antifungal susceptibilities from a multicenter study in China. *J Clin Microbiol* 57:e01505-18. <https://doi.org/10.1128/JCM.01505-18>.
- Hazirolan G, Koçak N, Karagöz A. 2018. Sequence-based identification, genotyping and virulence factors of *Trichosporon asahii* strains isolated from urine samples of hospitalized patients (2011–2016). *J Mycol Med* 28:452–456. <https://doi.org/10.1016/j.mycmed.2018.06.006>.
- Arendrup MC, Boekhout T, Akova M, Meis JF, Cornely OA, Lortholary O, European Society of Clinical Microbiology and Infectious Diseases Fungal Infection Study Group, European Confederation of Medical Mycology. 2014. ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. *Clin Microbiol Infect* 20 (Suppl 3):76–98. <https://doi.org/10.1111/1469-0691.12569>.
- Padovan ACB, Rocha W. P d S, Toti A. C d M, Freitas de Jesus DF, Chaves GM, Colombo AL. 2019. Exploring the resistance mechanisms in *Trichosporon asahii*: triazoles as the last defense for invasive trichosporonosis. *Fungal Genet Biol* 133:103267. <https://doi.org/10.1016/j.fgb.2019.103267>.
- de Almeida AA, Crispim B.d.A, Grisolia AB, Svidzinski TIE, Ortolani LG, Pires de Oliveira KM. 2016. Genotype, antifungal susceptibility, and biofilm formation of *Trichosporon asahii* isolated from the urine of hospitalized patients. *Rev Argent Microbiol* 48:62–66. <https://doi.org/10.1016/j.ram.2015.11.005>.
- Ruan S-Y, Chien J-Y, Hsueh P-R. 2009. Invasive trichosporonosis caused by *Trichosporon asahii* and other unusual *Trichosporon* species at a medical center in Taiwan. *Clin Infect Dis* 49:e11–e17. <https://doi.org/10.1086/599614>.
- Kontoyiannis DP, Torres HA, Chagua M, Hachem R, Tarrand JJ, Bodey GP, Raad II. 2004. Trichosporonosis in a tertiary care cancer center: risk factors, changing spectrum and determinants of outcome. *Scand J Infect Dis* 36:564–569. <https://doi.org/10.1080/00365540410017563>.
- Sugita T, Nakajima M, Ikeda R, Matsushima T, Shinoda T. 2002. Sequence analysis of the ribosomal DNA intergenic spacer 1 regions of *Trichosporon* species. *J Clin Microbiol* 40:1826–1830. <https://doi.org/10.1128/jcm.40.5.1826-1830.2002>.
- do Espírito Santo EPT, Monteiro RC, da Costa ARF, Marques-da-Silva SH. 2020. Molecular identification, genotyping, phenotyping, and antifungal susceptibilities of medically important *Trichosporon*, *Apiotrichum*, and *Cutaneotrichosporon* species. *Mycopathologia* 185:307–317. <https://doi.org/10.1007/s11046-019-00407-x>.
- Cho O, Matsukura M, Sugita T. 2015. Molecular evidence that the opportunistic fungal pathogen *Trichosporon asahii* is part of the normal fungal microbiota of the human gut based on rRNA genotyping. *Int J Infect Dis* 39:87–88. <https://doi.org/10.1016/j.ijid.2015.09.009>.
- Sellami H, Trabelsi H, Neji S, Amouri I, Cheikhrouhou F, Makni F, Ayadi A. 2017. First genotype identification of *Trichosporon asahii* in Sfax, Tunisia. *J Med Microbiol* 66:397–401. <https://doi.org/10.1099/jmm.0.000442>.
- Montoya AM, Sánchez González A, Palma-Nicolás JP, Gómez-Treviño A, González JG, González GM. 2015. Genotyping, extracellular compounds, and antifungal susceptibility testing of *Trichosporon asahii* isolated from Mexican patients. *Med Mycol* 53:505–511. <https://doi.org/10.1093/mmy/myv009>.
- Yang Y-L, Liu Y-W, Chen H-T, Tsai M-S, Chu W-L, Lo H-J. 2013. Genotype analysis based on intergenic spacer 1 sequences of *Trichosporon asahii* collected in Taiwan. *Med Mycol* 51:880–883. <https://doi.org/10.3109/13693786.2013.800240>.
- Mekha N, Sugita T, Ikeda R, Nishikawa A, Autthateinchai R, Poonwan N,

- Sawanpanyalert P. 2010. Genotyping and antifungal drug susceptibility of the pathogenic yeast *Trichosporon asahii* isolated from Thai patients. *Mycopathologia* 169:67–70. <https://doi.org/10.1007/s11046-009-9225-5>.
19. Kalkanci A, Sugita T, Arikani S, Yucesoy M, Ener B, Otag F, Kiraz N, Kustimur S, Sancak B, Evcı C, Emektas G. 2010. Molecular identification, genotyping, and drug susceptibility of the basidiomycetous yeast pathogen *Trichosporon* isolated from Turkish patients. *Med Mycol* 48:141–146. <https://doi.org/10.3109/13693780902977984>.
 20. Rodriguez-Tudela JL, Diaz-Guerra TM, Mellado E, Cano V, Tapia C, Perkins A, Gomez-Lopez A, Rodero L, Cuenca-Estrella M. 2005. Susceptibility patterns and molecular identification of *Trichosporon* species. *Antimicrob Agents Chemother* 49:4026–4034. <https://doi.org/10.1128/AAC.49.10.4026-4034.2005>.
 21. Sugita T, Ikeda R, Nishikawa A. 2004. Analysis of *Trichosporon* isolates obtained from the houses of patients with summer-type hypersensitivity pneumonitis. *J Clin Microbiol* 42:5467–5471. <https://doi.org/10.1128/JCM.42.12.5467-5471.2004>.
 22. Iturrieta-González IA, Padovan ACB, Bizerra FC, Hahn RC, Colombo AL. 2014. Multiple species of *Trichosporon* produce biofilms highly resistant to triazoles and amphotericin B. *PLoS One* 9:e109553. <https://doi.org/10.1371/journal.pone.0109553>.
 23. Arabatzis M, Abel P, Kanellopoulou M, Adamou D, Alexandrou-Athanasoulis H, Stathi A, Platsouka E, Milioni A, Pangalis A, Velegraki A. 2014. Sequence-based identification, genotyping and EUCAST antifungal susceptibilities of *Trichosporon* clinical isolates from Greece. *Clin Microbiol Infect* 20:777–783. <https://doi.org/10.1111/1469-0691.12501>.
 24. Taverna CG, Córdoba S, Murisengo OA, Vivot W, Davel G, Bosco-Borgeat ME. 2014. Molecular identification, genotyping, and antifungal susceptibility testing of clinically relevant *Trichosporon* species from Argentina. *Med Mycol* 52:356–366. <https://doi.org/10.1093/mmy/myt029>.
 25. Rastogi V, Honnavar P, Rudramurthy SM, Pamidi U, Ghosh A, Chakrabarti A. 2016. Molecular characterisation and antifungal susceptibility of clinical *Trichosporon* isolates in India. *Mycoses* 59:528–534. <https://doi.org/10.1111/myc.12511>.
 26. Xia Z, Yang R, Wang W, Cong L. 2012. Genotyping and antifungal drug susceptibility of *Trichosporon asahii* isolated from Chinese patients. *Mycopathologia* 173:127–133. <https://doi.org/10.1007/s11046-011-9478-7>.
 27. Liao Y, Lu X, Yang S, Luo Y, Chen Q, Yang R. 2015. Epidemiology and outcome of *Trichosporon* fungemia: a review of 185 reported cases from 1975 to 2014. *Open Forum Infect Dis* 2:ofv141. <https://doi.org/10.1093/ofid/ofv141>.
 28. Ewing B, Green P. 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. *Genome Res* 8:186–194. <https://doi.org/10.1101/gr.8.3.186>.
 29. Gordon D. 2003. Viewing and editing assembled sequences using Consed. *Curr Protoc Bioinformatics* Chapter 11:Unit11.2. <https://doi.org/10.1002/0471250953.bi1102s02>.
 30. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425. <https://doi.org/10.1093/oxfordjournals.molbev.a040454>.
 31. Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120. <https://doi.org/10.1007/BF01731581>.
 32. Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874. <https://doi.org/10.1093/molbev/msw054>.
 33. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797. <https://doi.org/10.1093/nar/gkh340>.
 34. Gouy M, Guindon S, Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol Biol Evol* 27:221–224. <https://doi.org/10.1093/molbev/msp259>.
 35. Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25:1451–1452. <https://doi.org/10.1093/bioinformatics/btp187>.
 36. Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16:37–48. <https://doi.org/10.1093/oxfordjournals.molbev.a026036>.
 37. Clinical and Laboratory Standards Institute. 2017. Reference method for broth dilution antifungal susceptibility testing of yeasts. CLSI document M27-4th ed. Clinical and Laboratory Standards Institute, Wayne, PA.